Randomized Study of Didanosine Monotherapy and Combination Therapy With Zidovudine in Hemophilic and Nonhemophilic Subjects With Asymptomatic Human Immunodeficiency Virus-1 Infection

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To evaluate the safety and efficacy of didanosine (ddI) monotherapy and three different combinations of zidovudine (ZDV) and ddI in asymptomatic human immunodeficiency virus-1 (HIV-1) infection, we conducted an open-label, phase I/II study in 126 asymptomatic HIV-1-infected hemophilic and nonhemophilic subjects with a CD4 count of 200 to 500/mm³ stratified for prior zidovudine treatment and baseline CD4 count. Study arms included arm A, low-dose combination (ZDV 150 mg and ddI 134 mg, daily); arm B, moderate-dose combination (ZDV 300 mg and ddI 334 mg, daily); arm C, high-dose combination (ZDV 600 mg and ddI 500 mg, daily), and arm D, ddI monotherapy (ddI 500 mg, daily). Earlier, more frequent hepatotoxicity was experienced by hemophilic subjects (P = .008), but there were no differences in toxicity between treatment arms (P = .51), nor were there any differences in the rate of development of clinical endpoints by treatment (P = .41). Smaller median CD4 increases occurred over the first 12 weeks for arms A and D, 44/mm³ and 42/mm³, than arms B and C, 105/mm³ and 114/mm³, respectively, (P = .015). Hemophilia status (P = .0004) and prior ZDV experience (P = .044) independently predicted weaker CD4 responses during the first 12 weeks of treatment. Using a regression model and adjusting for hemophilia status, prior ZDV treatment, and baseline CD4, there was a significant reduction in quantitative viral load from baseline by week 12 for all treatment arms combined (P = .0001), with significantly lower median percent reduction for arm A (56.3%) than arms B, C, and D (94.6%, 98.5%, and 91.9%, respectively, P = .015). Although greater hepatotoxicity and weaker CD4 responses occur in hemophilic subjects, didanosine monotherapy and combination therapy with zidovudine are safe and effective in asymptomatic HIV-1–infected patients.

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prior history of pancreatitis, recent alcohol abuse, seizures within 6 months, peripheral neuropathy, past or current heart disease, history of gout, or patients receiving ZDV for greater than 13 months were excluded from study. The use of other anti-HIV drugs, biologic response modifiers, prophylaxis for Pneumocystis carinii pneumonia other than aerosolized pentamidine, systemic corticosteroids, or potentially neurotoxic drugs was not allowed. Persons of reproductive potential were counseled to practice adequate birth control.

Subjects were recruited from five hemophilia treatment centers and six AIDS Clinical Trials Group sites. All subjects gave written, informed consent approved by local institutional review boards.

Study Design

The study was an open-label, randomized, phase I/II trial. The only patients eligible for study during the first 6 weeks of enrollment were hemophiliacs and their HIV-infected sexual partners, some of whom had completed ACTG 036, after which eligible nonhemophiliac subjects were enrolled with the intent to compare safety and efficacy. Subjects were randomized to one of four treatment regimens, arm A (low-dose combination), 50 mg ZDV orally every eight hours (at 07:00, 14:00, and 22:00 hours) plus 67 mg ddI, as buffered solution orally every 12 hours (at 07:00 and 19:00); arm B (moderate-dose combination), 100 mg ZDV orally every eight hours plus 167 mg ddI as buffered solution every 12 hours; arm C (high-dose combination), 200 mg ZDV orally every eight hours plus 250 mg ddI, as buffered solution orally every 12 hours; or arm D (ddI alone), 250 mg ddI, as buffered solution orally every 12 hours. (Note that the administration of the study drug ddI in sachet formulation, 67 mg, 167 mg, and 250 mg, are equivalent to the licensed formulation of ddI in tablet form, 50 mg, 125 mg, and 200 mg, respectively.) Didanosine was administered on an empty stomach, at least 1 hour before or 2 hours after meals. Patients were randomized using permuted blocks with dynamic balancing by institution. Patients were stratified by CD4 count at entry (either <300/mm³ or ≥300/mm³) and by prior ZDV treatment (either "ZDV naive" [those not previously treated with ZDV] or "ZDV experienced" [those receiving ZDV for ≥13 months]). Subjects were advised not to drink alcohol during the study.

For serious treatment-related toxicity, treatment drugs were interrupted until the abnormal lab values or symptoms resolved or decreased. For any symptoms of pancreatitis, hyperamylasemia, or peripheral neuropathy, study medication was permanently discontinued. If serious toxic events persisted or recurred after two separate dose reductions or if life-threatening toxic events occurred, the study medication was permanently discontinued.

