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

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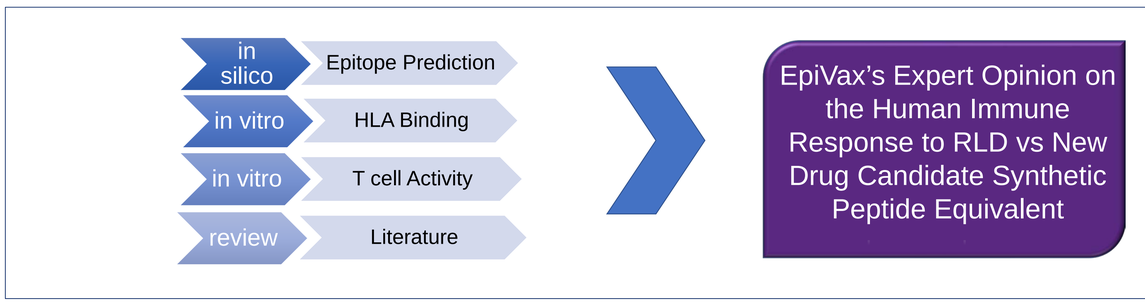
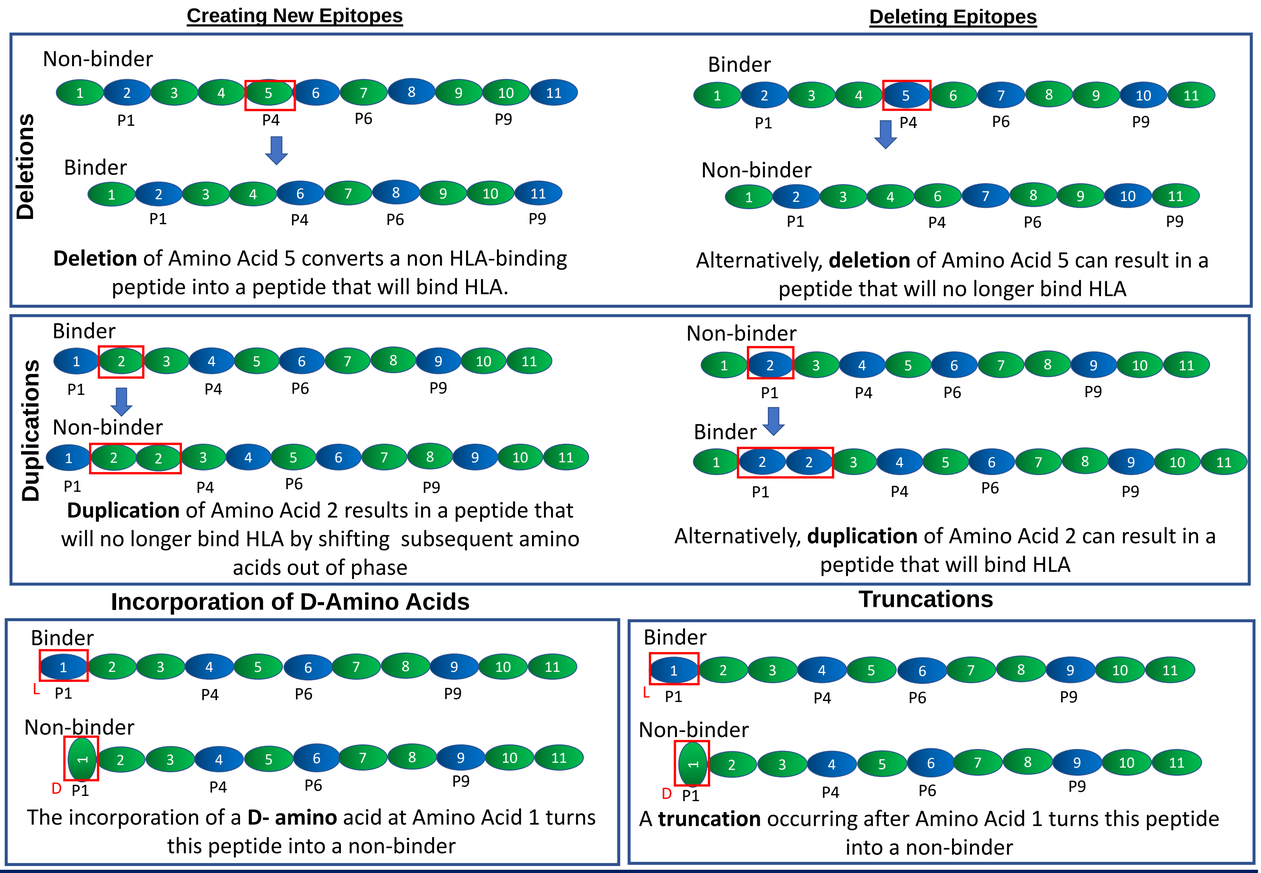
## Abstract


- The US Food and Drug Administration (FDA) recently released a new draft guidance enabling generic manufacturers of peptide drugs to file an Abbreviated New Drug Application (ANDA) for synthetic peptide drug products.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.

