HLA Binding Assay Design: Impact of HLA binding motif centering on HLA binding results and T cell response; Relevance to Overlapping Peptide Analysis

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Introduction

Purpose

- Two retrospective analyses of recent publications demonstrate the likely effect of off-centered HLA binding motifs on binding and T cell assays.
- Repeating the binding assay with properly centered peptides, and/or removing off-centered motifs **improved correlations between in silico predictions and in vitro findings**.
- These findings are relevant for developing accurate predictive tools and for proper interpretation of vaccination studies.

Background

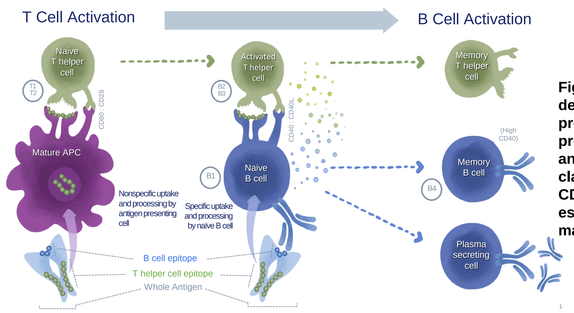


Figure 1: T cell epitopes that are derived from (self or foreign) proteins are processed and presented on the surface of antigen presenting cells (APCs) by class II HLA molecules, priming CD4+ T cells that provide the essential cytokines for B cell maturation

- Class II MHC have an open-ended binding groove[1] and can accommodate longer peptides (15-20 aa in length).
  - As a result, class II T cell epitopes can shift within the binding groove.

Importance of Peptide Flanking Residues (PFR)

- Nelson et al[2] propose that flanking residues at the ends of the core epitope, particularly the amino end, make contacts with the MHC molecule, **increasing stability of the pMHC complex**.
- Some studies have also identified the role of **PFRs and their interaction with TCR facing residues**, diminishing or enhancing the intensity of the T cell response.[3,4]

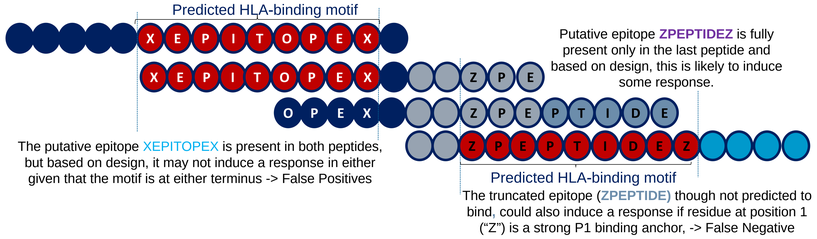


Figure 2: Overlapping Peptide Design and predicted epitopes; figure above depicts 15mers overlapping by ten amino acid residues.

Overlapping Peptide Library: What are the issues?

- **Poorly centered HLA-binding motifs** (at the N- or C- terminal of the binding peptide, Figure 2) may result in **absence of binding or T cell response.**
- A common issue with peptide synthesis processes such as solid phase peptide synthesis is N-terminal truncation, which arises by the use of capping to prevent deletion events[5].
  - If the HLA binding motif is present at the N-term, a truncation could lead to false positive outcomes.
- Synthesizing OL peptide libraries is **costly and time-consuming.**

Retrospective Analysis: Hamze et al. 2017[6]

Summary of the study:

- CD4 T cell epitopes from the variable regions of two chimeric antibodies, Infliximab and Rituximab, were identified using overlapping peptides in healthy donors.
  - These peptides were characterized using HLA-DR binding assays, IFNγ ELISpot assays and MHC-associated peptide proteomics (MAPPs).
  - Nine CD4 T cell epitopes were identified for each antibody.

