Immunologic and Virologic Effects of Subcutaneous Interleukin 2 in Combination With Antiretroviral Therapy

A Randomized Controlled Trial

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Context While interleukin 2 (IL-2) is capable of inducing a marked expansion of the CD4 T-lymphocyte pool, limited data exist on whether IL-2 treatment can add significantly to the immunologic and virologic effects of potent antiretroviral therapy (ART).

Objective To determine the rate and magnitude of CD4 cell recovery and viral suppression when using a combination therapy of IL-2 and ART compared with ART alone.

Design and Setting Randomized, controlled multicenter trial conducted from April 1996 through April 1998 at 8 clinical sites in the United States.

Patients Eighty-two adult outpatients who were infected with human immunodeficiency virus (HIV) and had baseline CD4 cell counts of 200×10^6/L to 500×10^6/L and baseline RNA levels of fewer than 10000 copies/mL were randomized; 78 completed the study.

Interventions Thirty-nine patients were randomly assigned to receive a combination therapy of subcutaneous IL-2 (administered in 5-day courses every 8 weeks at a starting dosage of 7.5 mIU twice per day) and ART; 43 were to receive ART therapy alone.

Main Outcome Measures Interleukin 2 safety and differential effects on CD4 cell counts, CD4 cell percentages, and plasma HIV RNA levels.

Results The mean (SD) percentage increase in CD4 cell counts at 1 year for patients who received IL-2 was 112% (113%) compared with 18% (35%) in recipients of ART alone ($P<.001$). Both groups had mean (SD) increases in CD4 cell percentage: from 20.4% (6.3%) to 32.3% (12.4%) for the combination therapy group compared with 20.4% (5.1%) to 23.0% (7.2%) for recipients of ART alone ($P<.001$). Using a sensitive viral RNA assay, mean viral load changes were –0.28 and 0.09 $\log_{10}$ copies for IL-2 recipients and control patients, respectively ($P=.03$). Twenty (67%) of 30 evaluable patients receiving IL-2 achieved final viral loads of fewer than 50 copies/mL compared with 13 (36%) of 36 control patients ($P=.02$). Toxic effects were common among patients who received IL-2 and were managed with antipyretics, hydration, rest, and dosage reduction as needed.

Conclusions Intermittent therapy with IL-2 and ART produced a substantially greater increase in CD4 cells and was associated with a larger decrease in viral load than ART alone. Clinical end-point trials will be necessary to determine whether the enhanced viral suppression and CD4 cell increases associated with IL-2 therapy will translate into improved clinical outcomes.

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USE OF POTENT MULTIDRUG combination antiretroviral treatment (ART) can suppress human immunodeficiency virus type 1 (HIV-1) RNA in plasma to extremely low levels for an extended period. The major hallmark of these potent regimens is a dramatic reduction in the incidence of death and acquired immunodeficiency syndrome (AIDS)–defining events.[1] Despite these gains, the ability of ART to restore immunocompetence to a level approaching the preinfection state appears incomplete. These medications present challenges to the patient and clinician, including reduced adherence over time, short- and long-term toxic effects, quality-of-life concerns, and the ongoing threat of emerging viral resis-

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tance.[2] Nonetheless, overall, the benefits of ART continue to strongly outweigh the disadvantages. However, any complementary strategies that could integrate well with existing antiretroviral agents and augment immune restoration might provide significant additional benefit.

Interleukin 2 (IL-2) has been investigated as an adjunct to ART to enhance immunologic activity.[3,4] Beginning in the pre–highly active ART (HAART) era, several phase 1 and phase 2 studies clearly documented the ability of intermittent therapy with either continuous intravenous or subcutaneous IL-2 with ART to induce marked, sustained increases in CD4 cell counts and CD4 cell percentages in HIV-infected individuals.[5-11] Furthermore, while dosage-dependent adverse effects are common and can be dosage-limiting, IL-2 is safe and generally well tolerated for the long term.[7,11]

We report here the results of a multicenter, randomized controlled clinical trial comparing the efficacy of combination treatment with IL-2 and ART vs ART alone. We sought to determine the rate and magnitude of CD4 cell recovery and whether such combination therapy might produce greater viral suppression than ART alone.

METHODS

Study Design

The trial was conducted from April 1996 through April 1998 at 8 geographically diverse medical centers in the United States. Adult patients with serologic evidence of HIV-1 infection were eligible if they met the following criteria: a baseline CD4 cell count between 200×10^6/L and 500×10^6/L and either a minimum CD4 cell percentage of 14% or a viral burden of fewer than 10 000 HIV-1 RNA copies/mL; no prior treatment with IL-2; and no history of AIDS-defining clinical conditions or Centers for Disease Control and Prevention category B symptoms (1993 criteria), with the exception of oral candidiasis, hairy leukoplakia, or herpes zoster.[12] Systemic corticosteroids, chemotherapy, and other experimental cytotoxic agents were not permitted in the 4 weeks prior to beginning study therapy. Subjects were required to be receiving a stable regimen of either standard-of-care–approved ART or experimental antiretroviral agents available through expanded access for at least 2 weeks prior to study entry. Choice of standard-of-care ART was made at the discretion of prescribing physicians and their patients at each medical center. All patients provided written informed consent as approved through the institutional review board of each participating center.

