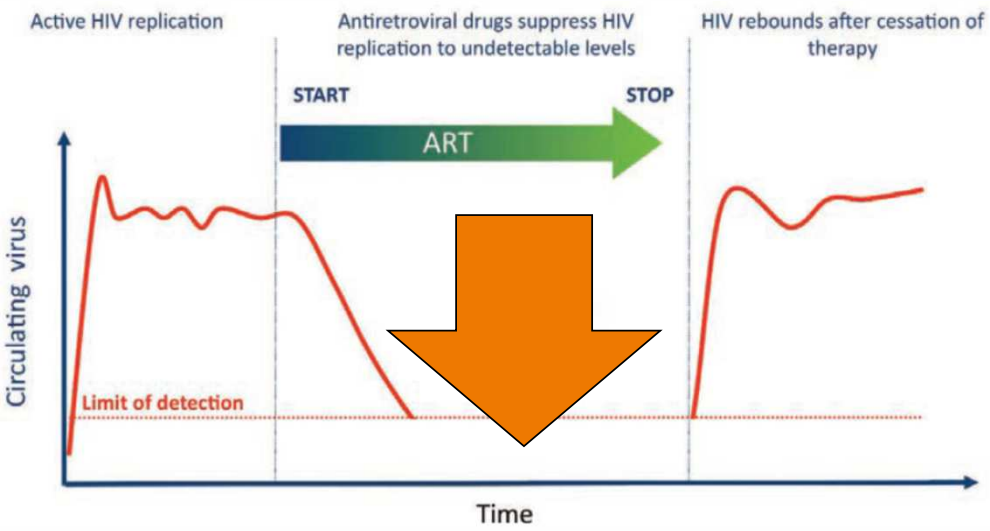
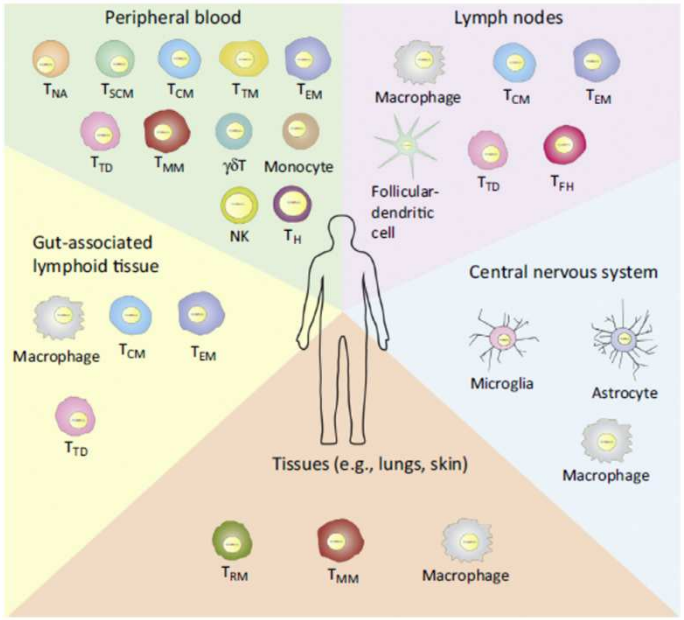
Sequencing HIV: Significance and Impact





Barton et al. Trends in Microbiology, 2016

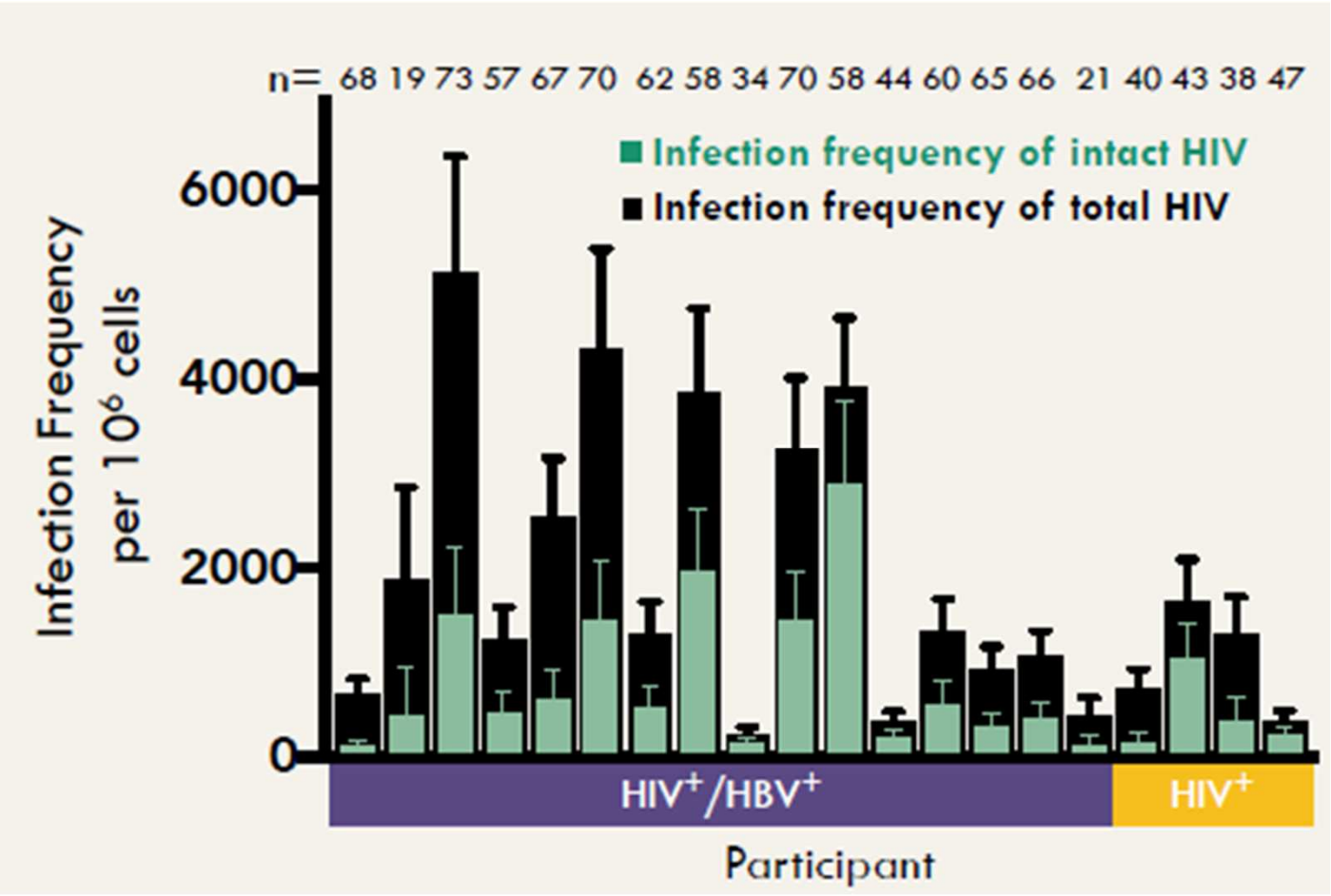
Where does HIV Persist <u>During Therapy?</u>

What cell types and cellular mechanisms contribute to persistent HIV during effective therapy?

Which cells contain genetically intact "replication-competent" HIV?

To help answer these questions full-length HIV DNA sequencing methods have been developed.

High number of genetically-intact proviruses identified during pretherapy



Thai Red Cross AIDS Research Center

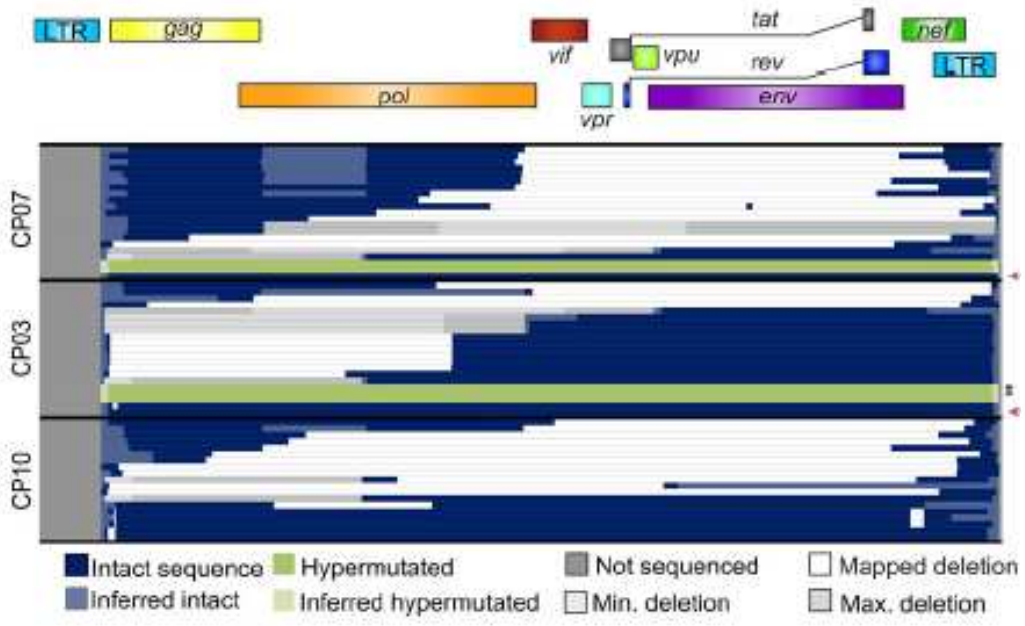
Untreated HIV-infected Participants full-length HIV DNA sequencing of CD4 T cells from peripheral blood (AE subtype):