Evaluation of Subjects

Pretreatment evaluation included a medical history, physical examination, and laboratory measurement of hematocrit, hemoglobin, white blood count, differential, platelet count, serum chemistry parameters, renal function tests, liver function tests, urinalysis, chest roentgenogram, 12-lead electrocardiogram, T-lymphocyte subset enumeration, serum HIV p24 antigen, and quantitative viral cultures. Subjects were reevaluated every other week for the first 4 weeks, every 4 weeks until week 16, and every 8 weeks thereafter for development of adverse events and clinical endpoints. Those subjects who withdrew from study medication were followed for progression of HIV infection and survival. Subjects withdrawing from study within the first 4 weeks for reasons other than the occurrence of toxicity or clinical endpoints were replaced. Although the 10 patients who withdrew were included in the analysis, they did not contribute substantively because of their short follow-up. Additional replacement patients were required to maintain statistical power.

Clinical endpoints for this study were defined as the progression to ARC or AIDS by Center for Disease Control (CDC) case definition, or a decrease in CD4 cell count defined as follows: either a decrease in CD4 cell count to below 200/mm³ in those with baseline CD4 greater than 250/mm³ at entry, or a decrease in CD4 cell count of at least 50/mm³ in those patients with baseline CD4 ≥ 250/mm³. The criteria for CD4 endpoints were based on the premise that a CD4 count below 200/mm³, whether arrived at by a decrease from greater than 250/mm³ or ≥ 250/mm³, indicated clinical progression of disease, as recognized by the associated greater risk of opportunistic infections below a CD4 count of 200/mm³. For those subjects with baseline CD4 near 200/mm³, to assure that a significant change in CD4 occurred, a decrease of at least 50/mm³ was required.

Laboratory Assays

Serum p24 assay. Two baseline samples and sequential post-treatment sera from each patient were tested in duplicate by Abbott “A” HIV Ag-I monoclonal assay by p24 immune complex dissociation according to manufacturer’s instructions. A standard curve was constructed with samples containing known amounts of HIV p24 antigen, and optical densities of the test sera were quantitated by computerized automatic data reduction analysis, against the known p24 standards.

Quantitative microculture assay. Six serial dilutions of Ficol-Hypaque–separated peripheral blood mononuclear cells (PBMCs), starting at a concentration of 1 × 10⁶ cells/mL, were cocultured with phytohemagglutinin (PHA)–stimulated donor PBMCs in RPMI 1640 growth medium containing 20% fetal bovine serum, 5% natural human interleukin-2, and antibiotics with or without 0.001% DEAE-dextran (or 2 µg/mL polybrene), as described by Fiscus et al.24 Half of the growth medium was removed and replaced after one week with 1.0 mL fresh growth medium containing 5.0 × 10⁶ PHA-stimulated donor cells. Culture supernatants were tested for the presence of HIV p24 antigen (Abbott, Dupont, or Coulter), and results were expressed as infectious units per million cells (IFU/M). During the first year of study, several cell dilution schemes were tried (i.e., twofold, tenfold, fivefold) and p24 antigen tested at days 14 and 21. However, the dilution schema did not affect results, as they were expressed in infectious units per million cells.

Statistical Analysis

All statistical analyses used an intent-to-treat approach, and all reported P values were two-sided. Time-to-event distributions for toxicity data and clinical CD4 endpoints were estimated by the method of Kaplan and Meier25 and were compared using the log-rank statistic.24 Changes in CD4 cell counts and other continuous measurements were compared among treatment arms using the Wilcoxon-Mann-Whitney test.25 Exact contingency table tests were used to compare proportions.26

For each patient, the CD4 response was fitted using a two-part linear regression model to obtain two slopes, one pertaining to the first 12 weeks and one for the period subsequent to week 12, with continuity at week 12. The two-part model was used because an initial increase followed by a gradual decrease in CD4 count is a typical pattern of response for antiretroviral therapies, and our data appeared to fit this pattern well. We chose week 12 as the dividing point for the two slopes because it was the median time at which peak responses occurred among all patients. The individual patient slopes so attained were then analyzed, using multiple regression, adjusting for treatment arm, prior ZDV experience (naive versus experienced), baseline CD4 value (below 300/mm³ versus 300/mm³).
or greater), and hemophilia status, with separate models for the first 12 weeks and for the period subsequent to week 12. To examine the overall change from baseline CD4, the two slopes for each patient were combined, using a weighted average. The weights used depended on the specific time point of interest. For example, equal weights (12:12) corresponded to week 24 and weights of 12:44 corresponded to week 56.