Patients were randomly assigned to treatment groups using a centralized randomization process through Chiron Corp, Emeryville, Calif. Randomization was computer generated and stratified by study site and used a block size of 4 for the first 2 blocks and subsequent block sizes of 2. To accomplish the randomization, site coordinators contacted a biostatistician, who randomly assigned treatment groups. The biostatistician who implemented the randomization scheme was not involved in subsequent data analysis.

Patients were randomly assigned to receive either 6 cycles of IL-2 (at a dosage of 7.5 mIU every 12 hours for 5 days approximately every 8 weeks), administered subcutaneously, and ART; or ART alone. After enrollment, IL-2 recipients were evaluated daily during IL-2 cycles as well as monthly during the first 4 months of the study; thereafter, they were evaluated at least every other month, at the beginning of each subsequent IL-2 cycle. CD4 cell counts and other assessments were performed immediately prior to each cycle. After baseline evaluation, patients receiving ART alone were evaluated serially at study weeks 14, 22, 30, 38, and 48. Safety, immunologic, and virologic assessments were completed at these specified points. Toxic effects were graded 1 through 4 on the National Cancer Institute Common Toxicity Scale. For IL-2 recipients, preemptive treatment with ibuprofen or acetaminophen on a scheduled basis was recommended during each day of the 5-day cycle, and maintenance of adequate hydration was emphasized. An initial dosage reduction of 3 mIU per injection was permitted for any significant (at least grade 3) toxic effects or patient request based on tolerance of IL-2. Dosages were subsequently reduced by 1.5 mIU per injection.

Flow cytometry and plasma HIV-1 RNA (branched DNA assay, version 2.0, Bayer Diagnostics, Norwood, Mass; sensitivity threshold, 500 copies/mL) assessments were performed serially in real time.[13-15] After 1 year, available frozen plasma samples from all sites were analyzed in batched fashion at a single central reference laboratory by the version 3.0 branched DNA assay for HIV-1 RNA levels (sensitivity threshold, 50 RNA copies/mL). Laboratory staff were not aware of treatment arm assignment at the time of specimen processing.

Statistical Analysis

The study was designed to have 90% power to detect a treatment difference of 30% or more in the mean percentage change in CD4 cell counts (the primary efficacy variable). The baseline CD4 cell count was defined as the mean of all CD4 cell counts obtained within 30 days of day 1 of the first cycle of treatment. For IL-2 patients who completed the trial, the final value was the mean of the last 2 values obtained within 1 month following the sixth cycle of treatment. For IL-2 patients who terminated treatment early, the mean of the 2 final posttrafficking CD4 values (or the last available value, if only 1 existed) was used for evaluation. For recipients of ART alone, the final value was the mean of the last 2 postbaseline CD4 cell counts. The primary analysis for the determination of effectiveness was an analysis of variance of percentage change in CD4 cell counts. Because of outlying observations and their possible effect on the results of the usual analysis of variance, the statistical analysis was based on ranked observations.[16] A single ranking of all patients' values (combined groups) was used. Independent factors in the model were treatment arm, study site, and

their interaction. In the primary analysis, the IL-2 and control groups were compared. All analyses used 2-sided tests. Secondary analyses examined the effects of baseline CD4 cell count, viral burden, and changes in ART on CD4 cell count. The proportion of patients with final HIV-1 RNA levels below and above the detection limit, stratified by baseline HIV-1 RNA levels, was analyzed by the Cochran-Mantel-Haenszel test. This test also was used to determine the proportion of protease inhibitor users with final HIV-1 RNA levels below and above assay detection limits, stratified by baseline HIV-1 RNA levels. Safety analysis included the incidence of clinical and laboratory adverse events as well as dosage-limiting toxic effects for the IL-2 regimen.

RESULTS

A total of 82 patients were enrolled at the 8 participating centers, of whom 39 were randomized to combination therapy with IL-2 and ART and 43 to ART alone (TABLE 1 and FIGURE 1). Of these 82 patients, 2 withdrew from the study prior to receiving study drug and 2 additional patients did not provide blood samples after baseline, leaving 78 patients who were evaluable for the primary efficacy end point. Baseline characteristics were similar in both groups, although prior exposure to protease inhibitors was more common (86% vs 56%) among the group receiving IL-2.

