# Lamivudine in combination with zidovudine, stavudine, or didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial

**Daniel R. Kuritzkes*, Ian Marschner†, Victoria A. Johnson‡, Roland Bassett†, Joseph J. Eron§, Margaret A. Fischl‖, Robert L. Murphy¶, Kenneth Fife**, Janine Maenza††, Mary E. Rosandich*, Dawn Bell‡‡, Ken Wood§§, Jean-Pierre Sommadossi‡, Carla Pettinelli‖‖ and the National Institute of Allergy and Infectious Disease AIDS Clinical Trials Group Protocol 306 Investigators**

**Objective:** To study the antiviral activity of lamivudine (3TC) plus zidovudine (ZDV), didanosine (ddI), or stavudine (d4T).

**Design:** Randomized, placebo-controlled, partially double-blinded multicenter study.

**Setting:** Adult AIDS Clinical Trials Units.

**Patients:** Treatment-naive HIV-infected adults with 200–600 $\times 10^6$ CD4 T lymphocytes/l.

**Interventions:** Patients were openly randomized to a d4T or a ddI limb, then randomized in a blinded manner to receive: d4T (80 mg/day), d4T plus 3TC (300 mg/day), or ZDV (600 mg/day) plus 3TC, with matching placebos; or ddI (400 mg/day), ddI plus 3TC (300 mg/day), or ZDV (600 mg/day) plus 3TC, with matching placebos. After 24 weeks 3TC was added for patients assigned to the monotherapy arms.

**Main outcome measure:** The reduction in plasma HIV-1 RNA level at weeks 24 and 48.

---

From the *University of Colorado Health Sciences Center and Veterans Affairs Medical Center, Denver, Colorado, the †Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, Massachusetts, the ‡University of Alabama at Birmingham School of Medicine and Birmingham Veterans Affairs Medical Center, Birmingham, Alabama, the §University of North Carolina at Chapel Hill, North Carolina, the ‖University of Miami, Miami, Florida, the ¶Northwestern University, Chicago, Illinios, **Indiana University, Indianapolis, Indiana, the ††Johns Hopkins University School of Medicine, Baltimore, Maryland, the ‡‡Adult AIDS Clinical Trials Group Operations Center, Rockville, Maryland, the §§Frontier Science & Technology Research Foundation, Amherst, New York and the ‖‖Division of AIDS, NIAID, NIH, Bethesda, Maryland, USA.

Sponsorship: Supported in part by the AIDS Clinical Trials Group (AI-38858), Statistical and Data Management Center (AI-38855) and AIDS Clinical Trials Unit awards from the NIAID (AI-32770, AI-32775) and by Virology Advanced Technical Laboratory Awards from the Adult AIDS Clinical Trials Group (subcontracts of AI-38858). Additional support was received from General Clinical Research Grants RR-00046 and RR-00051 and the Flow Cytometry Core Laboratory of the University of Colorado Cancer Center (CA-46934). Victoria Johnson was supported in part by a research grant from the NIH (AI-40876).

Note. Presented in part at the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, February 1–5, 1998. Abstract 1.

Requests for reprints to: D.R. Kuritzkes, Division of Infectious Disease, University of Colorado Health Sciences Center, 4200 E. Ninth Ave B-168, Denver, CO 80262, USA.

Date of receipt: 9 November 1998; revised: 25 January 1999; accepted: 1 February 1999.

**Results:** Two hundred ninety-nine patients were enrolled. After 24 weeks the mean reduction in plasma HIV-1 RNA copies/ml from baseline was 0.49 $\log_{10}$ (d4T monotherapy) versus 1.03 $\log_{10}$ (d4T plus 3TC; $P = 0.001$), and 0.68 $\log_{10}$ (ddI monotherapy) versus 0.82 $\log_{10}$ (ddI plus 3TC; $P > 0.22$). After 48 weeks the mean reduction was 1.08 $\log_{10}$ (d4T plus 3TC) versus 1.01 $\log_{10}$ (ZDV plus 3TC) in the d4T limb ($P = 0.66$), and 0.94 $\log_{10}$ (ddI plus 3TC) versus 0.88 $\log_{10}$ (ZDV plus 3TC; $P = 0.70$) in the ddI limb.

**Conclusions:** 3TC added significantly to the virologic effects of d4T, but not ddI, in treatment-naive patients. 3TC plus d4T produced virologic changes comparable to those of 3TC plus ZDV. These results support the use of 3TC with either ZDV or d4T as a component of initial combination antiretroviral therapy.



*AIDS* 1999, **13**:685–694

**Keywords: HIV, lamivudine, zidovudine, stavudine, combination therapy, antiretroviral therapy**

## Introduction

Current treatment guidelines for the treatment of HIV-1 infection recommend the use of a combination of nucleoside analogue inhibitors of reverse transcriptase together with a protease inhibitor or non-nucleoside reverse transcriptase inhibitor [1,2]. Most studies cited in support of these guidelines have used the combination of zidovudine (ZDV) and lamivudine (3TC) as the nucleoside component of such triple-therapy regimens. The combination of 3TC plus ZDV results in greater virologic and immunologic improvement than either drug alone and confers significant clinical benefit despite the rapid emergence of 3TC resistance [3–8]. Resistance to 3TC is conferred by a point mutation in the HIV-1 reverse transcriptase gene that results in a methionine to valine substitution at codon 184 (M184V) [9–12]; within weeks of initiating 3TC, wild-type HIV-1 in the plasma is replaced by variants carrying this mutation [13]. Introduction of the M184V mutation restores ZDV susceptibility in HIV-1 strains that carry ZDV resistance mutations [11,14,15]. Moreover, the frequency of ZDV resistance mutations in HIV-1 isolates from patients receiving ZDV plus 3TC is significantly lower than that seen during ZDV monotherapy [4,16]. Therefore it has been proposed that the sustained activity of 3TC in combination with ZDV is due to the prevention or reversal of ZDV resistance [16].