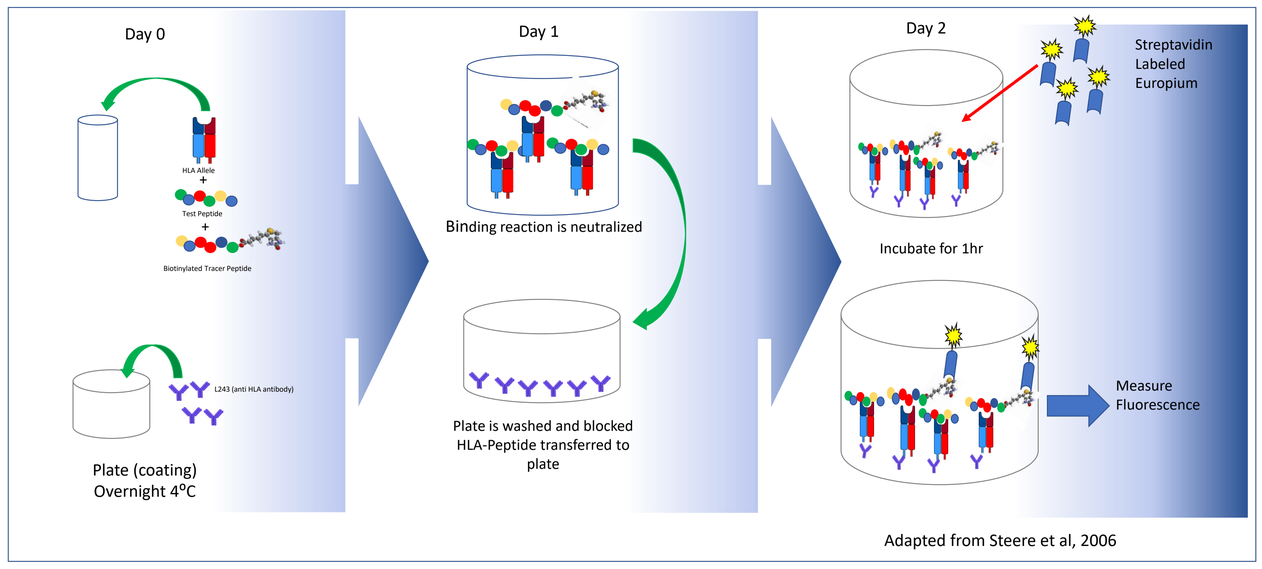

## PANDA Overview




## Impact of Manufacturing Impurities on T cell Epitopes

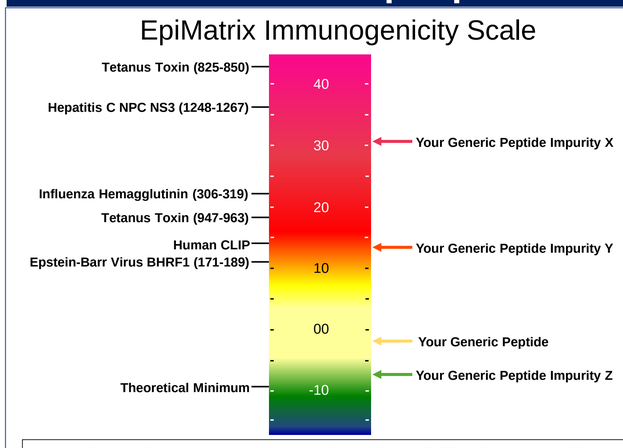



## HLA Class II Binding Assay

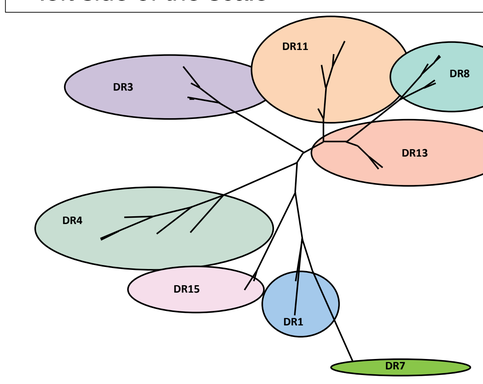



**HLA-Class II Binding Assay**: Peptides that are predicted to bind HLA are synthesized and assayed over a range of 7 concentrations in our HLA-binding assay. In brief, peptides are incubated overnight with soluble HLA and a biotin labeled competitor of moderate affinity. On day 2, the reaction is halted 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

