

TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION WITH SAQUINAVIR, ZIDOVUDINE, AND ZALCITABINE

ANN C. COLLIER, M.D., ROBERT W. COOMBS, M.D., PH.D., DAVID A. SCHOENFELD, PH.D.,
ROLAND L. BASSETT, M.S., JOSEPH TIMPONE, M.D., ALICE BARUCH, M.D., PH.D., MICHELLE JONES, M.SC.,
KAREN FACEY, PH.D., CAROLINE WHITACRE, PH.D., VINCENT J. MCAULIFFE, M.D.,
HARVEY M. FRIEDMAN, M.D., THOMAS C. MERIGAN, M.D., RICHARD C. REICHMAN, M.D.,
CAROL HOOPER, M.D., AND LAWRENCE COREY, M.D., FOR THE AIDS CLINICAL TRIALS GROUP

Abstract Background. In patients with human immunodeficiency virus (HIV) infection, combined treatment with several agents may increase the effectiveness of antiviral therapy. We studied the safety and efficacy of saquinavir, an HIV-protease inhibitor, given with one or two nucleoside antiretroviral agents, as compared with the safety and efficacy of a combination of two nucleosides alone.

Methods. In this double-blind trial, patients with HIV infection were randomly assigned to receive either saquinavir (1800 mg per day) plus both zidovudine (600 mg per day) and zalcitabine (2.25 mg per day) or zidovudine plus either saquinavir or zalcitabine. The 302 patients enrolled had CD4+ counts of 50 to 300 cells per cubic millimeter and had previously received zidovudine for a median of 27 months. The study lasted 24 weeks, with an optional double-blind extension period of an additional 12 to 32 weeks.

Results. Ninety-six percent of the patients completed the 24-week study. In all three treatment groups, CD4+

cell counts rose at first and then fell gradually. The normalized area under the curve for the CD4+ cell count was greater with the three-drug combination than with either saquinavir and zidovudine ($P=0.017$) or zalcitabine and zidovudine ($P<0.001$). There were significantly greater reductions in plasma HIV with the three-drug combination than with the other regimens when peripheral-blood mononuclear cells were cultured for HIV and HIV RNA was assessed, and there were greater decreases in serum neopterin and beta₂-microglobulin levels. There were no major differences in toxic effects among the three treatments.

Conclusions. Treatment with saquinavir, zalcitabine, and zidovudine was well tolerated. This drug combination reduced HIV-1 replication, increased CD4+ cell counts, and decreased levels of activation markers in serum more than did treatment with zidovudine and either saquinavir or zalcitabine. Studies are warranted to evaluate whether the three-drug combination will reduce morbidity and mortality. (N Engl J Med 1996;334:1011-7.)

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ANTIRETROVIRAL therapy is associated with delayed progression of disease and prolonged survival in patients with advanced human immunodeficiency virus (HIV) type 1 infection, but the duration of both clinical benefit and viral suppression is limited, and viral strains with decreased susceptibility emerge.¹⁻¹⁰ Although several reverse-transcriptase inhibitors have clinical usefulness, the disease eventually progresses to the acquired immunodeficiency syndrome (AIDS) despite the use of these agents.¹¹⁻¹³

One strategy for improving antiretroviral therapy is to use a combination of agents that inhibit different steps in the HIV life cycle. HIV protease acts late in the life cycle of the virus inside the cell and cleaves a polyprotein into structural proteins required for the assembly of infectious virions.¹⁴ In vitro, protease inhibitors reduce the infectivity of chronically infected cells.¹⁵⁻¹⁸ Saquinavir is a hydroxyethylamine transition-state analogue of the HIV-protease cleavage site that has potent in vitro inhibitory activity against a wide variety of laboratory and clinical isolates of HIV.¹⁸ In vitro, saquinavir, zidovudine, and zalcitabine have additive or syner-

gistic anti-HIV activity.¹⁹⁻²¹ Phase I studies demonstrated an oral bioavailability of 4 percent for saquinavir, and there was a mean peak plasma concentration eight times the 90 percent inhibitory concentration of HIV with a dose of 600 mg of saquinavir three times per day.^{22,23} These studies also suggested improvement in CD4+ cell counts and favorable antiviral responses, especially with the combination of saquinavir and zidovudine.^{24,25} We hypothesized that a combination of saquinavir and two nucleosides would provide more effective antiviral activity than does therapy with saquinavir and a single nucleoside or with a combination of two nucleosides alone.

