Induction and Maintenance Therapy with Intermittent
Interleukin-2 in HIV-1 Infection

Claire E. Farel¹, Doreen G. Chaitt¹, Barbara K. Hahn²,
Jorge A. Tavel¹, Joseph A. Kovacs², Michael A. Polis¹, Henry Masur², Dean A.
Follmann¹, H. Clifford Lane¹, and Richard T. Davey, Jr.¹

1. Laboratory of Immunoregulation, National Institute of Allergy and
Infectious Diseases (NIAID), NIH, DHHS, Bethesda, Maryland.
2: Critical Care Medicine Department, The Warren G. Magnuson
Clinical Center, NIH, DHHS, Bethesda, Maryland.

author to whom correspondence and reprint requests should be directed:
Richard T. Davey, Jr., M.D. Telephone: 301-496-8029
Building 10 Room 11C-103 Fax: 301-402-4097
National Institutes of Health Email: rdavey@niaid.nih.gov
Bethesda, Maryland 20892-1880

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Kovacs) are listed as co-inventors on this patent.
Abstract

Studies establishing that intermittent sc interleukin-2 (IL-2) therapy can lead to substantial CD4 cell increases in many HIV-infected patients have generally been of limited duration. We studied 77 patients participating in active longitudinal studies of sc IL-2 therapy at our center in order to determine the long term feasibility of this approach. Following initial induction, patients in each trial were eligible to receive intermittent 5-day cycles of sc IL-2 treatment at individualized doses and frequencies capable of maintaining CD4 counts at post-induction levels. The mean duration of study participation to date is 5.9 (range: 1.0 –9.3) years. Mean baseline CD4 cell count and CD4% values of 521 cells/microLiter and 27% have risen to 1005 cells/µl and 38%, respectively, at 90 months. The mean number of sc IL-2 cycles required to achieve and maintain these increases was 10 (range: 3-29) cycles, and the current mean interval of cycling required to maintain these elevations is 39 (median = 35, range: 2-91) months. We conclude that sc IL-2 therapy is capable of maintaining CD4 cell increases for an extended period using a remarkably low frequency of intermittent cycling. These observations may contribute to patients’ acceptance of sc IL-2 as a favorable long-term treatment strategy.
Introduction

While initially developed and ultimately licensed in the United States for the therapy of specific forms of refractory malignancy, interleukin-2 treatment has also been studied in the context of reversing the immunologic effects of HIV-1 infection. Indeed, experimental treatment of HIV-infected individuals with interleukin-2 (IL-2) in various forms has been under study in this country since the early 1980’s even prior to the etiologic identification of AIDS. Over the ensuing years, but in particular since the early 1990’s, a robust international phase II database of intermittent IL-2 treatment experience has accumulated. This database provides strong evidence that, for many patients receiving concurrent antiretroviral therapy (ART), an unprecedented rise in CD4+ cell counts can occur as a consequence of a scheduled series of intermittent IL-2 treatment cycles. Building upon these observations, the international phase III SILCAAT and ESPRIT trials were launched and are currently exploring whether these CD4 count changes translate into demonstrable clinical benefit for IL-2 recipients.

While these endpoint trials are ongoing, an acknowledged limitation of the current database is that most published studies to date have involved fairly limited treatment schedules without extensive long-term follow-up. For this reason most clinicians remain familiar with only the short-term effects of IL-2 therapy and have little direct knowledge of how this agent could potentially be employed within the context of a long-term treatment strategy.

In this report we summarize our long-term experience with three cohorts of HIV-infected individuals and their longitudinal response to IL-2 therapy. We explore, in particular, whether an “induction-maintenance” model using this cytokine can reasonably be applied to the overall management of these patients.

Methods
Patients included in this analysis were those who had enrolled in the extension phase of three separate trials of subcutaneous IL-2 plus ART. The initial phase of each trial had a distinct study design, dose and schedule of sc IL-2 administration, and entry CD4 cell count requirement (Table 1). These trials enrolled patients during a time period spanning 1993 through early 1997.

