

CHANGES IN PLASMA HIV-1 RNA AND CD4+ LYMPHOCYTE COUNTS AND THE RISK OF PROGRESSION TO AIDS

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Abstract Background. Clinical trials of antiretroviral drugs can take years to complete because the outcomes measured are progression to the acquired immunodeficiency syndrome (AIDS) or death. Trials could be accelerated by the use of end points such as changes in CD4+ lymphocyte counts and plasma levels of human immunodeficiency virus type 1 (HIV-1) RNA and β_2 -microglobulin, but there is uncertainty about whether these surrogate measures are valid predictors of disease progression.

Methods. We analyzed data from the Veterans Affairs Cooperative Study on AIDS, which compared immediate with deferred zidovudine therapy. Patients' plasma levels of HIV-1 RNA and β_2 -microglobulin were measured in stored plasma.

Results. Among the 129 patients in the immediate-treatment group, 34 had disease that progressed to AIDS, as compared with 57 of the 141 patients in the deferred-treatment group ($P=0.03$). Progression to AIDS correlated strongly with base-line CD4+ lymphocyte counts

($P=0.001$) and plasma levels of HIV-1 RNA ($P<0.001$), but not with base-line levels of β_2 -microglobulin ($P=0.14$). A decrease of at least 75 percent in the plasma level of HIV-1 RNA over the first six months of zidovudine therapy accounted for 59 percent of the benefit of treatment, defined as the absence of progression to AIDS (95 percent confidence interval, 13 to 112 percent). Plasma β_2 -microglobulin levels and CD4+ lymphocyte counts explained less of the effect of treatment. A 75 percent decrease in the plasma HIV-1 RNA level plus a 10 percent increase in the CD4+ lymphocyte count could explain 79 percent of the treatment effect (95 percent confidence interval, 27 to 145 percent).

Conclusions. Treatment-induced changes in the plasma HIV-1 RNA level and the CD4+ lymphocyte count, taken together, are valid predictors of the clinical progression of HIV-related disease and can be used to assess the efficacy of zidovudine and possibly other antiretroviral drugs as well. (N Engl J Med 1996;334:426-31.)

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ASSASSEMENTS of the efficacy of antiretroviral therapy based on clinical end points in patients with early or intermediate stages of human immunodeficiency virus (HIV) infection typically take many years.¹⁻⁵ As new antiretroviral agents become available, retaining patients in randomized, prospective trials for long periods will be increasingly difficult. Thus, to accelerate the evaluation of new drugs, determining whether other markers of HIV type 1 (HIV-1) infection can be used to predict an effect of treatment is a matter of great interest.⁶

There are several candidate markers for HIV-1 infection.⁷⁻⁹ The CD4+ lymphocyte count is the best characterized and is currently used as a guide for antiretroviral therapy. Although this count often increases after the initiation of effective antiretroviral therapy, counts can differ between treated and placebo (or control) patients even in the absence of a significant treatment ef-

fect.^{2,5} Neopterin and β_2 -microglobulin levels are non-specific markers of immune activation and tend to increase progressively during the course of HIV-1 disease,¹⁰⁻¹³ although their response to treatment and relation to the clinical progression of disease have not been extensively studied.

A more direct measure of antiviral response may be the decrease in levels of circulating virus.¹⁴ The measurement of plasma HIV-1 RNA is more sensitive than the quantitation of either HIV-1 p24 core antigen in plasma or infectious virus in blood.¹⁵⁻¹⁷ Plasma levels of HIV-1 RNA decrease profoundly after the start of antiretroviral therapy.¹⁸⁻²⁰

The usefulness of quantitative methods to assess the efficacy of antiretroviral therapy against HIV-1 must be validated in a longitudinal, controlled trial that includes a treatment benefit for the markers to explain.²¹ We performed a retrospective study to determine whether changes in plasma levels of HIV-1 RNA, plasma levels of β_2 -microglobulin, and CD4+ lymphocyte counts could predict the likelihood of progression to the acquired immunodeficiency syndrome (AIDS) in patients in the Veterans Affairs trial of zidovudine therapy.