CD4 Cell Counts

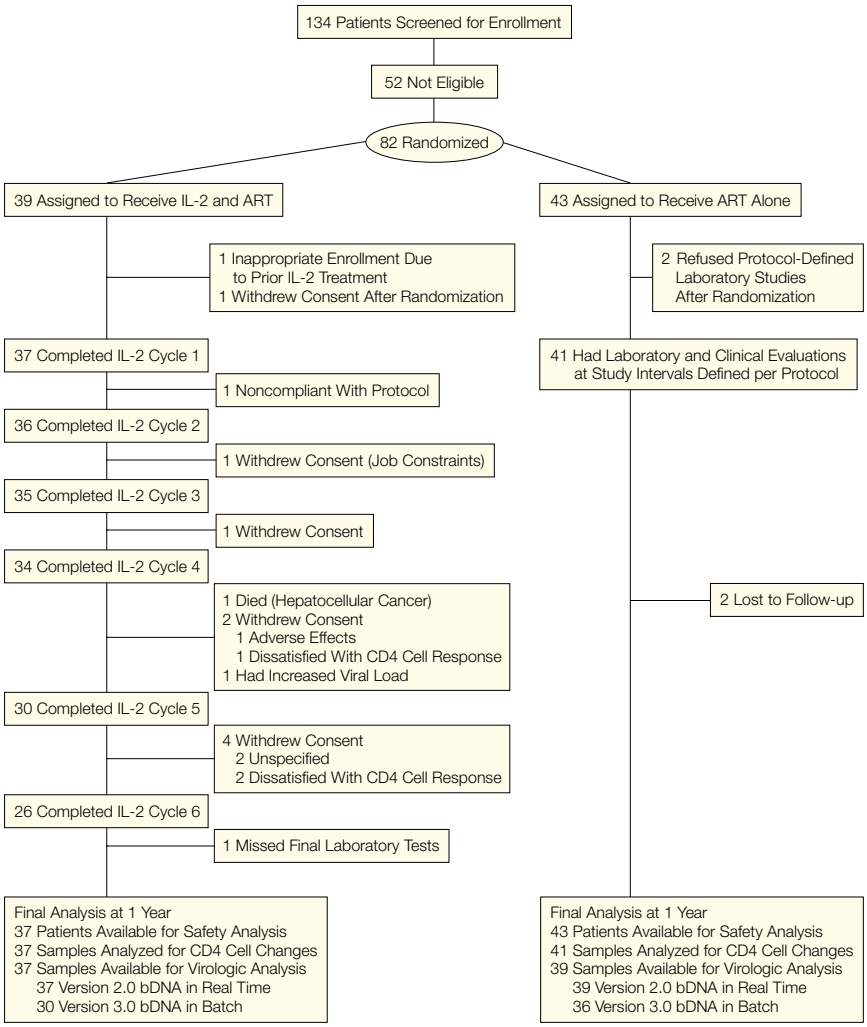
At 1-year follow-up, the mean (SD) percentage increase in CD4 cell count above baseline in IL-2 recipients (n=37) was 112% (113%) (median, 81%; range, –34% to 550%; interquartile range [IQR], 40%-147%) compared with 18% (35%) (median, 12%; range, –52% to 131%; IQR, –8% to 42%) in recipients of ART alone (n=41) ($P<.001$). These percentage increases corresponded to a mean (SD) increase in absolute numbers of CD4 cells of 384 (344) $\times 10^6$/L (median, 279 $\times 10^6$/L; range, –112 to 1057 $\times 10^6$/L; IQR, 149-698 $\times 10^6$/L) for the IL-2 arm compared with 64 (131) $\times 10^6$/L (median, 50 $\times 10^6$/L; range, –189 to 629 $\times 10^6$/L; IQR, –22 to 137 $\times 10^6$/L) for the control arm ($P<.001$). Changes in CD4 cell percentages for the 2 groups followed a similar pattern; among patients receiv-

Table 1. Demographics of the Study Population*

	Treatment Group	
	Subcutaneous IL-2 and ART (n = 39)	ART Alone (n = 43)
Age, mean (range), y	41 (25-68)	38 (23-56)
Sex, male, %	100	91
Known duration of HIV-positive status, mean (range), mo	83 (8-150)	79 (5-144)
Baseline CD4 cell count, mean (range), $\times 10^6$/L	347 (186-523)	341 (203-553)
Baseline plasma HIV-1 RNA load, mean (range), copies/mL	3.08 (2.70-5.11)	3.14 (2.70-4.67)
Use of protease inhibitors by evaluable patients, No. (%)		
Baseline	32 (86)	23 (56)
During study	33 (89)	33 (80)

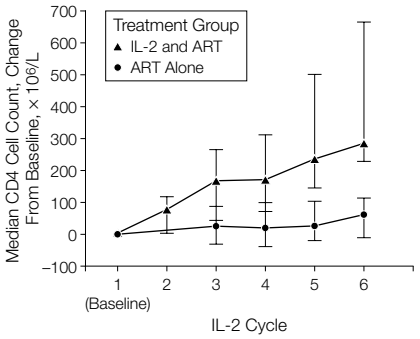
*IL-2 indicates interleukin 2; ART, antiretroviral therapy; and HIV-1, human immunodeficiency virus type 1.

