Post-Treatment Control is Associated with Unique Proteomic Signature before and during ATI

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Background

- Post-treatment controllers (PTCs) are people with HIV (PWH) who achieved low/no viremia in analytical treatment interruption (ATI).
- Multiple mechanisms could potentially account for PTC.
- In this study, we leveraged ACTG ATI studies to evaluate proteomic signatures of PTCs and non-controllers (NCs) before and during ATI.

Methods

- Cohorts and participants: We included participants from historic ACTG cohorts (Control of HIV After Antiretroviral Medication Pause [CHAMP] Study[1]) and A5345 cohorts[2] where participants were treated with contemporary antiretroviral therapy (ART).
- Definition for PTC:
  - Remained off ART for ≥24 weeks
  - Maintained viral loads ≤400 copies/mL for at least two-thirds of the time points.
- Proteomics: Olink Inflammatory I and Flex panels to measure 381 proteins in cryopreserved plasma.

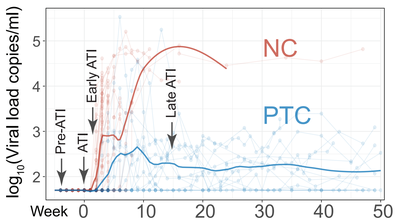


Figure 1. Viral load before and during ATI.

Time points:

Pre-ATI: Before stopping ART

Early ATI: 0-4 weeks after ATI initiation. Before or at early phase of viral rebound

Late ATI: Late phase of viral rebound before ART re-initiation

- Statistics: Multivariate linear models using protein levels as dependent variables and PTC status, age, sex, acutely/early-treated HIV as independent variables.
  - Proteomic changes within groups between different time points compared using paired T-test.
  - False detection rate (FDR) was calculated with the Benjamini-Hochberg method.
  - We used Gene Set Enrichment Analysis (GSEA) to evaluate differential pathways, using Hallmark and GOBP as reference.
  - We used Elastic Net for feature selection and sparse Partial Least Squares Discriminant Analysis (sPLS-DA) to illustrate and select final groups of proteins that can distinguish PTC from NC.

Results

- Participants

Characteristics	Overall N = 40	NC N = 25	PTC N = 15	P value
ART initiation timing, n (%)				0.4
Acute/Early	15 (38)	8 (32)	7 (47)	
Chronic	25 (63)	17 (68)	8 (53)	
Age, median (IQR)	41 (34, 47)	42 (35, 49)	39 (29, 42)	0.13
Sex, n (%)				0.3
Female	3 (7.5)	3 (12)	0 (0)	
Male	37 (93)	22 (88)	15 (100)	

IQR, interquartile range; ART, antiretroviral therapy.

Conclusions

- **PTCs and NCs had distinct proteomics pattern even before ATI.**
- **In Early ATI, PTCs upregulated IFN-β response before viral rebound.**
- **In Late ATI, NCs upregulated complement, coagulation and IFN-γ response, after viral rebound already occurred.**
- **Early coordinated innate and adaptive immune responses differentiated PTCs from NCs.**

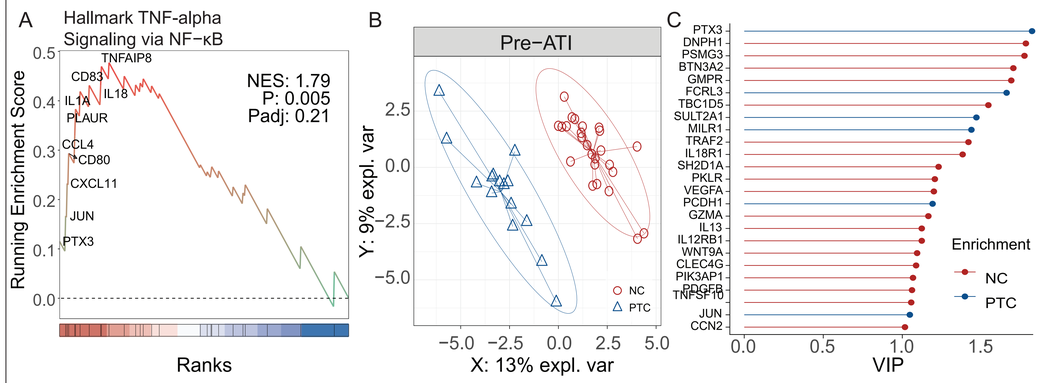


Figure 2. PTCs and NCs had distinctive proteomics pre-ATI. There was no significant differentially expressed proteins (DEPs) after multiple comparison adjustment (Padj>0.25). (A) PTC is associated with up-regulated TNF-α signaling. (B) Pre-ATI proteomics could distinguish PTCs from NCs after Elastic Net selection and sPLS-DA projection. (C) Proteins with Variable importance in projection (VIP)>1.0. NES, normalized enrichment score. Viral load was suppressed in all participants.

