# Measurement of Antibody Epitope Signatures to Determine Recency of HIV Infection

Kathryn E. Stephenson[1,2,3], Lisa M. Howe[1], Dan H. Barouch[1,2,3], Steven Deeks[4], Sheila Keating[5], Christopher Pilcher[4], Timothy J. Henrich[4]

[1]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA; [2]Harvard Medical School, Boston, MA;
[3]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA; [4]UCSF, San Francisco, CA; [5]Blood Systems Research Institute, San Francisco, CA

CROI 2018
Poster 1004

## BACKGROUND

### New Assays to Estimate HIV Incidence are Needed

- One of the challenges in HIV epidemiology is the inability to reliably estimate HIV incidence in cross-sectional surveys
- HIV incidence can be measured in longitudinal studies of HIV-negative persons, but such studies are expensive, prone to over-estimation, and difficult to generalize
- Accurate incidence estimates are critical to identify high risk populations, model transmission, and monitor the outcome of public health interventions
- Most tests for recent HIV infection measure either the magnitude or avidity of HIV-specific antibodies: both parameters are sensitive but not specific metrics
- Current tests for recent HIV infection must be supplemented with HIV RNA testing and/or CD4 T cell counts to be accurate; these assays are expensive and cumbersome
- New assays have emerged showing that HIV viral diversity can be a biomarker of HIV incidence, but these assays cannot be used on samples with low HIV RNA
- There is a critical need to develop a rapid, inexpensive, and accurate single assay that can be used to estimate HIV incidence anywhere in the world and on any sample

### Antibody Epitope Signatures May Be An Alternate Metric of HIV Disease Duration

- Studies of the developmental pathways of antibodies that neutralize HIV have shown that viral diversification profoundly impacts antibody specificity over time
- Antigen-specific antibodies can also persist long after the stimulating antigen is gone or suppressed
- We hypothesize that antibody epitope specificity – as measured by the diversity of antibody binding to HIV peptides – can serve as a biomarker of different stages of HIV infection
- To test this hypothesis, we compared the number of positive peptide responses in individuals with recent vs. non-recent HIV-1 infection using a global HIV peptide microarray

## METHODS

### Study Population

- Plasma samples were obtained from the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA)
- Three groups were represented: ART-naïve individuals with recent (<12 mo) and non-recent (>12 mo) infection, and ART-suppressed individuals with non-recent infection

### Antibody Epitope Signature by HIV-1 Peptide Microarray

- IgG binding to HIV-1 epitopes was measured with microarrays containing 4,032 overlapping 15 amino acid peptides covering global Env sequences
- Plasma was incubated with microarray and Alexa Fluor 647-conjugated Anti-Human IgG; readout and image processing was performed with Genepix 4300A scanner/software
- Signal intensity was corrected by subtracting values from matched peptides on control microarrays
- The threshold for positivity was >5x noise distribution of sample

## RESULTS

### Characteristics of Study Population

- The demographic and virologic characteristics of the study population are shown in Table 1.

**Table 1**

| | Recent | Non-Recent | |
|---|---|---|---|
| HIV Infection Stage | <12 months | >12 months | >12 months |
| ART Status | Naive | Naive | On ART |
| Viral Load Status | Viremic | Viremic | Suppressed |
| | | | |
| N | 17 | 13 | 15 |
| Male | 17 | 12 | 15 |
| Female | 0 | 1 | 0 |
| Mean Age | 38 | 39 | 44 |
| White | 14 | 8 | 9 |
| Hispanic/Latino | 3 | 3 | 2 |
| Asian | 0 | 2 | 0 |
| African American | 0 | 0 | 3 |
| Multi-ethnic | 0 | 0 | 1 |
| | | | |
| Mean Viral Load | 83,265 | 136,291 | <40 |
| Mean CD4 Count | 581 | 445 | 668 |
| Mean Duration of Infection* | 75 days | 1564 days | 2239 days |

### Differences Between HIV-1 Env Peptide Responses in ART-naïve Individuals with Recent versus Non-Recent Infection

- To localize antibody binding to particular Env regions, we plotted the magnitude of IgG binding to linear gp160 peptides by peptide start position in recently and non-recently infected individuals (Figure 1).
- The recently infected group had low peptide responses across Env. The non-recently infected group who were ART naïve and viremic appeared to have a marked increase in peptide responses, particularly at variable loop 3 (V3).

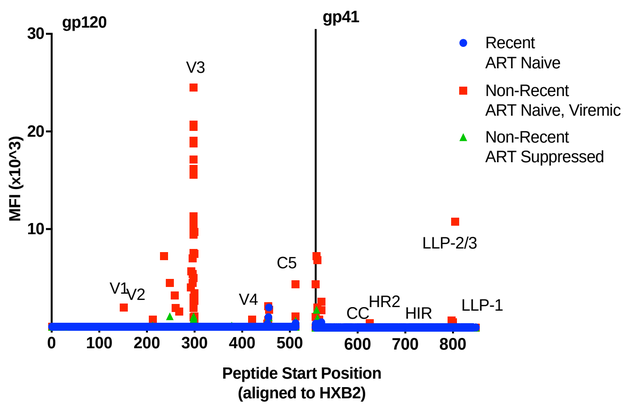



**Figure 1.** Detailed immunoglobulin G (IgG) responses to linear human immunodeficiency virus (HIV)-1 peptides in HIV-infected subjects at different stages of HIV infection. Mean fluorescent intensity (MFI) of IgG binding to glycoprotein (gp)120 and gp41 is plotted by peptide start position (ie, peptide location on HXB2 reference strain). Each symbol represents the median MFI in each group for an individual peptide.