Because a number of HIV-1-infected individuals cannot tolerate ZDV it was important to determine whether 3TC could be used in combination with other available nucleoside analogues. However, if the benefits of 3TC are due solely to suppression of ZDV resistance, the rapid emergence of 3TC resistance could limit the activity of treatment regimens in which 3TC is combined with other nucleoside analogues such as didanosine (ddI) or stavudine (d4T) for which similar favorable resistance interactions have not been described. Several uncontrolled pilot studies suggest that the combination of 3TC plus d4T produces sustained virologic and immunologic improvement [17,18]. Studies to examine the combination of 3TC with ddI, a nucleoside analogue that also confers significant clinical benefit, have not been performed. The AIDS Clinical Trials Group (ACTG) protocol 306 tested the hypothesis that 3TC was uniquely effective in combination with ZDV and superior to 3TC in combination with either d4T or ddI.

## Methods

This multicenter, randomized, placebo-controlled, double-blind trial was conducted by 21 collaborating units of the Adult ACTG. The protocol was reviewed and approved by the institutional review boards of: the Northwestern University, University of North Carolina, Indiana University, University of Colorado Health Sciences Center, John Hopkins University, University of Alabama at Birmingham School of Medicine, University of Rochester Medical Center, Stanford University, Ohio State University, University of Washington, University of California, San Diego, University of Puerto Rico, University of California, Los Angeles, Washington University, St. Louis, University of Miami, University of Pennsylvania, University of Hawaii, Case Western Reserve University, Beth Israel Deaconess Medical Center, Vanderbilt University Medical Center. Informed consent was obtained from all subjects participating in this study. Conduct of this study conformed to human experimentation guidelines of the US Department of Health and Human Services.

### Patients

Individuals with documented HIV-1 infection, at least 12 years of age with CD4 T-lymphocyte counts of

200–600 × $10^6$/l and less than 7 days of antiretroviral nucleoside analogue treatment were eligible. Eligibility criteria also included a Karnofsky score ≥ 80%, a hemoglobin of ≥ 90 g/l for men and ≥ 85 g/l for women, a neutrophil count of ≥ 1000 × $10^6$ cells/l, a serum creatinine concentration ≤ 1.5 times the upper limit of normal, serum concentrations of alanine aminotransferase and aspartate aminotransferase ≤ 5 times the upper limit of normal, and a serum amylase concentration ≤ 1.5 times the upper limit of normal (unless the serum lipase concentration was ≤ 1.5 times the upper limit of normal). Patients were excluded if they had unexplained temperature elevations to ≥ 38.5°C for 7 consecutive days within 30 days prior to randomization, had a history of acute or chronic pancreatitis, had moderate peripheral neuropathy [≥ grade 2 according to the National Institute of Allergy and Infectious Disease (NIAID) Division of AIDS Table for Grading Adult Adverse Experiences], or were pregnant. Patients were also excluded if they had received any antiretroviral agents within 90 days of randomization or had malignancies that required systemic cytotoxic chemotherapy.

## Study design and treatment regimens

Patients entering step 1 of the study were first randomized openly to either a d4T or ddI limb, then randomized within each limb in a double-blind fashion to receive one of three treatments in a 1.5 : 1.5 : 1 ratio, respectively (Fig. 1). The allotted sample size was greater in the combination therapy arms as it was expected that the variability of virologic response would be greater [3,19]. In the d4T limb, patients received either ZDV plus 3TC with d4T placebo, d4T plus 3TC with ZDV placebo, or d4T with ZDV and 3TC placebos. In the ddI limb, patients received either ZDV plus 3TC with ddI placebo, ddI plus 3TC with ZDV placebo, or ddI with ZDV and 3TC placebos. At study week 24, patients entered step 2 of the study: patients on monotherapy had 3TC added to their regimen in a blinded fashion (beyond week 24 these arms are referred to as the d4T/ or ddI/delayed 3TC arms, respectively). Patients on combination therapy remained on their originally assigned regimens. Step 2 continued for an additional 24 weeks for a total study duration of 48 weeks. A subset of patients at four sites were also enrolled in a pharmacology substudy to evaluate pharmacokinetic interactions of 3TC with either ZDV, d4T or ddI, which will be reported separately.

ZDV (Retrovir) and 3TC (Epivir) were provided by GlaxoWellcome, Research Triangle Park, North Carolina, USA; d4T (Zerit) and ddI (Videx) were provided by Bristol-Myers Squibb, Wallingford, Connecticut, USA. ZDV was given as three 100-mg tablets and 3TC as one 150-mg tablet twice daily. d4T was given as one 40-mg tablet and ddI as two 100-mg chewable tablets twice daily for patients weighing ≥ 60 kg and d4T as one 30-mg tablet and ddI as one 100-mg tablet and one 25-mg tablet twice daily for patients weighing < 60 kg. Patients could receive ddI (or matching placebo) as an oral suspension of the equivalent dose made up from the pediatric oral formulation.

## Measurements and follow-up

Study visits included plasma HIV-1 RNA titer, CD4 T-lymphocyte counts, a clinical assessment and safety laboratory tests at pre-entry and study entry, and at weeks 2, 4, 8, 12, 16, 20, 24, 28, 36, 44, and 48.

