Nadir CD4⁺ T Cell Count Predicts Response to Subcutaneous Recombinant Interleukin-2

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Community Program for Clinical Research on AIDS 059 was a multicenter study conducted among human immunodeficiency virus (HIV)–infected individuals with CD4⁺ cell counts ≥300 cells/mm³ who were randomly assigned to receive antiretroviral therapy with or without intermittent subcutaneously administered recombinant interleukin-2 (rIL-2). To identify factors associated with a response to IL-2, a secondary analysis was performed that included the subset of rIL-2 recipients who were able to complete all 3 initial treatment cycles. Predictors of a change in CD4⁺ cell count between baseline and 1 month after the start of treatment cycle 3 were examined in a multivariate model that included sex, race, body surface area, rIL-2 dose, HIV load, and both baseline and nadir CD4⁺ cell count. The combination of race and sex (P = .027) and the nadir CD4⁺ cell count (P = .005) were significant predictors of mean CD4⁺ cell count response. Baseline CD4⁺ cell count had no significant effect. The strong association between nadir CD4⁺ cell count and CD4⁺ cell count response suggests that immunologic losses resulting from HIV-mediated CD4⁺ cell depletion may be irreversible.

 Although the intermittent administration of IL-2 to HIV-infected individuals can frequently induce a significant elevation in the number of circulating CD4⁺ lymphocytes, it is unknown whether this response is a surrogate for clinical benefit [1, 2]. Nevertheless, the failure to achieve an improvement in CD4⁺ cell count has been a reason to increase the IL-2 dose, extend the number of treatment cycles, or discontinue its use. Given the toxicity of IL-2, the probability of response has been an important consideration in the decision to initiate or continue therapy. Previous reports have emphasized that the likelihood and the magnitude of an increase in the CD4⁺ cell count directly relate both to the dose of IL-2 and to the pretherapy CD4⁺ cell count [3–5]. More-recent data suggest that active antiretroviral therapy can lower the CD4⁺ cell count threshold above which a response can be expected [6, 7]. This observation is supported by the findings of 2 previous studies of IL-2 therapy that showed an inverse relationship between HIV load and CD4⁺ cell response [8, 9].

Community Program for Clinical Research on AIDS (CPCRA) 059 was a large, multicenter, randomized, open-label trial designed to compare the virologic and immunologic impact of active antiretroviral therapy with or without recombinant IL-2 (rIL-2) among HIV-infected persons with CD4⁺ cell counts ≥300 cells/mm³ [10]. After 12 months of follow-up, in an intent-to-treat analysis, patients randomly assigned to receive antiretroviral therapy and rIL-2 experienced a mean CD4⁺ cell count increase of 276.7 cells/mm³, compared with 22.5 cells/mm³ among control subjects randomly assigned to receive antiretroviral therapy alone (P < .001). We made an on-treatment analysis of rIL-2 recipients who were able to complete ≥3 cycles of therapy, to reexamine factors predictive of a CD4⁺ lymphocyte response to rIL-2.

METHODS

Trial design. The Terry Beirn CPCRA is a National Institute for Allergy and Infectious Diseases–funded...
clinical trials group that consisted of 17 units throughout the United States at the time that CPCRA 059 was conducted. Overall, 15 units participated in this phase 2, open-label, randomized clinical trial that compared the effects of antiretroviral therapy with those of antiretroviral therapy and subcutaneous rIL-2 on plasma HIV RNA levels and the number of CD4+ cells in peripheral blood. Participants were randomly assigned to receive 1 of 2 doses of rIL-2 (4.5 or 7.5 million IU [MIU]) or no IL-2, in a 1:1:2 allotment. Administration of rIL-2 began within 2 weeks after randomization and was to be continued for at least the required 3 cycles. Protocol guidelines also stated that additional cycles of therapy should be given to maintain CD4+ cell counts at twice the baseline level or >1000 cells/mm^3. CD4+ cell counts were measured at baseline, every 4 months after enrollment, and on day 29 after the start of each rIL-2 cycle. Subjects were followed up for at least 12 months.

**Patient selection.** All patients ≥18 years of age were eligible for this study, provided they were receiving or initiating combination antiretroviral therapy and had CD4+ cell counts ≥300 cells/mm^3. Exclusion criteria included previous receipt of IL-2 therapy; any CNS disorder that required the use of antiseizure medication; receipt of corticosteroids or cytotoxic agents (including hydroxyurea) within 4 weeks before randomization; history of an AIDS-defining illness; history of Crohn disease, psoriasis, or other autoimmune diseases; and pregnancy or breast-feeding. Medical records were also reviewed for CD4+ cell counts, and the lowest, or nadir, CD4+ cell count documented was recorded on the baseline case report form. Informed consent was obtained from all patients, and the study was approved by the institutional review boards at each participating center. The guidelines for human experimentation of the US Department of Health and Human Services and each participating center were followed in conducting the clinical research.