## RESULTS, continued

### HIV-1 Env Peptide Responses Increase Significantly After 12 Months of Infection

- To determine if the increase in positive responses after 12 mos was a significant change, we tallied the total number of positive Env responses and compared median values (Figure 2).
- We found that there was a significant difference in the median number of positive Env peptides in recent infection compared to non-recent infection among ART-naïve participants (50 vs. 138, P=0.0002, Wilcoxon signed rank test).
- There was also a trend towards a significant difference in the number of positive Env peptides in recent infection in ART-naïve versus non-recent infection in ART-suppressed participants (50 vs. 82, P=0.0554, Wilcoxon signed rank test).

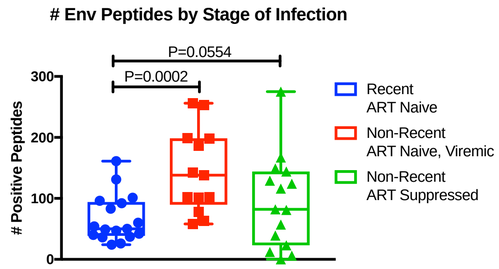



**Figure 2.** Number of HIV-1 Env peptides that are bound by participant plasma IgG plotted by group: recently infected participants who are ART naïve and viremic (N=17), non-recently infected participants who ART naïve and viremic (N=13), and non-recently infected participants who are virologically suppressed on ART (N=15).

### Increases in Env Peptide Responses are Focused on the Variable Region 3 (V3) Loop

- To confirm that increases in Env responses after 12 mos were largely attributable to increased V3 binding, we plotted the total number of positive Env responses by variable region (Figure 3).
- We observed that V3 was essentially the only variable region that had any shift in positive responses. V3 is involved in co-receptor binding and may be particularly engaged during the first year of infection.

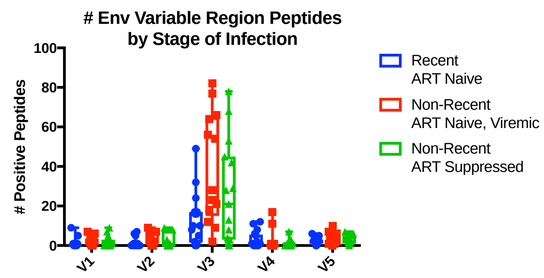



**Figure 3.** Number of peptides by Env region that are bound by participant plasma IgG plotted by group: recently infected participants who are ART naïve and viremic (N=17), non-recently infected participants who ART naïve and viremic (N=13), and non-recently infected participants who are virologically suppressed on ART (N=15).

## RESULTS, continued

### HIV-1 Env Peptide Responses Significantly Correlate with the Number of Days an ART-naïve Person is Infected

- We then asked whether Env peptide responses increased steadily over time or in a step-wise fashion at 12 months.
- We found that the number of positive Env peptides was significantly and strongly correlated with the duration of HIV-1 infection among ART-naïve participants (P<0.0001, R=0.67 by Spearman test), with duration of infection measured as the number of days from latest plausible date of detectable infection (DDI) to sample draw (Figure 4A).
- The number of positive Env peptides was also significantly correlated with duration of HIV-1 infection when ART-suppressed participants were included in the analysis (P=0.0078, R=0.39 by Spearman test; Figure 4B).

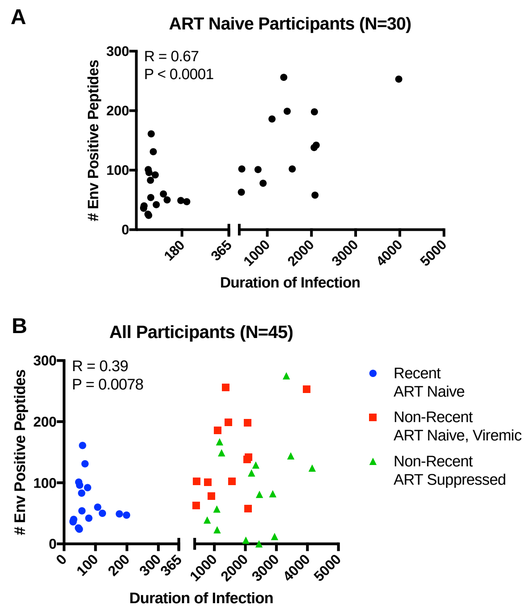



**Figure 4.** Number of HIV-1 Env peptides plotted against the number of days from latest plausible date of detectable infection (DDI) to sample draw among (A) ART naïve participants (N=30) and (B) all participants (N=45).

## CONCLUSIONS

- Recent HIV infection was associated with a narrow epitope signature, while non-recent HIV infection (even among persons with low HIV RNA) was associated with a broader and more diverse epitope signature.
- This data suggests that the global HIV peptide microarray can be used to measure epitope signatures associated with recent and non-recent HIV infection, independent of HIV RNA.
- Our data suggest that further optimization of the microarray in larger cohorts may allow for high throughput applications.

## ACKNOWLEDGEMENTS

- This study was funded by NIH/NIAID R21AI122938 (KES, TJH).