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IAS Pathways to an HIV cure meeting 2022:
Cure Advances Globally

Virology of the reservoir: a 15 minute summary
After this, the infected cell dies, or is eliminated by the immune system.
HIV can persist inside cells as an integrated viral genome.
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These **viral reservoirs** are the main barrier to HIV remission and cure:

- > antiretroviral therapies do not eliminate these cells
- > these cells are *largely* invisible to the immune system
- > these cells can persist for years
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- these cells are *largely* invisible to the immune system
- these cells can persist for years
- these cells can clonally expand, producing daughter cells that also contain integrated HIV within them\(^1\)-\(^5\)

\(^1\) Maldarelli et al 2014 PMID 24968937; \(^2\) Wagner et al 2014 PMID 25011556; \(^3\) Simonetti et al 2016 PMID 26858442; \(^4\) Hosmane et al 2017 PMID 28341641; \(^5\) Lorenzi et al 2016 PMID 27872306
Viral reservoirs can reactivate at any time to produce infectious HIV
Reservoir dynamics: a two-minute summary

Following transmission, HIV replicates and mutates, producing a genetically diverse viral population.
Seeding of the reservoir begins immediately following infection.

Reservoir cells can persist for years.
Reservoir dynamics: a two-minute summary

Seeding of the reservoir begins immediately following infection.

Reservoir cells can persist for years.

Clonal expansion is a major way in which the reservoir sustains itself.
Reservoir cells persist for years, but not forever. By the time that ART is initiated, many early lineages have been eliminated.
Reservoir dynamics: a two-minute summary

On ART, reservoir decay is SLOW
Estimated half-life = 44 months (3.7 years)

Figure: Siliciano et al, Nat Med 2003, PMID 12754504
Also: Golob et al, AIDS 2018, PMID 30005008
Peluso et al, JCI 2020, PMID 32045386
Gandhi et al, JID 2021, PMID 32823274
Reservoir dynamics: a two-minute summary

When ART is stopped, HIV re-emerges
Only ~2% proviruses persisting during long-term ART are genetically intact.
**Reservoir** = cell harboring intact, replication-competent HIV

Persistently infected cell

Slide by Natalie Kinloch
Cells with defective provirus may produce HIV transcripts/proteins\textsuperscript{1-3}, but they don’t meet the definition of “reservoir”.

\textsuperscript{1}Imamichi et al 2016 PMID 27432972; \textsuperscript{2}Imamichi et al 2020 PMID 32029589; \textsuperscript{3}Pollack et al 2018 PMID 29355843

Slide by Natalie Kinloch
Studying the reservoir: key methods

1. Methods for reservoir *quantification*

2. Methods for (genetically) *characterizing reservoir cells*
Reservoir quantification #1: Quantitative Viral Outgrowth Assay (QVOA)

Resting CD4+ T-cells → Limiting dilution culture → Stimulate to reactivate HIV → Add more cells to propagate virus in culture → HIV p24 ELISA to determine Infectious HIV units per million CD4+ T-cells (IUPM)

Adapted from Laird et al PLoS Pathogens 2013; PMID 23737751
Resting CD4+ T-cells

Limiting dilution culture

Stimulate to reactivate HIV

Add more cells to propagate virus in culture

HIV p24 ELISA to determine Infectious HIV units per million CD4+ T-cells (IUPM)

- Laborious
- Requires a lot (~200mL) of blood
- Underestimates reservoir, because not all cells reactivate in vitro

Adapted from Laird et al PLoS Pathogens 2013; PMID 23737751
Detection of both targets jointly discriminates >90% of defective proviruses

Target 1 ($\psi$)

Target 2 (env)

Reservoir quantification #2: The Intact Proviral DNA Assay (IPDA)

Starts with DNA!

Bruner et al, Nature 2019; 566(7):120

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Detection of both targets jointly discriminates >90% of defective proviruses

Target 1 ($\psi$)

Target 2 (env)

The Intact Proviral DNA Assay (IPDA)

Droplet digital PCR

1. MAKE
   Sample is partitioned into 20,000 droplets

2. CYCLE
   Run PCR cycles in all droplets simultaneously

3. READ
   Measure fluorescence intensity in each droplet
   Calculate concentration from number of positive droplets

Bruner et al, Nature 2019; 566(7):120
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Target 1 (ψ)
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Results expressed as intact proviral genomes per million CD4+ T-cells

Bruner et al, Nature 2019; 566(7):120
Reservoir quantification #2: The Intact Proviral DNA Assay (IPDA)

Detection of both targets jointly discriminates >90% of defective proviruses.

Results expressed as intact proviral genomes per million CD4+ T-cells.

• primers/probes don’t capture all HIV variants.

Bruner et al, Nature 2019; 566(7):120
Reservoir genetic characterization: Full-length individual proviral sequencing

DNA extraction → 96 well plate → Visualize amplicons → Intactness inference

- Limiting dilution
- Genome-intact provirus
- Defective provirus
- Human DNA
- PCR
- Sequencing

Figure adapted from Lee, Viruses 2021 PMID 34578455 and Patro et al, Viruses 2021 PMID 34960744. Also see Lee GQ et al, J Clin Investig 2017 PMID 28628034; Hiener et al Cell Rep 2017 PMID 29045846. Also Q4PCR: Gaebler et al, J Exp Med 2019 PMID 31350309
Reservoir genetic characterization: Full-length individual proviral sequencing

LIMITATION: This approach does not allow simultaneous study of other reservoir attributes (e.g. integration site)

