# Ex Vivo Antigen Stimulation Induces Viral Outgrowth from a Pericentromeric Provirus

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**Angelica Camilo-Contreras**[1], Hao Zhang[1], Daniel Smith[1], Arturo Casadevall[1], Janet D. Siliciano[1], Robert F. Siliciano[1], Stuart Ray[1], Luis J. Montaner[2] and Francesco R. Simonetti[1]
[1]Johns Hopkins University, Baltimore, MD, US, [2] University of Pennsylvania, Philadelphia, PA, US

## BACKGROUND

- Intact proviruses integrated into heterochromatic loci are progressively enriched during long-term ART and in elite controllers.[1-6]
- These proviruses may evade immune recognition due to lower viral gene expression, but their clinical significance upon ART interruption remains unclear.
- Recent findings indicate that cognate antigen engagement can increase HIV-1 RNA expression.[7]
- We sought to determine whether stimulation with cognate antigens from the commensal fungus *C. albicans* could result in viral outgrowth from a CD4+ T cell clone carrying a centromeric provirus .

## METHODS

**Case Report:**

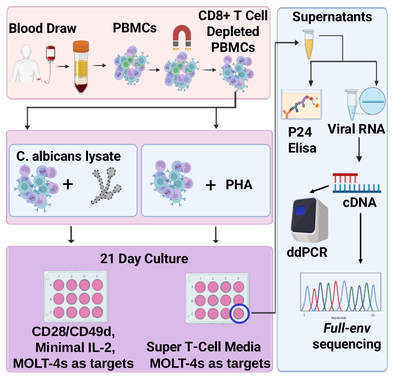
- One participant (Delspc-12) on long-term ART.

**Characterizing proviruses in CD4+ T cells responsive to a commensal fungus**

- Isolated CD4+ T cells responsive to lysate of the commensal fungus *Candida albicans* (CA) based on activation-induced markers (CD69, CD154, CD137).
- HIV DNA in CA-responsive cells analyzed by IPDA, matched integration and proviral sequencing, and integrant-specific digital PCR.

**Antigen Specific Quantitative Viral Outgrowth Assay (agQVOA):**

- CD8-depleted PBMCs were stimulated with CA lysate for 3 days, followed by co-culture with MOLT-4 cells for 21 days.
- Basal culture medium (10% human serum, 10 U/mL IL-2) was used, omitting irritated feeders and T-cell activation media.
- Phytohemagglutinin (PHA) combined with T-cell activation media served as a non-specific positive control.
- Characterized viral outgrowth using p24 ELISA, digital PCR, and full-env sequencing.



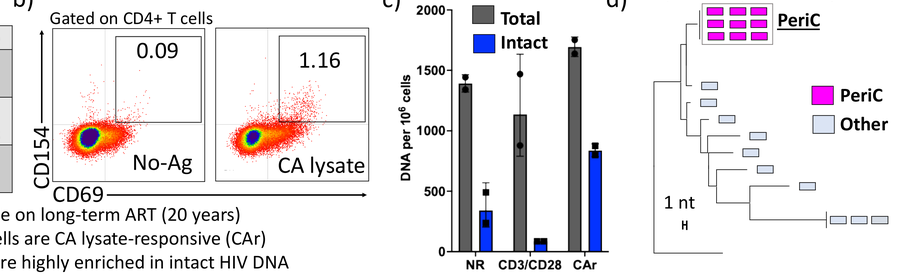

# **Cognate antigen recognition** by CD4+ T cells **reactivates** provirus integrated in a region associated with **deeper latency.**

## RESULTS

**Fig 1 – Participant Delspc-12 has a large CA-responsive clone containing an intact provirus**

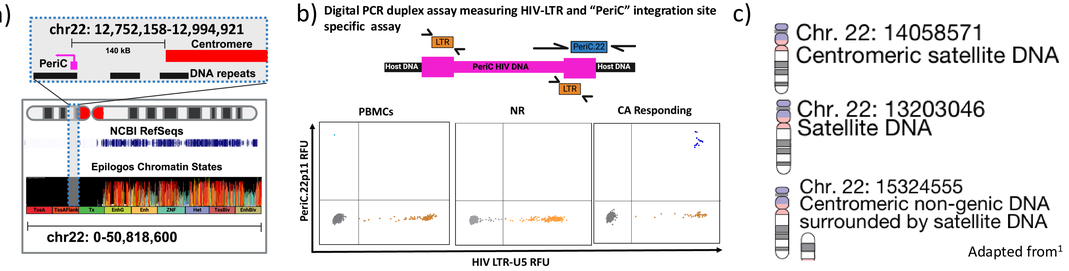
a)

| Sex | Male |
|---|---|
| Race | AA |
| Time on ART | 20 years |
| CD4 Nadir (cells/µL) | 6 |