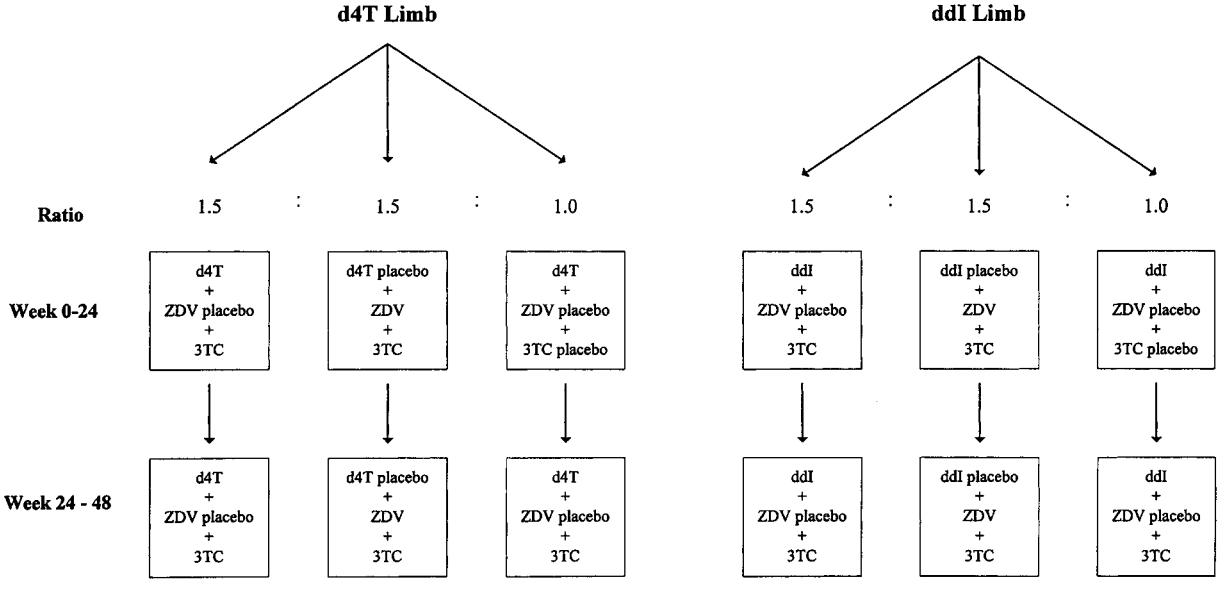


**Fig. 1.** Schema showing study design and treatment allocation for ACTG protocol 306. ZDV, zidovudine; d4T, stavudine; ddI, didanosine; 3TC, lamivudine.

Patients who prematurely discontinued study treatment were encouraged to return for all scheduled study visits and were included in all analyses. For the primary analyses for step 1 and step 2, all plasma HIV-1 RNA assays were performed in batch for each step by the branched DNA assay (HIV-1 Quantiplex Version 2.0, Chiron Corp., Emeryville, California, USA; limit of detection, 500 copies/ml) in a single laboratory (University of Colorado Health Sciences Center). As a secondary analysis, plasma HIV-1 RNA assays were repeated on samples from baseline, weeks 20 and 24, and weeks 44 and 48 using the 'ultrasensitive' modification of a reverse transcriptase (RT)–PCR assay (Amplicor HIV-1 Monitor, Roche Molecular Systems, Branchburg, New Jersey, USA; limit of detection, 50 copies/ml). Lymphocyte subsets were enumerated at each participating site by using a standardized, quality-controlled flow cytometry protocol [20].

The primary endpoints for the study were mean change from baseline in plasma HIV-1 RNA at weeks 24 and 48, the maximum decrease in plasma HIV-1 RNA within the first 8 weeks, and the occurrence of serious adverse events leading to treatment discontinuation. Secondary endpoints included increases in mean CD4 T-lymphocyte count from baseline at weeks 24 and 48, and the proportion of patients with plasma HIV-1 RNA levels below the limit of detection at weeks 24 and 48. HIV-1 RNA levels and CD4 T-lymphocyte counts at week 24 and at week 48 were defined as the average of the results at weeks 20 and 24, or 44 and 48, respectively. Additional secondary endpoints were change in CD4 cell count within the first 8 weeks; time to return to 50% reduction of plasma HIV-1 RNA from baseline, and area under the curve below baseline for plasma HIV-1 RNA levels and area under the curve above baseline for CD4 T-lymphocyte count.

### Statistical analysis

CD4 T-lymphocyte counts were analyzed on an absolute scale, whereas a $\log_{10}$ transformation was used in the analysis of HIV-1 RNA levels. Descriptive analyses and all statistical comparisons of change in the HIV-1 RNA levels were first performed by replacing any measurement below 500 copies/ml using the branched DNA assay by the value of 500 copies/ml. Because this approach can introduce bias into the treatment comparisons by underestimating the true magnitude of the HIV-1 RNA reduction, additional analyses were performed that adjusted for the effects of censoring by treating the change in HIV-1 RNA level as a censored normally distributed outcome variable [21]. Analyses of the primary endpoint were also repeated adjusting for baseline HIV-1 RNA level and CD4 T-lymphocyte counts; however, these additional analyses are not reported because the results were similar to analyses which did not adjust for baseline HIV-1 RNA level and CD4 T-lymphocyte counts. Secondary analyses that used plasma HIV-1 RNA data generated by the ultrasensitive RT–PCR assay were performed as for the branched DNA assay data, with the exception that the lower limit of detection was taken to be 50 copies/ml, and an upper limit of detection was imposed of 50 000 copies/ml.

All analyses were conducted using an intent-to-treat approach. In all cases, time was measured from the date of dispensation of protocol treatment. Analyses of changes in immunologic and virologic parameters used an analysis of variance (both with and without adjusting for censoring). Differences in proportions below the limit of detection of plasma HIV-1 RNA were compared among treatments using Fisher's exact test [22]. For the analysis of the proportion of patients with plasma HIV-1 RNA levels below the limit of detection at weeks 20/24 and weeks 44/48 it was required that measurements at both weeks were below the limit of detection to be considered undetectable (patients with only one measurement were classified as detectable or undetectable on the basis of the available measurement). Time-to-event distributions were estimated using the method of Kaplan and Meier and compared between treatment arms using the log-rank test [23]. Analyses of safety data were censored at 56 days after the date of the last dose of protocol treatment, except that all safety follow-up was censored at 50 weeks after the date of treatment dispensation.

## Results

### Patient disposition and follow-up

Between December 1995 and July 1996 299 patients were randomly assigned to treatment . Three patients in each limb were randomized but never started treatment, and one patient was randomized to the study and found to be ineligible due to prior ZDV use. These patients were excluded from analyses.

Table 1 contains the baseline characteristics for patients by treatment arm. In the d4T limb, 86% of the patients were male, 60% were white, non-Hispanic and the median age was 36 years. The median CD4 T-lymphocyte count was $407 \times 10^6$/l, and the median HIV-1 RNA was 11 137 (4.05 $\log_{10}$) copies/ml. In the ddI limb, 84% of the patients were male, 55% were white non-Hispanic, and the median age was 33 years. The median CD4 T-lymphocyte count was 391 × /l, and the median HIV-1 RNA level was 11 422 (4.06 $\log_{10}$) copies/ml. Baseline characteristics were well balanced across treatment arms and limbs.

