Pegylated IFNα-2b decreases latent HIV measures in ART-suppressed subjects.

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Abstract

Background
Data from NCT00594880 support that administration of pegylated interferon (Peg-IFN)-α2a results in viral suppression and reduction in integrated proviral HIV DNA in ART-suppressed subjects undergoing analytical ART interruption (ATI). NCT01935089 tested the hypothesis that treatment with Peg-IFN-α2b, in the presence of HIV reactivation via ATI, would decrease the levels of latent viral reservoir in chronic HIV infection.
Methods
Study design: 20 individuals with chronic HIV infection (on ART, VL <50 copies/ml) received 1 μg/kg Peg-IFN-α2b sc for 20 weeks, with a 4 week ATI after 5 weeks of IFN treatment. In addition to safety monitoring, HIV measures (see Table 1) were assessed at baseline and end of treatment (EOT).
Statistical analysis: we used Wilcoxon Signed rank test to test differences between time points; exact Fisher tests to compare frequency of viral suppression during ATI; Spearman tests, mixed effect models and hierarchical clustering to test relationships between HIV reservoir measurements.
Results
Of 20 subjects receiving treatment, 18 subjects completed treatment, with 7 grade 3-4 AEs and no reportable SUSAR.
Peg-IFN-α2b suppressed plasma HIV RNA during a 4 week ATI in 52.6% of participants, similar to Peg-IFN-α2a (NCT00594880: 66.6%, p= 0.5197), higher than ATI only (historical controls: 12.5%, p= 0.0127)
At EOT the number of HIV RNA-expressing GALT cells was significantly reduced (p= 0.012); integrated HIV DNA in circulating CD4 also showed a decrease trend (p= 0.079†).
Despite some baseline correlations across HIV measures, their changes over time were not correlated.
The change in GALT RNA was strongly correlated with baseline values. Similar significant associations were also detected for integrated DNA, TILDA, p24 and 2LTR.
Plasma HIV RNA rebound during ATI was not associated with changes in reservoir measures.
Conclusions
We conclude that treatment with Peg-IFN-α2b (20 weeks with a 4-week ATI) is safe, well tolerated and can reduce the load of cells expressing viral RNA in GALT; additional viral reservoir measures may be reduced in subjects with higher baseline levels.
Implications for future study design: the observed discordance between the tests performed suggests that multiple assessments should be incorporated in future trial design. In addition, randomized designs with no-treatment arm, robust test standardization and repeated sampling at both baseline and EOT will be required extend these observations and to rule out regression to the mean biases.

Methods

Cohort description. 20 adult individuals with chronic HIV-1 infection receiving suppressive combination ART (cART) were recruited in the Philadelphia, PA area
Study type. Single arm, open label clinical trial
Treatment. Pegylated IFN-α-2b (Pegintron, Merck Inc.)
Demographic characteristics. The study population was 23% female, 71% African American, median age 47 y.o.
Visit Schedule and study disposition. See section 1
Collected data. Variables with missingness > 30% were excluded from the analysis
Assays performed
Clinical labs: All clinical assessments were performed by Quest Diagnostics
PBMC (from whole blood or aphaeresis)

- Integrated proviral DNA (whole blood PBMC)
  - Alu-Gag PCR. The integration assay was performed on CD4+ T cells isolated from peripheral blood-derived cryopreserved PBMC by negative magnetic selection. Briefly, Alu-gag–based polymerase chain reaction with repetitive sampling techniques was used to increase the sensitivity and accuracy of the assay in order to measure low levels of integration in patient samples. Results are expressed as number of integrated HIV DNA copies per number of CD4+ T cells (12).
- In vitro latency reactivation (Aphaeresis-derived PBMC)
  - Tat/rev induced limiting dilution assay (TILDA). CD4+ T cells were isolated and stimulated with PMA and ionomycin or DMSO (control); 24 hrs later, cells were counted, re-suspended in PBS and added to 384 well plate (4 dilutions, 24 replicates per stimulation). RT-PCR was performed after diluting each well with TE (limiting dilution). At/rev amplicons were detected in individual cells after 40 amplification cycles (13).
  - HIV p24 Single Molecule Array (Simoa). Supernatants were collected from the cultures described above after 24 and 48 hrs of stimulation; Cell pellets were also collected by centrifugation and tested at 48h. HIV P-24 was detected using the Quanterix method, with a limit of detection of 4 fg/ml

