HIV persistence on ART

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MHRP
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No conflict of interest to disclose

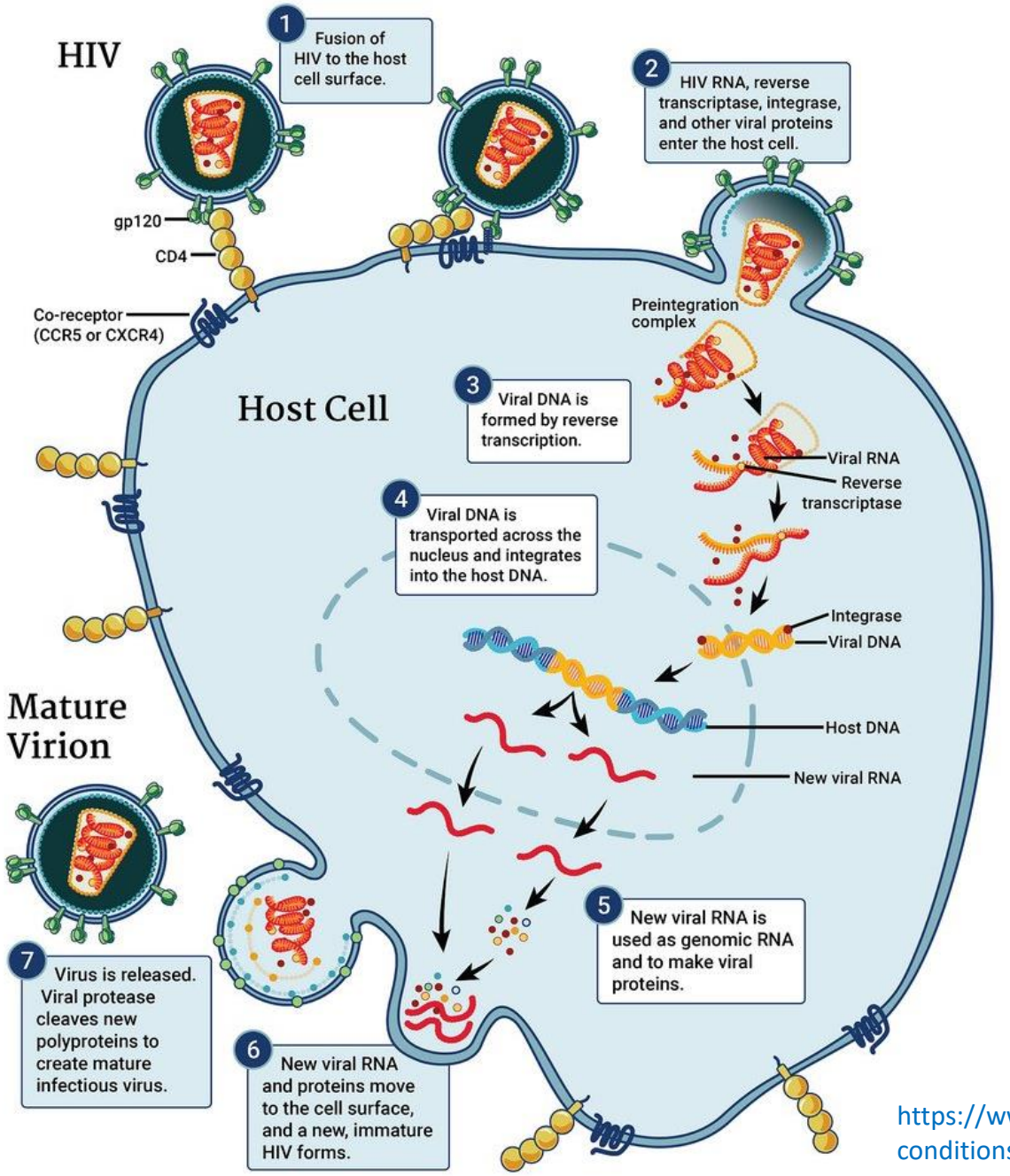
- Despite the success of ART, viral rebound is inevitable in almost all individuals when treatment is interrupted.

- The main obstacle to HIV cure is the persistence of HIV despite ART and plasma viral suppression to undetectable levels on commercial assays.