METHODS

Study Design

The study (AIDS Clinical Trials Group protocol 229) was a randomized, double-blind, phase 2 trial of three treatment regimens. The study patients received either 1800 mg per day of saquinavir (Invirase, formerly Ro 31-8959, Hoffmann-La Roche, Nutley, N.J.), 2.25 mg per day of zalcitabine (Hivid, Hoffmann-La Roche), or both agents in combination at these doses, along with 600 mg per day of open-label zidovudine (Retrovir, Glaxo-Wellcome, Research Triangle Park, N.C.). Each drug was given three times per day in divided doses. The period of primary treatment was 24 weeks; all the patients were allowed to continue receiving the same study regimen in a blinded fashion for an additional 12 to 32 weeks, until the study ended in March 1994.

Patients were enrolled at 10 participating AIDS Clinical Trials Units sponsored by the National Institute of Allergy and Infectious Diseases. Patients were required to be at least 13 years old and to have HIV infection, one CD4+ count of 50 to 300 cells per cubic millimeter obtained within 30 days before entry into the study, and at least 4 months of prior zidovudine therapy. The entry requirements also included a granulocyte count of at least 1000 cells per cubic mil-

From the University of Washington School of Medicine, Seattle (A.C.C., R.W.C., C.H., L.C.); the Harvard School of Public Health, Boston (D.A.S., R.L.B.); the National Institute of Allergy and Infectious Diseases, Bethesda, Md. (J.T.); Hoffmann-LaRoche, Nutley, N.J. (A.B.); Roche Products, Welwyn Garden City, United Kingdom (M.J., K.F.); Ohio State University, Columbus (C.W.); New York University, New York (V.J.M.); the University of Pennsylvania, Philadelphia (H.M.F.); Stanford University, Palo Alto, Calif. (T.C.M.); and the University of Rochester, Rochester, N.Y. (R.C.R.). Address reprint requests to Dr. Collier at 1001 Broadway, Suite 218, Seattle, WA 98122.

Supported by grants (AI-27664, AI-27658, and RR00044) from the National Institutes of Health.

limeter, a hemoglobin level of at least 8.5 g per deciliter (5.3 mmol per liter), a platelet count of at least 50,000 per cubic millimeter, a creatinine level not exceeding 2 times the upper limit of normal, aminotransferase and alkaline phosphatase levels not exceeding 5 times the upper limit of normal, a bilirubin level not exceeding 2.5 times the upper limit of normal, and an amylase level not exceeding 1.5 times the upper limit of normal. Patients were excluded from the study if they had lymphoma, visceral Kaposi's sarcoma, severe chronic diarrhea, peripheral neuropathy, pancreatitis, or an active untreated opportunistic infection; if they were dependent on transfusions; if they were pregnant or nursing; or if they were taking immunomodulatory or other experimental medications.

The study protocol was approved by the review boards of the participating institutions. All the patients gave written informed consent. They underwent standardized clinical and laboratory evaluations every two weeks for the first eight weeks (except the first 60 patients, who were seen weekly for the first four weeks), then monthly until week 24 and every two months thereafter. Patients who terminated their study therapy early continued on the same schedule of visits through week 24. Symptoms, signs, and laboratory results were graded on the standardized rating scale of the AIDS Clinical Trials Group. The doses of the study medications were modified if either severe or persistent and moderate adverse effects developed. The study drugs were discontinued permanently if there were life-threatening adverse effects; if foscarnet, ganciclovir, or chemotherapy was required; or if the patient was unable to receive the study drugs for more than 30 consecutive days. AIDS-defining diagnoses were verified by the chairperson of the study before the treatment assignments were unblinded.

Laboratory Analysis

CD4+ and CD8+ cells in samples of peripheral blood were counted with the use of monoclonal antibodies and flow cytometry on three occasions before therapy began; at weeks 4, 8, 12, 16, and 24; and every eight weeks thereafter.²⁶ Peripheral-blood mononuclear cells (PBMCs) were cultured for HIV by a quantitative technique of microculture.^{27,28} Plasma HIV RNA levels were determined in thawed plasma samples (stored at -70°C , with acid citrate dextrose used as an anticoagulant) by two analytic methods: quantitative polymerase-chain-reaction (PCR) amplification by the reverse-transcriptase method (Roche Molecular Systems, Alameda, Calif.) and branched-chain DNA (bDNA) signal amplification (Chiron, Emeryville, Calif.).²⁹⁻³⁴ The reverse-transcriptase PCR assays were performed at Roche Molecular Systems, and the bDNA assays were performed at the University of Washington. HIV RNA copy numbers were determined on the basis of the manufacturers' reference standards. The lower level of sensitivity of the reverse-transcriptase PCR assay was 400 RNA copies per milliliter, and that of the bDNA assay was 10,000 RNA equivalents per milliliter.^{29,31} The HIV RNA assays for a given patient were performed in one batch. The quantitative virologic assays were performed twice before entry into the study and at weeks 4, 8, 12, 16, and 24. PBMCs were cultured and reverse-transcriptase PCR assays also performed every eight weeks during extended treatment. The immunology and virology laboratories at each site were certified by quality-control programs of the AIDS Clinical Trials Group.

