

# Anti-HIV Antibody Responses Reflect the Quantifiable HIV Reservoir Size

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## Background

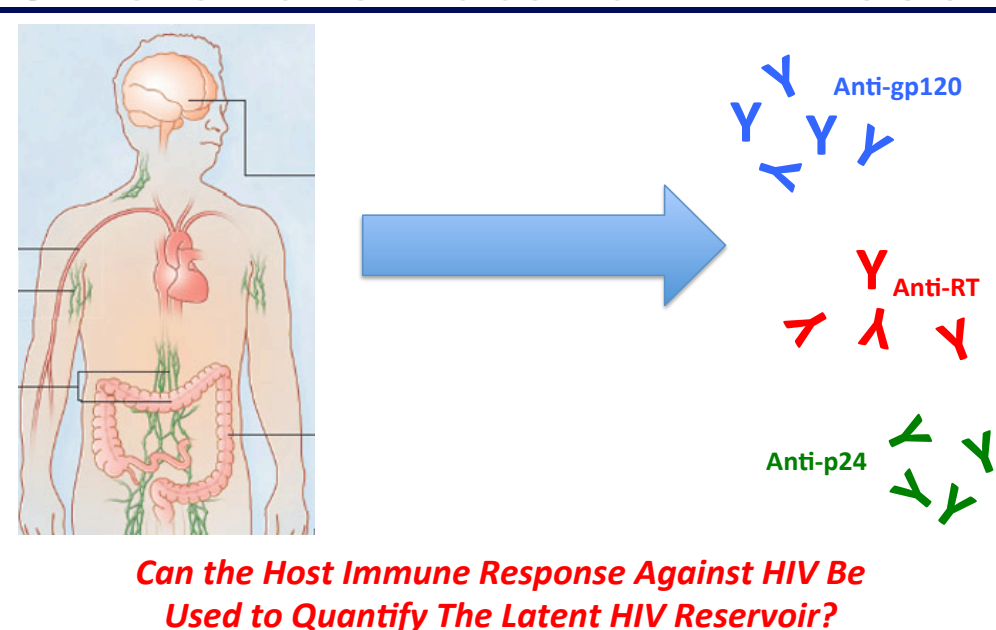
•A major challenge to HIV eradication strategies is accurate measurement of the latent HIV reservoir (Eriksson PLoS Pathogens 2013, Ho Cell 2013).

•There is a need for scalable assays that reflect the latent HIV reservoir to measure the success of HIV cure treatments.

•Our group has previously demonstrated that anti-HIV antibody levels differentiate HIV patient groups (Burbelo JID 2014).

•We performed a pilot study to assess whether anti-HIV antibody levels reflect the size of the HIV reservoir and may be a sensitive measure of HIV persistence.

### Do Anti-HIV Antibody Levels Reflect the Size of the Latent HIV Reservoir?



## Methods

•61 HIV+ SCOPE participants who initiated antiretroviral therapy (ART) during chronic infection.

•12 HIV-1 reservoir measures from two studies:

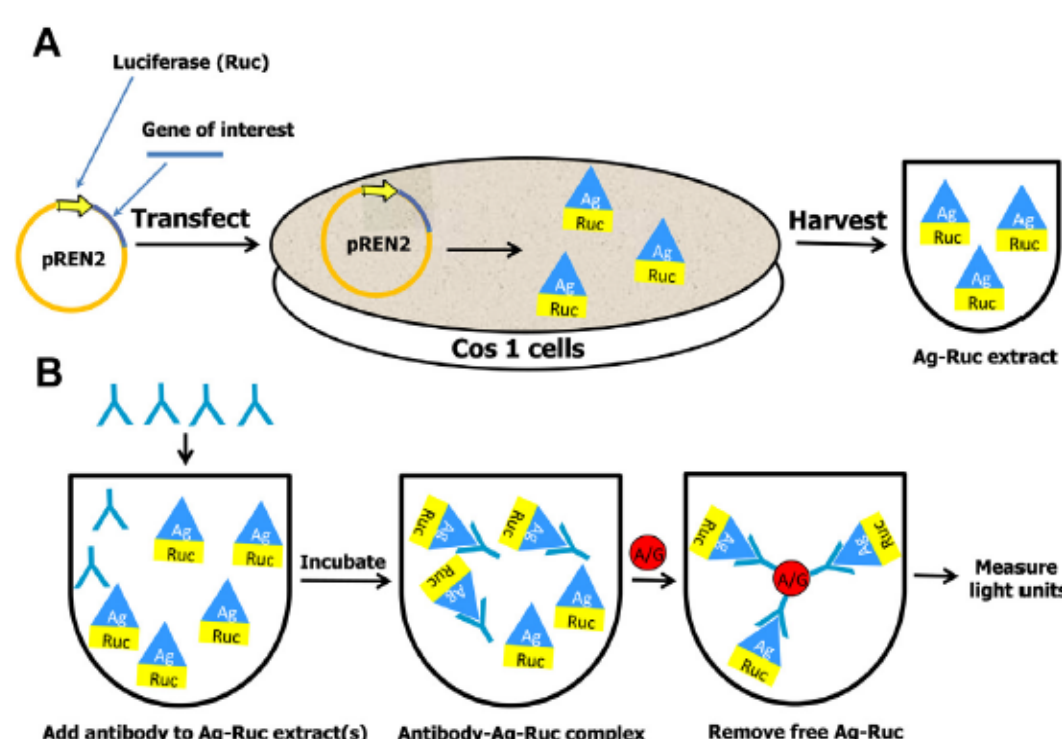
- Study 1: total, integrated, 2-LTR DNA (by rtPCR, N=48); unspliced RNA (by rtPCR, N=44).
- Study 2: total, 2-LTR DNA (by droplet digital PCR, N=27); integrated DNA (by *alu*PCR, N=16); infectious units (by viral outgrowth assay, VOA, N=27), and plasma HIV RNA (by single copy assay, SCA, N=27).