- Understanding the mechanisms of HIV persistence is essential for the development of strategies to induce HIV remission.

Summary of presentation

- Detection/quantification of HIV persistence
- Establishment of HIV persistence
- Effect of ART on HIV persistence
- Mechanisms of viral persistence on ART

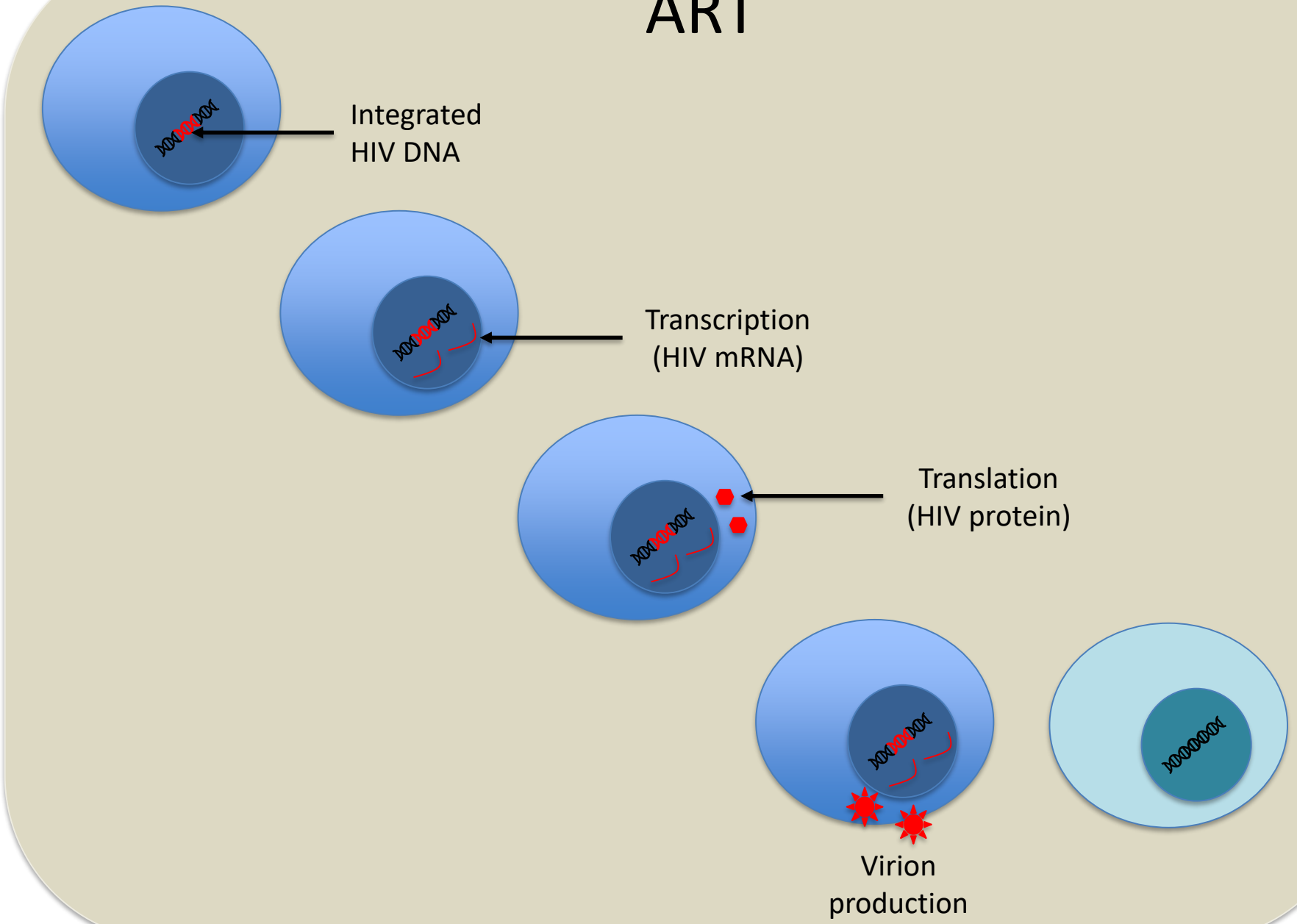


https://www.niaid.nih.gov/diseases-conditions/hiv-replication-cycle

- HIV persistence on ART is due to the existence of

1. Latent viral reservoir
    - made up of infected cells that harbor HIV DNA
    - during latency, these cells are transcriptionally silent but can later become activated and produce infectious HIV virion.
2. Ongoing transcription and virus production

