

No Effect of Pegylated Interferon- α on Total HIV-1 DNA Load in HIV-1/HCV Coinfected Patients

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Pegylated interferon-alpha (pIFN- α) is suggested to lower human immunodeficiency virus type-1 (HIV-1) DNA load in antiretroviral therapy (ART)-treated patients. We studied kinetics of HIV-1 DNA levels in 40 HIV-1/hepatitis C virus (HCV) coinfecting patients, treated with pIFN- α for HCV and categorized into 3 groups according to start of ART: chronic HIV-1 infection ($n = 22$), acute HIV-1 infection ($n = 8$), no-ART ($n = 10$). Total HIV-1 DNA levels in 247 peripheral blood mononuclear cell samples were stable before, during, and after pIFN- α treatment in all groups. Our results question the benefit of pIFN- α as an immunotherapeutic agent for reducing the HIV-1 reservoir.

Keywords. HIV-1 DNA; HCV; pegylated interferon alpha; antiretroviral treatment; latent reservoir.

Eradication of human immunodeficiency virus type 1 (HIV-1) infection is not attainable by antiretroviral drugs alone, due to the HIV-1 reservoir formed early during acute infection [1]. Pegylated interferon alpha (pIFN- α), used to treat hepatitis C virus (HCV) infection, represents an interesting candidate to reduce the HIV-1 reservoir due to its variable antiviral and immune-stimulating properties against HIV-1 [2, 3]. However, effects of pIFN- α on total HIV-1 DNA levels, a well-validated marker of the HIV-1 reservoir [4], in HIV-1-infected patients

receiving antiretroviral therapy (ART) is not fully understood and there are conflicting results concerning the effects and mechanisms of pIFN- α .

To examine benefits of pIFN- α treatment in reducing total HIV-1 DNA levels, we conducted a study including 40 HIV-1/HCV-coinfecting patients from the Swiss HIV Cohort Study (SHCS) and the Zurich Primary HIV Infection Study (ZPHI). Unique to our study, we included patients from 3 groups: (1) HIV-1 chronic; (2) HIV-1 acute (both stratified according to time to initiation of ART after HIV-1 infection); and (3) a no-ART HIV-1-infected group, all with long follow-up times post pIFN- α treatment. We hypothesized that pIFN- α would lead to sustained reduction of the total HIV-1 DNA.

METHODS

Study Design

This was a retrospective study using prospectively collected clinical information and blood samples obtained within protocols of the ZPHI and SHCS (Supplementary material 1) and stored in respective biobanks. Complete inclusion and exclusion criteria can be found in the Supplementary material 2. In brief, inclusion criteria for HIV-1-infected, ART-treated patients were: (1) HCV-coinfection and a history of pIFN- α /ribavirin (RBV) treatment for ≥ 24 weeks; (2) no history of virological failure; (3) no history of ART interruption; (4) on suppressive combination ART; and (5) documented ART adherence of $\geq 95\%$ [5]. Exclusion criteria were: ≥ 1 blip during ART before start of pIFN- α /RBV. Inclusion criteria for no-ART HIV-1-infected patients were: (1) HCV coinfection and history of pIFN- α /RBV treatment for ≥ 24 weeks; and (2) ART-naive at time of pIFN- α /RBV initiation, during and ≥ 1 year after pIFN- α /RBV treatment.

Patient groups were defined as chronic when ART was started ≥ 6 months after the estimated date of infection, acute when ART was started < 6 months after the estimated date of infection, and no-ART when patients were not on ART as defined above. Information on pIFN- α dosage and administration is in Supplementary material 3.

Quantitation of Total HIV-1 DNA

Genomic DNA was isolated from ≥ 2 million cryopreserved peripheral blood mononuclear cells (PBMCs) using DNeasy Blood and Tissue Kits (Qiagen) according to the manufacturer's description except that 2 elution steps, each with 45 μ L of preheated (70°C) AE elution buffer, were applied. Total HIV-1 DNA was quantified in duplicates by quantitative polymerase chain reaction (qPCR) with cycling profile: 95°C 5 minutes, 50 \times (95°C 15 seconds, 60°C 30 seconds) using forward and reverse primers H7 and H1, alongside the probe mf381tq. To

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measure genomic input, the single-copy reference gene CC chemokine receptor 5 (*CCR5*), was quantified using primers mf51 and mf52 with the probe mf73tq (Supplementary Table 1). Quantification was done using an in-house HIV-1 all-in-one standard (Supplemental material 4; Supplementary Figure 1A and 1B).