GALT (from colorectal mucosal biopsies)

- Cell-associated HIV DNA and RNA: Cryo-preserved cells derived from colorectal mucosa biopsies were evaluated for viral measures including
  - Cell-associated HIV DNA [total HIV DNA (pol copies) and Episomal 2-long terminal repeat (2-LTR) circles] by droplet digital PCR (ddPCR) as previously described (14).
  - Cell-associated HIV unspliced RNA (gag), multiply spliced RNA (tat/rev) and poly-A tailed transcripts (PolyA), by reverse transcriptase-ddPCR as previously described (14).
- Total HIV RNA
  - HIV RNA was detected in formalin-fixed, paraffin embedded tissue sections using full-length DNA probes, according to a previously reported *in situ* hybridization technique (15,16).

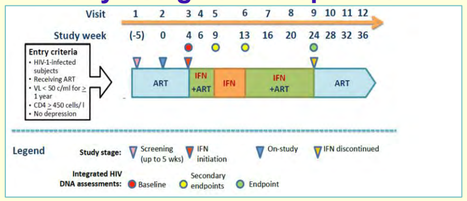
Statistical analysis

- Visual inspection: exploratory graphing, distributions
- Assessment of data missingness
- Differences between time points: Wilcoxon signed rank test
- Relations between variables: Spearman correlation test, linear regression and mixed effect linear models, Fisher's exact test, Hierarchical clustering (Ward method) with missing data imputation

Introduction

. HIV eradication and/or functional cure approaches require strategies aimed at reducing or eliminating latent HIV reservoirs located in all body sites (1). To this end, a number of strategies have been tested (2-4), ranging from Latency Reversing Agents (LARs, 5) to immunological approaches (6,7), stem cell transplantation (8) and genetic engineering (9) , with very limited success. In addition, the issue of what assay best measures the real size of the latent HIV reservoir remains open (10). Data from our NCT00594880 clinical trial (11) support that administration of pegylated interferon (Peg-IFN)-α2a (Pegasys) results in viral suppression and reduction in integrated proviral HIV DNA in ART-suppressed subjects undergoing analytical ART interruption (ATI). The present study, NCT01935089, was designed to test the hypothesis that treatment with Peg-IFN-α2b (PegIntron), combined with a brief Analytical treatment interruption (ATI) intended to induce HIV reactivation, would decrease the levels of latent viral reservoir in ART suppressed individuals with chronic HIV infection.

1. Study design and disposition



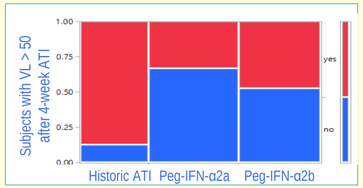
Disposition	N
Screened	22
Enrolled	20
Screening failures	2
Exposed	20
Adverse experiences *	8
Dose reductions	2
Premature discontinuation of study drug	3
Premature termination	2
Reached endpoint	18
Elected additional visit	16

** 1: depression; 7: neutropenia*

Baseline characteristics%		
Age: Median 47 (42-53 IQR)		
Race: 71% Afr. Am.; 29% Cauc.		
Gender: 24% female (n=5)		
Main clinical Labs		
Test	mean	SD
CD4 count (cells/μl)	777	187
CD8 count (cells/μl)	772	275
WBCC (10^6 cells/μl)	5.4	1.5
Lymph. count	2001	453
ANC	2892	1315
RBC	4.6	.56
Viral load (copies/μl)	<20	n.a.