Quantitative viral culture data were used to estimate the number of IUPM by the method of maximum likelihood. Whereas most IUPM values could be estimated uniquely, others could not. For example, 57 of 91 (63%) at week 12 showed an endpoint, ie, values for IUPM were positive at initial dilutions and became negative at subsequent dilutions, whereas 34 of 91 (37%) at week 12 showed no endpoint, either because values at all dilutions were negative (in 33) or values at all dilutions were positive (in 1). For the latter nonunique values, when all values were positive, IUPM was estimated to be higher than the highest concentration used in the assays. or, in the case of all values negative, IUPM was estimated to be lower than the lowest concentration used in the assay. To account for these nonunique values, patients were categorized as either "improved," defined as a decrease of at least one-half log IUPM from baseline, "worsened," defined as an increase of at least one-half log IUPM from baseline, or "unchanged," defined as neither improved nor worsened. A Kruskal-Wallis test was used to analyze these trichotomized culture data. The procedure PROC LIFEREG (SAS, Cary, NY) was used to evaluate the changes in viral titer from baseline to week 12 and to evaluate the rate of change subsequent to week 12.

RESULTS

Study Subjects

A total of 126 subjects were enrolled on study between September, 1990 and May, 1991. This number includes replacements for the ten subjects removed from study before completing 4 weeks of therapy for reasons other than toxicity or disease progression. These included eight voluntary withdrawals, one recent alcohol abuse, and one inappropriate enrollment. The latter subject was enrolled on arm B, immediately found to be ineligible, and removed from study. Thirty-one subjects each were randomized to arms A, B, and C, and 33 to arm D. Ninety-two percent of patients were male, 52% had no prior ZDV experience, and 65% had baseline CD4 cell counts between 300 and 500/mm³ with a median count of 342 cells/mm³ (Table 1). The median age was 31.5 years and 32% of patients had hemophilia. The treatment groups were well balanced with respect to these baseline patient characteristics.

The median duration of study treatment was 98 weeks, with 101 weeks for hemophilic and 96 weeks for nonhemophilic subjects. Twenty percent of patients had gone off study therapy by week 16. Subsequently, patients discontinued therapy at a steady rate of approximately 3% every 8 weeks through week 100. Although there were no significant differences between the rates of treatment discontinuation among the treatment arms (P = .20), there did appear to be a higher rate of withdrawal from arm A compared with the other arms (1-year rate, 48% for arm A v 26% to 29% for the other three arms).

The reasons for discontinuation from study treatment were toxic reactions (16%: 4 on arm A, 4 on arm B, 5 on arm C, and 7 on arm D), voluntary withdrawal by the subject (17%; 11 on arm A, 3 on arm B, 2 on arm C, 5 on arm D), development of a clinical endpoint in 6 (6%: 2 on arm A, none on arm B, 3 on arm C, 1 on arm D), and nonserious thrombocytopenia requiring ZDV before surgery (1%: 1 patient on arm D).

The median length of follow-up, using time from randomization to last contact date, was 108 weeks, with 103 weeks for hemophilic and 104 weeks for nonhemophilic subjects. Ninety percent of patients had at least 64 weeks of follow-up and 80% had at least 90 weeks of follow-up. Nine patients were lost to follow-up because they refused further contact (7%: 4 on arm A, 1 on arm B, none on arm C, and 4 on arm D). Arm A had the highest rate of withdrawal from study (1-year rate of 16% v 11%, 8%, and 3% for arms B, C, and D, respectively), but these differences were not significant (P = .61). The median duration of follow-up for CD4 counts, 96 weeks, was shorter than the overall follow-up: 87% had CD4 measurements at week 24, 64% at week 56, and 53% at week 88.

Adverse Events

A total of 21 patients (17%) experienced serious (grade 3 or 4) liver dysfunction with SGOT and/or SGPT greater than five times the ULN, including 7 on arm A, 5 on arm B, 3 on arm C, and 6 on arm D. There were no significant treatment arm differences in the rates of development of hepatotoxicity (P = .51). Twelve of those with serious hepatotoxicity were hemophiliacs, in whom the time to onset was shorter and the frequency greater than in nonhemophilic subjects (P = .008) (Fig 1). The rate of serious hepatotoxicity by one year was 25% for hemophilic and 11% for nonhemophilic subjects, and by 2 years was 34% versus 11%, respectively.

The development of severe (grade 3 or 4) hepatotoxicity was significantly greater among those with baseline liver function (SGOT and/or SGPT) $\geq$ 1.25 times the upper limit of normal, ULN, (the definition of a grade 1 or higher hepatotoxicity) as compared with those with normal baseline liver function. For the group overall, 13 of 40 with baseline liver function $\geq$ 1.25 ULN developed severe hepatotoxicity, compared with only 8 of 86 with normal baseline liver function (P = .0009, log-rank test). For the subset with hemophilia, none of 20 with baseline liver function $\geq$ 1.25 ULN developed severe hepatotoxicity, compared with 3 of 20 with normal baseline liver function (P = .028). No significant differences were observed for nonhemophilic subjects: 4 of 20 with baseline liver function $\geq$ 1.25 ULN, and 5 of 66 with normal baseline liver function developed severe hepatotoxicity (P = .11). Hepatotoxicity did not resolve with dose reduction in over half of the subjects (11 of 21), necessitating cessation of study drug (2 on arm A, 2 on arm B, 3 on arm C, and 4 on arm D).

