Virologic and CD4⁺ Cell Responses to New Nucleoside Regimens: Switching to Stavudine or Adding Lamivudine after Prolonged Zidovudine Treatment of Human Immunodeficiency Virus Infection

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ABSTRACT

Clinical benefit of zidovudine alone in the treatment of HIV infection wanes after several years, with decreasing CD4⁺ cell numbers and increasing HIV RNA in plasma. To develop treatment strategies following prolonged zidovudine treatment, 92 subjects from the AIDS Clinical Trials Group (ACTG) 175 study after a median of 3.6 years of zidovudine monotherapy were randomized to treatment with stavudine or zidovudine and lamivudine. Evaluation of long-term changes, the average of 40- and 48-week HIV plasma RNA, demonstrated that lamivudine and zidovudine provided significantly greater virologic suppression compared with stavudine (mean decrease 0.70 versus 0.18 log₁₀ copies/ml, \( p = 0.003 \)). Twenty-nine percent of zidovudine plus lamivudine recipients had HIV RNA levels below 500 copies per milliliter at 48 weeks as compared with 4% of stavudine recipients (\( p = 0.02 \)). Both regimens significantly increased CD4⁺ cell numbers, the means of weeks 40 and 48 rose to 49 and 36 CD4⁺ cells per cubic millimeter among zidovudine plus lamivudine and stavudine recipients, respectively. Treatments were well tolerated and only 3 of 92 subjects died or developed AIDS within 48 weeks. In zidovudine-experienced subjects, addition of lamivudine resulted in significantly decreased plasma HIV RNA levels at 48 weeks compared with treatment with stavudine alone.

INTRODUCTION

The aims of antiretroviral therapy in early human immunodeficiency virus infection are prolongation or restoration of immunocompetence and the prevention of clinical disease. With the advent of highly active antiretroviral therapies and methods to quantify circulating HIV RNA, suppression of viremia to levels below the limits of detection in current assays is a goal of antiretroviral therapy. 1,2 This is based largely on associations between reduction in virus load, increased or stable CD4⁺ cell numbers, and decreased risk of clinical disease in subjects receiving antiretroviral therapies 3,4 The remarkable decrease in deaths and hospitalizations of HIV-infected individuals in the last 2 years in the United States provides evidence of the effectiveness of aggressive antiretroviral therapy. 5 Measurement of HIV plasma RNA, a surrogate for virus replication, allows in vivo evaluation and comparison of antiretroviral drug activity without dependence on clinical end points or disease progression. 6 Zidovudine treatment has demonstrated reduction in disease

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progression compared with placebo. However, the Concorde study showed that despite an early advantage among subjects receiving zidovudine, after 2 years of treatment there was little clinical benefit from early therapy with zidovudine alone. In zidovudine-experienced subjects, continued monotherapy is less effective than nucleoside combinations. Disease progression among subjects with 200 to 500 CD4+ cells per cubic millimeter, assessed as a 50% fall in CD4+ cells, AIDS, or death among subjects assigned to zidovudine in the AIDS Clinical Trials Group (ACTG) 175 study, was nearly 10% per year and 57% of subjects initially assigned to zidovudine discontinued drug because of intolerance or disease progression during 2 to 4 years of follow-up.

In 1995, prior to the era of highly active antiretroviral therapies, we initiated a clinical trial among subjects who had been treated with zidovudine alone for more than 3 years in the ACTG 175 study. Subjects had 200 to 500 CD4+ cells per cubic millimeter at enrollment in ACTG 175 in 1991–1992 and most were asymptomatic. Nucleoside therapies for zidovudine-experienced subjects in 1995 included addition of lamivudine (2'-deoxy-3'-thiacytidine) or substitution of stavudine (2',3'-dideoxy-3'-deoxymethylidine) for zidovudine treatment. Lamivudine is a nucleoside analog reverse transcriptase inhibitor used extensively in combination with zidovudine in naive and zidovudine-experienced subjects. Stavudine has also been shown to increase CD4+ cell numbers, decrease plasma HIV RNA, and reduce the rate of clinical progression among zidovudine-experienced subjects.

To develop a strategy for the continued treatment of patients with long-term zidovudine exposure, 92 subjects from the zidovudine limb of ACTG 175 were enrolled in a blinded, randomized controlled trial of new nucleoside reverse transcriptase inhibitor regimens, stavudine (d4T) alone, or the addition of lamivudine (3TC) to zidovudine. The primary objectives of the study were to compare short- and long-term changes (at 4 or 8 weeks, and at 40 and 48 weeks, respectively) in HIV plasma RNA and CD4+ cell numbers for the two regimens.

