

# Virologic and CD4<sup>+</sup> 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<sup>+</sup> 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 log<sub>10</sub> 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<sup>+</sup> cell numbers, the means of weeks 40 and 48 rose to 49 and 36 CD4<sup>+</sup> 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.<sup>1,2</sup> This is based largely on associations between reduction in virus load, increased or sta-

ble CD4<sup>+</sup> cell numbers, and decreased risk of clinical disease in subjects receiving antiretroviral therapies.<sup>3,4</sup> 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.<sup>5</sup> 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.<sup>6</sup>

Zidovudine treatment has demonstrated reduction in disease

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progression compared with placebo.<sup>7,8</sup> 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.<sup>9</sup> In zidovudine-experienced subjects, continued monotherapy is less effective than nucleoside combinations.<sup>6,10-12</sup> Disease progression among subjects with 200 to 500 CD4<sup>+</sup> cells per cubic millimeter, assessed as a 50% fall in CD4<sup>+</sup> 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.<sup>9</sup>

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<sup>+</sup> cells per cubic millimeter at enrollment in ACTG 175 in 1991-1992 and most were asymptomatic.<sup>6</sup> Nucleoside therapies for zidovudine-experienced subjects in 1995 included addition of lamivudine (2'-deoxy-3'-thiacytidine) or substitution of stavudine (2',3'-didehydro-3'-deoxythymidine) for zidovudine treatment. Lamivudine is a nucleoside analog reverse transcriptase inhibitor used extensively in combination with zidovudine in naive and zidovudine-experienced subjects.<sup>11,12</sup> Stavudine has also been shown to increase CD4<sup>+</sup> cell numbers, decrease plasma HIV RNA, and reduce the rate of clinical progression among zidovudine-experienced subjects.<sup>13-16</sup>

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<sup>+</sup> 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<sup>+</sup> cell numbers were measured twice before starting study treatment, at 4 weeks (CD4<sup>+</sup> cell count only), 8 weeks (HIV RNA only), 16 weeks (CD4<sup>+</sup> 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<sub>10</sub> RNA and

CD4<sup>+</sup> cell count were the means of the two pretreatment measurements. Short-term change from baseline was to the week 4 CD4<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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.<sup>17</sup> 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<sup>18</sup>) were compared between treatments by the log-rank test<sup>19</sup> stratified by nucleoside experience prior to entry into ACTG 175. Analyses were intent-to-treat<sup>17</sup> 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<sup>+</sup> cell count for these subjects declined from 399 CD4<sup>+</sup> cells per cubic millimeter to 302 CD4<sup>+</sup> 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<sub>10</sub> copies per milliliter compared with a mean value of 4.34 log<sub>10</sub> copies per milliliter in plasma for 79 subjects in ACTG 302 at study entry.

TABLE 1. SELECTED BASELINE CHARACTERISTICS BY TREATMENT

		Total (N = 92)	Stavudine (N = 46)	Zidovudine plus Lamivudine (N = 46)
Sex: number (%)	Male	75 (82%)	38 (83%)	37 (80%)
Race: number (%)	White non-Hispanic	68 (74%)	35 (76%)	33 (72%)
	Black non-Hispanic	13 (14%)	6 (13%)	7 (15%)
	Hispanic	8 (9%)	4 (9%)	4 (9%)
	Other	3 (3%)	1 (2%)	2 (4%)
IV drug use: number (%)	Previously or currently	2 (2%)	1 (2%)	1 (2%)
Hemophiliac: number (%)	Yes	8 (9%)	3 (7%)	5 (11%)
HIV symptoms: number (%)	Symptomatic <sup>a</sup>	11 (12%)	5 (11%)	6 (13%)
Age (years):	Mean (S.D.)	40 (9)	39 (8)	41 (10)
Prior ZDV use (years):	Median (Q1–Q3)	4.1 (3.5–5.7)	4.0 (3.5–6.1)	4.3 (3.4–5.0)
CD4 (cell/mm <sup>3</sup> ) at ACTG 175 baseline:	Mean (S.D.)	399 (106)	400 (102)	399 (112)
CD4 (cell/mm <sup>3</sup> ):	Mean (S.D.)	302 (137)	313 (138)	292 (137)
HIV-1 RNA level <sup>b</sup> (log <sub>10</sub> copies/mL):	Mean (S.D.)	4.34 (0.09)	4.41 (0.12)	4.27 (0.13)

<sup>a</sup>Symptomatic was defined having candidiasis, oral hairy leukoplakia or herpes zoster.

