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

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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- The US Food and Drug Administration (FDA) recently released a new draft guidance enabling generic manufacturers of peptide drugs to file an Abbreviated New Drug Application (ANDA) for synthetic peptide drug products.
- Manufacturers are required to show that their synthetic peptide product does not contain process-related impurities that increase the risk of immunogenicity that could result in the development of anti-drug antibodies compared to the Reference Listed Drug (RLD)
- In response to the FDA's draft guidance, EpiVax has developed an innovative multi step protocol for the assessment of generic peptide drugs and their impurities that we call the Peptide Abbreviated New Drug Application or PANDA.
- Taspoglutide is a glucagon-like peptide-1 agonist used for the treatment of type 2 diabetes. In 2010, phase III clinical trials were stopped due to hypersensitivity reactions and GI side effects.
- Follow up analysis identified several manufacturing related peptide impurities believed to result in the observed hypersensitivity in an HLA-dependent manner. Retrospective analysis by EpiMatrix was able to predict increased immunogenic risk of duplication impurities that could contribute to increased risk in HLA DR7 and DR11.

PANDA Process Overview

in silico — Epitope Prediction

in vitro — HLA Binding

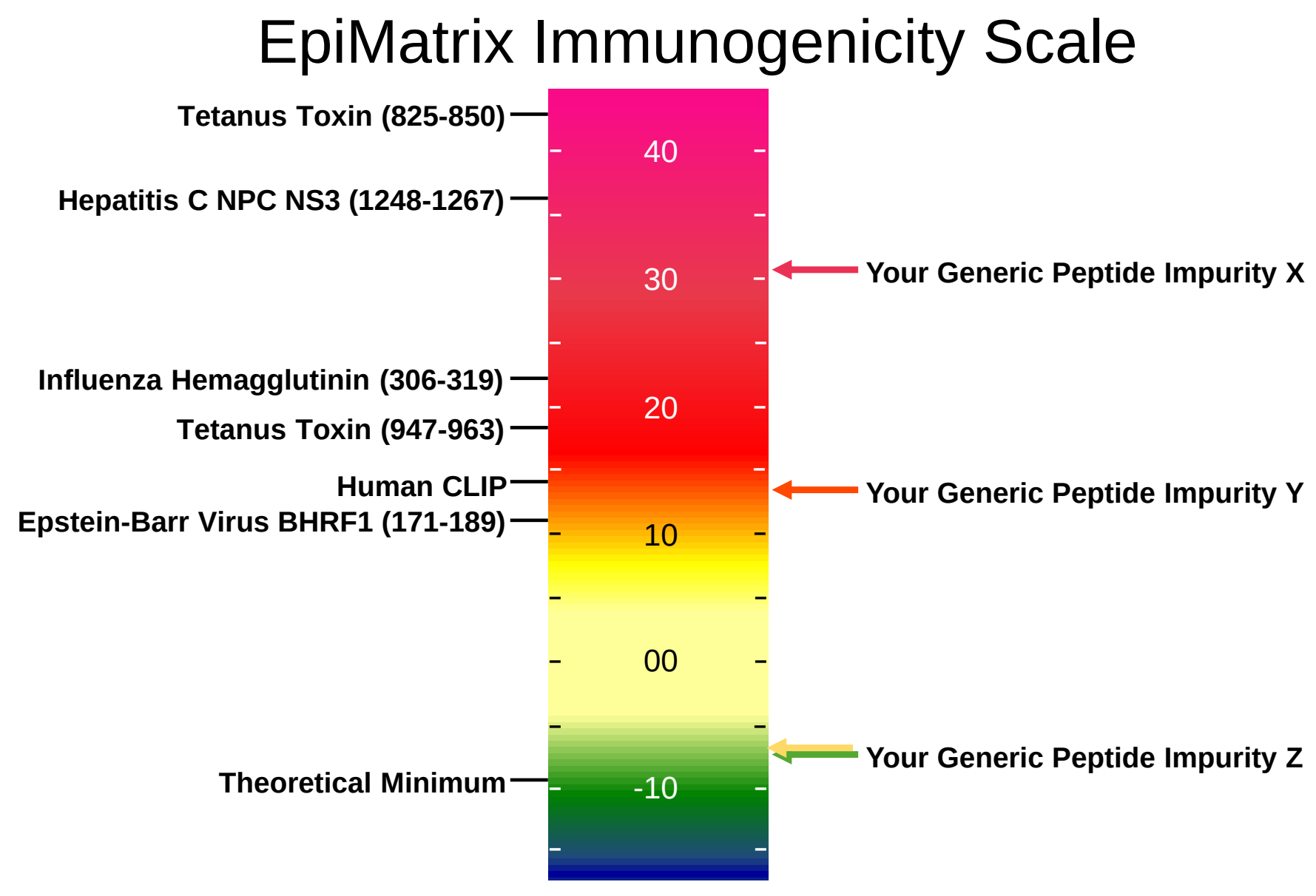
in vitro — T cell Activity

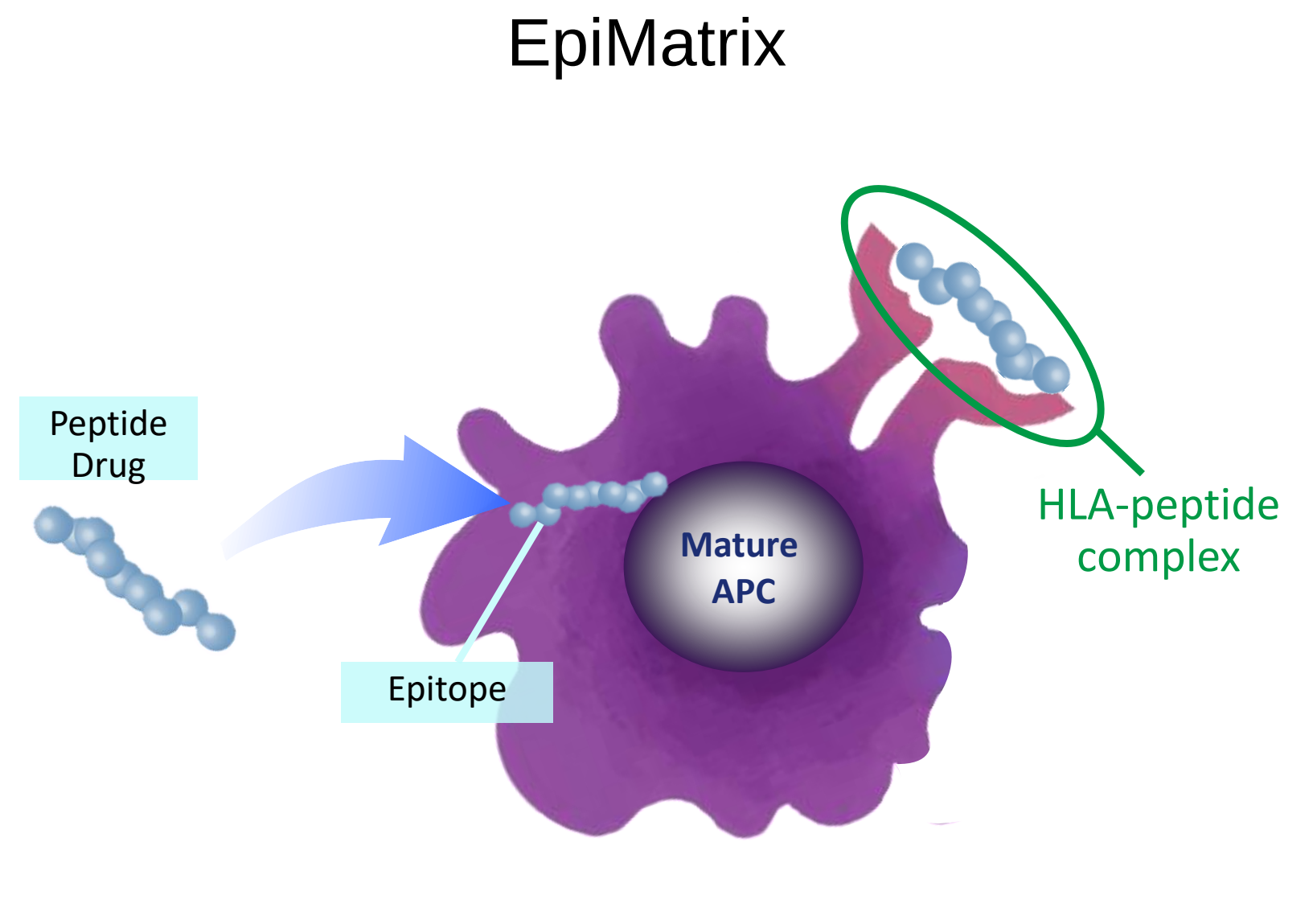
review — Literature

EpiVax's Expert Opinion on the Human Immune Response to RLD vs New Drug Candidate Synthetic Peptide Equivalent

