Characterizing the Intact and Defective Reservoir in a Large Cohort Living with Subtype C HIV

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Shorok Sebaa[1], Bianca Abrahams[1], Sherazaan Ismail[1], Sharana Mahomed[2], Nigel Garrett[2,5], Carolyn Williamson[1,4], Thomas Quinn[3], Andrew D. Redd[3], Melissa-Rose Abrahams[1,4]

[1]University of Cape Town, Cape Town, South Africa, [2]Centre for the AIDS Programme of Research in South Africa, Durban, South Africa, [3]Division of Intramural Research, NIAID, NIH
[4]Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa, [5]Desmond Tutu HIV Centre, Cape Town, South Africa

BACKGROUND

Antiretroviral therapy (ART) has significantly improved the lives of people living with HIV. However, a cure remains elusive due to the formation of a pool of latently infected cells, known as the latent reservoir. While most of the reservoir is defective, a small fraction is replication-competent and can give rise to viral rebound upon ART interruption. We characterized the proviral DNA reservoir in a cohort of South African women followed from pre-acquisition up to five years post-ART, using the Cross-Subtype Intact Proviral DNA Assay (CS-IPDA)[1].

METHODS AND EXPERIMENTAL DESIGN

- 283 Peripheral blood mononuclear cells (PBMC) samples from196 CAPRISA 002 cohort women with sustained viral suppression of at least 1.5 years were used (**Figure 1**).

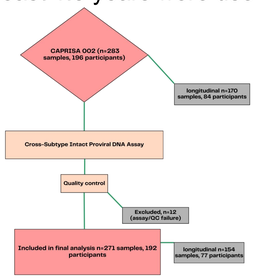


Figure 1. Flow diagram illustrating CAPRISA 002 sample processing and exclusion

- CS-IPDA was performed using minimally sheared genomic DNA. Digital droplet PCR (BioRad QX200) was used to conduct CS-IPDA assay with reaction mixes for HIV targets (**LTR-*gag***, ***pol*** and ***env***) and cellular reference genes (***RPP30*** and **deltaD**) (**Figure 2**).

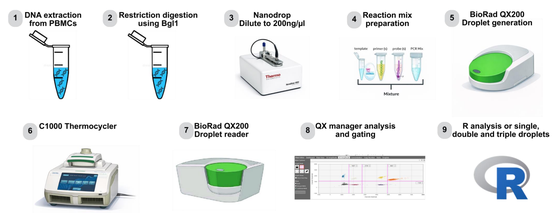


Figure 2. Experimental design for CS-IPDA protocol

- Spearman's rank correlation analysis was conducted along with univariate linear regression to compare intact proviral copies with cumulative viral load (AUC), nadir CD4 T-cell count, time from infection to ART initiation (in months), and time on ART (in months).

Cumulative viral load and CD4 nadir impact intact HIV reservoir size

RESULTS

Women were ART naïve for a median of 3.7 years (IQR:1.7–5.6). Among 271 samples analyzed, the median percentage of intact proviruses relative to total HIV copies/ 10^6 T cells was 0.73% (IQR: 0.00–3.17%). Intact reservoir measurements were below the limit of detection in 44.28% of samples, with ***gag*** most frequently undetected.

A positive correlation was observed between log-transformed cumulative viral load from HIV acquisition to ART initiation and intact proviral DNA copies/10^6 T cells (n=164, r = 0.43, $p < 0.001$) (**Figure 3a**). In a univariate linear regression model, log10 cumulative viral load (AUC) was positively associated with intact proviral HIV copies (β = 19.82, 95% CI 11.74–27.89, $p < 0.001$, n = 164). Conversely, a negative correlation was found between log-transformed nadir CD4 T-cell count and intact proviral DNA copies /10^6 T cells (n=191, r = -0.39, $p < 0.001$) (**Figure 3b**). In univariate linear regression, Log10 CD4 nadir was inversely associated with intact proviral HIV copies (β = −0.081, 95% CI −0.115 to −0.046, $p < 0.001$).