Quantitation of HIV-1 Viral Load

Values for HIV-1 RNA copies/mL plasma were obtained from patients' clinical history. These values were measured using Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 assay versions 1 and 2. Detection limits were 40 and 20 HIV-1 RNA copies/mL plasma for version 1 and 2, respectively.

Cell Counts

CD4⁺, CD8⁺, and CD3⁺ T cells were quantified by flow cytometry following routine laboratory protocols.

Statistical Analysis

Wilcoxon matched-pairs signed rank test were applied to assess changes in total HIV-1 DNA, HIV-1 viral load, and cell counts using GraphPad Prism Version 5.04 (La Jolla, CA).

RESULTS

Patient Population and HCV Treatment

Forty HIV-1/HCV-coinfected individuals were categorized into 3 patient groups according to their stage of HIV-1 infection at the time of ART initiation: (1) chronic HIV-1 infection (*n* = 22); (2) acute HIV-1 infection (*n* = 8); and (3) no-ART (*n* = 10). Baseline demographics and clinical characteristics are given in Table 1.

Total HIV-1 DNA Levels Remain Stable During pIFN- α Treatment

We quantified total HIV-1 DNA in 247 PBMC samples with a mean number of time points (range) per patient of 6.6 (2–12) in the chronic, 5.6 (2–10) in the acute, and 5.7 (3–9) in the no-ART groups. The average follow-up times after pIFN- α treatment are given in Table 1.

We analyzed total HIV-1 DNA levels in PBMCs obtained prior to pIFN- α treatment (Figure 1). Values were grouped according to time of pIFN- α treatment and median values were calculated. We found that total HIV-1 DNA levels (median HIV-1 DNA copies/10⁶ genomic equivalents [range]) were 0.15 log₁₀ higher in the chronic (1652 [67.4–9807]) versus the acute group (1156 [50.2–2516.7], *P* = .07), as well as 0.74 log₁₀ higher than in the no-ART group (298.1 [6.9–3635], *P* = .06).

We evaluated the effect of pIFN- α /RBV treatment on total HIV-1 DNA. We observed no decrease in total HIV-1 DNA levels during administration of pIFN- α /RBV in any of the 3 patient groups (Figure 1). In fact, in some patients across all groups (chronic *n* = 8, acute *n* = 4, no-ART *n* = 1) there was a moderate increase in total HIV-1 DNA levels during pIFN- α treatment (*P* > .05). After pIFN- α treatment, total HIV-1 DNA returned to the

same levels as before pIFN- α treatment in the acute and chronic groups. In the no-ART group, total HIV-1 DNA levels after pIFN- α treatment were higher (*P* = .02) than total HIV-1 DNA levels before pIFN- α treatment (individual total HIV-1 DNA profiles provided in Supplementary Figures 2A–2C). As interferon is known to cause general lymphopenia, we calculated the total HIV-1 DNA levels normalized to CD4⁺ T-cell counts, but observed no change in the total HIV-1 DNA kinetics. Of note, 4 patients (C6, C19, C20, and A4) underwent repeated pIFN- α treatments due to nonresponse to previous HCV treatment. In all 4 patients, no sustained decrease in total HIV-1 DNA levels was detected (Supplementary Figures 2A and 2B).

pIFN- α Decreases the HIV-1 Viral Load in the no-ART Group

As IFN- α is known to have antiviral effects against HIV-1, we studied effects of pIFN- α HIV-1 on viral load in the no-ART group. The mean viral load decreased by 0.8 log₁₀ (*P* = .004) during treatment as compared to pretreatment levels. After pIFN- α treatment cessation, viral load returned to pretreatment levels (Figure 1D). All patients in the chronic and acute groups maintained undetectable viral loads (<20–40 HIV-1 RNA copies/mL plasma) during pIFN- α treatment (Supplementary Figure 3).

pIFN- α -induced Lymphopenia Observed in T-cell Subsets

As expected, during pIFN- α treatment we observed a significant drop in mean values (cell count/ μ L of blood) of CD4⁺ (*P* = <.0001), CD8⁺ (*P* = <.0001), and CD3⁺ (*P* = <.0001) T cells and in absolute lymphocyte counts in comparison to pre-pIFN- α levels. After treatment stop, all lymphocyte subsets returned to pretreatment levels (Supplementary Figure 4).