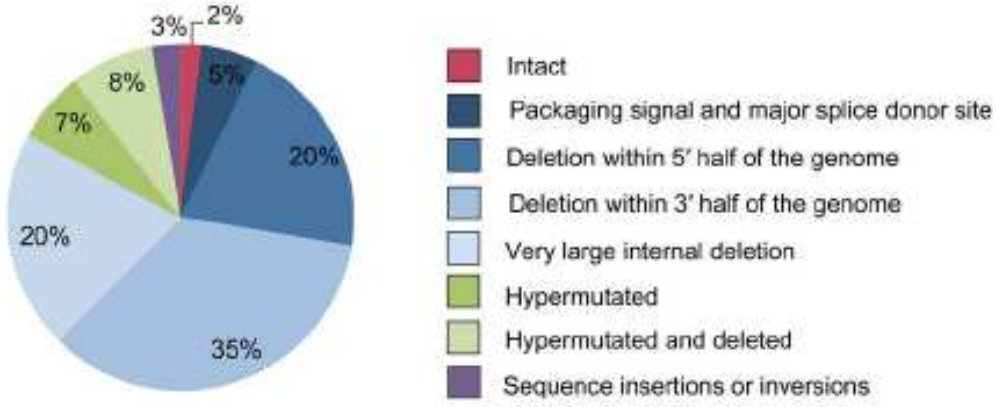
60 to 3,000 genetically-intact proviruses per 10^6 CD4 T cells

Participants on ART:
0 to 26 genetically-intact proviruses per 10^6 CD4 T cells

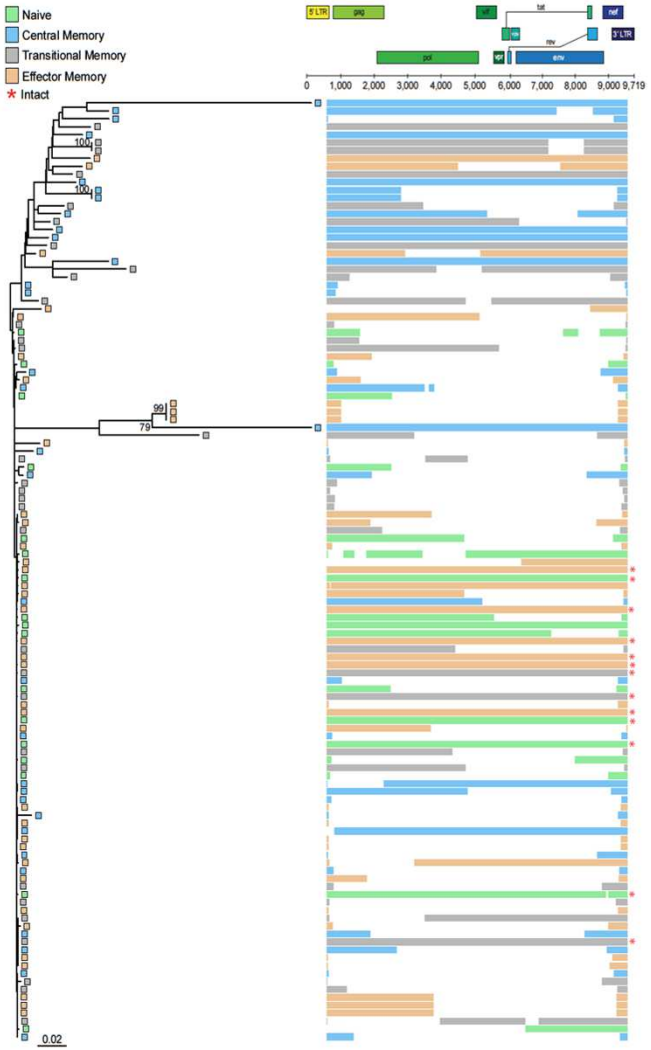
Wang et al. JVI 2022

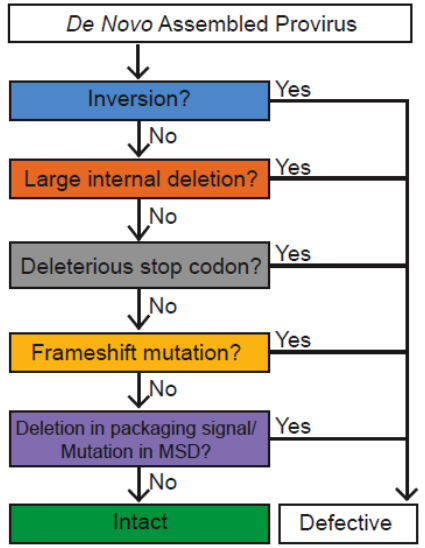
Majority of proviruses are genetically defective during ART