**MATERIALS AND METHODS**

**Study design**

ACTG 302 was a randomized, double-blinded clinical trial enrolling subjects with prolonged prior experience with either zidovudine or didanosine monotherapy; this article concerns only the zidovudine recipients. Subjects who continued to receive their originally assigned therapy in ACTG 175 were eligible to participate if they met requirements for hematology and serum chemistry measures and did not have evidence of an active infection within 14 days of randomization, or a neoplasm (other than minimal Kaposi sarcoma) or an AIDS-defining opportunistic infection. Plasma HIV RNA and CD4+ cell numbers were measured twice before starting study treatment, at 4 weeks (CD4+ cell count only), 8 weeks (HIV RNA only), 16 weeks (CD4+ cell count only), and at 24, 40, and 48 weeks. Subjects were randomized to switch from zidovudine (200 mg three times daily) to stavudine (40 mg twice daily) or to add lamivudine (150 mg twice daily) to zidovudine with appropriate placebos in a blinded fashion. Baseline log_{10} RNA and CD4+ cell count were the means of the two pretreatment measurements. Short-term change from baseline was to the week 4 CD4+ cell count and the week 8 HIV RNA determination; long-term change was to the mean of the week 40 and 48 determinations for both markers. The study objective was to compare short- and long-term plasma HIV RNA and CD4+ cell changes in the two treatments.

**Plasma HIV RNA measurements**

HIV-1 RNA was measured in citrated plasma, separated within 6 hr of phlebotomy and stored at −70°C. All samples from each study participant were run in a single assay, including standards containing 15,000 and 150,000 copies of HIV RNA. Assays were performed at the conclusion of the study in three laboratories, certified in the performance of the Roche (Nutley, NJ) Amplicor RNA monitor test, by Roche as well as the Virology Quality Assurance program (supported by the Division of AIDS, NIAID, NIH).

**Statistical methods**

All analyses of HIV RNA were undertaken after log base 10 transformation. Changes in HIV RNA and CD4+ cell count were analyzed by linear regression with adjustment for center and nucleoside experience prior to entry into ACTG 175 (none versus experienced), and used maximum likelihood methods for censored data to handle HIV RNA values outside the range of quantification of the assay. The proportion of subjects with HIV RNA levels below 500 copies/ml was analyzed by the Fisher exact test, and logistic regression was used to evaluate predictors of suppression. Times to loss to follow-up, treatment discontinuation, and to the development of signs and symptoms or laboratory abnormalities of grade 3 or higher (according to the NIAID toxicity-grading tables) were compared between treatments by the log-rank test stratified by nucleoside experience prior to entry into ACTG 175. Analyses were intent-to-treat including all randomized subjects and all available follow-up to 48 weeks after starting study treatment, except that analyses of adverse effects were censored at 8 weeks after study treatment discontinuation if this was before 48 weeks.

**RESULTS**

**Baseline characteristics of subjects**

Ninety-two subjects who completed treatment in ACTG 175 were randomized to zidovudine plus lamivudine or stavudine (46 subjects each). They had received zidovudine monotherapy for a median of 4.1 years (interquartile range, 3.3 to 5.7 years). Demographic, virologic, and immunologic characteristics of the subjects are shown in Table 1. Between entering ACTG 175 and entering ACTG 302, a median of 3.5 years, the mean CD4+ cell count for these subjects declined from 399 CD4+ cells per cubic millimeter to 302 CD4+ cells per cubic millimeter while receiving zidovudine. At entry into ACTG 175, 57 subjects with available samples for measurement presented a mean HIV serum HIV RNA of 3.96 log_{10} copies per milliliter compared with a mean value of 4.34 log_{10} copies per milliliter in plasma for 79 subjects in ACTG 302 at study entry.
Follow-up and treatment status

Seven of the 92 subjects (8%) were lost to follow-up: 5 assigned stavudine versus 2 assigned zidovudine and lamivudine ($p = 0.23$). Twenty-two subjects discontinued study treatment prior to 48 weeks: 14 versus 8, respectively ($p = 0.16$). Only one subject (assigned stavudine) was withdrawn because of protocol-defined toxicity.

RNA and CD4 changes with new nucleoside therapies

Subjects assigned zidovudine and lamivudine had a mean short-term (8 week) reduction in HIV RNA of 0.84 log$_{10}$ copies per milliliter ($p < 0.001$), and a long-term (weeks 40 and 48) reduction of 0.70 log$_{10}$ copies per milliliter ($p < 0.001$), compared with 0.17 and 0.18 log$_{10}$ copies per milliliter ($p = 0.27$ and $p = 0.13$) among subjects assigned stavudine (Fig. 1).