20 Individuals with HIV-1 infection (18-65 y.o., 135-300 lbs, on ART for > 1year, HIV VL<50 copies/ml, CD4 count ≥ 450 cells/μl, absent pathological conditions that would contraindicate the administration of type-I IFNs) were treated with a 20-week course of peg-IFN-α2b, with a 4-week analytical treatment interruption (ATI) from week 9 to week 13. Blood was collected approximately every 4 weeks; leukaphaeresis and rectal mucosa biopsies were collected at baseline (wk. 0) and endpoint (wk. 24). Study disposition and baseline characteristics of the cohort are represented in section 1: no SUSAR was reported, and the rate of AEs was as anticipated based on the product's information package and our prior clinical experience

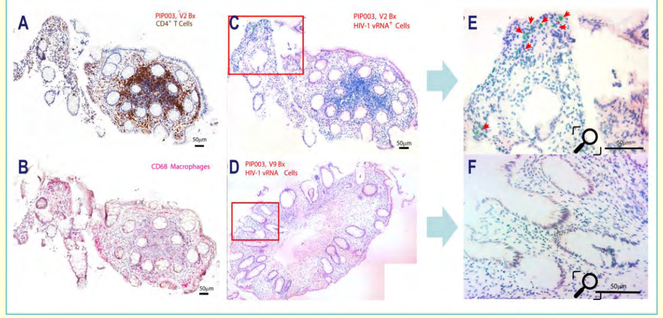
2. Suppression of HIV replication on ATI

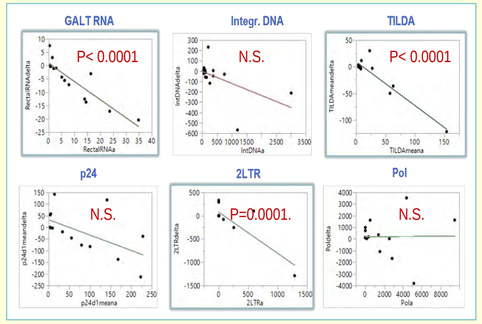


At the end of the 4-week ATI, 9 of 19 (52.6%) participants experienced a VL ≥ 50 copies/ml, similar to our prior results using Peg-IFN-α2a (NCT00594880: 66.6%, p= 0.5197), but significantly higher than ATI conducted in the absence of immunotherapy (historical controls: 12.5%, p= 0.0127), confirming the antiviral effect of Peg-IFN-α2a.

3. Change in HIV measures upon exposure to Peg-IFN-α2b

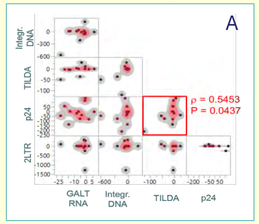
Method	Baseline *(med; IQR)*	20 weeks *(med; IQR)*	p
Alu-Gag PCR	109.5 *(58.5; 286)*	98 *(62; 377)*	0.08
Tat/rev TILDA	8.00 *(4; 27.5)*	6.00 *(4; 22.5)*	n.s.
HIV p24 SIMOA	55.5 *(7.5; 124.3)*	14.4 *(5.0; 60.2)*	n.s.
2-LTR ddPCR	1.4 *(1.4; 61)*	1.4 *(1.4; 1.4)*	n.s.
HIV Pol ddPCR	959 *(39; 2776)*	994 *(326; 1946)*	n.s.
GALT ISH	2.4 *(0.7; 12.4)*	0.8 *(0.7; 3.2)*	0.012

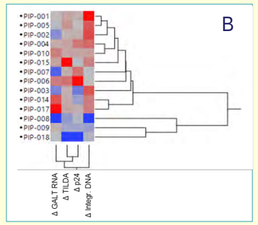


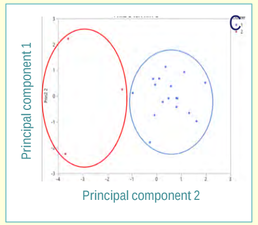


LEFT PANEL: Viral measurements were assessed at baseline and endpoint (20 weeks on treatment, median and interquartile ranges). Changes over time were assessed using the Wilcoxon signed rank test. A significant change in these variables was observed only in the number of HIV-1 RNA-positive cells in GALT biopsies. We also observed a decline trend in integrated HIV DNA (Alu-Gag PCR, p=.08). No significant change between time points was observed for the other viral measurements. CENTER PANEL: adjacent sections of colorectal mucosa biopsies, were used to assess the location of CD4+ T cells (A); and CD68+ macrophages (B) by immunohistochemistry, and to detect HIV RNA (full length) by in situ hybridization at baseline (C, magnified in E) and endpoint (D, magnified in F). Results suggest that a) HIV RNA+ cells are detected in GALT derived from ART-suppressed individuals, mostly outside typical T cell zones, and b) 20 weeks of peg-IFN-α2b exposure lead to a significant decrease in HIV-1 RNA positivity. RIGHT PANEL: a strong correlation was observed between baseline values and change over time in treatment for GALT RNA, CD4+ T cell TILDA and cell-associated GALT 2 LTR DNA, suggesting that a meaningful change in these variables would be observed mostly in subjects that have relatively high initial values.