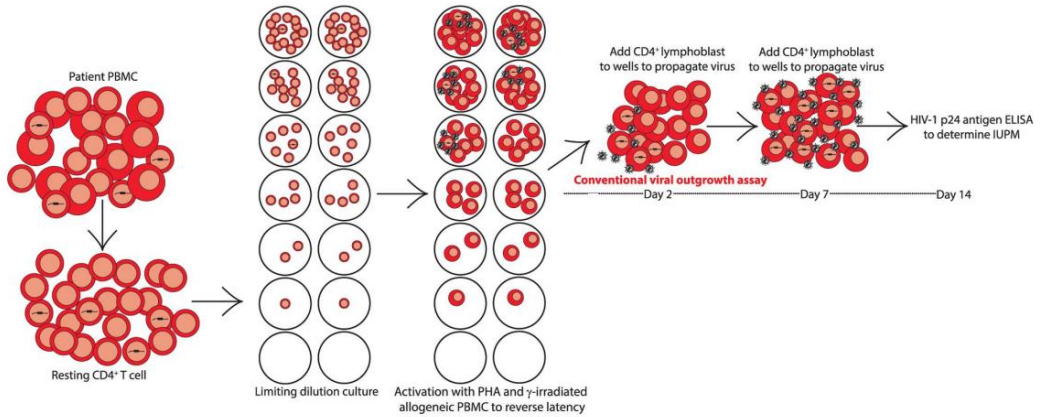
ART


Integrated
HIV DNA
Transcription
(HIV mRNA)
Translation
(HIV protein)
Virion
production

Detecting/Quantifying HIV persistence

Detecting and quantifying the latent HIV reservoir

- QVOA (quantitative viral outgrowth assay)

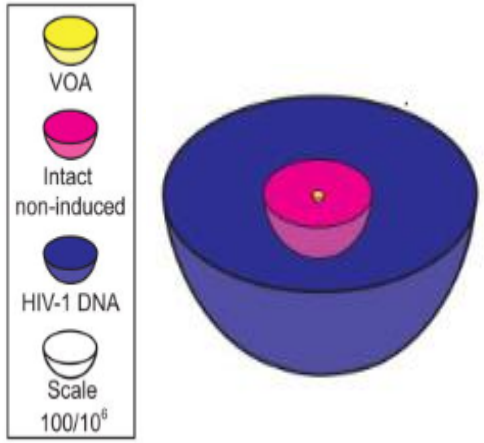


Laird et al. HIV Protocols. Methods in Molecular Biology. 2016

- HIV DNA
  - Single-region PCR-based assay

- TILDA (Tat-/Rev-induced limiting dilution assay)

	QVOA	HIV DNA
Cell numbers required	~100 million PBMC	~5 million PBMC
Time required	~2 weeks	1 day
Indicate replication competency	Yes	No
	Under estimates	Over estimates



Ho et al. Cell. 2013

- Intact proviral DNA assay (IPDA)
  - Digital droplet PCR assay detects intact, cell-associated, full-length genomic HIV DNA

