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Instability of the HLA-E Peptidome of HIV Presents a Major Barrier to Therapeutic Targeting

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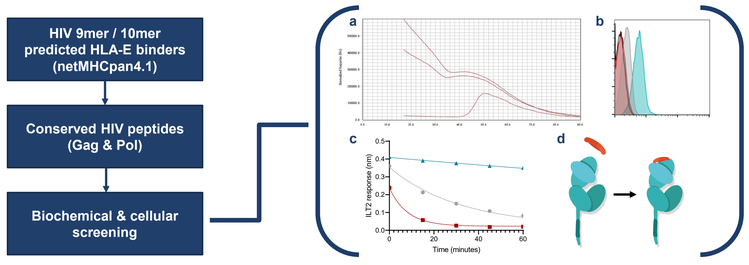
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Introduction

- HLA class 1a-restricted peptides are well characterised targets for TCR-based biologics in development for infectious diseases, including HIV. However, the diversity of MHC-encoded HLA allotypes within the human population limits the potential for broad clinical application.
- The non-polymorphic HLA class 1b molecule, HLA-E, has two functional alleles (HLA-E*01:01 and HLA-E*01:03) that differ by a single amino acid found outside the binding groove, providing an opportunity for universal coverage with a single TCR therapeutic.
- MHC-E restricted CD8+ T cells have been shown to play a crucial role in eliminating a pathogenic SIV challenge in a rhesus CMV-SIV vaccination model[1]. This observation provided a rationale for searching the HIV proteome for HLA-E binding peptides as potential drug targets.
- **We performed a systematic search for stably presented HLA-E ligands in the HIV proteome using both unbiased and targeted approaches. A known HLA-E binding Mtb enoyl reductase-derived peptide, inhA$_{53-61}$, was used throughout as a positive HLA-E control.**

Predicted HLA-E binding HIV peptides form unstable complexes with HLA-E in solution and on cells

Figure 1. Workflow for bioinformatic prediction of putative HLA-E binding HIV peptides and stability assessment using biochemical & cellular assays



Candidate HLA-E ligands (from bioinformatic predictions and mass spectrometry discovery) were assessed by (a) thermostability[2] (Tm, stable: higher Tm), (b) cell surface stabilization (stable: shift to right), (c) ILT2 binding[2] (bio-layer interferometry, stable: longer time), and / or (d) refold yield (stable: able to refold, higher yield).

Table 1. Top 8 HIV HLA-E ligands ranked by stability (1+ stability assay passed, n > 80 total)

Peptide	Tm (°C) ND = not able to determine	ILT2 binding (min)	Cell surface stabilisation	Refold
RLPAKAPLL (Mtb inhA$_{53-61}$)	48.2	231.4	YES – 100%	YES
RMYSPTSIL (Gag$_{275-283}$)[3]	Not tested	21.0	NO	YES
RMYSPVSIL (Gag$_{275-283}$)	ND	11.4	NO	YES
RFAVNPGLL (HIV Gag)	ND	24.6	NO	NO
RFALNPGLL (HIV Gag)	40	ND	NO	Not tested
TLNAWVKVI (HIV Gag)	40.3	ND	NO	Not tested
AISPRTLNA (HIV Gag)	ND	ND	NO	YES
MLTQLGCTL (HIV Pol)	41.3	ND	NO	NO
KLLRGAKAL (HIV Pol)	39.6	ND	NO	Not tested

References

1. Hansen S et al. Broadly targeted CD8(+) T cell responses restricted by major histocompatibility complex E. Science. 2016; 351:714-720.
2. Barber C et al. Structure-guided stabilization of pathogen-derived peptide-HLA-E complexes using non-natural amino acids conserves native TCR recognition. Euro J Immunol. 2022;0:1-15.
3. Yang H et al. HLA-E restricted Gag specific CD8+ T cells can suppress HIV-1 infection, offering vaccine opportunities. Sci Immunol. 2021; 6(57): eabg1703.