METHODS

Study Population

Patients with symptomatic HIV-1 infection and 200 to 500 CD4+ lymphocytes per cubic millimeter were enrolled in Veterans Affairs Cooperative Studies Program trial 298, a blinded, randomized comparison of immediate with deferred zidovudine therapy. The results of part 1 of that study (conducted from January 1987 through January 1991) have been published.¹ All the patients in the immediate-therapy

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group received open-label zidovudine for the entire study period, whereas those in the deferred-therapy group received placebo until their CD4+ lymphocyte counts fell below 200 cells per cubic millimeter or an AIDS-defining illness developed,²² when they were switched to open-label zidovudine. In addition, after part 1 of the study, there was a three-year follow-up period (from January 1991 to January 1994) during which all the patients were offered open-label zidovudine.²³ In the current study, we evaluated a subgroup of the original study population that included all patients for whom we had base-line plasma samples and at least one follow-up sample obtained during the six months after randomization. To record additional instances of clinical progression of disease and permit effects of treatment to be shown in our subgroup, we extended the observation period to include the first year of the three-year follow-up period.

Plasma Samples

At intervals of one to four months during the trial, plasma specimens were collected, cryopreserved, and stored at repositories in Baltimore and Durham, North Carolina. Because the collection and storage procedures at the two repositories differed, we used samples from only one repository for each patient (73 percent were from Baltimore, where they were stored at -20°C , and 27 percent were from Durham, where they were stored at -70°C). All the samples for a given patient were retrieved at the same time from various freezer boxes and analyzed in a batch at the Roche Biomedical Laboratory, Research Triangle Park, North Carolina. For the quantitation of HIV-1 RNA in plasma, RNA was extracted from the plasma sample by the method of Boom et al.²⁴ and the reverse-transcriptase polymerase chain reaction (PCR) was performed with the GeneAmp kit (Roche Diagnostics, Nutley, N.J.).²⁵ Plasma β_2 -microglobulin was measured by radioimmunoassay (β_2 -micro RIA, Kabi Pharmacia Diagnostics, Uppsala, Sweden). The methods used to count CD4+ lymphocytes have been described elsewhere.¹

Statistical Analysis

The Wilcoxon rank-sum test and the chi-square test were used to compare groups with regard to continuous and discrete variables, respectively.²⁶ In the time-to-event analyses, life-table methods that included Cox models were used, with stratification according to the place where the specimens were stored.²⁷ The mean change from base line over the six-month period after randomization was our primary analytic measure of each marker. This change was calculated for each patient as the mean of the measurements obtained during the six months after randomization, minus the base-line measurement. Plasma levels of HIV-1 RNA were expressed as the \log_{10} of the number of copies of RNA per milliliter of plasma in order to account for the wide range of values, which encompassed more than five orders of magnitude. The value for each marker was expressed as the percentage of change from base line on the arithmetic scale.

The method proposed by Freedman et al.²⁸ was adapted to validate the use of the markers as surrogate end points. A sequence of four Cox proportional-hazards models that related each marker and the effect of treatment to the risk of progression to AIDS²⁷ was used, with adjustment in each model for base-line values. Model 1 measured the effect of treatment; model 2, the change in the marker; and model 3, both the effect of treatment and the change in the marker. In model 4, a term for the interaction between the effect of treatment and the change in the marker was added to model 3. The coefficients for the treatment effect in the model that also included the change in the marker (model 3) were compared with those in the model for treatment only (model 1) in order to calculate the ability of the change in the marker to "explain" the effect of treatment; these values were expressed as percentages. Bootstrap methods were used to calculate 95 percent confidence intervals for these percentages, conditional on an effect of treatment.

Conditional relative risks and 95 percent confidence intervals for the magnitude of the effects of the markers were calculated from the Cox models, which included base-line values and treatment effects. Patients were classified in one of four categories according to their responses to treatment during the study period, as determined by meas-

urement of the markers. The classifications were correlated with the progression to AIDS by means of life-table methods and validated with use of three indicator variables as the marker in the first three steps of the modeling procedure. All analyses in this study were performed according to the intention-to-treat approach, and all reported P values are two-tailed.