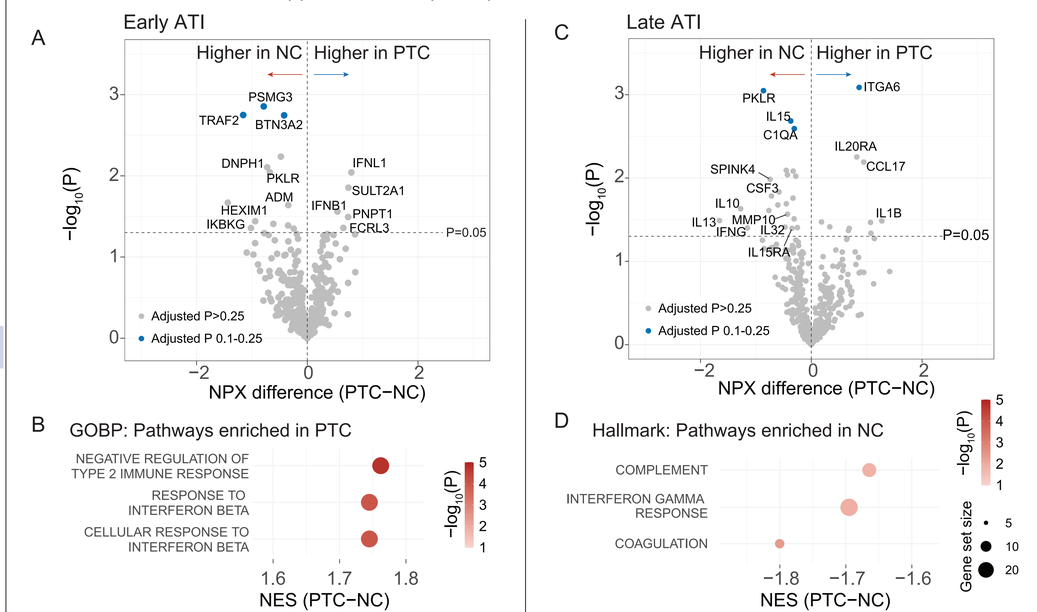


Figure 3. DEPs and differentially regulated pathways in early and late ATI. (A) DEPs in early ATI. Of note, median VL in PTCs and NCs were both <50 copies/ml. 4% vs. 0% in NCs vs. PTCs had VL>1000 copies/ml. (B) PTCs showed upregulated Interferon (IFN) β pathway signals compared to NC. No significant Hallmark pathways in early ATI. (C) DEPs in late ATI. Median VL in PTCs and NCs were 62 and 8365 copies/ml, respectively. (D) Complement, IFN-γ, and coagulation pathways were upregulated in NCs. No significant GOBP pathways in late ATI. Only pathways with Padj<0.05 were included.

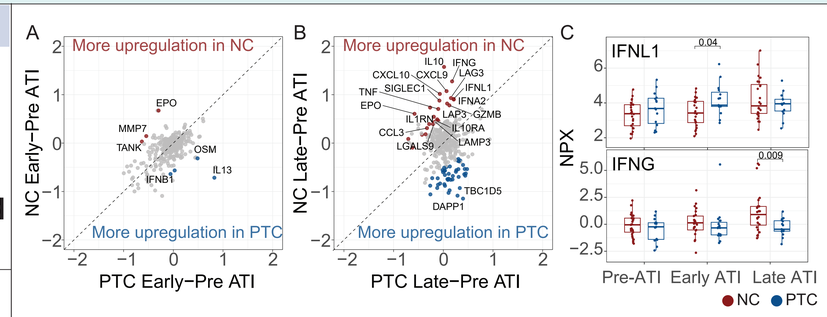


Figure 4. Longitudinal proteomic changes. Protein level changes in early ATI (A) and and late ATI (B) compared to pre-ATI. Only proteins with $|\Delta NPX_{PTC}-\Delta NPX_{NC}|>0.5$ and P<0.05 were labeled with color codes. Compared to PTCs, NCs had type I and II IFN, TNF, as well as immune regulatory proteins (IL10, IL10RA, LGALS9) upregulated. (C) Differential temporal trends of IFN-λ and IFN-γ in PTCs and NCs.

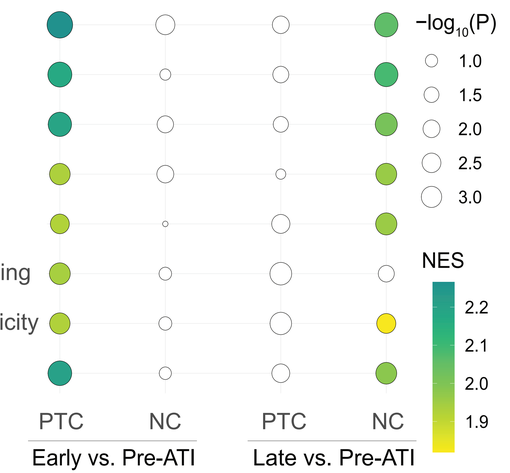


Figure 5. Temporally differentially regulated pathways in PTCs and NCs. PTCs up-regulated T cell activation and NK killing pathways early in ATI compared to pre-ATI while NCs did not upregulate these pathways until late in ATI, while PTCs already downtregulated these pathways after reaching ART-free viral control.

Limitations

- Limited sample size.
- Require validations from other cohorts.

References

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