Discussion

In 40 well-characterized HIV-1/HCV-coinfected patients, we longitudinally assessed effects of pIFN- α treatment on total HIV-1 DNA levels in PBMCs, a surrogate marker for the HIV-1 reservoir [4]. We observed that total HIV-1 DNA levels remained stable during and after pIFN- α in comparison to pretreatment levels; this also occurred in patients who underwent repeated pIFN- α treatments.

To dissect potential effects of pIFN- α with regard to the timing of ART initiation, we analyzed 3 different HIV-1/HCV-coinfected patient groups: (1) HIV-1-infected patients treated with ART during chronic HIV-1 infection; (2) HIV-1-infected patients treated with ART during acute HIV-1 infection; and (3) untreated HIV-1-infected patients. Baseline total HIV-1 DNA levels in the acute group were 0.15 log₁₀ lower compared to patients treated during the chronic stage of infection. This observation is in line with several studies showing that patients treated in the early stage of HIV-1 infection have substantially lower total HIV-1 DNA levels compared to individuals treated during chronic phase [6]. The low total HIV-1 DNA levels observed in the no-ART group are most likely due to selection

Table 1. Swiss HIV Cohort Study Patient Characteristics at Baseline (Before IFN- α /Ribavirin Treatment)

Characteristic	Chronic Group (n = 22)	Acute Group (n = 8)	No-ART Group (n = 10)	Chronic vs Acute (<i>P</i> Value)
Age (y) at time of pIFN- α treatment, mean (range)	45 (37–55)	38 (27–45)	42 (32–53)	.005
Gender, No. (%)				
Male	21 (95.5)	8 (100)	7 (70)	>.9
Female	1 (4.5)	0 (0)	3 (30)	>.9
Ethnicity, No. (%)				
Caucasian	22 (100)	8 (100)	10 (100)	NA
Risk group, No. (%)				
MSM	5 (22.7)	7 (87.5)	4 (40)	.003
HET	1 (4.5)	1 (12.5)	1 (10)	.46
IDU	11 (50)	0	2 (20)	.01
IDU or HET	3 (13.6)	0	3 (30)	.54
IDU or MSM	2 (9.1)	0	0	>.9
HIV-1 subtype, No. (%)				
B	22 (100)	7 (87.5)	8 (80)	.27
Non-B	0	1 (12.5)	1 (10)	.27
Unknown	0	0	1 (10)	
Time under suppressive ART, ^a months (range)	42.4 (7–110)	39.4 (4–98)	NA ^b	.9
HCV genotype				
1	9	3	1	>.9
2	0	0	1	>.9
3	9	1	6	.21
4	2	2	0	.28
Unknown	2	2	2	NA
HCV treatment				
pIFN- α	1	0	0	>.9
pIFN- α + RBV	21	7	10	.47
IFN- α + RBV	0	1	0	.27
Treatment duration, mean weeks (range)	48.1 (24–68)	40.5 (24–48)	38.7 (20–51)	.04
SVR 12 or 24 ^c with IFN- α (%)				
Yes	19 (86.4)	7 (87.5)	9 (90)	>.9
No	1 (4.5)	1 (12.5)	1 (10)	.46
Unknown	2 (9.1)	0	0	>.9
Follow-up, ^d mean months (range)	46.6 (3–112)	13.3 (0–34)	43.9 (3–99)	.003

Significant *P* values are italicized.

Abbreviations: ART, antiretroviral therapy; HCV, hepatitis C virus; HET, heterosexual; HIV-1, human immunodeficiency virus type-1; IDU, intravenous drug use; IFN- α , interferon alpha; MSM, men who have sex with men; pIFN- α , pegylated interferon alpha; RBV, ribavirin; SVR, sustained viral response.

^aSuppressive ART defined as treatment controlling HIV-1 RNA <50 copies/mL plasma.

^bNA, not applicable as were not on antiretroviral treatment at the time of interferon administration.

^cSVR against HCV.

^dAfter stop of interferon- α treatment.

of patients controlling HIV-1 well in absence of ART, which is associated with low total HIV-DNA levels [7]. Six of the 10 patients in this group showed viral loads of <5000 HIV-1 RNA copies/mL plasma before pIFN- α treatment, with 1 being an elite controller (Figure 1D) [7]. Viral load was assessed in the no-ART group before, during, and after pIFN- α treatment and a reduction of 0.8 log₁₀ was observed. This finding is in line with the results of several clinical trials reporting an approximately 0.5–1 log₁₀ decrease in viral load when pIFN- α is administered to HIV-1-infected persons in absence of ART [8].

