MAJOR ARTICLE

Safety, Tolerability, and Mechanisms of Antiretroviral Activity of Pegylated Interferon Alfa-2a in HIV-1–Monoinfected Participants: A Phase II Clinical Trial

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Background. To our knowledge, the antiviral activity of pegylated interferon alfa-2a has not been studied in participants with untreated human immunodeficiency virus type 1 (HIV-1) infection but without chronic hepatitis C virus (HCV) infection.

Methods. Untreated HIV-1–infected volunteers without HCV infection received 180 μg of pegylated interferon alfa-2a weekly for 12 weeks. Changes in plasma HIV-1 RNA load, $CD4^+$ T cell counts, pharmacokinetics, pharmacodynamic measurements of 2′,5′-oligoadenylate synthetase (OAS) activity, and induction levels of interferon-inducible genes (IFIGs) were measured. Nonparametric statistical analysis was performed.

Results. Eleven participants completed 12 weeks of therapy. The median plasma viral load decrease and change in $CD4^+$ T cell counts at week 12 were 0.61 $\log_{10}$ copies/mL (90% confidence interval [CI], 0.20–1.18 $\log_{10}$ copies/mL) and −44 cells/μL (90% CI, −95 to 85 cells/μL), respectively. There was no correlation between plasma viral load decreases and concurrent pegylated interferon plasma concentrations. However, participants with larger increases in OAS level exhibited greater decreases in plasma viral load at weeks 1 and 2 ($r = -0.75$ [90% CI, −0.93 to −0.28] and $r = -0.61$ [90% CI, −0.87 to −0.09], respectively; estimated Spearman rank correlation). Participants with higher baseline IFIG levels had smaller week 12 decreases in plasma viral load (0.66 $\log_{10}$ copies/mL [90% CI, 0.06–0.91 $\log_{10}$ copies/mL]), whereas those with larger IFIG induction levels exhibited larger decreases in plasma viral load (−0.74 $\log_{10}$ copies/mL [90% CI, −0.93 to −0.21 $\log_{10}$ copies/mL]).

Conclusion. Pegylated interferon alfa-2a was well tolerated and exhibited statistically significant anti–HIV-1 activity in HIV-1–monoinfected patients. The anti–HIV-1 effect correlated with OAS protein levels (weeks 1 and 2) and IFIG induction levels (week 12) but not with pegylated interferon concentrations.

Trial registration. ClinicalTrials.gov identifier: NCT00078442.

Interferon α is produced predominantly by B lymphocytes, null lymphocytes, macrophages, and dendritic cells after exposure to foreign eukaryotic, tumor, or virus-infected cells. Interferons have potent and diverse immunoregulatory effects, which include induction of other cytokines, activation of macrophages and dendritic cells, augmentation of natural killer cell cytotoxicity, antibody-dependent cellular cytotoxicity and T

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cell cytotoxicity, and alterations of cell trafficking [1–3]. Studies of both RNA and DNA viruses indicate that inhibition of translation and virion assembly appears to be the principal mode of the antiviral effects of interferons [4–6]. Several enzyme systems that are induced by interferon (interferon-inducible genes [IFIGs]) have been shown to interfere with viral replication. These include, among others, 2′,5′-oligoadenylate synthetase (OAS), which catalyzes the synthesis of oligonucleotides that activate the endoribonuclease RNAse L, which in turn produces cleavage of viral RNA.

Interferon α inhibits both early human immunodeficiency virus type 1 (HIV-1) replication and integration [7] and late-stage assembly and packaging of viral particles [8]. Over 30 different IFIGs have been implicated as playing a potential role in the inhibition of various viruses [5, 6, 9, 10]. Measurement of IFIGs has provided insight into the antiviral effects of interferon therapy in the setting of treatment of hepatitis C virus (HCV) infection and may play a role in predicting response to treatment [11].

Pegylated interferon alfa-2a (peginterferon alfa-2a) is a commercial preparation of recombinant interferon alfa-2a covalently attached to a branched mobile 40-kDa polyethylene glycol moiety, which inhibits enzymatic degradation of interferon alfa-2a and allows for weekly administration. The pegylation increases the half-life of interferon alfa-2a for a sustained virological response, compared with nonpegylated interferon alfa-2a [12, 13]. Peginterferon alfa-2a is approved for the treatment of HCV and hepatitis B virus (HBV) infections and has a more favorable pharmacokinetic and safety profile than those of previously available interferon alfa formulations [14, 15]. Pegylated interferon alfa-2b and peginterferon alfa-2a have only been tested for the treatment of HIV-1 infection in the setting of acute HIV-1 infection in conjunction with highly active antiretroviral therapy [16, 17]. This study was therefore undertaken to test the antiviral activity, safety, and tolerability of peginterferon alfa-2a (Pegasys) in HCV-uninfected, HIV-1–infected volunteers who are not currently receiving antiretroviral therapy.

MATERIALS AND METHODS

Participants were eligible to enroll in the AIDS Clinical Trials Group Protocol 5192 if they had a $CD4^+$ T cell count of ⩾300 cells/μL, had a plasma HIV-1 RNA load of ⩾5000 copies/mL, and were antiretroviral therapy–naive or were antiretroviral therapy–experienced but currently not receiving therapy for at least 12 weeks. The patients must have tested negative for HBV surface antigen and HCV antibody and have had transaminase levels of grade <1 at entry. Exclusion criteria included a history of severe psychiatric illness or any history of a chronic illness, such as a cardiopulmonary disorder, that could be worsened by interferon therapy or its known toxicities. All participants expressed a willingness to defer initiation (or reinitiation) of antiretroviral therapy until after the completion of the study, although a safety clause for withdrawal from the study for a $CD4^+$ T cell count of ⩽200 cells/μL was stipulated in the toxicity management section of the protocol. Filgrastim (Neupogen; provided by Amgen), a granulocyte colony-stimulating factor analogue, was available for the treatment of neutropenia through the study for providers to use according to local standard-of-care practices. Written informed consent was obtained from all participants.