<sup>b</sup>Available for 79 subjects (38 on stavudine and 41 on zidovudine plus lamivudine).

### 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<sub>10</sub> copies per milliliter ( $p < 0.001$ ), and a long-term (weeks 40 and 48) reduction of 0.70 log<sub>10</sub> copies per milliliter ( $p < 0.001$ ), compared with 0.17 and 0.18 log<sub>10</sub> copies per milliliter ( $p = 0.27$  and  $p = 0.13$ ) among subjects assigned stavudine (Fig. 1). The differences between treatments in short-term and long-term mean change in HIV plasma RNA were 0.65 and 0.53 log<sub>10</sub> copies per milliliter ( $p < 0.001$  and  $p = 0.003$ , respectively). Short- and long-term changes in HIV RNA were analyzed to evaluate whether the differences between treatments was associated with CD4<sup>+</sup> cell count, HIV RNA, prior duration of ZDV use, HIV-related symptoms, or the isolation of syncytium-inducing virus at baseline. For baseline CD4<sup>+</sup> cell count there was significant evidence that the decrease in short-term HIV RNA associated with treatment with zidovudine and lamivudine compared with stavudine was greater for subjects with higher counts than lower counts: ranging from 1.15 log<sub>10</sub> copies per milliliter greater for subjects with CD4<sup>+</sup> cell counts greater than 400 CD4<sup>+</sup> cells per cubic millimeter to 0.36 log<sub>10</sub> copies per milliliter for subjects with counts less than 200 CD4<sup>+</sup> cells per cubic millimeter. A corresponding trend was seen for long-term changes: ranging from 1.03 to 0.31 log<sub>10</sub> copies per milliliter ( $p = 0.08$ ).

A reduction in HIV RNA below 500 copies per milliliter was seen in 10 of 35 subjects (29%) assigned zidovudine and lamivu-

dine at week 48, compared with 1 of 24 subjects (4%) assigned stavudine ( $p = 0.02$ ). Lower plasma HIV RNA, higher CD4<sup>+</sup> cell percentage, and increasing trends in CD4<sup>+</sup> cell count during follow-up in ACTG 175 were all significantly associated with suppression below 500 copies/ml at week 48 (Table 2).

There were significant short- and long-term mean increases in CD4<sup>+</sup> cell count from baseline in both treatment arms (Fig. 2). The mean short- and long-term increases were 44 and 59 CD4<sup>+</sup> cells per cubic millimeter, respectively, among subjects assigned zidovudine and lamivudine ( $p = 0.002$  and  $p < 0.001$ ) compared with 28 and 36 CD4<sup>+</sup> cells per cubic millimeter among subjects assigned stavudine ( $p = 0.023$  and  $p = 0.010$ ). However, there was no significant difference between treatments ( $p = 0.16$  and  $p = 0.30$ , respectively).