For the determinations of serum beta₂-microglobulin and neopterin, samples of whole blood were collected twice before entry into the study and at weeks 8, 16, and 24; the samples were protected from light and stored at -20°C . The samples from all centers were assayed simultaneously at the Clinical Immunology Research Laboratory of the University of California at Los Angeles. Neopterin levels were quantitated by a commercial radioimmunoassay (IMMU test Neopterin, Henning, Berlin, Germany). Levels of beta₂-microglobulin were measured by an automated microparticle enzyme immunoassay (IMx, Abbott Diagnostics, Abbott Park, Ill.).

Statistical Analysis

The primary end points indicative of efficacy were changes in absolute CD4+ cell counts and quantitative HIV titers in cultures of PBMCs during the first 24 weeks of treatment.³⁵ The trial was designed to detect a difference equal to half the standard deviation of the measurements of outcome. On the basis of available data, for CD4+ cells this difference was 50 cells per cubic millimeter. For HIV

titers in cultures of PBMCs, the trial had an 80 percent power to detect a similar difference, which was a log titer of 0.8. The analyses used an intention-to-treat approach that included all observations, even when the study therapy was discontinued prematurely. Patients for whom no assessments were made after base line were excluded from all the analyses. If a test for the overall difference among the three treatment groups showed a significant difference, the three-drug combination was tested pairwise against each double combination. No adjustment was made for the testing of multiple measures. All reported P values are two-sided. The geometric mean of all pretreatment values was used as the base-line value. The logarithmic transformations used base 10.

CD4+ Cell Counts

CD4+ cell counts were log-transformed before analysis. The normalized area under the log-transformed curve for the CD4+ count was calculated for each patient, and the areas were compared between treatment groups by the Kruskal-Wallis test. The area under the curve was calculated by subtracting the base-line log CD4+ count from the log CD4+ count at each time point and calculating the area under the resulting curve by the trapezoidal method. The resulting area was normalized according to the proportion of the 24-week period that the patient had completed. The units used were the log CD4+ counts times the number of days. The proportions of patients whose CD4+ cell counts had returned to base line at 48 weeks were compared by a test based on Kaplan-Meier estimates.

Virologic Analysis

In the quantitative analyses of HIV in PBMCs, the titer of infectious units per million cells was calculated for each sample.²⁸ The analyses of PBMC data and viral RNA used a mixed-model analysis of variance to compare the values obtained during treatment with the base-line value. The dependent variable was the difference between the log-transformed value at each interval of follow-up and the base-line value. The effects analyzed in the model were the base-line value, the treatment assignment, and a random patient-related effect. Changes in titer were estimated with least-squares means. The correlation between the measurements obtained by the reverse-transcriptase PCR assays and those obtained by the bDNA assays was estimated with a censored regression model, to account for the difference in censoring with these two assays at the lower limit of detection.³⁶

Serum Levels of Activation Markers

The log-transformed levels of neopterin and beta₂-microglobulin were compared among the treatment groups by analysis of variance. The frequency of adverse events was compared among treatment groups by Fisher's exact test.³⁷ The time to death or the first new occurrence of an AIDS-defining opportunistic infection was compared among treatment groups by the method of Kaplan and Meier.

RESULTS

A total of 302 patients were enrolled from March 1993 through July 1993. Five patients were excluded from the analyses; one (who had active tuberculosis) was enrolled by error, two never received the study therapy, and two were lost to follow-up after day 1. The treatment groups were well balanced with regard to the base-line characteristics of the patients who could be evaluated (Table 1).

