Comparative Analysis of Measures of Viral Reservoirs in HIV-1 Eradication Studies

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Disclosures: None
Slow decay of latently infected CD4+ T cells

Time to eradication $> 73.4$ years

Finzi et al., Nature Med., 1999
Virus culture assay for latent HIV-1 in resting CD4+ T cells

180-200 ml blood

Purified resting CD4+ T cells

5x10^6 10^6 2x10^5 4x10^4 8x10^3 1.6x10^2 Negative control

Finzi et al, Science 1997
Finzi et al., Nature Med., 1999
Virus culture assay for latent HIV-1 in resting CD4+ T cells

- Detects individual latently infected cells
- Detects cells with latent viruses capable of robust growth in vitro in primary CD4+ T cells
- Does not detect cells with defective viruses
- No other approach has given a higher frequency of cells with replication competent virus
- BUT labor-intensive and costly

Finzi et al, Science 1997
Finzi et al., Nature Med., 1999
Comparison of reservoir assays

- SCOPE, Chronic, n=20
- Options, Acute, n=10

180 ml blood → GALT biopsy → HIV RNA:DNA ratios

160 ml → 20 ml → Ancillary studies

Separate: plasma PBMC

Send to JHU
Send to Karolinska
Send to UCSD

Single copy assay for RV, single genome sequencing
Digital droplet PCR

Purified resting CD4+ T cells

Total and integrated HIV-1 DNA
Viral outgrowth assay

Sites:
- UCSF
- JHU
- Karolinska
- UCSD
- U Penn

Correlation between culture and PCR assays

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<tr>
<th></th>
<th>r</th>
<th>P</th>
<th>n</th>
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<tbody>
<tr>
<td>Combined</td>
<td>0.20</td>
<td>0.29</td>
<td>30</td>
</tr>
<tr>
<td>Chronic</td>
<td>-0.04</td>
<td>0.87</td>
<td>18</td>
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<tr>
<td>Acute</td>
<td>0.46</td>
<td>0.18</td>
<td>20</td>
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HIV DNA (copies/10^6 PBMC)

Viral outgrowth (IUPM)

Infected cell frequency

Viral outgrowth

Total HIV DNA

2 LTR circles

Integrated HIV DNA

Total HIV DNA

Residual viremia

**Assay for persistent HIV in patients on HAART**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Viral outgrowth</th>
<th>Total HIV DNA</th>
<th>2 LTR circles</th>
<th>Integrated HIV DNA</th>
<th>Total HIV DNA</th>
<th>Residual viremia</th>
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<tbody>
<tr>
<td>Cell/tissue</td>
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<td>Resting CD4</td>
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<td>PBMC</td>
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Plasma HIV RNA (copies/ml)

**Eriksson et al, PLoS Pathogens, 2013**
Ratio of infected cell frequencies by PCR and culture assays

Infected cell frequency (per 10^6)

Viral outgrowth

Total HIV DNA

2 LTR circles

Integrated HIV DNA

Total HIV DNA

Residual viremia

Assay for persistent HIV in patients on HAART

Which assay should be used?

Non-induced proviruses

Resting CD4+ T cells

Non-induced proviruses

PHA + irradiated allogeneic PBMC

d2:CD4+ blasts from HIV-donors
d7:CD4+ blasts from HIV-donors

5x10^6 10^6 2x10^5 4x10^4 8x10^3 1.6x10^2 Negative control

Full length, single genome analysis

Non-induced ≠ non-inducible

Ho et al, Cell, 2013
Clonal analysis of non-induced proviruses

9.1 KB limiting dilution PCR

Nested gag PCR to establish clonality

Direct sequencing of PCR products
No cloning!

Ho et al, Cell, 2013
Non-induced proviral clones (n=213)

Hypermutated 32.4%

TGG → TAG
Trp → Stop

Ho et al, Cell, 2013
32.4% of non-induced proviruses have G→A hypermutation

ATG → ATA
M→I
start codon mutation

B.FR.83.HXB2_LAI_IIIB_BRU_K034
9CC3_31E5_gag_hypermut
9CC3_31E11_gag_hypermut
20CB4_36D12_gag_hypermut
20TB1_33C3_gag_hypermut
20TB1_33C9_gag_hypermut
20TB3_33G10_gag_hypermut

ATGGTGGAGAGCGTGTTATTAGCGGGGGAATGATCGAGGGAAAAATTCTGTTAACCCAGGGGAA

A..........................A.............A............................A.......C...........

TGG → TAA, TAG, TGA
Tryptophan → stop codon
nonsense mutation

B.FR.83.HXB2_LAI_IIIB_BRU_K034
9CC3_31E5_gag_hypermut
9CC3_31E11_gag_hypermut
20CB4_36D12_gag_hypermut
20TB1_33C3_gag_hypermut
20TB1_33C9_gag_hypermut
20TB3_33G10_gag_hypermut

MGARASVLSSGELDEWVKIIRPGKGGKYLKHIVWASRELFAVNPGLLETSACRCQILGQLPML
 IS....................I.R...............Q.K..................L.*.CK.............S.A...R....
 I....................Q...............R.N.R......................AG........E...A.
 I....................Q...............R.N.R......................AG........E...A.
 I....................Q...............R.N.R......................AG........E...A.
 I....................R.Q..............E.N.R......................K............AG...E...A.
Non-induced proviral clones (n=213)

Large internal deletion 45.5%

Hypermутated 32.4%

Ho et al, Cell, 2013
45.5% of non-induced proviruses have large internal deletions

Ho et al, Cell, 2013
Non-induced proviral clones (n=213)

- Nonsense mutations/INDELS 3.8%
- Deletion in ψ/MSD site 6.5%
- 11.7% Intact genome
- Large internal deletion 45.0%
- Hypermutated 32.4%

Ho et al, Cell, 2013
Replication-competence of non-induced proviruses

Non-induced proviral sequence

pNL4-3

LTR

Transfection into 293T cells

Virus production

Infection of primary CD4+ T lymphoblasts
Replication of non-induced proviruses clones

Ho et al, Cell, 2013
Non-induced proviruses have functional LTRs except for hypermutated clones.

NF-κB

Sp1-III

Sp1-II

Sp1-I

HXB2

NF-κB

Hypermutated
Clonal analysis of DNA methylation

Patient 20

Cells from p24 negative co-culture well

Single genome bisulfite sequencing

Ho et al, Cell, 2013
Non-induced proviruses integrate into active transcription units

- Location
  - Intron, 82.9%
  - Exon, 5.7%
  - Other, 4.3%
  - Intergenic space, 7.1%

- Activity of genes

Transcription units: 92.9% (65/70)

Ho et al, Cell, 2013
Intact vs induced proviruses

62 fold

Ho et al, Cell, 2013
Can intact non-induced proviruses be induced?

180-200 ml blood

Purified resting CD4+ T cells

PHA + irradiated allogeneic PBMC

Recover cells from negative wells

5x10^6  10^6  2x10^5  4x10^4  8x10^3  1.6x10^2  Negative control

d2: add CD4+ lymphoblasts from HIV- donors

d7: add CD4+ lymphoblasts from HIV- donors

Ho et al, Cell, 2013

p24 Ag
Can intact non-induced proviruses be induced?

Ho et al, Cell, 2013
Thanks

Collaborators
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Doug Richman             Joe Wong
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