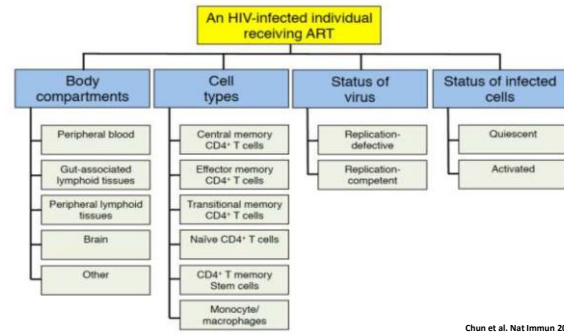
Westmead Millennium Institute for Medical Research

# Measuring the HIV reservoirs: new and improved methods

THE UNIVERSITY OF SYDNEY

## Measuring Persistent HIV Infection




Chun et al. Nat Immun 2015



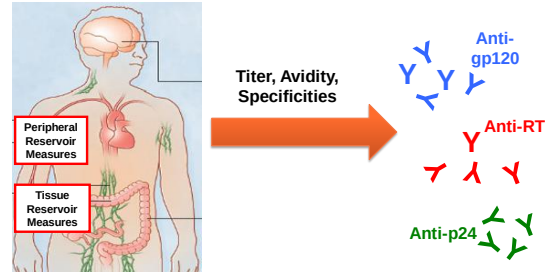
## Current Methods For Evaluating Curative Strategies

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



## New Methods For Evaluating Curative Strategies

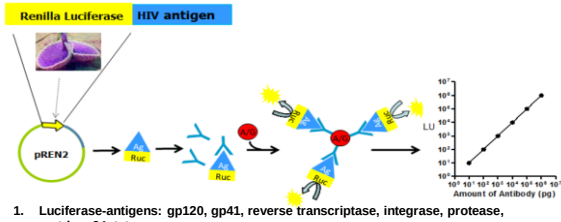
1) **Can the Host Immune Response Against HIV Be Used to Quantify The Latent HIV Reservoir?**




courtesy of Michael Busch, Blood Systems Research Institute

## Luciferase Immunoprecipitation Assay (LIPS) to Detect HIV Antibody Levels

Peter Burbelo, NIH



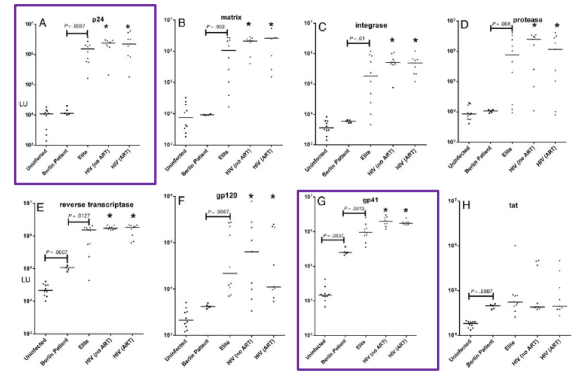

1. Luciferase-antigens: gp120, gp41, reverse transcriptase, integrase, protease, matrix, p24, tat
2. Add diluted sample
3. Add IgG beads – binds Ag-bound Ab
4. Measure luciferase

*Burbelo Curr Opinion Rheum Rev 2014, Burbelo Translational Res 2015*

courtesy of Michael Busch, Blood Systems Research Institute

## 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

| | Env | | Pol | | | Gag | |
|---|---|---|---|---|---|---|---|
| | gp120 | gp41 | RT | INT | PR | MA | p24 |
| All HIV Reservoir Measures* | | | | | | | |
| Correlation (R) | 0.80 | 0.73 | 0.82 | 0.70 | 0.60 | 0.54 | 0.41 |
| P | 0.009 | 0.04 | 0.007 | 0.05 | 0.20 | 0.34 | 0.68 |

** Driven by strong correlations with total (ddPCR) or integrated (aluPCR) HIV DNA*

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

Adjustment for age, nadir CD4+ T cell count, proximal CD4+ T cell count, years of ART, pre-ART viral load did not significantly alter these results

courtesy of Michael Busch, Blood Systems Research Institute

2)

## Full HIV Genome Sequencing

*Using Sanger Sequencing*

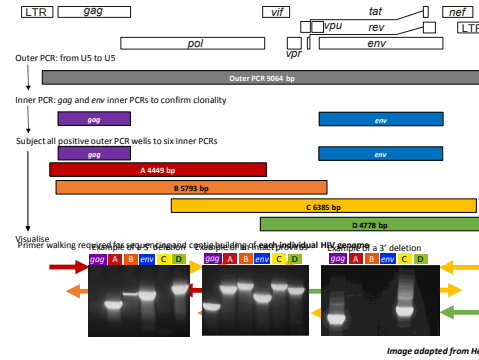
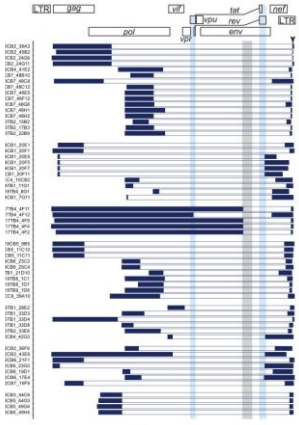



