Innovative Preclinical Assessment Tools for Safety and Efficacy of Protein and Peptide Therapeutics … Of Peptides and P-ANDAS

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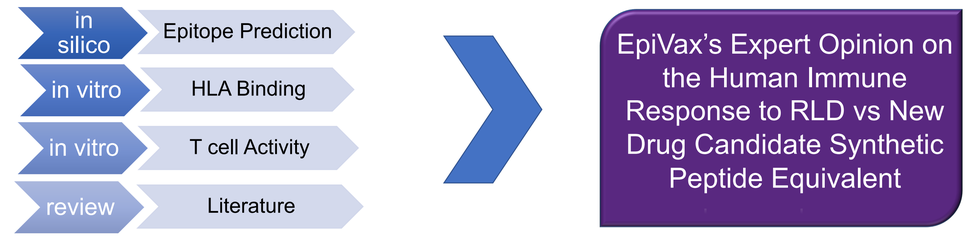
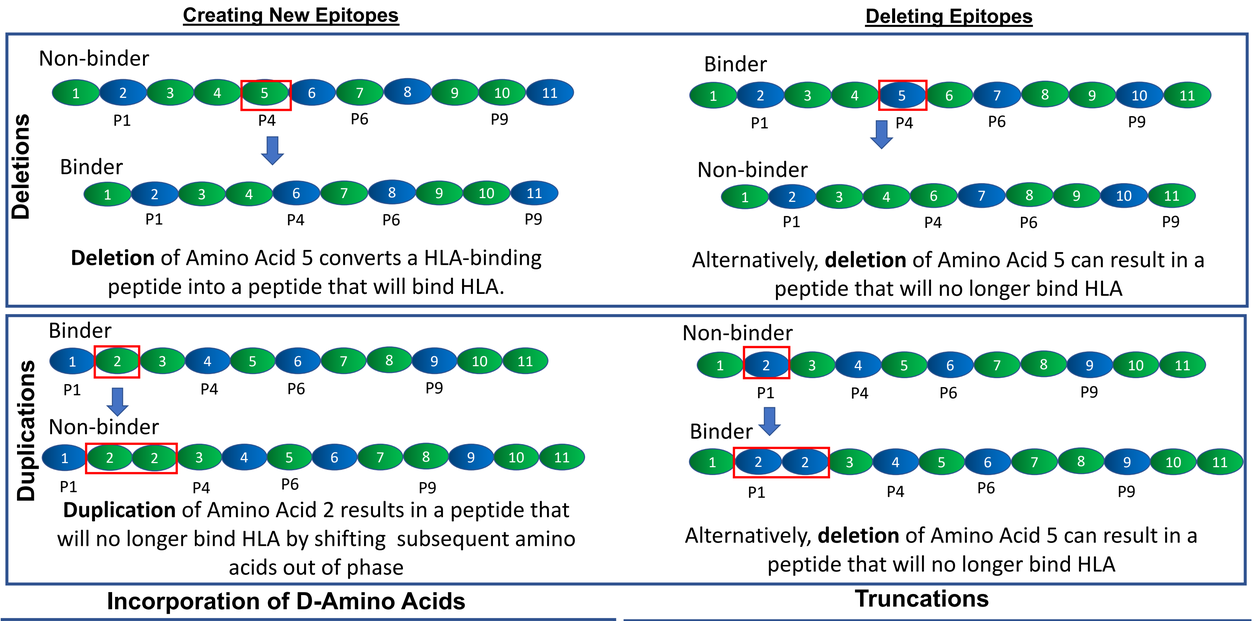
Abstract

- The US Food and Drug Administration (FDA) recently released a new draft guidance enabling generic manufacturers of peptide drugs to file an Abbreviated New Drug Application (ANDA) for synthetic peptide drug products.
- Processes for manufacturing the generic and reference drug (RLD) are not equivalent, leading to manufacturing related impurities.
- Manufacturers are required to prove that the synthetic peptide product does not contain impurities with an increased risk of immunogenicity that could result in the development of anti-drug antibodies.
- We use both in silico analysis and in vitro validation assays to perform immunogenicity risk assessment of peptide generics. This process is referred to as the **PANDA assay** which can be used to support generic peptide drug equivalency in an ANDA application.