Of the 146 patients who began study treatment in the d4T limb, 146 (100%) remained on study and 136

**Table 1.** Baseline characteristics by treatment arm.

| Characteristic | Treatment arm | | | Total |
|---|---|---|---|---|
| Stavudine (d4T) limb | ZDV/3TC | d4T/3TC | d4T/delayed 3TC | |
| n | 54 | 57 | 35 | 146 |
| Male (%) | 87 | 86 | 83 | 86 |
| White non-Hispanic (%) | 59 | 63 | 57 | 60 |
| Median age (years) | 35 | 37 | 35 | 36 |
| Median weight (kg) | 77 | 77 | 80 | 78 |
| Median (range) CD4 T lymphocytes ($\times 10^6$/l) | 401 (122–675) | 405 (216–716) | 424 (212–630) | 407 (122–716) |
| Median (range) plasma HIV-1 RNA ($\log_{10}$ copies/ml) | 4.13 (2.70–5.41) | 4.15 (2.70–5.46) | 4.00 (2.70–5.50) | 4.05 (2.70–5.51) |
| Didanosine (ddI) limb | ZDV/3TC | ddI/3TC | ddI/delayed 3TC | |
| n | 54 | 55 | 37 | 146 |
| Male (%) | 87 | 80 | 84 | 84 |
| White non-Hispanic (%) | 59 | 47 | 59 | 55 |
| Median age (years) | 33 | 35 | 31 | 33 |
| Median weight (kg) | 80 | 78 | 74 | 76 |
| Median (range) CD4 T lymphocytes ($\times 10^6$/l) | 386 (201–624) | 387 (150–663) | 398 (206–689) | 391 (150–689) |
| Median (range) plasma HIV-1 RNA ($\log_{10}$ copies/ml) | 4.08 (2.70–5.85) | 4.01 (2.70–5.78) | 4.08 (2.70–5.41) | 4.06 (2.70–5.85) |

ZDV, Zidovudine; 3TC, lamivudine.

(94.4%) remained on study treatment through week 24; 128 (87.7%) patients remained on study and 118 (80.8%) remained on study treatment through week 48. In the ddI limb, of the 146 patients who began study treatment 144 (98.6%) remained on study and 135 (92.5%) remained on study treatment through week 24; 121 (82.9%) patients remained on study and 106 (72.6%) remained on study treatment through week 48. There were no significant differences in the rate of premature discontinuation of study treatment.

## Virologic endpoints

A pronounced initial decline in HIV-1 RNA level from baseline that was sustained out to 24 weeks was evident in all treatment arms (Fig. 2). After week 24 the addition of 3TC to d4T monotherapy led to an additional mean reduction in plasma HIV-1 RNA level of 0.26 $\log_{10}$ ($P = 0.096$), which increased to 0.44 $\log_{10}$ after adjustment for censoring ($P = 0.042$). The addition of 3TC to ddI monotherapy led to an additional reduction of 0.42 $\log_{10}$ in plasma HIV-1 RNA [$P = 0.002$; 0.79 $\log_{10}$ after adjustment for censoring ($P = 0.021$)]. At week 48 the combination treatment arms in both limbs all showed sustained reductions from baseline in HIV-1 RNA level [on the order of 1.0 $\log_{10}$ (unadjusted)].

The mean change in plasma HIV-1 RNA level from baseline to week 20/24 was significantly greater for the d4T/3TC arm than the d4T monotherapy arm before and after adjustment for censoring (0.54 $\log_{10}$ before and 1.04 $\log_{10}$ after adjustment, respectively; $P \leq 0.001$ in each case; Fig. 2A). There was a greater decrease in mean plasma HIV-1 RNA level in the d4T/3TC arm than in the ZDV/3TC arm at week 20/24 (0.22 $\log_{10}$) that was not statistically significant in the unadjusted analysis ($P = 0.14$), but which approached significance after adjustment for censoring (0.54 $\log_{10}$; $P = 0.052$). However, no significant difference in virologic response between the d4T/3TC and ZDV/3TC arms was observed at week 44/48 ($P > 0.66$; Table 2).

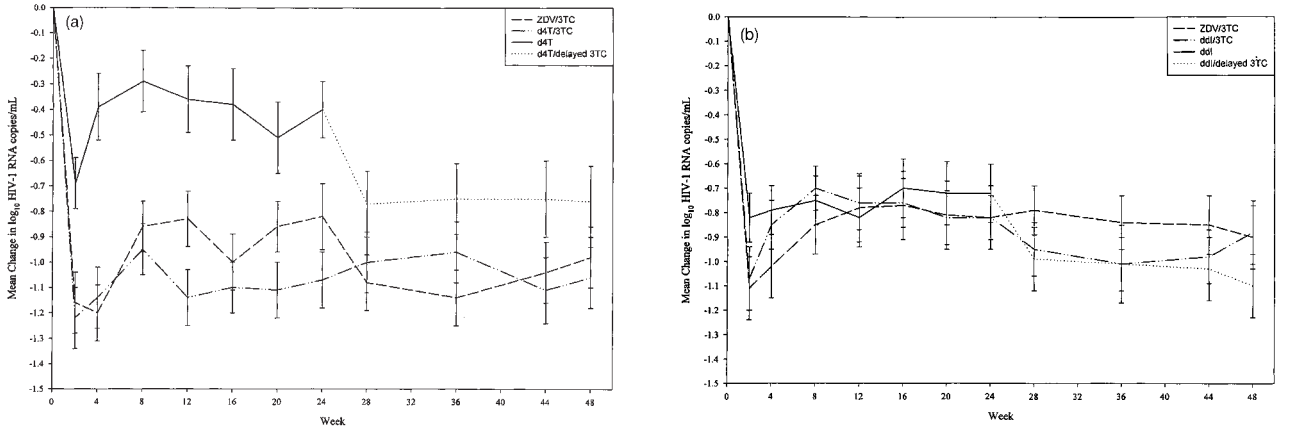


**Fig. 2.** Mean change from baseline in plasma HIV-1 RNA level. (a) Stavudine limb; (b) didanosine limb. ZDV, zidovudine; d4T, stavudine; ddI, didanosine; 3TC, lamivudine.

