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CTL Epitopes From Structurally Important HIV Proteins Are Identified In Rebound HIV

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BACKGROUND

During an analytic treatment interruption (ATI), the elimination of HIV-infected cells by HIV-specitic cytotoxic CD8 T cells (CTLs) is required to control viral infection.
(Jones & Walker 2016 J Clin Invest; Collins et al., 2020 Nature Reviews Immunology)

However, unless treatment is initiated during acute infection, the HIV reservoir becomes dominated by CTL escape variants resistant to immune response.
(Deng et al., 2015 Nature)

CTL epitopes within structurally important (i.e., highly networked) regions of HIV proteins are associated with viral control in elite controllers.

The highly networked HIV protein regions are essential for HIV protein structure and function. Therefore, these regions are unlikely to mutate and evade immunesurveillance.
(Gaiha et al., 2019 Science; Kaseke et al., 2021 Cell Reports)

STUDY AIMS

Using an *in-silico* model, select T cell epitope-derived peptides from structurally important (i.e., highly networked) regions of HIV protein, Gag p24.

Determine if the selected peptides are genetically-conserved across global HIV subtypes and circulating recombinant forms.

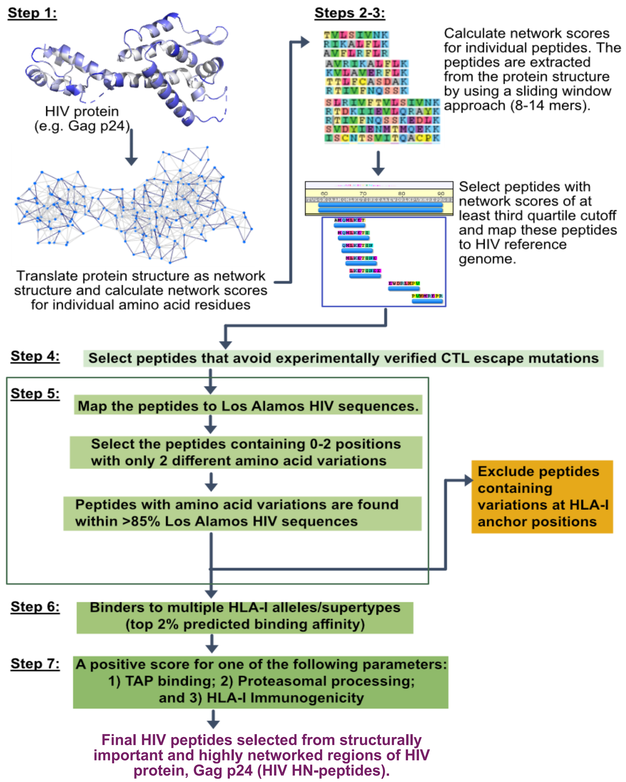
Determine if the selected peptides are found within rebound HIV during an ATI.

KEY POINTS

- A total of 33 HIV HN-peptides were identified within structurally important regions of Gag p24 by applying the IMAP.
- These peptides were selected within the genetically-conserved protein regions that lack experimentally verified CTL immune escpae mutations.
- The HIV HN-peptides were found within rebound virus derived from 5 participants who underwent an ATI.
- The HN-peptides are predicted binders to multiple HLA-I alleles, including the alleles of the HIV-infected individuals who underwent an ATI.

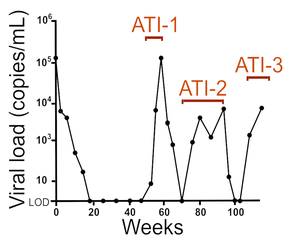
METHODS

Immunoinformatics Analysis Pipeline (IMAP)



Sequence comparison: Peptides vs Rebound HIV during an ATI

A total of five HIV-infected individuals who underwent multiple ATIs was included in this genetic comparison (Fisher et al., 2022 JVI).