RESULTS

There were sufficient cryopreserved plasma samples for 270 of the 338 patients in the original study population to be assessed (80 percent); of the 270, 129 were assigned to immediate therapy and 141 to deferred therapy. The number of copies of HIV-1 RNA and the β_2 -microglobulin level were measured in 2202 plasma samples (median number of samples per patient, 9; range, 2 to 17). The characteristics of the 270 patients before treatment did not differ from those of the whole group of 338 patients, nor did the characteristics differ between treatment groups. Among the patients studied, HIV-1 disease progressed to AIDS in 34 members of the immediate-therapy group and 57 members of the deferred-therapy group (relative risk associated with immediate vs. deferred therapy, 0.63; 95 percent confidence interval, 0.43 to 0.92; $P=0.03$). The relative risk of death when the immediate-treatment group was compared with the deferred-treatment group was not significant at any time in this study.

Effect of Base-Line Values on Outcome

When the base-line values for all three markers were analyzed independently, each was related to the occurrence of progression to AIDS, regardless of treatment group. In a multivariate Cox regression analysis, however, the base-line plasma level of β_2 -microglobulin was not significant ($P=0.14$), although there remained strong relations between both the base-line plasma level of HIV-1 RNA (relative risk for each increase of 0.5 log in the level, 1.27; $P<0.001$) and the base-line CD4+ lymphocyte count (relative risk for each increase of 35 CD4+ lymphocytes per cubic millimeter, 0.85; $P=0.001$) and the progression to AIDS. In a bivariate Cox regression analysis, the base-line plasma level of HIV-1 RNA (relative risk, 1.25; $P<0.001$) and base-line CD4+ lymphocyte count (relative risk, 0.82; $P<0.001$) were also strongly related to the incidence of death. Since the base-line values for both markers were important for outcome, all analyses of treatment or treatment-induced changes in either marker have been adjusted for the base-line values of that marker.

Changes in Markers over Time

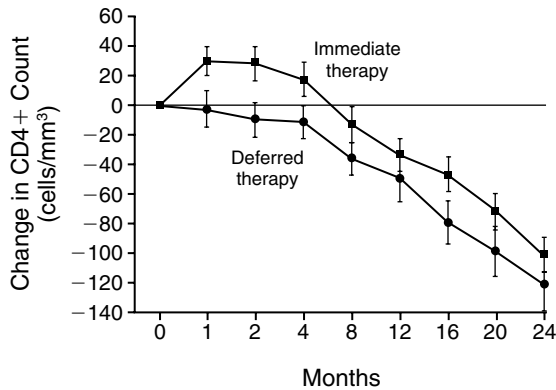
The changes from base line in the CD4+ lymphocyte count and the plasma level of HIV-1 RNA are shown in Figure 1. The treatment-induced increase in the CD4+ lymphocyte count was smaller and more short-lived than the corresponding decrease in the plasma HIV-1 RNA level, which did not return to the base-line value for 12 months. Levels of β_2 -microglobulin had a substantial treatment-induced decline, similar to the decline in plasma HIV-1 RNA (data not shown). The values in the de-

ferred-therapy group were affected by the crossover of patients from placebo to open-label treatment with zidovudine, which occurred a mean of 16 months (range, 1 to 24) after the start of the study in 114 of the 141 patients.