Clinical Events

The total follow-up in the study was 2861 person-months, 1529 in the first 24 weeks and 1332 in the extension period. Among the 297 patients, 284 (96 percent) completed the 24-week study period; among the remaining patients, 7 were lost to follow-up, 2 died, 2 withdrew because of side effects, 1 did not comply with the study protocol, and 1 withdrew after an as-

Table 1. Base-Line Characteristics of the Study Patients.*

CHARACTERISTIC	THREE DRUGS (N = 98)	SAQUINAVIR- ZIDOVUDINE (N = 99)	ZALCITABINE- ZIDOVUDINE (N = 100)	ALL PATIENTS (N = 297)
Mean (±SD) age — yr	37.8±7.9	38.6±9.2	37.8±8.2	38.1±8.4
Male sex — no. (%)	89 (91)	87 (88)	94 (94)	270 (91)
Race — no. (%)				
White	79 (81)	78 (79)	77 (77)	234 (79)
Black	6 (6)	11 (11)	16 (16)	33 (11)
Hispanic	11 (11)	9 (9)	6 (6)	26 (9)
Other	2 (2)	1 (1)	1 (1)	4 (1)
Duration of prior zidovudine — mo				
Median	27	30	27	27
Range	4–75	4–68	4–98	4–98
Prior zalcitabine or zidovudine				
Patients — no. (%)	41 (42)	41 (41)	32 (32)	114 (38)
Duration of use — mo				
Median	6	7	9	7
Range	<1–23	<1–25	<1–25	<1–25
HIV status — no. (%)				
AIDS	13 (13)	10 (10)	10 (10)	33 (11)
Symptoms, no AIDS	52 (53)	52 (53)	49 (49)	153 (52)
No symptoms	33 (34)	37 (37)	41 (41)	111 (37)
CD4+ count — cells/mm ³				
Median	145	156	171	156
Range	25–311	31–394	32–361	25–394
HIV titer in PBMCs — infectious units per million				
Median	38	26	26	27
Range	0.2–1223	0.2–1026	0.2–2899	0.2–2899
Mean (±SE) beta ₂ -microglobulin — mg/liter†	2.6±0.08	2.7±0.08	2.6±0.07	2.6±0.04
Mean (±SE) neopterin — nmol/liter	15.9±0.6	16.2±0.8	14.9±0.6	15.7±0.4

*There were no statistically significant differences among the three groups. PBMC denotes peripheral-blood mononuclear cell.

†To convert values to nanomoles per liter, multiply by 84.75.

sault. Twenty-four other patients (8 percent) discontinued the study medication before week 24 — 6 in the three-drug group, 8 in the group assigned to saquinavir and zidovudine, and 10 in the group assigned to zalcitabine and zidovudine. Treatment was discontinued early in these patients because of toxic effects (13 patients), the request of the patient (4), noncompliance (4), and a need for drugs or chemotherapy not permitted according to the study protocol (3). A total of 244 patients (82 percent) received therapy during the extension period for a median of 6 additional months (range, 1 to 7.5). In all, 218 patients (73 percent) were treated until the end of the study — 75 in the three-drug group, 74 in the group assigned to saquinavir and zidovudine, and 69 in the group assigned to zalcitabine and zidovudine. There were no significant differences in pretreatment characteristics between the patients who completed the 24 weeks of the study and those who did not, except that the former were slightly older (mean age, 38 vs. 32 years), or between those who received extended treatment and those who did not.

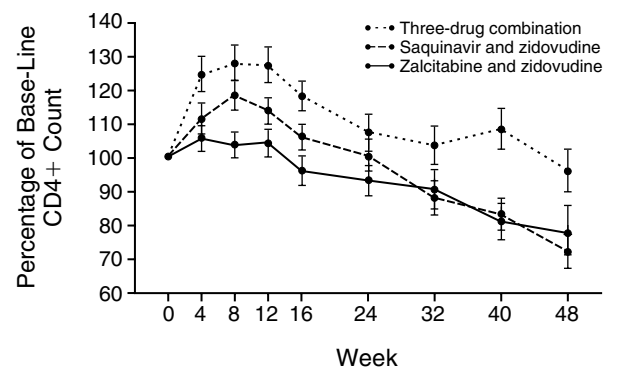
Thirteen patients had AIDS-defining illnesses or died during the first 24 weeks, and four more did so during the extension period. Of these 17, 3 were in the three-drug group, 8 in the saquinavir–zidovudine group, and 6 in the zalcitabine–zidovudine group. There was no statistically significant difference among the three groups in the time to the occurrence of a clinical event, either during the 24-week treatment period (P=0.22) or overall

(P=0.32). Two patients died of undiagnosed processes involving the central nervous system during the first 24 weeks, having discontinued the study therapy after 3 days and 3 weeks. Three other patients died at weeks 32, 40, and 52 of AIDS-related complications, having discontinued therapy at study weeks 3, 14, and 20, respectively.