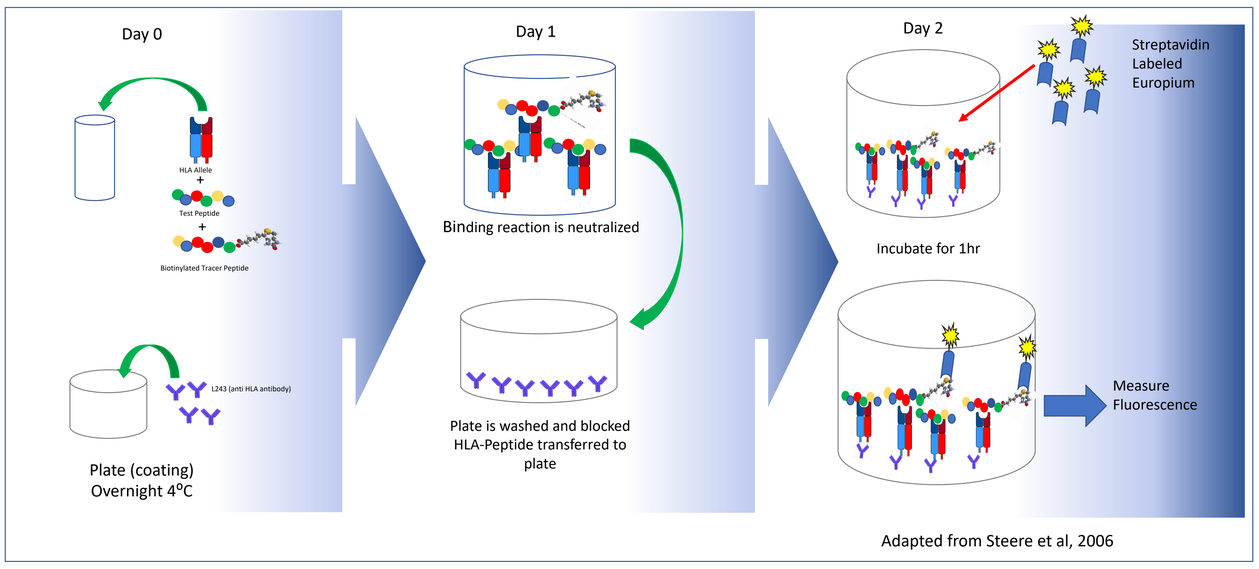
PANDA Overview



Impact of Manufacturing Impurities on T cell Epitopes

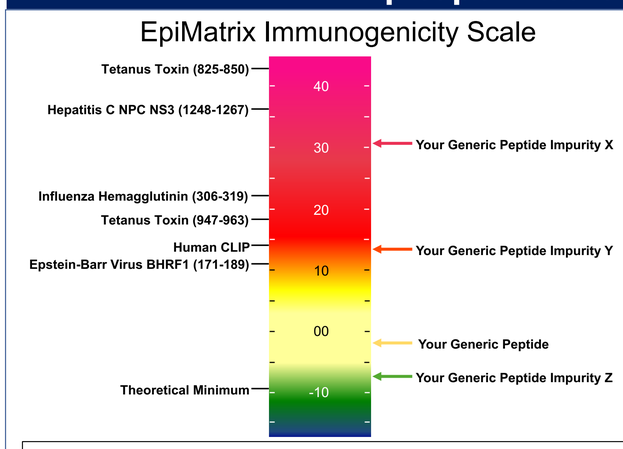


HLA Class II Binding Assay

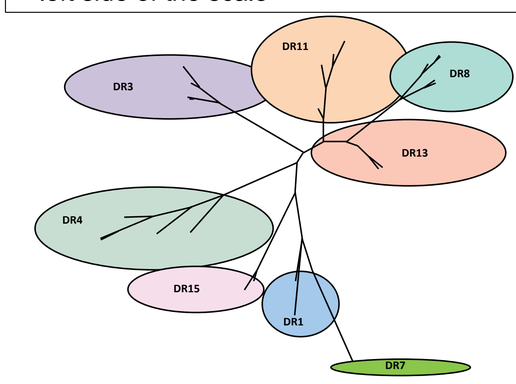


HLA-Class II Binding Assay: Peptides that are predicted to bind HLA are synthesized and assayed over a range of 7 concentrations in our HLA-binding assay. In brief, peptides are incubated overnight with soluble HLA and a biotin labeled competitor of moderate affinity. On day 2, the reaction is halted and the mixture is transferred to a plate coated with a pan anti-HLA antibody. On day 3, plates are developed by the addition of streptavidin-Europium and fluorescence is measured.

