

Low-Dose Daily Subcutaneous Interleukin-2 in Combination with Highly Active Antiretroviral Therapy in HIV+ Patients: A Randomized Controlled Trial

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Purpose: Previous studies with intermittent interleukin-2 (IL-2) therapy using intermediate and high levels of IL-2 have demonstrated significant increases in the CD4+ T cell count in HIV-infected patients. Intermittent regimens are amenable to outpatient use, but severe adverse events are frequently experienced with intermediate- and high-dose levels of IL-2. Therefore in this study, the effect of daily, subcutaneous low-dose IL-2 therapy on safety and immunological endpoints was investigated to determine whether immunological benefit could be achieved without toxicity in HIV-infected patients also receiving highly active antiretroviral therapy (HAART). **Method:** A total of 115 patients were enrolled in the trial. Fifty-six asymptomatic HIV-infected patients who had CD4+ T cell counts less than 300 cells/ μ L at screening and a stable HIV viral load received low-dose IL-2 (1.2 million IU [MIU]/m² beginning dose) once daily in conjunction with HAART (IL-2 group). Fifty-nine patients received HAART alone (control group). **Results:** A dramatic effect of IL-2 on the natural killer (NK) cell population was observed with mean increases of 156 cells/ μ L in the IL-2 group compared to 19.93 cells/ μ L in the control group ($p < .001$). Additionally, IL-2-treated patients experienced a statistically significant increase in the mean percentage of CD4+ T cells (3.52% increase) when compared to control patients (1.33% increase) ($p < .001$). The expanded CD4+ T cell population was primarily of the naive phenotype, with mean increases of 4.53% for the IL-2 group and 0.31% for the control group ($p < .001$ for between-group difference). In addition, a higher proportion of IL-2-treated patients (67%) compared to control patients (33%) achieved increases of greater than 50% in the CD4+ T cell count ($p = .08$). Adverse events of grade 3 or grade 4 toxicity were infrequent in the current study and were substantially lower by comparison to those in studies of intermittent dose IL-2 therapy. Also, negligible changes in the HIV viral load from baseline to final measurement were observed in both groups. A trend toward a reduced number of modifications of antiretroviral therapy was apparent in the IL-2 group when compared to control patients. **Conclusion:** Daily, low-dose subcutaneous IL-2 therapy in conjunction with HAART is safe and well tolerated and is effective in expanding lymphocyte cell types including NK cells and naive T cells in individuals who have <300 CD4+ T cells. **Key words:** *interleukin-2, human immunodeficiency virus, naive CD4+ T cells*

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Human immunodeficiency virus (HIV) disease is characterized by a progressive decline in CD4⁺ T cells and an increase in HIV viral load. Together with the decrease in the total number of CD4 T cells, the balance between the numbers of CD4⁺CD45RA⁻45RO⁺ T cells (memory CD4⁺ T cells) and CD4⁺CD45RA⁺45RO⁻ T cells (naive CD4⁺ T cells) is dramatically altered during HIV disease progression.¹⁻³ Although both memory and naive CD4⁺ T cells are lost during disease progression, preferential loss in the naive CD4⁺ T cell population is observed.^{1,3} The loss of naive CD4⁺ T cells is thought to compromise the ability of an HIV-infected individual to respond to newly encountered infectious agents or indeed to mount a sustained immunological reaction against HIV.⁴ Treatment with antiretroviral combination therapy* generally results in decreases in viral load and corresponding increases in the CD4⁺ T cell concentration.³ This therapy has been found to result in relatively small increases in the total number of CD4⁺ T cells, and particularly in the number of naive CD4⁺ T cells, at an approximate rate of only 4 cells/ μ L/month. The increases in the total CD4⁺ T cell count and naive CD4⁺ T cell count often reach a plateau at levels that are considerably less than normal, indicating that antiretroviral combination therapy alone does not accelerate recovery of the HIV-induced immune deficiency.³

Interleukin-2 (IL-2) is a pleiotropic immune system cytokine that is crucial for the generation of immune responses to foreign agents by stimulating the proliferation and differentiated function of antigen-selected CD4⁺ T cells and CD8⁺ T cells. Additionally, IL-2 expands the natural killer (NK) cell population and increases the cytolytic activity of NK cells against a variety of target cells.⁵⁻⁸ Treatment with IL-2 and antiretroviral combination therapy or highly active antiretroviral therapy (HAART) has been studied in a number of clinical trials to test whether IL-2 therapy can accelerate the recovery of the immune system.⁹⁻¹² In these studies, significant dose-dependent increases in

the concentration of circulating CD4⁺ T cells have been reported in HIV-infected patients who received either subcutaneous or intravenous treatment with IL-2 together with antiretroviral combination therapy. Moreover, a large part of the increase in total CD4⁺ T cells is attributed to expansion of the naive CD4⁺ T cell subset while lesser increases are found in the number of memory CD4⁺ T cells.¹¹⁻¹⁴

In most of the prior studies, an intermittent dosing schedule for IL-2 was followed in which patients received 5-day cycles of IL-2 (e.g., 7.5 million IU [MIU] twice a day for 5 days) every 4 to 8 weeks.^{8,10} The intermittent dosing regimen for IL-2 is found to be tolerable and can be carried out on an outpatient basis, although adverse events of grade 3 toxicity (according to the National Cancer Institute Common Toxicity Criteria) are frequently observed.^{8,10} In addition, the high-dose intermittent IL-2 regimen was not efficacious in participants who most need immune enhancement, that is, those individuals with low concentrations of circulating CD4⁺ T cells. However, in a pilot study of 16 HIV-infected patients with CD4⁺ T cell counts between 200 and 500 cells/ μ L, daily subcutaneous administration of IL-2 at low doses (from 0.31 MIU/ m^2 /day to 1.2 MIU/ m^2 /day) increased the number of CD4⁺ T cells and NK cells without causing systemic toxicity, even at the grade 1 level.¹⁵ These data suggest that the low-dose daily IL-2 regimen is efficacious with respect to immunological endpoints and might be better tolerated than the high-dose intermittent schedule.