CD4+ Cell Counts

CD4+ cell counts rose initially in all three treatment groups (Fig. 1). The analyses of the areas under the curve for the CD4+ count demonstrated the superiority of the three-drug combination; the mean (±SE) normalized area under the curve in the first 24 weeks for the three-drug group was 12.2±2.0, as compared with 5.1±2.1 for the saquinavir–zidovudine group and -0.33±2.2 for the zalcitabine–zidovudine group (P<0.001). In pairwise comparisons, the CD4+ cell response was significantly better in the three-drug group than in either the saquinavir–zidovudine group (P=0.017) or the zalcitabine–zidovudine group (P<0.001).

At 24 weeks, 70 percent of the patients in the three-drug group had CD4+ cell counts that had remained above the base-line count, as compared with 63 percent of the saquinavir–zidovudine group and 45 percent of the zalcitabine–zidovudine group (P<0.004). In pairwise tests, significantly more patients had CD4+ cell counts above base line at 24 weeks in the three-drug group than in the zalcitabine–zidovudine group (P<0.001), but not the saquinavir–zidovudine group (P=0.24). The analysis of changes in



No. of patients 297 287 280 266 273 270 228 211 177

Figure 1. Mean (±SE) CD4+ Cell Counts, Expressed as Percentages of the Base-Line Count, According to Study Week.

The numbers below the graph are the numbers of patients studied in the weeks shown.

CD4+ cell counts from base line to week 48 showed that the three-drug combination was better than either saquinavir and zidovudine ($P=0.004$) or zalcitabine and zidovudine ($P=0.047$).

Virologic Data

Quantitative HIV Culture

The three-drug combination suppressed HIV titers in cultures of PBMCs more than did either two-drug regimen, and it produced the most sustained antiviral response ($P<0.001$ for the three-way comparison) (Table 2). In pairwise comparisons, the three-drug combination lowered titers in the first 24 weeks more than did either saquinavir and zidovudine ($P<0.001$) or zalcitabine and zidovudine ($P=0.003$). The mean titer of HIV in PBMCs decreased by 0.8 log in the three-drug group, as compared with no change in the saquinavir-zidovudine group and a change of less than 0.4 log in the zalcitabine-zidovudine group. Zalcitabine and zidovudine lowered titers more than did saquinavir and zidovudine ($P=0.004$). The patients assigned to three-drug therapy had titers that remained below base line longer than those of the patients assigned to saquinavir and zidovudine, although over time, even in the three-drug group, there was a gradual return toward the base-line titer. With the three-drug therapy, mean titers remained below the base-line levels through week 48 (Table 2), although there was no longer a significant difference from the other groups.

Viral RNA

The mean titer of HIV RNA in plasma decreased with all regimens (Fig. 2). HIV RNA levels measured by the bDNA and reverse-transcriptase PCR assays were highly correlated ($r=0.83$). The decrease in the titer and the durability of suppression were greater with the three-drug combination than with the two-drug regimens ($P<0.001$). In pairwise comparisons, the three-drug combination lowered HIV RNA copy numbers more than did either saquinavir and zidovudine

Table 2. Quantitative HIV Titers in Peripheral-Blood Mononuclear Cells.

WEEK	HIV TITER		
	THREE DRUGS	SAQUINAVIR-ZIDOVUDINE	ZALCITABINE-ZIDOVUDINE
	<i>log IUPM (no. of patients tested)*</i>		
0	1.37±0.09 (96)	1.25±0.09 (98)	1.20±0.09 (100)
4	0.53±0.10 (89)	1.28±0.10 (93)	0.88±0.09 (97)
8	0.62±0.10 (95)	1.24±0.11 (87)	0.77±0.10 (94)
12	0.72±0.11 (87)	1.27±0.10 (92)	0.86±0.10 (92)
16	0.74±0.11 (90)	1.37±0.09 (90)	1.00±0.10 (95)
24	0.81±0.12 (86)	1.44±0.10 (86)	0.93±0.11 (87)
32	0.76±0.11 (81)	1.54±0.13 (69)	1.00±0.12 (68)
40	0.87±0.12 (66)	1.38±0.11 (69)	0.88±0.13 (64)
48	0.83±0.13 (57)	1.27±0.12 (60)	0.98±0.13 (54)

*IUPM denotes infectious units per million. Plus-minus values are means ±SE.

