High-Dose Interferon-α2a Exerts Potent Activity against Human Immunodeficiency Virus Type 1 Not Associated with Antitumor Activity in Subjects with Kaposi’s Sarcoma

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Anti–human immunodeficiency virus type 1 (HIV-1) activity was assessed in HIV-1–infected homosexual and bisexual men receiving 18–36 MIU/day of recombinant interferon (IFN)-α2a for Kaposi’s sarcoma (KS). The median baseline HIV-1 RNA level was 4.99 log10 copies/mL. Seventeen subjects (68%) showed an RNA decline ≥ 5 log10/mL, with a maximum at week 4 (median decline = 1.91, range = 3.64–1.15; P = .0007), after which RNA levels stabilized. Eight subjects (32%) with lower median initial CD4+ T cell counts (60 vs. 350 × 106/L; P = .01) did not show RNA responses. Neither RNA nor KS responses were negatively affected by IFN-α2a dose modifications. Anti-HIV responses of KS responders (n = 15) and nonresponders (n = 10) did not differ. High-dose IFN-α can exert potent anti-HIV activity that is not associated with anti-KS activity.

Interferon (IFN)-α is a cytokine that is mainly produced by peripheral blood mononuclear cells after stimulation with various viral and nonviral stimuli. It is involved in the regulation of many cellular functions, resulting in antiviral, antitumor, and immunomodulatory activities [1]. Soon after it was discovered that human immunodeficiency virus (HIV) causes AIDS, the antiretroviral capacity of IFN-α was demonstrated in vitro [2, 3]. The mechanisms of action of IFN-α in HIV infection are not fully elucidated. An important inhibitory action of IFN-α on HIV replication is interference with assembly and budding of new HIV virions [4].

Clinical results with IFN-α for the treatment of HIV infection have been discouraging. After the demonstration in vitro of synergistic anti-HIV activity with IFN-α plus zidovudine [5], combinations of low-dose INF-α and zidovudine have been used in different clinical settings with different primary end points and viral parameters [6–9]. However, the largest of these studies showed no evidence of superior clinical benefit from combination therapy: It compared, in a randomized and controlled fashion, a combination of low-dose INF-α and zidovudine with zidovudine monotherapy in treatment-naive HIV-infected subjects [8]. These disappointing clinical results might be due to the use of low-dose IFN-α, because high-dose IFN-α monotherapy for the treatment of HIV-related Kaposi’s sarcoma (KS) has been reported to suppress HIV-1 p24 antigen and provide clinical benefit [10, 11].

Therefore, we conducted a study to explore the anti-HIV activity of high-dose recombinant IFN-α2a, using serum HIV-1 RNA quantification as the most reliable and exact viral parameter. We used stored serum samples from subjects who had participated in a KS treatment study with high-dose recombinant IFN-α monotherapy (27–36 MIU/day) in 1988; all had demonstrated clear antitumor activity and an anti-HIV response [10]. In addition, that study found evidence of an association between antitumor and anti-p24 responses, which was later confirmed by others [11].

Materials and Methods

Study population and design. Twenty-five HIV-1 seropositive homosexual or bisexual men with histologically confirmed and progressive KS showing measurable lesions were included. Additional inclusion criteria are described in detail elsewhere [10]. Clinical and laboratory assessments were conducted at four weekly intervals. The major KS response evaluation was performed at week 8. Subjects with a complete or a partial KS response or with stable KS disease were called KS responders; subjects with progressive disease were called KS nonresponders. Stable disease was categorized as a KS response because an important inclusion criterion was the presence of progressive KS (i.e., new or growing lesions). KS responses were defined according to guidelines described in detail elsewhere [10]. Subjects could not have received any previous treatment for HIV infection and were not allowed to use any known antiretrovirals or immunomodulatory agents during the study. For the purpose of the current study, serum samples up to 12 weeks after initiation of IFN-α2a therapy were available for
quantitation of HIV RNA: Patients received IFN-α2a daily during the first 8 weeks; during the subsequent 4 weeks, regimens were modified according to the KS response at week 8. An HIV RNA response was defined as a decrease from baseline of at least $0.5 \log_{10}$ copies/mL at two consecutive time points (or for at least 1 month).