Figure 1. Patient Flow Diagram



IL-2 indicates interleukin 2; ART, antiretroviral therapy; and bDNA, branched DNA.

Figure 2. Comparative Changes in CD4 Cell Counts Throughout Study



Subcutaneous interleukin 2 (IL-2) cycles were administered no less than every 8 weeks apart. Samples were obtained on day 0 of each cycle, prior to the first IL-2 injection. Serial changes in CD4 cell counts for recipients of antiretroviral therapy (ART) alone are shown according to comparable assessments throughout the study. CD4 cell counts were not measured in recipients of ART alone during a period comparable with the beginning of cycle 2 for IL-2 recipients. Error bars indicate interquartile range.

ing IL-2, the mean (SD) CD4 cell percentage increased from 20.4% (6.3%) at baseline to 32.3% (12.4%) at 1 year follow-up compared with corresponding changes from 20.4% (5.1%) to 23.0% (7.2%) among control patients ($P<.001$). In contrast, mean absolute CD8 cell counts in these 2 groups did not change substantially during this same time span, increasing from a baseline mean (SD) of 1016 (399) $\times 10^6$/L (median, 1049 $\times 10^6$/L; range, 340-3217 $\times 10^6$/L; IQR, 732-1237 $\times 10^6$/L) to a final value of 1056 (522) $\times 10^6$/L (median, 1000 $\times 10^6$/L; range, 320-2700 $\times 10^6$/L; IQR, 706-1309 $\times 10^6$/L) in the IL-2 group compared with a corresponding increase from 955 (336) $\times 10^6$/L (median, 870 $\times 10^6$/L; range, 447-1983 $\times 10^6$/L; IQR, 765-1085 $\times 10^6$/L) to 1001 (340) $\times 10^6$/L (median, 935 $\times 10^6$/L; range, 270-1813 $\times 10^6$/L; IQR, 815-1104 $\times 10^6$/L) in the control group ($P=.67$).

The CD4 cell count in the IL-2 group increased following the first IL-2 cycle and continued to increase with subsequent cycles (FIGURE 2). In addition, CD4 cell count increased from baseline with increasing IL-2 dosage. When analyzed by the average IL-2 dose administered during the first 6 cycles, patients who received less than 3.0 mIU (n=8), 3.0 to 4.4 mIU (n=10), 4.5 to 5.9 mIU (n=6), and 6.0 to 7.5 mIU (n=13) had mean (SD) absolute increases from baseline CD4 cell counts of 176 (116), 106 (127), 446 (344), and 697 (291) $\times 10^6$/L, respectively. These corresponded to mean (SD) percentage increases from baseline CD4 cell counts of 39% (34%), 42% (50%), 119% (84%), and 207% (125%), respectively.

While absolute increases for CD4 cell counts were greater among patients with higher CD4 cell counts at baseline, percentage increases in CD4 cell counts from baseline among patients receiving IL-2 were generally similar across the entry CD4 cell range of 200 to 500 $\times 10^6$/L. Thus, for IL-2 recipients with baseline CD4 cell counts of less than 300 $\times 10^6$/L, 301 to 400 $\times 10^6$/L, and >400 $\times 10^6$/L, mean percentage increases from baseline values were 115% (149%), 101% (96%), and 116% (87%), respectively.

The 2 treatment groups included similar proportions of patients who received protease inhibitors at any time during the study (33 [89%] of 37 patients receiving IL-2 compared with 33 [80%] of 41 control patients). Among patients who received protease inhibitors, the mean (median) proportion of study time these participants received 1 or more protease inhibitor during the first year was also similar (99% [100%] in IL-2 patients compared with 92% [100%] in control patients). The percentage increase (SD) from baseline in CD4 cell counts at 1-year follow-up was 123% (86%) among IL-2 patients who did not receive protease inhibitors and 111% (117%) among IL-2 patients who did receive protease inhibitors; corresponding values for control patients were 15% (21%) and 19% (38%), respectively.

