Measuring the HIV-1 latent reservoir: Lessons from single cell analyses

Lillian Cohn, PhD
CZ Biohub
San Francisco, CA
## Disclosure

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The major barrier to HIV-1 cure is the **latent reservoir**

\[ t_{1/2} = 44 \text{ months} \]

*HBV also has a long half-life*

The major barrier to HIV-1 cure is the **latent reservoir**

- Established early
- Primarily rare, long-lived memory CD4+ T cells
- Transcriptionally silent and no validated surface marker
- Mechanism for persistence unknown
Using single-cell methods to identify and characterize latently infected cells

Challenges inherent to analysis of primary samples

Strategies to study cells and virus in the reservoir

Comparing circulating reservoir viruses to plasma rebound

*HBV therapy has similar pattern on HBV viremia*
Strategies to characterize virus and infected cells from HIV-1 infected donors

**Nucleic acid**

1. Virus sequence
   ...AGACCCTTTTAGTCAGTGAGAAATC...

2. Provirus sequence
   ...CAGTGTGGAAAATCTCTTAGCAactgccgttagccaggcta...

   Integration site
   ...actgccgttagccagctaggactgacctaggct...
   ...ctaggctaggactgaccaggttttttcactaggac...

**Cell-based assay**

3. Surface protein expression

Host transcriptome
...actgccgttagccagctaggactgacctaggct...
Viral RNA
AGACCCTTTTAGTCAGTGAGAAATC
Single cell integration site analysis reveals clones of infected cells

Provirus  CD4 genome  Linker

Semi-nested PCR

Illumina deep sequencing and analysis

Cohn et al., *Cell*. 2015.

Identifying intact virus by PCR

1. Nested near-full length PCR amplification of proviral DNA followed by sequencing of viral genome
   Frequency and sequence, low throughput. Ho et al., Cell 2013.

2. Pair of qPCR primers quantify likely intact virus by digital droplet PCR (IPDA)

3. Near-full length PCR, digital PCR for 4 viral segments, nested PCR and sequencing
Replication competent virus persists in clones of infected cells

Longitudinal analysis of replication competent clones

Lorenzi et al., PNAS. 2016.
Hosmane et al., JEM. 2017.

Wang et al., PNAS. 2018.
Methods for characterizing primary infected cells

HIV-Flow


Latency Capture


HIV-1 SortSeq

Ho Lab, unpublished
Recently reactivated latent cells expression a unique set of genes

Latency Capture

HIV-Flow
- Surface marker expression

HIV-1 SortSeq
- Integration site
- Splicing
- Gene expression

Ho Lab, unpublished
Technical advances enable high-throughput single cell analyses

- Seq-Well, 10x Genomics, other droplet-based systems
- 10,000s of cells
- Enormous datasets
- Challenge to integrate sequencing data with effective analysis techniques

Minority of CD4+ T cells are in circulation

Adapted from Farber et al., Nat Rev Immunol. 2014.
How relevant are infected cells in circulation to viral rebound *in vivo*?

**Conundrum:**
Latent virus from circulating cells does not represent plasma rebound.

However,
Some rebound sequences were found to be likely recombinants of viruses identified in circulation.

Conclusions

1. Single cell analyses reveals clonally expanded infected cells
   Majority harbor defective viruses, but cells with replication competent virus can also divide
2. Analysis of primary single-cells reveals gene/protein expression differences
3. Challenges for the field:
   Single cell analysis modalities
   Analysis of rebound-relevant populations
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Study participants

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