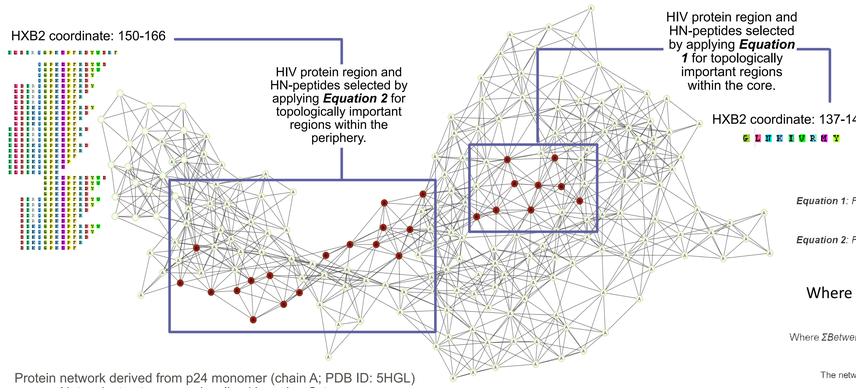


HIV RNA (~8.3 kb, *gag*-3') was sequenced using plasma-derived RNA using long-range sequencing assay (PRLS) during ATIs.

A total of 184 HIV RNA sequences were inlcuded to determine whether these sequences contain the HIV HN-peptides.

RESULTS

IMAP identifies highly networked regions within Gag p24 core and periphery



Protein network derived from p24 monomer (chain A; PDB ID: 5HGL)
Network structure was visualized by using Cytoscape

Mathematical equations designed to identify protein core and periphery regions containing physicochemical interactions that contribute to side-chain clusters (C-β network), and protein folding (atom pair network) were included in the IMAP (Kannan & Vishveshwara, 1999 J. Mol. Biol; Greene & Higman, 2003 J.Mol.Bio; Aflabuddin & Kundu, 2007 Biophys. J.; Chkrabarty & Parekh, 2016 Nucleic Aicds Research).

In addition, these core and periphery regions were delineated by performing centroid network analysis which identifies buried and exposed amino acid residues (Alves &Nartinez, 2007 Physica A; Cusak, 2007 PLoS One; Chkrabarty & Parekh, 2016 Nucleic Aicds Research).

Equation 1: *For topologically important regions identified within the core of an HIV-1 protein:*

$$NS_{core,\ aa} = \ln[Prod_{aa,\ Atom\ pair\ network}] + \ln[Prod_{aa,\ C\beta\ network}] + \ln[Prod_{aa,\ Centroid\ network}]$$

Equation 2: *For topologically important regions identified within the surface of an HIV-1 protein.*

$$NS_{surface,\ aa} = \ln[Prod_{aa,\ Atom\ pair\ network}] + \ln[Prod_{aa,\ C\beta\ network}] - 2\ln[Prod_{aa,\ Centroid\ network}]$$

Where

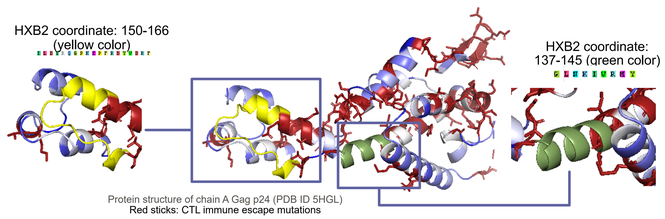
$$Prod_{aa} = Degree_{aa} \times Closeness_{aa} \times (\Sigma Betweeness_{aa}) \times Eigenvector_{aa}$$

Where $\Sigma Betweness_{aa} = \Sigma$[*Ratio of all the shortest paths passing through a node to the total number of shortest paths in the network*]

The network score for each peptide (8-14 mer length) is derived from either *Equation 1* or *2* by using the equation below:

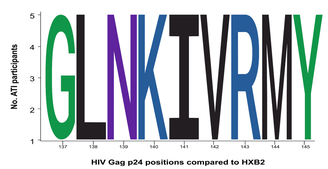
$$NS_{core\ or\ surface,\ peptide} = (\Sigma NS_{core\ or\ surface,\ aa\ within\ a\ peptide}) \div (Peptide\ length)$$

HN-peptides selected by the IMAP avoid experimentally verified CTL immune escape mutations

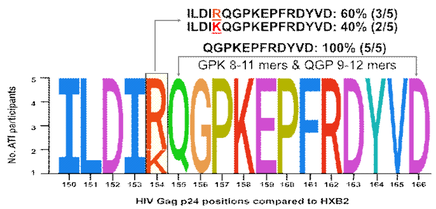


Protein structure of chain A Gag p24 (PDB ID 5HGL)
Red sticks: CTL immune escape mutations

HN-peptides from Gag p24 were found within viral sequences derived from HIV-infected individuals who underwent an ATI



All ATI-derived HIV sequences isolated from plasma samples of 5 out of 5 participants contained the HN-peptide selected by applying *Equation 1*.