The primary end points were change in plasma HIV-1 RNA load from baseline to week 12 and safety and tolerability of peginterferon alfa-2a (provided by Roche Pharmaceuticals) at 180 μg given subcutaneously weekly by study personnel for 12 weeks. A post hoc decision was made to include analyses at weeks 1 and 2, when the largest decreases in HIV-1 RNA load were observed. Secondary objectives included assessment of HIV-1–specific $CD4^+$ T cell immunity while receiving treatment, compared with baseline levels, by measurement of lymphocyte proliferation response to p24Ag and whole inactivated HIV-1 antigen; measurement of weekly and end-of-study serum trough levels of peginterferon alfa-2a and OAS; durability of the virological and immunological responses to peginterferon alfa-2a therapy 6 weeks after discontinuation of the study drug (week 18); and correlations among baseline and concurrent week-specific changes in plasma HIV-1 RNA load, $CD4^+$ T cell count, and OAS level and concurrent absolute concentrations of peginterferon alfa-2a and OAS. Post hoc examinations of the effect of missed doses on viral load change were performed. After the correlations between OAS level and HIV-1 RNA load changes were observed, a substudy was designed to explore the induction of IFIGs.

Viral load measurements were performed by means of the Amplicor HIV-1 Monitor polymerase chain reaction assay (Roche Diagnostics) before entry, at entry, and at weeks 1, 2, 3, 4, 6, 8, 10, and 12 just prior to each weekly injection of study drug, as well as at weeks 13 and 18 (2 and 7 weeks after therapy had been discontinued). Except at week 1, $CD4^+$ T cell counts were measured at the same times.

Conventional lymphocyte proliferation assays were performed on freshly obtained peripheral blood mononuclear cells within 24 h of collection by use of the following stimulants: tetanus toxin, *Candida*, phytohemaglutinin, HIV-1 p24 antigen, and whole inactivated HIV-1 antigen. The lymphocyte proliferation response is described in terms of the stimulation index, which is defined as the median counts per minute in the stimulated replicates divided by the median counts per minute in the appropriate control replicates. If an observed stimulation index was <1, a value of 1 was imputed for the analyses. If the phytohemaglutinin (positive control) stimulation index was <5, then the lymphocyte proliferation response panel was excluded from the analysis.

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Table 1. Demographics and Baseline Clinical Characteristics of Human Immunodeficiency Virus Type 1 (HIV-1)–Infected Study Participants

Participant ID	Sex	Age, years	Race/ethnicity	$CD4^+$ T cell count, cells/μL (%)	HIV-1 RNA load, $\log_{10}$ copies/mL
1	Male	62	Black non-Hispanic	357 (30.5)	4.47
2	Male	35	Black non-Hispanic	367.5 (35.5)	4.37
3	Male	45	White non-Hispanic	480 (23.5)	4.49
4	Male	37	White non-Hispanic	929.5 (27)	4.10
5	Male	46	White non-Hispanic	650 (33.5)	3.57
6	Male	37	Black non-Hispanic	372 (30.5)	4.39
7	Female	36	White non-Hispanic	510 (21.5)	4.56
8	Male	42	White non-Hispanic	325.5 (13)	4.72
9	Male	30	Black non-Hispanic	298 (16)	3.86
10	Male	32	Black non-Hispanic	612 (27.5)	3.84
11	Male	45	American Indian/Alaska Native	359.5 (26)	4.76
Median	…	37	…	372 (27)	4.39

Peginterferon alfa-2a concentrations were measured by Quest Pharmaceutical Services (Newark, DE) by means of an enzyme-linked immunosorbent assay that has been used elsewhere [18], with lower limits of quantification of 0.250 ng/mL for interferon alfa. The assay is specific for pegylated interferon and does not recognize nonpegylated (endogenous) interferon up to a concentration of 25 ng/mL. Posttreatment interferon levels that were reported as being below the lower limit of quantification (0.250 ng/mL) were assigned a value 0.125 ng/mL (one-half the lower limit of quantification). The mean steady-state trough interferon concentration was calculated as the mean of the concentrations at weeks 6, 8, 10, and 12 (a priori definition). For participants for whom peginterferon alfa-2a concentrations were available at week 0 and at least 1 other week, the area under the pegylated interferon trough concentration–time curve (AUC) was calculated from week 0 to week 12 by use of the linear trapezoidal rule as an estimate of total drug exposure after multiple weekly doses. Peginterferon alfa-2a clearance was calculated by assuming complete absorption from subcutaneous injections as the dosing rate (135 μg/week for participants with reduced doses; 180 μg/week for all other participants) divided by mean steady-state trough concentration, divided by 168 to obtain the value in units of liters per hour. Weight-adjusted clearance was calculated as clearance divided by the participant's pretreatment weight (in kilograms).

The activity level of OAS in serum was measured in duplicate by Quest Pharmaceutical Services by use of a radioimmunoassay kit (obtained from Eiken Chemical and distributed by Alpco Diagnostic) that measures the amount of adenosine triphosphate converted into oligoadenylate. OAS levels that were reported as undetectable (<10 pmol/dL) were assigned a value of 5 pmol/dL. Three distinct isoforms of OAS exist in human cells: small, medium and large. On the basis of the principle of the radioimmunoassay used, it was assumed that this assay measured the total activity level of OAS.

Peripheral blood mononuclear cells stored at weeks 0, 3, 6, 12, and 18 were used to measure IFIG levels. Quantitation was performed using a novel customized branched DNA multiplex assay capable of detecting the expression of 35 genes [11]. The expression level of individual genes was measured in relation to that of housekeeping genes—that is, in a 1:1 relationship. The IFIG levels reported herein represent the average mean fluorescence intensity across the individual measured genes.