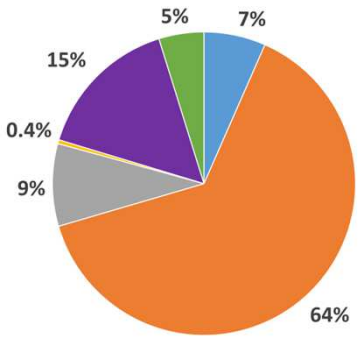




Ho et al. Cell 2013; Bruner et al. Nat Med 2016

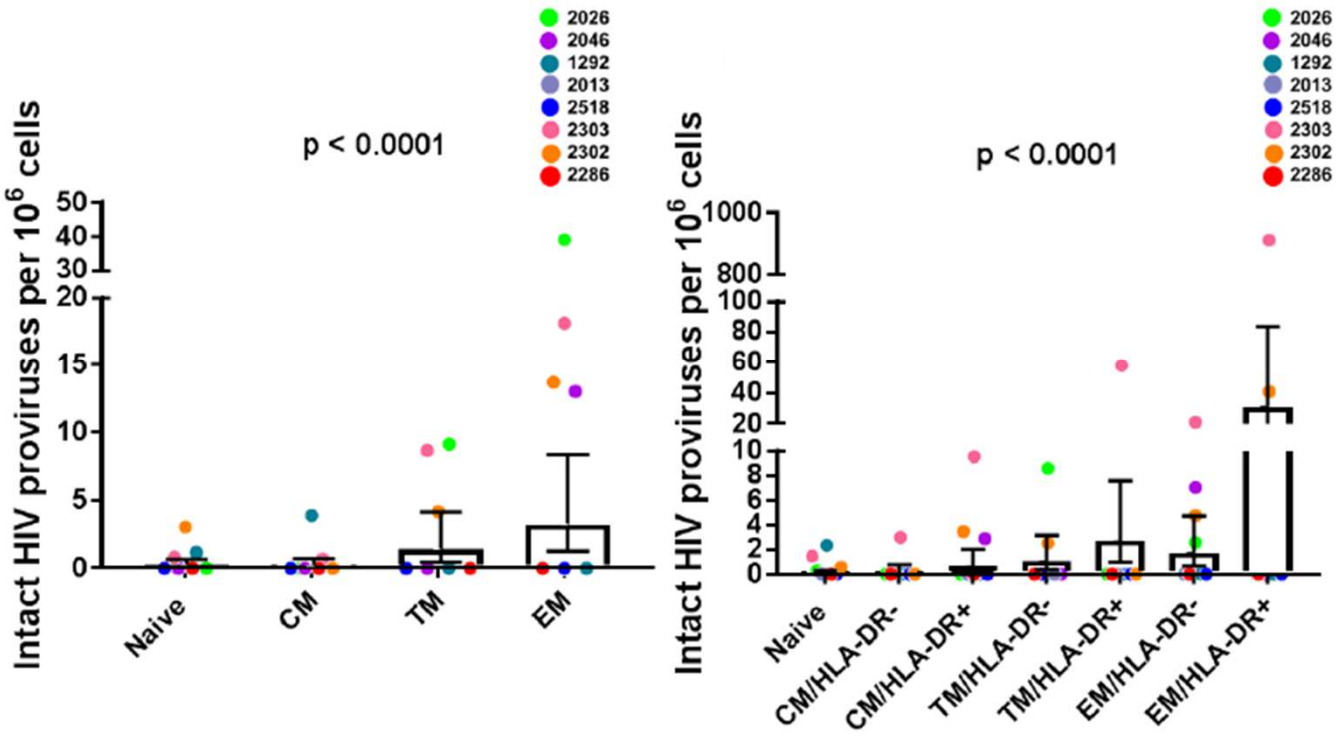


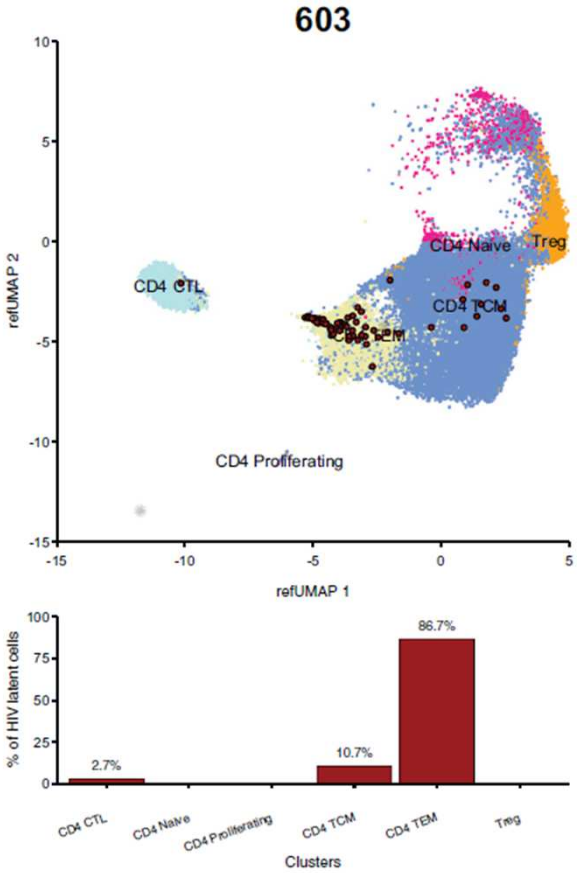




Hiener et al. Cell Reports 2017; Lee et al. JCI 2017

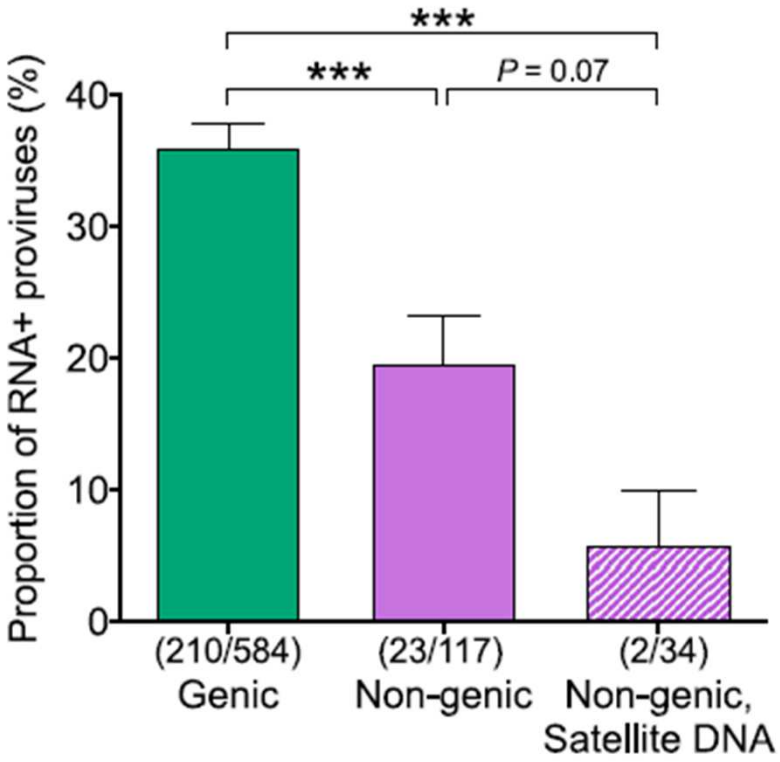
Genetically-intact proviruses are unequally distributed in CD4+ T cell subsets

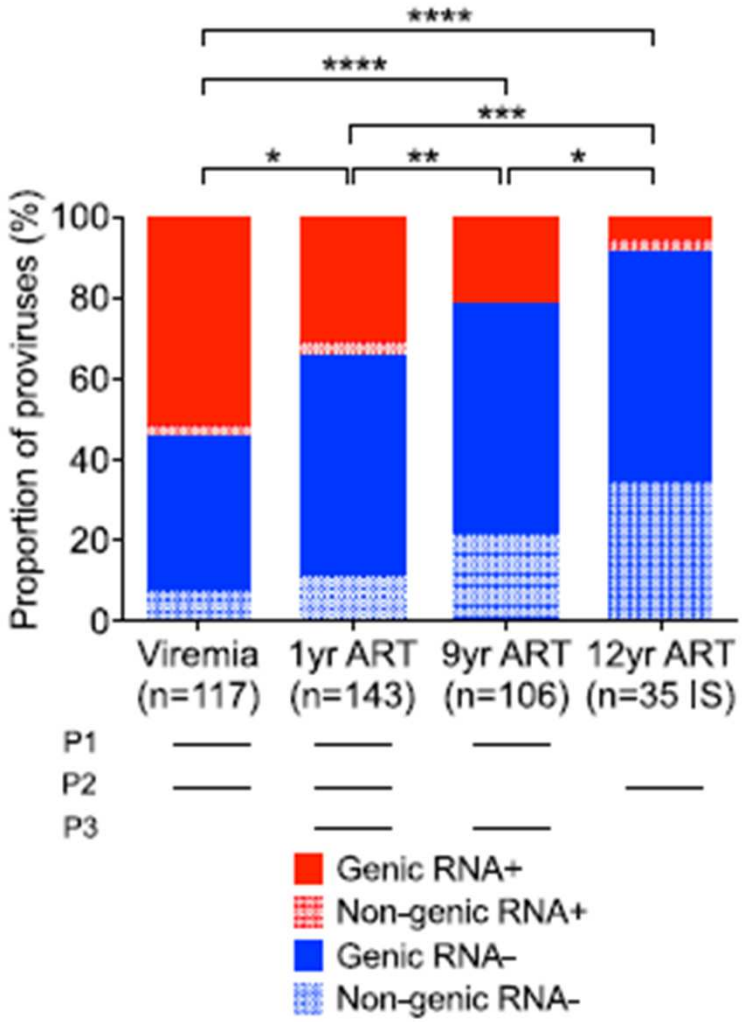




Hiener et al. Cell Reports 2017; Rullo et al. JCI Insight 2020; Neidleman et al. elife 2020; Horsburgh et al. JID 2021; Duette/Hiener et al. JCI 2022; Weymar et al. Cell Reports 2022

Parallel analysis of transcription, integration, and sequence of single HIV-1 proviruses





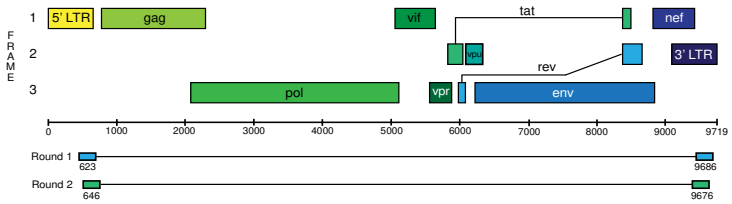
- **Transcriptionally active proviruses were actively selected against during prolonged ART**
- **Transcriptionally active proviral clones can persist long-term during ART due to elevated cell turnover rates**

Einkauf et al. Cell 2022

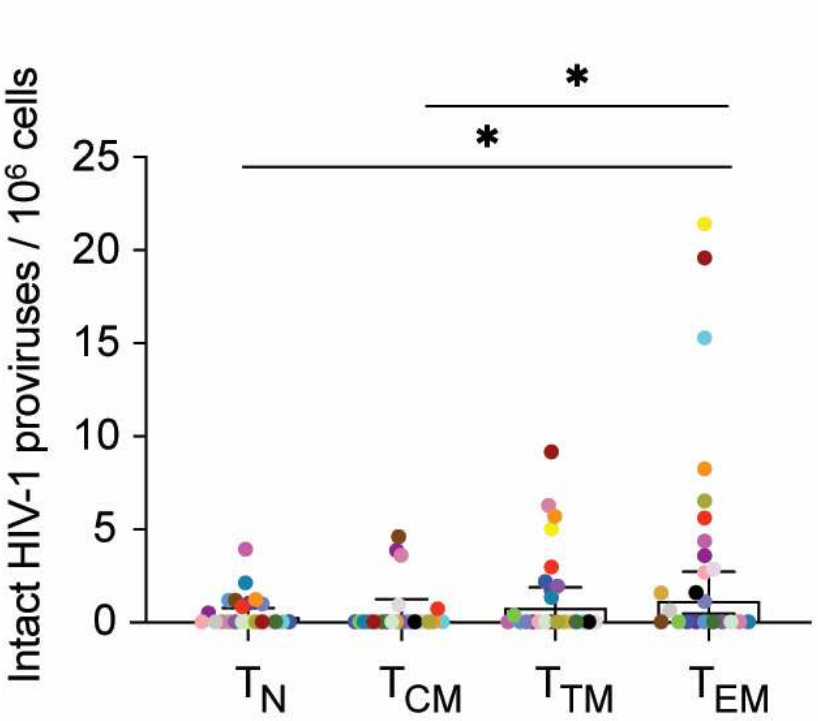
Proviral landscape in T cell subsets

We analysed 2730 near-full-length HIV-1 proviral sequences from:

- 24 ART supressed participants
  - On ART 2-22 years
- T_N, T_{CM}, T_{TM} and T_{EM} CD4+ T-cells
- Compared the genetic landscape of persistent HIV-1 between these cell subsets



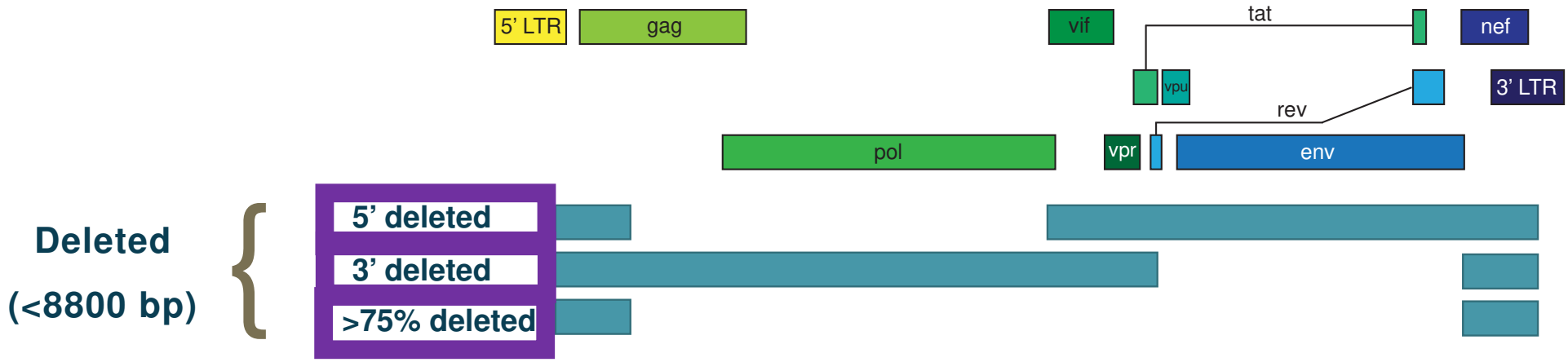
Full-Length Individual Proviral Sequencing (FLIPS) assay



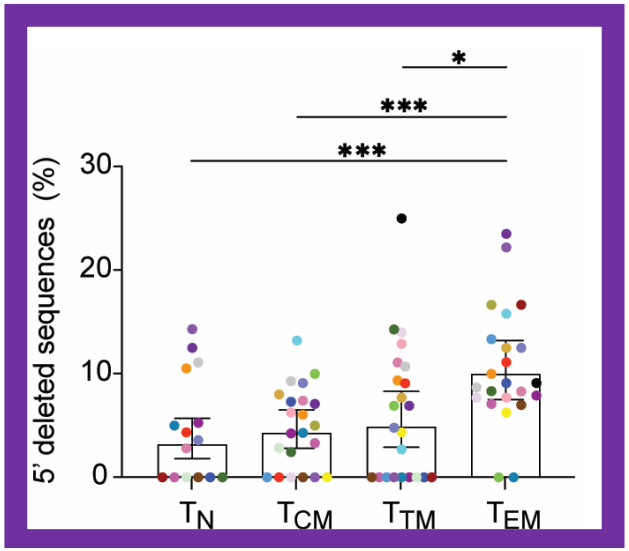
*p≤0.05

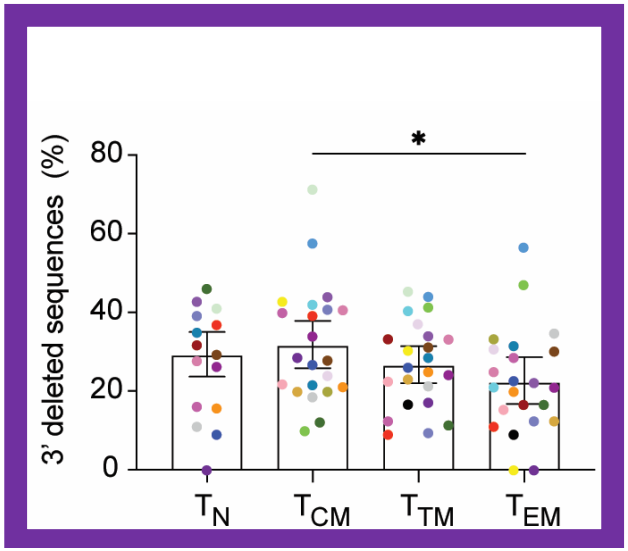
Duette/Hiener et al. JCI 2022

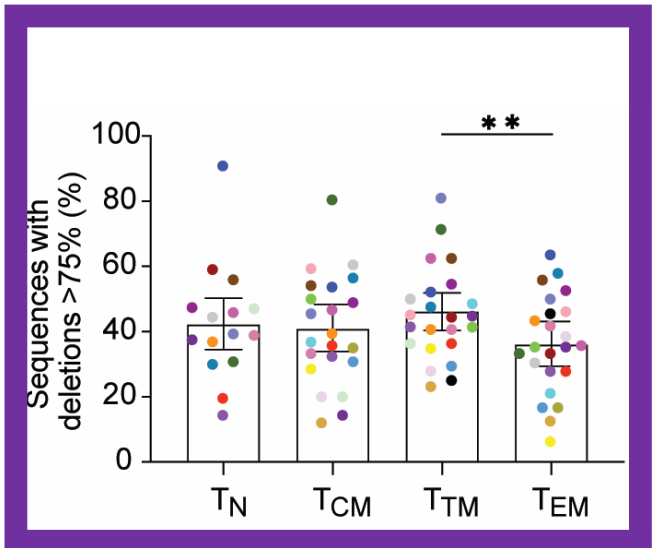
The proviral landscape is different between subsets