The first subcutaneous IL-2 trial ("sc IL-2") was designed as an initial 12-month, dose escalating study aimed at determining the maximum tolerated dose for sc IL-2 in patients with CD4 counts ≥ 200 cells/microliter. Fourteen patients were enrolled between 9/14/93 and 6/7/94. The second subcutaneous IL-2 trial ("sc500") was an initial 6-month randomized, open-label trial comparing the immunologic effects of two different doses of subcutaneous IL-2, and a monthly versus bimonthly schedule of administration, in a cohort of HIV-infected patients with less advanced disease. A total of 53 patients with CD4 counts ≥ 500 cells/microliter were enrolled between 5/17/95 and 3/7/97. The third trial ("msc IL-2") was an initial 12-month multicenter randomized, open-label trial comparing the effectiveness of subcutaneous IL-2 with standard-of-care ART compared to standard-of-care ART alone in patients with CD4 counts between 200 and 500 cells/microliter. Patients were enrolled between 4/18/96 and 3/19/97.

An additional minimum study participation of 6 months following the initial formal study period was required for inclusion in the long term (or "extension") analysis of these three trials. In the extension phase the administration of sc IL-2 was guided through a common algorithm: serial monitoring of the CD4 cell count and an individualization of sc IL-2 cycle frequency designed to maintain the CD4 cell count at or above a predefined threshold. For all patients this threshold was the achievable post-induction CD4 count, on average approximately twice the baseline count, present at the time of the individual’s entry into the extension phase. Patients were encouraged throughout the study to cycle as often as necessary to maintain their CD4 counts in the post-induction range. However, exceptions were granted for those patients whose post-induction CD4 counts extended high into the supra-physiologic range for this parameter; in such cases
a minimum CD4 count in the range of 1000-1200 cells/µl was generally adopted as the cycling threshold. In addition, over time those IL-2 recipients requesting lowering of their individualized thresholds by a few hundred cells were generally permitted to do so as long as a substantial increment over their baseline counts was preserved. As before, sc IL-2 cycles consisted of twice daily injections for five days at the maximally-tolerated dose (≤ 7.5 MIU bid) for each individual. For analysis extension data were normalized to a mutual time point 0 (Day 1, cycle 1 of the initial study period). The data set was closed on 1/15/03. Individualized end of study dates were used for those patients who ended extension phase participation prior to 1/15/03.

All patients provided written informed consent as approved through the NIAID institutional review board.

Immunologic and virologic (bDNA assay version 3.0, Bayer Diagnostics, Tarrytown, NY) assessments were performed as previously described. For analytical purposes, all viral loads below the lower limit of detection (< 50 copies/ml.) were assigned a value of 49 copies/ml.

Results

Of a total of 97 patients originally enrolled in the 3 trials, 77 patients continued to receive sc IL-2 treatment during the extension phase of each study. Of the 20 patients who did not continue IL-2 therapy, 2 were enrolled in the original study but subsequently declined participation following randomization, 10 did not complete the formal study period, 7 chose to discontinue participation after the formal study period, and 1 never received any IL-2 treatment (Figure 1).

61 patients currently remain on active follow-up. Four patients in this cohort were diagnosed with an AIDS-defining condition at some point during their study participation (Table 2) but remain on active follow-up. Of the 16 patients no longer participating in the extension phase, 1 patient died, 1 patient developed non-Hodgkin’s lymphoma, 8 patients chose to explore other treatment options,
and 6 patients experienced CD4+ T cell count declines that did not readily respond to IL-2 salvage therapy.