This study was designed to provide 80% power (with 12 evaluable participants) to detect a change in viral load of 0.56 $\log_{10}$ copies/mL at week 12, assuming use of a 1-sided t test with α set to 5% and a standard deviation of 0.88 $\log_{10}$. After the study was designed, the decision was made to use nonparametric methods and 2-sided confidence intervals (CIs) with a significance level of 0.10 without adjustments for multiple testing. Continuous measures are summarized by medians and associated 90% CIs. Rank-based Spearman correlations, adjusted for bias using the Fisher z transformation, assessed associations between continuous variables. The baseline viral load was defined as the mean of preentry and entry levels.

RESULTS

Thirteen HIV-1–infected volunteers enrolled. Two participants discontinued therapy early in the course of the trial, one because of a central nervous system lymphoma that in retrospect was present prior to enrollment and the other because of travel difficulties; these 2 participants were included only in the safety analysis. The remaining 11 participants, for whom Table 1 presents baseline characteristics, completed 12 weeks of weekly peginterferon alfa-2a injections and 2 posttreatment follow-up visits to 6 weeks after treatment.

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Safety and tolerability. The treatment was generally well tolerated, with only 1 participant experiencing grade 2 treatment-related depression. The most common treatment-related (or possibly treatment-related) adverse events were grade 1 or 2 absolute neutrophil count decreases (11 [85%] of 13 participants) and fatigue (6 [46%] of 13 participants). There were 3 cases of grade 3 treatment-related toxicity: fatigue in 1 participant and decreased absolute neutrophil count in 2 participants. Fatigue was also experienced by 5 other participants: 4 with grade 2 fatigue and 1 with grade 1 fatigue reported at some point during the treatment period. Filgrastim (1 dose) was administered to 1 of the participants who experienced toxicity, and all 3 participants who experienced toxicity completed the trial at reduced doses according to protocol dosage-adjustment schemes (Figure 1*A*–1*B*). Overall, the median (first quartile, third quartile) change from week 0 to week 12 for white blood cell count, absolute neutrophil count, and platelet count was -2.0×10^3 (-2.6×10^3, -0.8×10^3) cells/μL, -1345 (1870, -372) cells/μL, and -64×10^3 (79×10^3, -28×10^3) cells/μL, respectively.

Virological responses. HIV-1 RNA load changes from baseline ranged from a decrease of 1.47 to an increase of 0.20 $\log_{10}$ copies/mL at week 1, from -1.82 to -0.18 $\log_{10}$ copies/mL at week 2, and from -1.58 to $+0.03$ $\log_{10}$ copies/mL at week 12 (Figure 1*A*–1*B*). The largest median decrease from baseline was at week 2 (change in viral load, -1.30 $\log_{10}$ copies/mL [90% CI, -1.75 to -0.58 $\log_{10}$ copies/mL]). At the primary end point at week 12, the median change in viral load was -0.61 $\log_{10}$ copies/mL (90% CI, -1.18 to -0.20 $\log_{10}$ copies/mL). There were statistically significant decreases from baseline to each of weeks 1–13 (all CIs excluded a value of 0); however, at week 18, the viral load change from baseline was not statistically significant (90% CI, -0.10 to 0.50 $\log_{10}$ copies/mL).

Correlations between preentry viral load and changes in viral load from entry to weeks 1, 2, and 12 were not statistically significant. Correlations between viral load changes at weeks 1, 2, and 12 and baseline $CD4^+$ T cell count (Figure 4*B*) and baseline OAS level (data not shown) were also not statistically significant.

Immunological responses. Changes in $CD4^+$ T cell count from baseline were not statistically significant except for small increases at weeks 2 and 4 (week 2 median change in $CD4^+$ T cell count, 37.8 cells/μL [90% CI, 28–76 cells/μL]; week 4 median change in $CD4^+$ T cell count, 13.5 cells/μL [90% CI, 7–57 cells/μL]) (Figure 1*C*). At week 12, the median change in $CD4^+$ T cell count from baseline was -44 cells/μL (90% CI, -95 to 85 cells/μL). Most participants had $CD4^+$ percent increases from baseline while receiving treatment, and the increases were statistically significant at week 4 (median change, 4.5% [90% CI, 2.5%–5.5%]) and week 12 (median change, 1.5% [90% CI, 1.0%–4.0%]). There was no evidence of a relationship between the corresponding $CD4^+$ T cell count change and viral load change from baseline to week 2 ($r = -0.21$ [90% CI, -0.68 to 0.39]) or from baseline to week 12 ($r = 0.23$ [90% CI, -0.34 to 0.67]).

There was no statistically significant association between baseline $CD4^+$ T cell count and change in viral load from baseline to week 12 ($r = -0.40$ [90% CI, -0.76 to 0.16]) (Figure 4*B*) or between baseline $CD4^+$ T cell count and baseline viral load ($r = -0.41$ [90% CI, -0.77 to 0.15]). In addition, there was no statistically significant association between change in $CD4^+$ T cell count (from week 0 to week 12) with either absolute week 12 peginterferon alfa-2a level ($r = -0.13$ [90% CI, -0.61 to 0.42]) or AUC from week 0 to week 12 ($r = -0.10$ [90% CI, -0.59 to 0.45]).

The lymphocyte proliferation responses to all antigens tested during and after treatment were not different from the baseline levels (data not shown).

Figure 2*A* provides an overview of the study findings, showing median week-specific peginterferon alfa-2a concentrations and (scaled) median changes in OAS level, IFIG expression level, and plasma HIV-1 RNA load by study week. This graph demonstrates the relationships between these parameters and emphasizes the pharmacokinetic and pharmacodynamic effects that accompany the administration of peginterferon alfa-2a. The observed increases in IFIG levels with peginterferon alfa-2a dosing are expected, but they further confirm the appropriateness of the selected genes in the panel of IFIGs.

Pharmacokinetics. Peginterferon alfa-2a trough concentrations increased rapidly and remained elevated during treatment (through week 12) (Figure 2*A*). Steady state was achieved by week 6 and was maintained during the remainder of the treatment period. Weekly trough peginterferon alfa-2a levels were consistently stable in each participant after reaching steady state. At week 12, peginterferon alfa-2a trough concentrations were 10.2–27.5 ng/mL, and the coefficient of variation was 25%. Table 2 summarizes the week-specific peginterferon alfa-2a concentrations, the derived pharmacokinetic parameters, the AUC, and the estimated systemic clearance at steady state.