Epitope Prediction by EpiMatrix

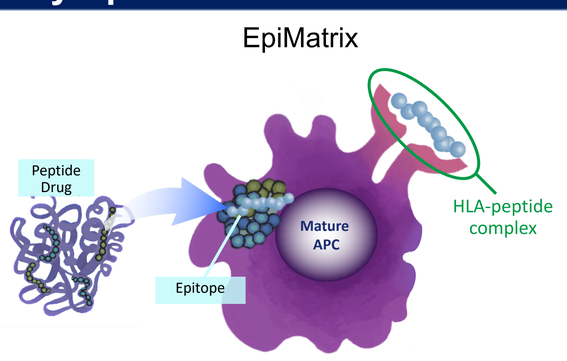


- EpiMatrix excess and shortfall in predicted aggregate immunogenicity relative to a random peptide standard
- EpiMatrix Cluster Scores above ten are comparable to those of known promiscuous Class II epitopes, commonly used as positive controls in T cell assays and included for reference on the left side of the scale

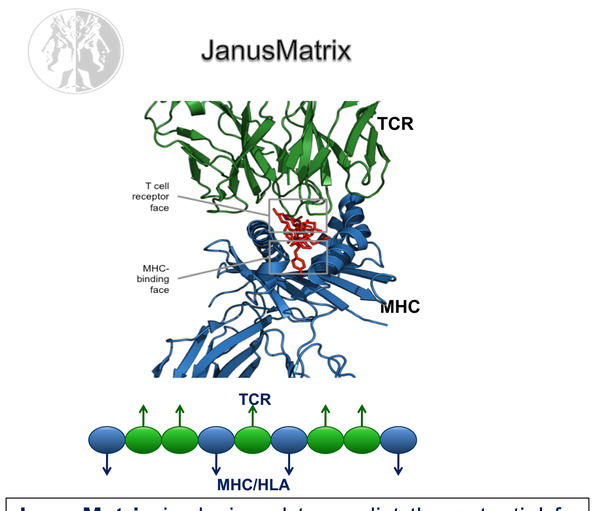


EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left.

This allows us to provide results that are representative of >95% of human populations worldwide** without the necessity of testing each haplotype individually.



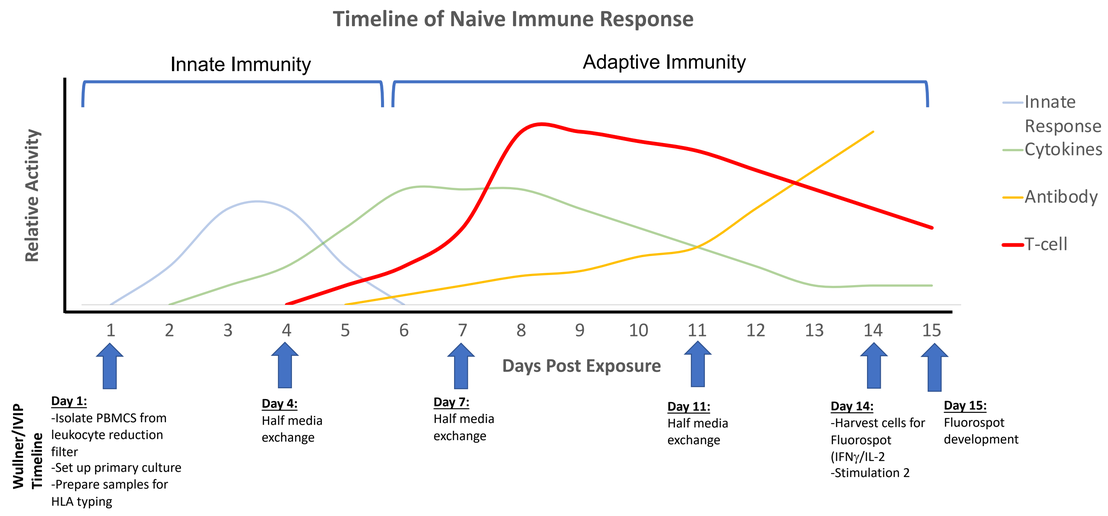
- EpiVax uses EpiMatrix to predict T cell epitopes
  - –EpiVax predicts both class I and class II HLA binding
  - – HLA binding is a prerequisite for immunogenicity
  - – Full suite of HLA-based predictions are available