With intermittent IL-2 therapy the CD4 cell counts for these 61 patients have been maintained in the target range for an extended period of time. Relative to mean values for CD4 cell count and CD4% of 521 cells/microLiter and 27% at study entry, at month 90 the cohort was still maintaining mean levels of 1005 cells/microLiter and 38%, respectively (Figure 2). Corresponding median [25%-75% IQR] values for CD4 cell count and CD4% at study entry were 521 [414-606] cells/microLiter and 27 [22-31] %, respectively, whereas these same parameters at month 90 were 979 [717-1228] cells/microLiter and 38 [30-42] %.

There was a corresponding drop in mean CD8% as the mean CD4% rose, while absolute CD8 counts changed only slightly: mean CD8% declined from 53% at study entry to 42% at 90 months, whereas the mean CD8 cell count changed from 1040 cells/microLiter to a level of 1132 cells/microLiter over this same time period (Figure 3). Corresponding median [25%-75% IQR] values for the CD8 cell count and CD8% at study entry were 1019 [800-1221] cells/microLiter and 53 [47-59] %, respectively, whereas these same parameters measured at month 90 were 1003 [814-1322] cells/microLiter and 42 [39-48] %.

Expression of the alpha chain (CD25) of the IL-2 receptor was induced by IL-2 therapy: mean CD4+/CD25+% rose from 5% at study entry to 17% at month 90. Corresponding median [25%-75% IQR] values for CD4+/CD25+% expression were 4 [3-6] % and 16 [11-20] % over this same time period. Similar, both naïve (RO-) and memory (RO+) phenotype expression on CD4+ cells was induced by IL-2: mean RO+ CD45% rose from 17% at study entry to 23% at month 90, while mean RO- CD45% rose from 10% at study entry to 14% at month 90 (Figure 4). Corresponding median [25%-75% IQR] values for RO+ CD45% were 16 [13-20] % and 22 [18-26] % at study entry and month 90, respectively, whereas RO-
CD45% expression increased from 9 [7-13] % to 13 [9-21] % over this same time period.

Viral load did not increase in study participants during the extension period. Using the bDNA version 3.0 assay, the mean HIV log VL at baseline was 3.53 log copies/mL and declined to 2.61 log copies/mL at 90 months (Figure 5). The corresponding median [25%-75% IQR] values for viral load at study entry and at month 90 were 3.73 [3.00-4.33] log copies/mL and 1.69 [1.69-3.25] log copies/mL, respectively.

791 sc IL-2 cycles (mean=10, median=9, range: 3 – 29 per patient) were administered to 77 patients. The ratio of IL-2 cycles per patient decreased from 3.5:1 at study entry to 0.1:1 at 90 months (Figure 6). The mean minimal interval since the last cycle was required until closure of the data set was 39 (median 35, range: 2 – 91) months.

In order to evaluate the effect of improved viral suppression through the introduction of highly active antiretroviral therapy (HAART) on CD4+ T cell counts in our patient population, we also examined CD4 count trends for each patient 12 months prior to the start of HAART, at the start of HAART, and after 12 months of HAART. Patients on the three studies began HAART therapy at a mean of 11 months (median 10, range -25 to 58 months) following initiation of IL-2 therapy. The mean CD4+ T cell counts 12 months prior to HAART initiation, at the time of HAART initiation, and 12 months afterwards were 866, 903, and 1004 cells/microLiter, respectively.

The potential effects of patient attrition were examined by assigning baseline CD4 values as maximums to all subsequent data points for each patient of the original 97 for whom follow-up data were unavailable. The mean CD4 counts at 48 weeks and at 78 weeks using this method would be 867 and 751
cells/microLiter, respectively, versus values of 1040 and 1029 cells/microLiter when the analysis is limited to patients participating in the extension phase.

The mean baseline CD4 count for the 61 patients who enrolled in the extension phase was 549 cells/microLiter (27%). The 36 patients who did not enroll in the extension phase or were lost to follow-up had a mean baseline CD4 count of 496 cells/microLiter (26%). The difference in the mean baseline CD4 count between these two groups of patients was not statistically significant (p=0.23).