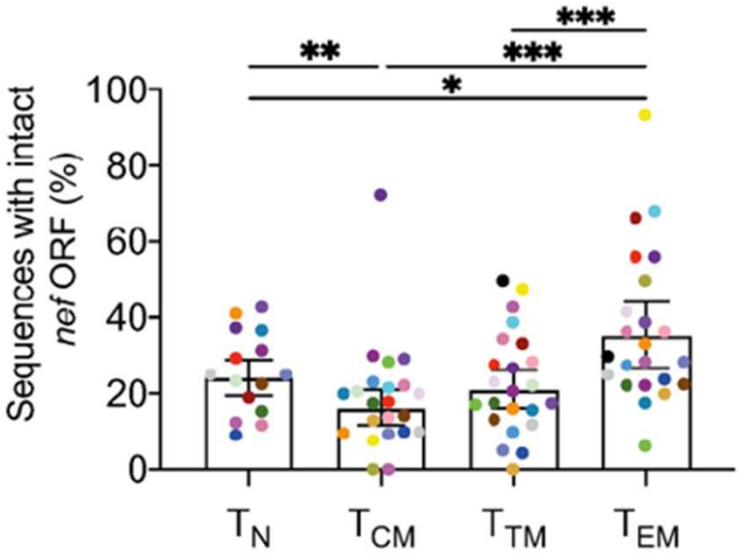
*p≤0.05
**p≤0.01
***p≤0.001

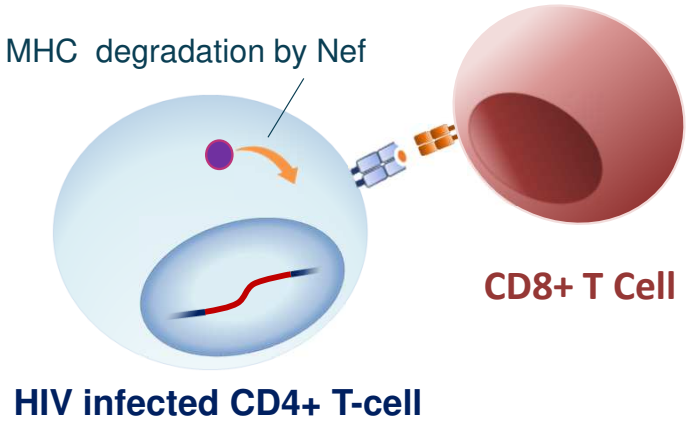


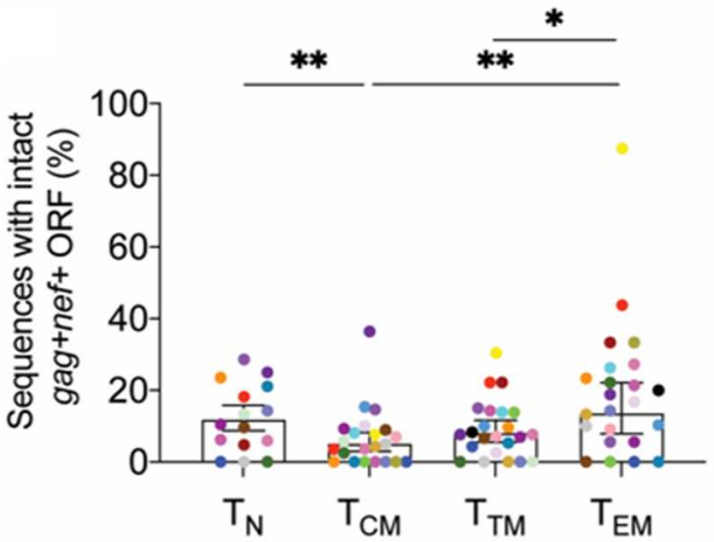




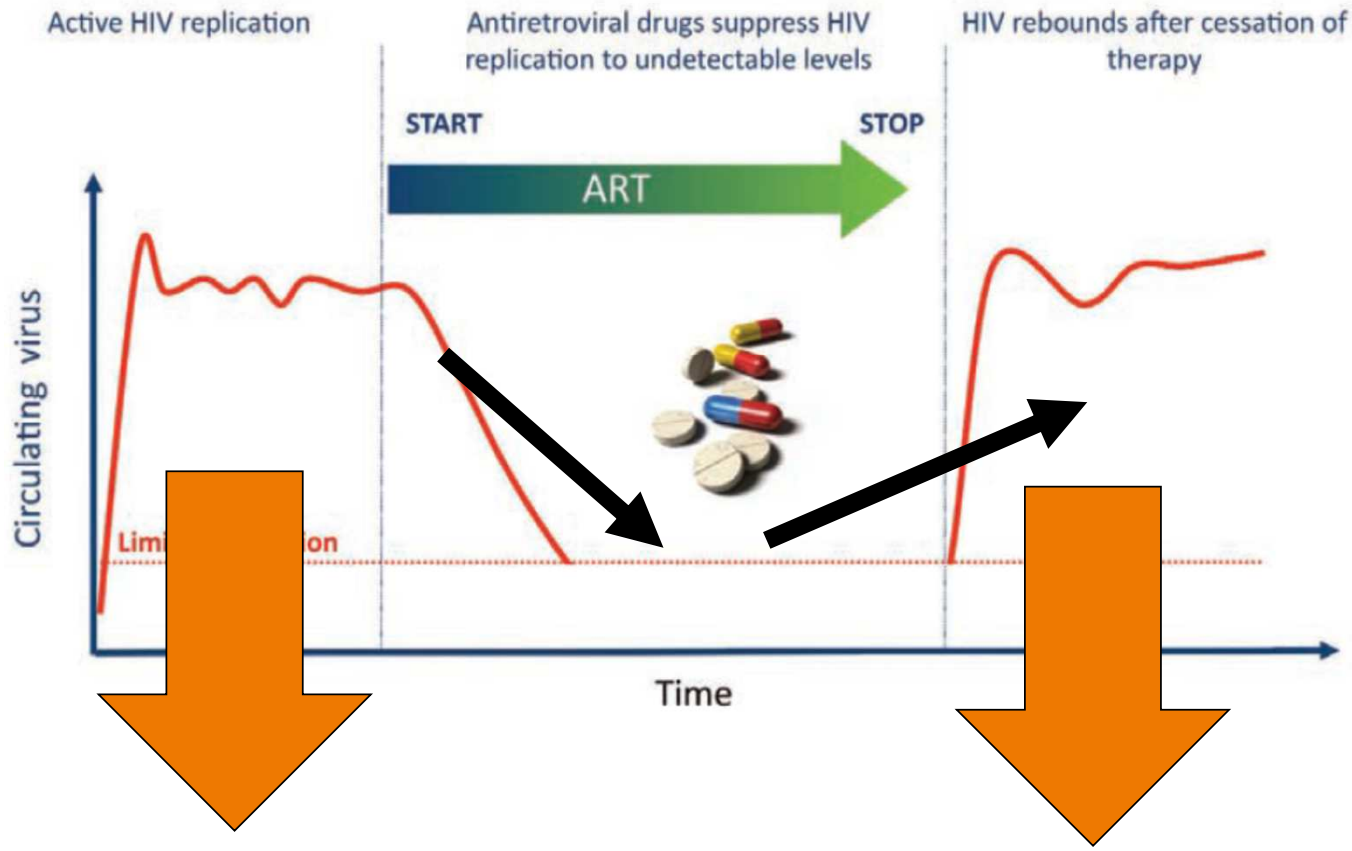
Nef may protect cells from clearance







Dirk et al. Scientific reports 2016; Blagoveshchenskaya et al. Cell 2002; Duette/Hiener et al. JCI 2022



Pre-therapy virions plasma-derived HIV RNA

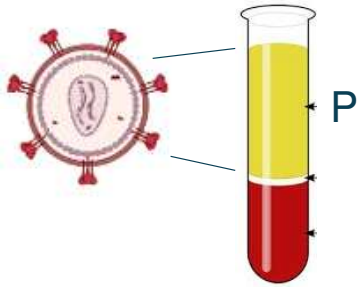
Rebound virus plasma-derived HIV RNA

How do Plasma Virions Contribute to Persistent HIV?

1) **How does pre-therapy plasma-derived virions contribute to the HIV reservoir in cells?**

2) **What does rebound virus look like and which cells contribute to this rebound virus?**

PRLS (plasma-derived HIV-1 RNA using long-range sequencing) Assay



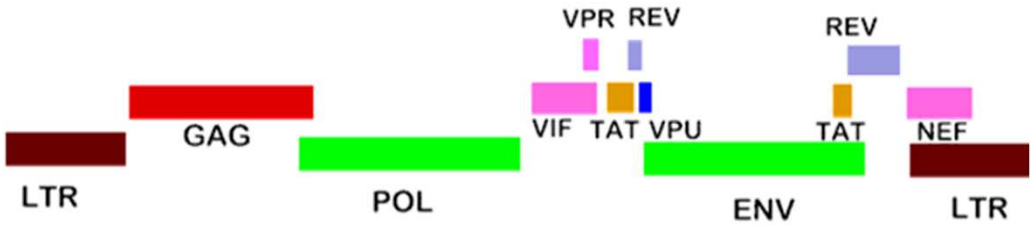
Plasma

RNA Extraction

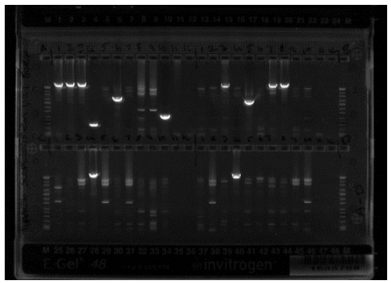
cDNA synthesis

gag-3' (~8.3kb)

int-3' (~5kb)



Full-Length Individual Proviral Sequencing (FLIPS) (9 kb)



PCR Amplification (limiting dilution)

Next Generation Sequencing

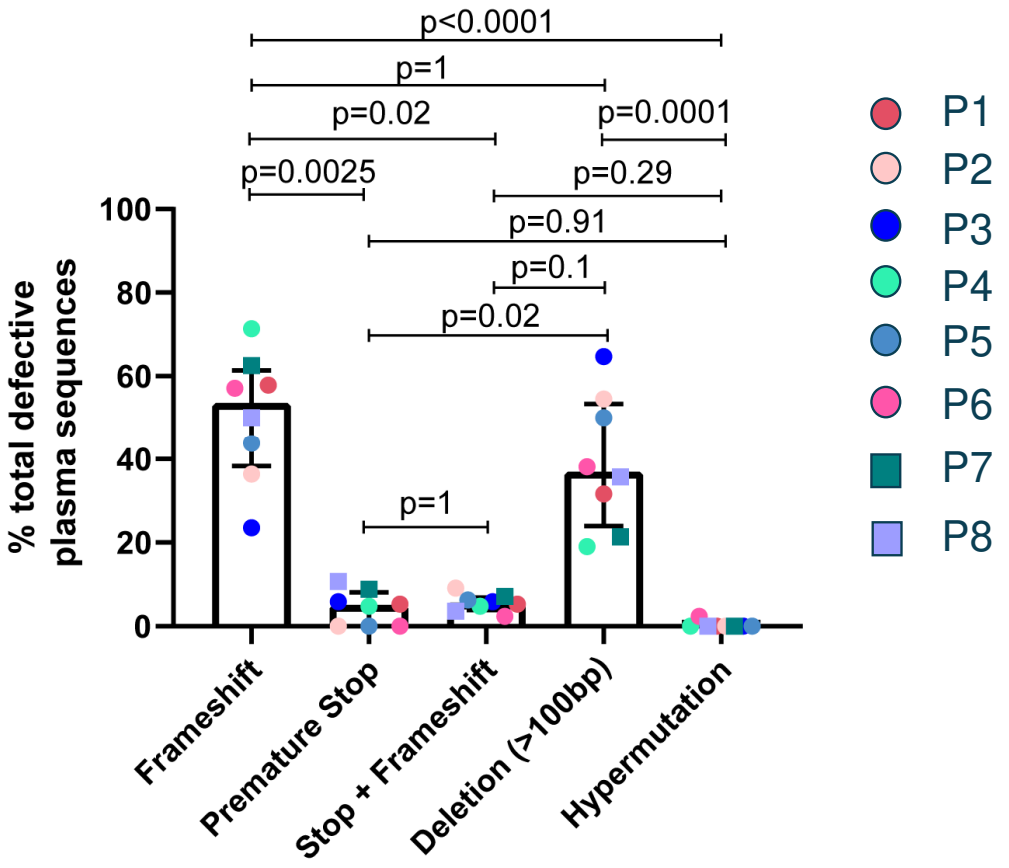
Fisher et al. JVI 2022

Modified SGS (Palmer et al JCM 2005) and FLIPS assays (Hiener et al Cell Reports 2017)