Cell surface expression of CD4/CD25, the α chain of the high-affinity IL-2 receptor, was measured in 26 IL-2 patients and 27 control patients prior to initial treatment and at 1-year follow-up. Mean (SD) numbers of CD4 cells expressing CD25 increased almost 4-fold (from 22.1 [25.8] $\times 10^6$/L at baseline to 82.2 [99.4] $\times 10^6$/L at 1 year) among IL-2 recipients but remained essentially unchanged (from 29.7 [40.3] $\times 10^6$/L at baseline to 29.2 [45.7] $\times 10^6$/L at 1 year) in control patients during this period ($P<.001$). In contrast, expression of the activation markers CD8/CD25, CD4-DR, and CD8-DR in this same subset of patients did not differ significantly (data not shown).

HIV-1 RNA Levels

Plasma samples were available from 76 patients for real-time analysis of viral load using version 2.0 branched DNA methods. At 1-year follow-up, mean (SD) viral load changes relative to baseline using the version 2.0 assay were –0.16 (0.76) and –0.09 (0.95) $\log_{10}$ copies/mL for IL-2 recipients and control patients, respectively ($P=.61$). Stored samples were available from 66 patients for repeat HIV-1 RNA determinations using more sensitive RNA detection technology. Demographics, mean baseline CD4 cell counts, prestudy exposure to protease inhibitors, and mean baseline HIV-1 RNA levels of this subcohort of 66 patients were similar to those of the entire cohort (data not shown). Changes in HIV-1 RNA detected by the more sensitive assay were significantly different for IL-2 patients compared with control patients (–0.28 [0.81] and 0.09 [1.23] $\log_{10}$ copies/mL, respectively; $P=.03$). The final HIV-1 RNA level was fewer than 50 copies/mL for 20 (67%) of 30 patients receiving IL-2 compared with 13 (36%) of 36 control patients ($P=.02$) (TABLE 2). Furthermore, this difference was maintained if the analysis was restricted to only patients who used protease inhibitors (20 [71%] of 28 patients receiving IL-2 and ART including a protease inhibitor vs 13 [43%] of 30 ART-only patients who received a protease inhibitor demonstrated an HIV-1 RNA level <50 copies/mL; $P=.04$). To determine if the immediacy of treatment with potent ART influenced anti-HIV activity associated with IL-2 therapy, the analysis was further restricted to include only patients receiving protease inhibitors

Table 2. Final Viral Load Determinations by Baseline Viral Load*

Baseline HIV-1 RNA Determination, Copies/mL	Final HIV-1 RNA Determination, Copies/mL											
	All Patients (n = 66)				Patients Receiving Protease Inhibitors (n = 58)				Patients Receiving Protease Inhibitors Continuously During 90 Days Prior to Final Viral Load Determination (n = 53)			
	IL-2 and ART		ART Alone		IL-2 and ART		ART Alone		IL-2 and ART		ART Alone	
	<50	≥50	<50	≥50	<50	≥50	<50	≥50	<50	≥50	<50	≥50
<50, No.	11	1	8	3	11	1	8	2	11	1	8	2
≥50, No.	9	9	5	20	9	7	5	15	7	7	4	13
Total, No. (%)	20 (67)	10 (33)	13 (36)	23 (64)	20 (71)	8 (29)	13 (43)	17 (57)	18 (69)	8 (31)	12 (44)	15 (56)
P value†	.02				.04				.09			

*HIV-1 indicates human immunodeficiency virus type 1; IL-2, interleukin 2; and ART, antiretroviral therapy. Version 3.0 branched DNA analyses were limited to patients with frozen plasma samples available at both the baseline and final HIV-1 RNA determinations.
†*P* values represent the difference between patients receiving IL-2 and ART vs patients receiving ART alone, stratified by baseline branched DNA.

within 90 days of the final viral load determination; the proportion of IL-2 patients with fewer than 50 copies/mL of HIV-1 RNA was still higher (18 [69%] of 26) than the proportion of patients randomized to potent ART with a protease inhibitor (12 [44%] of 27) but the difference did not reach statistical significance ($P=.09$). Of note, when analyzed by either the magnitude of the net viral load change or the relative percentage of IL-2 recipients achieving fewer than 50 copies/mL of HIV-1 RNA, no dose-response relationship was evident between the average IL-2 dose delivered and viral load differences at study end (data not shown).

Table 3. Most Common Serious Adverse Events*

	Treatment Group, No. (%)	
	Subcutaneous IL-2 and ART (n = 37)	ART Alone (n = 41)
Any grade 3 or higher adverse event	20 (54)	7 (16)
Asthenia	8 (22)	0 (0)
Fever	6 (16)	0 (0)
Arthralgia	5 (14)	0 (0)
Myalgia	5 (14)	0 (0)
Increase in		
Alanine aminotransferase level	8 (22)	1 (2)
Aspartate aminotransferase level	5 (14)	3 (7)
Bilirubin level	15 (41)	10 (24)

*Serious adverse events were defined as grade 3 or higher on the National Cancer Institute Common Toxicity Scale. IL-2 indicates interleukin 2; ART, antiretroviral therapy.