Clinical endpoints for this patient population were defined as AIDS-defining illnesses or death. One patient with long-standing short term memory loss was diagnosed with possible mild HIV encephalopathy. Two patients developed cutaneous Kaposi sarcoma and, as mentioned above, one patient was diagnosed with Non-Hodgkin’s lymphoma (Table 2). Three patient deaths were reported: two patients, both IL-2 non-responders, died of opportunistic infections prior to completion of the initial study phase, and one patient on the extension phase died from cardiac disease two years after study discontinuation. 12 patients enrolled in the extension phase were diagnosed with either subclinical or mild clinical hypothyroidism; 10 of these patients received synthetic thyroid hormone treatment at some point during their participation.
Discussion

Concerns over the unknown specter of long-term toxicities, costs, and substantial inconvenience associated with combination antiretroviral therapies have continued to promote interest in alternative methods of treating HIV-1 infection. Even with the advent of once daily regimens to facilitate adherence, such considerations as the necessity of a lifelong reliance on these drugs and the ever-present threat of emerging drug resistance have fueled the search for alternative strategies. As primarily a progressive immunodeficiency disease, albeit one with a viral etiology, it has long seemed logical that immunostimulatory therapy should play a larger, more substantive role in the clinical approach to this disorder. Nonetheless, the scope of immune-based treatments being studied for this condition has been comparatively minor in relation to those treatments aimed at direct interference with viral replication. The absence of a broad panoply of suitable candidate agents, our incomplete understanding of the nature of an effective immune response against the virus, the costs and inconveniences associated with largely parenteral therapies, and the absence of well-accepted surrogates for gauging the effectiveness of clinically meaningful immune enhancement remain major obstacles to further development in this field.

Despite these hurdles, at least one immune-based treatment has been under study for a number of years and continues to hold substantial promise either as an adjunct to conventional antiretroviral therapy or potentially even as a stand-alone treatment. As our data show, using the strategy of administering intermittent cycles of recombinant IL-2 at moderate doses to patients receiving antiretroviral medications, it is often possible to elevate the baseline CD4 cell counts in the majority of recipients (although clearly not in all) to levels generally not obtainable through the use of HAART alone. Moreover, this increase occurs in the absence of any adverse effect upon plasma virus levels. Increased CD4/CD25+ expression accompanies these CD4 count increases, and measurement of CD45 isoforms suggests that both phenotypically naïve and memory CD4 cell subsets are included in these elevations. As such,
these results are wholly consistent with recent \textit{in vivo} kinetic labeling studies involving either bromodeoxyuridine or deuterium incorporation that suggest that chronic IL-2 administration results in a sustained increase in the survival both of CD25+ naïve and central memory CD4 cell subsets.\textsuperscript{19} While this non-randomized longitudinal follow-up was not intended to address the overall “functionality” of these CD4 cells, and whereas the subsequent introduction of HAART would confound the interpretation of those results regardless, the notable paucity of significant AIDS-defining events in these long-term IL-2 responders at least provides no evidence of a strong discordance between their CD4 count elevations and the risk of clinical disease progression. Acknowledgment must be made, however, that the high proportion of patients achieving sustained virologic suppression would be expected to lead to a comparatively low rate of disease progression regardless, and that a larger trial involving more advanced patients at higher risk of progression would be required to address this question fully. The results of the SILCAAT trial presently underway may be particularly instructive in this regard.

In this longitudinal follow-up to three phase II trials of sc IL-2, we believe we have demonstrated that this agent can be adapted successfully for use according to an induction-maintenance strategy of long-term treatment. Following an initial series of sc IL-2 cycles to elevate the CD4 count substantially above baseline, a remarkably low frequency of intermittent IL-2 therapy is then required in order to maintain these counts in the physiologic, or even supra-physiologic, range for an extended period of time. At the time the database was closed for this analysis, the mean interval between cycles required to maintain this effect was already in excess of three years for that majority of patients continuing in active follow-up. While IL-2 treatment can be associated with transient but significant dose-related side effects even in long-term recipients, a cycling requirement of that low frequency should add considerably to the clinical appeal of this treatment strategy.