There was no correlation between viral load decreases and concurrent peginterferon alfa-2a concentrations for weeks 1, 2, and 12 or between AUC and viral load at weeks 1, 2, or 12. Three participants (2, 6, and 11) did not take full doses (Figure 1*B*, *red dashed lines*; Figure 3, *gray triangles*). The total number of doses administered to participants 2, 6, and 11 was 9.25 (ie, 4 doses of 180 μg followed by 1 dose being held and 7 doses of 135 μg), 10.25, and 10.75 of a possible 12. Although the participants whose dose of peginterferon alfa-2a was reduced were among those with the lowest viral load decreases, both their absolute peginterferon alfa-2a concentrations at week 12 (Figure 3*A*) and their AUCs (Figure 3*B*) covered the spectrum of values observed among study participants.

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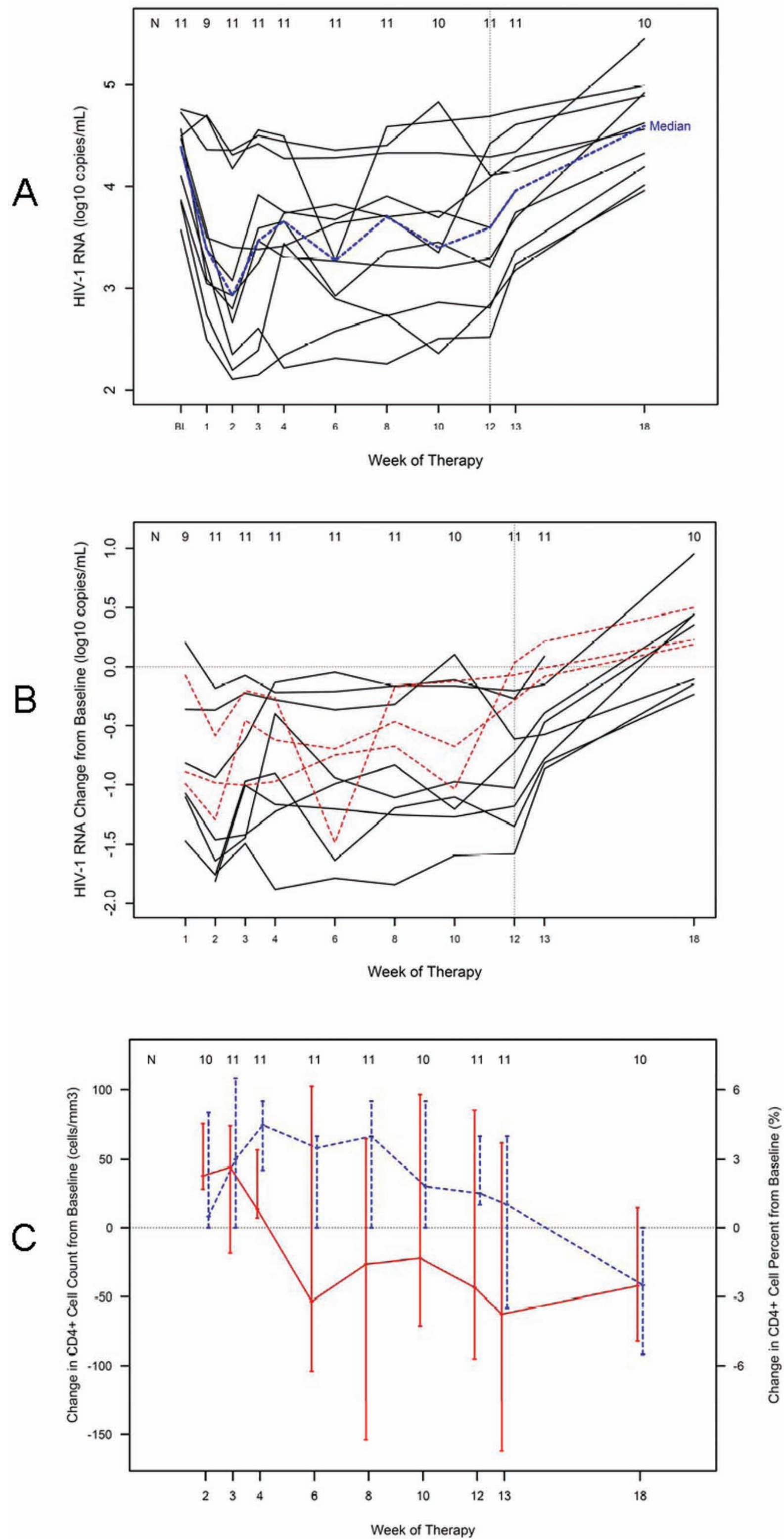

Figure 1. *A*, Plasma human immunodeficiency virus type 1 (HIV-1) RNA load of study participants receiving pegylated interferon alfa-2a. Black lines show the participant-specific trajectories, and the blue line shows the median values. The vertical line shows the study week at which the final injection of pegylated interferon occurred. The number of participants with available data for each week is shown at the top of the graph. *B*, Plasma HIV-1 RNA load changes from baseline. Red lines show the trajectories of participant-specific changes in 3 participants with reduced dosing, and black lines show those in all other participants. The horizontal line at 0 indicates no change from baseline; the vertical line indicates the final weekly injection of the study drug. *C*, Median changes in $CD4^+$ T cell count (*solid red lines*) and percent (*dashed blue lines*) by study week. Error bars represent 90% confidence intervals.