Adverse experiences and clinical progression

Only eight subjects experienced signs and symptoms of grade 3 or 4: three subjects assigned stavudine and lamivudine compared with five subjects assigned stavudine ($p = 0.44$). Similarly, few subjects experienced laboratory abnormalities of grade 3 or higher: eight subjects versus six subjects, respectively ($p = 0.79$). There was 1 death and 2 AIDS-defining events observed within 48 weeks of starting treatment among the 92 subjects. The death, due to bacterial sepsis, occurred in a subject randomized to receive stavudine, although the relationship of this death to HIV infection was unknown. AIDS-defining events, both in subjects assigned to zidovudine and lamivudine, were a diagnosis of HIV dementia complex and a new clinical diagnosis of cutaneous Kaposi sarcoma limited to the ankle.

**DISCUSSION**

HIV-infected individuals, after prolonged zidovudine monotherapy, who added lamivudine had significantly greater...
FIG. 1. Changes in log HIV-1 RNA from baseline over 48 weeks in subjects treated with stavudine compared to zidovudine and lamivudine.

TABLE 2. SELECTED BASELINE CHARACTERISTICS BY WEEK 48 RNA RESULTS SUBJECTS ON ZDV PLUS LAMIVUDINE

<table>
<thead>
<tr>
<th>RNA at Week 48</th>
<th>≤500 (N = 10)</th>
<th>&gt;500 (N = 25)</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: number (%)</td>
<td>Male 8 (80%) 20 (80%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Race: number (%)</td>
<td>White non-Hispanic 9 (90%) 17 (68%)</td>
<td>0.205</td>
<td></td>
</tr>
<tr>
<td>Black non-Hispanic</td>
<td>0 5 (20%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (10%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>IV drug use: number (%)</td>
<td>Preceding or currently 0</td>
<td>1 (4%)</td>
<td>0.982</td>
</tr>
<tr>
<td>Hemophiliac: number (%)</td>
<td>Yes 2 (20%)</td>
<td>0</td>
<td>0.973</td>
</tr>
<tr>
<td>HIV symptoms: number (%)</td>
<td>Symptomatic&lt;sup&gt;b&lt;/sup&gt; 0</td>
<td>5 (20%)</td>
<td>0.959</td>
</tr>
<tr>
<td>Age (years):</td>
<td>Mean (S.D.)</td>
<td>39 (9)</td>
<td>41 (11)</td>
</tr>
<tr>
<td>Prior ZDV use (years):</td>
<td>Median (Q1–Q3)</td>
<td>4.6 (3.3–6.9)</td>
<td>4.4 (3.4–5.0)</td>
</tr>
<tr>
<td>Change in CD4 per year:&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mean (S.D.)</td>
<td>9 (30)</td>
<td>29 (24)</td>
</tr>
<tr>
<td>CD4 (cell/mm&lt;sup&gt;3&lt;/sup&gt;) at ACTG 175 baseline:</td>
<td>Mean (S.D.)</td>
<td>429 (136)</td>
<td>388 (116)</td>
</tr>
<tr>
<td>CD4 (cell/mm&lt;sup&gt;3&lt;/sup&gt;):</td>
<td>Mean (S.D.)</td>
<td>371 (148)</td>
<td>261 (134)</td>
</tr>
<tr>
<td>Percent CD4:</td>
<td>Mean (S.D.)</td>
<td>26 (5)</td>
<td>18 (8)</td>
</tr>
<tr>
<td>HIV-1 RNA level&lt;sup&gt;d&lt;/sup&gt; (log&lt;sub&gt;10&lt;/sub&gt; copies/mL):</td>
<td>Mean (S.D.)</td>
<td>3.52 (0.20)</td>
<td>4.59 (0.15)</td>
</tr>
</tbody>
</table>

<sup>a</sup>p value from logistic regression model.
<sup>b</sup>Symptomatic was defined having candidiasis, oral hairy leukoplakia or herpes zoster.
<sup>c</sup>Based on a median of 3 years of follow-up in ACTG 175.
reduction in short- and long-term plasma HIV-1 RNA levels compared with individuals changing therapy to stavudine. Increased mean CD4\(^+\) cell numbers were observed in both treatments, although the differences between zidovudine plus lamivudine treatment and stavudine alone were not significant. Addition of lamivudine provided mean suppression of HIV RNA of 0.70 log\(_{10}\) copies per cubic milliliter with a rise in mean CD4\(^+\) cell count of 59 cells per cubic millimeter at 40 to 48 weeks relative to study entry. In contrast, substitution of stavudine for zidovudine resulted in only a mean 0.18 log\(_{10}\) copies per cubic millimeter decrease in RNA, although this was accompanied by a mean increase of 36 cells per cubic millimeter. Continued zidovudine treatment with addition of lamivudine demonstrated significantly greater virologic activity compared with stavudine following prolonged zidovudine monotherapy in intermediate HIV disease. Despite the greater virologic activity of lamivudine and zidovudine compared with stavudine, there were significant increases in mean CD4\(^+\) cell numbers in both treatment arms.