Bruner et al. Nat. 2019

Detecting and quantifying ongoing transcription

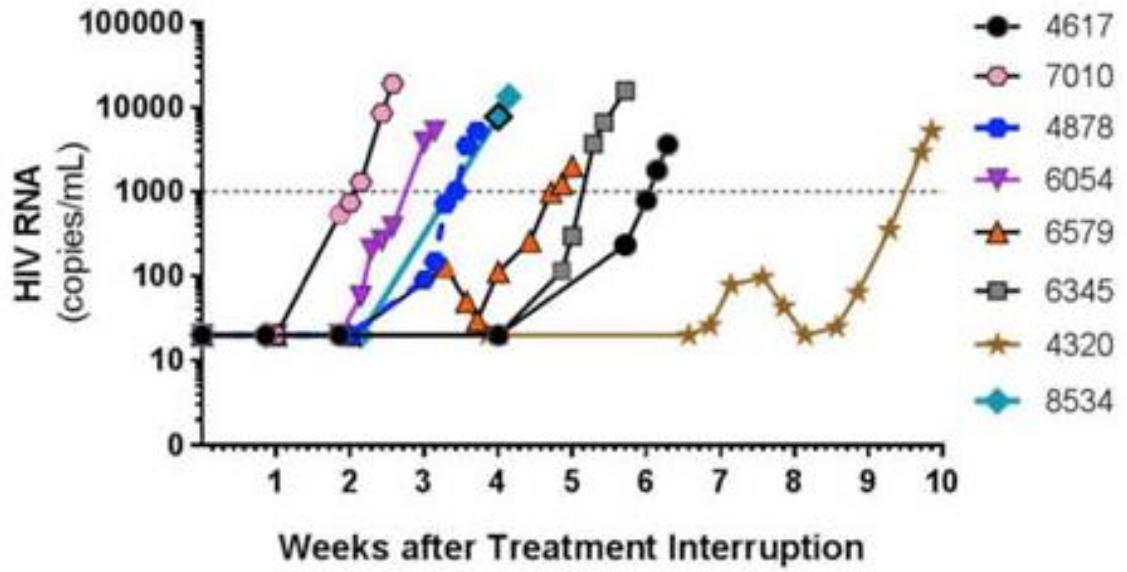
- Detects HIV RNA
  - Cell associated HIV-RNA
    - Presence does not necessarily imply productive replication
  - Single copy assay
    - Plasma (cell-free) RNA
    - Majority of individuals on ART with undetectable VL on conventional assay still have detectable HIV RNA on SCA

Malderelli et al. Plos Pathogen. 2007; Palmer et al. PNAS. 2008

Establishment of HIV persistence

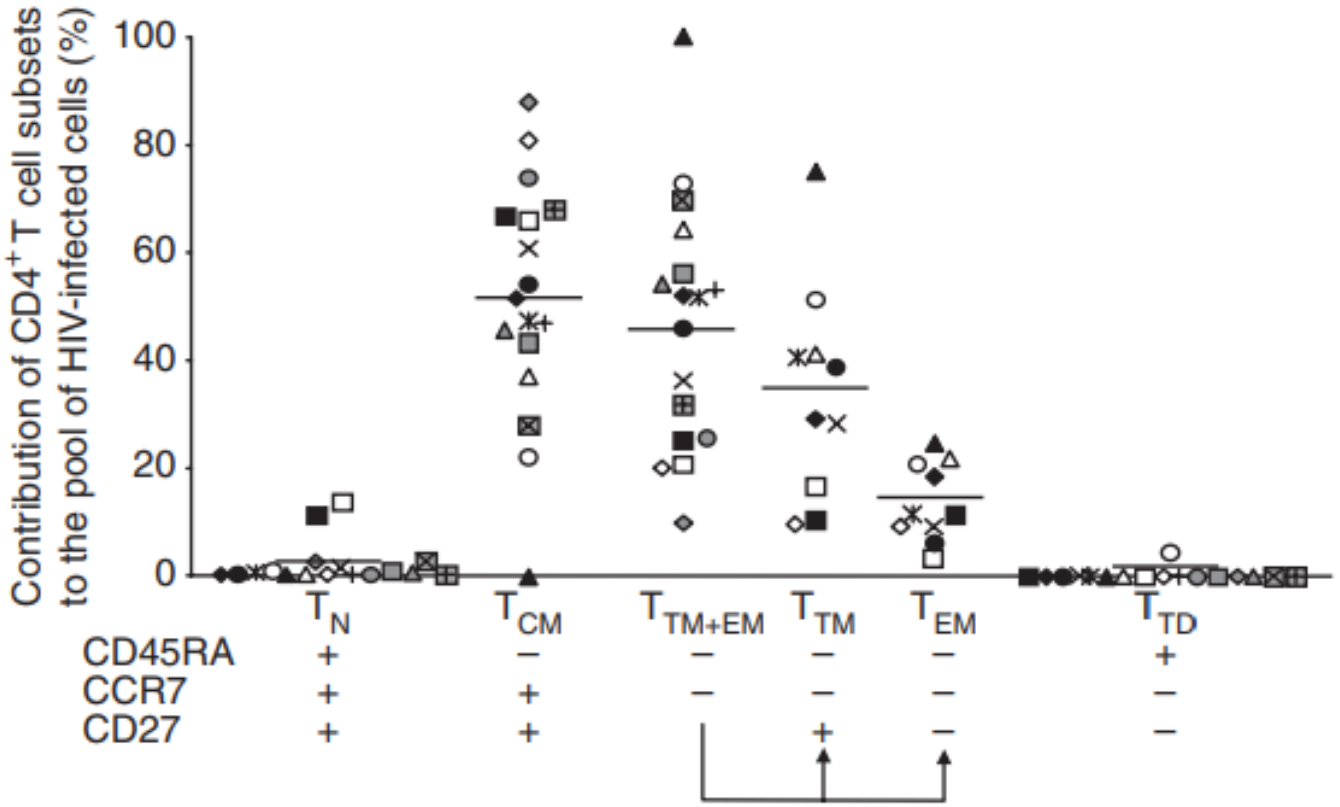
The HIV reservoir is established very early