### Adverse experiences and clinical progression

Only eight subjects experienced signs and symptoms of grade 3 or 4: three subjects assigned zidovudine 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

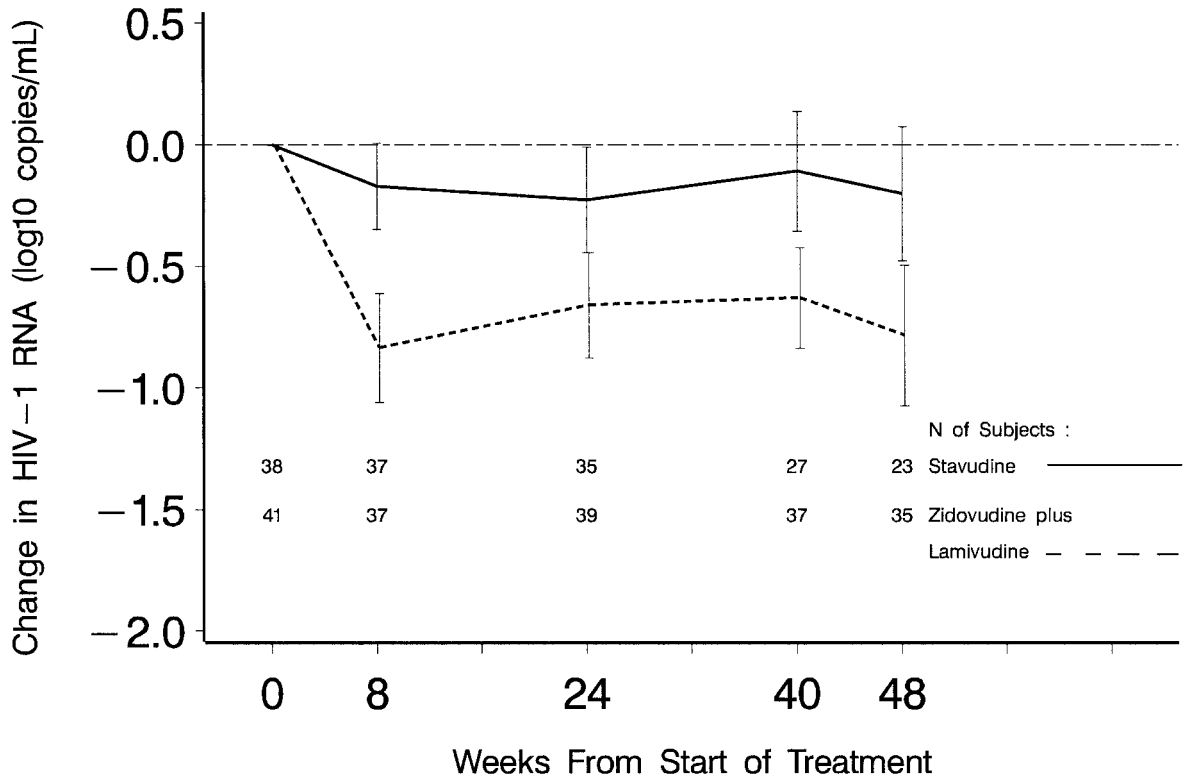


FIG. 1. Changes in log HIV-1 RNA from baseline over 48 weeks in subjects treated with stavudine compared to zidovudine and lamivudine.

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

		RNA at Week 48		p Value <sup>a</sup>
		≤ 500 (N = 10)	> 500 (N = 25)	
Sex: number (%)	Male	8 (80%)	20 (80%)	1
Race: number (%)	White non-Hispanic	9 (90%)	17 (68%)	0.205
	Black non-Hispanic	0	5 (20%)	
	Hispanic	1 (10%)	2 (8%)	
	Other	0	1 (4%)	
IV drug use: number (%)	Previously or currently	0	1 (4%)	0.982
	Yes	2 (20%)	0	0.973
HIV symptoms: number (%)	Symptomatic <sup>b</sup>	0	5 (20%)	0.959
Age (years):	Mean (S.D.)	39 (9)	41 (11)	0.531
Prior ZDV use (years):	Median (Q1–Q3)	4.6 (3.3–6.9)	4.4 (3.4–5.0)	0.330
Change in CD4 per year: <sup>c</sup>	Mean (S.D.)	9 (30)	-29 (24)	0.005
CD4 (cell/mm <sup>3</sup> ) at ACTG 175 baseline:	Mean (S.D.)	429 (136)	388 (116)	0.377
	Mean (S.D.)	371 (148)	261 (134)	
Percent CD4:	Mean (S.D.)	26 (5)	18 (8)	0.023
HIV-1 RNA level <sup>c</sup> (log <sub>10</sub> copies/mL):	Mean (S.D.)	3.52 (0.20)	4.59 (0.15)	0.007

<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.