The pilot study described previously provided a rationale for further studies of the use of daily low-dose IL-2 in the treatment of HIV-infected individuals. Additional support is provided in the observation that the level of IL-2 in plasma after low-dose IL-2 treatments is sufficient for saturation of high-affinity IL-2 receptors expressed by activated T cells, B cells, and a minor subset of NK cells but is insufficient for the occupation of intermediate affinity receptors expressed by the majority of NK cells.¹⁵ As a result, the release of inflammatory cytokines by NK cells after low-dose IL-2 therapy is minimal and the toxicity of IL-2 is avoided.

To further investigate the safety and efficacy of low-dose daily IL-2, a randomized, controlled, open-label, multicenter study was carried out in individuals with low levels (below 300 cells/ μ L) of circulating CD4⁺ T cells. The results of this study,

*Antiretroviral combination therapy refers to therapy with two antiretroviral drugs (e.g., zidovudine and didanosine) or highly active antiretroviral therapy (HAART). HAART is defined as at least triple drug therapy with at least one protease inhibitor and/or one nonnucleoside reverse transcriptase inhibitor.

described here, reveal that daily low-dose IL-2 administration in this setting is safe and results in significant increases in NK cells and both naive CD4+ and CD8+ T cells during 26 weeks of therapy. In addition, the percentage of CD4+ T cells increased while the percentage of CD8+ T cells decreased, resulting in an improvement in the CD4+/CD8+ T cell ratio.

METHOD

Study Design

This was a multicenter, phase II, pilot, open-label, controlled, randomized study in HIV-infected individuals who were currently receiving HAART and who had CD4+ T cell counts less than 300 cells/ μ L at screening and a stable HIV viral load. A stable HIV viral load was defined as less than 500 HIV mRNA copies/mL on two occasions within 8 months prior to screening. A total of 115 patients were enrolled into the study. All patients who were enrolled in the study signed a written informed consent form. All of the procedures followed were in accordance with the ethical standards of the Institutional Review Board at each center and the Declaration of Helsinki (as most recently amended by the 48th General Assembly, Somerset West, Republic of South Africa, October 1996).

To eliminate assignment bias, patients were randomly assigned to treatment groups by a centralized randomization process. Randomization was done prospectively in blocks and stratified by study site. On day 1, the beginning of the on-treatment study period, patients were randomized to continue with their current HAART regimen (control group) or to receive IL-2 plus HAART (IL-2 group) for 6 months. Those who were randomized to the IL-2 group self-administered low-dose IL-2 (1.2 MIU/ m^2) every day by subcutaneous injection. Upon completion of the 6-month, on-treatment study period, all remaining patients (regardless of prior study treatment regimen) were given the opportunity to receive open-label IL-2 for an additional 6 months as part of an extension study designed to monitor safety. The data from the extension study are not included in this article.

Each vial of IL-2 was initially reconstituted with 1.2 mL USP unit of sterile water for injection. The resulting solution was a clear, colorless liquid.

When reconstituted as directed, each milliliter contained 18 MIU of IL-2. For subcutaneous use, IL-2 was further diluted to a final volume of 6 mL with 5% dextrose solution and a final concentration of 220 μ g/mL (3.6 MIU/mL). Prefilled syringes with sterile 25-gauge needles were dispensed to each patient. Patients or their caretakers administered IL-2 after being properly trained. The sites of administration were typically on the thighs and abdomen and were rotated daily.

After a patient received 1.2 MIU/ m^2 of IL-2 daily for at least 2 weeks and experienced no dose-limiting toxicity (defined as National Cancer Institute grade 2 or greater), the dose of IL-2 was escalated at the discretion of the investigator. The investigator was allowed to increase a patient's IL-2 dose by increments of 0.3 MIU/ m^2 every 2 weeks until the patient experienced a grade 2 or greater toxicity. In the event that a patient experienced a grade 2 or greater toxicity, IL-2 treatment was withheld until the toxic symptoms or signs resolved. IL-2 treatment was then resumed at the highest dosage that did not result in dose-limiting toxicity.

During the on-treatment study period, patients visited the clinic 10 times for the assessment of efficacy and safety variables and/or to obtain IL-2. CD4+ and CD8+ T cell counts and NK cell counts were measured at screening, at day 1, and at weeks 4, 8, 16, and 26. Additional immunological phenotyping was performed to quantify CD4+ and CD8+ T cell subsets (e.g., naive and memory CD4+ or CD8+ T cells) at day 1 and at weeks 4, 8, 16, and 26. A central laboratory performed all cell counts and cell phenotyping¹⁶ (Covance Central Laboratory Services, Indianapolis, IN, USA).

Additional laboratory tests consisted of hematology, blood chemistries, and urinalysis. A central laboratory performed all laboratory tests. Vital signs were measured at screening, day 1, and weeks 1, 4, 8, 16, and 26. Plasma was collected for HIV viral load assessment at screening, at day 1, and weeks 4, 8, 16, and 26. HIV viral load was measured at Chiron Corporation (Emeryville, CA, USA), using the Quantiplex bDNA assay (bDNA Version 3.0).

Statistical Methods

Treatment differences in the change from baseline analyses for T cell, NK cell, and HIV viral load data were assessed by analysis of variance

(ANOVA) of ranked observation, with factors for treatment, center, and Treatment x Center interaction. A Cochran-Mantel-Haenszel statistic was used in calculating the proportion of patients in each treatment group with final HIV viral load value (i.e., HIV mRNA copy number) below or above the assay detection limit, after adjusting for baseline HIV viral load. For laboratory data, within treatment group changes from baseline were analyzed using the Wilcoxon signed rank test, and between treatment group changes from baseline were analyzed using the Wilcoxon rank sum test. Comparisons between treatment groups were analyzed in the intent-to-treat population. The intent-to-treat population included patients with baseline measurements and at least one assessment during the treatment period. The sample size determination was based on the following assumptions. The percent changes in CD4+ T cell count were normally distributed with a common standard deviation, $\sigma = 50\%$. The null hypothesis was that the means of the percent changes in CD4+ T cell count were equal. The test was two-sided and was conducted at the 5% significance level.

Under these assumptions, 30 evaluable patients per arm were required in order to have 86% power to detect a 40% higher mean percent change in CD4+ T cell count in the IL-2 group than in the control group.