**Table 2.** Change in $\log_{10}$ plasma HIV-1 RNA copies/ml from baseline to weeks 20/24 and 44/48.

| | 20/24 Weeks | | | | | 44/48 Weeks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | Mean change | *P* unadjusted | Mean change adjusted* | *P* adjusted | n | Mean change | *P* unadjusted | Mean change adjusted* | *P* adjusted |
| Stavudine (d4T) limb | | | | | | | | | | |
| ZDV/3TC | 51 | –0.81 | 0.14[†] | –1.05 | 0.052[†] | 50 | –1.01 | 0.66[†] | –1.50 | 0.95[†] |
| d4T/3TC | 53 | –1.03 | 0.001[‡] | –1.59 | 0.001[‡] | 52 | –1.08 | 0.081[‡] | –1.50 | 0.089[‡] |
| d4T/delayed 3TC | 34 | –0.49 | – | –0.55 | – | 30 | –0.76 | – | –0.96 | – |
| Didanosine (ddI) limb | | | | | | | | | | |
| ZDV/3TC | 50 | –0.80 | 0.92[§] | –1.13 | 0.64[§] | 43 | –0.88 | 0.70[§] | –1.27 | 0.78[§] |
| ddI/3TC | 51 | –0.82 | 0.33[‖] | –1.08 | 0.31[‖] | 44 | –0.94 | 0.34[‖] | –1.15 | 0.14[‖] |
| ddI/delayed 3TC | 36 | –0.68 | – | –0.90 | – | 33 | –1.10 | – | –1.61 | – |

*Adjusted for censoring. [†]Zidovudine (ZDV)/lamivudine (3TC) versus d4T/3TC. [‡]d4T/3TC versus d4T/delayed 3TC. [§]ZDV/3TC versus ddI/3TC. [‖]ddI/3TC versus ddI/delayed 3TC.

**Table 3.** Maximum change in $\log_{10}$ plasma HIV-1 RNA copies/ml from baseline in the first 8 weeks.

| | n | Mean change | *P* unadjusted | Mean change adjusted* | *P* adjusted |
|---|---|---|---|---|---|
| Stavudine (d4T) limb | | | | | |
| ZDV/3TC | 52 | –1.28 | 0.72[†] | –3.59 | 0.60[†] |
| d4T/3TC | 57 | –1.23 | 0.003[‡] | –3.56 | 0.001[‡] |
| d4T/delayed 3TC | 34 | –0.74 | – | –1.20 | – |
| Didanosine (ddI) limb | | | | | |
| ZDV/3TC | 52 | –1.20 | 0.82[§] | –3.77 | 0.13[§] |
| ddI/3TC | 54 | –1.16 | 0.068[‖] | –1.94 | 0.003[‖] |
| ddI/delayed 3TC | 36 | –0.90 | – | –1.36 | |

*Adjusted for censoring. [†]Zidovudine (ZDV)/lamivudine (3TC) versus d4T/3TC. [‡]d4T/3TC versus d4T/delayed 3TC. [§]ZDV/3TC versus ddI/3TC. [‖]ddI/3TC versus ddI/delayed 3TC.

In the ddI limb there was little difference between the three treatment arms with respect to changes in HIV-1 RNA level at week 20/24 ($P > 0.20$ in all cases, with or without adjustment for censoring; Fig. 2B; Table 2). Similarly, there were no statistically significant differences between the two combination arms (ddI/3TC and ZDV/3TC) with respect to changes in HIV-1 RNA level at week 44/48, with or without adjustment for censoring ($P \geq 0.14$ in all cases, Table 2).

## Maximum reductions in HIV-1 RNA during the first 8 weeks

The d4T/3TC combination achieved a maximum reduction in mean plasma HIV-1 RNA level that was 0.49 $\log_{10}$ greater than observed in the d4T monotherapy arm during this initial period ($P \leq 0.003$; Table 3). There was evidence of a moderate difference in maximum HIV-1 RNA decrease between the ddI and ddI/3TC arms. Prior to adjustment for censoring, the difference was 0.26 $\log_{10}$ in favor of the ddI/3TC arm; after adjustment this difference increased to 0.58 $\log_{10}$ ($P = 0.068$ and 0.003, respectively; Table 3).

## Results of ultrasensitive plasma HIV-1 RNA testing

With the exception of the d4T monotherapy arm, median plasma HIV-1 RNA levels at weeks 20 and 24 and weeks 44 and 48 were equal to or less than the threshold of detection for the branched DNA assay (500 copies/ml). In view of the high proportion of patients below the threshold of detection, additional analyses were performed using plasma HIV-1 RNA levels determined by an ultrasensitive RT–PCR assay. Less than 20% of patients in any treatment arm achieved a plasma HIV-1 RNA level below the limits of detection of this assay (< 50 copies/ml). Treatment

**Table 4.** Estimated change in $\log_{10}$ plasma HIV-1 RNA copies/ml from baseline using ultrasensitive reverse transcriptase–PCR assay.

| | 20/24 Weeks | | | 44/48 Weeks | | |
|---|---|---|---|---|---|---|
| | n | Estimated mean change | *P** | n | Estimated mean | *P** |
| Stavudine (d4T) limb | | | | | | |
| ZDV/3TC | 52 | –1.57 | 0.77[†] | 52 | –1.51 | 0.47[†] |
| d4T/3TC | 54 | –1.64 | 0.0001[‡] | 52 | –1.51 | 0.064[‡] |
| d4T/delayed 3TC | 34 | –0.47 | – | 30 | –1.19 | – |
| Didanosine (ddI) limb | | | | | | |
| ZDV/3TC | 44 | –1.40 | 0.15[§] | 40 | –1.23 | 0.79[§] |
| ddI/3TC | 53 | –1.16 | 0.52[‖] | 47 | –1.27 | 0.17[‖] |
| ddI/delayed 3TC | 35 | –1.32 | – | 30 | –1.78 | – |

*Adjusted for upper and lower censoring. [†]Zidovudine (ZDV)/lamivudine (3TC) versus d4T/3TC. [‡]d4T/3TC versus d4T/delayed 3TC. [§]ZDV/3TC versus ddI/3TC. [‖]ddI/3TC versus ddI/delayed 3TC.