Two patients, one on each of the arms containing high-dose ddl, developed pancreatitis, including one on arm C at week 31 with clinical pancreatitis and one on arm D at week 8 with elevated amylase and clinical pancreatitis. Pancreatitis resolved in both subjects once treatment was stopped. Peripheral neuropathy occurred in two subjects on the high-dose ddl arms, including one on arm C occurring at week 12 and one on arm D occurring at week 42. In both, the
neuropathy was sensory, but atypical, occurring in the upper distal extremities, associated with numbness and cold intolerance in the former. Symptoms resolved in both subjects after treatment was stopped. Serious grade 3 neutropenia (<750/mm³) occurred in only one patient at week 4 on arm D, and resolved without dose modification. One patient each had hyperuricemia (arm A) and hypertriglyceridemia (arm B), both of which were asymptomatic.

Clinical Endpoints

A total of 19 subjects developed 31 clinical endpoints, defined as AIDS [1987 definition21], ARC, or a CD4 endpoint (Fig 2), with no difference between treatment arms (P = .41). These 19 subjects included 6 on arm A, 2 on arm B, 6 on arm C, and 5 on arm D. Of these 19 subjects, four developed AIDS (1987 definition), three of whom also developed ARC and a CD4 endpoint, one developed ARC alone, and 14 developed a CD4 endpoint alone.

The four subjects who progressed to AIDS (1987 definition) included 1 on arm A who developed cryptococcosis at week 68 and atypical mycobacteria at week 98, and 3 on arm C, 2 of whom developed candida esophagitis at weeks 29 and 56, respectively, and 1 other with herpes simplex ulcerations at week 96. Four subjects (2 on arm A and 2 on arm C) developed AIDS-related complex (ARC), defined as a decrease in CD4 below 200/mm³ together with one of the following: recurrent oral candidiasis, hairy leukoplakia, and/or loss of greater than 10% body weight. The rates of development of AIDS and/or ARC endpoints were not significantly different among the four treatment arms (P = .11). Because of the small number of observed clinical endpoints, outcome measure evaluations relied on CD4 endpoint comparisons among the four treatment arms.

Of the 17 subjects (13%) who developed a CD4 endpoint, 6 of 20 (30%) had a decrease in CD4 count by ≥ 50/mm³ from a baseline CD4 count of ≤ 250/mm³, and 11 of 106 (10%) had a decrease in CD4 count below 200/mm³ from a baseline CD4 count > 250/mm³. The estimated 2-year rates

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Table 1. Characteristics of Study Subjects by Treatment Arm

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Arm A</th>
<th>Arm B</th>
<th>Arm C</th>
<th>Arm D</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Zidovudine/didanosine dose (mg/d)</td>
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<td>300/334</td>
<td>600/500</td>
<td>-/500</td>
<td>-/500</td>
</tr>
<tr>
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<td>31</td>
<td>31</td>
<td>33</td>
<td>126</td>
</tr>
<tr>
<td>Median age (yr)</td>
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<td>29.9</td>
<td>28.4</td>
<td>35.9</td>
<td>31.5</td>
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<td>No. males/females</td>
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<td>27/4</td>
<td>30/1</td>
<td>30/3</td>
<td>118/10</td>
</tr>
<tr>
<td>Risk</td>
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<td>13</td>
<td>10</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>No. hemophilic subjects</td>
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<td>15</td>
<td>17</td>
<td>18</td>
<td>66</td>
</tr>
<tr>
<td>No. nonhemophilic subjects</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>No. CD4+ count</td>
<td>20</td>
<td>21</td>
<td>20</td>
<td>21</td>
<td>82</td>
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<tr>
<td>Mean (no./mm³)</td>
<td>301</td>
<td>384</td>
<td>340</td>
<td>340</td>
<td>342</td>
</tr>
<tr>
<td>Median (no./mm³)</td>
<td>301</td>
<td>384</td>
<td>340</td>
<td>340</td>
<td>342</td>
</tr>
</tbody>
</table>

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Fig 1. Kaplan Meier distribution of time to hepatic toxicity in hemophilic subjects (---) versus nonhemophilic subjects (-). Fig 2. Kaplan Meier distribution of time to clinical endpoints (AIDS, ARC, or CD4 endpoint) by treatment arm: arm A, low-dose combination ZDV andddl (- -); arm B, moderate-dose combination ZDV and ddl (---); arm C, high-dose combination ZDV and ddl (- - -); and arm D, ddl monotherapy (---).
for development of a CD4 endpoint were 21% for arm A, 7% for arm B, 27% for arm C, and 16% for arm D (P = .60). A decrease in the CD4 count below 200/mm³ accounted for 83% (10 of 12) of CD4 endpoints on the three combination arms (arms A, B, C), whereas a decrease in CD4 count by more than 50/mm³ accounted for 80% (4 of 5) of the CD4 endpoints on the ddI monotherapy arm (arm D).