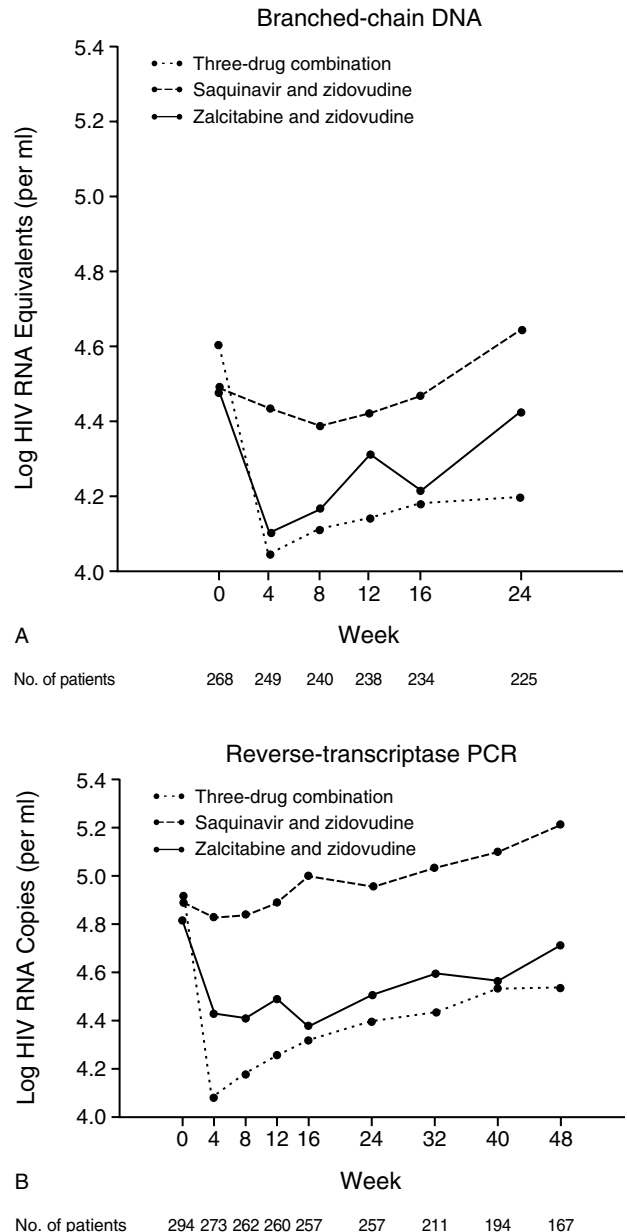


Figure 2. Median Plasma Levels of HIV RNA in the Study Patients as Determined by Two Methods, According to Treatment Group and Study Week.

The data in Panel A were obtained by branched-chain DNA signal amplification, and those in Panel B by reverse-transcriptase PCR amplification. The numbers below the graphs are the numbers of patients studied in the weeks shown.

($P<0.001$) or zalcitabine and zidovudine ($P<0.001$). At week 48, there was a significant difference in the change from base line in the HIV RNA titer among the three groups ($P=0.001$), with the three-drug combination producing the largest decrease.

Serum Levels of Activation Markers

Mean values for serum beta₂-microglobulin and neopterin are shown in Table 3. Levels of both serum

Table 3. Serum Levels of Neopterin and Beta₂-Microglobulin.

WEEK	THREE DRUGS	SAQUINAVIR-ZIDOVUDINE	ZALCITABINE-ZIDOVUDINE	P VALUE*
<i>serum level (no. of patients tested)†</i>				
Neopterin (nmol/liter)				
0	15.9±0.6 (96)	16.2±0.8 (99)	14.9±0.6 (100)	NS
8	14.4±0.7 (89)	14.1±1.0 (83)	14.2±0.7 (88)	0.13
16	13.3±0.6 (85)‡	14.7±1.1 (85)	14.1±0.6 (93)	0.021
24	13.3±0.7 (86)§	14.7±1.1 (80)	15.3±0.9 (83)	0.001
Beta ₂ -microglobulin (mg/liter)¶				
0	2.6±0.08 (96)	2.7±0.08 (99)	2.6±0.07 (100)	NS
8	2.4±0.08 (89)	2.5±0.08 (83)	2.5±0.07 (88)	0.11
16	2.4±0.08 (85)	2.5±0.08 (85)	2.5±0.07 (93)	0.072
24	2.4±0.08 (86)	2.6±0.1 (80)	2.6±0.1 (83)	0.006

*By three-way comparison. NS denotes not significant.
 †Plus-minus values are means ±SE.
 ‡P=0.006 for the comparison with zalcitabine and zidovudine.
 §P=0.001 for the comparison with zalcitabine and zidovudine.
 ¶To convert values to nanomoles per liter, multiply by 84.75.
 ||P=0.021 for the comparison with saquinavir and zidovudine, and P=0.03 for the comparison with zalcitabine and zidovudine.

activation markers were reduced by the three-drug therapy, as well as by saquinavir and zidovudine; the reductions were greater with the three-drug regimen.