**Medication.** The protocol mandated that all participants receive combination antiretroviral therapy with at least 2 agents. Otherwise, management of antiretrovirals was left to the discretion of the treating clinician. Administration of rIL-2 (provided by Chiron) was initiated at the randomization dose of 4.5 or 7.5 MIU b.i.d., subcutaneously administered for 5 consecutive days every 8 weeks. To manage toxicity, treating clinicians were able to reduce the rIL-2 dosage in decrements of 1.5 MIU per dose. Thereafter, dosage could be adjusted stepwise in either direction but was not to exceed the originally assigned dose or to decrease to <1.5 MIU per dose. All dosage modifications and missed doses were reported for each patient. Twenty-nine days after the first subcutaneous rIL-2 dose of each cycle, the CD4+ cell count was measured.

**Statistical analysis.** Only those participants able to complete at least 3 cycles of subcutaneous rIL-2 and for whom the CD4+ cell count was measured on day 29 after the third cycle were included in this analysis. A site-stratified analysis of covariance was used to compare the absolute change in CD4+ cell count from baseline to day 29 after the start of cycle 3. The covariates considered in this analysis include randomized rIL-2 dose, sex, race, undetectable plasma HIV RNA level (<50 copies/mL) at baseline, baseline CD4+ cell count, nadir CD4+ cell count, body surface area, age, and duration of antiretroviral therapy at baseline, with the latter 5 treated as continuous covariates.

**RESULTS**

**Patient population.** From September 1998 through July 1999, 511 patients were enrolled in this study; 256 participants were randomly assigned to receive rIL-2. One hundred (76.9%) of 130 persons assigned to the 4.5-MIU arm and 95 (75.4%) of 126 persons assigned to the 7.5-MIU arm were able to complete at least 3 cycles of rIL-2 and had a CD4+ cell count measured on day 29 after the third cycle. Of the 61 rIL-2 recipients who were excluded from analysis, 7 never began a treatment cycle, and 14 completed 3 cycles but did not have a CD4+ cell count measured after the third cycle.

Baseline characteristics were similar in both dose groups (Table 1). The majority of subjects were male, white, and men who have sex with men. The median baseline and nadir CD4+ cell counts were 527 and 290 cells/mm^3, respectively. Virus load (measured by bDNA analysis) was <50 copies/mL in 58.8% of the patients, with a median value of 1.71 log_{10} copies/mL among those with a detectable virus load. No significant differences between the 2 dose groups were noted in height, weight, or body surface area. Patients were highly antiretroviral experienced (they had received a median of 33 months of previous therapy); all were receiving at least 2 antiretroviral agents at the time of initiation of rIL-2. There were no significant differences between dose groups in the use of specific agents or in the type of regimen prescribed. Approximately 57% of patients were given combinations of protease inhibitors and nucleosides; 21% received nonnucleoside reverse-transcriptase inhibitors and nucleosides, and 14% received at least 1 drug from all 3 classes.

**Administration of rIL-2.** In the 4.5-MIU group, 62 (62.0%) of 100 patients received all 30 injections without dosage reduction, 15 patients (15.0%) missed doses of rIL-2 but did not have dosage reduction, and 23 patients (23.0%) had dosage reduction. Dosage adjustment was more common in the 7.5-MIU arm. Only 43 (45.3%) of 95 patients received all 30 injections without dosage modification; 18 patients (19.0%) missed doses but did not require dosage modification, and 34 patients (35.8%) required dosage reduction. The mean administered cumulative dose in the third cycle of rIL-2 treatment was 41.8 MIU in the 4.5-MIU group (maximum, 45 MIU),...
Table 1. Characteristics of HIV-infected patients, by IL-2 dose group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-2 dose</th>
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<tbody>
<tr>
<td></td>
<td>4.5 MIU</td>
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<tr>
<td>No. of patients</td>
<td>130</td>
</tr>
<tr>
<td>No. of patients who completed treatment cycle 3</td>
<td>100</td>
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Demographic characteristics

<table>
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<tr>
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<th>IL-2 dose</th>
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<tr>
<td>Age, mean years</td>
<td>40.5</td>
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<tr>
<td>Female sex, % of patients</td>
<td>6.0</td>
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<tr>
<td>Race, % of patients</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>74.0</td>
</tr>
<tr>
<td>Black</td>
<td>15.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9.0</td>
</tr>
<tr>
<td>Other</td>
<td>2.0</td>
</tr>
<tr>
<td>Body surface area, mean m²</td>
<td>2.0</td>
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<tr>
<td>Duration of previous antiretroviral therapy, median months</td>
<td>36</td>
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</table>