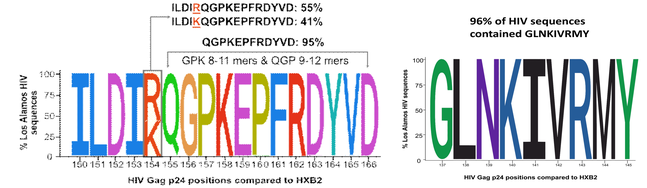


All HIV RNA sequences derived from 3 participants had arginine (R) within the peptide selection region determined by the *Equation 2*.

All viral sequences derived from 2 participants had lysine (K) within this peptide selection region.

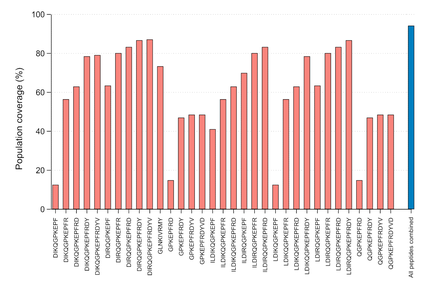
The HIV-peptides (n=32) overlapping within HXB2 positions 150-166, either R or K form, were found within the rebound HIV within the 5 HIV-infected individuals who underwent an ATI.

HN-peptides selected from Gag p24 are found within 95-96% of viral sequences derived from Los Alamos database



The R/K variations within the HN-peptides were not found at the sites important for HLA-I anchoring.

HN-peptides selected from Gag p24 are predicted binders to many HLA-I alleles, including the alleles of HIV-infected individuals who underwent an ATI.



The HN-peptides bind to mutliple HLA-A, -B and -C alleles. These peptides combined have worldwide population coverage of 94.2%.

These HN-peptides are also predicted to bind to HLA-I alleles of HIV-infected individuals who underwent an ATI. As a pool, these peptides can be effective to induce CD8 T cell immune response within all five ATI participants.

HLA	PT ID	Allele	
HLA-A	103	HLA-A*02:01	
		HLA-A*68:01	✓
	104	HLA-A*11:01	✓
		HLA-A*32:01	✓
	202	HLA-A*02:01	
		HLA-A*11:01	✓
	206	HLA-A*02:06	
		HLA-A*24:02	
	304	HLA-A*02:01	
		HLA-A*03:01	✓
		HLA-A*44:02	✓
HLA-B	103	HLA-B*44:03	
	104	HLA-B*07:02	✓
		HLA-B*35:01	✓
	202	HLA-B*40:01	
		HLA-B*44:02	✓
	206	HLA-B*55:02	✓
		HLA-B*55:02	✓
	304	HLA-B*39:06	
		HLA-B*44:03	
HLA-C	103	HLA-C*07:04	
		HLA-C*16:01	
	104	HLA-C*04:01	
		HLA-C*07:02	✓
	202	HLA-C*03:04	
		HLA-C*05:01	
	206	HLA-C*01:02	
		HLA-C*01:02	
	304	HLA-C*07:02	✓
		HLA-C*16:01	

✓ HN-peptide pool contains peptides that are predicted binders to HLA-I alleles of participants who underwent an ATI.

Conclusions

Applying the IMAP, we defined novel HIV peptides within a structurally important region of the Gag protein (n = 33). These peptides were genetically conserved, avoided known CTL escape mutations, and were identified within ATI-derived plasma HIV RNA sequences, indicating these peptides as a pool are highly promising for eliciting a CD8 T-cell immune response during treatment interruption.

Acknowledgment

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