LONGITUDINAL CHARACTERIZATION OF HIV PROVIRUSES IN PEOPLE ON SUPPRESSIVE ART

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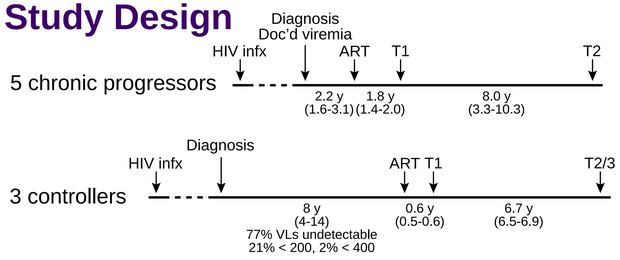
Background

- Clonal proliferation of HIV-infected cells is a major contributor to viral persistence in people on ART.
- Clones can harbor intact or defective HIV proviruses.
- Indirect evidence supports cytotoxic T lymphocyte (CTL) recognition of cells harboring both defective and intact proviruses.
- If this occurs, the efficacy of shock and kill therapeutics may be diluted by the excess of defective proviruses.

Central Questions

- Do CTLs shape the HIV proviral landscape over time in suppressed patients?
- How does clonal proliferation shape the HIV provirus landscape over time on ART?

Study Design



Chronic progressors: Undetectable viral loads checked at least every six months, with minor exceptions. Avg 57 provirus sequences/sample. 1 woman, 4 men, all cisgender.

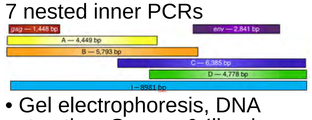
Controllers: Included due to presumed strong CTL responses. Did not lose natural control of virus prior to ART. Undetectable viral loads after ART start. Avg 13 provirus sequences/sample. 2 women, 1 man, all cisgender.

Methods

Cryopreserved PBMCs → Resting $CD4^+$ T cells

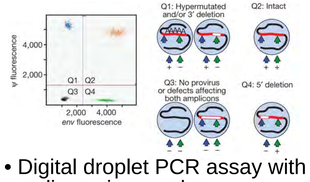
Near-full length provirus sequencing

Outer PCR at limiting dilution



- Gel electrophoresis, DNA extraction, Sanger & Illumina MiSeq, & hand-align sequences.
- Clone-finding by sequence identity.
- Identify dominant CTL epitopes in Gag, Pol, & Nef based on HLA type and published data. Classify whether provirus seq at epitope is wild-type, escaped, deleted.

Intact Proviral DNA Assay (IPDA)



- Digital droplet PCR assay with amplicons in ψ and *env*.
- Measures frequency of intact and defective proviruses.

Results

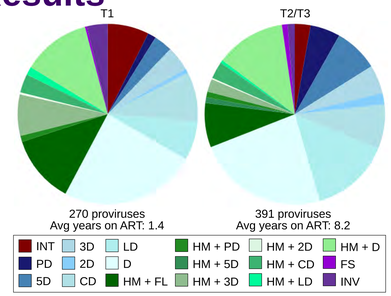


Fig. 1. Distribution of provirus types is similar early and late on suppressive ART.

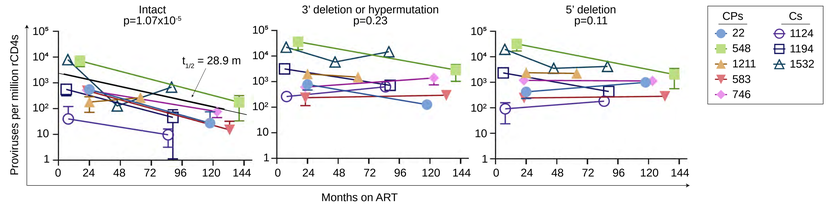


Fig. 2. IPDA demonstrates decay of intact proviruses during ART with half-life of 28.9 months.

Key for Fig. 1: INT - intact, PD - Ψ deletion, 5D/3D - deletion within 5'/3' half of genome, 2D - 2+ deletions, CD - central deletion, LD - deletion spans >75% of genome, D - unmapped deletion, HM - hypermutated, FS - full-length with 1+ frameshifts. INV - large deletion with inverted sequence.