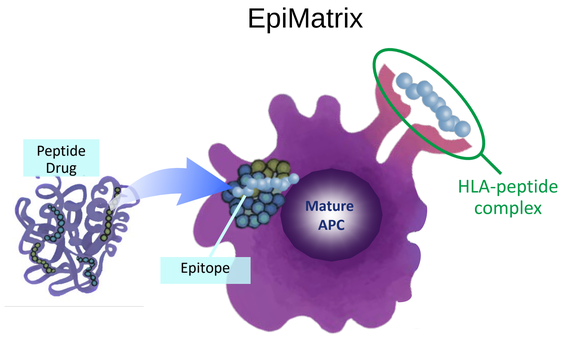



- EpiMatrix excess and shortfall in predicted aggregate immunogenicity relative to a random peptide standard
- EpiMatrix Cluster Scores above ten are comparable to those of known promiscuous Class II epitopes, commonly used as positive controls in T cell assays and included for reference on the left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

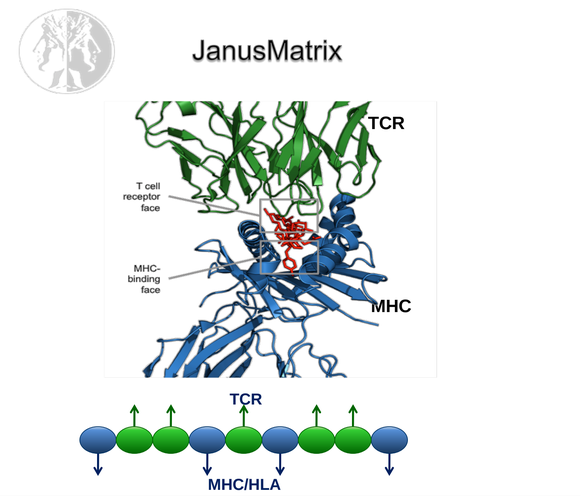



EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left.

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



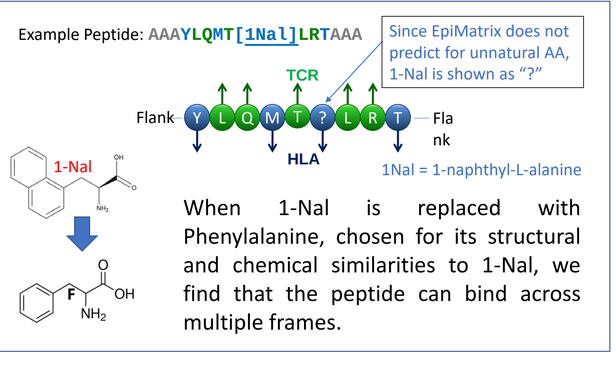

- EpiVax uses EpiMatrix to predict T cell epitopes
  - –EpiVax predicts both class I and class II HLA binding
  - – HLA binding is a prerequisite for immunogenicity
  - – Full suite of HLA-based predictions are available




**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.

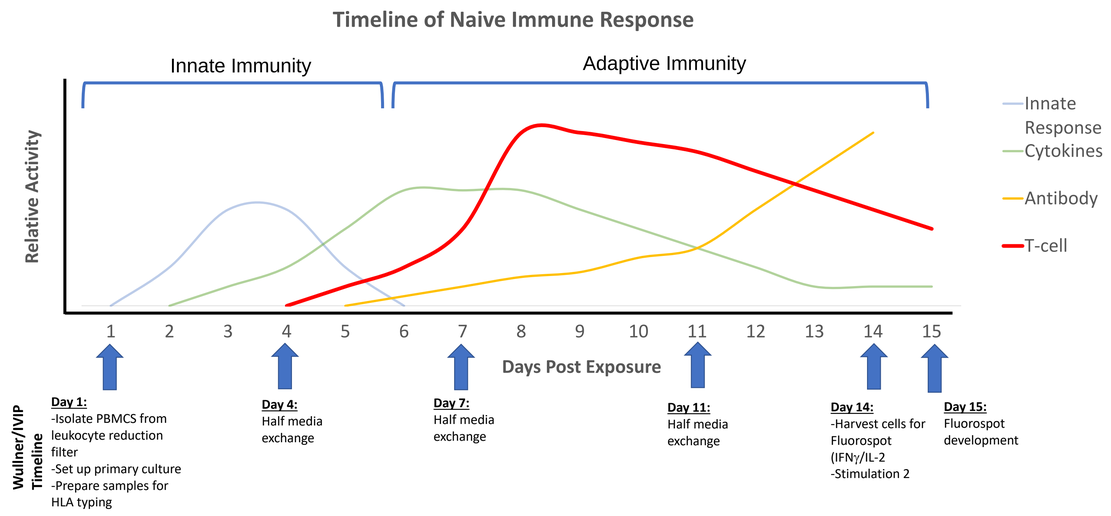
### Modeling Unnatural Amino Acids

- 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




## *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 compared to several controls** including HSA (protein neg control), KLH (protein positive control) and a CEFT (peptide pool positive control).
- **14 days post exposure, cells are harvested and plated into pre-coated 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 B

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

* 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.

## References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- Wullner D, Zhou L, Bramhall E, et al. Considerations for optimization and validation of an in vitro PBMC derived T cell assay for immunogenicity prediction of biotherapeutics. *Clin Immunol* 2010; 137: 5–14.
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