**Intervention.** The IFN-α used in this study was recombinant human IFN-α2a (Roferon; Roche, Basel, Switzerland) and was self-administered by subcutaneous injection. For days 1–5, subjects received doses that increased daily by stepwise increments (i.e., 3, 9, 18, 27, and 36 MIU, respectively). Thereafter, for the first 8 weeks, subjects received 36 MIU/day if their body weight was $\geq 60$ kg and 27 MIU if it was $<60$ kg. After 8 weeks, IFN-α2a was administered every other day to patients with a partial KS response or stable disease. For those with a complete KS response or progressive disease, treatment was discontinued.

**HIV-1 RNA quantification.** Serum was stored at $-70^\circ$C. Serum HIV-1 RNA was quantitated in once-thawed serum, using nucleic acid–based sequence amplification (Organon Teknika, Boxtel, Netherlands) [12]. The variation of quantitative results is within $0.5 \log_{10}$ copies/mL. The lower limit at which RNA can be reliably quantified is 1000 copies/mL ($3 \log_{10}$ copies/mL).

**T cell subpopulations and HIV-1 p24 antigen.** Peripheral blood CD4$^+$ and CD8$^+$ T lymphocyte subpopulations were enumerated using dual-color immunofluorescence and flow cytometry. Serum HIV-1 p24 antigen was measured by a sandwich-type ELISA (Abbott Laboratories, Abbott Park, IL).

**Statistics.** Laboratory values for CD4$^+$ cells and RNA were not normally distributed, partially due to censoring of the data (RNA). HIV-1 RNA results were log-transformed, and values below the lower quantification limit were considered to contain $3 \log_{10}$ RNA copies/mL. Baseline laboratory values are the means of two pretreatment values (one assessment within 4–8 weeks before entry and one at the start of treatment). Fifteen subjects (60%) had only one pretreatment RNA value available. Comparisons between groups were conducted at weeks 0, 4, and 8, using the Mann-Whitney U test. The HIV RNA response over time in the total study population was tested by the Wilcoxon signed rank test for paired observations. Comparisons of the proportion of either HIV-1 RNA or KS responders and nonresponders stratified by CD4$^+$ cell counts below or above $200 \times 10^6$/L were tested by the $\chi^2$ test. The correlation between serum HIV-1 RNA and p24 antigen concentrations was tested by Spearman’s rank correlation coefficient. All tests were two-sided, and significance was set at $P < .05$.

**Results**

**Study population.** At baseline, the median CD4$^+$ cell count was $280 \times 10^6$/L (range = 10–670 $\times 10^6$), and the median serum HIV-1 RNA concentration was $4.99 \log_{10}$ copies/mL. Three subjects with only one baseline serum sample available had HIV-1 RNA concentrations below the lower quantification limit of the RNA assay. In the 14 subjects (56%) with p24 antigenemia ($\geq 30$ pg/mL), no correlation between serum HIV-1 RNA and p24 antigen concentrations was found at baseline or at any analyzed time point thereafter (data not shown).

**HIV-1 RNA response.** In all subjects, the maximum change in serum HIV-1 RNA concentrations during IFN-α2a treatment was observed at week 4 (median = $-0.79 \log_{10}$/mL, range = $-3.64$ to $+2.62; P = .001$). However, this group contained fewer RNA responders ($n = 17; 68\%)$ as well as nonresponders ($n = 8; 32\%$). Seventeen HIV RNA responders showed RNA declines of $\geq 1 \log_{10}$ copies/mL, whereas the 8 RNA nonresponders showed clear increases relative to baseline (median RNA increase = 1.3 and $1.5 \log_{10}$/mL at weeks 4 and 8, respectively). The RNA responders showed a median RNA decrease from baseline of $1.91 \log_{10}$/mL ($P = .0007$) at week 4 and $1.78 \log_{10}$/mL ($P = .005$) at week 8 (figure 1). For 5 subjects (20%) at week 4 and 4 subjects (21%) at week 8 after the start of IFN-α2a therapy, serum HIV-1 RNA levels were below the quantification limit of the assay ($3 \log_{10}$/mL).