Anti–IL-2 Antibody Formation

Serial plasma samples from 75 patients were analyzed for the development of IgG or IgM antibodies reactive with recombinant IL-2. Plasma from 7 patients (9.3%; 6 IL-2 recipients and 1 control patient) contained IgM antibody reactive with IL-2, although in 4 of these patients this activity was present prior to exposure to study therapy. Plasma containing IgG anti–IL-2 activity was identified in 11 patients (14.7%; in 9 IL-2 recipients and 2 control patients), 3 of whom (all IL-2 recipients) also had IgM reactivity detected at 1 or more points. Four of these 11 patients had IgG reactivity at baseline. This reactivity persisted in subsequent plasma samples in only 3 of the 9 IL-2 recipients. IgM- and IgG-mediated anti–IL-2 reactivity did not appear to correlate with the CD4 cell increases induced by IL-2 therapy.

Adverse Events

Clinical and laboratory safety data were available from 37 IL-2 recipients (2 patients in this group withdrew early) and 43 control patients. Although there were no new or unexpected toxic effects attributable to study drugs in either arm, IL-2 recipients experienced more adverse events than recipients of ART alone. The most common toxic effects experienced by IL-2–treated patients were constitutional symptoms of fever, fatigue, and myalgias of varying severity. Per protocol-defined guidelines, mild-to-moderate symptoms were managed by scheduled administration of alternating acetaminophen and ibuprofen, oral hydration, oral narcotics to control rigors, and rest. More serious or sustained symptoms were managed by omitting a scheduled dose, dosage reduction, or both, as required. Despite these measures, serious (at least grade 3) adverse events occurred in 20 (54%) of 37 evaluable IL-2 recipients and 7 (16%) of 43 ART recipients. The most common (≥10%) serious adverse events are shown in TABLE 3. Three grade 4 adverse clinical events (thrombophlebitis, increased bilirubin, and acute exacerbation of mania) occurred singly in 3 different recipients of IL-2, whereas none occurred in control patients. One patient in the IL-2 group died during the study from complications of hepatocellular carcinoma believed to be secondary to chronic hepatitis B infection. Another IL-2 patient developed

hyperthyroidism during the study and required synthetic thyroid hormone supplementation after becoming chemically hypothyroid following cycle 4 of IL-2 therapy. Of 70 patients remaining in active follow-up at participating sites, no episodes of any clinical AIDS-defining events were reported in patients from either arm during the 18 months following study completion.

COMMENT

In this prospective, randomized, controlled multicenter trial, the addition of IL-2 to standard ART significantly improved CD4 cell response in patients at an intermediate stage of HIV-1 infection. This improvement was observed with the initial IL-2 treatment cycle, and IL-2–associated increases in CD4 cells were observed throughout the CD4 cell range at entry of 200 to 500×10^6/L. The IL-2–associated CD4 cell increase continued throughout the study. Furthermore, IL-2 therapy was relatively well tolerated overall; adverse events were consistent with previously defined toxic effects associated with intermittent use of this agent at the dosages tested. These results are consistent with other small, randomized controlled trials of either intravenous or subcutaneous intermittent IL-2 in demonstrating that IL-2 can increase CD4 cell counts safely in HIV-infected patients to the normal or even supraphysiologic range.[17-21]

Long-term IL-2 therapy may require dosage adjustment in many participants over the course of a year, primarily on the basis of subjective toxic effects such as constitutional symptoms. However, these toxic effects are largely predictable and do not diminish the rationale for selecting a starting dosage of proven efficacy in this range, especially since the CD4 cell response in this trial was strikingly dose-dependent. Patients who were able to tolerate an outpatient treatment dosage closest to 7.5×10^6 IU twice per day throughout the 6 cycles had the greatest overall CD4 cell increases. Recently, concern has been raised about the appropriateness of treating patients with intermittent 5-day cycles of IL-2 at dosages known to induce transient adverse effects, particularly compared with the theoretical potency of experimental regimens involving low-dosage daily injections administered on an extended basis.[22] Unfortunately, randomized controlled studies validating comparable efficacy with the latter approach have not been reported to date. Thus, while low-dosage continuous IL-2 treatment strategies are clearly better tolerated, it remains unclear whether such regimens will be able to reproduce the profound CD4 cell increases associated with intermittent high-dosage therapy.