There was no correlation between intact proviral DNA copies/10^6 T cells and time from acquisition to ART initiation (in months) (n=191, r = 0.01, p = 0.947) yet a weak correlation was seen with time from ART initiation to sample collection (in months) (n=191, r = -0.1683, $p = 0.0199$) (**Figure 3c**).

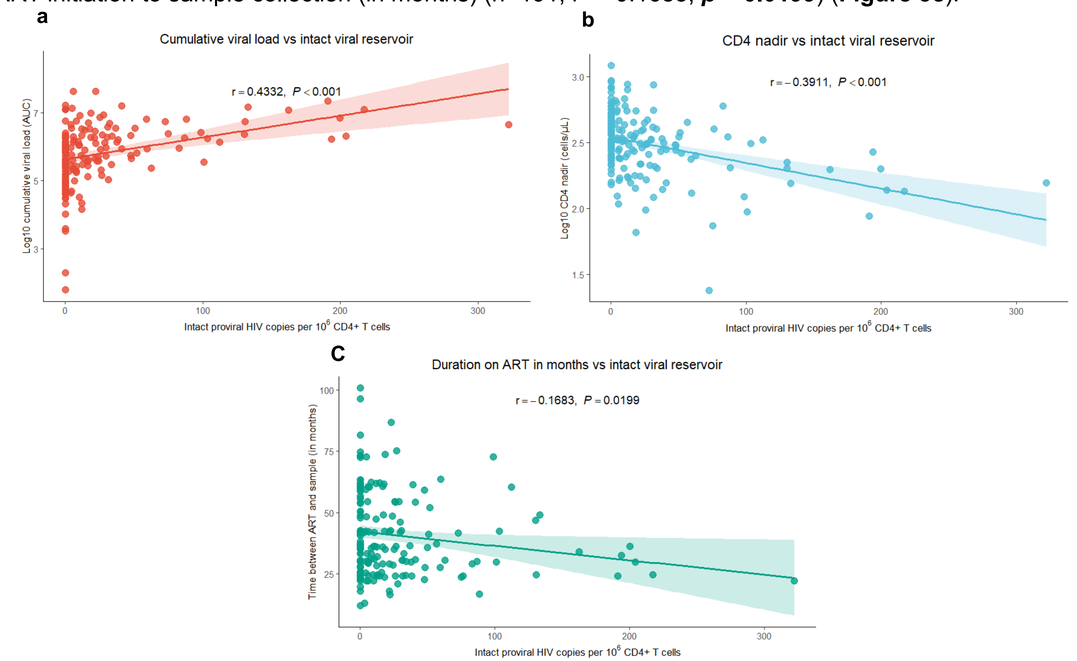


Figure 3. Correlation analysis between intact proviral HIV reservoir size (copies per million T cells) and (a) cumulative viral load, (b) CD4 nadir, and (c) duration on ART (months).

DISCUSSION

We optimized, validated, and implemented the CS-IPDA in a large cohort of women living with HIV-1 subtype C. Cumulative plasma viral load and lower CD4 nadir prior to ART initiation were both associated with the size of the intact HIV reservoir, highlighting the impact of severe immunological damage on viral persistence. A high proportion of participants exhibited undetectable reservoir levels, suggesting substantial heterogeneity in reservoir establishment and/or potential limitations of target region detection.

CONCLUSION

To our knowledge, this is the largest reservoir sizing study conducted in a population of African women. Our results validate earlier research showing that pre-ART viral dynamics and immune depletion are key determinants of long-term reservoir size and persistence.

REFERENCES

1.Cassidy, N. A. J. et al. HIV reservoir quantification using cross-subtype multiplex ddPCR. iScience 25, (2022).

AUTHOR INFORMATION

Affiliation: Division of Medical Virology, Faculty of Health Sciences, University of Cape Town
Email address: SBXSHO001@myuct.ac.za

ACKNOWLEDGMENTS

NIH PRF Polymyalitis Research Foundation CAPRISA

Higher levels of HIV in blood combined with loss of immune function prior to starting HIV treatment can shape outcomes of viral persistence in African women.