While encouraging, these results highlight at least two major deficits in our optimal use of IL-2 therapy. To begin with, it remains unclear why a
minority of patients with early to moderately advanced disease who undergo aggressive induction with IL-2 treatment do not respond immunologically to this approach despite having otherwise similar baseline CD4 counts and viral loads. Of the 77 patients who originally entered this long-term follow-up study, at least 6 patients experienced no sustained CD4 benefit from subsequent intermittent use of SC IL-2 therapy. Of the 8 additional patients who eventually chose to pursue other treatment options, it is also possible that personal dissatisfaction with the robustness of the CD4 response may have played some role in their decision to discontinue IL-2 therapy. Thus, the positive findings in the majority of IL-2 recipients who remained in active long-term follow-up may represent some degree of survivor bias in the sense that non-responders were more likely to terminate their participation earlier. Regardless, it is possible that ongoing in vivo lymphocyte labeling experiments may shed additional insight concerning the kinetics of CD4 cell turnover in this subset of patients and how this parameter may differ from that of IL-2 responders.

Secondly, if IL-2 induction in the setting of antiretroviral suppression produces CD4 count elevations of this magnitude whose durability is then easily maintained with intermittent cycling, such results beg the obvious question of whether similar increases could either be induced or maintained in the absence of concomitant antiretroviral suppression. That is, could IL-2 therapy be adapted for use as a potential HAART-sparing strategy to obviate or delay the need for initiating antiretroviral treatment in HIV-infected patients? The latter prospects are indeed intriguing, and several pilot trials to address this question are either planned or presently underway.20
Acknowledgment:

The participation of the patient volunteers, the numerous contributions of the Clinic 8 nursing staff, and the ongoing scientific guidance of Dr. Anthony S. Fauci, Director NIAID, throughout the performance of these trials are acknowledged with gratitude.
### Table 1. Demographics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>SCIL2 (n=18) [ref. 4]</th>
<th>SC500 (n=53) [ref. 3]</th>
<th>MSCIL2 (n=26) [ref. 5]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study Purpose</strong></td>
<td>Determine maximally tolerated dose of sc IL-2</td>
<td>Compare sc IL-2 dosing and schedule</td>
<td>sc IL-2+ ARV vs ARV alone</td>
</tr>
<tr>
<td><strong>Study Design</strong></td>
<td>Dose escalating</td>
<td>Randomized, open label</td>
<td>Randomized, open label</td>
</tr>
<tr>
<td><strong>Formal Study Period</strong></td>
<td>12 months</td>
<td>6 months</td>
<td>12 months</td>
</tr>
<tr>
<td><strong>CD4 cell count requirement</strong></td>
<td>≥ 200 cells/microLiter</td>
<td>≥ 500 cells/microLiter</td>
<td>200 – 500 cells/microLiter</td>
</tr>
<tr>
<td><strong>% protease inhibitor use at baseline</strong></td>
<td>0%</td>
<td>15%</td>
<td>77%</td>
</tr>
<tr>
<td><strong>Definition for Inclusion in Long Term Follow-up</strong></td>
<td>&gt;18 months</td>
<td>&gt;12 months</td>
<td>&gt;18 months</td>
</tr>
<tr>
<td><strong>Number enrolled in extension phase</strong></td>
<td>14</td>
<td>43</td>
<td>20</td>
</tr>
<tr>
<td><strong>Age in years (mean)</strong></td>
<td>38</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>93% male</td>
<td>100% male</td>
<td>100% male</td>
</tr>
<tr>
<td><strong>Baseline mean CD4 cell count (%)</strong></td>
<td>371 cells/microLiter (22%)</td>
<td>655 cells/microLiter (32%)</td>
<td>425 cells/microLiter (24%)</td>
</tr>
<tr>
<td><strong>Baseline mean HIV log_{10} viral load</strong></td>
<td>4.14</td>
<td>3.51</td>
<td>3.15</td>
</tr>
<tr>
<td><strong>Total number of IL-2 cycles</strong></td>
<td>212</td>
<td>417</td>
<td>162</td>
</tr>
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</table>
**Table 2. AIDS-Defining Illnesses in the Active Study Population**