With regard to effects of pIFN- α administration on the total HIV-1 DNA levels, our findings differ from previous studies. Sun et al reported a moderate reduction of CD4⁺ T-cell-associated total and integrated HIV-1 DNA in 12 HIV-1/HCV-coinfected

individuals treated with pIFN- α for a median of 51 weeks [9]. In their study, reductions of HIV-1 DNA occurred mainly in patients without treatment-associated lymphopenia, suggesting that the decline in HIV-1 DNA levels did not result simply from lymphocellular toxicity of pIFN- α treatment. In our study, all patients exhibited pIFN- α -associated lymphopenia and no reduction in HIV-1 DNA levels from PBMC was observed. A recent study [10] found results more in line with ours as the authors did not detect any change in total HIV-1 DNA levels in 10 patients receiving a short-term pIFN- α treatment of 28 days.

A possible explanation for the absence of a HIV-1 DNA decrease is that our assay measures total HIV-1 DNA; however, this is not only a measure of replication-competent viruses [11]. The study by Morón-López et al [10], where the

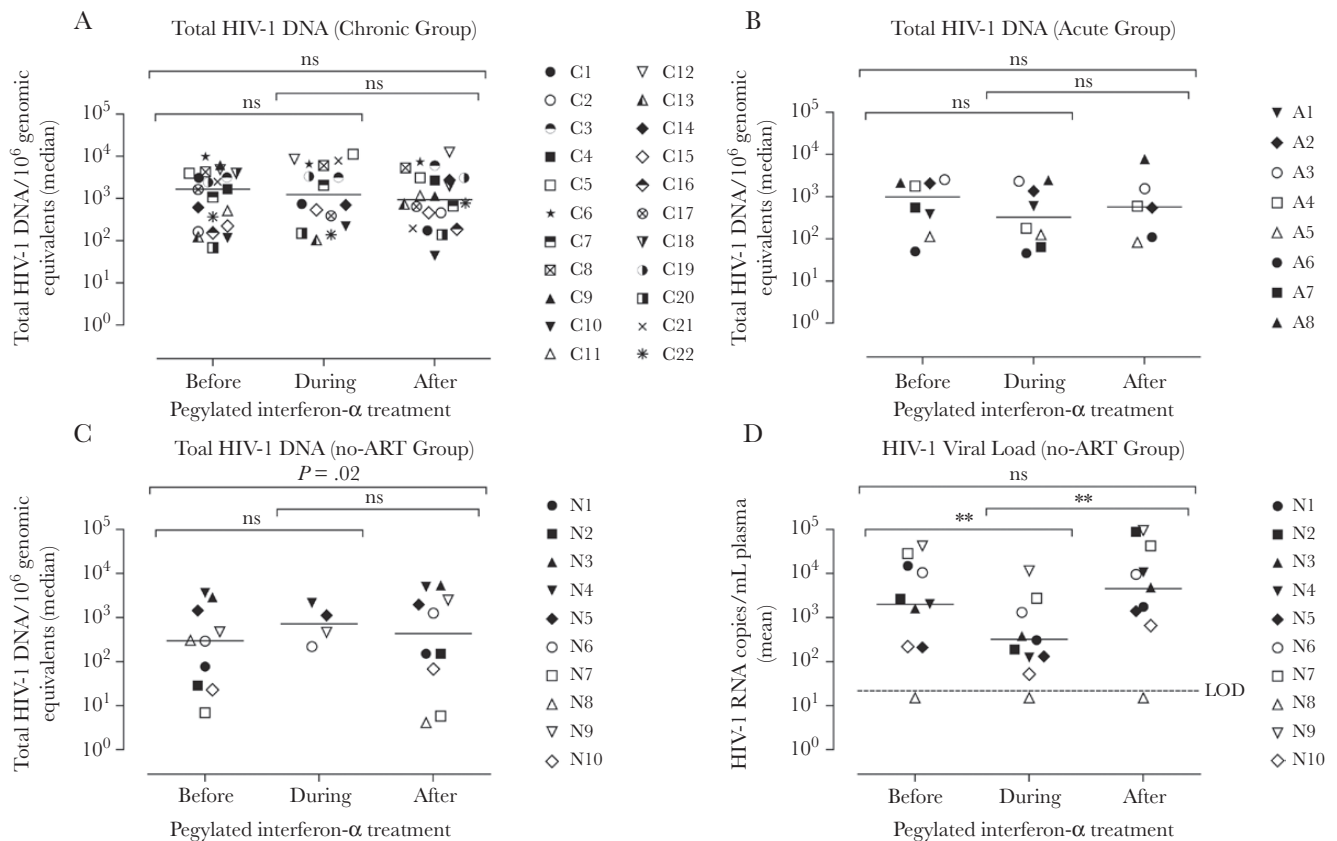


Figure 1. Total human immunodeficiency virus type-1 (HIV-1) DNA levels remain stable before, during, and after pegylated interferon-alpha (pIFN-α) treatment periods in all 3 groups (chronic, acute, and no-antiretroviral therapy [ART]) of HIV-1/ hepatitis C virus (HCV) coinfecting patients with the HIV-1 viral load decreasing in the no-ART group. Total HIV-1 DNA values were calculated to 10⁶ genomic equivalents (*CCR5*). **A**, Patients who started ART during the chronic stage of HIV-1 infection and later received pIFN-α for an HCV infection. **B**, Patients who started ART during the acute stage of HIV-1 infection and later received pIFN-α for an HCV infection. **C**, Patients who received no ART at the time of pIFN-α administration. **D**, pIFN-α decreases the HIV-1 viral load in the no-ART group. HIV-1 viral load (RNA copies/mL plasma) is plotted as mean values for before, during, and after pIFN-α treatment periods. The samples below the limit of detection (LOD; 40 and 20 HIV-1 RNA copies/mL plasma for Roche Cobas Taqman HIV-1 assay version 1 and 2, respectively) appear below the dashed line. ** $P \leq 0.01$; ns, not significant. Individual patients are depicted by a symbol and corresponding identification codes. Abbreviations: A, acute; C, chronic; N, no-ART.

replication-competent reservoir was measured by viral outgrowth assay (VOA) in patients receiving pIFN-α/RBV, showed no change in the number of infectious units per million CD4⁺ T cells from patients on ART. The absence of an HIV-1 DNA decrease is unlikely to have resulted from insufficient pIFN-α treatment as: (1) all our patients exhibited lymphopenia; (2) the no-ART group showed an RNA decline of 0.8 log₁₀; and (3) 90% had a sustained virological response of the HCV infection.