HIV peptides are not detected in HLA-E complexes immunopurified from virus-infected cells using liquid chromatography-tandem mass spectrometry

Figure 2. Workflow for immunoprecipitation of HLA-E peptide complexes from HIV-infected cell lysates

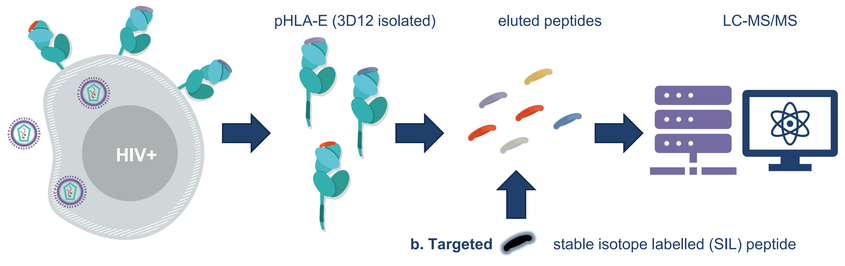
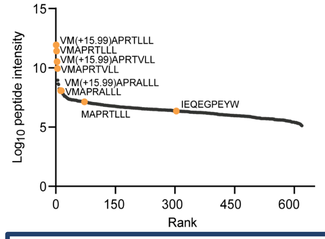


Figure 3. HLA class I leaders found in HLA-E (3D12) fraction



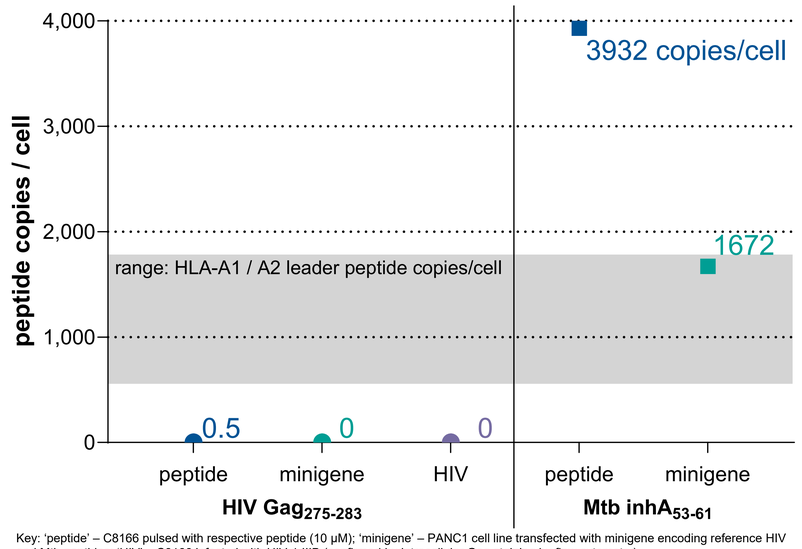
43,741 unique peptides (95% 8-14mer)

22 HIV peptides (HLA-E fraction)
{57 HIV peptides (pan-HLA class I fraction)}

15 8-14mer peptides

0 stable binders

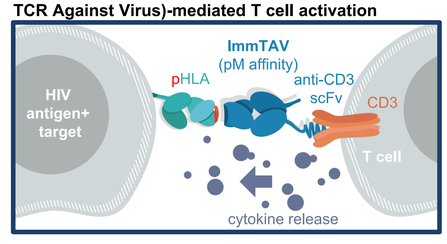
Figure 4. Quantification of HLA-E bound peptides from antigen positive cell lines using SIL-labelled HIV and Mtb peptides

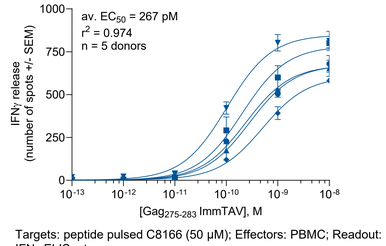


Key: 'peptide' – C8166 pulsed with respective peptide (10 µM); 'minigene' – PANC1 cell line transfected with minigene encoding reference HIV and Mtb peptides; 'HIV' – C8166 infected with HIV-1 IIIB (confirmed by intracellular Gag staining by flow cytometry).