- Initiated ART during Fiebig I acute HIV infection
- On ART for a median of 2.8 years
- All experienced rapid viral load rebound following analytical treatment interruption



Colby et al. Nat Med. 2018

Majority of the latent reservoir is in memory CD4+ cells



Chomont et al. Nature Medicine. 2009

Other cell types

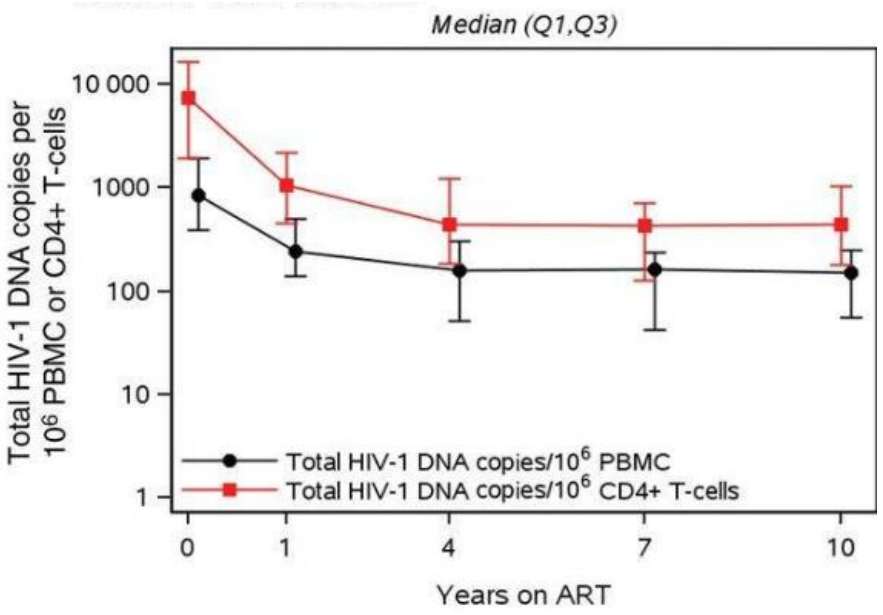
- Monocytes, tissue macrophages and dendritic cells
  - Are susceptible to HIV infection
    - Express CD4, CCR5 and CXCR4
  - Relatively long-lived
- Harbor HIV DNA in the setting of plasma viral suppression with ART

