The Plasma Cell-Free DNA Methylome Predicts Intact HIV Reservoir Decay During Antiretroviral Therapy

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Genome-wide methylation of cell-free DNA reliably predicts latent HIV reservoir decay during ART.
Decay of the latent reservoir is likely an epigenetically regulated process driven by the silencing of host molecular pathways.
Cell-free DNA "liquid biopsy" may be a convenient, non-invasive method to determine the efficacy of HIV cure strategies.

BACKGROUND

- Antiretroviral therapy (ART) fails to cure HIV due to the persistence of viral reservoirs that fuel rapid HIV rebound upon treatment interruption.
- A recent study of people with HIV (PWH) on ART demonstrates that plasma Galectin-9 levels predict intact HIV reservoir decay[1,2], reflecting immune processes that influence reservoir persistence.
- Building on the oncology-derived liquid biopsy concept, we recently revealed that plasma cell-free DNA (cfDNA) features predict HIV rebound kinetics and post-treatment control following ART cessation[3,4].
- Here, we profiled the plasma cfDNA methylome to gauge its potential as a biomarker to track HIV reservoir decay and guide cure strategies.

METHODS

- Longitudinal plasma samples (n=203) were collected retrospectively from 73 people with HIV (PWH) on ART enrolled in the UCSF SCOPE cohort who maintained viral suppression for >2 years.
- The HIV reservoir was measured by intact proviral DNA assay (IPDA), and reservoir decay rates were computed using linear spline models[1,2].
- Whole-genome enzymatic methyl sequencing was performed on cfDNA (Fig. 1).

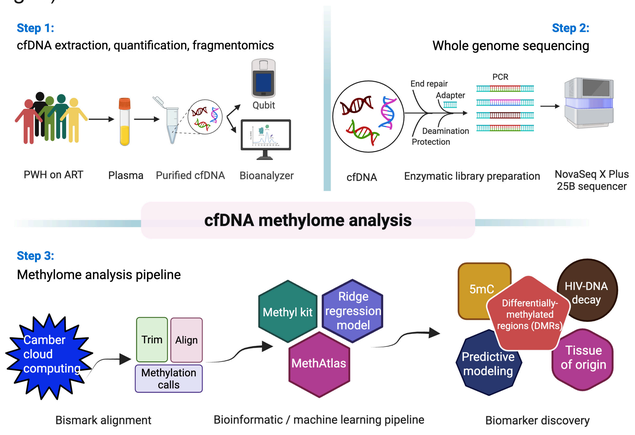


Fig. 1. Schematic framework of methodology.

RESULTS

- **The cfDNA size distribution shows mono, di, and tri-nucleosome bound DNA generally observed in plasma (Fig. 2).**

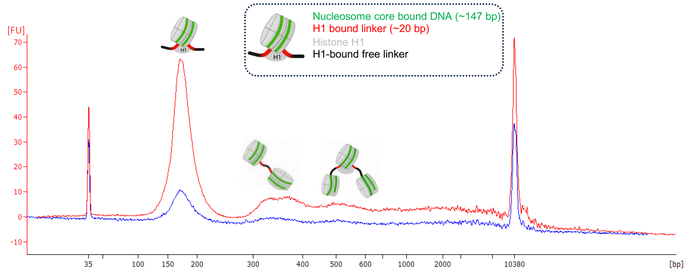


Fig. 2. Typical cfDNA fragment size distribution in Bioanalyzer.

- **Plasma cfDNA methylation patterns are strongly associated with latent HIV reservoir decay during ART, and differentially-methylated regions include established HIV host factors and cell morphogenesis / survival programs (Fig. 3).**