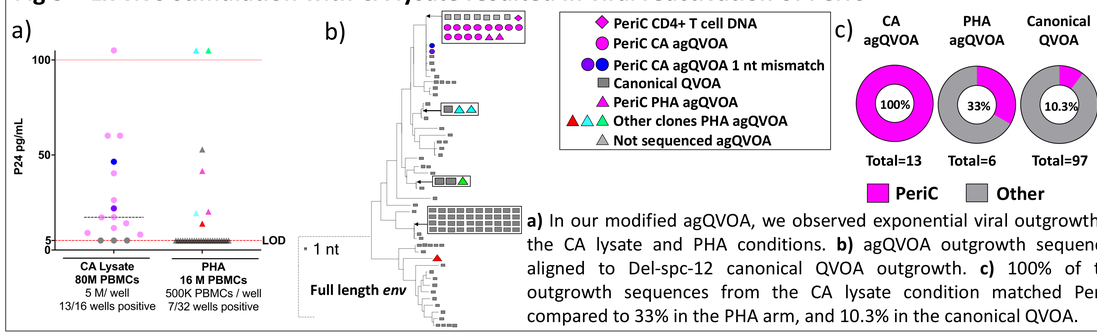

**a)** Delspc-12 is AA male on long-term ART (20 years)
**b)** 1.16 % of CD4+ T cells are CA lysate-responsive (CAr)
**c)** Sorted CAr cells were highly enriched in intact HIV DNA
**d)** CAr cells contained a large HIV infected clone "PeriC" composing 50% of all env sequences.

**Fig 2 – PeriC is integrated in a pericentromeric region and is abundant in CAr cells**




**a)** Integration site analysis showed this provirus is in a pericentromeric satellite repeat in Chr22:12813186. Publicly available epigenetic and gene expression data suggests this is an inactive region of the genome.[8,9] **b)** PeriC has a frequency of 930 copies per million CA-responsive cells. **c)** Other intact proviruses have been reported in this arm of Chr 22.[1]

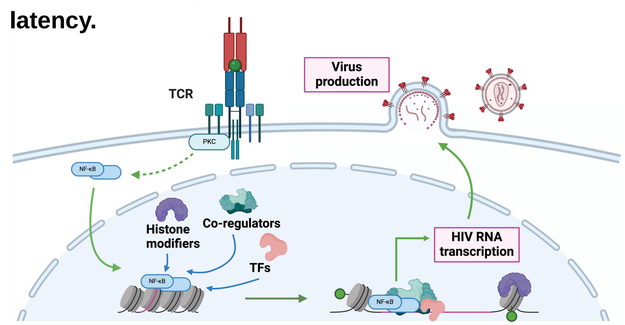
**Fig 3 – Ex vivo stimulation with CA lysate resulted in viral reactivation of PeriC**




**a)** In our modified agQVOA, we observed exponential viral outgrowth in the CA lysate and PHA conditions. **b)** agQVOA outgrowth sequences aligned to Del-spc-12 canonical QVOA outgrowth. **c)** 100% of the outgrowth sequences from the CA lysate condition matched PeriC, compared to 33% in the PHA arm, and 10.3% in the canonical QVOA.

## CONCLUSIONS

In this participant, antigens from the commensal fungus *Candida albicans* contribute to the persistence of a replication-competent and inducible provirus during long-term ART. Additionally, we demonstrate that cognate antigen stimulation can reactivate this provirus, despite its pericentromeric integration site. **While limited to an ex vivo culture, these findings suggest that CD4+ T cell recognition of ubiquitous antigens can reactivate proviruses in regions previously associated with deeper latency.**




**Impact:** **While these proviruses are enriched in individuals on long-term ART and elite controllers, epigenetically repressed proviruses, despite their lower inducibility, may still contribute to viral rebound if the cell encounters its cognate antigen.**

## Sources:

1. **Jiang et al, 2020** PMID: 32848246
2. **Huang et al, 2021** PMID: 34636876
3. **Clark et al, 2023** PMID: 39060113
4. **Sun et al, 2023** PMID: 36599977
5. **Lian et al, 2023** PMID: 36596305
6. **Armani-Tourret et al, 2024** PMID: 38367616
7. **Moskovljevic et al, 2024** PMID: 39612916
8. **http://genome.ucsc.edu** PMID 39460617
9. **https://epilogos.net/**

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**Figures generated in Biorender.**

**Author Contact Information: acamilo3@jh.edu**