**Immunologic Data**

There was a significant increase in CD4 lymphocyte number between week 0 and week 12 for treatment arms B, C, and D (P = .0003), and a marginally significant increase for arm A (P = .065), using a model adjusting for hemophilia status, prior ZDV treatment (naive versus experienced), and baseline CD4 count. There were significant treatment arm differences in the increase in CD4 counts over the first 12 weeks (P = .015), according to this model. This result is consistent with the Kruskal-Wallis test of differences between treatment arms at week 12 (P = .02) (Table 2, Fig 3). Arms A and D appeared to have smaller increases in CD4 count compared with arms B and C (A v B: P = .16; A v C: P = .0097; B v D: P = .072; C v D: P = .043). The median CD4 increases at week 12 were 44, 105, 114, and 42 cells/mm³ for arms A through D, respectively.

Patients naïve to ZDV had a significantly greater increase in CD4 counts than those with ZDV experience (P = .044; median, 89/mm³ v 44/mm³). Hemophiliac subjects had a significantly smaller increase in CD4 counts than nonhemophiliac subjects (P = .0004, median 25/mm³ v 86/mm³), although for the subset of patients with hemophilia, there was no difference between those treated with high-purity (monoclonal, recombinant) products (n = 23) as compared with those treated with lesser-purity products (n = 17) (P = .46). The baseline CD4 count did not have a significant effect on the change in CD4 count between baseline and week 12 (P = .26) (Fig 3).

Subsequent to week 12, there was a significant decline in CD4 counts of approximately 56 cells/mm³ per year (P = .007). There were no significant treatment arm differences in this rate of CD4 decrease (P = .23). In addition, the rate of CD4 decrease was not associated with baseline CD4 count (P = .27), prior ZDV experience (P = .77), or hemophilic status (P = .80). However, there was a significant association between the height of the CD4 increase in the first 12 weeks and the rate of decrease in CD4 counts over the subsequent 12 weeks (P = .011); those who showed the greatest increase in CD4 over the first 12 weeks decreased at the fastest rate.

The overall change in CD4 counts from baseline to week 24, examined by combining the two fitted regression slopes for each patient, was significantly different among treatment arms (P = .0048). Overall, arms B and C showed a greater increase in CD4 from baseline by week 24 than arms A and D, although this occurred to a lesser extent with arm C than arm B. These differences appeared to persist at week 56, but were marginally significant (P = .084).

Similar results were observed when the three combination arms (arms A, B, and C) were compared, and when the high-dose ddI arms (arms C and D) were compared. There was a significantly greater increase in CD4 counts over the first 12 weeks with higher-dose combination ZDV and ddI (P = .012), but there was no difference in the subsequent rate of decrease (P = .25). When the high-dose ddI arms were compared, there was a significantly greater increase in CD4 counts over the first 12 weeks for high-dose ddI in combination (arm C) as compared with monotherapy (arm D) (P = .043), but no significant difference in the subsequent rate of decrease (P = .78).
Virologic Data

p24 antigen. Seventy-two of 126 (57%) patient samples were available for serum p24 antigen testing at weeks 0, 12, and 24. Of these, 23 of the 72 subjects (32%) were p24 antigen positive at baseline by the method of acid dissociation. The latter included 5 of 15 (33%) in arm A, 4 of 20 (20%) in arm B, 8 of 16 (50%) in arm C, and 6 of 21 (29%) in arm D. Of this small sample, 52%, or 12 of 23 initially p24 antigen positive, remained positive at both weeks 12 and 24. The proportion of hemophilic subjects at baseline who were acid-dissociated p24 antigen positive was 5 of 14 (36%), similar to the proportion in nonhemophilic subjects (18 of 58, 31%). The proportion of ZDV-naive subjects who were acid-dissociated p24 antigen positive was 12/37 (32%), similar to the proportion in ZDV-experienced subjects (11 of 35, 31%). Small numbers precluded definitive between-arm comparisons.

Quantitative Viral Load

Overall, 64% of subjects experienced a significant reduction in quantitative viral load, defined as at least a one-half log reduction in IUPM by week 12. A few patients (8%) had at least a one-half log increase in viral titer, and the remaining 29% had no substantial change from baseline. The percentage of patients showing a substantial reduction in viral load was 43% on arm A, 71% on arm B, 78% on arm C, and 61% on arm D (Table 3). There were no significant differences between treatment arms at week 12 using a Kruskal-Wallis test (P = .11). Arm A had a noticeably smaller percentage of patients showing a substantial improvement at week 12 and at all subsequent weeks. The treatment difference was significant at week 40 (P = .014), but not at other weeks using the trichotomized outcomes. However, other tests that are more powerful showed that the smaller percentage with improvement on arm A was significantly different from the other treatment arms, as shown below.