RESULTS

Patient Demographics

A total of 115 patients were enrolled into study; 56 were randomized to the IL-2 group and 59 patients were randomized to the control group (Figure 1). The first patient was enrolled in July 1998 and the last patient completed the study in September 1999. Premature terminations from the study were reported for 24 patients from the IL-2 group and 4 patients from the control group. In the IL-2 treatment group, the most common reasons for premature termination were withdrawal of consent (10 patients) and adverse events (10 patients), for which the investigator or patient decided on discontinuation of study.

The adverse events and serious adverse events leading to premature study termination are listed in Table 1. In 6 of 10 patients, the adverse events leading to premature study termination were

found to be mild or moderate in severity (grade 1 or 2). Serious adverse events were experienced by four patients who prematurely withdrew from the study. Of these four serious adverse events, two were not related and two were possibly related to IL-2 therapy.

Demographic and baseline characteristics such as age, sex, Karnofsky status, prestudy mean CD4+ T cell count, prestudy mean HIV mRNA copy number, and mean number of antiretroviral medications were similar between treatment groups (Table 2). Also, there were few notable between-group differences in the reported medical history, HIV history, and antiretroviral medication history.

Safety Profile

Dose modifications according to protocol were permitted during the course of the trial. An increase from the initial dose of 1.2 MIU/m²/day was reported for 44% of patients and a decrease from the initial dose was reported for 31% of patients. The mean (median) dose of IL-2 (1.21[1.18] MIU/m²/day) did not differ substantially from the initial dose level of IL-2. The adverse events reported for the majority of patients in the IL-2 and control groups were generally characterized as mild or moderate (i.e., toxicity grade 1 or 2; Table 3). In general, the number and frequency of patients with any grade adverse event were similar in IL-2-treated patients and control patients. The most common adverse events for patients in the IL-2 group were injection site reactions (73% of patients), asthenia (53%), flu syndrome (46%), nausea (36%), and diarrhea (27%). Less than 13% of patients in the control group experienced each of these adverse events.

Severe (grade 3) and life-threatening adverse events (grade 4) were infrequent in both treatment groups (Table 3). The numbers and percentages of patients who experienced any grade 3 adverse event were similar between treatment groups, with grade 3 adverse events observed in 18% of patients in the IL-2 group and 15% of patients in the control group. Grade 4 adverse events were reported for 2% of the IL-2 treatment group and 5% of the control group. Grade 3 adverse events related to IL-2 therapy or to the subcutaneous route of administration, such as injection site problems, flu syndrome, and asthenia, were observed in only 4%, 2%, and 4% of IL-2-treated patients, respectively.

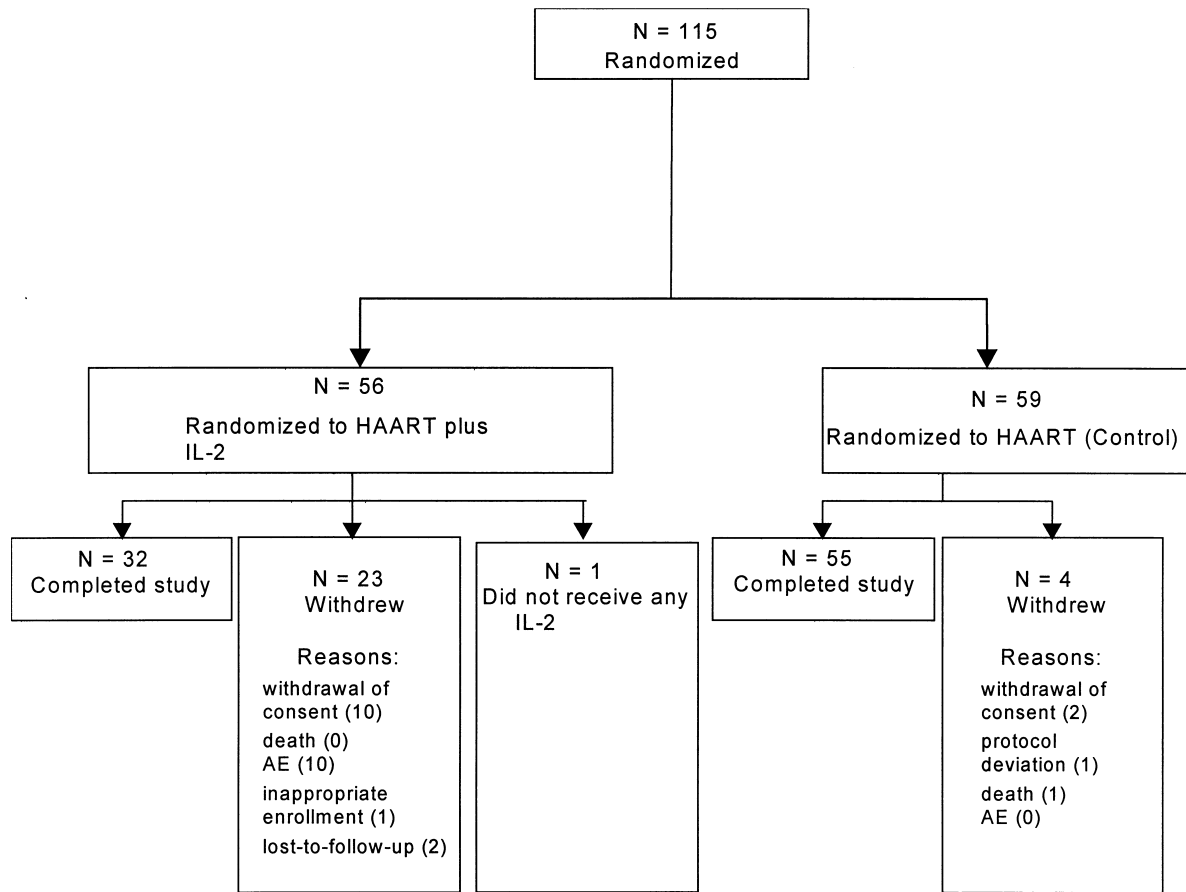


Figure 1. Study scheme.