A unique finding from this study was the effect of IL-2 together with ART on HIV RNA levels. As first documented in studies conducted in the pre-HAART era, the use of IL-2 can be associated with transient bursts of acute plasma viremia.[4,7] Nonetheless, these same studies have documented that no long-term deleterious effects on plasma viral load result from such bursts. Indeed, several trials have described either no HIV viral load differences between patients receiving and not receiving IL-2 or even a trend toward an overall reduction in plasma viremia or a reduction in proviral DNA copy number among IL-2 recipients.[6,10,11,23,24] In the present study, we demonstrate a trend toward decreased HIV RNA at the end of 1 year of treatment. However, when we used an assay for plasma viral load with an enhanced sensitivity threshold, there was a statistically significant decrease in plasma virus among IL-2–treated patients compared with control patients. The proportion of IL-2 recipients who achieved viral suppression (67%) was also significantly higher than the proportion of patients in the control arm who did so (36%). This observation suggests that the enhanced immune effects (increased CD4 cell count) associated with IL-2 therapy may lead to greater control of viral replication than that afforded by ART alone. This is the first randomized controlled trial of IL-2 therapy demonstrating that IL-2 administered with potent ART is associated with enhanced viral suppression.

There are at least 2 potential limitations that must be considered when interpreting these findings. First, a smaller number of appropriately stored plasma samples were available for batch analysis using the more sensitive assay compared with samples available for the study-specified assay. However, the baseline characteristics of the 66 patients for whom these stored samples were available did not differ significantly from those of the entire cohort. Furthermore, even if the analysis using the less sensitive HIV-1 RNA assay is restricted to just these 66 patients, the same trend in favor of the IL-2–treated patients is still present. Thus, it is unlikely that the selected patient population significantly influenced the observation that IL-2 was associated with greater suppression of viral RNA. Second, a slightly higher percentage (89% vs 80%) of IL-2 recipients were also recipients of protease inhibitors during the study than were control patients. However, when the viral load analysis was restricted to only patients using protease inhibitors, the same trend is still evident. Thus, this difference is also unlikely to account for the more potent viral suppression observed among patients receiving IL-2.

These considerations notwithstanding, the magnitude of the difference in viral load outcomes between the 2 groups was not large and may reflect the detection limits of even the sensitive assay used. Extensive use of HAART induced vigorous direct viral inhibition that likely limited the ability to detect additional suppression induced by IL-2 through immunologic means. That is, it may be unrealistic to expect any agent lacking direct antiretroviral properties to produce a dramatic difference in plasma HIV RNA when viral load is well suppressed with potent ART. The advent of even more sensitive assays of plasma RNA potentially may provide greater discrimination in outcomes. Until then, since limiting viral replication to levels below the sensitivity threshold of existing assays has been strongly associated with long-term suppression.[25,26] The relative percentages of patients achieving this goal may provide a

more meaningful comparison in this regard.

The results of 2 ongoing randomized trials of IL-2 therapy involving hundreds of patients, namely, CPCRA Trial 059 (Terry Biern Community Programs for Clinical Research on AIDS) and AIDS Clinical Trials Group trial 328, will help determine whether the viral load differences observed in the present study can be reproduced in larger clinical studies. In addition, 2 larger and longer international phase 3 clinical end-point trials of IL-2 (the SILCAAT [Chiron Corp] and ESPRIT [NIH-sponsored] studies) have recently begun and should eventually provide definitive data to determine the clinical efficacy of IL-2 and the value of immunologic mechanisms to enhance CD4 cell count and suppress viral replication. From an immunologic perspective, it will be instructive to determine whether the CD4 cell count differences likely to be established early in these studies will, in turn, translate into substantial viral load differences between treatment arms. It will be especially important to determine whether these viral load differences, if present, are any more predictive of potential differences in ultimate clinical outcomes than the expected CD4 cell gains associated with IL-2 therapy. Our data support the possibility that IL-2 therapy may be associated with enhanced and durable HIV suppression as well as significant increases in CD4 cell responses. The clinical correlate of these changes must await the results of the clinical end-point trials currently under way.

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REFERENCES

1. Palella FJ Jr, Delaney KM, Moorman AC, et al, for the HIV Outpatient Study Investigators. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J Med.* 1998;338:853-860.

2. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet*. 1999;353:2093-2099.

3. Schwartz DH, Skowron G, Merigan TC. Safety and effects of interleukin 2 plus zidovudine in asymptomatic individuals infected with human immunodeficiency virus. *J Acquir Immune Defic Syndr*. 1991;4:11-23.

4. Kovacs JA, Baseler M, Dewar RJ, et al. Increases in CD4 T lymphocytes with intermittent courses of interleukin 2 in patients with human immunodeficiency virus infection: a preliminary study. *N Engl J Med.* 1995;332:567-575.

5. McMahon DK, Armstrong JA, Huang X-L, et al. A phase I study of subcutaneous recombinant interleukin-2 in patients with advanced HIV disease while on zidovudine. *AIDS*. 1994;8:59-66.

6. Kovacs JA, Vogel S, Albert JM, et al. Sustained increases in CD4 counts with intermittent interleukin 2 therapy in HIV-infected patients with greater than 200 CD4 cells/mm^3: results of a randomized, controlled trial. *N Engl J Med*. 1996;335:1350-1356.

