Reduced rectal HIV RNA after Peg-IFN-α2b added to ART together with ART interruption

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Abstract

Background: The effects of Peg-IFN-α2b immunotherapy on gut tissue HIV-1 RNA remain unknown. We hypothesized that in antiretroviral therapy (ART) chronically suppressed HIV-1$^+$ individuals addition of Peg-IFN-α2b to ART, including of a short ART interruption (to induce HIV reactivation), would decrease rectal tissue HIV levels by triggering anti-HIV mechanisms.

Methods: 20 ART chronically suppressed (<50 copies/ml) HIV-1$^+$ subjects received 20 weeks of Peg-IFN-α2b. ART was continued for the first 5 weeks, followed by a 4-week interruption, and resumption from week 9-20. Viral load (VL) and CD4$^+$ T cell levels were monitored throughout the treatment. Parameters assessed at baseline (BL) and after 20 weeks of treatment (EoT) included: a) immune activation markers (e.g. CD38, CD69, NKG2A, NKG2C, KIR) and IFN-responsive molecules (e.g. CD169, Tetherin) on cryopreserved peripheral blood mononuclear cells (PBMC) by flow cytometry, b) soluble markers of immune activation (CD14, CRP, D-dimer, IL-6, CD163) in plasma by ELISA, and c) HIV RNA in rectal mucosa biopsies by in situ hybridization (confirmed by reverse transcriptase-ddPCR). Differences between BL and EoT were tested, using non-parametric Wilcoxon Sign-Rank test or paired t-tests, depending on data distribution. A p<0.05 was considered significant.

Results: 17/20 subjects completed the treatment; 3 subjects were withdrawn prematurely due to adverse event (AEs; neutropenia). 9/17 subjects had VL>50 copies/ml during ART interruption, returning to undetectable upon ART re-initiation. Coupled (BL-EoT) rectal biopsies were available for 15/17 subjects with 7 of them having detectable tissue in situ HIV RNA at BL. Of these, 7/7 had a decrease in tissue HIV RNA at EoT, with a complete loss of detectable tissue HIV RNA in 5/7. 1 subject had detectable tissue HIV RNA at EoT, but not at BL. We observed a decrease in CD4$^+$ T cell count and an increase in CD4$^+$ and CD8$^+$ T cell activation (CD38, HLA-DR, PD1) and Tetherin expression between BL and EoT. Higher frequencies of CD56^{bright} and lower of CD56^{dim}CD16$^+$ NKs at EoT were also found, as well as an increase in CD38 and NKG2A in both NK subsets. In the myeloid compartment, we noted an increase in CD169 and Tetherin, and in plasma levels of CD163. No changes in plasma CD14, CRP, D-dimer, or IL-6 were found.

Conclusion: 20 weeks of combined Peg-IFN-α2b+ART, including a 4-week ART interruption, result in a decrease in tissue HIV RNA and an increase in peripheral innate and adaptive immune activation.

Introduction

Antiretroviral therapy (ART) reduces HIV viremia to undetectable levels, yet the persistence of ART resistant reservoirs[1,2] is a major barrier to HIV eradication. Data from our recently completed R01 AI065279 trial support that *in vivo* administration of Peg-IFN-α2a results in decrease in HIV reservoir measures after HIV reactivation triggered by ART removal[3], with IFN-induced NK cytotoxicity been a correlate of *in vivo* suppression and reductions in integrated HIV. Peg-IFN-α2a and Peg-IFN-α2b have equivalent clinical response outcomes[4], and there is clinical evidence suggesting that Peg-IFN-α2b is better tolerated and achieves larger tissue volume distribution than Peg-IFN-α2a[5]. In ART-suppressed chronically HIV-1-infected subjects, we investigated the effects of addition of Peg-IFN-α2b to ART, in the presence of a short ART interruption, on anti-HIV mechanisms and gut tissue HIV-1 RNA.

Hypothesis

In chronically HIV-infected, immune-reconstituted individuals, addition of Peg-IFN-α2b to ART, in the presence of HIV reactivation (i.e.: short ART interruption), will decrease rectal tissue HIV levels by triggering anti-HIV mechanisms.

Subjects, Materials & Methods

Study subjects

A total of 20 ART chronically suppressed (<50 copies/ml) HIV-1$^+$ subjects received Peg-IFN-α2b for a 20-week period. ART was continued for the first 5 weeks of the 20-week period, followed by a 4-week ART interruption, and ART resumption from week 9 to week 20. 17/20 subjects completed the treatment; 3 subjects were withdrawn prematurely due to adverse event (AEs; neutropenia). Study schema and characteristics of patients who completed the treatment are shown in Sections 1-2.

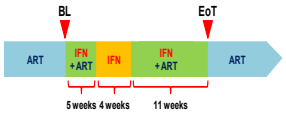
Methods

Viral load (VL) and CD4$^+$ T cell levels were monitored throughout the treatment. Parameters assessed at baseline (BL) and after 20 weeks of treatment (EoT) included: a) immune activation markers (e.g. CD25, CD38, CD69, HLA-DR, NKG2A, NKG2C, KIR, PDL1) and IFN-responsive molecules (e.g. CD169, Tetherin) on cryopreserved peripheral blood mononuclear cells (PBMC) and fresh cells from rectal mucosa biopsies by flow cytometry[6,7], b) soluble markers of immune activation (CD14, CRP, D-dimer, IL-6, CD163) in plasma by ELISA, and c) HIV RNA in rectal mucosa biopsies by *in situ* hybridization (confirmed by reverse transcriptase-ddPCR).