Nine patients reported serious adverse events on-treatment. Five of the nine serious adverse events were in the IL-2 population, three of which were judged to be possibly related to IL-2 therapy. These included myocardial infarction, optic neuritis, and pneumonia. One patient in the control group died from a cardiac arrest during the study.

In general, chemistry and hematology laboratory data were at the minimal (grade 1) or moderate (grade 2) toxicity level. Some parameters were found to have statistically significant differences between treatment groups, including the plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine phosphokinase (CPK) ($p < .05$; Table 4). It is interesting to note that total cholesterol and triglyceride levels were decreased in the IL-2-treated patients compared to controls ($p < .05$; Table 4).

As would be predicted, the total eosinophil cell count increased in the IL-2 group ($p < .001$; Table 4).

However, the hematocrit and hemoglobin values decreased during the study in patients receiving IL-2, and the differences in the change in these values relative to baseline between treatment groups were statistically significant ($p < .001$).

Virological Assessment

HIV mRNA copy number (viral load) was compared between the IL-2 and control populations. After stratification of patients according to undetectable (<50 copies/mL) or detectable (≥ 50 copies/mL) levels of baseline HIV mRNA copies, the number of patients below or above the level of detection (50 copies/mL) at final measurement was similar in the IL-2 and control groups ($p = .68$ for between-group difference). This is consistent with the findings of other studies of intermediate- or low-dose IL-2 therapy in HIV-infected patients.

To quantify and compare the HIV viral load be-

Table 1. Adverse events leading to premature study termination in interleukin-2–treated patients

Patient number	Adverse event	Severity grade
02/003	Skin carcinoma*	3
03/001	Myocardial infarction*	3
04/001	Optic neuritis*	2
06/002	Diarrhea	2
	Pruritis	2
	Urticaria	2
07/004	Nausea	1
	Vomiting	1
07/006	Flu syndrome	1
	Amnesia	1
	Hallucination	2
07/007	Abdominal pain	2
	Dyspepsia	1
	Dizziness	2
07/012	Abdominal pain	2
	Flu syndrome	2
	Nausea	2
09/005	Myocardial infarction*	4
11/007	Chills	2
	Fever	1
	Nausea	2

*Serious adverse event.

tween treatment groups, we assigned the lowest detectable value to patients with HIV mRNA copy number/mL below the limit of detection for the bDNA assay (50 copies/mL). The mean baseline values for \log_{10} (HIV mRNA copy number/mL) were 1.86 ($n = 49$) and 1.88 ($n = 58$) for the IL-2 and control groups, respectively. The viral load increased slightly for both treatment groups during the on-treatment study period, and the mean \log_{10} (HIV mRNA copy number/mL) values at final measurement were 1.98 ($n = 49$) and 1.95 ($n = 58$) for the IL-2 and control patients, respectively. There was no statistically significant difference between groups in the absolute change of \log_{10} HIV mRNA copy number/mL from baseline to final ($p = .85$).

The antiretroviral therapy for a proportion of patients in both treatment populations was altered during the study. Combination antiretroviral therapy was modified for a substantially lower number of patients in the IL-2 treatment group when compared to the control treatment group. The combination of antiretroviral agents was

changed in 5 of 56 IL-2 patients and in 12 of 59 control patients ($p = 0.11$ for the between-group difference). There were 9 total modifications in combination therapy in the 5 IL-2 patients and 21 total changes in the 12 control patients.

Immunology

Low-dose IL-2 therapy resulted in remarkable increases in the number of NK cells (cells expressing CD16 and CD56 [CD16+56+]) at study weeks 4, 8, 16, and 26. The difference between treatment groups in the increase from baseline in NK cell count was apparent at week 4 ($p < .001$; Figure 2). The NK cell count increased further for IL-2–treated patients at week 8, and the differences in the increases in NK cell count in IL-2–treated patients relative to control patients were similar at weeks 8, 16, and 26 ($p < .001$ at weeks 8, 16, and 26). The mean change in NK cell count from baseline to final visit was 156.30 cells/mL ($n = 52$) for the IL-2 treatment group compared to a mean change of 19.93 cells/ μ L ($n = 57$) in the control treatment group ($p < .001$ for between-group difference).

IL-2–treated patients experienced an expansion of the naive CD4+ T cell population (cells expressing CD3, CD4, and 45RA, and not expressing 45RO markers [CD3+4+45RO-45RA+ cells]) that was not observed in the control group (Figure 3). The naive CD4+ T cell percentage increased in both treatment groups, but the increase was greater in IL-2–treated patients. Progressive increases in the naive CD4+ T cell percentage were evident for patients treated with IL-2 during the course of the study. The differences in the increases in naive CD4+ T cell percentage between the IL-2 group and control group were first apparent at study week 8, and greater differences in the increases in this cell type were observed at study weeks 16 and 26 ($p = .062$, $p < .001$, and $p < .001$ at study weeks 8, 16, and 26, respectively). The changes from baseline to final evaluation in the mean percentages of naive CD4+ T cells were 4.53% ($n = 49$) and 0.31% ($n = 51$) in the IL-2 and control treatment groups, respectively ($p < .001$ for the between-group difference).

In contrast to the effect of IL-2 on the naive CD4+ T cell population, the percentage of memory CD4+ T cells (cells expressing CD3, CD4, and 45RO, and not expressing 45RA [CD3+4+45RO+45RA- cells]) in the IL-2–treated patients was found to decrease during the course of the study (Table 5).

Table 2. Summary of demography: All randomized patients

Demographics	Interleukin-2 (n = 56)	Control (n = 59)
Age (years)		
Mean	40.6	42.2
Median	41.5	42.0
SD	7.9	7.3
Sex		
Male	53 (95%)	58 (98%)
Female	3 (5%)	1 (2%)
Baseline Karnofsky status		
Mean	92.3	92.7
Median	90.0	90.0
SD	7.6	7.2
Prestudy mean CD4+ count*		
Mean	178.43	180.71
Median	183.50	184.00
SD	62.38	72.99
Number of antiretroviral medications taken at enrollment		
Mean	3.2	3.2
Median	3	3
SD	0.9	0.7
Percent of patients with ≥ 4 agents	33%	33%
Number of patients on protease inhibitors		
Any protease inhibitor	55	57
Ritonavir	8	18
Prestudy mean \log_{10} (HIV mRNA copy number/mL) [†]		
Mean	1.856	1.881
Median	1.700	1.700
SD	0.335	0.394

* Average of a patient's screening and study entry (day 1) CD4+ count.