Using a regression model adjusting for prior ZDV treatment, hemophilic status, and baseline CD4 count, there was a significant decrease in IUPM between week 0 and week 12 for all treatment arms combined (P = .0001). There were significant treatment differences in the change in viral titer during the first 12 weeks (P = .015), with median percentage decreases at week 12 of 56.3% for arm A, 94.6% for arm B, 98.5% for arm C, and 91.9% for arm D (Fig 4). Arm A, the low-dose combination arm, had smaller reductions in viral titer compared with arm B (P = .0068), arm C (P = .0067), and arm D (P = .036). There were no significant differences between the other treatment arms. Subsequent to week 12, there were no significant differences between the treatment arms in the rate of change in viral titer (P = .74). Although arm D appeared to have a greater reduction in viral titer than the other treatment arms, this may be somewhat related to smaller sample sizes at later time points. Those with greater initial viral burdens (above the median baseline value of 1.02 log titer) had significantly greater decreases in viral titer than those with lower values. The median reductions in log titer were 98.4% and 82.0% for these two groups, respectively (P = .0053). However, adjustment for baseline titer did not alter the treatment comparisons appreciably.

There was a tendency for hemophilic subjects to experience a smaller decrease in viral titer by week 12 than nonhemophilic subjects (median decrease, 83.9% v 96.5%, respectively, a difference of 0.67 log titer). Similarly, patients naive to ZDV tended to have a smaller decrease in viral titer than those with prior ZDV experience (median decrease of 93.2% v 97.8%, a difference of 0.50 log titer), and those with baseline CD4 count > 300/mm³ tended to have a marginally significant smaller decrease than those <300/mm³, 91.3% v 99.2%, a difference of 1.05 log titer. By univariate analysis,
Table 3. Proportion of Subjects Showing Change in Viral Titer From Baseline by Treatment Arm

<table>
<thead>
<tr>
<th>Study Week</th>
<th>Status</th>
<th>Arm A</th>
<th>Arm B</th>
<th>Arm C</th>
<th>Arm D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Improved</td>
<td>9 (43%)</td>
<td>17 (71%)</td>
<td>18 (78%)</td>
<td>14 (61%)</td>
<td>(P = .11)</td>
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<tr>
<td></td>
<td>No change</td>
<td>10 (48%)</td>
<td>5 (21%)</td>
<td>4 (19%)</td>
<td>7 (30%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>2 (9%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
<td>2 (9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21</td>
<td>24</td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Improved</td>
<td>9 (50%)</td>
<td>16 (70%)</td>
<td>19 (79%)</td>
<td>20 (77%)</td>
<td>(P = .16)</td>
</tr>
<tr>
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<td>No change</td>
<td>6 (33%)</td>
<td>5 (22%)</td>
<td>4 (17%)</td>
<td>4 (15%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>3 (17%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18</td>
<td>23</td>
<td>24</td>
<td>26</td>
<td></td>
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<tr>
<td>40</td>
<td>Improved</td>
<td>6 (38%)</td>
<td>14 (70%)</td>
<td>18 (82%)</td>
<td>17 (74%)</td>
<td>(P = .014)</td>
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<td>No change</td>
<td>6 (38%)</td>
<td>5 (25%)</td>
<td>3 (14%)</td>
<td>6 (26%)</td>
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</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>4 (25%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
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<tr>
<td></td>
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<tr>
<td>56</td>
<td>Improved</td>
<td>6 (46%)</td>
<td>15 (79%)</td>
<td>12 (71%)</td>
<td>14 (78%)</td>
<td>(P = .14)</td>
</tr>
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<td></td>
<td>No change</td>
<td>5 (39%)</td>
<td>4 (21%)</td>
<td>3 (17%)</td>
<td>4 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>2 (15%)</td>
<td>0 (0%)</td>
<td>2 (12%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>13</td>
<td>19</td>
<td>17</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>Improved</td>
<td>3 (30%)</td>
<td>8 (44%)</td>
<td>11 (69%)</td>
<td>11 (69%)</td>
<td>(P = .21)</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>6 (60%)</td>
<td>8 (44%)</td>
<td>3 (19%)</td>
<td>4 (25%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>1 (10%)</td>
<td>2 (12%)</td>
<td>2 (12%)</td>
<td>1 (6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10</td>
<td>18</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>Improved</td>
<td>3 (30%)</td>
<td>9 (56%)</td>
<td>8 (53%)</td>
<td>8 (66%)</td>
<td>(P = .40)</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>3 (30%)</td>
<td>2 (13%)</td>
<td>4 (27%)</td>
<td>2 (17%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>4 (40%)</td>
<td>5 (31%)</td>
<td>3 (20%)</td>
<td>2 (17%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10</td>
<td>16</td>
<td>15</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Improved</td>
<td>2 (29%)</td>
<td>9 (64%)</td>
<td>6 (48%)</td>
<td>5 (66%)</td>
<td>(P = .54)</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>3 (42%)</td>
<td>2 (14%)</td>
<td>4 (31%)</td>
<td>2 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worsened</td>
<td>2 (29%)</td>
<td>3 (22%)</td>
<td>3 (23%)</td>
<td>2 (22%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7</td>
<td>14</td>
<td>13</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