Relation between Treatment-Induced Changes in Markers and Progression to AIDS

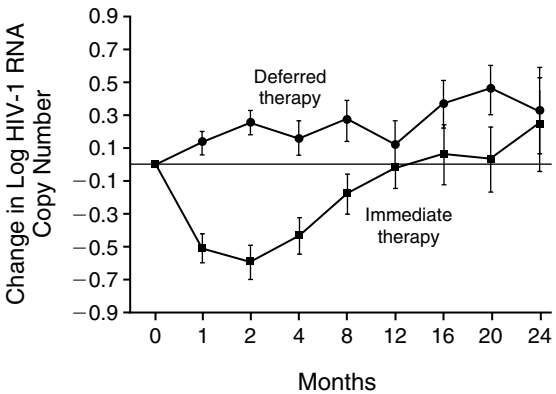
We used Cox models to summarize the relation between the mean changes in markers during the six months after randomization and the progression to AIDS. In the univariate model, the relative risk for each increase of 0.5 log in the plasma level of HIV-1 RNA

was 1.5 (95 percent confidence interval, 1.23 to 1.83; $P < 0.001$). For each increment of 35 CD4+ lymphocytes per cubic millimeter, the relative risk was 0.83 (95 percent confidence interval, 0.76 to 0.91; $P < 0.001$), and for each increment of 0.33 μg per milliliter in the plasma level of β_2 -microglobulin, the relative risk was 1.11 (95 percent confidence interval, 0.99 to 1.24; $P = 0.08$). We also performed these analyses using changes of various magnitudes, including decreases in plasma levels of HIV-1 RNA by factors of three to seven, 5 to 25 percent increases in the CD4+ lymphocyte count, and 5 to 25 percent decreases in the plasma level of β_2 -microglobulin. Table 1 shows the results of the univariate and multivariate analyses in which the values closest to the mean change for each marker were used as thresholds: a decrease of 75 percent (0.6 log) in the plasma level of HIV-1 RNA, a 10 percent increase in the CD4+ lymphocyte count, and a 15 percent decrease in the plasma level of β_2 -microglobulin. These data show the importance of the relations between the progression to AIDS and these changes in the plasma HIV-1 RNA level and the CD4+ lymphocyte count. For the other specified degrees of change in each marker, the results were similar.



A

NO. OF PATIENTS TESTED	
Deferred therapy	141 125 130 127 115 111 110 108 98
Immediate therapy	129 115 108 110 100 90 93 84 80



B

NO. OF PATIENTS TESTED	
Deferred therapy	141 42 101 102 77 66 48 39 22
Immediate therapy	129 43 86 86 62 62 40 36 21

Figure 1. Changes from Base Line in the CD4+ Lymphocyte Count (Panel A) and the Log₁₀ Number of Copies of HIV-1 RNA in Plasma (Panel B) in the Two Treatment Groups.

Positive numbers indicate an increase, and negative numbers a decrease. When multiple measurements were made in a patient close to a given time, only the measurement made closest to that time is shown. The numbers of patients shown do not decrease linearly over time because not all patients were tested at each time point. Vertical bars denote 95 percent confidence intervals.

Validation with Mean Values during the Six Months after Randomization

Detailed results of the validation analyses of the effect of a 75 percent decrease in the plasma level of HIV-1 RNA and a 10 percent increase in the CD4+ lymphocyte count from the base-line values are provided for both markers in Table 2. A significant benefit of treatment in terms of the progression to AIDS was found in model 1 for each marker ($P = 0.03$). A 75 percent decrease in the plasma level of HIV-1 RNA during the six months after randomization markedly reduced the risk of progression to AIDS, as shown in model 2 ($P < 0.001$), regardless of treatment group. When treatment assignment and the change in the plasma level of HIV-1 RNA were considered together in model 3, the treatment assignment was no longer significant ($P = 0.33$), but a decrease in the plasma level of HIV-1 RNA remained significant ($P = 0.004$), indicating the importance of such a decrease as a predictor of the progression to AIDS. The interaction between treatment and either marker, shown in model 4, was not significant.