Methods:

- A preliminary analysis of the overlapping peptides using EpiMatrix (Figure 3) revealed discordance between in silico predictions and HLA-DR binding assays.
- After review with the EpiMatrix system, a subset of the original peptides was re-synthesized and centered versions of these peptides were also produced.
  - These peptides were assayed for HLA class II binding at EpiVax (Figure 4)

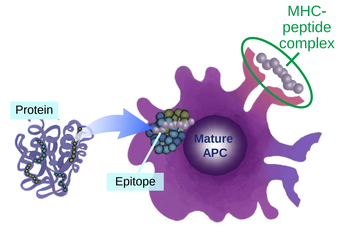


Figure 3: EpiMatrix models the interaction between a 9-mer peptide and an MHC molecule.

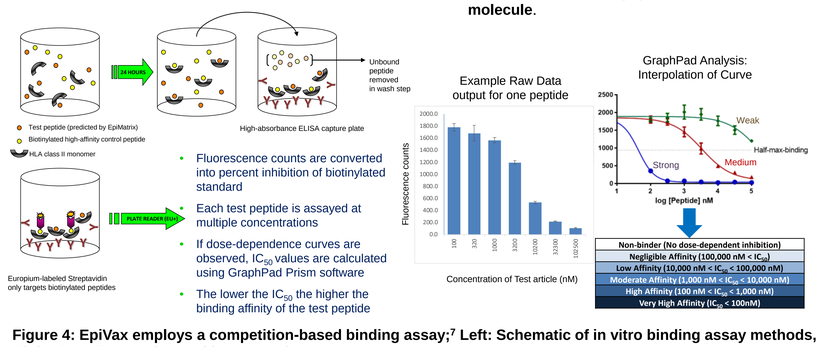
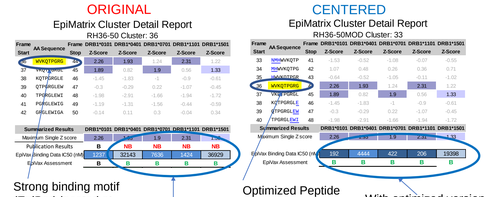


Figure 4: EpiVax employs a competition-based binding assay;[7] Left: Schematic of in vitro binding assay methods, Right: Data Processing and interpretation

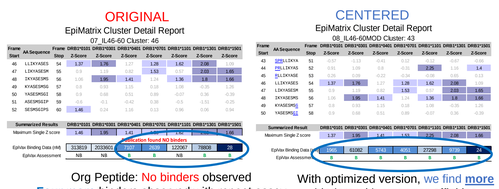
Results:



Publication Results					
Peptides	HLA-DR alleles				
	DR1	DR4	DR7	DR11	DR15
RH36-50					
IH41-55					
IH46-60					
IL31-45					
IH51-65					
IL46-60					
RL56-70					
RL46-60	Three binders found (unspecified)				

Positive Predictive Value (excluding RL46-60*): **22%**

True Positive or True Negative
False Negative (top 10% EpiMatrix hit)
False Positive/False negative



Centered Peptides Results (EpiVax)					
Peptides	HLA-DR alleles				
	DR1	DR4	DR7	DR11	DR15
RH33-48					
IH44-59					
IH43-62					
IL32-46					
IH49-67					
IL43-58					
RL56-72					
RL43-57					

Positive Predictive Value (including RL46-60): **83%**

In most cases, centering binding motifs yields **more binders with stronger affinities** ✓

Note: Only a subset of the data is shown here and we are only reporting data for optimized peptides.
*RL46-60 was excluded from Positive Predictive Value analysis as allele specific binding data for this peptide is not reported in the publication

Retrospective Analysis: Kennedy and Poland, 2010[8]

Summary of the study:

- An overlapping peptide library of four vaccinia membrane proteins (A33R, A27L, B5R and L1R), known to induce a humoral response in vaccinated individuals, was synthesized and tested in IFNγ ELISpot assays using the PBMCs of 29 recent smallpox vaccine recipients.
  - 19 Class II T cell epitopes and 17 Class I/Class II T cell epitopes were identified

Methods:

- The EpiMatrix and JanusMatrix[9] tools (Figure 5) were leveraged to correlate in vitro results with in silico predictions.
  - **Q1: Are predicted epitope content and putative cross-conservation with self significant predictors of a positive T cell response?**
- An overlapping peptide "library" for non-responding peptides was created:
  - Peptides not reported as positive (CD4 or CD8) were assumed to be negative.