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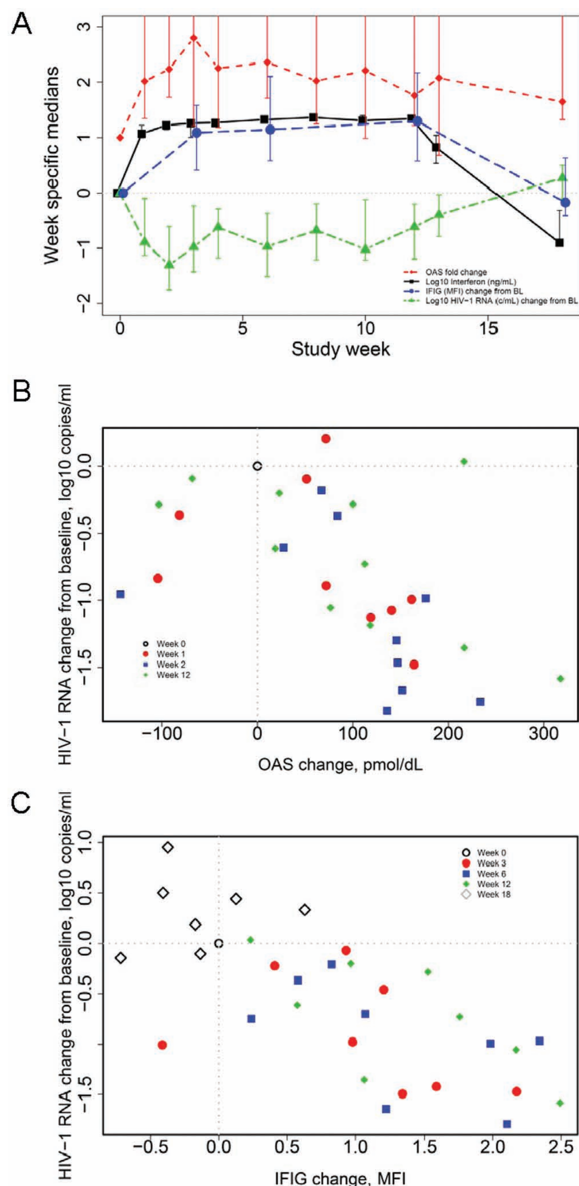

Figure 2. *A*, Median interferon concentration, median 2′,5′-oligoadenylate synthetase (OAS) fold change from baseline, median interferon-inducible gene (IFIG) level change from baseline (measured as mean fluorescence intensity [MFI]), and median plasma human immunodeficiency virus type 1 (HIV-1) RNA load change from baseline plotted against study week. Error bars represent 90% confidence intervals (CIs). The *y*-axis is truncated at 2.5. The CIs for OAS fold change are as follows: week 1, 1.3–4.8; week 2, 1.7–8.0; week 3, 1.2–5.7; week 4, 1.2–5.0; week 6, 1.7–4.4; week 8, 1.2–7.7; week 10, 1.0–7.7; week 12, 1.2–4.5; week 13, 0.7–3.9; and week 18, 1.3–3.4. *B*, Scatterplot of participant-specific changes in viral load against concurrent changes in OAS protein level at week 0, week 1 (r = –0.75 [90% CI, −0.93 to −0.28]), week 2 (r = −0.61 [90% CI, −0.87 to −0.09]), and week 12 (r = −0.51 [90% CI, −0.81 to 0.02]; estimated Spearman rank correlations). *C*, Scatterplot of participant-specific changes in viral load against changes in IFIG level at week 0, week 3 (r = −0.62 [90% CI, −0.90 to 0.02]), week 6 (r = −0.64 [90% CI, −0.90 to −0.02]), week 12 (r = −0.74 [90% CI, −0.93 to −0.21]), and week 18 (r = 0.07 [−0.64 to 0.71]; estimated Spearman rank correlations).

Pharmacodynamic responses. Pretreatment OAS concentrations were 14.9–588.9 pmol/dL; the coefficient of variation was 59% when a single outlier was excluded. Figure 2*A* shows OAS concentrations increasing until week 3 and remaining elevated during treatment. Participant-specific means over weeks 6–12 were 112–470 pmol/dl (coefficient of variation, 48%; data not shown). At week 12, OAS concentrations were 79.8–485.6 pmol/dL. OAS levels exhibited larger variability than did peginterferon alfa-2a concentrations; the coefficient of variation at week 12 was 57%. Changes in OAS were not statistically significantly correlated with concurrent peginterferon alfa-2a concentrations at weeks 1, 2, or 12.

Baseline OAS levels and baseline viral loads or $CD4^+$ T cell counts were not statistically significantly correlated; however, at weeks 1 and 2, participants with larger increases in OAS level tended to have larger decreases in viral load (week 1 $r = -0.75$ [90% CI, −0.93 to −0.28]; week 2 $r = -0.61$ [90% CI, −0.87 to −0.09]), whereas the correlation at week 12 was not statistically significant (Figure 2*B*). Participants with higher baseline $CD4^+$ T cell count tended to have larger increases in OAS level (week 12 $r = 0.63$ [90% CI, 0.15–0.87]; week 1 and 2 correlations were also statistically significant) (Figure 4*A*).

Correlations between viral load changes and IFIG level changes from baseline are shown in Figure 2*C*; a consistent relationship was observed between level of IFIG induction and change in viral load over the treatment period. At weeks 6 and 12, correlations between decrease in viral load and level of IFIG induction were statistically significant.

Baseline IFIG levels were also highly predictive of changes in viral load at weeks 2 and 12 (but not at week 1): participants with higher IFIG expression levels prior to therapy showed poorer responses to therapy. At both weeks 2 and 12, the estimated correlations between baseline IFIG levels and viral load changes were 0.66 (90% CI, 0.06–0.91). (The estimated correlation between baseline IFIG level and week 1 viral load change was also large [$r = 0.65$], but it did not reach statistical significance [90% CI, −0.05 to 0.92].) Thus, participants with higher baseline IFIG levels tended to have either increases or smaller decreases in viral load, as was observed for OAS level.

Baseline IFIG levels were inversely correlated with baseline $CD4^+$ T cell counts (r = –0.81 [90% CI, −0.95 to −0.38]) (Figure 4*C*). Changes in IFIG levels at week 12 were positively correlated with baseline $CD4^+$ T cell counts ($r = 0.76$ [90% CI, 0.26–0.94]) Figure 4*D*).