Laboratory findings

<table>
<thead>
<tr>
<th></th>
<th>IL-2 dose</th>
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<tbody>
<tr>
<td>CD4⁺ cell count at baseline, median cells/mm³</td>
<td>499.5</td>
</tr>
<tr>
<td>Nadir CD4⁺ cell count, median cells/mm³</td>
<td>265.0</td>
</tr>
<tr>
<td>HIV RNA load &lt;50 copies/mL at baseline, % of patients</td>
<td>57.6</td>
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</table>

NOTE. MIU, million IU.

compared with 64.9 MIU in the 7.5-MIU group (maximum, 75 MIU). After 3 cycles, the mean cumulative doses of rIL-2 were 127.7 MIU (95% of maximum) and 202.9 MIU (90% of maximum) in the 4.5- and 7.5-MIU groups, respectively.

**CD4⁺ cell count response.** In a stratified analysis of covariance, the nadir CD4⁺ cell count emerged as the strongest predictor of rIL-2 response. Every 100-cell/mm³ increment in nadir value accounted for an increase of 67 cells/mm³ (95% CI, 20.9–113.6 cells/mm³; \( P = .005 \)). Table 2 presents the relationship between nadir CD4⁺ cell count and CD4⁺ cell count change from baseline to day 29 of treatment cycle 3 for 2 response criteria: (1) increase of >200 cells/mm³ from baseline and (2) achievement of the study-defined CD4⁺ cell count goal of an increase of ≥2-fold in baseline CD4⁺ cell count or an increase to >1000 cells/mm³. For example, after 3 cycles of subcutaneous rIL-2, the proportions of individuals who had an increase from baseline of >200 CD4⁺ cells/mm³ were 52.1%, 73.6%, and 78.7% for those with nadir CD4⁺ cell counts of <200, 200–300, and >300 cells/mm³, respectively. Approximately 31% of those with nadir counts of <200 CD4⁺ cells/mm³, compared with ~69% of those with nadir values of >300 CD4⁺ cells/mm³, attained the study CD4⁺ cell count goal.

Figure 1 depicts the mean change in CD4⁺ cell count from baseline to day 29 of cycle 3 by nadir CD4⁺ cell count stratum. For patients with nadir CD4⁺ cell values of <200, 200–300, and >300 cells/mm³, the mean changes were 322, 427, and 598 cells/mm³, respectively (\( P < .01 \), in a univariate analysis of trend).

The rate of mean CD4⁺ cell count change per cycle was determined on the basis of the slopes of the curves shown in figure 1. For the lowest- to highest-nadir group, these slopes were 106, 137, and 199 cells/mm³ per cycle (\( P < .01 \), in a univariate analysis of trend). In pairwise comparisons, however, the slopes of the lowest 2 nadir strata were not statistically significantly different. After adjustment for the other covariates in the model, baseline CD4⁺ cell count was not a significant predictor of CD4⁺ cell count change at day 29 of cycle 3 (\( P = .623 \)).

The magnitude of the change from baseline CD4⁺ cell count was assessed at day 29 after the start of cycle 1 for the ability to predict third-cycle response. Among the 162 patients who experienced a response of ≥50 cells/mm³ during cycle 1, 80% had a third-cycle response of >200 cells/mm³, and 62% attained the study target of an increment at least double the baseline CD4⁺ cell value or >1000 CD4⁺ cells/mm³ by the end of cycle 3. Among the 28 patients with smaller first-cycle changes, third-cycle responses of >200 cells/mm³ were observed in 29%, and 14% achieved the study goal. Patients who achieved an increase of ≥50 CD4⁺ cells/mm³ after cycle 1 of rIL-2 were 8.9 times more likely than those who did not to experience an increase of >200 CD4⁺ cells/mm³ (\( P = .0001 \)) and 7.1 times more likely to attain the study CD4⁺ cell count target by the end of cycle 3 (\( P = .003 \)).

Other covariates of interest were sex/race combinations, body surface area, randomized dose group, and baseline virus load. After adjustment for other covariates, nonwhite men experienced...
a mean CD4⁺ cell increase that was 178 cells/mm² larger than that observed for white men (95% CI, 21.8–334.2 cells/mm²; \( P = .027 \)). Numbers were too small to allow for comparisons between female sex and nonwhite racial groups taken individually. The effect of body surface area on response did not attain statistical significance; a 0.1-m² increase resulted in a decrease of 33 cells/mm² in mean CD4⁺ cell count change from baseline (\( P = .056 \)). However, among 38 patients with a body surface area of <1.84 m², the mean change in CD4⁺ cell count was 635.4 cells/mm². In comparison, 47 patients with a body surface area \( \geq 2.09 \) m² experienced a mean gain of 461.6 cells/mm².