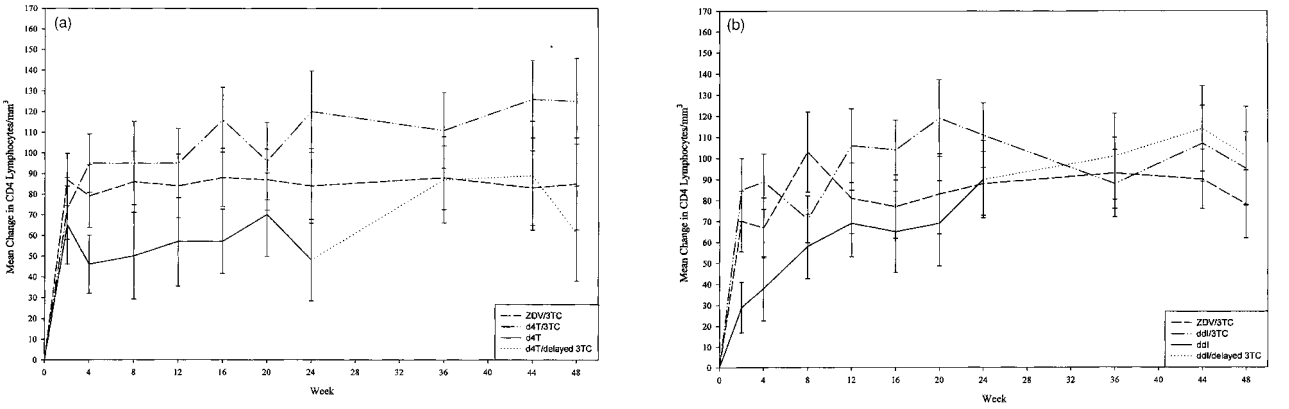


**Fig. 3.** Mean change from baseline in CD4 T-lymphocyte count. (a) Stavudine limb; (b) didanosine limb. ZDV, zidovudine; d4T, stavudine; ddI, didanosine; 3TC, lamivudine.

arms did not differ with respect to the proportion of patients below the limit of detection at weeks 20 and 24 and weeks 44 and 48 ($P \geq 0.095$ in all cases).

Table 4 summarizes analyses of the mean change in HIV-1 RNA levels at weeks 20/24 and 44/48 using results of the ultrasensitive RT–PCR assay adjusted for the upper and lower measurement limits (50 000 and 50 copies/ml, respectively). Within the d4T limb, the difference between the d4T/3TC and d4T/delayed 3TC arms was highly significant at week 20/24 ($P < 0.0001$) and marginally significant at week 44/48 ($P = 0.064$). The difference between the ZDV/3TC and d4T/3TC arms was insignificant at both week 20/24 ($P = 0.77$) and 44/48 ($P = 0.47$), as were the differences at weeks 20/24 ($P = 0.15$) or 44/48 ($P = 0.79$) between the ZDV/3TC and ddI/3TC arms, or between the ddI/3TC and ddI/delayed 3TC arms ($P = 0.52$ and $P = 0.17$ for weeks 20/24 and 44/48, respectively).

### Change in CD4 T-lymphocyte count

In all treatment arms there was an initial increase in mean CD4 T-lymphocyte count, which was sustained out to 48 weeks (Fig. 3). In the d4T limb, the mean increase in CD4 T-lymphocyte count at week 44/48 ranged from $80 \times 10^6$ CD4 T lymphocytes/l (d4T/delayed 3TC arm) to $118 \times 10^6$ CD4 T lymphocytes/l (d4T/ 3TC arm). In the ddI limb, the mean increase in CD4 T-lymphocyte count at week 44/48 ranged from $77 \times 10^6$ CD4 T lymphocytes/l (ZDV/3TC arm) to $109 \times 10^6$ CD4 T lymphocytes/l (ddI/delayed 3TC arm). None of the statistical comparisons of change in CD4 T lymphocytes were significant in either limb, although in both limbs the ZDV/ 3TC arm had CD4 T-lymphocyte increases that were approximately 30 cells less than the other combination therapy arm.

### Adverse events

Study treatment was tolerated well overall. In the d4T limb, a severe (grade 3) or worse toxicity occurred in 17 out of 54 (31%) patients on ZDV/3TC, 13 out of 57 (23%) patients on d4T/3TC, and 10 out of 35 (29%) patients on d4T/delayed 3TC ($P = 0.39$ for the two combination arms; $P = 0.62$ for d4T/3TC versus d4T/delayed 3TC). Of patients on the ddI limb, seven out of 54 (13%) patients on ZDV/3TC, 14 out of 55 (25%) patients on ddI/3TC, and 11 out of 37 (30%) patients on ddI/delayed 3TC experienced a severe or worse toxicity ($P = 0.14$ for ZDV/3TC versus ddI/3TC; $P = 0.81$ for ddI/3TC versus ddI/delayed 3TC). The most frequent severe or worse laboratory abnormalities were abnormalities of liver function tests and neutropenia; the most frequent severe or worse signs or symptoms included aches and pains, malaise or fatigue, and gastrointestinal complaints. Fourteen out of 292 (4.8%) patients permanently discontinued study treatment as a result of severe or worse toxicity. The time to first severe or worse laboratory toxicity or severe or worse sign or symptom did not differ significantly between treatment arms in either limb.

One patient experienced clinical progression to AIDS during the study. This patient, who was assigned to ddI/3TC, died at week 16 because of cytomegalovirus infection; the patient had discontinued study therapy at the time of developing the infection. Two other patients died during the course of the study of causes unrelated to HIV-1 infection or study treatment.

## Discussion

This study was a randomized, double-blind, placebo-controlled comparison of the virologic and immunologic activity of 3TC in combination with either ZDV, d4T or ddI in previously untreated HIV-1-infected individuals with $200–600 \times 10^6$ CD4 T lymphocytes/l. The combination of d4T plus 3TC had significantly greater virologic activity than d4T alone, and was comparable to the combination of ZDV plus 3TC. The

ddI/3TC combination resulted in greater virologic and immunologic responses during the first 8 weeks of treatment as compared to ddI monotherapy, but this difference was diminished (and was insignificant) by week 24. There were no significant differences in virologic and immunologic response at weeks 24 and 48 for the ZDV/3TC and ddI/3TC combinations. Although the combination therapy arms tended to have greater increases in CD4 T-lymphocyte counts than the initial monotherapy/delayed 3TC arms, these differences were not statistically significant.

Results of the d4T limb of our trial are the first to demonstrate, in a controlled fashion, comparable antiviral effects of d4T/3TC and ZDV/3TC, extending the results of uncontrolled pilot studies [17,18]. Our results show conclusively that 3TC adds significantly to the activity of d4T, and that the combination of d4T plus 3TC has virologic activity that is similar to that of ZDV plus 3TC. Thus, the hypothesis that 3TC is uniquely active in combination with ZDV was disproved. Factors other than initial antiretroviral activity such as toxicity, ease of administration, or cost need to be considered in choosing whether to use d4T/3TC or ZDV/3TC as part of initial potent combination regimens.