DISCUSSION

To our knowledge, this clinical trial represents the first study of peginterferon alfa-2a monotherapy for the treatment of HIV-1 infection in the absence of chronic viral hepatitis or opportunistic malignancies. After 12 weeks of treatment, participants experienced a median decrease in HIV-1 RNA level of 0.61 $\log_{10}$

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Table 2. Statistical Summary of Pharmacokinetic and Pharmacodynamic Parameters of Pegylated Interferon Alfa-2a

Parameter, week(s)	Median (90% CI)	CV, %	Range
Interferon concentration, ng/mL			
1 ($n = 10$)	11.80 (9.94–16.60)	28	8.52–19.20
2 ($n = 11$)	16.70 (13.80–19.40)	32	3.84–21.20
3 ($n = 11$)	18.10 (10.10–20.40)	42	2.96–27.50
6 ($n = 11$)	21.00 (19.50–23.40)	22	9.95–27.80
12 ($n = 11$)	21.90 (19.30–25.20)	25	10.20–27.50
13 ($n = 11$)	6.69 (3.42–11.00)	68	3.01–22.10
18 ($n = 10$)	0.13 (0.13–0.49)	83	0.13–0.77
Mean steady-state interferon concentration, ng/mL			
6, 8, 10, and 12 ($n = 11$)	21.53 (20.08–24.25)	20	11.28–26.58
Interferon clearance, L/h			
0–12 ($n = 11$)	0.047 (0.043–0.053)	21	0.037–0.071
Weight-adjusted interferon clearance, L/h/kg			
0–12 ($n = 11$)	0.0006 (0.0005–0.0007)	31	0.0004–0.0012
Interferon AUC from week 0 to week 12, ng · week/mL			
0–12 ($n = 11$)	219.45 (183.34–242.15)	17	147.67–275.96
OAS concentration, pmol/dL			
0 ($n = 11$)	86.74 (43.01–148.06)	125	14.91–588.91
1 ($n = 10$)	202.03 (132.99–304.46)	64	5.00–484.60
2 ($n = 10$)	212.92 (170.56–310.71)	39	150.71–445.50
3 ($n = 11$)	194.69 (128.57–346.88)	54	104.45–492.49
6 ($n = 11$)	234.57 (120.64–293.92)	50	79.38–456.52
12 ($n = 11$)	177.11 (114.62–285.04)	57	79.82–485.65
13 ($n = 11$)	166.69 (123.70–282.89)	49	62.17–359.07
18 ($n = 10$)	107.52 (80.68–200.89)	46	71.71–242.99
Change in OAS concentration from baseline, pmol/dL			
1 ($n = 10$)	71.84 (51.48–161.52)	…	−104.31 to 164.07
2 ($n = 10$)	140.69 (67.13–176.22)	…	−143.41 to 233.46
3 ($n = 11$)	127.57 (31.50–170.47)	…	−96.42 to 294.49
6 ($n = 11$)	134.02 (61.69–193.39)	…	−132.40 to 222.97
12 ($n = 11$)	100.04 (18.78–216.45)	…	−103.26 to 317.49
13 ($n = 11$)	93.72 (−29.51 to 123.67)	…	−229.84 to 271.07
18 ($n = 10$)	51.75 (19.66–103.52)	…	−60.86 to 114.15
IFIG concentration, MFI			
0 ($n = 8$)	4.94 (4.53–6.04)	13	4.46–6.16
3 ($n = 8$)	6.21 (5.80–6.57)	6	5.63–6.70
6 ($n = 8$)	6.44 (6.24–6.75)	5	5.85–6.87
12 ($n = 8$)	6.45 (6.27–6.73)	4	6.16–6.95
18 ($n = 7$)	5.04 (4.61–6.02)	15	3.74–6.02
Change in IFIG concentration from baseline, MFI			
3 ($n = 8$)	1.09 (0.41–1.59)	…	−0.41 to 2.17
6 ($n = 8$)	1.15 (0.58–2.10)	…	0.24–2.34
12 ($n = 8$)	1.30 (0.57–2.17)	…	0.23–2.49
18 ($n = 7$)	−0.17 (−0.41 to 0.63)	…	−0.72 to 0.63

NOTE. AUC, area under the pegylated interferon trough concentration–time curve; CI, confidence interval; CV, coefficient of variation; IFIG, interferon-inducible gene; MFI, mean fluorescence intensity; OAS, 2′,5′-oligoadenylate synthetase.

copies/mL, which is comparable with the virological effect of many antiretroviral agents, particularly nucleoside reverse-transcriptase inhibitors. However, no apparent HIV-1 RNA load response was observed with respect to individual drug exposure. Although the virological response of interferon alfa therapy is dose-dependent in the therapeutic setting of HCV disease, this relationship does not appear to hold in the setting of HIV-1 disease [19]. This study was not designed to address the effect