Figure adapted from Lee, Viruses 2021 PMID 34578455 and Patro et al, Viruses 2021 PMID 34960744.
Also see Lee GQ et al, J Clin Investig 2017 PMID 28628034 ; Hiener et al Cell Rep 2017 PMID 29045846
Also Q4PCR: Gaebler et al, J Exp Med 2019 PMID 31350309
SOLUTION:
“Multiply” your DNA to allow multi-parameter characterization

**DNA extraction**

- Genome-intact provirus
- Defective provirus
- Human DNA

**96 well plate**

- Limiting dilution

**Amplify the entire DNA contents of each well by Multiple Displacement Amplification (MDA)**

Figure adapted from Lee, viruses 2021 PMID 34578455 and Patro et al, Viruses 2021 PMID 34960744. Also see Lee GQ et al, J Clin Investig 2017 PMID 28628034; Hiener et al Cell Rep 2017 PMID 29045846

**Also QPCR:** Gaebler et al, J Exp Med 2019 PMID 31350309
SOLUTION: “Multiply” your DNA to allow multi-parameter characterization

DNA extraction  

96 well plate

Amplify the entire DNA contents of each well by Multiple Displacement Amplification (MDA)

Multiparameter characterization

Figure adapted from Lee, viruses 2021 PMID 34578455 and Patro et al, Viruses 2021 PMID 34960744. Also see Lee GQ et al, J Clin Investig 2017 PMID 28628034 ; Hiener et al Cell Rep 2017 PMID 29045846

Also Q4PCR: Gaebler et al, J Exp Med 2019 PMID 31350309
Where is the reservoir? The answer is multidimensional

1. Where in the cell’s genome did the provirus integrate?

2. What cell types harbor HIV reservoirs?

3. What tissues in the body harbor HIV reservoirs?

Deeks et al Nat Med 2021; PMID 34848888
Where is the reservoir? The answer is multidimensional

1. Where in the cell’s genome did the provirus integrate?

2. What cell types harbor HIV reservoirs?

3. What tissues in the body harbor HIV reservoirs?

These locations influence reservoir longevity, reactivation, and genetic composition

Deeks et al Nat Med 2021; PMID 34848888
Intact proviruses in expanded CD4+ T-cell clones were preferentially integrated within KRAB domain-containing zinc finger (ZNF) genes.

Huang et al JEM 2021 PMID 34636876
Intact proviruses in expanded CD4+ T-cell clones were preferentially integrated within KRAB domain-containing zinc finger (ZNF) genes.

Integration into certain genomic sites may help reservoir cells persist following clonal expansion.
Integration site influences the likelihood of persistence

In Elite Controllers, clonally expanded proviruses tend to be integrated into transcriptionally inactive regions (e.g. centromeric satellite DNA)
Integration site influences the likelihood of persistence

In Elite Controllers, clonally expanded proviruses tend to be integrated into transcriptionally inactive regions (e.g. centromeric satellite DNA)

This is not because HIV preferentially integrates into these regions.

It is because their immune systems eliminate cells with proviruses integrated into more transcriptionally active regions.
Cell type matters

CD4+ T-cell subsets differ in the degree of clonal expansion

Longevity and stability as a reservoir

Effector memory cells are enriched in clonally expanded proviral sequences

1 Hiener et al 2017 PMID 29045846; 2 De Scheerder et al 2019 PMID 31471273; 3 Jones et al 2020 PMID 31776273
Cell type matters

CD4+ T-cell subsets differ in the degree of clonal expansion

- naive
- central memory
- transitional memory
- effector memory

Longevity and stability as a reservoir

**Effector memory** cells are enriched in clonally expanded proviral sequences

Macrophages as distinctive reservoirs

- Found in all tissues
- Long-lived; relatively resistant to immune and HIV-mediated killing
- Reside in sites with reduced ART penetration (e.g. CNS).

These properties can yield genetically distinctive proviral populations in certain tissues

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Proviral diversity in blood is generally representative of that in tissues, and genetic compartmentalization in tissue is generally limited.

How (genetically) distinctive are reservoirs in different tissues?

LG03

Tissue
- Blood/PBMC
- Gastrointestinal tract
- Liver
- Lymphatic tissue
- CNS
- Pancreas
- Heart
- Genito–urinary tract

IC₅₀ to various nAbs
- ≤ 0.1
- ≤ 1
- ≤ 10
- > 10

Wang et al, Clin Infect Dis 2022 PMID 35234862
Proviral diversity in blood is generally representative of that in tissues, and genetic compartmentalization in tissue is generally limited.

BUT some tissues may harbor proviral populations with distinctive genetic and functional features (e.g. the brain).

How (genetically) distinctive are reservoirs in different tissues?

Wang et al, Clin Infect Dis 2022 PMID 35234862
Methods innovation: single-cell reservoir profiling

**HIV SORT-seq**

- CD4+ T-cells
- Activate
- Hybridize
- Sort

Liu et al, Sci Trans Med 2020, PMID 32404504

**HIV STIP-seq**

- Activate
- HIV p24 stain
- Sort
- MDA

Cole et al, Nat Comm 2020, PMID 34140517

**ECCITEseq**

- Surface protein
- Cellular RNA Transcriptome
- HIV-1 RNA
- T cell receptor (TCR)

Collora et al, Immunity 2022, PMID 35320704

These techniques “fish” out individual reservoir cells for characterization!
Summary

- Intact, replication-competent HIV proviruses persist throughout the body during ART, and represent the main barrier to cure.
- Clonal expansion is a major mechanism that sustains the reservoir.
- A provirus’ location (genomic, cellular, tissue) can influence its ability to persist.
- Methods for reservoir characterization continue to be innovated.
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Without you, research would not be possible.