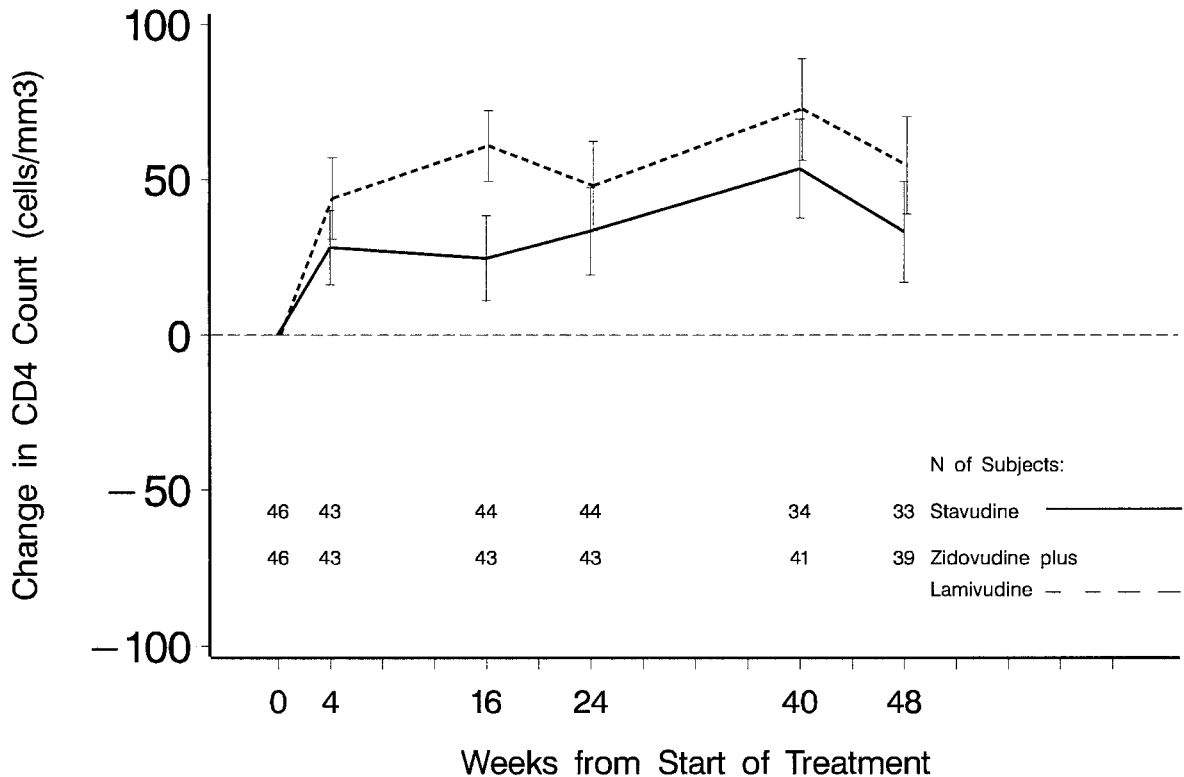


FIG. 2. Changes in CD4<sup>+</sup> T lymphocytes over 48 weeks in subjects treated with stavudine compared to zidovudine and lamivudine.