[†] Average of a patient's screening and study entry (day 1) HIV mRNA, as measured by bDNA version 3.0 assay.

Statistically significant increases in the percentage of CD4+ T cells in the total population of lymphocytes were observed for IL-2-treated patients by study week 4, and similar increases were sustained through week 26. A significant change in the percentage of CD4+ T cells from baseline was apparent at the week 4 time point for the IL-2 treatment group (Figure 4). The increases in the percentage of CD4+ T cells from baseline for the IL-2 group appeared to plateau at week 4, as similar increases were observed at weeks 8 and 16; a further increase from baseline was observed at week 26. The increases in the percentage of CD4+ T cells for control patients were negligible at weeks 4 and

8, and slight increases were observed at weeks 16 and 26. *P* values for between-group differences in the change in percentage of CD4+ T cells were $<.001$ at each time point. The increases from baseline to final evaluation in the mean percentages of CD4+ T cells were 3.52% ($n = 52$) and 1.33% ($n = 58$) for patients treated with IL-2 and for control patients, respectively ($p < .001$ for between-group difference).

Increases in the absolute CD4+ T cell count were observed in both treatment groups during the course of the study. However, greater increases in the number of CD4+ T cells were observed in IL-2-treated patients than in control patients. The effect

Table 3. Summary of all adverse events, by worst grade

Adverse event	Grade 1		Grade 2		Grade 3		Grade 4		Any grade	
	IL-2 n (%)	Control n (%)	IL-2 n (%)	Control n (%)	IL-2 n (%)	Control n (%)	IL-2 n (%)	Control n (%)	IL-2 n (%)	Control n (%)
Any adverse event	15 (27%)	21 (36%)	28 (51%)	25 (42%)	10 (18%)	9 (15%)	1 (2%)	3 (5%)	55 (100%)*	57 (97%)
Body as a whole	24 (44%)	26 (44%)	22 (40%)	12 (20%)	6 (11%)	0	0	0	53 (96%)	38 (64%)
Flu syndrome	18 (33%)	3 (5%)	5 (9%)	0	1 (2%)	0	0	0	25 (46%)	3 (5%)
Injection site reactions	31 (56%)	0	6 (11%)	0	2 (4%)	0	0	0	40 (73%)	0
Asthenia	12 (22%)	4 (7%)	15 (27%)	0	2 (4%)	0	0	0	29 (53%)	4 (7%)
Cardiovascular system	4 (7%)	5 (8%)	0	0	1 (2%)	0	1 (2%)	1 (2%)	6 (11%)	6 (10%)
Digestive system	24 (44%)	11 (19%)	15 (27%)	13 (22%)	0	0	0	1 (2%)	39 (71%)	25 (42%)
Nausea	14 (25%)	3 (5%)	6 (11%)	3 (5%)	0	0	0	0	20 (36%)	6 (10%)
Diarrhea	12 (22%)	4 (7%)	3 (5%)	3 (5%)	0	0	0	0	15 (27%)	7 (12%)
Endocrine system	0	0	0	1 (2%)	0	0	0	0	0	1 (2%)
Hemic and lymphatic system	14 (25%)	4 (7%)	4 (7%)	4 (7%)	1 (2%)	3 (5%)	0	0	19 (35%)	11 (19%)
Metabolic and nutritional disorders	16 (29%)	12 (20%)	3 (5%)	6 (10%)	0	4 (7%)	0	1 (2%)	19 (35%)	23 (39%)
Musculoskeletal system	14 (25%)	2 (3%)	4 (7%)	3 (5%)	0	0	0	0	18 (33%)	5 (8%)
Nervous system	15 (27%)	6 (10%)	14 (25%)	2 (3%)	0	1 (2%)	0	0	29 (53%)	9 (15%)
Respiratory system	19 (35%)	15 (25%)	5 (9%)	3 (5%)	1 (2%)	1 (2%)	0	0	25 (45%)	19 (32%)
Skin and appendages	14 (25%)	15 (25%)	8 (15%)	1 (2%)	1 (2%)	0	0	0	23 (42%)	16 (27%)
Special senses	1 (2%)	7 (12%)	2 (4%)	1 (2%)	0	0	0	0	3 (5%)	8 (14%)
Urogenital system	6 (11%)	9 (15%)	0	2 (3%)	0	0	0	0	6 (11%)	11 (19%)

*The total number of evaluable patients for safety was 55 for the interleukin-2 (IL-2) group and not 56 (see Table 2), because one patient was randomized to the IL-2 group but did not receive treatment.

Table 4. Laboratory abnormalities

Laboratory test	Mean (median) change from baseline	
	Interleukin-2	Control
Alanine aminotransferase (ALT) (U/L)	-12.25 (-3.00) [*]	7.07 (2.50)
Aspartate aminotransferase (AST) (U/L)	-10.25 (-6.00)	8.17 (1.00)
Creatinine phosphokinase (CPK) (U/L)	-125.69 (-46.00)	416.28 (-10.05)
Triglyceride (mg/dL)	-70.50 (-13.00)	61.71 (48.00)
Total cholesterol (mg/dL)	-30.85 (-27.50)	8.69 (11.00)
Eosinophil count ($\times 10^3/\mu\text{L}$)	0.37 (0.23) [†]	0.00 (-0.01)
Hematocrit (%)	-1.79 (-2.00)	2.05 (2.00)
Hemoglobin (g/dL)	-0.94 (-1.10)	0.51 (0.45)

^{*} $p < .05$ for between-group differences in the levels of ALT, AST, CPK, triglyceride, and total cholesterol.