4. Observed patterns of change in HIV measures







To test the hypothesis that the antiviral response to peg-IFN-α2b treatment was limited to a discrete group of subjects in our cohort (i.e.: responders vs. non-responders), we assessed:

1. The correlation between the change over time (delta) in 5 of the HIV latent reservoir markers (see panel 3): a Spearmen test indicated that only TILDA DNA and p24 were significantly correlated, as anticipated since both tests are derived from the same in vitro stimulation assay (panel 4A).
2. Hierarchical clustering based on measures of latent HIV reservoir: this was performed based on a minimal set of 4 variables (addition of 2LTR and/or Pol did not improve the clustering). Based on this test we identified 3 subjects that clustered separately from the others (panel 4B; see also PC analysis in panel C). However, there were no significant differences in latent reservoir variables, clinical labs, baseline values or ability to suppress HIV replication at week 4 of ATI between clusters.
3. The relationship between viral control on ATI and changes in markers of HIV replication or latent reservoir: no significant difference was detected between individuals who suppressed to < 50 copies/ml during ATI and those who became viremic (not shown).

Conclusions

We have assessed the effect of 20-weeks exposure to peg-IFN-α2b (PegIntron), coupled with a brief ATI, on measures of latent HIV reservoir in 18 ART-suppressed individual with chronic HIV-1 infection.

- Our results indicate that treatment with peg-IFN-α2b:
  - was safe and tolerable.
  - resulted in the control of viral replication to < 50 copies/ml during a 4-week ATI in 53% of the study subjects, similar to peg-IFN-α2a (67%), and significantly different from historical studies in subjects undergoing ATI without type-1 IFNs .
  - resulted in significant decrease or complete loss of RNA-positive cells in the GALT was the most clearly affected,
  - resulted in a trend in loss of integrated HIV DNA in circulating CD4+ T cells, consistent with our previous reports.
  - did not significantly alter other viral measures.
- High baseline levels were associated with greater change over time for three of the variables assessed. Further testing in larger cohorts with multiple time point assessments will be required to confirm this finding.
- We did not observe significant correlations between independent HIV latency/replication measures in tissue or PBMC.
- We conclude that eradication studies are currently best monitored by assessing multiple HIV latency and replication measures.

Acknowledgements:
We thank Mr. K. Lynn (Univ. of PA Medical Center), Ms. A. Kapalko and L. Lalley-Chareczko (Philadelphia FIGHT), and all Study Participants for their invaluable contribution to this study.
This study was supported by: NIAID grant U01AI110434 to L. Montaner, the BEAT-HIV Delaney Collaboratory UM1AI126620, co-funded by NIAID, NIMH, NINDS, and NIDA ; The Philadelphia Foundation Robert I. Jacobs Fund; Core services and support from the Penn Center for AIDS Research (CFAR), an NIH-funded program (P30 AI 045008); Merck, Inc;