Comparison of the baseline characteristics of the HIV RNA responders and nonresponders revealed that RNA nonresponders had significantly lower median CD4$^+$ T cell numbers than did the responders (60 vs. $350 \times 10^6$/L; $P = .01$). Five of the RNA responders (29%) versus 6 of the RNA nonresponders (75%) had baseline CD4$^+$ cell counts of $< 200 \times 10^6$/L, whereas 12 RNA responders (71%) versus 2 nonresponders (25%) had baseline CD4$^+$ cell counts of $\geq 200 \times 10^6$/L ($P = .032$). Other baseline variables, such as age, p24 antigenemia, KS response, and RNA concentrations did not show differences (data not shown). During IFN-α2a therapy, CD4$^+$ cell responses (percentages and numbers) relative to baseline responses did not show different patterns between patient response groups at any analyzed time point (data not shown). The RNA nonresponder group included 2 of 3 subjects with censored baseline RNA levels of $3 \log_{10}$ copies/mL whose RNA concentrations became detectable during therapy.

**KS response.** Fifteen subjects (60%) showed a KS response, whereas 10 subjects (40%) showed progressive KS disease. At baseline, mean age (35.2 vs. 39.7 years) and median baseline CD4$^+$ cell counts (230 vs. $350 \times 10^6$/L) were lower.

**Figure 1.** Serum levels of HIV RNA relative to baseline levels in patients whose RNA levels declined in response to treatment with IFN-α2a.
in the group of KS nonresponders than in the responders, but the difference was not significant. All subjects with HIV-1 RNA concentrations below the assay quantification limit at any time point belonged to the group of KS responders. RNA concentrations did not show different patterns between KS responders and nonresponders during the first 8 weeks of daily IFN-α2a administration: at week 4, the median RNA concentration relative to baseline was $-1.91 \log_{10} \text{copies/mL}$ ($-3.64$ to $+2.62$) versus $-1.47 \log_{10} \text{copies/mL}$ ($-2.28$ to $+1.9$); at week 8, the median RNA concentration relative to baseline was $-1.41 \log_{10} \text{copies/mL}$ ($-3.64$ to $+2.33$) versus $-1.48 \log_{10} \text{copies/mL}$ ($-1.92$ to $+1.65$), respectively.

Within the group of KS responders, RNA responses of subjects with either complete ($n = 2$) or partial response ($n = 8$) or with stable disease ($n = 5$) were indistinguishable by RNA response (data not shown). At weeks 4 and 8, median CD4$^+$ T cell counts relative to baseline were higher in the KS responders than in the nonresponders, although these differences did not reach significance ($+10$ vs. $-40 \times 10^3/\mu\text{L}$; $P = .09$ at week 4; $0$ vs. $-40 \times 10^3/\mu\text{L}$; $P = .14$ at week 8). Concomitant KS and RNA responses were found in 10 subjects (40%), whereas a combined KS and RNA nonresponse was observed in 3 (12%). All other subjects ($n = 12; 48\%$) had divergent KS and RNA responses (table 1).

**Dose-response relation.** HIV RNA and KS responses in relation to the dose of IFN-α2a are listed in table 1. Full-dose therapy was considered to be treatment with 36 MIU IFN-α2a daily, whereas all other dosages were considered to be a reduced IFN-α2a dose. Five subjects (20%) had dose modifications before week 8. Of them, 4 had toxicity-related dose reductions to 18 MIU daily and 1 had a short-lasting treatment interruption. Table 1 shows that neither the HIV RNA response nor the KS response seems to be affected by a reduced IFN-α2a dose.

After the KS response evaluation at week 8, treatment was stopped for 12 subjects because of progressive KS ($n = 8$) or personal request, mainly because of side effects ($n = 4$). Thirteen subjects (52%), including 2 with progressive KS (protocol violation), continued to use IFN-α2a every other day. Twelve of these subjects had serum samples available at week 12. Between week 8 and 12, RNA increased ($>5 \log_{10} \text{copies/mL}$) in 4 subjects (33%), including both subjects with progressive KS, whereas RNA declined ($>5 \log_{10} \text{copies/mL}$) in 2 subjects (17%). In the other subjects ($n = 6; 50\%$), changes in RNA levels were within $5 \log_{10} \text{copies/mL}$.