The proportion of the effect of treatment or trend toward reduction in the rate of progression to AIDS that was explained by the plasma level of HIV-1 RNA was estimated by dividing the difference between the coefficients for model 3 and model 1 by the coefficient for model 1; this estimate was 59 percent (95 percent confidence interval, 13 to 112 percent). The percentage of the treatment effect that was explained by decreases from base line in plasma HIV-1 RNA levels by factors of three, five, and seven was also approximately 60 percent, whereas a decrease by a factor of 10 explained 42 percent of the treatment effect. In these analyses of changes of varying magnitudes in the plasma level of

Table 1. Cox Models Relating Changes in Markers and Antiretroviral Treatment to the Risk of Progression to AIDS.*

VARIABLE	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	RELATIVE RISK (95% CI)	P VALUE	RELATIVE RISK (95% CI)	P VALUE
75% decrease in HIV-1 RNA	0.40 (0.22–0.75)	0.004	0.44 (0.23–0.81)	0.009
10% increase in CD4+ count	0.45 (0.27–0.77)	0.003	0.48 (0.28–0.82)	0.007
15% decrease in β_2 -microglobulin	0.64 (0.37–1.10)	0.11	0.72 (0.42–1.24)	0.24
Treatment	0.62 (0.43–0.92)	0.03	1.08 (0.66–1.77)	0.76

*CI denotes confidence interval.

HIV-1 RNA, the differences between the treatment groups were significant in each case ($P < 0.005$ for all).

The proportion of the treatment effect accounted for by a 10 percent increase in the CD4+ lymphocyte count was 31 percent (95 percent confidence interval, 4 to 58 percent). The effect of a given increase in the CD4+ lymphocyte count ranged from 22 percent of the treatment effect (for a 5 percent increase) to 37 percent (for an increase of either 15 or 25 percent) ($P < 0.01$ for all these changes). The proportion of the effect of treatment explained by a 15 percent decrease in the plasma level of β_2 -microglobulin was 28 percent, but the effect of this or any other degree of change that was tested for this variable was not significant ($P > 0.05$ for all). When continuous changes in plasma HIV-1 RNA levels were analyzed, the percentage of the treatment effect that was explained was higher than when thresholds of change were analyzed. Thus, we found that a decrease in the plasma level of HIV-1 RNA was a better predictor of outcome than was either an increase in the CD4+ lymphocyte count or a decrease in the plasma level of β_2 -microglobulin.

Classification of Patients and Combinations of Markers

We analyzed pairwise combinations of markers, using six-month mean changes of at least a 75 percent decrease in the plasma level of HIV-1 RNA, a 10 percent increase in the CD4+ lymphocyte count, and a 15 percent decrease in the plasma level of β_2 -microglobulin. The effect of treatment was best explained by a decrease of at least 75 percent in the plasma level of HIV-1 RNA and an increase of at least 10 percent in the CD4+ lymphocyte count. We then classified the patients into four groups, as follows: group 1 included patients with a decrease in plasma HIV-1 RNA of 75 percent or more

and an increase in the CD4+ lymphocyte count of 10 percent or more; group 2, patients with a decrease in plasma HIV-1 RNA of 75 percent or more and an increase in the CD4+ lymphocyte count of less than 10 percent; group 3, patients with a decrease in plasma HIV-1 RNA of less than 75 percent and an increase in the CD4+ lymphocyte count of 10 percent or more; and group 4, patients with a decrease in plasma HIV-1 RNA of less than 75 percent and an increase in the CD4+ lymphocyte count of less than 10 percent. When we validated this classification system as a marker of the risk of progression to AIDS, 79 percent of the effect of treatment was explained by its use (95 percent confidence interval, 27 to 145 percent). Although this is more than the 59 percent obtained with the use of HIV-1 RNA levels alone, there is no statistical method available with which to test the difference between the percentages. Combinations of markers that did not include the plasma level of HIV-1 RNA had consistently lower predictive value than those that included that variable.

We performed a life-table analysis using this classification system, without taking treatment assignment into account. The patients in group 1 had the best outcome, whereas the patients in group 4 had the worst. In the other two groups the results were intermediate. Figure 2 shows Kaplan–Meier curves for this analysis, which demonstrate that the plasma level of HIV-1 RNA and the CD4+ lymphocyte count, used in combination, are a good predictor of the progression to AIDS.