Results of the ddI limb are consistent with those of other studies that have compared ddI to other nucleosides in the treatment of HIV-1 infection. Two studies by the ACTG, one in children and one in adults, found that the clinical benefits of ZDV plus ddI were not superior to those of ddI alone [24,25]. Moreover, in the adult study, ACTG Protocol 175, the combination of ZDV plus ddI was associated with greater reduction in plasma HIV-1 RNA titers than was ddI monotherapy at week 8, but these differences were not significant at week 56 [26]. Similarly, we found that 3TC plus ddI was associated with greater suppression of plasma HIV-1 RNA than was ddI alone at week 8, but this difference was not significant by week 24. Although the difference in antiviral activity of the ddI monotherapy and ZDV/3TC arms was not formally tested, the two arms had comparable antiviral activity. This result appears to contradict results of another study ACTG Protocol 300, that showed a significant reduction in progression to AIDS and death for HIV-1-infected children, who received ZDV plus 3TC as compared with ddI alone [27]. In that study, the difference in treatment benefit was observed only in children under 3 years of age. However, the very small number of clinical endpoints observed in children > 3 years of age may have precluded detection of any treatment differences in older children.

Treatment in the present study was well-tolerated in all arms. Only 14 out of 292 (4.8%) patients permanently discontinued therapy as a result of severe or worse toxicity. Addition of 3TC to d4T or ddI did not result in any unexpected toxicities or an increase in toxicity. Thus, this study demonstrates that 3TC can safely be added to ZDV, d4T or ddI.

A limitation of the current study was the moderately low plasma HIV-1 RNA level of the participants at study entry (around 4.0 $\log_{10}$ copies/ml). As the branched DNA assay has a threshold for detection of 500 copies/ml, the maximum observable decrease in plasma HIV-1 RNA for half of the patients was 20-fold (1.3 $\log_{10}$) or less. These factors tended to underestimate the true magnitude of suppression of HIV-1 by the study treatments and may have limited our ability to discern differences among the treatment arms. This difficulty was avoided to some extent by the use of statistical methods that treated values that fell below 500 copies/ml as censored data. Moreover, the qualitative results of the study were unchanged when analyzed using the results of the ultrasensitive RT–PCR assay and were comparable to previously reported results of ZDV/3TC trials [3,5].

In summary, results of the present study, which may represent the last opportunity to compare activities of nucleoside combinations that contribute to potency of currently recommended three-drug regimens, suggest that 3TC can be combined safely and effectively with either ZDV or d4T. 3TC can also safely be combined with ddI, but this combination did not provide a significant advantage over ddI monotherapy. Further exploration of the combination of 3TC and ddI is warranted, since the prolonged intracellular half-lives of the respective nucleoside triphosphates might make it possible to dose both drugs once a day. These combinations should be used as part of potent regimens that include a protease inhibitor or non-nucleoside reverse transcriptase inhibitor as a third agent [2].

## References


1. Centers for Disease Control and Prevention: **Report of the NIH panel to define principles of therapy of HIV infection and guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents.** *MMWR* 1998, **47**:No.RR-5.
2. Carpenter CJ, Fischl MA, Hammer SM, *et al.*: **Antiretroviral therapy for HIV Infection in 1998.** *JAMA* 1998, **280**:78–86.
3. Eron JJ, Benoit SL, Jemsek J, *et al.*: **Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter.** *N Engl J Med* 1995, **333**:1662–1669.
4. Kuritzkes DR, Quinn JB, Benoit SL, *et al.*: **Drug resistance and virologic response in NUCA 3001, a randomized trial of lamivudine (3TC) versus zidovudine (ZDV) versus ZDV plus 3TC in previously untreated patients.** *AIDS* 1996, **10**:975–981.
5. Katlama C, Ingrand D, Loveday C, *et al.*: **Safety and efficacy of lamivudine–zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy.** *JAMA* 1996, **276**:118–125.
6. Bartlett JA, Benoit SL, Johnson VA, *et al.*: **Lamivudine plus zidovudine compared with zalcitabine plus zidovudine in patients with HIV infection. A randomized, double-blind, placebo-controlled trial.** *Ann Intern Med* 1996, **125**:161–172.