Adverse Events

The three treatments were tolerated equally well. Table 4 summarizes the most common toxic effects. No statistically significant differences were found among the three regimens with respect to any clinical or laboratory measure during either the first 24 weeks or the overall study. A total of 19 patients (6 percent) terminated the study therapy permanently because of toxic effects. Thirty-three patients (11 percent) had one or more severe symptoms that may have been related to treatment. Seven of these patients were assigned to the three-drug combination, 12 to saquinavir and zidovudine, and 14 to zalcitabine and zidovudine — 7, 12, and 14 percent of the respective groups (P=0.29). Forty-eight patients (16 percent of the total number) had severe laboratory abnormalities that resulted in a decrease in the dose of study medication; of these, 13 were assigned to the three-drug combination, 12 to saquinavir and zidovudine, and 23 to zalcitabine and zidovudine. Overall, 62 patients (21 percent) had either severe clinical symptoms that may have been related to the study treatment or laboratory abnormalities that resulted in a dose decrease; 17 were assigned to the three-drug combination, 20 to saquinavir and zidovudine, and 25 to zalcitabine and zidovudine.

DISCUSSION

This phase 2 study relied on laboratory end points to determine the virologic, immunologic, and clinical tolerance to saquinavir-containing treatment regimens of patients with relatively advanced HIV infection. The data suggest that saquinavir has in vivo anti-HIV activity at a dose of 1800 mg per day when combined with zidovudine and zalcitabine. Among patients with extensive previous zidovudine therapy, the use of three anti-

retroviral agents lowered cell-associated infectious titers of HIV, plasma titers of viral RNA, and levels of neopterin and beta₂-microglobulin, and the three-drug therapy raised CD4+ cell counts more than did the use of either saquinavir or zalcitabine in combination with zidovudine. Because this study was not designed with sufficient power to detect differences in clinical events, it is not known whether the favorable effects on laboratory markers with the three-drug combination will translate into a reduced progression of disease and improved survival.

This study used several quantitative HIV assays that measured different aspects of the viral burden. Which measure is the most clinically relevant is not known. The three-drug combination was superior to the other two regimens with regard to the primary virologic measure of cell-associated infectious virus, as well as that of plasma HIV RNA titers. Reduction in these titers with antiviral therapy has been associated with a reduced risk of clinical progression of HIV disease.³⁸⁻⁴⁰ One of the interesting observations was that the suppressive effect of the three-drug combination on viral load, as measured by quantitative microculture of PBMCs, HIV RNA titers, and effects on serum activation markers, appeared to be more durable than the elevation of CD4+ counts. That the antiviral response was sustained longer than the CD4+ cell response

Table 4. Clinical Symptoms and Laboratory Abnormalities Leading to Alterations in the Dose of the Study Drugs.

VARIABLE	PATIENTS WITH ≥1 EPISODE OF TOXICITY			
	THREE DRUGS (N = 98)	SAQUINAVIR-ZIDOVUDINE (N = 99)	ZALCITABINE-ZIDOVUDINE (N = 100)	ALL PATIENTS (N = 297)
	<i>no. of patients (%)</i>			
Fatigue				
Moderate	17 (17)	16 (16)	26 (26)	59 (20)
Severe	2 (2)	2 (2)	2 (2)	6 (2)
Diarrhea				
Moderate	5 (5)	12 (12)	9 (9)	26 (9)
Severe	1 (1)	1 (1)	2 (2)	4 (1)
Nausea				
Moderate	9 (9)	11 (11)	15 (15)	35 (12)
Severe	0	0	1 (1)	1 (<1)
Any clinical symptom*				
Moderate	57 (58)	59 (60)	59 (59)	175 (59)
Severe	7 (7)	12 (12)	14 (14)	33 (11)
Creatine phosphokinase†				
Moderate	3 (3)	3 (3)	3 (3)	9 (3)
Severe	4 (4)	4 (4)	4 (4)	12 (4)
Neutropenia‡				
Moderate	1 (1)	2 (2)	1 (1)	4 (1)
Severe	3 (3)	2 (2)	7 (7)	12 (4)
Any laboratory abnormality§				
Moderate	11 (11)	12 (12)	14 (14)	37 (12)
Severe	13 (13)	12 (12)	23 (23)	48 (16)