[†] $p < .001$ for between-group differences in the eosinophil count, hematocrit percentage, and hemoglobin concentration.

of IL-2 on the change in CD4+ T cell count was pronounced at study weeks 4 and 8 (Figure 5). The mean changes in the IL-2 group were five to eight times higher than in the control group at these time

points (p values for the between-group differences at study weeks 4 and 8 were $<.001$). However, from weeks 16 to 26, the change in CD4+ T cell count increased gradually for control patients and de-

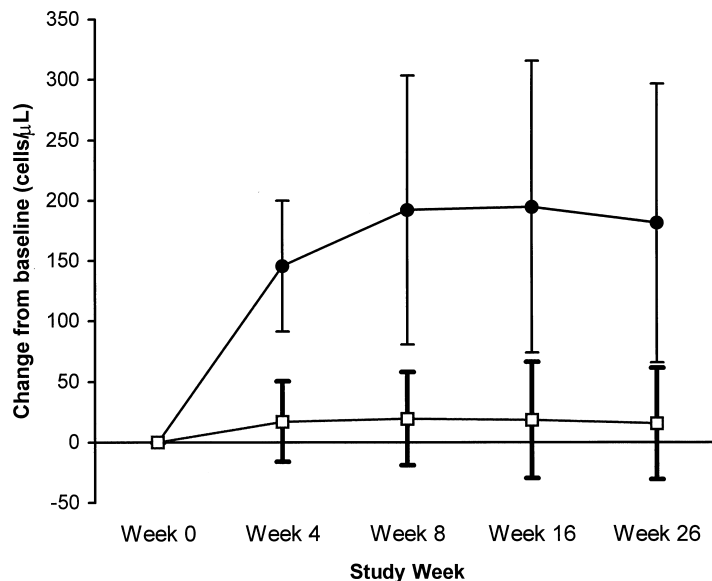


Figure 2. Change from baseline in the NK (CD16+56+) cell count, by study week. Filled circles (—●—) and open squares (—□—) represent the IL-2 and control groups, respectively. The mean changes in the NK cell count from baseline \pm SD at study weeks 4, 8, 16, and 26 are presented. The standard deviation is illustrated by lines above and below the data point. N values are 47 and 56 at week 4; 45 and 55 at week 8; 42 and 51 at week 16; and 35 and 52 at week 26 for the IL-2 and control groups, respectively.

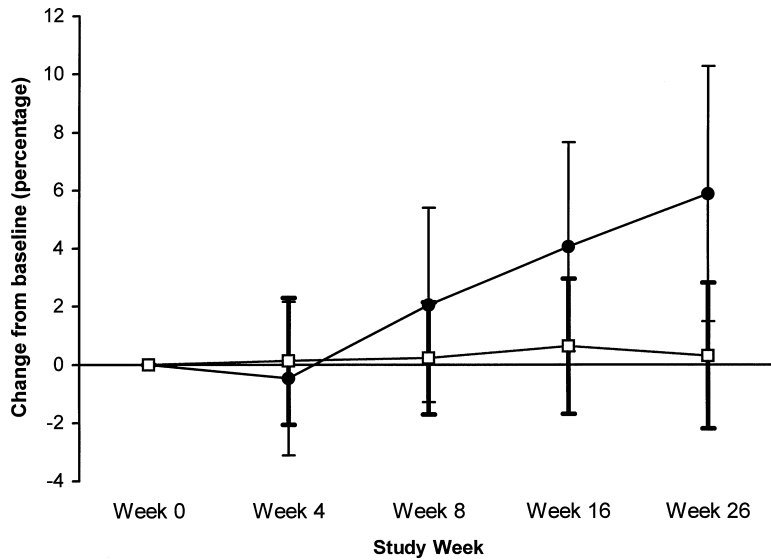


Figure 3. Change from baseline in the percentage of naive CD4+ T cells (CD3+4+45RO-45RA+), by study week. Filled circles (—●—) and open squares (—□—) represent the IL-2 and control groups, respectively. The mean changes in the percentage of naive CD4+ T cells from baseline \pm SD at study weeks 4, 8, 16, and 26 are presented. The standard deviation is illustrated by lines above and below the data point. *N* values are 45 and 50 at week 4; 40 and 49 at week 8; 39 and 45 at week 16; and 33 and 45 at week 26 for the IL-2 and control groups, respectively.

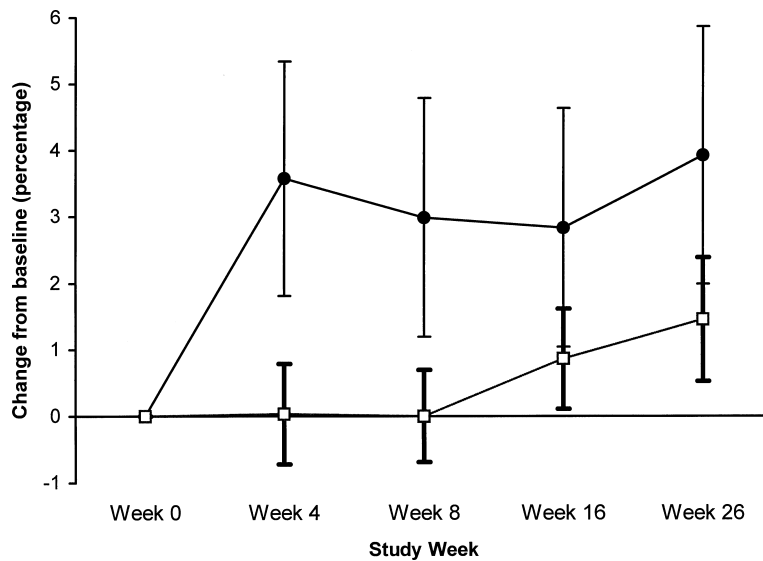


Figure 4. Change from baseline in the percentage of CD4+ T cells, by study week. Filled circles (—●—) and open squares (—□—) represent the IL-2 and control groups, respectively. The mean changes in the percentage of CD4+ T cells from baseline \pm SD at study weeks 4, 8, 16, and 26 are presented. The standard deviation is illustrated by lines above and below the data point. *N* values were 47 and 57 at week 4; 45 and 56 at week 8; 42 and 52 at week 16; and 35 and 53 at week 26 for the IL-2 and control groups, respectively.