Zalar et al. Antiviral Research. 2010; Ganor et al. Nat Microbiol. 2019

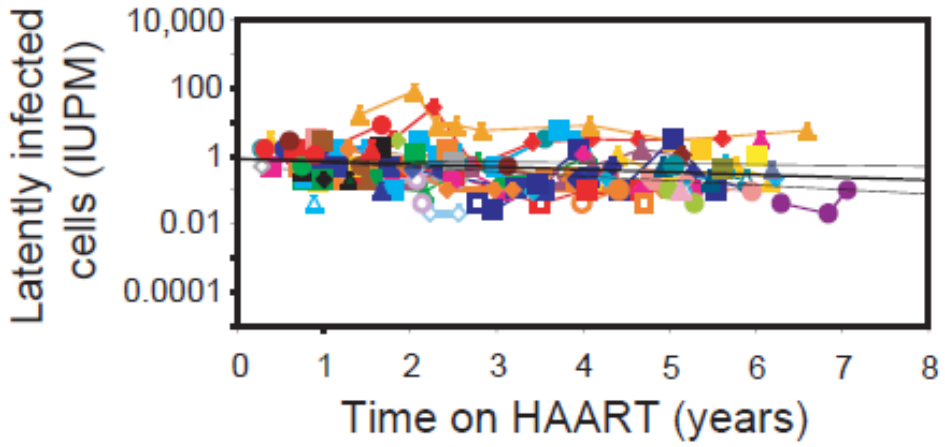
- Their contribution to the latent reservoir is still unclear

