Translation-competent HIV reservoirs are highly expanded and phenotypically diverse

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Clonal expansion of **inducible** and **translation-competent** proviruses in latently infected CD4+ T cells contribute to the **phenotypic diversification** of HIV reservoirs.

BACKGROUND

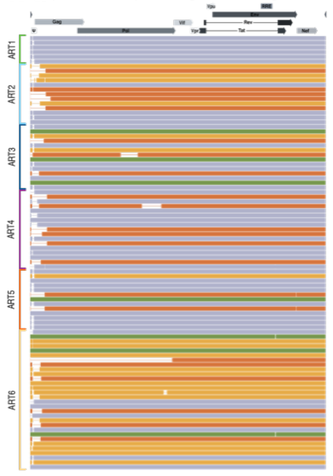
Understanding the dynamics of viral persistence in people living with HIV (PLWH) receiving ART is a key step to develop a cure for HIV. HIV persists as an integrated provirus in a small pool of latently infected cells. Of note, only a fraction of these cells produce viral products when activated. We combined HIV-Flow with a modified FLIPS assay **to assess the integrity and the clonality of translation-competent and inducible proviruses compared to non-induced genomes**.

METHODS

CD4+ T cells from 6 PLWH on ART were stimulated for 24h with PMA/Ionomycin

- **p24+ cells** were **individually index-cell sorted** using a panel of markers (CD45RA, CCR7, PD-1, TIGIT, ICOS, HLA-DR, α4, β1)
- **p24- bulk cells** containing non-induced proviruses were simultaneously sorted
- Individual proviruses from single-sorted p24+ cells and serial-diluted bulk p24- cells were **amplified by nested PCR and sequenced using PacBio**
- p24+ proviruses **integrity and clonality** were **associated with the expression levels** of each cellular marker

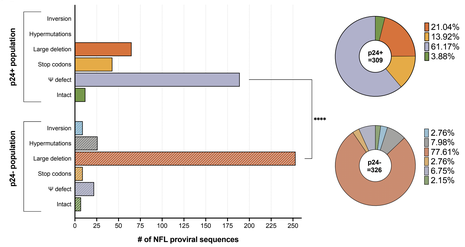
1A. p24+ cells - proviral genomes alignment



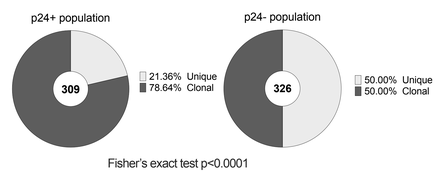
1B. p24- cells - proviral genomes alignment