Table 5. Mean change from baseline for various lymphocyte cell types

Cell types	Week 4	Week 8	Week 16	Week 26	Final
CD8+ T cells[†]					
Interleukin-2	-67.72 (n = 47)	-39.02 (n = 45)	-185.41 (n = 42)	-131.26 (n = 35)	-114.19 (n = 52)
Control	50.04 (n = 57)	61.35 (n = 56)	48.86 (n = 52)	40.12 (n = 53)	41.56 (n = 58)
P value for between-group difference	.072	.21	<.001	.013	.006
Memory CD4+ T cells[†]					
Interleukin-2	0.06% (n = 45)	-2.71% (n = 40)	-4.24% (n = 39)	-5.92% (n = 33)	-4.49% (n = 49)
Control	0.12% (n = 50)	-0.95% (n = 49)	-0.40% (n = 45)	-1.26% (n = 45)	-1.11% (n = 51)
P value for between-group difference	.920	.028	.002	.001	.001
Naive CD8+ T cells					
Interleukin-2	1.08% (n = 45)	2.10% (n = 40)	1.96% (n = 40)	2.73% (n = 33)	2.16% (n = 49)
Control	0.23% (n = 50)	-0.32% (n = 49)	-0.03% (n = 45)	-1.36% (n = 45)	-1.70% (n = 51)
P value for between-group difference	.830	.250	.120	.029	.007
Memory CD8+ T cells					
Interleukin-2	-1.09% (n = 45)	-3.73% (n = 40)	-3.62% (n = 40)	-4.53% (n = 33)	-3.2% (n = 49)
Control	0.97% (n = 50)	0.38% (n = 49)	1.46% (n = 45)	-2.16% (n = 45)	-1.81% (n = 51)
P value for between-group difference	.41	.10	.009	.27	.37

* Data expressed as the mean change from baseline in cell number (cells/ μ L).

† Data expressed as the mean change from baseline in percentage of this cell type in the total population of lymphocytes (% value).

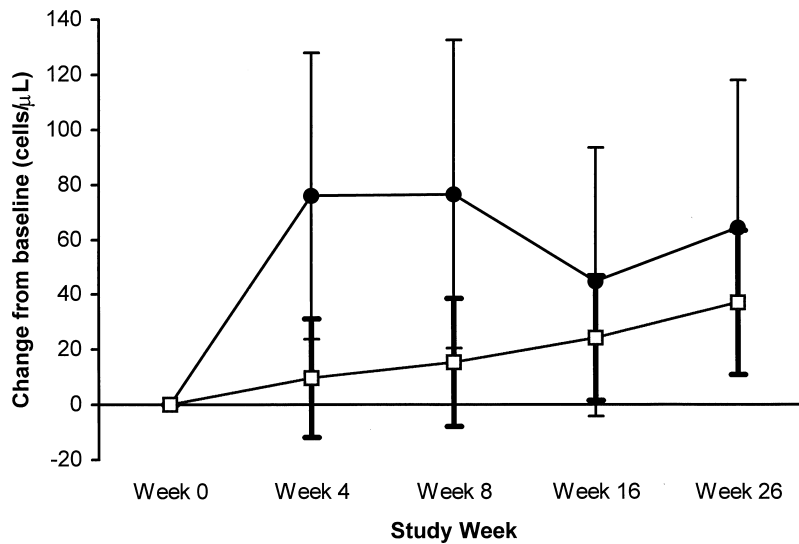


Figure 5. Change from baseline in the absolute CD4+ T cell count, by study week. Filled circles (—●—) and open squares (—□—) represent the IL-2 and control groups, respectively. The mean changes in CD4+ T cell count from baseline \pm SD at study weeks 4, 8, 16, and 26 are presented. The standard deviation is illustrated by lines above and below the data point. *N* values were 47 and 57 at week 4; 45 and 56 at week 8; 42 and 52 at week 16; and 35 and 53 at week 26 for the IL-2 and control groups, respectively.

creased slightly for IL-2-treated patients. The differences between treatment groups were less at these time points and did not reach statistical significance; however, the mean changes in CD4+ T cell count for the IL-2 group were up to two times greater than the mean changes observed for the control group.

The CD4+/CD8+ T cell ratio, which grossly measures the health of the immune system, increased from baseline and final assessment for IL-2-treated patients. The mean CD4+/CD8+ T cell ratio at baseline was 0.24 ($n = 52$) and 0.24 ($n = 58$) for the IL-2 and control treatment groups, respectively. For patients in the control group, this ratio increased slightly, to a final mean value of 0.27. However, patients treated with IL-2 experienced a significant increase in the CD4+/CD8+ T cell ratio to a final mean value of 0.38. This difference between treatment groups from baseline to final assessment in the CD4+/CD8+ T cell ratio was statistically significant ($p < .001$).

When baseline and final measurements were compared, the percentage of patients with increases of greater than 50% in CD4+ T cell count was 2-fold higher in the IL-2 treatment group (67%,

$n = 52$) than in the control group (33%, $n = 58$) ($p = .08$). The percentage of patients with increases of greater than 25% were the same for the IL-2 (50%, $n = 52$) and control (50%, $n = 58$) treatment groups.

When the CD8+ T cell number was analyzed during the course of the study, decreases in the CD8+ T cell count were observed in IL-2-treated patients and increases were observed in control patients at each of the time points analyzed (Table 5). The difference between treatment groups in the change in CD8+ T cell count from baseline to final evaluation was statistically significant ($p = .006$).

Similar to the results described earlier for the change in the percentage of naive CD4+ T cells during the study, the percentage of naive CD8+ T cells (CD3+8+45RO⁻45RA⁺ cells) increased from baseline at study weeks 4, 8, 16, and 26 for the IL-2 group (Table 5). A significant difference in the percentage of naive CD8+ T cells between treatment groups was observed with respect to the changes from baseline to week 26 and to final assessment. In contrast, the percentages of memory CD8+ T cells (CD3+8+45RO⁺45RA⁻ cells) were found to decrease from baseline for IL-2-treated patients at weeks 4, 8, 16, and 26 (Table 5).