*Image adopted from Ho et al. Cell 2013*



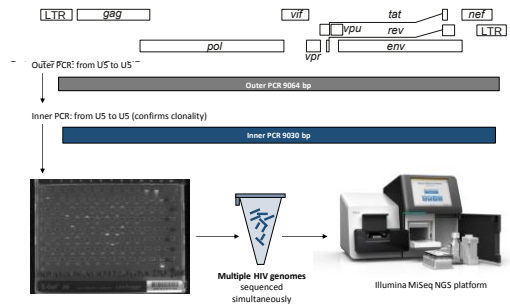

## Identifying Defective HIV-1

- Resting Memory CD4+ T cells ($4x10^4$ to $2x10^5$)
- 88% noninduced viruses 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

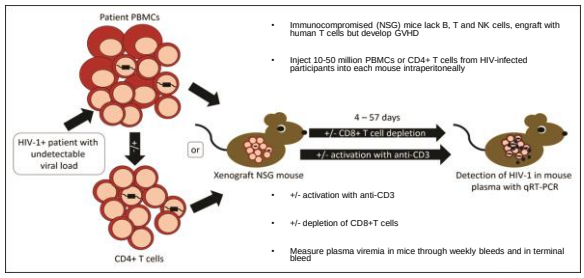
*Image adopted from Ho et al. Cell 2013*

## Full HIV Genome Sequencing

*Using Next Generation Sequencing*



# 3) The murine viral outgrowth assay (MVOA)

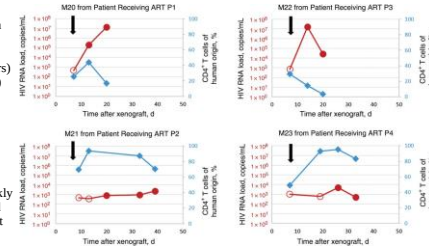



Kelly A. Metcalf Pate et al. J Infect Dis. 2015;infdis.jiv230

Courtesy of Kelly A. Metcalf Pate and Joel Blankson

# MVOA Detects HIV-1 from Participants on Suppressive ART

- Injected 25 to 55 million PBMCs from 4 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

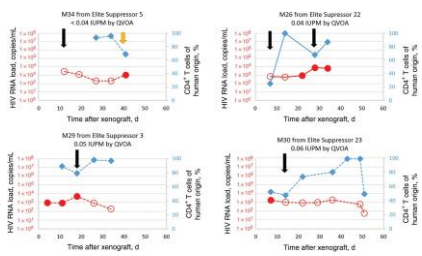



Kelly A. Metcalf Pate et al. J Infect Dis. 2015;infdis.jiv230

Courtesy of Kelly A. Metcalf Pate and Joel Blankson

# MVOA Detects HIV-1 from Elite Controllers with Undetectable Viral Loads

- Injected 66 million PBMCs from 1 EC OR 20 – 26 million purified CD4 T cells from EC IP into NSG mice
- On day 7 anti-CD8 mAb was given IP for the mice engrafted with PBMCs
- For the mice engrafted with purified CD4s, anti-CD8 was given as needed
- 2 mice were treated with anti-CD3 mAb (OKT3) IP
- Mice were bled weekly to evaluate viral load and CD4 T cell count

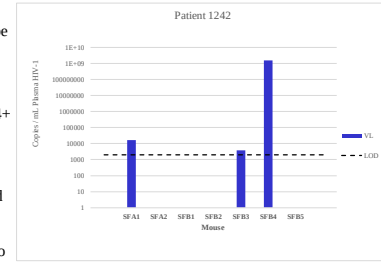



Kelly A. Metcalf Pate et al. J Infect Dis. 2015;infdis.jiv230

Courtesy of Kelly A. Metcalf Pate and Joel Blankson

# MVOA Detects HIV-1 from Elite Controllers on Suppressive ART

- Viremic controller on cART
- IUPM not determined by QVOA, but assumed to be low (Chun T et al JID 2013)
- Obtained 1 billion cells from leukopak
- Isolated 350 million CD4+ T cells
- Engrafted 7 NSG mice with 50 million CD4+ T cells each
- Gave anti-CD8 as needed
- Treated with anti-CD3 mAb (OKT3) IP
- Mice were bled weekly to evaluate viral load and CD4 T cell count




Courtesy of Kelly A. Metcalf Pate and Joel Blankson

# 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.**



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