Results

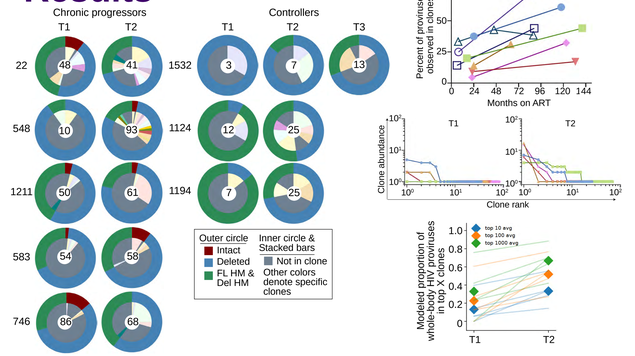


Fig. 6. HIV proviruses concentrate into extremely large clones over time on ART.

- During long-term viral suppression on ART:
  - Intact proviruses decay slowly.
  - Distribution of defective provirus types does not change dramatically.
  - No enrichment of escaped/unrecognized CTL epitopes seen.
  - HIV proviruses are concentrated into a small number of extremely large clones.
- Cells harboring intact proviruses appear as likely to clonally proliferate as cells harboring defective proviruses.
- Clones are not enriched in escaped/unrecognized CTL epitopes.

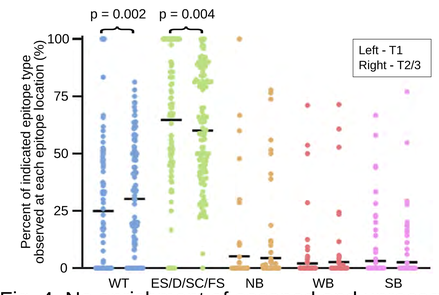


Fig. 4. No enrichment of escaped and unrecognized CTL epitopes with time on suppressive ART. WT - wild-type, ES - escaped epitope, D - deletion at epitope site, SC/FS - stop codon or frameshift between start codon and epitope. NB/WB/SB - nonbinder/weak binder/strong binder of uncharacterized sequence at epitope site to relevant HLA allele via NetMHC 4.0.

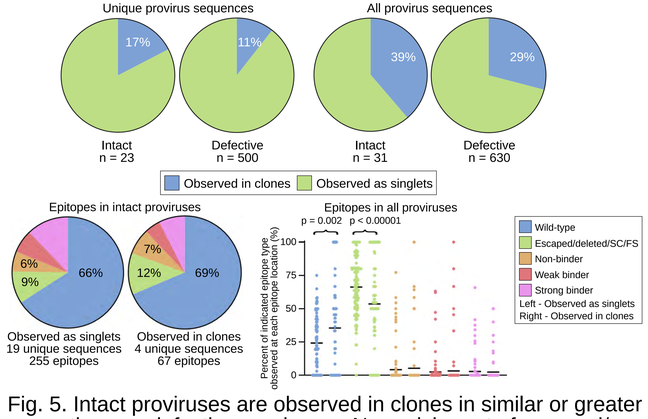


Fig. 5. Intact proviruses are observed in clones in similar or greater proportions as defective proviruses. No enrichment of escaped/unrecognized CTL epitopes in clones vs singlets.

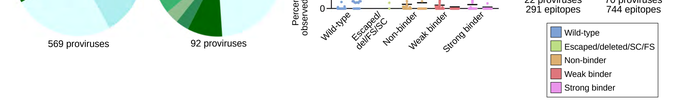


Fig. 7. Controllers on ART have a similar defective provirus landscape as progressors on ART. No enrichment of escaped or unrecognized CTL epitopes is seen in controllers on ART.

Conclusions

- During long-term viral suppression on ART:
  - Intact proviruses decay slowly.
  - Distribution of defective proviruses does not change dramatically.
  - No enrichment of escaped or unrecognized CTL epitopes occurs.
  - Proviruses become concentrated into a small number of extremely large clone families
- Cells harboring intact proviruses appear as likely to clonally proliferate as cells harboring defective proviruses.
- Clones are not enriched in escaped or unrecognized CTL epitopes.
- Controllers on ART have a similar defective provirus distribution as progressors on ART.
- Controllers on ART do not demonstrate enrichment of escaped or unrecognized CTL epitopes.

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