7. Phillips AN, Eron J, Bartlett J, *et al*.: **Correspondence between the effect of zidovudine plus lamivudine on plasma HIV level/CD4 lymphocyte count and the incidence of clinical disease in infected individuals.** *AIDS* 1997, **11**:169–175.
8. CAESAR Coordinating Committee. **Randomized trial of addition of lamivudine plus loviride to zidovudine-containing regimens for patients with HIV-1 infection: the CAESAR trial.** *Lancet* 1997, **349**:1413–1421.
9. Gao Q, Gu Z, Hiscott J, Dionne G, Wainberg MA. **Generation of drug-resistant variants of human immunodeficiency virus type 1 by in vitro passage in increasing concentrations of 2′,3′-dideoxycytidine and 2′,3′-dideoxy-3′-thiacytidine.** *Antimicrob Agents Chemother* 1993, **37**:130–133.
10. Boucher CAB, Cammack N, Schipper P, *et al*.: **High-level resistance to (–) enantiomeric 2′-deoxy-3′-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase.** *Antimicrob Agents Chemother* 1993, **37**:2231–2234.
11. Tisdale M, Kemp SD, Parry NR, Larder BA. **Rapid *in vitro* selection of human immunodeficiency virus type 1 resistant to 3′-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase.** *Proc Natl Acad Sci USA* 1993, **90**:5653–5656.
12. Schinazi RF, Lloyd RM, Nguyen MH, *et al*.: **Characterization of human immunodeficiency viruses resistant to oxathiolane–cytosine nucleosides.** *Antimicrob Agents Chemother* 1993, **37**:875–881.
13. Schuurman R, Nijhuis M, van Leeuwen R, *et al*.: **Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC).** *J Infect Dis* 1995, **171**:1411–1419.
14. Johnson VA, Wagner SF, Overbay CB, *et al*.: **Drug resistance and viral load in NUCA 3002:A comparative trial of lamivudine (3TC) (high or low dose)/zidovudine (ZDV) combination therapy vs ZDV/dideoxycytidine (ddC) combination therapy in ZDV-experienced (>24 weeks) patients (CD4 cells 100–300/mm$^3$).** *4th International Workshop on HIV Drug Resistance*. Sardinia, July 1995 [abstract 55].
15. Gass RJA, Shugarts D, Young R, Allen M, Rosandich M, Kuritzkes DR. **Emergence of dual resistance to zidovudine (ZDV) and lamivudine (3TC) in clinical HIV-1 isolates from patients receiving ZDV/3TC combination therapy.** *Antiviral Therapy* 1998, **3**:97–102.
16. Larder BA, Kemp SD, Harrigan PR. **Potential mechanism for sustained antiretroviral efficacy of AZT–3TC combination therapy.** *Science* 1995, **269**:696–699.
17. Rouleau D, Conway B, Raboud J, *et al*.: **Stavudine plus lamivudine in advanced human immunodeficiency virus disease: a short-term pilot study.** *J Infect Dis* 1997, **176**:1156–1160.
18. Katlama C, Valantin M-A, Matheron S, *et al*.: **Efficacy and tolerability of stavudine plus lamivudine in treatment-naive and treatment-experienced patients with HIV-1 infection.** *Ann Intern Med* 1998, **129**:525–531.
19. D'Aquila RT, Hughes MD, Johnson VA, *et al*.: **Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection.** *Ann Intern Med* 1996, **124**:1019–1030.
20. Gelman R, Cheng SC, Kidd P, Waxdal M, Kagan J. **Assessment of the effects of instrumentation, monoclonal antibody, and fluorochrome on flow cytometric immunophenotypic: a report based on 2 years of the NIAID DAIDS flow cytometry quality assessment program.** *Clin Immunol Immunopathol* 1993, **66**:150–162.
21. SAS Institute Inc.: *SAS/STAT User's Guide, Version 6. 4th Edn*. Carey: SAS Institute Inc.; 1989:997–1025.
22. Altman DG. *Practical Statistics for Medical Research*. London: Chapman & Hall; 1991.
23. Collett D. *Modeling Survival Data in Medical Research*. London: Chapman & Hall; 1994.
24. Hammer SM, Katzenstein DA, Hughes MD, *et al*.: **A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter.** *N Engl J Med* 1996, **335**:1081–1090.
25. Englund JA, Baker CJ, Raskino C, *et al*.: **Zidovudine, didanosine, or both as the initial treatment for symptomatic HIV-infected children.** *N Engl J Med* 1997, **336**:1704–1712.
26. Katzenstein DA, Hammer SM, Hughes MD, *et al*.: **The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter.** *N Engl J Med* 1996, **335**:1091–1098.
27. McKinney Jnr RE, Johnson GM, Stanley K, *et al*.: **A randomized study of combined zidovudine-lamivudine versus didanosine monotherapy in children with symptomatic therapy-naive HIV-1 infection. The Pediatric AIDS Clinical Trials Group Protocol Study Team.** *J Pediatrics* 1998, **133**:500–508.


## Acknowledgements

Dr. Johnson acknowledges core research facilities of the University of Alabama at Birmingham, Center for AIDS Research, and the Birmingham Veterans Affairs Medical Center. ZDV and 3TC were provided by GlaxoWellcome, Research Triangle Park, North Carolina, USA; ddI and d4T were provided by Bristol-Myers Squibb, Wallingford, Connecticut, USA. In addition, GlaxoWellcome and Bristol-Myers Squibb provided financial support for plasma HIV-1 RNA testing in this protocol.

Additional members of the ACTG 306 protocol team included A. Martinez and M. G. Ray. Assistance of the following individuals in performance of the ultrasensitive RT–PCR assays is gratefully acknowledged: Beth Israel Deaconess Medical Center: S. Hammer, V. Blais, R. Gee Nowak; Johns Hopkins University: B. Jackson, M. A. Bailey, J. Parsons, E. Piwowar-Manning; University of Rochester: L. Demeter, L. Berrios; ACTG Statistical and Data Analysis Center, Harvard School of Public Health: T. Ramacciotti. The authors also thank K. Kohler for expert editorial assistance.

This study could not have been completed without the participation of the following persons: Northwestern University and Rush Medical College: N. French, B. Sha, J. Pulvirenti; University of North Carolina and Carolinas Medical Center: J. Horton, J. Connell, T. Lane, C. Marcus; Indiana University: J. Black, S. Ryan, D. Heise; University of Colorado Health Sciences Center: S. Cannman, D. Torre, S. Johnson, R. Schooley; John Hopkins University: D. Baker, P. Brooks, R. Becker; University of Alabama at Birmingham School of Medicine: K. McPheeters, S. DeLoach, B. Hill, M. Saag; University of Rochester Medical Center: R. Reichman, M. Shoemaker, R. Hewitt, M. Lewis; Stanford University: T. Merigan, D. Katzenstein, P. Cain, D. Slamowitz; Ohio State University: M. Para, R. Fass; University of Washington: A. Collier, R. Vasquez, K. Novak, N. J. Conley; University of California, San Diego: D. Havlir, K. Nuffer; University of Puerto Rico: G. J. Vazquez, M. Cruz-Ortiz, I. Lopez, V. Ramirez; University of California, Los Angeles: S. Miles, A. Johiro, S. Chafey, S. McCarthy; Washington

University, St. Louis: W. Powderly, P. Tebas, T. Stiffler, M. Royal; University of Miami: A. Rodriguez, E. Scerpella, T. Tanner; University of Pennsylvania: S. Gluckman, H. Friedman, E. Donaghy, C. Helker; University of Hawaii: C. Shikuma, D. Ogata-Arakaki, M. Millard, S. Souza; Case Western Reserve University: R. Kalayjian, N. Frantz; Beth Israel Deaconess Medical Center: M. Albrecht, C. Wanke, C. Koziol, M. Hong; Beth Isreal Medical Center, Mt. Sinai: D. Mildvan, R. Yarrish; Vanderbilt University Medical Center: D. Haas, V. Harris, J. McKinsey, S. Raffanti; Frontier Science Technology and Research Foundation: C. Ranney; Glaxo-Wellcome: S. Benoit, G. Boone, N. Graham, J. Rooney, F. Rousseau; Bristol-Meyers Squibb: C. McLaren, J. Skovronski, M. Stevens.