reduction in short- and long-term plasma HIV-1 RNA levels compared with individuals changing therapy to stavudine. Increased mean CD4<sup>+</sup> 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<sub>10</sub> copies per cubic milliliter with a rise in mean CD4<sup>+</sup> 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<sub>10</sub> copies per cubic milliliter 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<sup>+</sup> 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.<sup>20-22</sup> Studies of zidovudine-experienced subjects treated with stavudine have shown reduced antiretroviral activity among zidovudine-experienced as compared with naive subjects.<sup>16</sup> 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.<sup>23</sup> Reduced activity of zidovudine and stavudine treatment in combination<sup>24</sup> 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<sup>4,11,25</sup> and combination therapy with stavudine and lamivudine in zidovudine-experienced subjects.<sup>25,26</sup> Lamivudine treatment is associated with development of an M184V mutation resulting in decreased susceptibility to lamivudine.<sup>27,28</sup> 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.<sup>29</sup> 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.<sup>30</sup> However, HIV with M184V in association with T215Y/F, M41L, and other zidovudine resistance mutations demonstrates phenotypic resistance to both drugs.<sup>31,32</sup> Zidovudine and lamivudine resistance with T215Y and M184V mutations have been identified in recent seroconverters and

their partners, providing evidence that dually resistant virus may be transmitted.<sup>33,34</sup>

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.<sup>1,2</sup> 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.<sup>35</sup> Subjects with lower HIV RNA levels and higher CD4<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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.

### ACKNOWLEDGMENTS

The following institutions and investigators participated in ACTG 302: Harvard University (Meg Guthro-White, R.N., and Clyde Crumpacker, M.D.); Northwestern University Medical School (Robert Murphy, M.D.); Rush-Presbyterian St. Luke's Medical Center (Harold Kessler, M.D.); Cook County Hospital (Joseph Pulvirenti, M.D.); Case Western University (Hernan Valdez, M.D. and Ronald Johnson, R.N.); The Ohio State University College of Medicine and Public Health (Michael F. Para, M.D., Carmellia Jackson, R.N., and Robert J. Fass, M.D.); University of California at San Diego (Richard Haubrich, M.D. and Cindy Jacobsen, R.N.); University of Minnesota (Henry H. Balfour, M.D. and Nancy V. Reed, R.N., M.S., R.N.P.); Indiana University School of Medicine (Kenneth Fife, M.D.); University of Texas at Galveston (Michael Borucki, M.D. and Gerianne Casey, R.N.); University of North Carolina (Timothy W. Lane, M.D. and Pam Mentley, R.N., B.S.N., C.C.R.N., James Horton, M.D., and Joan Connell, R.N.); University of Alabama (Michael Saag, M.D. and Donna Davis, R.N.); Stanford University (Thomas C. Merigan, M.D., Mary Rinki, R.N., and Pat Cain, R.N.); University of Miami School of Medicine (Allen

Rodriguez, M.D. and Margaret A. Fischl, M.D.); University of Washington, Seattle (Ann C. Collier, M.D., Becky A. Royer, P.A.-C., and Kim Marquis, M.D.); Washington University, St. Louis (William G. Powderly, M.D., Tom Stiffler, R.N., and Michael Klebert, R.N.P.); MSKCC/Cornell (Kent Sepowitz, M.D. and Laura Ponticello, R.N.); University of Colorado (Wheaton Williams, M.D. and M. Graham Ray, M.S.N.); Johns Hopkins University (John G. Bartlett, M.D. and Melody Higgins, R.N., M.S.); Mount Sinai University (Henry S. Sacks, M.D., Beverly Simpson, R.N., and Donna Mildvan, M.D.); UCLA Medical Center (Ronald Mitsuyasu, M.D., Ann Johiro, A.C.R.N., C.S., M.N., F.N.P.-C, Gildon Beall, M.D., and Sandra Baker); University of California (UCSF), San Francisco (Donald Abrams, M.D. and David Gary, R.N.); University of Cincinnati (Peter Frame, M.D. and Deborah Neumann, R.N.); University of Pennsylvania (Ian Frank, M.D. and Isabel Mattozzo, R.N.); New York University (Mary Vogler, M.D. and Victoria Rosenwald, R.N., M.P.H.); Puerto Rico (Guillermo Vazquez, M.D. and Virginia Ramirez, B.S.N., M.P.H.); University of Southern California (USC) (P. Jan Geiseler, M.D., Connie Olson, R.N., and John M. Leedom, M.D.).