<table>
<thead>
<tr>
<th>Study</th>
<th>Date</th>
<th>CD4 (%)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCIL2</td>
<td>Nov-98</td>
<td>543 (33%)</td>
<td>Mild HIV encephalopathy, NARS 1</td>
</tr>
<tr>
<td>SC500</td>
<td>Jan-96</td>
<td>1041 (49%)</td>
<td>Cutaneous Kaposi sarcoma with multiple recurrences, first diagnosis with CD4 1082 (44%)</td>
</tr>
<tr>
<td>SCIL2</td>
<td>Apr-99</td>
<td>209 (18%)</td>
<td>Cutaneous Kaposi Sarcoma</td>
</tr>
<tr>
<td>SC500</td>
<td>Dec-99</td>
<td>1565 (46%)</td>
<td>Non-Hodgkin's lymphoma, right lower quadrant mass</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. **Patient Flow Diagram.** The course of the 97 patients originally enrolled in three separate trials of SC IL-2 is outlined.

Figure 2. **Mean CD4+ T cell count (solid line) and mean CD4% (dashed line) response over time.** Standard errors are indicated for these two parameters at each time point. The number of evaluable patients at each time point are indicated.

Figure 3. **Mean CD8+ T cell count (solid line) and mean CD8% (dashed line) response over time.** Standard errors are indicated for these two parameters at each time point. The number of evaluable patients at each time point are indicated.

Figure 4. **Panel A: Mean percentage of naïve (CD45 RO-, solid line) and memory (CD45RO+, dashed line) CD4 cell subsets over time.**
**Panel B: Mean percentage CD4/CD25+ T cells over time.** In each panel standard errors are indicated for these parameters at each time point.

Figure 5. **Changes in mean plasma HIV-1 log copies over time.** Standard errors are indicated for these two parameters at each time point. The number of evaluable patients at each time point are indicated.

Figure 6. **The mean ratio of IL-2 cycles per patient over time.** This is shown in six month intervals according to the number of IL-2 cycles for all participants (solid line) and for each individual study: scIL2 (dashed line, solid circle marker), sc500 (dashed line, solid square marker) and MscIL2 (dashed line, open triangle marker). The number of evaluable patients at each time point are indicated.
References


<table>
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<tr>
<th>Patients enrolled in three SC IL-2 Trials (1993-1997) (n=97)</th>
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<tbody>
<tr>
<td>declined participation in initial trial after enrollment (n=2)</td>
</tr>
<tr>
<td>did not complete initial trial (n=10)</td>
</tr>
<tr>
<td>elected not to participate in long-term follow-up (n=7)</td>
</tr>
<tr>
<td>never received SC IL-2 in initial trial (n=1)</td>
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<table>
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<tr>
<th>Patients initially enrolled in long-term follow-up (n=77)</th>
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<tr>
<td>patient death (n=1)</td>
</tr>
<tr>
<td>developed non-Hodgkin's lymphoma (n=1)</td>
</tr>
<tr>
<td>elected to pursue other treatment options (n=8)</td>
</tr>
<tr>
<td>experienced CD4 decline despite SC IL-2 (n=6)</td>
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<tr>
<th>Patients remaining in active long-term follow-up (2003-2004) (n=61)</th>
</tr>
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Figure 1
Figure 2

![Graph showing mean CD4 cell count and mean CD4+ percentage over months of study with error bars.](image-url)
Figure 3
Figure 4

A

Percentage CD4+/CD45+ RO Cells (%)

Month of Study

B

Mean % CD4/CD25+ T Cells

Month on Study
Figure 5
Figure 6
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