High affinity soluble TCR bispecific molecules (ImmTAV) confirm the poor stability of Gag$_{275-283}$ in HLA-E

Figure 5. ImmTAV (Immune mobilising monoclonal TCR Against Virus)-mediated T cell activation





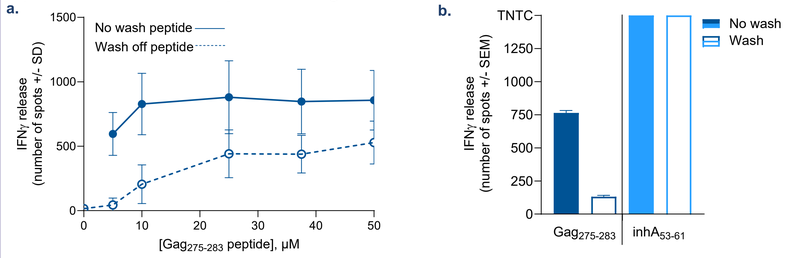
Targets: peptide pulsed C8166 (50 µM); Effectors: PBMC; Readout: IFNγ ELISpot

Table 2. Affinity enhancement of Gag$_{275-283}$ specific TCR

	Gag$_{275-283}$ (6V)		Gag$_{275-283}$ (6T)	
TCR	K_D (nM)	t ½ (s)	K_D (nM)	t ½ (s)
Wildtype	2,830	5.1	12,500	1.7
Affinity enhanced	0.03	56,928	0.09	31,920

Exogenous source of peptide

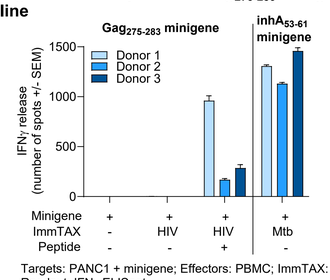
Figure 6. ImmTAV-mediated T cell activation by peptide pulsed targets is negatively impacted by peptide concentrations <25 µM & washing conditions



Targets: peptide pulsed C8166 +/- wash (a – titration; b – 10 µM); Effectors: PBMC; ImmTAV: 10 nM; Readout: IFNγ ELISpot

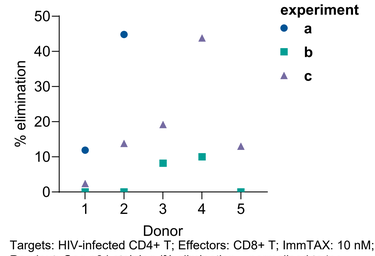
Endogenous source of peptide

Figure 7. No response to Gag$_{275-283}$ minigene cell line



Targets: PANC1 + minigene; Effectors: PBMC; ImmTAX: 10 nM; Readout: IFNγ ELISpot

Figure 8. Sporadic killing of HIV-infected T cells



Targets: HIV-infected CD4+ T; Effectors: CD8+ T; ImmTAX: 10 nM; Readout: Gag p24 staining (% elimination – normalised to 'no ImmTAV' control)

Conclusions

- We systematically interrogated the HIV proteome using bioinformatic and immunopeptidomic approaches and identified no HIV peptides that are stably presented on HLA-E.
- In T cell redirection assays with a high affinity soluble TCR bispecific (ImmTAV) against the previously reported HIV HLA-E epitope (Gag$_{275-283}$), consistent T cell responses were only detected when the targets were supplied with exogenous peptide, at micromolar concentrations (peptide pulsing).
- This study highlights the instability of the HLA-E peptidome of HIV as a major challenge for drug or vaccine development.