Statistical analysis

Data were described as medians, 25th and 75th percentiles. Differences between BL and EoT were tested, using Wilcoxon Signed-Rank or paired t-tests depending on data distribution. P-values <0.05 were considered statistically significant. All statistics were performed with JMP Pro11 (SAS Institute, Cary, NC). Results are shown in Sections 3-4.

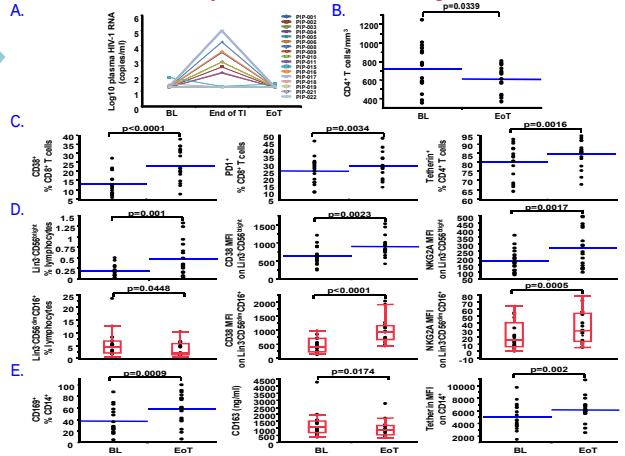
1 Study schema



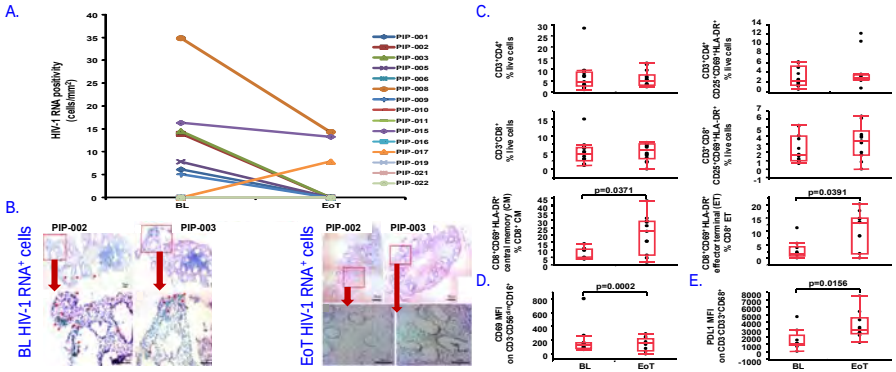
2 Patient Characteristics

Patient	Year of Birth	Sex	Race
PIP-001	1970	Female	Black
PIP-002	1980	Male	Black
PIP-003	1954	Female	Black
PIP-004	1979	Male	Black
PIP-005	1966	Male	White
PIP-006	1979	Male	Black
PIP-008	1961	Male	Black
PIP-009	1953	Male	White
PIP-010	1970	Male	Black
PIP-011	1966	Female	Black
PIP-015	1956	Female	Black
PIP-016	1969	Male	Black
PIP-017	1967	Male	White
PIP-018	1965	Male	Black
PIP-019	1972	Male	Black
PIP-021	1960	Male	White
PIP-022	1967	Male	Black

3 Peripheral blood changes in HIV VL, CD4$^+$ T cell count, T cells, NK cells and myeloid cells activation during treatment



4 Rectal tissue changes in cell-associated HIV, T cells, NK cells and myeloid cells activation during treatment



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Conclusions

20 weeks of combined Peg-IFN-α2b+ART, including a 4-week ART interruption, resulted in the following changes:

I) Peripheral blood:

- Increase in plasma HIV-1 RNA >20 copies/ml during a 4 week ART interruption (in the presence of Peg-IFN-α2b+ART) in 10/17 patients, followed by re-suppression to <20 copies/ml at EoT.
- Decrease in CD4$^+$ T cell count and increase in markers of activation and Tetherin on T cells at EoT.
- Increase in CD56^{bright} NK and decrease in CD56^{dim}CD16$^+$ NK cells, together with an increase of markers of activation in both cell subsets at EoT.
- Increase in CD169 and Tetherin on myeloid cells, together with no change in soluble markers of inflammation with the exception of an increase in CD163 plasma levels at EoT.

II) Rectal tissue:

- Decrease in tissue HIV RNA at EoT in 7/7 subjects with detectable tissue HIV RNA at BL, with a complete loss of detectable tissue HIV RNA in 5/7; Detectable tissue HIV RNA at EoT in 1 subject, despite lack of detectable tissue HIV RNA at BL.
- No change in the frequency of T cells, and of activated or resting T cells at EoT.
- Increase in markers of activation on T cell memory subsets, NK cells, and macrophages.

Interpretation

20 weeks of combined Peg-IFN-α2b+ART, including a 4-week ART interruption, result in a decrease in tissue HIV RNA and an increase in peripheral and tissue-associated T cell, NK and myeloid activation.

Future Direction

Comparative analysis of changes in tissue-associated HIV by measurement of the frequency of peripheral blood CD4$^+$ T cells with inducible multiply-spliced HIV RNA by (Tat/rev Induced Limiting Dilution Assay; TILDA), and by measurement of cell-associated integrated HIV DNA.

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