•7 anti-HIV antibody measures using a novel luciferase immunoprecipitation systems (LIPS) assay.

•We performed permutation testing to analyze the association between antibody and reservoir measures, adjusted for multiple comparisons.

•Univariate correlations and multivariate linear regressions were performed using log-transformed, standardized predictor and outcome variables. Multivariate analyses included covariates for age, proximal CD4+ T cell count, nadir CD4+ T cell count, years of ART suppression, and pre-ART viral load

### Novel Luciferase Immunoprecipitation Systems (LIPS) Assay Detects Conformational Antibodies



Burbelo Curr Opin Rheum Rev 2014, Burbelo Translational Res 2015

## Results

### Descriptive Statistics of Study Participants

Characteristics	N=61
Gender (Male)	59 (96%)*
Age (years)	56 (50-61)
Pre-ART HIV RNA (log <sub>10</sub> copies/mL)	3.9 (3.5-4.1)
Nadir CD4+ T cell count (cells/mm <sup>3</sup> )	215 (124-343)
Duration of ART (years)	6 (3-11)
Recent CD4+ T cell count (cells/mm <sup>3</sup> )	670 (541-809)

Median and Interquartile Range shown  
\*Number (%)

### Measures of CA HIV-1 DNA and RNA but Not Plasma RNA are Correlated with Anti-HIV Antibody Levels

	Env		Pol			Gag	
	gp120	gp41	RT	INT	PR	MA	p24
<b>Total HIV DNA</b>							
CD4+ T cells (rtPCR)*	0.16 0.07	-0.03 0.46	0.16 0.14	0.18 0.11	0.17 0.15	0.11 0.19	-0.08 0.69
PBMCs (ddPCR)	<b>0.44</b> 0.01	<b>0.48</b> 0.01	<b>0.30</b> 0.14	<b>0.40</b> 0.04	<b>0.40</b> 0.04	<b>0.23</b> 0.21	<b>0.16</b> 0.31
Resting CD4+ T cells (ddPCR)	<b>0.51</b> 0.02	<b>0.28</b> 0.13	<b>0.62</b> 0.05	<b>0.57</b> 0.03	<b>0.60</b> 0.04	<b>0.54</b> 0.06	<b>0.29</b> 0.26
<b>Integrated HIV DNA</b>							
CD4+ T cells (rtPCR)*	0.21 0.03	0.07 0.25	0.20 0.07	0.11 0.19	0.27 0.23	0.06 0.33	0.06 0.35
PBMCs (aluPCR)	<b>0.80</b> <i>p</i> <0.001	<b>0.73</b> 0.002	<b>0.76</b> 0.003	<b>0.70</b> 0.003	<b>0.54</b> 0.04	<b>0.53</b> 0.04	<b>0.41</b> 0.09
Resting CD4+ T cells (aluPCR)	<b>0.50</b> 0.05	<b>0.72</b> 0.01	<b>0.82</b> <i>p</i> <0.001	<b>0.50</b> 0.08	<b>0.25</b> 0.38	<b>0.34</b> 0.21	<b>0.25</b> 0.27
<b>2-LTR HIV DNA</b>							
CD4+ T cells (rtPCR)*	0.05 0.25	-0.12 0.70	-0.10 0.69	-0.10 0.69	0.14 0.23	0.17 0.14	-0.07 0.65
PBMCs (ddPCR)	<b>0.47</b> 0.06	<b>0.26</b> 0.26	<b>0.33</b> 0.32	<b>0.56</b> 0.08	<b>0.46</b> 0.24	<b>0.39</b> 0.24	<b>0.01</b> 0.65
Resting CD4+ T cells (ddPCR)	-0.05 0.57	<b>0.14</b> 0.36	<b>0.09</b> 0.41	<b>0.01</b> 0.50	<b>0.02</b> 0.45	<b>0.29</b> 0.39	<b>-0.14</b> 0.66
<b>Unspliced HIV RNA</b>							
CD4+ T cells (rtPCR)*	<b>0.40</b> 0.001	0.15 0.13	0.09 0.27	0.12 0.20	0.05 0.38	0.07 0.34	0.10 0.26
<b>Plasma HIV RNA</b>							
Plasma (rtPCR single copy assay)	-0.03 0.63	-0.24 0.90	-0.15 0.58	-0.10 0.53	-0.16 0.52	-0.06 0.41	-0.01 0.31
<b>Infectious Units (Per Million Cells)</b>							
Resting CD4+ T cells (VOA)	<b>0.45</b> 0.01	<b>0.43</b> 0.01	0.20 0.20	0.31 0.08	0.30 0.06	0.25 0.11	0.02 0.47