Defective genomes are found in plasma of untreated participants

- **PRLS analysis of 8 participants during untreated infection, revealed 65% (range 49-74%) of plasma-derived genomes were genetically-intact.**

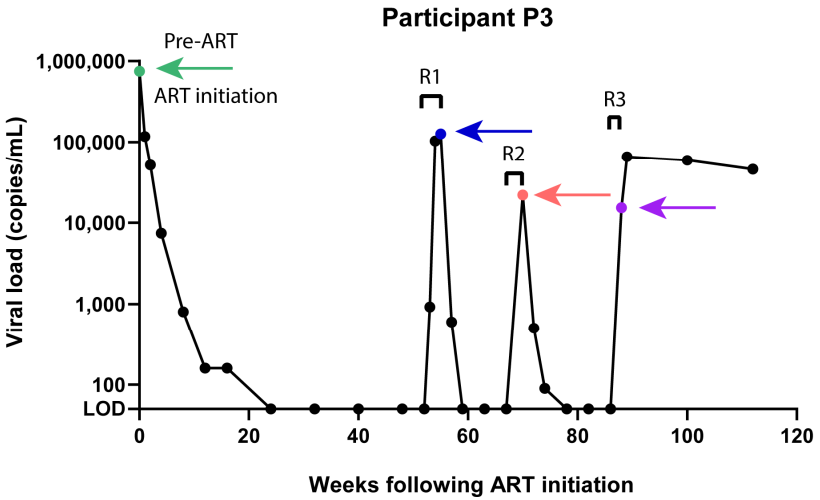
- **Frameshifts were the most common type of defect, followed by deletions of >100bp.**



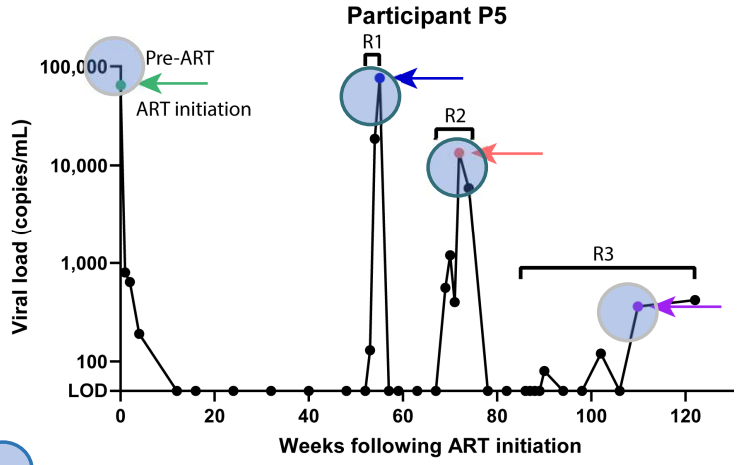
Fisher et al. JVI 2022

Viral rebound during multiple analytical treatment interruptions

Non-controllers



Transient controllers



Pulse Study Participants (Bloch et al. 2006)
Treated during acute/early infection

Initiated ART for 1 year, then interrupted and re-initiated ART three times

Therapy was restarted when viral load ≥5,000 copies/ ml

Viral Factors

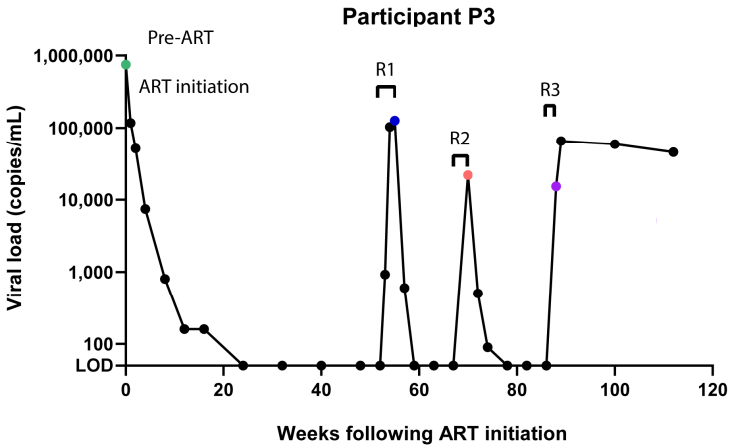
- CTL escape mutations
- Viral genes
  - HIV RNA sequencing
  - HIV DNA sequencing

Host Factors

- CD8 T cell repertoire
- CD8 T cell immunodominance
- CD8 T cell functionality
  - HIV clearance assay
  - ICS - Polyfunctionality
  - Immune exhaustion

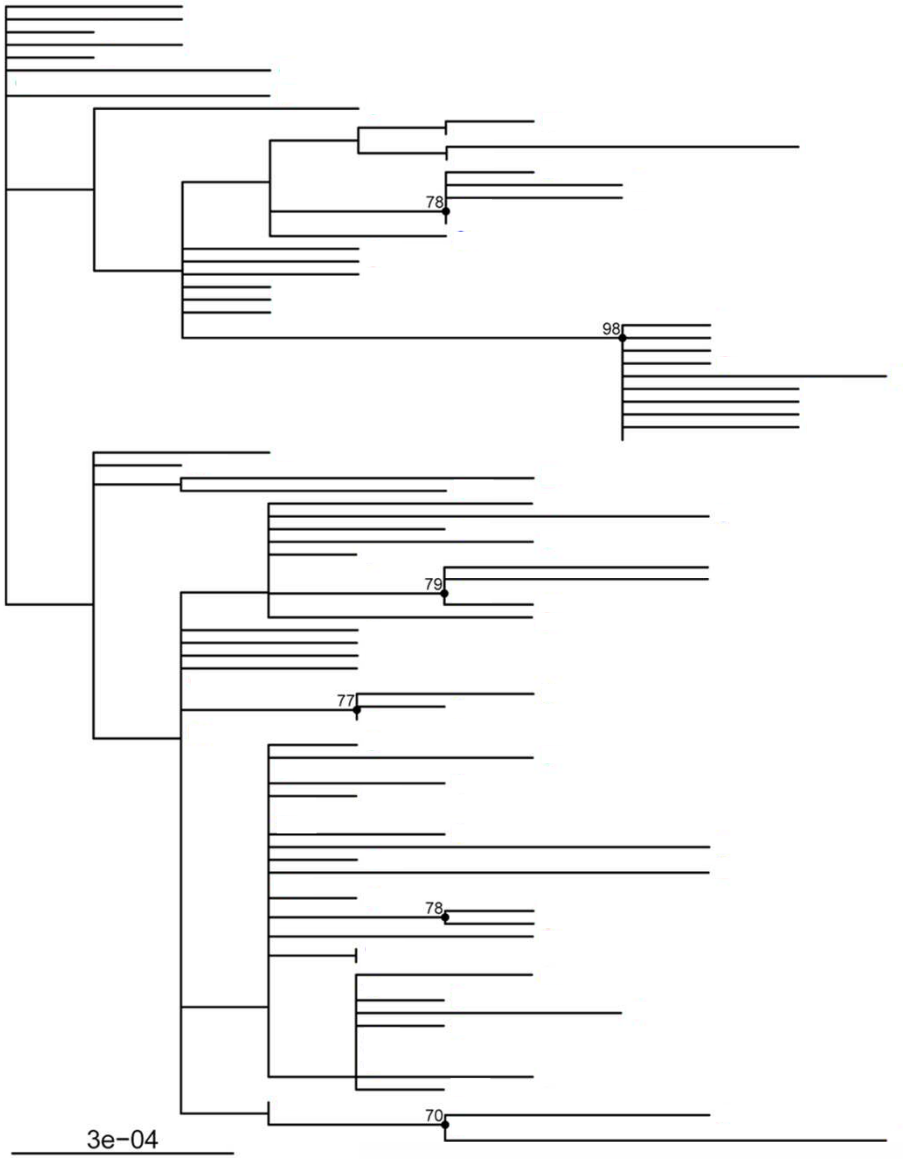
Participant P3

(genetically-intact sequences only)