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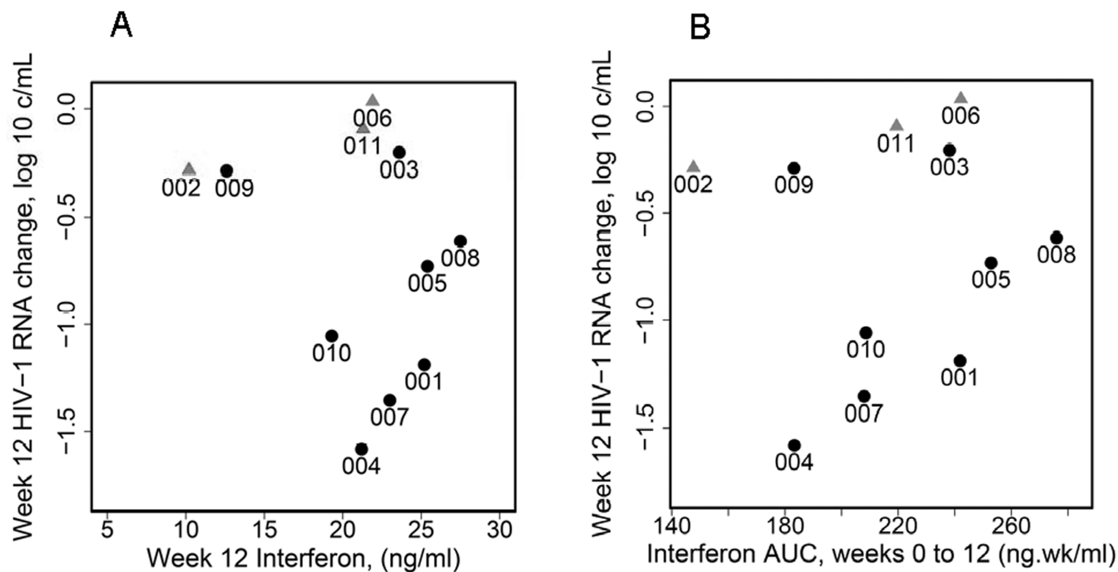

Figure 3. *A*, Week 12 plasma human immunodeficiency virus type 1 (HIV-1) RNA load changes from baseline (mean of preentry and entry on a $\log_{10}$ scale) plotted against week 12 plasma concentrations of pegylated interferon alfa-2a ($r = -0.11$ [90% CI, −0.60 to 0.44]; estimated Spearman rank correlation). Gray triangles indicate the 3 participants with reduced dosing; black circles indicate the remaining participants. *B*, Week 12 plasma HIV-1 RNA load changes from baseline (mean of preentry and entry on a $\log_{10}$ scale) plotted against area under the concentration-time curve (weeks 0–12) for interferon ($r = 0.16$ [90% CI, −0.39 to 0.63]; estimated Spearman rank correlation). Gray triangles indicate the 3 participants with reduced dosing; black circles indicate the remaining participants.

of multiple dosing regimens in this context. There was also no statistically significant association between changes in absolute $CD4^+$ T cell counts and peginterferon alfa-2a levels. This observation does not provide support for the hypothesis that type 1 interferons are pro-apoptotic molecules in this cellular compartment in the setting of HIV-1 infection [20]. In addition, there was no statistically significant association between decrease in viral load and changes in absolute $CD4^+$ T cell count, which suggests that the observed viral load decrease is not accounted for by fewer $CD4^+$ T cells producing virus.

Since the antiviral properties of interferon were first recognized in the late 1950s, efforts to identify additional viruses to target as well as better tolerated preparations have continued [21]. Early in the AIDS epidemic, researchers sought to learn whether interferon alfa could play a role in the therapeutic management of HIV-1 infection [22]. A potential anti–HIV-1 clinical benefit of interferon alfa therapy was recognized in the setting of its use for the treatment of Kaposi sarcoma [23–26]. Early accounts of the anti–HIV-1 effects of interferon alfa treatment presented by Lane et al [27] and de Wit et al [28] in this setting demonstrated a modest decrease in p24 antigen in a subset of patients. Several clinical trials have employed various interferon alfa preparations in combination with antiviral medications that supported the safety and potential efficacy of this approach [16, 17, 29–34].

Among the few trials of interferon alfa that have not treated participants with other antiviral agents is that of Skillman et al [35], in which interferon alfa-n3 was administered to 20 participants with $CD4^+$ T cell counts of >400 cells/μL, a cohort similar to that of the participants enrolled in our study. In that dose-ranging study, those participants who received the highest dose of a mean of 44 MIU/week showed a decrease in viral load of 1.86 $\log_{10}$ copies/mL at 60 d of treatment. As in our study, adverse events were uncommon and $CD4^+$ T cell count decreases were modest and associated with $CD4^+$ percent increases. Hatzakis et al [36] undertook a 28-d trial of recombinant interferon alfa-2b as monotherapy for HIV-1 infection. The baseline $CD4^+$ T cell count was 394 cells/μL. The mean viral load decrease was 0.89, 1.10, 1.03, and 0.97 $\log_{10}$ copies/mL on days 7, 14, 21, and 28, respectively. The adverse event profile noted grade 1 symptoms that were not dose-limiting.

The anti–HIV-1 properties of interferon alfa have been examined in the setting of treatment of HCV infection [37–39]. Neumann et al [38] examined the HIV-1 kinetics in 9 study participants with detectable HIV-1 RNA loads prior to initiation of interferon alfa-2b treatment. The decrease in HIV-1 RNA load was 1.1 $\log_{10}$ copies/mL after 1 week, and the changes in HIV-1 RNA load were not correlated with interferon concentrations. This is consistent with our findings, a point further emphasized by the lack of a statistically significant association between viral load decreases and interferon AUC measures. In the AIDS Pegasys Ribavirin International Coinfection Trial (APRICOT) study [39], among those participants with HIV and HCV coinfection who had detectable HIV RNA loads