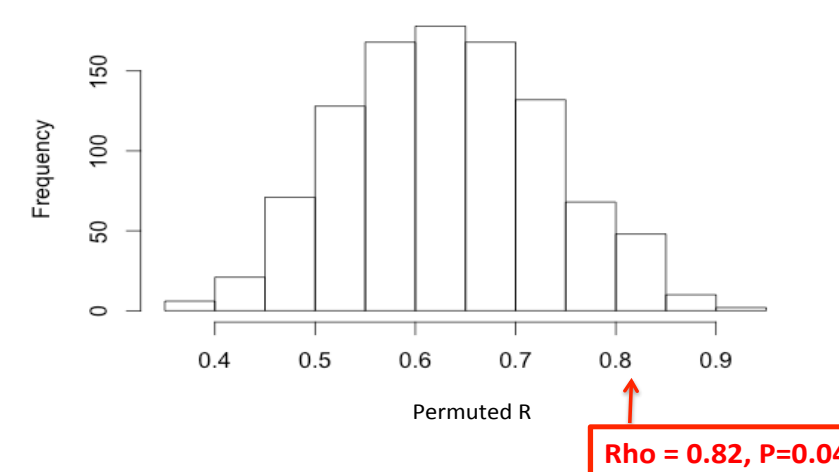
- CA= Cell-Associated
- Correlation coefficients between HIV reservoir measures and anti-HIV antibody levels shown in bold font. Permuted p-values shown below. Legend indicates the relative strength of the correlation (Red > Orange > Yellow).
- Multivariate analyses adjusted for age, nadir CD4+ T cell count, proximal CD4+ T cell count, years of ART, pre-ART viral load did not significantly alter these results.
- The four HIV reservoir measures marked with an asterisk (\*) were performed on a subgroup of different patient samples (Study 1) than the samples studied using the other eight HIV reservoir measures (Study 2).

### Measures of CA HIV-1 DNA and RNA but Not Plasma RNA are Correlated with Anti-HIV Antibody Levels

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

The maximum correlation between all HIV reservoir measures and each anti-HIV antibody level are shown in bold font with permuted p-values below.

### The Observed Maximum Correlation is Greater Than Expected by Chance



Histogram of thousands of permuted correlations between all HIV reservoir size and all anti-HIV antibody levels. The observed maximum correlation of R=0.82 is shown at the tail of the distribution of permuted correlations, P=0.04.

## Conclusions/Implications

- We observed a strong association between measures of the latent HIV reservoir and anti-HIV antibody levels.
- Anti-HIV antibody levels against RT, gp120, gp41, had the strongest association with the HIV reservoir size.

•We will be performing a larger follow-up study, including longitudinal samples and tissue HIV reservoir measures.

•Remaining Questions include:

- Can viral RNA or proteins be produced in cells with defective proviruses?
- Is there a preferential B cell response against certain HIV antigens?
- Are certain anti-HIV antibody levels more "stable over time"/decay slower?
- Why are measures of cell-associated HIV DNA and RNA but not plasma HIV RNA correlated with anti-HIV antibody levels? Do antibody levels more closely quantify the latent *tissue* HIV reservoir?