### REFERENCES

1. National Institutes of Health: Report of the NIH Panel to define principles of therapy of HIV infection. *Ann Intern Med* 1998; 128:1057-1078.
2. International AIDS Society-USA: Antiviral therapy for HIV infection in 1997: Updated recommendations of the International AIDS Society-USA. *JAMA* 1997;277:1962-1969.
3. Mellors JW, Munoz A, Giorgi JV, *et al.*: Plasma virus load and CD4 lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126:946-954.
4. Katzenstein DA, Hammer S, Hughes M, *et al.*: The relationship 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.
5. Palella F, Delaney K, Moorman A, *et al.*: Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J Med* 1998;338:853-860.
6. Hammer SM, Katzenstein D, Hughes M, *et al.*: Nucleoside monotherapy vs. combination therapy in HIV infected adults: A randomized, double blind, placebo controlled trial in persons with CD4 cell counts between 200 and 500 per cubic millimeter. *N Engl J Med* 1996;335:1081-1090.
7. O'Brien WA, Hartigan PM, Martin D, *et al.*: Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. *N Engl J Med* 1996;334:426-431.
8. Volberding PA, Lagakos SW, Koch MA, *et al.*: Zidovudine in asymptomatic human immunodeficiency virus infection: A controlled trial in persons with fewer than 500 CD4-positive cells per cubic milliliter. *N Engl J Med* 1990;322:941-949.
9. Concorde Coordinating Committee: Concorde MRC/ANRS randomized double blind controlled trial of immediate and deferred zidovudine in symptom-free HIV infection. *Lancet* 1994;343: 871-881.
10. Schooley RT, Ramirez-Ronda C, Lange JMA, *et al.*: Virologic and immunologic benefits of initial combination therapy with zidovudine and zalcitabine or didanosine compared with zidovudine monotherapy. *J Infect Dis* 1996;173:1354-1366.
11. Eron JJ, Benoit SL, Jemsek J, MacArthur RD, Santana 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.
12. Caesar Coordinating Committee: Randomized trial of addition of lamivudine or lamivudine plus loviridine to zidovudine-containing regimens for patients with HIV-1 infection: The Caesar trial. *Lancet* 1997;349:1413–1421.
  13. Sprunace SL, Pavia AT, Mellors JW, *et al.*: Clinical efficacy of monotherapy with stavudine compared with zidovudine in HIV-infected, zidovudine experienced patients. *Ann Intern Med* 1997;126:355–363.
  14. Griffith BP, Brett-Smith H, Kim G, *et al.*: Effect of stavudine on human immunodeficiency virus type 1 virus load as measured by quantitative mononuclear cell culture, plasma RNA and immune complex-disassociated antigenemia. *J Infect Dis* 1996;173:1252–1255.
  15. Ridder SA, Anderson R, and Mellors JW: Antiretroviral activity of stavudine (2',3'-didehydro-3'-deoxythymidine, D4T). *Antiretroviral Res* 1995;27:189–203.
  16. Villalba N, Soriano V, Gomez-Cano M, Castilla J, Mas A, and Gonzales-Lahoz J: Efficacy and safety of stavudine in pretreated HIV-1 infected patients. *Antiviral Ther* 1997;2:185–190.
  17. Kalbfleisch JD and Prentice RL: *The Statistical Analysis of Failure Time Data*. John Wiley & Sons, New York, 1980.
  18. Division of AIDS. Division of AIDS table for grading severity of adult adverse experiences. Rockville, MD. National Institute of Allergy and Infectious Diseases, 1996.
  19. Pocock SJ: *Clinical Trials*. John Wiley & Sons, Chichester, 1983.
  20. Kozal MJ, Shafer RW, Winters MA, *et al.*: HIV-1 syncytium inducing phenotype, virus burden, codon 215 reverse transcriptase mutation and CD4 cell decline in zidovudine treated patients. *J Acquir Immunodefic Syndr* 1994;7:832–838.