7. Davey RT Jr, Chaitt DG, Piscitelli SC, et al. Subcutaneous administration of interleukin-2 in HIV-1-infected persons. *J Infect Dis*. 1997;175:781-789.

8. De Paoli P, Zanussi S, Simonelli C, et al. Effects of subcutaneous interleukin-2 therapy on CD4 subsets and in vitro cytokine production in HIV+ subjects. *J Clin Invest*. 1997;100:2737-2743.

9. Emery S, Capra W, Vig P, et al. IL-2 therapy for HIV disease: a meta-analysis of three randomized, controlled trials. From: Fifth Conference on Retroviruses and Opportunistic Infections; February 1-5, 1998; Chicago, Ill. Abstract 608.

10. Carr A, Emery S, Lloyd A, et al, for the Australian IL-2 Study Group. Outpatient continuous intravenous interleukin-2 or subcutaneous, polyethylene glycol-modified interleukin-2 in human immunodeficiency virus-infected patients: a randomized, controlled, multicenter study. *J Infect Dis*. 1998;178:992-999.

11. Davey RT Jr, Chaitt DG, Albert JM, et al. A randomized trial of high- versus low-dose subcutaneous interleukin-2 outpatient therapy for early human immunodeficiency virus type 1 infection. *J Infect Dis*. 1999;179:849-858.

12. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep*. 1992;41(RR-17):4-19.

13. Revised guidelines for the performance of CD4$^+$ T-cell determinations in persons with human immunodeficiency virus (HIV) infections. *MMWR Morb Mortal Wkly Rep.* 1994;42(RR-3):1-21.

14. Urdea MS, Wilber JC, Yeghiazarian T, et al. Direct and quantitative detection of HIV-1 RNA in human plasma with a branched DNA signal amplification assay. *AIDS*. 1993;7(suppl 2):S11-S14.

15. Dewar R, Highbarger HC, Sarmiento MD, et al. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J Infect Dis*. 1994;170:1172-1179.

16. Pavur R, Nath R. Parametric versus rank transform procedures in the two-way factorial experiment: a comparative study. *J Stat Computation Simulation*. 1986;23:231-240.

17. Van Lunzen J, Tenner-Racs K, Racz P, Hufert FT, Lauer J, Stellbrink HJ. Effect of HAART +/– IL-2 on viral kinetics and T-cell reconstitution in lymphoid tissues of asymptomatic HIV-1 infected patients. *Int Conf AIDS*. 1998;12:787-788.

18. Hengge UR, Goos M, Esser S, et al. Randomized, controlled phase II trial of subcutaneous interleukin-2 in combination with highly active antiretroviral therapy (HAART) in HIV patients. *AIDS*. 1998;12:F225-F234.

19. Levy Y, Capitant C, Houhou S, et al, for the ANRS 048 Study Group. Comparison of subcutaneous and intravenous interleukin-2 in asymptomatic HIV-1 infection: a randomised controlled trial. *Lancet*. 1999; 353:1923-1929.

20. Zanussi S, Simonelli C, Bortolin MT, et al. Immunological changes in peripheral blood and in lymphoid tissue after treatment of HIV-infected subjects with highly active anti-retroviral therapy (HAART) or HAART + IL-2. *Clin Exp Immunol*. 1999;116:486-492.

21. Arno A, Ruiz L, Juan M, et al. Efficacy of low-dose subcutaneous interleukin-2 to treat advanced human immunodeficiency virus type 1 in persons with ≤250/μL CD4 T cells and undetectable plasma virus load. *J Infect Dis*. 1999;180:56-60.

22. Jacobson EL, Pilaro F, Smith KA. Rational interleukin 2 therapy for HIV-positive individuals: daily low doses enhance immune function without toxicity. *Proc Natl Acad Sci U S A*. 1996;93:10405-10410.

23. Clark AG, Holodmiy M, Schwartz DH, Katzenstein DA, Merigan TC. Decrease in HIV provirus in peripheral blood mononuclear cells during zidovudine and human rIL-2 administration. *J Acquir Immune Defic Syndr*. 1992;5:52-59.

24. Simonelli C, Zanussi S, Sandri S, et al. Concomitant therapy with subcutaneous interleukin-2 and zidovudine plus didanosine in patients with early stage HIV infection. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1999;20:20-27.

25. Izopet J, Salama G, Pasquier C, et al. Ultra sensitive detection of plasma HIV-1 RNA for predicting the durability of 3-drug antiretroviral therapy. In: Program and abstracts of the 5th Conference on Retroviruses and Opportunistic Infections; February 1-5, 1998; Chicago, Ill. Abstract 326.

26. Kempf DJ, Rode RA, Xu Y, et al. The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir. *AIDS.* 1998;12:F9-F14.