Although there was a trend toward a greater CD4⁺ cell response in the 7.5-MIU dose group, compared with the 4.5-MIU arm, the difference was not statistically significant, regardless of whether it was analyzed by randomized or cumulative IL-2 dose. Among those with nadir CD4⁺ cell counts <200 cells/mm², individuals in the 4.5-MIU arm had a mean increase of 240 cells/mm² after cycle 3, whereas those in the 7.5-MIU arm had a mean increase of 463 cells/mm² (95% CI for the difference, 121–323 cells/mm²; \( P = .006 \)), which suggests that dose had an effect on the CD4⁺ cell response in this group. Finally, no significant differences in response were found when patients who had <50 copies/mL of HIV RNA in plasma at baseline were compared with those who had \( \geq 50 \) copies/mL. However, given the large proportion of patients with baseline virus loads of <50 copies/mL, the power to detect such differences was limited.

**DISCUSSION**

The majority of published studies of the use of IL-2 for the treatment of HIV infection were conducted before the availability of HAART. In the present study, 195 patients who received combination antiretroviral therapy in conjunction with rIL-2 were evaluated to identify factors predictive of a CD4⁺ cell response after 3 cycles of rIL-2 administration. In a multivariate model based on change in absolute CD4⁺ cell count from baseline to day 29 of cycle 3, only the nadir CD4⁺ cell count and the combination of nonwhite race and male sex emerged as statistically significant predictors of response.

Previous studies have described a direct relationship between baseline CD4⁺ cell count and IL-2 response, and the poorest response rates have been observed among individuals with baseline values of <200 cells/mm² [3, 4, 11]. However, these studies did not report data on nadir CD4⁺ cell counts. Although baseline CD4⁺ cell count was associated with a CD4⁺ cell count increment in the present study, the association failed to achieve statistical significance when the additional covariate of nadir CD4⁺ cell count was introduced into the model. This study enrolled persons with relatively high baseline CD4⁺ cell counts. Most HIV-infected individuals with CD4⁺ cell counts of \( \geq 300 \) cells/mm² can be expected to respond to IL-2, even in the absence of combination antiretroviral therapy [2–5, 8, 12, 13]. Therefore, the ability to discern an effect of baseline CD4⁺ cell count may have been diminished by patient selection. Although several other studies have not shown a significant relationship between the absolute number of CD4⁺ cells at baseline and CD4⁺ cell count change after IL-2 therapy, those studies enrolled individuals with higher CD4⁺ cell counts or included too few subjects to demonstrate a difference [8, 14].

Nonetheless, the effect of the nadir CD4⁺ cell count on the CD4⁺ cell response after 3 cycles of IL-2 was highly significant and consistent across strata, with the best response observed among patients with nadir CD4⁺ cell counts of >300 cells/mm². Thus, the lowest CD4⁺ cell count, and not the higher baseline count, predicted response to IL-2 therapy. These findings strongly suggest that at least some aspects of the immune system are irreversibly changed by HIV infection.

IL-2 participates in the regulation of lymphocyte homeostasis at multiple levels, through action on the proliferative response to mitogens and antigens, cell survival, apoptosis, and the coordination of progression through the cell cycle [15–18]. The administration of IL-2 to HIV-infected persons leads to a brisk elevation in the rates of lymphocyte proliferation and, at least in the short term, of apoptosis [16, 17]. Although the balance between these 2 processes is important, the major mechanism

**Table 2. Change in CD4⁺ cell counts among HIV-infected patients after 3 cycles of IL-2, by nadir CD4⁺ cell count.**

<table>
<thead>
<tr>
<th>Nadir CD4⁺ cell count, cells/mm²</th>
<th>No. of patients</th>
<th>Patients who met goal⁰ after 3 cycles, %</th>
<th>Patients with an increase of &gt;200 cells/mm² after 3 cycles, %</th>
<th>Mean change in CD4⁺ cell count, cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>48</td>
<td>31.3</td>
<td>52.1</td>
<td>322</td>
</tr>
<tr>
<td>201–300</td>
<td>53</td>
<td>52.8</td>
<td>73.6</td>
<td>428</td>
</tr>
<tr>
<td>&gt;300</td>
<td>94</td>
<td>69.1</td>
<td>78.7</td>
<td>599</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>55.4</td>
<td>70.8</td>
<td>484</td>
</tr>
</tbody>
</table>