The \(P\) value is based on the Kruskal-Wallis test comparing arms A, B, C, and D at each study week. "Improved" indicates a decrease of at least one half log IUPM from baseline, "worsened" indicates an increase of at least one half log IUPM from baseline, and "unchanged" indicates neither "improved" nor "worsened."
Table 4. Comparison Between Change in Viral Titer and Change in CD4 Cell Count at Week 12

<table>
<thead>
<tr>
<th>Change in Viral Titer (IUPM)*</th>
<th>&lt;50/mm³ Increase†</th>
<th>50-99/mm³ Increase</th>
<th>&gt;100/mm³ Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Much improved</td>
<td>27%</td>
<td>20%</td>
<td>54%</td>
</tr>
<tr>
<td>Improved</td>
<td>47%</td>
<td>40%</td>
<td>13%</td>
</tr>
<tr>
<td>Not improved</td>
<td>54%</td>
<td>18%</td>
<td>29%</td>
</tr>
</tbody>
</table>

* "Much improved" indicates a decrease of at least one log IUPM from baseline, "improved" indicates a decrease of between one half and one log IUPM from baseline, and "worsened" indicates a decrease of less than one half IUPM from baseline, no change, or an increase in IUPM from baseline.
† A less than 50/mm³ increase in median CD4 cell count indicates an increase of between 0 and 50/mm³, no increase, or a decrease in CD4 cell count.

There was a significant association between change in viral titer (IUPM) and median change in CD4 cell count at week 12, P = .024, exact contingency test (Table 4). Over half, 54%, of those with a much improved viral response (a reduction of at least one log titer IUPM) showed a CD4 count increase of at least 100/mm³, whereas only 13% of those with an improved viral response (between one-half and one log reduction in IUPM) and 28% of those with a worsened viral response (less than one-half log reduction, no change, or an increase in viral titer) showed such an increase in CD4 response. The median increases in CD4 count were 115/mm³, 60/mm³, and 45/mm³ for these three categories, respectively.

DISCUSSION

Didanosine monotherapy and combination therapy with ZDV are safe, well-tolerated, and effective in increasing CD4 lymphocyte number and in reducing viral load in individuals with asymptomatic HIV infection. The durability of the antiviral response appears to be related to the level of CD4 increase and viral load reduction achieved in the first 12 weeks of antiviral therapy. After adjusting for hemophilia status, prior ZDV exposure, and baseline CD4, the moderate-dose arm, arm B, showed the greatest CD4 increase with the least toxicity of all treatment arms. Specifically, although arm B showed an increase in CD4 and reduction in viral load similar to arm C (the high-dose combination arm), arm B used lower doses of ZDV and ddI, had fewer clinical endpoints, less toxicity, and not as sharp a decrease in CD4 subsequent to week 12 as arm C.

The magnitude of the increase in CD4 with ddI monotherapy and combination therapy was related to two factors, prior ZDV treatment and hemophilia status. A significantly weaker CD4 response was observed in those who had received prior ZDV treatment and in those with hemophilia. However, this reduced CD4 response was not related to a lower baseline CD4. In addition, although not significant, there was a tendency to weaker viral suppression for those with prior ZDV treatment and hemophilia.

The reason for the weaker CD4 response in those with prior ZDV treatment is not known, but may be related to the development of resistance mutations in HIV reverse transcriptase, which increases with increasing duration of antiviral treatment and occurs in over 25% of asymptomatic HIV-infected subjects after 1 year of treatment. More recently, new resistance mutations have been observed in patients receiving combination therapy with ZDV and ddI. Thus, it is possible, but not proven, that the reduced CD4 response observed in previously ZDV-treated patients may be related to emergence of resistance. The relationship between development of viral resistance and CD4 response in these subjects is currently being analyzed.

The reduced CD4 response in subjects with hemophilia was an unexpected observation of this study. Although the majority (70%) of individuals with hemophilia participating in this study had received prior ZDV treatment, the difference persisted after adjusting for prior ZDV. Thus, the reduced CD4 response in hemophilic subjects is not related to prior ZDV. Most of those with hemophilia (88%) had received chronic treatment with clotting factor concentrate, which has been associated with depressed natural killer activity and CD4 number in both HIV-infected and uninfected hemophiliacs. Despite their weaker CD4 response, those with hemophilia experienced no greater proportion of clinical endpoints than nonhemophilic subjects. Thus, the clinical significance of weaker CD4 responses in hemophilic subjects is not known. The reported protective effect of higher-purity clotting factor products (monoclonally-purified, recombinant) in slowing the rate of decrease in CD4 number was not observed in this study; the type of blood-product treatment hemophilia patients received during this study had no effect on CD4 response.