  21. Koot M, Keet IPM, Vos AHV, *et al.*: Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. *Ann Intern Med* 1993;118:681–688.
  22. Rey D, Hughes M, Pi JT, Winters M, Merigan TC, and Katzenstein DA: Human immunodeficiency virus type 1 reverse transcriptase codon 215 mutation in plasma RNA: Immunologic and virologic responses to zidovudine. *J Acquir Immune Defic Syndr Hum Retroviruses* 1998;17:203–208.
  23. Sommadossi JP, Zhou XJ, Moore J, *et al.*: Impairment of stavudine (d4T) phosphorylation in patients receiving a combination of zidovudine (ZDV) and d4T (ACTG 290). In *5th National Conference on Retroviruses and Opportunistic Infections, Chicago, Illinois, February 1998*. [Abstract 3]
  24. Havlir D, Friedland G, Pollard R, *et al.*: Combination zidovudine (ZDV) and stavudine (d4T) therapy versus other nucleosides: Report of two randomized trials (ACTG290 and 291). Presented at the 5th Conference on Retrovirus and Opportunistic Infections, February 1–5, 1998, Chicago, Illinois.
  25. Calvez V, Descamps D, Valantin MA, *et al.*: Genotypic analysis of experienced patients treated by D4T/3TC combination (ALTIS 2). Presented at the 5th Conference on Retrovirus and Opportunistic Infections, February 1–5, 1998, Chicago, Illinois. [Abstract 676]
  26. Hammer SM, Squires KE, Hughes MD, *et al.*: A controlled trial of two nucleoside analogs plus indinavir in persons with human immunodeficiency virus infection and CD4 counts of 200 per cubic millimeter or less. *N Engl J Med* 1997;337:725–733.
  27. 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.
  28. Larder BA, Kemp SD, and Hatrigan R: Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. *Science* 1995;269:696–699.
  29. Tisdale M, Kemp SD, Parry N, and Larder BA: Rapid *in vitro* selection of human immunodeficiency virus type 1 resistant to 2',3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci USA* 1993;90:5653–5656.
  30. Arts EJ and Wainberg MA: Preferential incorporation of nucleoside analogs after template switching during human immunodeficiency virus reverse transcription. *Antimicrob Agents Chemother* 1994;38:1008–1016.
  31. Nijhuis M, Schuurman R, de Jong D, *et al.*: Lamivudine-resistant human immunodeficiency virus type 1 variants (184V) require multiple amino acid changes to become co-resistant to zidovudine *in vivo*. *J Infect Dis* 1997;176:398–405.
  32. Shafer RW, Winters MA, Palmer S, and Merigan TC: Multiple concurrent reverse transcriptase and protease inhibitor mutations and multi-drug resistance of HIV-1 isolates from heavily treated patients. *Ann Intern Med* 1998;128:906–911.
  33. Hecht FM, Grant RM, Petropoulos CJ, *et al.*: Sexual transmission of an HIV-1 variant resistant to multiple reverse transcriptase and protease inhibitors. *N Engl J Med* 1998;339:307–311.
  34. Yerly S, Kaiser L, Race E, Clavel F, and Perrin L: Reverse transcriptase and protease gene analysis at the time of primary HIV-1 infection. Presented at the 2nd International Workshop on HIV Drug Resistance and Treatment Strategies. Lake Maggiore, Italy 24–27, June 1998. [Abstract 107]
  35. Hill A, Gartland M, De Masi R, and Kuhn M, on behalf of the Avanti Steering Committee: Differential analyses give highly variable estimates of HIV-1 RNA undetectability and log<sub>10</sub> reduction in clinical trials. Presented at the 2nd International Workshop on HIV Drug Resistance and Treatment Strategies. Lake Maggiore, Italy 24–27, June 1998. [Abstract 76]

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