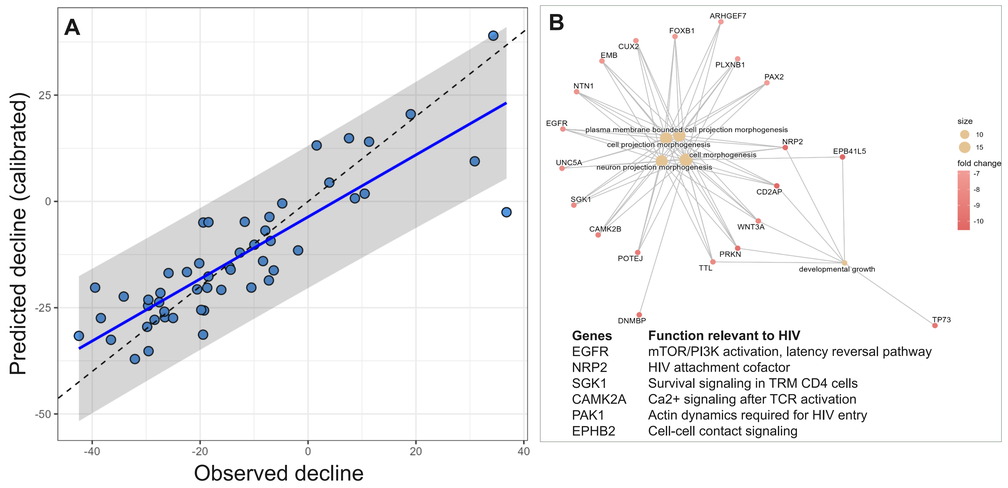


Fig. 3. Associations between the cfDNA methylome and HIV reservoir decay *in vivo*. A) A ridge regression (machine learning) model using genome-wide DNA methylation features (115,646 CpG motifs) predicts intact HIV DNA decay rates in CD4+ T cells (R^2 = 0.73, p = $1.75e^{-13}$, root mean-squared error (RMSE) = 9.43 post calibration and 5-fold cross validation). B) The negative normalized enrichment scores (NES< -2.3) reveal a coordinated downregulation of pathways. The dominant modules involve mTOR signaling and cell morphogenesis driven by cytoskeletal regulators, consistent with epigenetic control of cell–cell interaction and survival programs relevant to HIV persistence.

- **Plasma cfDNA exhibits a "set point" during ART, but cfDNA abundance and fragmentome do not associate with latent HIV reservoir decay (Fig. 3).**

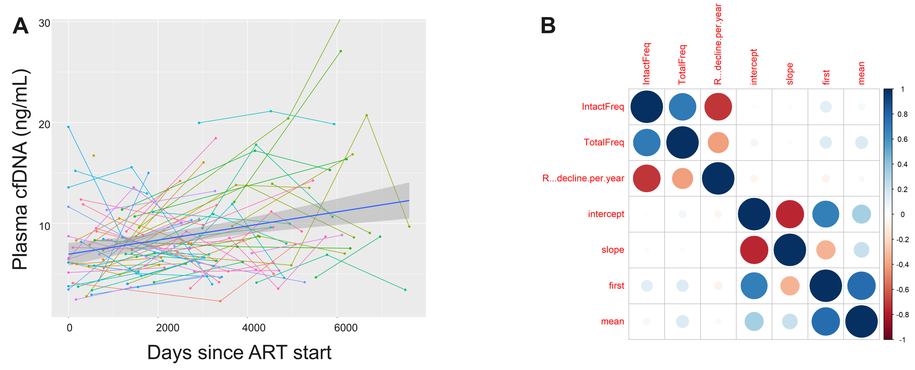


Fig. 4. Dynamics of plasma cfDNA during ART and exploratory associations with the latent HIV reservoir. A) During ART, plasma cfDNA in PWH exhibits a distinct "set point" over time; within-subject variation was significantly lower than between-subject variation (mean standard error 9.9 vs 19.1, P<0.01, Kruskal-Wallis test). B) Plasma cfDNA abundance-derived measures do not correlate with latent HIV reservoir size or decay (Pearson's test).

- **Plasma cfDNA in PLWH on ART is largely derived from innate immune cells (Fig. 5).**

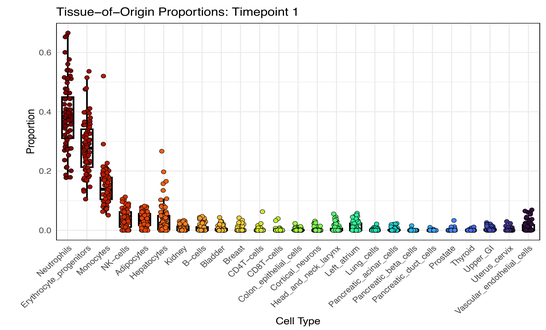


Fig. 5. Methylome-based prediction of cfDNA tissue of origin. The MethAtlas program utilizes the methylation ratios of CpG sites to perform cell type deconvolution and infer tissue of origin. The analysis was run using a 25 tissue, 7,000 CpG site matrix and the hg38 human genome.

CONCLUSIONS

- Plasma cfDNA genome-wide DNA methylation features enable accurate prediction of HIV reservoir decay rates during ART.
- Coordinated silencing of host morphogenesis, cytoskeletal, and cell survival pathways may modulate HIV reservoir decay.
- cfDNA tissue of origin likely reflects cell turnover within each anatomic compartment, and may enable non-invasive assessment of HIV reservoir distribution during ART.
- The relationship between epigenetic aging (inferred via cfDNA methylome analysis) and HIV persistence should be investigated.
- The cfDNA methylome should be explored as a biomarker in HIV cure-focused clinical trials.

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ADDITIONAL INFORMATION

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