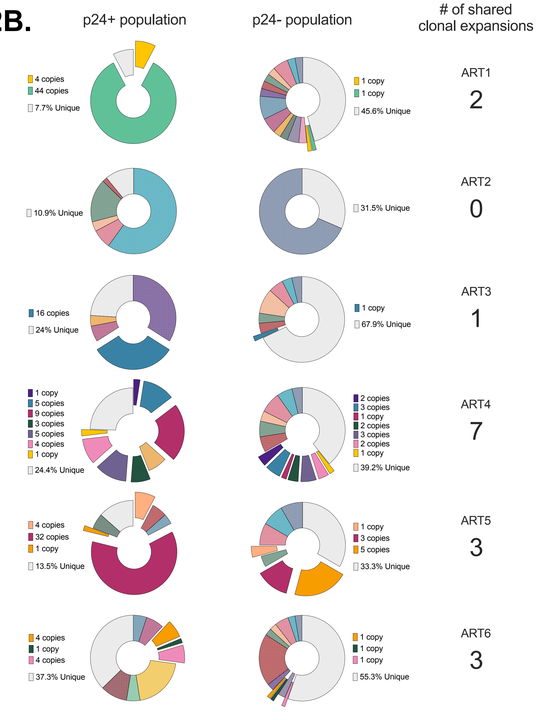
1C. Integrity of HIV genomes



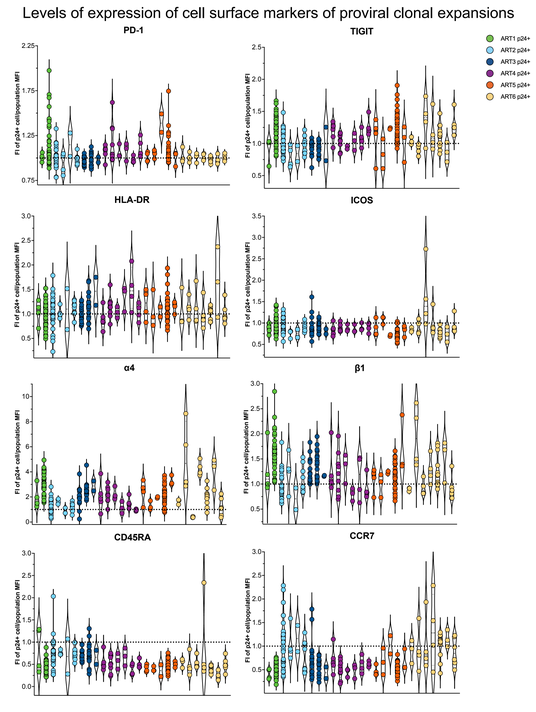
2A. Clonality of HIV genomes



2B.



3. Levels of expression of cell surface markers of proviral clonal expansions



RESULTS

We obtained 309 near-full length proviral sequences from single-sorted p24+ cells (**1A.**) and 326 from p24- bulk population **(1B.**) Despite their ability to express viral protein, 96% of the viral genomes obtained from p24+ single-sorted cells were defective, particularly in the packaging signal (64% of all defects), while the p24- bulk population included 98% of defective proviruses, mostly due to large internal deletions (79%) (**1C.**) This difference in the type of defects between p24+ and p24- cells was highly significant (p<0.0001).

Clonal expansions (cells sharing the exact same HIV genome) were more frequent (79% and 50%, respectively; p<0.0001) (**2A.**) and larger (41% and 11% of ≥5 copies per clones, respectively; p=0.0073) in p24+ cells compared to p24- cells. Importantly, identical proviruses shared between p24+ and p24- cells were observed in 5 out of 6 participants (**2B.**).

In p24+ cells, levels of expression of cellular markers greatly varied within a given proviral clone (**3.**)

CONCLUSIONS

Our results demonstrate that the **inducible and translation-competent reservoir** that persists during suppressive ART is **highly clonally expanded** and includes **only a small fraction of intact proviruses**. We also show that **proviral sequences are not the only driver of efficient reactivation** from latency. Finally, we highlight that identical proviruses are found in cells **displaying diverse phenotypes**, suggesting that **T cell proliferation**, which causes clonal expansion in the reservoir, **contributes** to the **phenotypic diversification** of the pool of **HIV reservoir cells.**

ACKNOWLEDGEMENTS

Chomont's lab
Nicolas Chomont
Rémi Fromentin
Amélie Pagliuzza
Alessandro Modica
Armando Espinosa Ortiz
Cesar Trifone
Isabelle Turcotte
Pierre Gantner

CRCHUM platforms
Dominique Gauchat
Philippe St-Onge
Gaël Dulude
Olfa Debbeche

Bioinformatics
Ben Murrell
Corentin Richard

McGill
Josée Girouard

U. of Sydney
Sarah Palmer
Bonnie Hiener

The study participants!

Université de Montréal
CRCHUM Centre de recherche
IRSC CIHR
NIH National Institutes of Health
CanCURE Together, we Can CURE HIV
Delivering AIDS Research Enterprise DARE to find a cure
Réseau SIDA/MI du FRQS
INNOVATION.CA Canada Foundation for Innovation / Fondation canadienne pour l'innovation