The strength of this work is that, to our knowledge, our study is to date the largest investigating the effects of pIFN-α on total HIV-1 DNA levels in people on suppressive antiretroviral therapy. The longitudinal nature is unprecedented, with patients being followed up for as long as 112 months post pIFN-α. Limitations are that, due to the retrospective nature of our study, we investigated only effects of pIFN-α on total HIV-1 DNA in cryopreserved patient PBMCs. As we did not investigate cells from other biological compartments, we may have missed tissue-specific pIFN-α effects. We also did not

differentiate between integrated and nonintegrated proviral HIV-1 DNA. However, it has been shown that after 1 year of ART almost no linear unintegrated HIV-1 DNA exists anymore [12] and our ART-treated patients were virologically suppressed for ≥1 year before pIFN-α administration. Moreover, we did not use the VOA, which is suggested as the gold standard for HIV-1 latency, although it fails to detect a considerable fraction of inducible latently infected cells [13]. Finally, our study population did not include patients who received pIFN-α administration close to the time of HIV-1 acquisition. Thus, a potential beneficial pIFN-α effect on the establishment of the HIV-1 reservoir, which is formed early after HIV-1 infection [14], cannot be ruled out. The only study exploring pIFN-α together with antiretroviral treatment in 12 acutely HIV-1-infected patients, however, found a total HIV-1 DNA decay that was similar to that found in acutely infected ART-treated patients alone [12, 15]. Thus, pIFN-α would probably need to be administered much earlier, which is very difficult to achieve in clinical practice.

In summary, our longitudinal study in different well-characterized patient groups did not reveal any substantial effect of pIFN- α on the HIV-1 reservoir as measured by a total HIV-1 DNA qPCR assay in PBMCs. Thus, our study does not support a sustainable effect of pIFN- α as an immunotherapeutic intervention to reduce the HIV-1 reservoir in patients who have initiated ART during acute or chronic HIV-1 infection or who are off ART.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. H. F. G., K. J. M., and D. L. B. conceptualized, designed, and supervised the study. Data acquisition was done by V. P. S., D. L. B., V. V., A. U. S., Y. L. K., R. D. K., M. S., A. R., K. D., M. H., K. J. M., and H. F. G. Data analysis was done by V. P. S., A. U. S., R. D. K., H. F. G., and K. J. M. A first draft of the manuscript was written by V. P. S., D. L. B., R. D. K., K. J. M., and H. F. G. All investigators contributed to data collection and interpretation of the data, reviewed drafts of the manuscript, and approved the final manuscript.

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