⁰ Doubling of baseline CD4⁺ cell count or increase to >1000 CD4⁺ cells/mm².
Figure 1. Mean change from baseline in CD4+ cell count, by nadir CD4+ cell count, for each cycle of subcutaneous recombinant IL-2 therapy among HIV-infected patients who completed 3 treatment cycles. The time interval between CD4+ cell count measurements is \( \sim 8 \) weeks. Bars represent 95% CIs. Nos. below the figure indicate the no. of patients for whom CD4+ cell count measurements were obtained at each time point. Note that the no. of patients in each CD4+ cell stratum at baseline and at cycle 3 are identical and represent the study cohort as defined by the inclusion criteria. However, CD4+ cell count measurements were not obtained for 5 persons after cycle 1 and for 11 persons after cycle 2.

Through which IL-2 mediates expansion of the CD4+ T cell pool appears to reside in the prolongation of CD4+ T cell survival [19, 20]. As HIV disease progresses, subpopulations of IL-2–responsive CD4+ T cells may be permanently lost. The nadir CD4+ cell count may represent a surrogate for the extent of these losses. Similarly, the nadir CD4+ T cell count has been shown to be an independent predictor of the CD4+ cell response to potent antiretroviral therapy [21]. Nevertheless, in CPCRA 059, the mean change in CD4+ cell count at each of the 4-month follow-up visits was significantly greater among IL-2 recipients than among control subjects who received only antiretroviral agents [10]. At month 12, for example, the mean difference between groups was 251 cells/mm³ \((P<.0001)\).

Among men, nonwhite subjects demonstrated a significantly greater CD4+ cell response to IL-2 than did white subjects. Although this observation has not been reported elsewhere, other studies have included highly homogeneous populations with an underrepresentation of nonwhite races [2, 4, 6–8, 12, 14, 19]. The basis for this difference in response is unexplained and awaits confirmation in larger studies. Sex and racial differences in response to immune-based therapy are not unknown and have been reported for IFN-\( \alpha \) in the treatment of hepatitis C (female patients are more likely and black patients are less likely to experience sustained virologic response) [22, 23].

Overall, the steepest increase in absolute CD4+ cell count occurred after the first cycle of IL-2, which is consistent with the findings of other studies [2–4, 14]. First-cycle response was predictive of subsequent third-cycle response. Discontinuation of IL-2 therapy after the first cycle for subjects in whom the baseline CD4+ cell count did not increase by \( \geq 50 \) cells/mm³ would have led to the exclusion of \( \sim 4% \) of those who ultimately attained the target CD4+ cell count. Conversely, those with a smaller first-cycle increment had a 14% chance of response.

CPCRA 059 was not designed to be a dose-finding study, and no significant differences in response were noted when examined by randomly assigned doses of IL-2, cumulative dos-
age received, or dose per square meter of body surface area. The higher frequency of missed and reduced doses of rIL-2 in the 7.5-MIU arm, compared with the 4.5-MIU arm, probably contributed to the lack of difference seen. In addition, the results of this study do not reflect response beyond the first 3 cycles of IL-2 therapy. However, in the context of other studies [2–5, 8, 10, 24] that have demonstrated a dose-response relationship and a benefit of dose escalation (weighed against the increased toxicity associated with higher IL-2 doses), some guidance can be gained from the observations made in the present study. Individuals with nadir CD4+ cell counts of <200 cells/mm3, those with first-cycle IL-2 responses of <50 cells/mm3, and, perhaps, those with a very large body surface area may be candidates for higher initial or subsequent escalation of IL-2 dosage.

However, the validity of this suggestion, as well as the roles of sex and race in dosage selection, requires further study. Although IL-2 can induce a remarkable increase in the absolute CD4+ cell count, its clinical benefit is not yet established. Virtually all individuals who receive IL-2 experience side effects that may negatively impact quality of life [2–5, 8]. Therefore, it is worthwhile to identify predictors of response to therapy, so that candidates for treatment can be selected more carefully. In this on-therapy analysis of CPCRA 059, nadir CD4+ cell count emerged as the dominant predictor of IL-2 response. Although this observation may have been biased by patient selection, it was sufficiently strong to suggest that it is biologically plausible that there is an inability to completely restore immune function after HIV-mediated CD4+ T lymphocyte depletion.

References

22. Huk Q, Vierling JM, Redeker AG. Viral, host, and interferon-related factors modulating the effect on interferon therapy for hepatitis C virus infection. J Viral Hepat 2001;8:1–18.