**Discussion**

In the present study, high-dose recombinant IFN-α2a exerted potent anti-HIV activity in a substantial subgroup of subjects treated with the agent for AIDS-related KS. A previous study established the anti-HIV activity of IFN-α alone, as measured by serum HIV-1 RNA concentrations [13]. However, direct comparison of the two studies is hampered by differences in study designs, IFN-α preparations (natural human IFN-α or IFN-α3, consisting of a mixture of different IFN-α species), and clinical parameters of the study populations (disease stage, CD4$^+$ cell counts, and HIV-1 RNA levels). Despite the present results, it should be emphasized that IFN-α2a exhibited no anti-HIV response in 32% of our subjects. This group of nonresponders might be slightly greater, considering that no serum samples were available at week 8 for 6 subjects. However, these subjects were considered to be RNA responders because of unequivocal RNA declines at week 4. In addition, only 1 of 8 RNA nonresponders showed a biphasic RNA response. Although the number of RNA nonresponders is small, the increase of HIV-1 RNA levels in some of these subjects could not be explained by noncompliance or intercurrent infections. The only significant difference between the RNA responders and nonresponders was the lower initial CD4$^+$ cell counts than KS nonresponders; however the difference was not significant, probably due to the small sample size. In various studies, CD4$^+$ cell counts $>200 \times 10^3/\mu\text{L}$ have been associated with favorable KS responses to IFN-α [10, 11] or suppression of serum HIV p24 antigen levels (or both) [11, 14]. Adequate anti-HIV and anti-KS activities of IFN-α appear to require a certain minimum level of cellular immunity.

To date, the minimal effective dose of IFN-α in HIV infection is still unclear. Within the dose range of the current study ($\geq 18$ MIU/day up to week 8) no dose-effect relationship could be demonstrated, possibly due to the small sample size. One study using a new natural IFN-α at different dose levels (1, 5, and 12.5–20 MIU three times a week) showed a dose-dependent anti-HIV effect [13]. In the highest dose group, 3 of 5 subjects had $>2.41 \log_{10} \text{copies/mL}$ RNA decline, whereas the decline of serum RNA concentrations was $<1 \log_{10} \text{copies/mL}$ with the two lower dose levels. Another study, using HIV p24 antigen as the viral parameter, demonstrated a dose-dependent p24 antigen suppression even with the use of low-dose IFN-α (2–6 MIU/day) [9].

**Table 1.** Distribution of 25 study subjects given different doses of IFN-α2a for treatment of Kaposi’s sarcoma (KS), according to size of dose received, HIV RNA response, and KS response.

<table>
<thead>
<tr>
<th>Dose size, KS response</th>
<th>RNA response</th>
<th>No RNA response</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 MIU/day ($n = 18$)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>KS responder</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>KS nonresponder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–27 MIU/day* ($n = 7$)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>KS responder</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of subjects. KS responders = subjects with complete or partial KS response or with stable KS disease; KS nonresponders = subjects with progressive disease. HIV RNA response was defined as decrease from baseline of at least $5 \log_{10} \text{copies/mL}$ at 2 consecutive time points (or for at least 1 month).

* 27 MIU was weight-adjusted dose (<60 kg).
An important observation in the current study was the lack of an association between the anti-HIV and anti-KS responses: Previous reports on IFN-α therapy in KS had suggested an association between the two responses [10, 11]; however, the anti-HIV response in those studies was assessed by the reduction of serum HIV p24 antigen levels. We and others [15] were unable to demonstrate a correlation between serum HIV p24 antigen and RNA concentrations. The anti-KS activity of IFN-α might be associated with inhibition of KS-associated herpes-like virus (KSHV). Development of viral quantitation techniques for KSHV might elucidate this question.

In conclusion, high daily doses of IFN-α can exert potent antiretroviral activity. Low CD4+ T cell counts seem to impair both the anti-HIV and the anti-KS response. These responses to IFN-α show no association. Despite disappointing previous clinical data from treatment with low-dose IFN-α, it is too early to completely abandon IFN-α for the treatment of HIV-infected subjects with moderate immune impairment. The dose and type of IFN-α with the best ratio between activity and toxicity, either in monotherapy or combination therapy, and the factors interfering with the response to IFN-α remain to be established.

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References