A Subset of People With HIV Continue to Have Decay in Intact Proviral DNA During Long-Term ART

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BACKGROUND

- Understanding intact proviral DNA (IPD) changes in people with HIV (PWH) on antiretroviral therapy (ART) informs HIV remission strategies.
- Studies have shown initial decreases in IPD in the first years after ART initiation (half-life of ~1-2 years; *Gandhi RT et al. OFID 2025*).
- IPD decay slows following an inflection point at ART year 4-7 *(Peluso M et al. JCI Insight 2020; Gandhi RT et al. JID 2021).*
- We aimed to identify factors associated with IPD levels and changes on long-term ART, examining:
  - Age, Sex, Low-level Viremia
  - Senescence-Associated Secretory Phenotype (SASP) Biomarkers

METHODS

- 55 participants with documented long-term HIV suppression followed in the ACTG A5321 cohort had proviral DNA (intact, defective, total) measured by IPDA® at 4-5 time points from years 5 to 25 on ART.
- Post-year 5 of ART time points are after the initial IPD decay phase
- SASP biomarkers in plasma were measured at IPD time points (*Li Y et al. AIDS, 2025*)
- Plasma HIV RNA by single copy assay (SCA) was measured at the first and last IPD time points.
- By-participant modeling of log-transformed IPD with censored-data linear regression. Rank-based analyses of estimated year 5 IPD levels and subsequent changes (Spearman, Wilcoxon).

RESULTS

- 55 participants (27% female, 29% Black). Pre-ART median age: 43 yrs (range 22-60; IQR 32-48). Median CD4 at first IPD: 641 cells/mm^3
- **IPD levels declined slowly after year 5 of ART (median half-life 8.6 years) whereas defective and total proviruses remained stable.**
- **Different patterns of IPD decay were observed:**
  - 32 participants (58%) had IPD slopes significantly different than zero and negative (indicating IPD decay; half-life 5.6 yrs).
  - 4 participants with rapid decay (**Figure 1**) had IPD half-life of 3.3 yrs (8.3-fold reduction over 10 years).
  - 23 participants (42%) did not have significantly negative slopes; most had flat or minimal changes in IPD (**Figure 1**).
  - 4 participants had increases in IPD during long-term ART (two described in *Brandt L et al. HIV Persistence Workshop 2024*)

Intact proviral DNA continues to decline in some PWH over 25 years of ART.

Aggregate analyses can mask these intact proviral DNA patterns.

Determining mechanisms of continued IPD decay is a priority.

Intact Proviral DNA Patterns on Long-Term ART

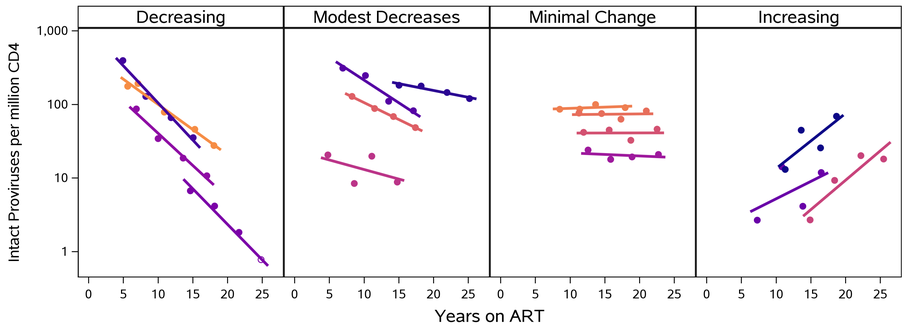


Figure 1 – Longitudinal trajectories of IPD in PWH on long-term ART measured by IPDA®

Intact proviral DNA was expressed as $\log_{10}$ intact proviruses per million CD4$^+$ T cells over years on suppressive ART. Each color represents an individual participant with repeated longitudinal measurements. Filled circles denote detected intact proviruses, and open circles indicate values below the limit of detection. Solid lines represent participant-specific linear trends over time. Example participants are grouped by overall longitudinal pattern into *Decreasing* (n=4), *Modest Decreases* (n=4), *Minimal Change* (n=4), and *Increasing* (n=3) categories.

