Measuring the HIV reservoirs: new and improved methods

### Current Methods For Evaluating Curative Strategies

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Advantages</th>
<th>Disadvantages</th>
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</thead>
<tbody>
<tr>
<td>Plasma-derived HIV RNA (SCA)</td>
<td>Relatively inexpensive</td>
<td>Does not detect the frequency of latently infected cells. Patients on long-term ART close to limit of assay detection.</td>
</tr>
<tr>
<td>Quantitative Viral Outgrowth Assay (QVOA)</td>
<td>Direct measurement of replication-competent virus, or number of proviruses capable of productive infection</td>
<td>Requires large quantities of cells, $$$, time consuming, limited dynamic range, does not detect all proviruses which pose a barrier to a cure</td>
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<tr>
<td>T cell Activation Assays with viral RNA readout</td>
<td>Less culture time required, no need for outgrowth of virus for measurement</td>
<td>May detect some defective viruses. Does not detect all proviruses which pose a barrier to a cure</td>
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<tr>
<td>Total HIV DNA qPCR/ddPCR</td>
<td>Inexpensive, easy, quick and for ddPCR absolute quantitation</td>
<td>Detects defective proviruses which may not pose a barrier to a cure</td>
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<tr>
<td>Integrated HIV DNA Alu-PCR</td>
<td>Excludes unintegrated HIV DNA, less error than VOA</td>
<td>Detects defective provirus, does not detect proviruses too far from alu sequence</td>
</tr>
<tr>
<td>Cell-associated HIV RNA/US RNA/ MS RNA</td>
<td>Inexpensive, easy, quick and measures intracellular HIV transcription</td>
<td>Detects aborted and defective HIV RNA transcripts which may not be translated into viral proteins</td>
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### New Methods For Evaluating Curative Strategies

1) Can the Host Immune Response Against HIV Be Used to Quantify The Latent HIV Reservoir?

- Titer, Avidity, Specificities

- Anti-gp120
- Anti-RT
- Anti-p24

courtesy of Michael Busch, Blood Systems Research Institute
Luciferase Immunoprecipitation Assay (LIPS) to Detect HIV Antibody Levels

Peter Burbelo, NIH

- Luciferase-antigens: gp120, gp41, reverse transcriptase, integrase, protease, matrix, p24, tat
- Add diluted sample
- Add IgG beads – binds Ag-bound Ab
- Measure luciferase

Antibody Profiles Against HIV Antigens

Burbelo et al. JID 2014

Measures of the HIV Reservoir are Correlated with anti-RT, -gp120 & -gp41 Levels by LIPS

<table>
<thead>
<tr>
<th>Env</th>
<th>Pol</th>
<th>Gag</th>
</tr>
</thead>
<tbody>
<tr>
<td>avg</td>
<td>avg</td>
<td>avg</td>
</tr>
<tr>
<td>0.88</td>
<td>0.73</td>
<td>0.70</td>
</tr>
<tr>
<td>0.70</td>
<td>0.60</td>
<td>0.54</td>
</tr>
<tr>
<td>0.41</td>
<td>0.34</td>
<td>0.28</td>
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- Driven by strong correlations with total (dRdPCR) or integrated (aliPCR) HIV DNA

Moderate correlations between:
VOA: gp120, gp41
CA: U5 RNA: gp120
2-LTR DNA: gp120

Adjustment for age, past CD4+ T cell count, present CD4+ T cell count, prior ART, pre-ART viral load did not significantly alter these results.

Full HIV Genome Sequencing

Using Sanger Sequencing

- Deletion junction not precisely defined
- Incorrect amplicon size – large internal deletion 45.5%

Full HIV Genome Sequencing

Using Next Generation Sequencing

- Allows for the direct calculation of defective versus replication-competent virus located in cells

Image adapted from He et al. Cell 2013

Image adapted from He et al. Cell 2013

Identifying Defective HIV-1

- Resting Memory CD4+ T cells
  (4x10^6 to 2x10^7)
- SR5 nonintegrated virus from VOA assay had obvious defects
- Locations of deletions shown in white horizontal bars
- Obvious to detect defective HIV-1
- Allows for the direct calculation of defective versus replication-competent virus located in cells

Image adapted from He et al. Cell 2013
3) The murine viral outgrowth assay (MVOA)

- Immunocompromised (NSG) mice lack B, T and NK cells, engraft with human T cells but develop GVHD
- Inject 10^7-50 million PBMCs or CD4+ T cells from HIV-infected participants into each mouse intraperitoneally
- +/- activation with anti-CD3
- +/- depletion of CD8+ T cells
- Measure plasma viremia in mice through weekly bleeds and in terminal bleed

MVOA Detects HIV-1 from Participants on Suppressive ART

- Injected 25 to 55 million PBMCs from 6 patients on suppressive cART regimens (1 to 6 years) intraperitoneally (IP) into NSG mice
- On day 7 anti-CD8 mAb IP was given
- Mice were bled weekly to evaluate viral load and CD4 T cell count

Conclusions

- New assays are being developed for measuring the HIV reservoir.
- However, additional assays are needed to differentiate between defective and replication-competent virus:
  - High throughput full-length proviral sequencing
- Must all potential reservoirs be analyzed? If not, are we confident that certain reservoirs are determinative of cure/remission?
- Looking ahead, to determine the effectiveness of curative strategies, our field will need to develop a more standardized assay system which is sensitive, efficient, less costly, and adoptable in local settings.

Acknowledgements

Centre for Virus Research-WMI
University of Sydney
E. Lee
B. Hiener
K. Barton
A. Winkelmann
S. Von Stockenström
M. Logan
T. Cunningham

For Slides and Discussion
Michael Busch
Katherine Bruner
Robert Siliciano
Kelly A. Metcalf Pate
Joel N. Blankson

We acknowledge with gratitude the study participants

[Images of graphs and diagrams related to MVOA and HIV-1 detection]