DISCUSSION

We evaluated plasma levels of HIV-1 RNA and β_2 -microglobulin and CD4+ lymphocyte counts as markers

Table 2. Sequential Cox Proportional-Hazards Models Relating Progression to AIDS to Antiretroviral Treatment and Either a 75 Percent Decrease from Base Line in the Plasma HIV-1 RNA Level or a 10 Percent Increase from Base Line in the CD4+ Lymphocyte Count.*

VARIABLE	PLASMA HIV-1 RNA (N = 270)			CD4+ LYMPHOCYTE COUNT (N = 263†)		
	COEFFICIENT	RELATIVE RISK	P VALUE	COEFFICIENT	RELATIVE RISK	P VALUE
Model 1 (treatment only)	–0.572	0.56	0.03	–0.473	0.62	0.03
Model 2 (marker only)	–1.019	0.36	<0.001	–0.878	0.42	<0.001
Model 3						
Treatment	–0.233	0.79	0.33	–0.327	0.72	0.14
Marker	–0.894	0.41	0.004	–0.809	0.45	0.003
Model 4						
Treatment	0.772	2.16	0.28	–0.435	0.65	0.53
Marker	0.502	1.65	0.60	–0.942	0.39	0.28
Treatment and marker	–0.850	0.43	0.14	0.089	1.09	0.87
Treatment effect‡						
Plasma HIV-1 RNA: $[(–0.572) – (–0.233)] \div (–0.572) = 59\%$ (95% confidence interval, 13% to 112%)						
CD4+ lymphocyte count: $[(–0.473) – (–0.327)] \div (–0.473) = 31\%$ (95% confidence interval, 4% to 58%)						

*Base-line values for each marker were included in the model.

†CD4+ lymphocyte counts from the first six months after randomization were not available for seven patients.

‡As described in the Methods section, the proportion of the treatment effect that was explained by each marker was estimated by dividing the difference between the coefficients for model 3 and model 1 by the coefficient for model 1.

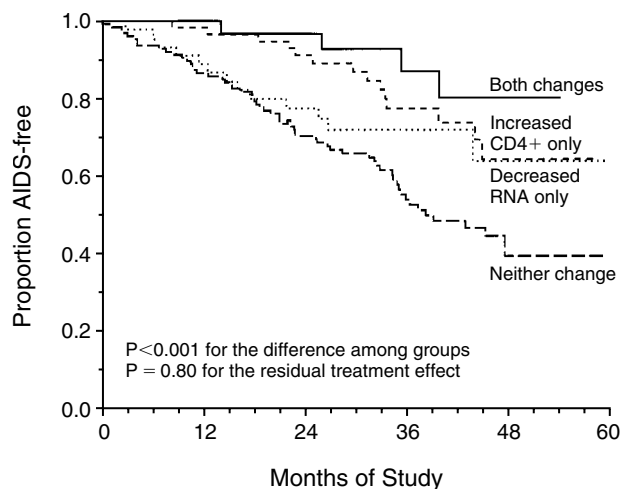


Figure 2. Kaplan-Meier Analysis of the Time to the Progression to AIDS in Patients Found to Have Both a Six-Month Mean Decrease of at Least 75 Percent in Plasma HIV-1 RNA and a Six-Month Mean Increase of at Least 10 Percent in the CD4+ Lymphocyte Count, One of These Changes, or Neither Change.

of HIV-1 disease because they are affected by viral or immune events associated with clinical progression. The results of the study showed that among these markers, changes in plasma HIV-1 RNA explain the effect of treatment on clinical outcome the most reliably and that changes in the CD4+ lymphocyte count provide additional information. Although there are extravascular sites of viral replication, it is probable that circulating levels of HIV-1 RNA best reflect the overall replication of the virus *in vivo*.^{29,30} This report shows that treatment-induced changes in HIV-1 RNA can account for the clinical outcome of HIV-1 infection in symptomatic patients at an intermediate stage of disease (with CD4+ lymphocyte counts of 200 to 500 cells per cubic millimeter).