Effect of ART on the HIV reservoir

Decay of the HIV reservoir is slow



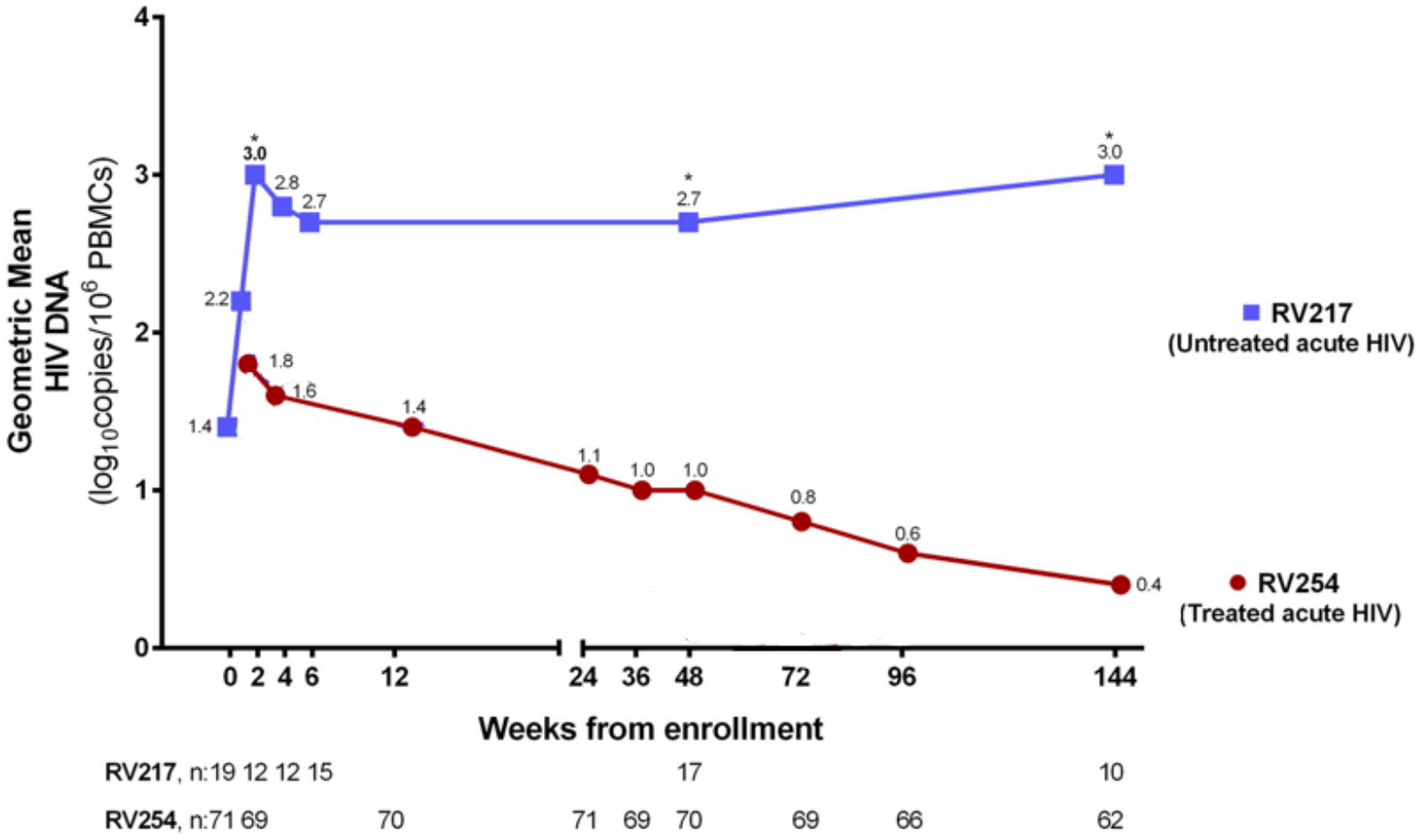
Besson et al. Clin Infect Dis. 2014



Siliciano et al. Nature Med. 2003

- t1/2 = 44 months in individuals on ART with plasma viral suppression
- Require 73 years of ART to eradicate a reservoir of 1 million cells

ART during acute infection reduces the size of the HIV reservoir



Ananworanich et al. E Biomedicine. 2016

HIV persistence on ART

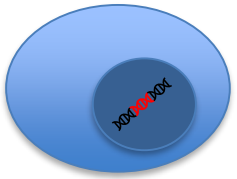
Anatomical Locations of HIV persistence on ART

Tissue	HIV DNA	HIV RNA
Lymph node	√	√
Gut	√	√
CNS	√	√
Spleen	√	√
Bone Marrow	√	
Lung	√	√
Kidney	√	√
Reproductive tract	√	√
Adipose tissue	√	√
Liver	√	

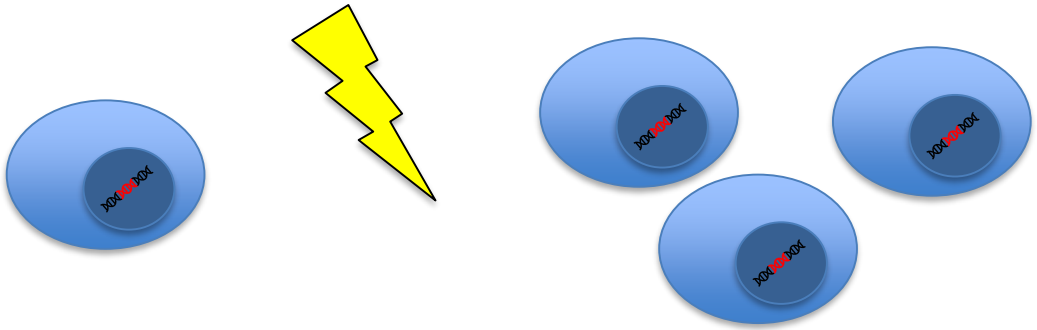
Wong et al. Curr Opin HIV AIDS. 2016

Sources of HIV persistence

- 1. Latently infected cells
  - Survival of long-lived latently infected cells
    - Potentially target with kick and kill strategy



  - Clonal expansion of latently infected cells
    - Populations of cells with identical HIV sequences and integration sites



    - Homeostatic proliferation
    - Antigen stimulation
    - Integration in genes that control growth and development of cells/ cancer associated genes

Chomont et al. Nature. 2009; Malderelli et al. Science. 2014; Wagner et al Science. 2014; Hosmane et al. J Exp Med. 2017

Clonal expansion

- >50% of the inducible, replication-competent HIV reservoir consists of $CD4^+$ T-cells carrying identical proviral sequences.

Bui et al. Plos pathogens. 2014

- Clonally expanded provirus can be intact and infectious

Simonetti et al. Proc Natl Acad Sci USA. 2016

- These clones can be a source of initial rebound viremia during ATI

Kearney. J Virol. 2015

- Potentially target with anti-proliferative therapies

2. Persistent viral expression on ART

- >80% of patients on ART for 60 wk have persistent viremia (median of 3.1 copies/ml) on single copy assay.

Malderelli et al. Plos Pathogen. 2007

- Potential sources

1) ongoing cycles of viral replication and infection (controversial)
   - Inadequate ART potency
   - Inadequate ART penetrance at sanctuary sites

2) ongoing virus production from integrated proviruses, but ongoing cycles on infection blocked by ART

Why is the differentiation important?