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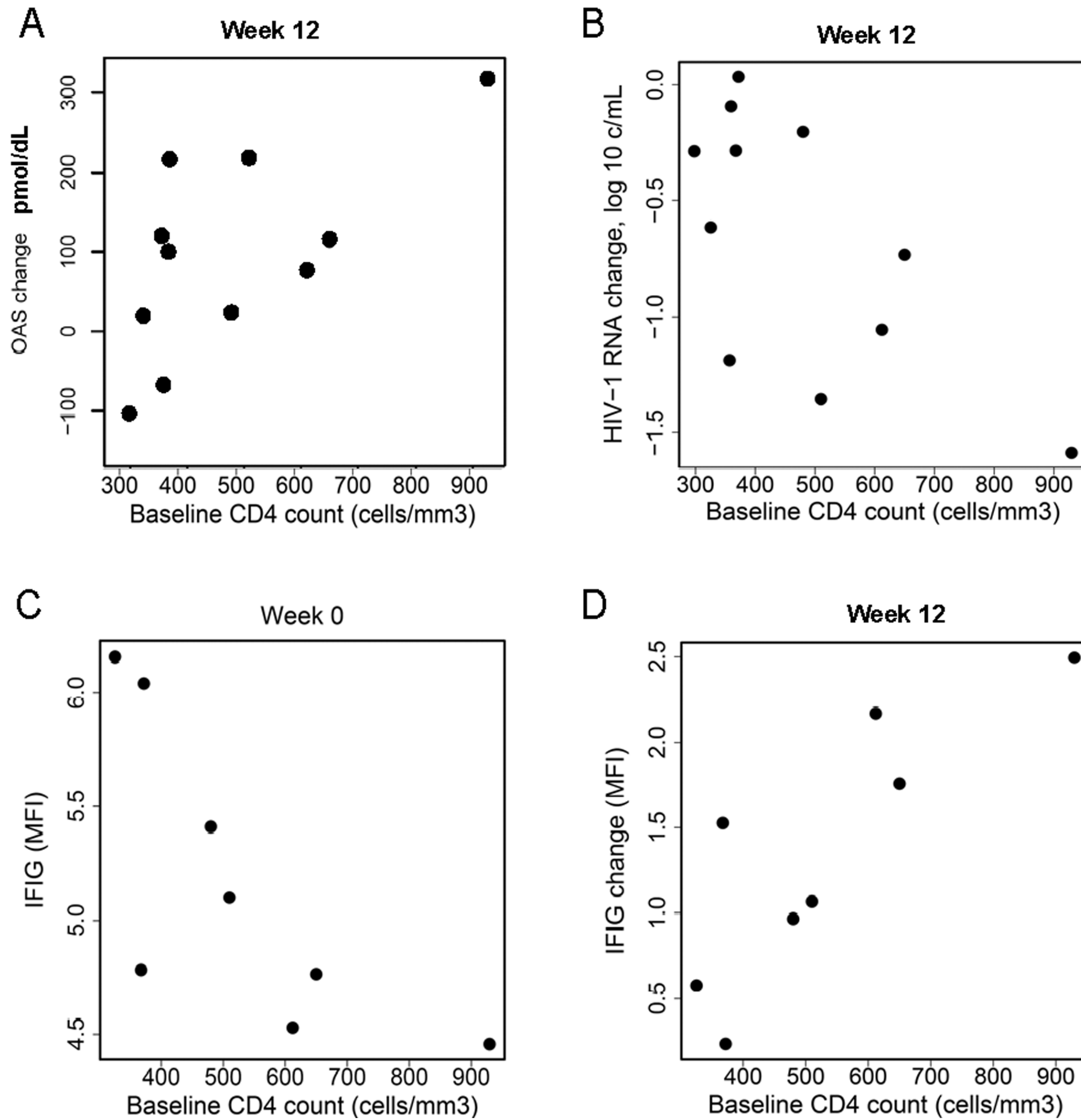

Figure 4. *A*, Week 12 serum 2′,5′-oligoadenylate synthetase (OAS) level change from baseline plotted against absolute CD4+ T cell count at baseline ($r = 0.63$ [90% CI, 0.15–0.87]; estimated Spearman rank correlation). *B*, Week 12 plasma human immunodeficiency virus type 1 (HIV-1) RNA load change from baseline plotted against absolute CD4+ T cell count at baseline ($r = -0.40$ [90% CI, −0.76 to 0.16]; estimated Spearman rank correlation). *C*, Interferon-inducible gene (IFIG) level at baseline (measured as mean fluorescence intensity [MFI]) plotted against CD4+ T cell count at baseline ($r = -0.81$ [90% CI, −0.95 to −0.38]; estimated Spearman rank correlation). *D*, IFIG level change from baseline at week 12 (measured as MFI) plotted against CD4+ T cell count at baseline ($r = 0.76$ [90% CI, 0.26–0.94]).

at entry, a decrease in HIV RNA load of 0.7 $\log_{10}$ copies/mL was observed at 48 weeks.

The observed peginterferon alfa-2a plasma concentrations (measured at the trough) were in the range of expected values for the doses of peginterferon alfa-2a administered in this study and were remarkably stable in all participants once steady state was achieved. Given a constant weekly dosing rate, this result also suggests constancy of pegylated interferon clearance in these participants over the 12-week study period. Our clearance estimates at steady state (dosing rate/mean trough steady-state concentration), in the range of 0.037–0.071 L/h, are somewhat lower than those previously reported using noncompartmental approaches (dose/AUC for 0–168 h) [40, 41]. This probably reflects our use of trough peginterferon alfa-2a concentrations, which underestimated the true mean steady-state concentration over a weekly dosing interval. However, a concentration-response relationship was not apparent between either peginterferon alfa-2a concentrations and plasma viral load changes or between pegylated interferon levels and OAS levels. This was also the case when analyzing the AUC trough from week 0 to

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week 12 as a measure of overall drug exposure and the response parameters of interest.

The findings in this study go beyond the primary objective of determining the viral load decrease that follows peginterferon alfa-2a administration in the absence of active HBV and HCV infections or other antiviral agents. Rather, this study explored correlates of antiviral activity. The results suggest that the anti–HIV-1 effect could be mediated by interferon-induced antiviral responses, on the basis of correlations with OAS and IFIG levels that influence the antiviral response. These factors are likely reflected in the observation that induction of the interferon response pathway prior to initiation of therapy results in refractoriness to an antivirological response following exogenously administered interferon.

This novel information about potential pathways for the antiviral effect raises several questions that were not addressed in the design of this study. The inverse relationship between baseline $CD4^+$ T cell counts and IFIG levels was observed among that subset of participants who were least likely to respond to exogenous interferon with a further increase in IFIG and OAS levels and decrease in viral load. The mechanism for these relationships is not known and may represent a unique feature of HIV-1 disease progression that has not been previously appreciated. Longitudinal studies will be required to identify which host and/or viral factors contribute most to this constellation of conditions.

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