Clinical and virologic failure among patients receiving prolonged zidovudine therapy has been closely associated with zidovudine resistance and genotypic changes in the polymerase (pol) and envelope (env) genes of HIV-1 resulting in phenotypic drug resistance and syncytium-inducing virus.\(^{20-22}\) Studies of zidovudine-experienced subjects treated with stavudine have shown reduced antiretroviral activity among zidovudine-experienced as compared with naive subjects.\(^{16}\) The waning activity of nucleoside therapies after prolonged treatment may be due to the development of high-level resistance to thymidine analog reverse transcriptase inhibitors, or host cell changes in intracellular phosphorylation of stavudine and lamivudine in individuals with long-term zidovudine experience.\(^{23}\) Reduced activity of zidovudine and stavudine treatment in combination\(^{24}\) provides a rationale for nonthymidine analog nucleosides such as didanosine, lamivudine, or abacavir for combination therapy in zidovudine-experienced subjects.

Reduction in mean HIV RNA levels after addition of lamivudine to zidovudine in this study was similar to the reductions in HIV RNA with the addition of didanosine or zalcitabine to zidovudine\(^{4,11,25}\) and combination therapy with stavudine and lamivudine in zidovudine-experienced subjects.\(^{25,26}\) Lamivudine treatment is associated with development of an M184V mutation resulting in decreased susceptibility to lamivudine.\(^{27,28}\) A study of zidovudine and lamivudine susceptibilities suggested that the M184V mutation could return zidovudine susceptibility, despite the presence of zidovudine resistance mutations at codons 70, 215, and 41 commonly associated with high-level zidovudine resistance.\(^{29}\) Other studies of the HIV reverse transcriptase suggested that the increase in fidelity, associated with changes in enzymatic processivity of the M184V mutant, might reduce the rate of evolutionary change in virus.\(^{30}\) However, HIV with M184V in association with T215Y/F, M41L, and other zidovudine resistance mutations demonstrates phenotypic resistance to both drugs.\(^{31,32}\) Zidovudine and lamivudine resistance with T215Y and M184V mutations have been identified in recent seroconverters and

**FIG. 2.** Changes in CD4\(^+\) T lymphocytes over 48 weeks in subjects treated with stavudine compared to zidovudine and lamivudine.
their partners, providing evidence that dually resistant virus may be transmitted.\textsuperscript{33,34}

On the basis of the evolution of drug resistance and disease progression, the goals of antiretroviral treatment in HIV infection have rapidly shifted to suppression of HIV replication to the lowest possible levels with combination antiretroviral therapy regimens.\textsuperscript{1,2} In this study, suppression of plasma HIV RNA to levels less than 500 copies per milliliter at 48 weeks was observed in 10 of 35 subjects (29\%) receiving zidovudine and lamivudine. Using a more stringent intent-to-treat analysis, only 10 of 46 subjects (22\%) achieved suppression to levels below 500 copies per milliliter at 48 weeks.\textsuperscript{35} Subjects with lower HIV RNA levels and higher CD4\textsuperscript{+} cell numbers at study entry were more likely to achieve prolonged suppression of HIV RNA.

These HIV RNA responses demonstrate that the addition of lamivudine provides significantly greater short- and long-term antiviral activity compared with stavudine alone. However, these results were observed in a highly selected group of HIV-infected subjects, long-term recipients of zidovudine monotherapy with little clinical evidence of progression. In 1999, with 14 antiretroviral drugs available, recommended strategies for the treatment of HIV include the use of at least 3 potent drugs to achieve suppression of HIV RNA. Among clinically stable subjects with low levels of HIV RNA and relatively preserved CD4\textsuperscript{+} cell numbers the HIV RNA response to lamivudine resulted in suppression of detectable HIV RNA in a minority of subjects. In contrast, substitution of stavudine for zidovudine, while safe, had little effect on plasma HIV RNA in highly zidovudine-experienced subjects. The substitution of stavudine for zidovudine in a failing antiretroviral regimen including zidovudine does not provide for a significant reduction in HIV RNA replication. However, the increased mean CD4\textsuperscript{+} cell numbers in both arms of this study provide support for the use of lamivudine and zidovudine with additional potent drugs in zidovudine-experienced patients.

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