*Numbers shown include all clinical symptoms or signs possibly related to treatment.
 †Moderate levels of creatine phosphokinase were defined as levels 2 to 4 times the upper limit of the normal range, and severe levels as those more than 4 times the upper limit.
 ‡Moderate neutropenia was defined as being present when there were 750 to 999 neutrophils per cubic millimeter, and severe neutropenia when the neutrophil count was less than 750 per cubic millimeter.
 §Numbers shown include all laboratory abnormalities that led to a decrease in the dose of the study drugs or an interruption in the study treatment.

raises intriguing questions about the association between quantitative measures of HIV, immune activation, and CD4+ cell counts. Nonetheless, these results suggest that the combination of saquinavir, zalcitabine, and zidovudine should be further investigated in long-term studies.

The double-drug combinations had similar effects on CD4+ cell counts, whereas the trends in the results of PBMC microculture for HIV and HIV RNA findings favored the combination of zalcitabine and zidovudine. The explanation for this discrepancy is not clear. There is a need to be cautious in comparing the double-drug regimens, because the study lacked sufficient power for rigorous comparisons. Favorable trends in viral suppression have been seen with saquinavir and zidovudine in patients with no prior antiretroviral therapy.²⁵ The results of studies of viral resistance and assays of the serum concentrations of the study medications may aid in understanding these data.

The type, severity, and frequency of adverse events appeared to be similar with the regimens we used. Toxic effects led to the discontinuation of the study therapy in 6 percent of patients. The toxicity profiles of zidovudine and zalcitabine are well established.^{10,41} No unexpected or unique adverse effects were associated with the regimens containing saquinavir. The lack of increased toxicity associated with the three-drug regimen contrasts with what might be expected when a third medication is added to two therapies that have recognized toxic effects. Mild-to-moderate clinical symptoms were common but were typical of the symptoms encountered in advanced HIV disease. One of the most common laboratory abnormalities was an asymptomatic elevation in creatine kinase, although the frequency of this finding was similar in all the treatment groups. Whether this abnormality was related to HIV or to long-term nucleoside therapy cannot be determined from this study, but it did not appear to be related to saquinavir. We did not see dose-limiting toxicity with saquinavir. Although our study suggests that 1800 mg of saquinavir per day has in vivo antiretroviral effects when zidovudine and zalcitabine are also given, higher doses of saquinavir may produce greater benefits.

The AIDS-defining events and deaths that occurred were typical for advanced HIV infection and appeared to be unrelated to the toxic effects of the drugs. The antiviral and CD4+ cell effects of the three-drug combination are promising, but the clinical benefits of regimens containing saquinavir remain to be determined.

We are indebted to Keith Bragman, M.D., Pearl Leung, Jeanne Conley, R.N., and Barbara Schock for assistance in the planning and execution of this study; to John Fahey, M.D., Ph.D., for performing the neopterin and beta₂-microglobulin assays; to Linda Page for preparing the manuscript; and to the following people for their part in the recruitment and follow-up of patients and laboratory support: University of Washington — Becky Royer, P.A.-C., Hilton Locke, Jason Paragas, and Hsin-Hung Yang; New York University — Victoria Rosenwald, R.N., Janet V. Forcht, R.N., and Fred Valentine, M.D.; University of Pennsylvania — Janice Jacovini, R.N., Debora Dunbar, M.S.N., C.R.N.P., Stephen Hauptman, D.O., and Lyle Jew, Pharm.D.; Stanford University — Mark Winters, M.S., Virginia Talman, R.N., M.A., Gretchen van Raalte, M.S., and Jeffrey Fessel, M.D.; University

of Rochester — Ross Hewitt, M.D., Donald Blair, M.D., Lisa Demeter, M.D., and Carol Greisberger, R.N., B.S.N.; Northwestern University — Frank Palella, M.D., Robert Murphy, M.D., Harold Kessler, M.D., and Joseph Pulvirenti, M.D.; University of Texas at Galveston — Richard Pollard, M.D., Michael J. Borucki, M.D., Tammy Becker, P.A.-C., and Karen Waterman, R.N.; University of Alabama — Michael Saag, M.D., Kathleen Squires, M.D., Robin Noles, R.N., and Judy Smith; and Ohio State University — Michael F. Para, M.D., Judith L. Neidig, M.S., R.N., and Robert J. Fass, M.D.

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