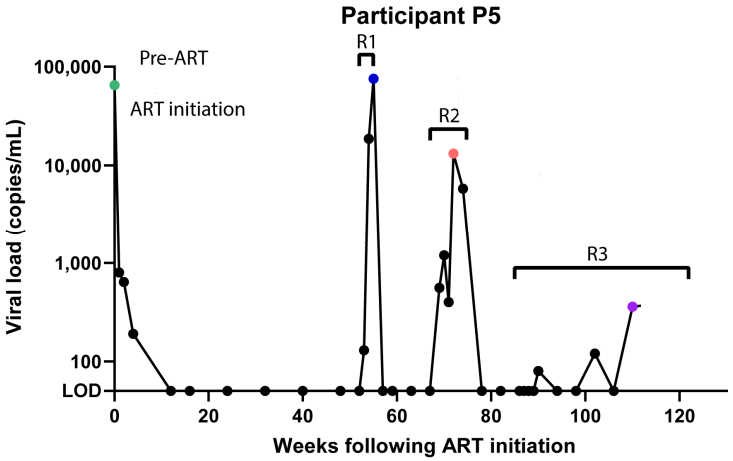
Tree topology and compartmentalization analyses (Tree-based: Bayesian model and Distance-based: Wrights: F_{ST})

- **No obvious separation of sequences from individual timepoints**
- **Some small groups of pre-ART and R3 sequences clustering separately (p=0.002)**
- **R1 and R2 sequences intermingled with other sequences (p>0.1 for all)**
- **Overall low evidence for compartmentalization by timepoint**



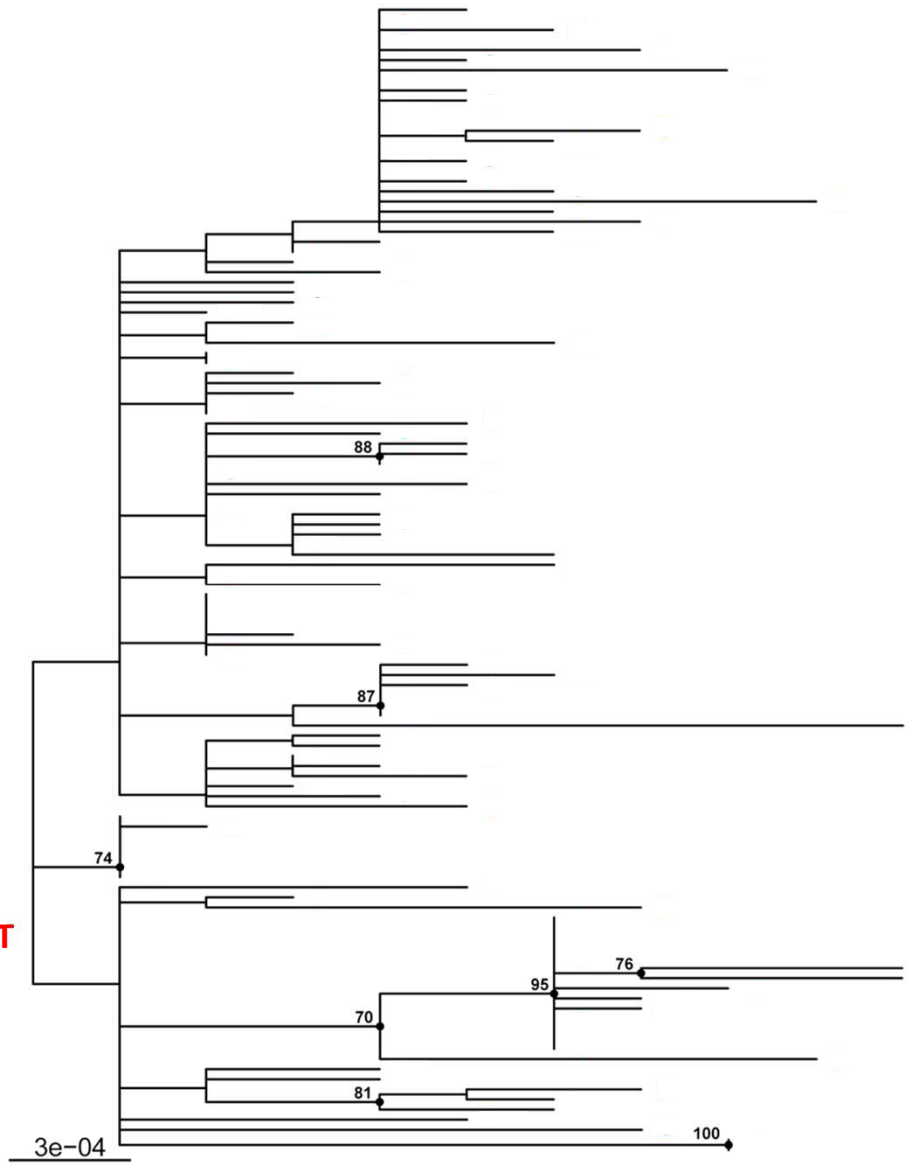
Participant P5

(genetically-intact sequences only)



- **Some groups of viral sequences cluster separately between pre-ART and R1 ($p<0.0005$)**
  - **But overall the viral sequences from pre-ART, R1 and R2 group together**
- **Strong evidence that the virus identified during R3 is genetically-different to the virus replicating prior to ART and the virus identified during the 1st and 2nd rebounds (all $p<0.0001$)**

What host and virological factors are contributing to the lower viral load and delayed viral rebound?



Conclusions

1) There is a difference in the proviral genetic landscape between cell subsets of memory CD4+ T cells.

2) Genetically-intact proviruses appear to be concentrated in specific memory T cell subsets.

3) Cellular proliferation contributes to HIV persistence during therapy; cells which are more proliferative contain more genetically-intact HIV.

4) Many proviruses are transcriptionally active; which allows the immune system to target these proviruses; however rapid cellular turn over rates counteract this host immune pressure.

5) Not all virions in the plasma are infectious; in fact up to 45% are defective.

6) Investigating the interplay between the virus and the host immune cell response will provide insights as to how some HIV-infected individuals control HIV during an analytical treatment interruption.

7) Understanding the viro-immunological mechanisms contributing to viral control will identify new therapeutic strategies to enhance the clearance of HIV-infected cells.

- **What does near full-length sequencing of HIV DNA and RNA tell us about HIV persistence?**

- **Most proviruses are defective. However, specific cellular mechanisms such as a short half-life and greater proliferative potential contribute to the maintenance of genetically-intact and potentially replication-competent HIV. In addition, expression of some viral proteins support genetically-intact provirus.**

- **Conduct viro-immunological studies to further understand the mechanims contributing to post-treatment control of viremia.**

CONFLICTS OF INTEREST

No Conflicts of Interest

ACKNOWLEDGEMENTS

Thanks...

We acknowledge with gratitude the participants of these studies

Palmer lab WIMR: Centre for Virus Research
G. Duette
B. Hiener
E. Lee
K. Fisher
B. Horsburgh
X. Q. Wang
M. Sharabas
A. Pereyra Casanova

University of Sydney
J-S Eden
T. Schlub
H. Morgan

Clinical Trials & Biorepository Group, St Vincent's Centre for Applied Medical Research
Kate Merlin
Bertha Fsadni
Sri Meka
Julie Jurczyluk

The Kirby Institute/UNSW
A. Kelleher
A. Shaik
S. Turville
V. Mathivanan

Department of Medicine UCSF
F. M. Hecht
S. G. Deeks
M. Somsouk
P. Hunt
E. Sinclair
P. Lewis
H. Hatano
L. Epling
M. Kilian
T. Ho
J. Milush
T. Liegler
J. Custer
L. Loeb
R. Hoh
L. Poole
S. Yukl

VGTIFL/Montreal
N. Chomont
R. Fromentin

The Peter Doherty Institute for Infection and Immunity
S. Lewin
J. Audsley
J. Zerbato
W. Zhao

The Thai Red Cross AIDS Research Center
A. Avihingsanon

Australian Government
National Health and Medical Research Council

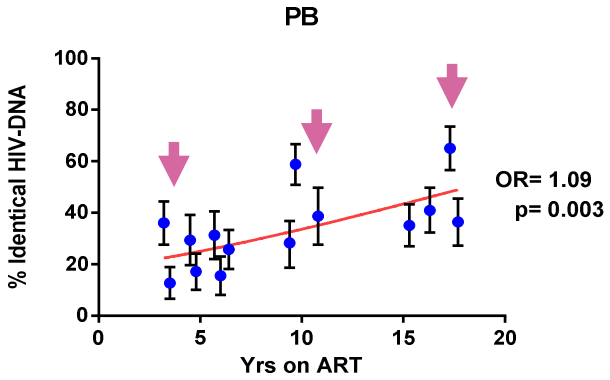
NIH National Institute of Allergy and Infectious Diseases