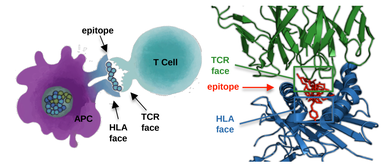
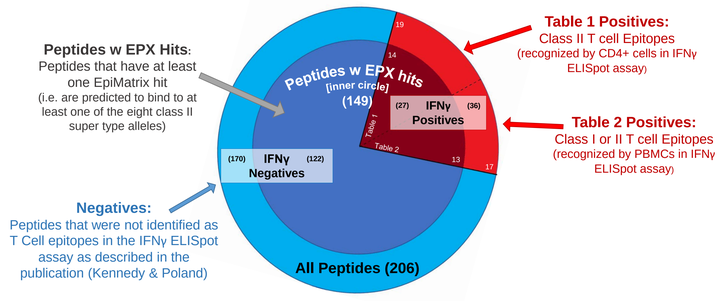


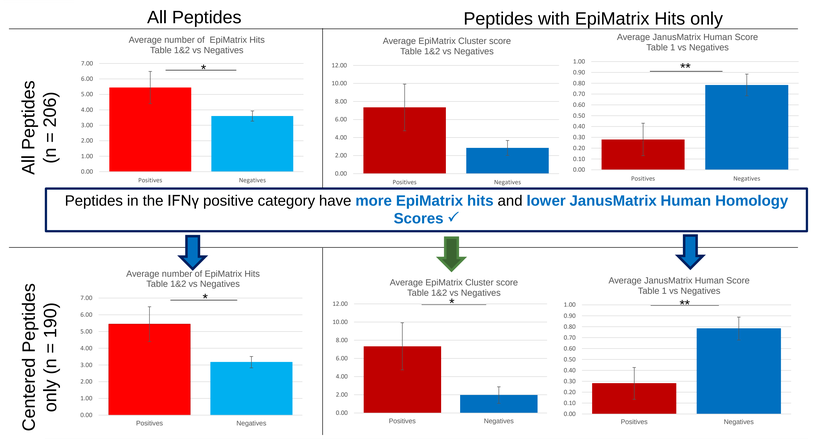
Figure 5: JanusMatrix performs cross-reactivity analyses and can identify regulatory T cell (Treg) epitopes. Cross-reactive sequences:
- **Are predicted to bind to the same MHC allele.**
- **Share same/similar T cell-facing residues.**

Population of Peptides in Study



- Peptides with off-center EpiBars in the **EpiMatrix Hit/IFNγ Negative (n=122)** category were identified and removed from the analysis.
  - Due to the design, we do not expect these peptides to have a strong T cell response.
  - **Q2: Does removal of off-center peptides improve correlations between in silico and in vitro results?**

Results:



Conclusions

- The studies described here highlight the impact of off-centered T cell epitope binding motifs in HLA binding and T cell assays:
  - For Hamze et al. we find that **centering binding motifs** in overlapping peptides yields **more binders** with **stronger affinities, improving association** of **in silico** predictions and **in vitro** findings**.**
  - For Kennedy and Poland, the preliminary analysis with all peptides indicates that **number of EpiMatrix hits** and **JanusMatrix Human Homology Scores** are **significant predictors** of positive response.
    - After removing off-center peptides, **EpiMatrix Cluster Score** is also **significantly correlated** with **IFNγ response.**
- Careful attention should be taken to design peptides with optimal features, such as centered HLA binding motifs, before their usage in in vitro and in vivo experiments.

References

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