JanusMatrix is designed to predict the potential for cross-conservation between epitope clusters and the human proteome, based on conservation **of TCR-facing residue**s in their putative HLA ligands.
This results in a **more in depth analysis** than typical alignment homology.

In Vitro Immunogenicity Protocol (IVIP)

- **The ability of the test article (new Generic and impurities) and the RLD to stimulate a *de novo* T-cell response is compare to several controls** including HSA (protein neg control), KLH (protein positive control) and a CEFT (protein pool positive control).
- **14 days post exposure, cells are harvested and plated into precoated IFNγ ELISpot plates.** Cells are restimulated and incubated overnight. On day 15, ELISpot plates are developed and sent to Zellnet Consulting Inc. for blind, independent analysis.



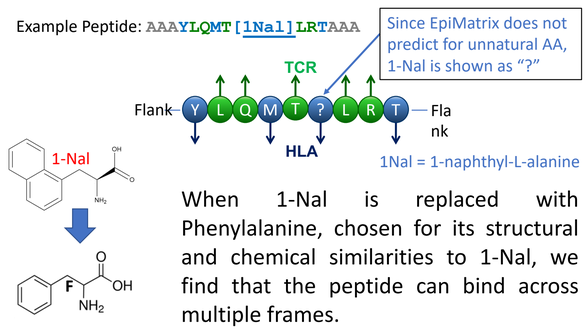
Summary of IFNγ Fluorospot responses across donors – RLD A

	DONOR	1	2	3	4	5	6	7	8	9	10	TOTAL # of Positive Responses*
	DRB1 Allele	04:03 15:01	01:01 07:01	07:01 07:01	03:01 16:01†	03:01 07:01	01:01 14:01†	03:01 13:03†	03:01 09:01†	01:02 07:01	03:01 15:01	
	EpiMatrix Hits: Allele	1 0	1 1	1 1	1 0	1 1	1 NA	1 NA	1 0	1 1	1 0	
RLD - A	1	-	-	-	-	-	-	-	-	-	-	0/10
RLD - A	2	+	-	-	+	-	-	-	-	-	-	2/10
Test Article - A	11	-	-	-	-	-	-	-	+	-	+	2/10
Test Article - A	12	-	+	-	-	-	+	-	-	-	-	2/10
Test Article - A	13	-	+	-	-	-	-	-	-	-	-	1/10

* A response is considered positive with >50 SFC/10^6 cells and a stimulation index ≥ 2
† Epitope predictions (EpiMatrix Hits) for HLA DRB1*16:01 were modeled on supertype relative DRB1*1101; predictions for HLA DBR1*14:01 and *13:03 are not available; predictions for HLA DRB1*0901 are available through EpiVax internal models not normally included in PreDeFT analysis.

Modeling Unnatural Amino Acids

- When EpiMatrix cannot model HLA binding for modifications found in the impurity, we use a sensitivity analysis to find the best proxy.
- The modified residue is replaced with a neutral placeholder "X". We then replace "X" with each of the natural 20 amino acids.
- The goal is to determine if any residue at these positions can lead to a significant increase or decrease in predicted HLA binding potential.
- We also compare the properties of the chemically modified residues with the naturally occurring amino acids and pick the "best-matched" residue as a proxy



When 1-Nal is replaced with Phenylalanine, chosen for its structural and chemical similarities to 1-Nal, we find that the peptide can bind across multiple frames.

References

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- **Southwood et al. Several Common HLA-DR Types Share Largely Overlapping Peptide Binding Repertoires. J Immunol. 1998; 160(7):3363–73.