Recent reports have demonstrated a dynamic interaction between the replication of HIV-1 and the destruction of CD4+ lymphocytes.^{19,20} When effective antiretroviral therapy is begun, levels of HIV-1 replication decline steeply over a period of one to two weeks and the CD4+ lymphocyte count increases correspondingly.^{18-20,31} The availability of assays with which to quantify HIV-specific RNA permits the assessment of treatment-induced changes in viral replication, which cannot be done by measuring levels of HIV-1 DNA in blood or using any other method of detecting HIV-1.^{18-20,31-34}

In our study, the base-line CD4+ lymphocyte count and plasma level of HIV-1 RNA were highly predictive of both the progression to AIDS and death, which suggests that both variables are useful markers of the disease stage. Recently, Mellors et al.³⁵ showed that plasma levels of HIV-1 RNA exceeding 10,000 genome equivalents per milliliter were strongly associated with the progression to AIDS over a mean period of follow-up lasting nearly five years. Those investigators found that plasma levels of HIV-1 RNA predicted clinical out-

come independently of CD4+ lymphocyte counts. This finding reinforces our contention that base-line plasma levels of HIV-1 RNA and CD4+ lymphocyte counts may be used together in determining clinical stages of disease and in therapeutic decision making. Although relative values can be compared, the relations between absolute plasma levels of HIV-1 RNA and outcome are difficult to determine from our study, because of the storage conditions and age of the samples; these associations should be examined with fresh samples.

Although the CD4+ lymphocyte count is a useful marker in determining the stage of HIV disease, it has not been particularly useful as a marker of the clinical response to antiretroviral therapy. A retrospective analysis of data presented by Volberding et al.³ showed that the base-line CD4+ lymphocyte count was highly correlated with the progression to AIDS ($P < 0.001$). Even so, only a small proportion of the effect of zidovudine on this progression (less than 37 percent) was statistically explained by the effect of the drug on the CD4+ lymphocyte count³⁶ — a finding similar to ours and to those of others.^{9,37} In another study, the duration of the increase in the CD4+ lymphocyte count in response to antiretroviral therapy appeared to be more important in predicting disease progression than the magnitude of the initial response.³⁸

The appropriate measure must be found with which to evaluate antiretroviral therapy in HIV-infected patients with various stages of disease, particularly those with different base-line levels of HIV-1 RNA in plasma. On the basis of our data, this should be a reduction by a factor of at least three (i.e., by 0.5 log) in the plasma level of HIV-1 RNA. The rapid appearance of resistance to some antiretroviral agents, such as the non-nucleoside inhibitors of reverse transcriptase,³⁹ and the short-lived response of plasma HIV-1 RNA levels to therapy suggest that the duration of the effect on plasma HIV-1 RNA should also be considered.^{31,40} It is likely that a longer-lasting reduction in the plasma level of HIV-1 RNA may be associated with greater clinical efficacy. The goal of antiretroviral therapy should be to reduce levels of circulating virus as much as possible, for as long as possible. The ability to use reverse-transcriptase PCR to measure the treatment-induced decline in plasma levels of HIV-1 could speed the evaluation of new therapies in prospective trials, while clinical data continue to be collected for subsequent correlation with measurements of changes in markers, including both the CD4+ lymphocyte count and virologic markers.

APPENDIX

In addition to the authors, the following members of the Veterans Affairs Cooperative Study Group on AIDS participated in this study: Houston Veterans Affairs Medical Center (VAMC) — C. Lahart and N. Wray; West Los Angeles VAMC — S.M. Finegold and W.L. George; Miami VAMC — G.M. Dickinson and N. Klimas; New York VAMC — G. Diamond and S.B. Zolla-Pazner; San Francisco VAMC — P.C. Jensen; Walter Reed Army Hospital, Washington, D.C. — C. Hawkes

and C. Oster; Washington, D.C., VAMC — F. Gordin and A.M. Labriola; Durham, N.C., VAMC — P. Spivey; Duke University Virology Center — T. Matthews and K. Weinhold; and University of Maryland Pharmacology Laboratory — G. Drusano and M.J. Egorin.

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