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Impact of IFNα-2a on the Replication-Competent HIV-1 Reservoir in CD4+ T Cells

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INTRODUCTION

- Combined antiretroviral therapy (cART) suppresses HIV-1 progression, but does not eliminate residual viral production of the reservoirs that perpetuate the infection, and constitute the limiting factor for HIV-1 cure.
- Previous data suggest that IFNα controls HIV-1 replication in patients without cART and decreases proviral DNA in peripheral CD4⁺ T cells[1,2].
- Our **aim** was **analyzing the effect of pegIFN-α in:**
  - Total HIV-1 reservoir
  - Replication-competent HIV-1 reservoir
  - Cell-associated HIV-1 RNA, and
  - Host restriction factors gene expression
  in CD4⁺ T cells from a cohort of HIV/HCV-coinfected patients on suppressive cART.

METHODS

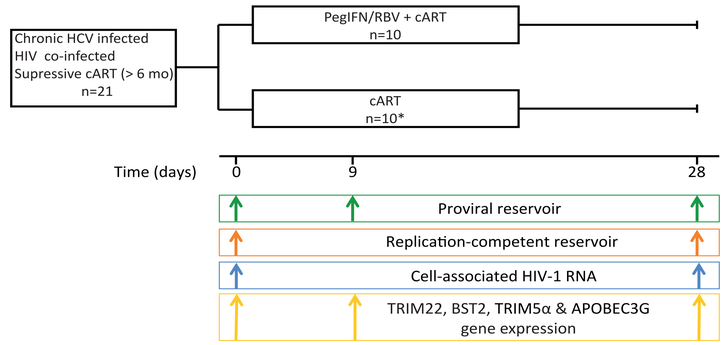


Figure 1. Timeline and study design. *One patient excluded from analysis (HIV RNA > 50 cp/ml at d0).

Subjects. **21 HIV-1 infected cART-suppressed** (HIV-RNA<25 copies/ml) **and HCV-coinfected patients** (HCV-RNA>10,000 IU/L):

- **IFN group** (n=10): treated with pegIFN-α (180µg/sc/week), and RBV (800-1200mg/d)
- **Control group** (n=10*): did not receive treatment against HCV

Proviral reservoir. Total HIV-1 DNA in circulating CD4⁺ T cells was measured by droplet digital PCR (ddPCR), as we previously described[3], at days 0, 9 and 28. Shortly, ddPCR was used to quantify the copy number of HIV DNA, by amplifying gag or 5'LTR region[4,5], and the RPP30 cellular gene[6], from CD4⁺T cell lysed extracts, to estimate the size of the proviral reservoir.

Replication-competent reservoir. We performed a limiting dilution virus culture assay, as previously described[7] with minor modifications (Fig 2).

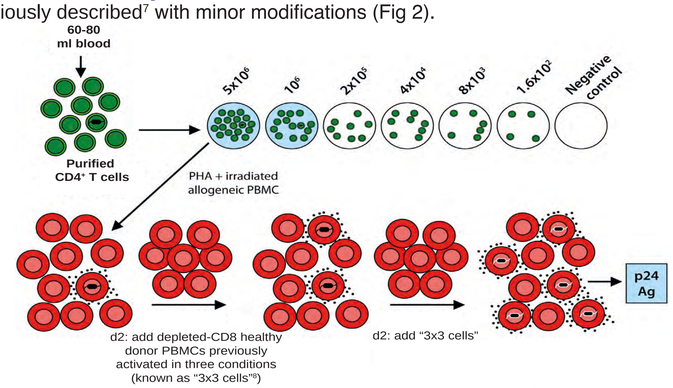


Figure 2. Quantitative viral outgrowth assay used, modified from[9].

Cell-associated HIV-1 mRNA. Viral transcription levels in circulating CD4⁺ T cells were measured by ddPCR, as we previously described[3], at days 0 and 28. Briefly, mRNA from CD4⁺ T cells was extracted using RNeasy Mini Kit (Qiagen). Gag or 5'LTR region[4,5] were amplyfied to quantify HIV-1 expression and normalised by the expression of the housekeeping gene TBP[5].

TRIM22, BST2, TRIM5α, APOBEC3G gene expression. Restriction factors expression was quantified by qPCR, at days 0, 9 and 28. Shortly, mRNA was extracted as in cell-associated HIV-1 mRNA. RNA was retro-transcribed into cDNA, amplified and quantified using TaqMan Gene Expression Assays.

RESULTS

Subject characteristics

Table 1. Demographic data of the control and the IFN groups.

	Control group (n=10)	IFN group (n=10)	p value between groups
Age (years), median[IQR][a]	46.5[45.2-52.8]	46.5[41.0-50.5]	0.4300
Females, n(%)[b]	1(10)	2(20)	1.0000
PI based treatment, n(%)[b]	8(80)	7(70)	1.0000
Time since diagnosis (years), median[IQR][a]	23.5[20.2-24.0]	24.0[19.8-25.2]	0.5700
Time of viral load suppression (years), median[IQR][a]	4.5[2.5-5.8]	4.5[2.2-7.8]	0.9400
CD4+ T cell count			
Absolute (cells/µl) day0, median[IQR][a]	447[295-670]	437[218-624]	0.8200
Absolute (cells/µl) day28, median[IQR][a]	570[480-650]	310[203-370]	0.0100
p value (d0 vs. d28)[c]	1.0000	0.0091	
CD8+ T cell count			
Absolute (cells/µl) day0, median[IQR][a]	1,050[1,010-1,200]	650[585-1,130]	0.3700
Absolute (cells/µl) day28, median[IQR][a]	1,190[660-1,310]	440[377-523]	0.0048
p value (d0 vs. d28)[c]	0.8125	0.0313	