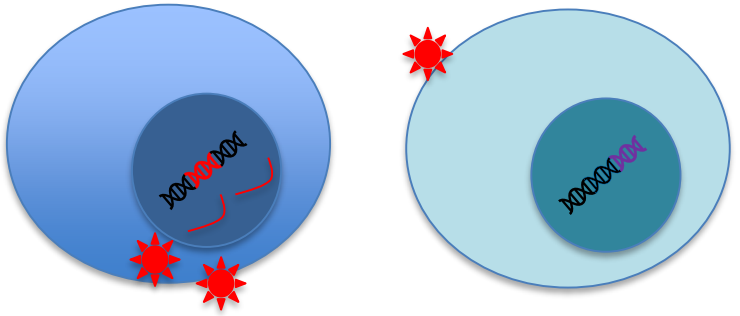
1) If there is ongoing cycles of viral replication and infection

   - Focus research on improving ART potency and penetration

2) If there is ongoing virus production from integrated proviruses

   - Not targeted by existing immune responses
   - Latency reversal agents will not affect this population
   - Block and lock strategy may have greater potential

1. Ongoing cycles of viral replication and infection



Mutated sequence and different integration site

- Unlikely in peripheral blood
  - Emergence of new variants whilst on ART is rare

    Kearney et al. Plos Pathogen. 2014

  - The majority of studies showed no reduction in the level of persistent viremia with treatment intensification

    Gandhi et al. JAIDS. 2012

Ongoing cycles of viral replication and infection

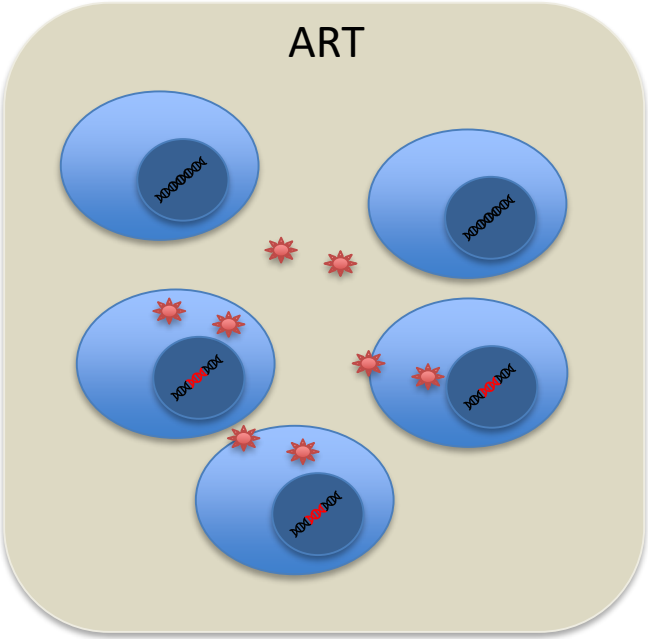
- May potentially occur in sanctuary sites

	Lymph Node	CNS
Reduced ART penetration	Fletcher. PNAS. 2014.	Letendre et al. Arch Neurol. 2008
Reduced immune mediated killing	Fukazawa. Nat Med. 2015.	
HIV RNA expression on ART	Estes. Nat Med. 2018.	Pérez-Valero et al. AIDS. 2019
Sequence evolution on ART	Lorenzo-Redondo et al. Nature. 2016	

2. Ongoing virus production from integrated provirus

- Clonally expanded intact provirus can produce sufficient virus to cause detectable low-level viremia

Simonetti et al. Proc Natl Acad Sci USA. 2016; Halvas et al. CROI. 2019



- Plasma viral sequences matches proviral sequences matches QVOA well sequences
- No ART resistance mutation
- No sequence evolution in the clones
- Mechanisms of escape are yet to be determined
- How to suppress these clones ?

Summary

- Understanding the mechanisms of HIV persistence is essential for the development of strategies to induce HIV remission.

- HIV persistence on ART is due to the existence of

  1. Latent viral reservoir
  2. Ongoing transcription and virus production

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