Didanosine monotherapy and a combination with ZDV were well tolerated, with only two instances each of peripheral neuropathy and pancreatitis/hyperamylasemia, occurring on the highest-dose ddI-containing arms (arm C, high-dose combination, and arm D, ddI monotherapy). In subjects with hemophilia, there was a significantly shorter time to and greater frequency of hepatotoxicity with ddI monotherapy and combination therapy than in non-hemophiliacs. The reason for this finding is not known, but may be related to chronic hepatitis infection (hepatitis B, HBV, and hepatitis C, HCV) and chronic liver disease, typical of the majority of hemophiliacs receiving chronic blood-product treatment. Recent studies of combination antiviral therapy with ZDV and zalcitabine and ZDV and ddI in more advanced HIV disease, have shown better, more sustained...
CD4 responses than observed in this study. This is the first randomized, controlled clinical trial of ddI monotherapy and combination therapy in asymptomatic HIV-infected patients, and it is likely that the higher baseline CD4 number and absence of baseline HIV-related symptoms will require a longer time of follow-up to determine the effect of antiviral therapy on disease progression. At the time this study was initiated, ddI had been shown to be efficacious as a single agent in advanced HIV disease,37,38 so we designed this pilot study to compare ddI monotherapy with a threefold dose-range of ddI in combination with ZDV in asymptomatic HIV-1 infection and to determine the safest, most effective arm for a larger phase III clinical trial (ACTG 175). For that reason, no ZDV monotherapy arm was included. Although the latter would have provided additional information, the safety and efficacy of ZDV monotherapy in asymptomatic HIV-1 infection,1 including in asymptomatic hemophilic subjects,39 were already known.

The correlation observed between CD4 and quantitative viral titers suggests that reduction in viral load is accompanied by an increase in CD4. Given the impression of CD4 as a surrogate marker for clinical HIV disease progression,40 it is possible that viral load quantitation, either alone or together with CD4, may provide a more predictive marker of disease progression and antiviral response. Ongoing clinical trials evaluating CD4 and quantitative viral load should help to prove this.

In summary, ddI monotherapy and combination therapy are well tolerated in asymptomatic HIV infection and result in significant CD4 increases and viral load reduction. Further study is needed to investigate the greater hepatotoxicity and lesser CD4 responses with antiviral therapy that occurred in hemophilic subjects. Finally, ongoing and future clinical trials will hopefully determine the role for combination therapy in individuals with asymptomatic HIV-1 infection.

ACKNOWLEDGMENT

We thank Bernard S. Landry for his contribution in protocol development and Karen Saban for her expertise in preparation of the manuscript.

APPENDIX

The following individuals, members of the AIDS Clinical Trials Group, participated in this trial: AIDS Clinical Trials Group (ACTG) Virology Subcommittee: Suraiya Rasheed, PhD, Chair, Susan Fiscus, PhD, Phalguni Gupta, PhD, Michael Katzman, MD, Robert Shafer, MD, William S. Meyer, PhD, and Robert W. Coombs, MD, PhD; Division of AIDS, Treatment Research Initiative: Bernard S. Landry, MPH; Administrative Center, NIAID, Division of AIDS: Patricia Kasdan, MS, Lynda Nerhood, BS, and Richel Stowell; Frontier Science and Technology Research Foundation: Karen Kazial, RN, BSN; National Hemophilia Foundation: Alan Brownstein, MSW, MPH, Amy S. Kramer, RN, and Joan Wasserman, RN, MBA; University of North Carolina: Susan Fiscus, PhD, and Karen Bloodgood, RN; University of Pittsburgh: Phalguni Gupta, PhD and Deborah McMahon, MD; Pennsylvania State University Hershey Medical Center: Michael Katzman, MD and Beth Superdock, RN; Stanford University: Robert Shafer, MD, Dennis M. Israelksi, MD, and Patricia Cain, RN; University of Pittsburgh Hemophilia Center: Elaine Carfagna, RN, MPH; George Washington University Hemophilia Center: Charlotte Quinlan, RN; George Washington University: Peter Hawley, MD and Susan F. LeLacheur, PA-C, MPH; University of Washington-Seattle Hemophilia Center: Leona Muench, RN; University of Washington-Seattle: Ann C. Collier, MD and Robert W. Coombs, MD, PhD; University of Southern California: Lily Cheng, MD and Xin Yan Li, MD; and University of Rochester: Gene Morse, PharmD.

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