[a] p value between groups: Kruskal-Wallis test. [b] p value between groups: Fisher Exact test. [c] p value (d0 vs. d28): Wilcoxon Signed Rank test

PegIFN-α does not impact proviral reservoir either at d9 or d28

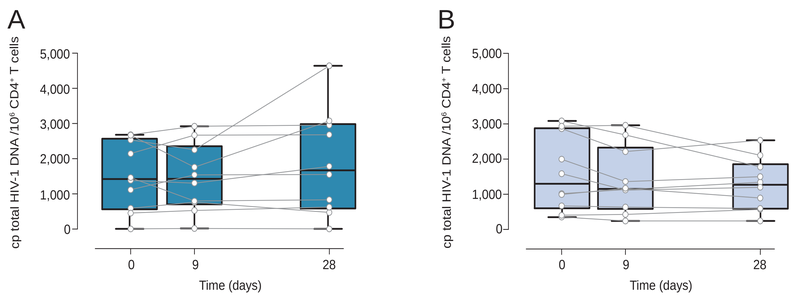


Figure 3. Copies of total HIV-1 DNA per Million CD4⁺ T cells for each study group at d0, 9 and 28. (A) IFN group and (B) Control group.

PegIFN-α down-regulates cell-associated HIV-1 RNA at d28

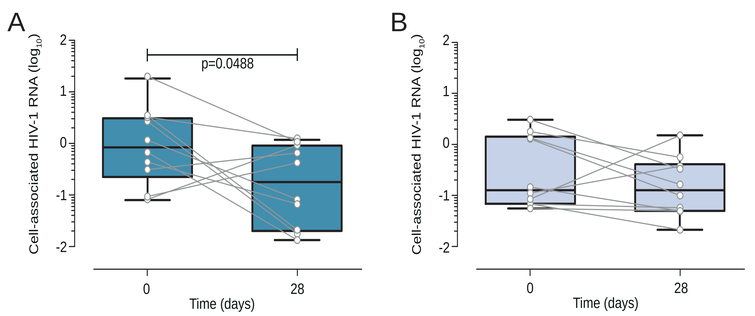


Figure 4. Cell-associated HIV-1 RNA (log_{10}) at d0 and d28. (A) IFN group and (B) Control group (Wilcoxon Signed Rank test, p=0.0488).

PegIFN-α perturbs replication-competent reservoir at d28

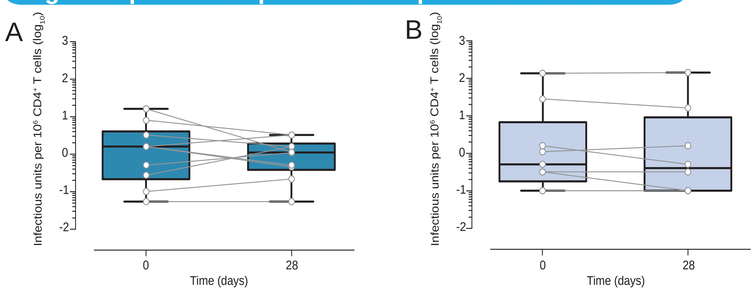


Figure 5. Infectious Units per Million CD4⁺ T cells (log_{10}) at d0 and d28. (A) IFN group and (B) Control group.

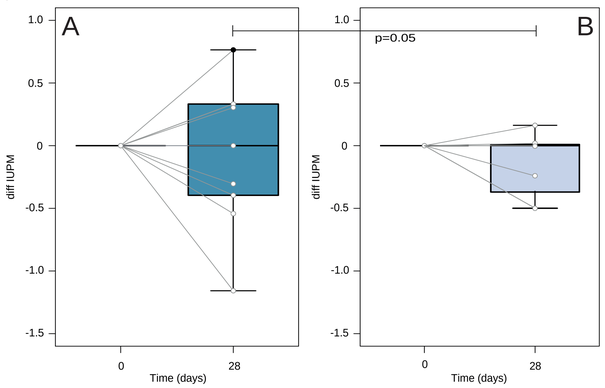


Figure 6. Difference between Infectious Units per Million CD4⁺ T cells (IUPM) at d0 and d28. (A) IFN group and (B) Control group (Levene test, p=0.05).

PegIFN-α up-regulates APOBEC3G, TRIM5α, BST2 and TRIM22

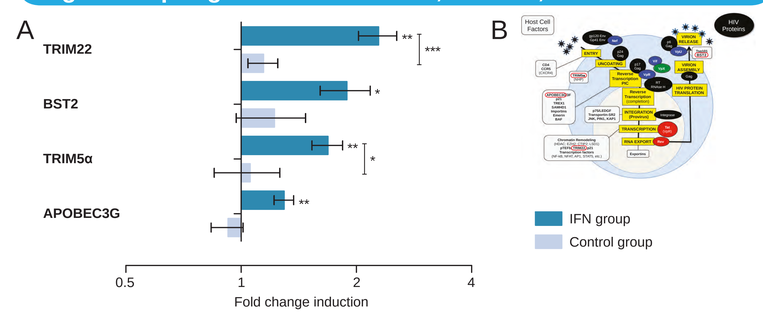


Figure 7. (A) Fold change induction (Wilcoxon Signed Rank test, p(d0 vs. d28); Wald test, p between groups; *p<0.05, **p<0.005, ***p<0.0005). (B) Host restriction factors functions.

CONCLUSIONS

- **PegIFN-α perturbs** the **replication-competent reservoir** in peripheral CD4⁺ T cells. **However**, after 28 days of treatment **no significant decrease was observed in replication-competent or in proviral DNA reservoir size.**
- **A concomitant decrease of cell-associated HIV-1 RNA is observed**, probably through the inhibition of HIV-1 transcription by TRIM22.

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