DISCUSSION

Daily, low-dose subcutaneous IL-2 therapy, in conjunction with HAART for a 6-month time interval, was safe and well tolerated in the outpatient setting. Adverse events were primarily minimal or moderate in nature. Excluding events related to subcutaneous injections (injection site reactions), the common adverse events in the IL-2 group, such as flu syndrome, were expected based on data from similar studies and were most likely due to activation of the immune system by exogenous IL-2.^{9,12}

However, by comparison with the higher doses of IL-2 used intermittently, the low-dose daily regimen was tolerated considerably better. An important finding in this study is the virtual absence of grade 3 toxicity in patients treated with the daily, low-dose IL-2 treatment regimen (Table 3). Even though grade 3 adverse events were observed, the frequency of grade 3 toxicity (18%; 10 of 55) in the IL-2 group was only slightly higher than in the control group (15%; 9 of 59). By comparison to patients treated with an intermittent-dose IL-2 therapy, the incidence of grade 3 adverse events in the current study was remarkably reduced.^{8,9,11} Previously, grade 3 adverse events were reported in 91% of patients treated with an intermittent schedule for IL-2 (5.0 MIU/cycle to 8.5 MIU/cycle) and in 10% of control patients.⁸

Of interest are the positive effects of IL-2 that were noted from the analysis of chemistry laboratory data. The decreases in the proportion of patients in the IL-2 group with above normal values for AST, ALT, and CPK at final assessment, when compared to baseline, suggest that IL-2 therapy may decrease the toxicity associated with HIV disease and HAART. Also, IL-2 therapy resulted in improvements in the total cholesterol and triglyceride levels. When comparing final to baseline measurements, substantial decreases in the number of patients with above normal levels of total cholesterol and triglyceride were observed in the IL-2 group relative to the control group.

Patients' viral load remained stable for the duration of the study, with slight increases in HIV mRNA copy number/mL for both treatment groups. Significant differences between treatment groups in the change in HIV mRNA copy number/mL were not observed, demonstrating that no detrimental effect of IL-2 on viral burden was apparent for the duration of this study ($p = .85$ for be-

tween-group difference). This result was important since IL-2 has been found to activate HIV replication in cell culture and in vivo.¹⁷ It is interesting that the number of changes in antiretroviral medications during the study was higher in control patients than in the IL-2-treated patients ($p = .11$). The less frequent modifications in antiretroviral medications in patients who received daily, low-dose IL-2 therapy indicates that IL-2 may have contributed to disease stabilization and improved the overall health of patients.

IL-2 treatment as an adjunct to HAART resulted in a positive effect on the CD4+ T cell count. Differences between the IL-2 and control populations were observed in the increase in percentage of CD4+ T cells ($p < .001$), the increase in CD4+/CD8+ cell ratio ($p < .001$), and the proportion of patients with greater than 50% increases in CD4+ T cell count ($p = .08$). In addition, remarkable differences were observed between treatment groups in the increases in the number and percentage of NK cells ($p < .001$) and in the percentage of naive CD4+ T cells ($p < .001$), demonstrating that the populations of these cell types had expanded during IL-2 treatment. NK cell activity is important for the destruction of cells infected with virus and for mediating antibody-dependent cytotoxicity.¹⁸ The mechanisms by which the replication and spread of HIV is inhibited by NK cells includes lytic activity against HIV-infected cells, the inhibition of CC-chemokine-mediated entry of HIV into uninfected cells, and the production of antiviral cytokines such as interferon- γ (IFN- γ).¹⁹ Therefore, increases in the NK cell count resulting from the low-dose IL-2-treatment regimen may benefit HIV-infected patients on a HAART regimen by enhancing the innate cellular immune response, thereby augmenting the suppression of HIV replication by HAART.

Connors et al.¹ demonstrated that in HIV-infected patients who were receiving protease inhibitor (indinavir) therapy with or without intermittent IL-2 treatments, the expansion of naive CD4+ T cells was not associated with an increase in the CD4+ T cell repertoire. The increases in naive CD4+ T cells were primarily from proliferation of naive CD4+ T cells that were present before therapy rather than proliferation of newly synthesized cells in the thymus. In our study, a significant increase in the percentage of naive CD4+ T cells and decrease in the percentage of activated

memory CD4+ T cells was evident in IL-2-treated patients relative to control patients. The increase in the percentage of naive CD4+ T cells in patients treated with daily, low-dose IL-2 was similar to the increase in this cell type that was observed in a recent study that used a high-dose intermittent regimen.⁹ This effect of daily, low-dose IL-2 on T cell subsets resulted in partial restoration of the normal balance between the percentages of naive and memory CD4+ T cells. An interesting future study might investigate whether treatment with daily, low-dose subcutaneous IL-2 leads to an expanded T cell repertoire or whether it will be similar to studies using an intermittent dosing schedule, where pre-existing naive CD4+ T cells proliferate in response to IL-2.¹

A notable decrease in the CD8+ T cell count was experienced by patients treated with IL-2 when compared to control patients ($p = .006$). However, relative to control patients, statistically significant increases in the percentage of naive CD8+ T cells were experienced by patients treated with IL-2 ($p = .007$). It is notable that the expansion of the naive CD8+ T cell subset has not been previously reported for patients treated with the intermittent IL-2 dosing schedule with or without antiretroviral therapy. It appears that administration of IL-2 using the daily, low-dose subcutaneous treatment regimen has a different effect than the intermittent schedule on the population of naive CD8+ T cells.

In summary, daily, low-dose subcutaneous IL-2 therapy in conjunction with HAART for a 6-month time interval was safe and well tolerated and appeared effective in expanding the population of NK cells and naive CD4+ and CD8+ T cells in HIV-infected individuals with less than 300 cells/ μ L. IL-2 therapy was effective at not only increasing the percentage of CD4+ T cells and increasing the CD4+/CD8+ T cell ratio but also significantly increasing the fraction of naive T cells. Also, the CD4+ T-cell count increased by greater than 50% for a larger proportion of patients treated with IL-2 when compared to control patients. These results indicate that daily, low-dose IL-2 therapy accelerates the recovery of several measurable immunological parameters. Therefore, the results described here provide the impetus for further study to determine whether restoration of immunocompetence in HIV-infected patients is observed after daily, low-dose IL-2 plus HAART combination therapy.

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