Baseline inducible HIV P24 informs viral control during interferon-α monotherapy

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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 prior studies (11) support that administration of pegylated interferon alpha results in viral suppression and reduction in integrated proviral HIV DNA in ART-suppressed subjects undergoing analytical ART interruption (ATI). We explored the relationships between HIV latent reservoir measurements and viral control off ART, with or without immunotherapy, in a subset of participants enrolled in the BEAT-HIV study (NCT02227277)

Methods

13 individuals randomized to arm 1 of **NCT02227277** (HIV VL < 50 copies/ml on ART, CD4 count > 450/µl) receiving 1 µg/kg of peg-IFNα-2b (Pegintron, Merck, Inc.) for 20 weeks, interrupting ART at week 4 and resuming it upon reaching VL > 50 copies/µl, or at week 20.

P24 SIMOA (iP24) was measured in CD4+ T cells cultured for 16-hour with medium or PMA/Ionomycin using single-molecule array (SIMOA, 13).

IPDA. Intact, 5' defective, 3' defective and total proviral DNA were measured by Accelevir, Inc. on CD4+ T cells.

Integrated HIV proviral DNA was assessed using Alu-gag RT-PCR on CD4+ T cells. Time to viremia: days to first VL > 50 copies/ml off ART.

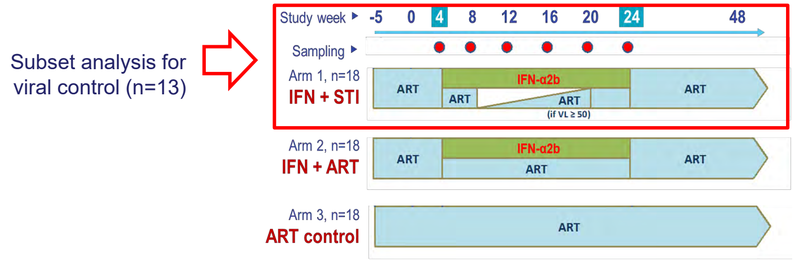
HIV-specific responses:

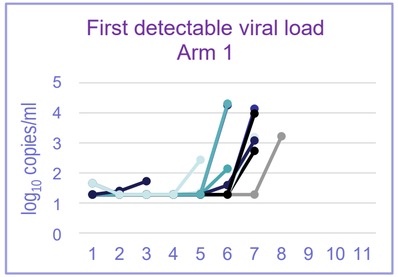
a) *T cells*: 6-hour cultures of PBMC with 15-mer gag peptides
b) *NK cells:* 4-hour co-cultures with anti- HIV sera with gp120-coated CEM NK^{res} targets (ADCC surrogate).

Multicolor flow cytometry was used to assess HIV-specific degranulation cytokine production.

Statistical analysis. Associations between variables were tested with Pearson or Spearman tests, and/or linear regression models.

1. Origin of the study population: the BEAT-HIV study





13 of the 18 participants enrolled in arm 1 of NCT02227277 (BEAT-HIV study) with available cryopreserved PBMC were assessed. All individuals analyzed became viremic during ART interruption (monotherapy with peg-IFN-α2b), and were assessed for correlates to viral measures (see section 2)

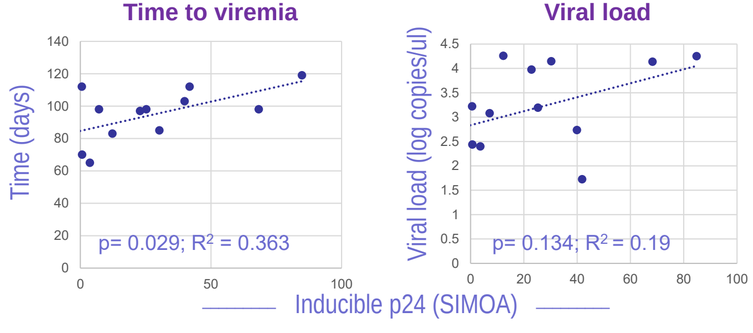
3. Association of baseline (on ART) viral measures with immune parameters

Baseline inducible HIV p24 and intact proviral DNA were associated ($p<0.05$) with T cell responses to HIV Gag peptides and NK cell gp120/Ab-dependent expression of intracellular cytokines (ADCC surrogate). in a subset of 8 participants

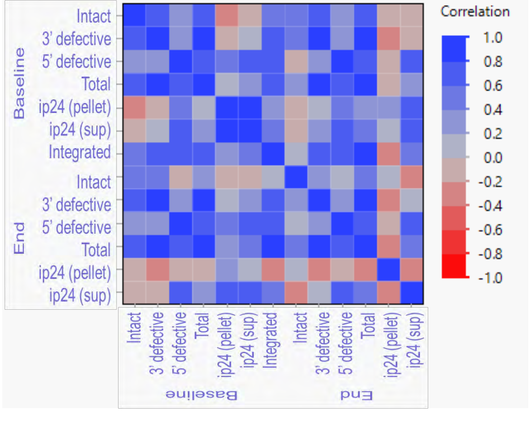
Cell type	Stimulation	Var. 1	Var. 2	Spearman r	P
T cells	HIV Gag 15-mer	i-p24 (SIMOA)	CD4+/Memory/CD107a+ Perf+	-0.7563	0.0414
			CD8+/Memory/CD107a/TNFa	-0.9027	0.0054
			CD8+/Memory/CD107a+ TNFa+	-0.7483	0.0429
		Intact Provirus (IPDA)	CD8+/Memory/CD107a/HLA-DR	0.7568	0.0351
			CD8+/Memory/CD107a/MIP-1b	-0.8649	0.022
			CD8+/Memory/CD107a+ MIP+	-0.7357	0.05
			CD8+/Memory/HLA-DR	0.75	0.0399
			CD8+/Memory/Ki67	0.9459	0.003
			CD8+/Memory/MIP-1b	-0.7702	0.0375
NK cells	anti-HIV Ab + gp120-coated CEM	i-p24 (SIMOA)	CD57+/CD107a+/CD107a+IFNg+	-0.8571	0.0107
			CD57-/CD107a+/CD107a+ MIPb+	0.8383	0.0131

2. Association of baseline (on ART) inducible HIV p24 production viral suppression

Baseline (week 0) inducible HIV p24 was positively associated with time to viremia and the first viral load detected during peg-IFN-α monotherapy. A positive association was also observed between the change (endpoint-baseline) in intact (p=0.048) or total (p= 0.007) provirus (IPDA) and the first detected VL. Other baseline PCR-based viral measures were not associated with time to viremia or first VL measured.



4. Associations between reservoir measures



As anticipated, we observed a number of significant correlations between proviral DNA measures across time points and study arms. However, we did not detect any significant correlation between time to viremia and baseline IPDA or integrated DNA.

Conclusions

We analyzed a group of HIV-infected individuals undergoing a period of ART interruption and peg-IFN-α2b monotherapy.

- Our data indicate that the baseline, pre-treatment amount of inducible HIV-1 p24 is associated with the time to viral control.
- Individuals with low inducible p24 experienced viremia earlier, suggesting that immune mechanisms triggered by p24 expression may be involved, and may be enhanced by peg-IFN-α2b, leading to increased viral control over time.
- The involvement of immune effectors, observed in our previous studies (14), is supported by the detected relationship between baseline inducible p24 or intact proviral DNA with antigen specific T cell responses and NK cell-dependent ADCC-related activity..

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