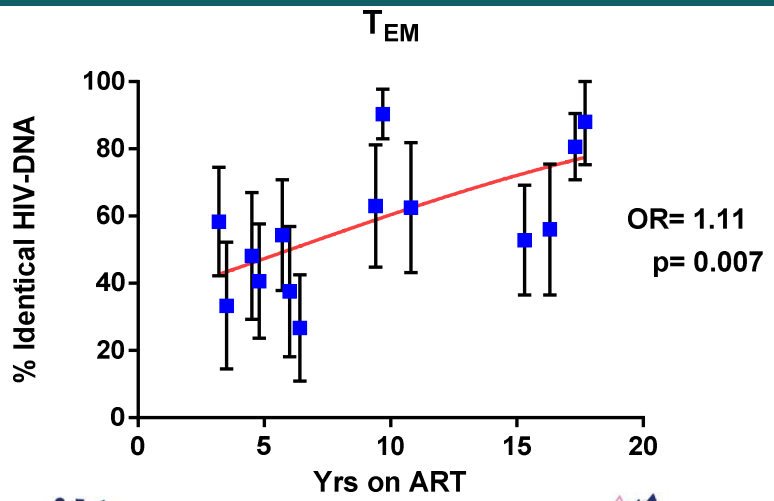
ARCHE amfAR Research Consortium on HIV Eradication

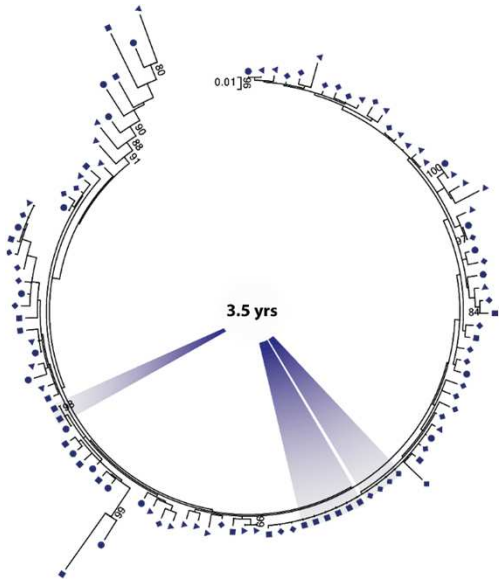
DARE

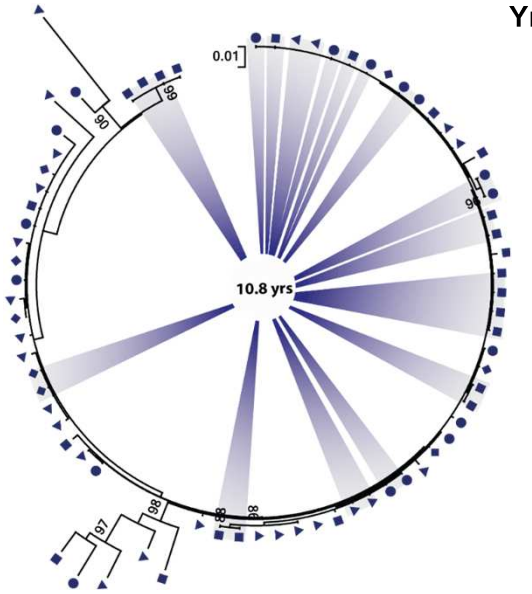
Sandra and David Ansley

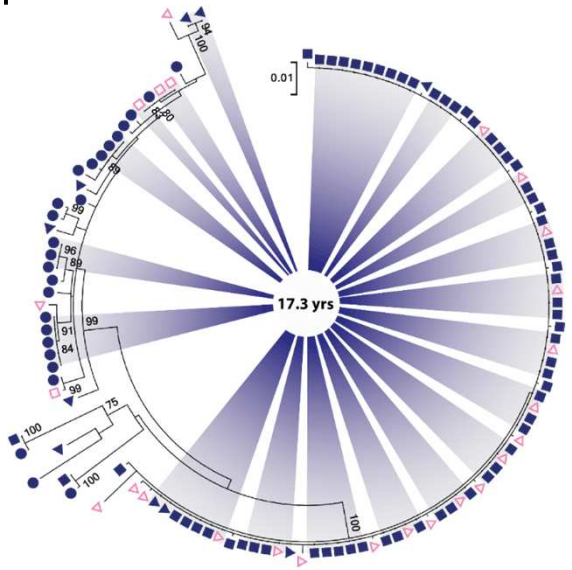
HIV persistence due to cellular proliferation





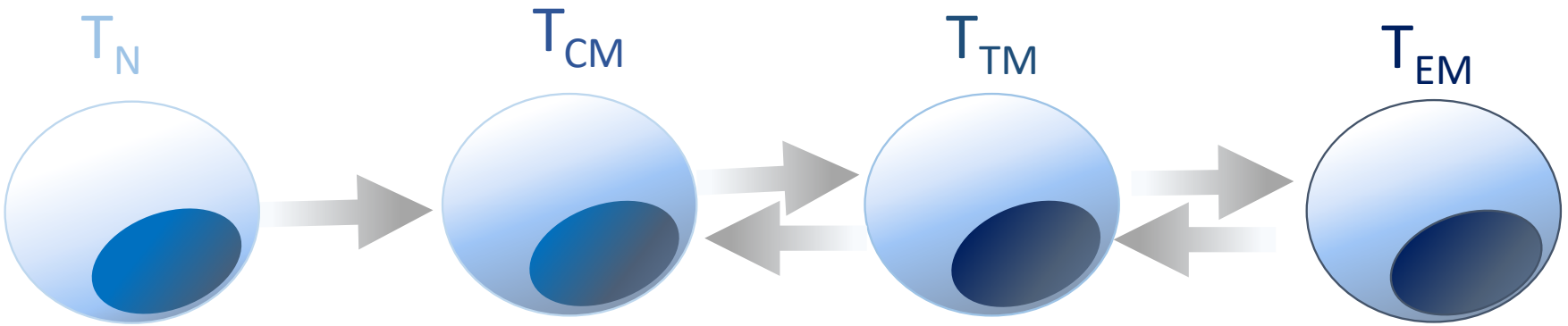






Lee et al. JVI 2020

CD4+ T cell subsets exhibit unique qualities that influence the proviral landscape



Half-life

Capacity for self renewal

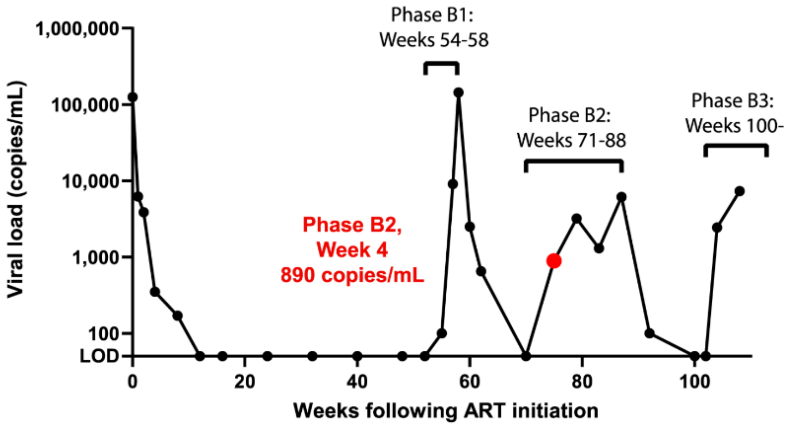
Resistance to clearance by CTL

Differentiation

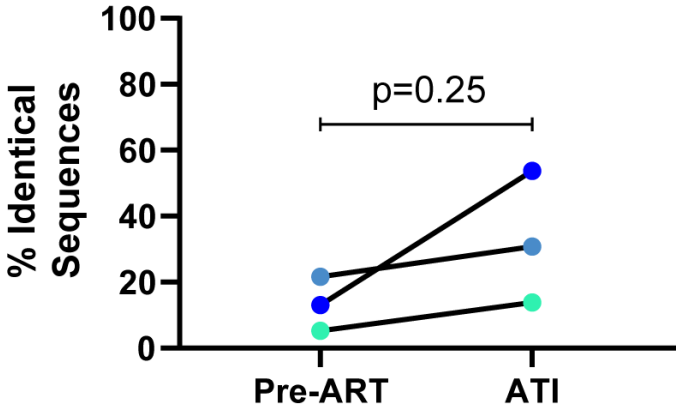
Proliferative

HIV-1 expression

Sequencing HIV RNA during an analytical treatment Interruption



Proportion 100% identical sequences:



Proportion of 100% identical sequences was higher in the ATI plasma sequences (median 30.8%) compared to the pre-ART plasma sequences (median 13.3%).

For 3 participants undergoing an ATI, phylogenetic analyses revealed an ATI plasma-derived sequence was 100% identical to a cluster of pre-ART plasma-derived sequences and PBMC-derived sequences.

Fisher et al. JVI 2022