Males have Higher Intact Proviral DNA at ART year 5, and Greater Subsequent Decline in IPD

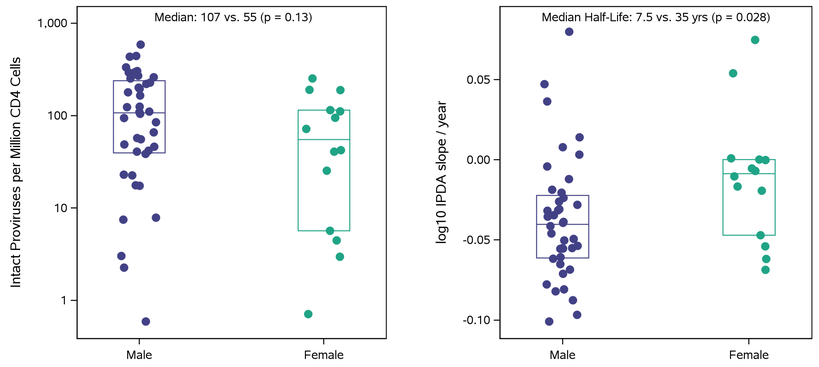


Figure 2 – Sex-based differences in intact proviral DNA levels and decay kinetics in PWH on long-term ART.

Left panel: Cross-sectional intact proviral DNA levels measured by IPDA® and expressed as $\log_{10}$ intact proviruses per million CD4$^+$ T cells in males and females on long-term suppressive ART. Each point represents an individual; box plots show the median and interquartile range.

Right panel: Longitudinal decay rates of IPD expressed as the $\log_{10}$ IPDA slope per year, derived from participant-specific linear models. Points represent individual participants, with box plots indicating the median and interquartile range.

RESULTS (Continued)

- **At year 5 of ART, males had higher IPD than females** (median 107 vs. 55 proviruses/million CD4 cells, p=0.13; **Figure 2**).
- **Subsequent IPD decay was faster in males** than females (half-life 7.5 vs. 35 years, p=0.028) **(Figure 2).** 65% of males vs. 40% of females had significantly negative IPD slope.
- Plasma HIV RNA by single copy assay (SCA) was positively correlated with IPD level (r=0.50, p<0.001, first time point, not shown).
- Levels of SASP biomarkers, MMP-2 and MMP-9, at the initial time point did not correlate with IPD level or with IPD slope. MMP-2 and MMP-9 levels did not correlate with age (not shown).

CONCLUSIONS

- IPD decays slowly in most PWH on long-term ART, with an overall half-life of 8.6 years.
- Aggregate data can mask substantial differences in IPD decay across individuals followed longitudinally up to 25 years on ART.
- While some PWH have minimal change in IPD after year 5 of ART, a subset continue to have significant IPD decay during long-term ART.
- Some people have increases in IPD, likely because of clonal expansion of infected cells
- Sex, but not age, associated with IPD decay. Females may have more quiescent HIV reservoirs than males during long-term ART (Tan TS et al, STM, 2025), which might explain why IPD decay is slower in females.
- **Determining why some people have continued IPD decay is a high priority to inform future HIV remission strategies.**

PLAIN LANGUAGE SUMMARY

People with HIV suppressed on treatment have HIV in their cells which remains despite >25 years of ART. Women and men differ in both levels and changes over time. Understanding why some people have larger decreases in HIV in their cells during ART can inform strategies to achieve HIV remission.

ADDITIONAL KEY INFORMATION

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Acknowledgement: Supported by NIH/NIAID UM1 AI068634, AI068636, AI106701, and a supplemental award from NIH/National Institute of Aging (NOT-AG-23-008)

We extend our sincere thanks to the study participants for their dedication and follow-up over 25 years. Thanks to the parent study teams, all the ACTG sites and staff, and NIH/NIAID and NIA for funding. We thank Sadie Cowan for her assistance.