References

1. Stein J, Storcksdieck Genannt Bonsmann M, Streeck H. Barriers to HIV Cure. HLA. 2016;88(4):155-63. doi: 10.1111/tan.12867. PubMed PMID: 27620852.
2. Delagreverie HM, Delaugerre C, Lewin SR, Deeks SG, Li JZ. Ongoing Clinical Trials of Human Immunodeficiency Virus Latency-Reversing and Immunomodulatory Agents. Open Forum Infect Dis. 2016;3(4):ofw189. doi: 10.1093/ofid/ofw189. PubMed PMID: 27757411; PubMed Central PMCID: PMCPMC5066458.
3. Spivak AM, Planelles V. HIV-1 Eradication: Early Trials (and Tribulations). Trends Mol Med. 2016;22(1):10-27. doi: 10.1016/j.molmed.2015.11.004. PubMed PMID: 26691297.
4. Cillo AR, Mellors JW. Which therapeutic strategy will achieve a cure for HIV-1? Curr Opin Virol. 2016;18:14-9. doi: 10.1016/j.coviro.2016.02.001. PubMed PMID: 26985878.
5. Rasmussen TA, Tolstrup M, Sogaard OS. Reversal of Latency as Part of a Cure for HIV-1. Trends Microbiol. 2016;24(2):90-7. doi: 10.1016/j.tim.2015.11.003. PubMed PMID: 26690612.
6. Pantaleo G, Levy Y. Therapeutic vaccines and immunological intervention in HIV infection: a paradigm change. Curr Opin HIV AIDS. 2016;11(6):576-84. doi: 10.1097/COH.0000000000000324. PubMed PMID: 27607591.
7. Stephenson KE, Barouch DH. Broadly Neutralizing Antibodies for HIV Eradication. Curr HIV/AIDS Rep. 2016;13(1):31-7. doi: 10.1007/s11904-016-0299-7. PubMed PMID: 26841901; PubMed Central PMCID: PMCPMC4791134.
8. Hutter G. Stem cell transplantation in strategies for curing HIV/AIDS. AIDS Res Ther. 2016;13(1):31. doi: 10.1186/s12981-016-0114-y. PubMed PMID: 27625700; PubMed Central PMCID: PMCPMC5020531.
9. Dey R, Pillai B. Cell-based gene therapy against HIV. Gene Ther. 2015;22(11):851-5. doi: 10.1038/gt.2015.58. PubMed PMID: 26079406.
10. Massanella M, Richman DD. Measuring the latent reservoir in vivo. J Clin Invest. 2016;126(2):464-72. doi: 10.1172/JCI80567. PubMed PMID: 26829625; PubMed Central PMCID: PMCPMC4731179.
11. Azzoni L, Foulkes AS, Papasavvas E, Mexas AM, Lynn KM, Mounzer K, et al. Pegylated Interferon-alpha2a mono-therapy results in suppression of HIV-1 replication and decreased cell-associated HIV DNA integration. J Infect Dis. 2012. Epub 2012/10/30. doi: 10.1093/infdis/jis663. PubMed PMID: 23105144.
12. Mexas AM, Graf EH, Pace MJ, Yu JJ, Papasavvas E, Azzoni L, et al. Concurrent Measures Of Total And Integrated HIV DNA Monitor Reservoirs And Ongoing Replication In Eradication Trials. Aids. 2012. Epub 2012/09/26. doi: 10.1097/QAD.0b013e32835a5c2f. PubMed PMID: 23014521.
13. Procopio FA, Fromentin R, Kulpa DA, Brehm JH, Bebin AG, Strain MC, et al. A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals. EBioMedicine. 2015;2(8):874-83. doi: 10.1016/j.ebiom.2015.06.019. PubMed PMID: 26425694; PubMed Central PMCID: PMCPMC4563128.
14. Lee SA, Bacchetti P, Chomont N, Fromentin R, Lewin SR, O'Doherty U, et al. Anti-HIV Antibody Responses and the HIV Reservoir Size during Antiretroviral Therapy. PLoS One. 2016;11(8):e0160192. doi: 10.1371/journal.pone.0160192. PubMed PMID: 27483366; PubMed Central PMCID: PMCPMC4970722.
15. Yuan Z, Kang G, Ma F, Lu W, Fan W, Fennessey CM, et al. Recapitulating Cross-Species Transmission of Simian Immunodeficiency Virus SIVcpz to Humans by Using Humanized BLT Mice. J Virol. 2016;90(17):7728-39. doi: 10.1128/JVI.00860-16. PubMed PMID: 27307566; PubMed Central PMCID: PMCPMC4988171.
16. Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, et al. Glycerol monolaurate prevents mucosal SIV transmission. Nature. 2009;458(7241):1034-8. doi: 10.1038/nature07831. PubMed PMID: 19262509; PubMed Central PMCID: PMCPMC2785041