Peptide Abbreviated New Drug Application

Epitope Prediction by EpiMatrix

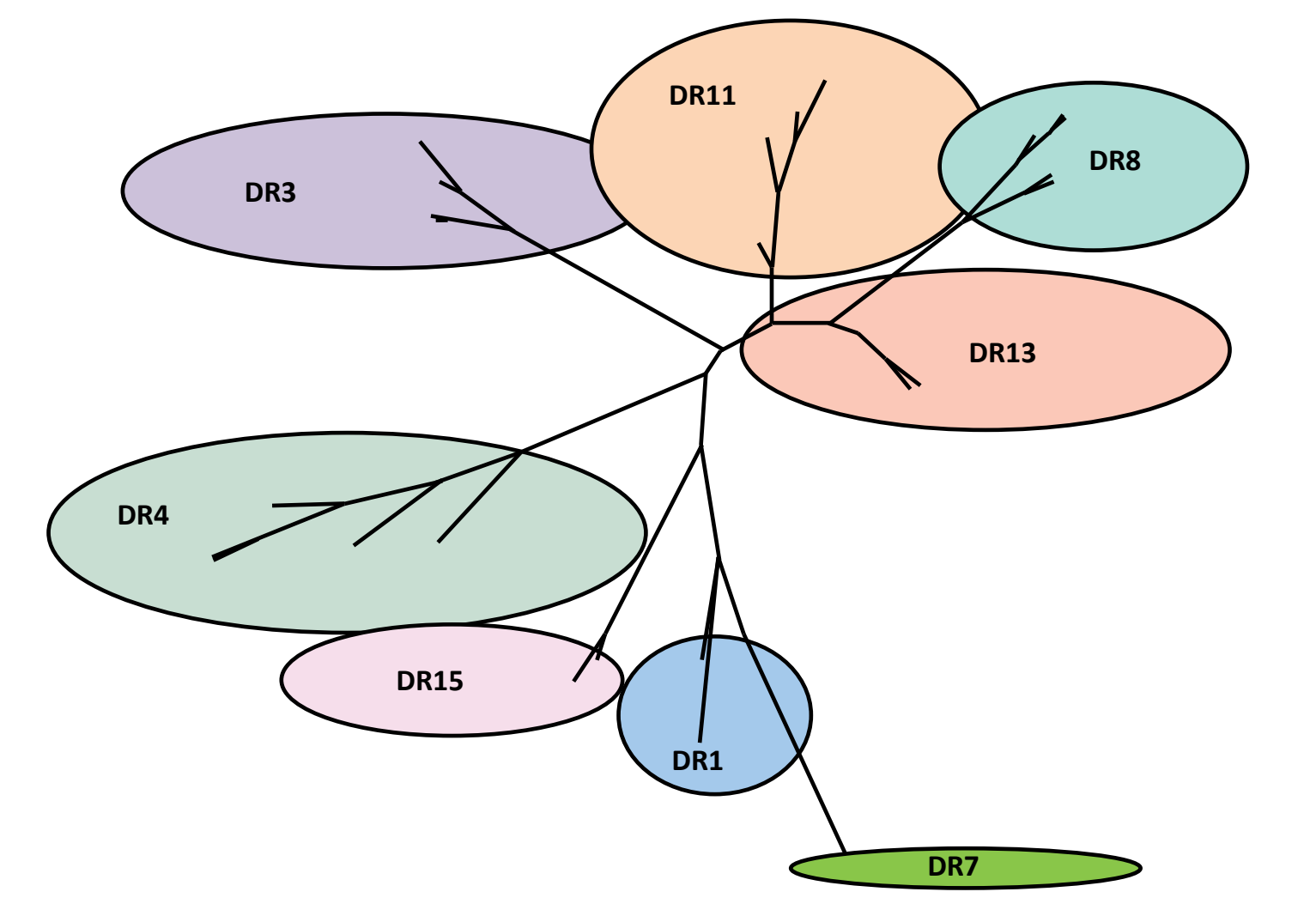


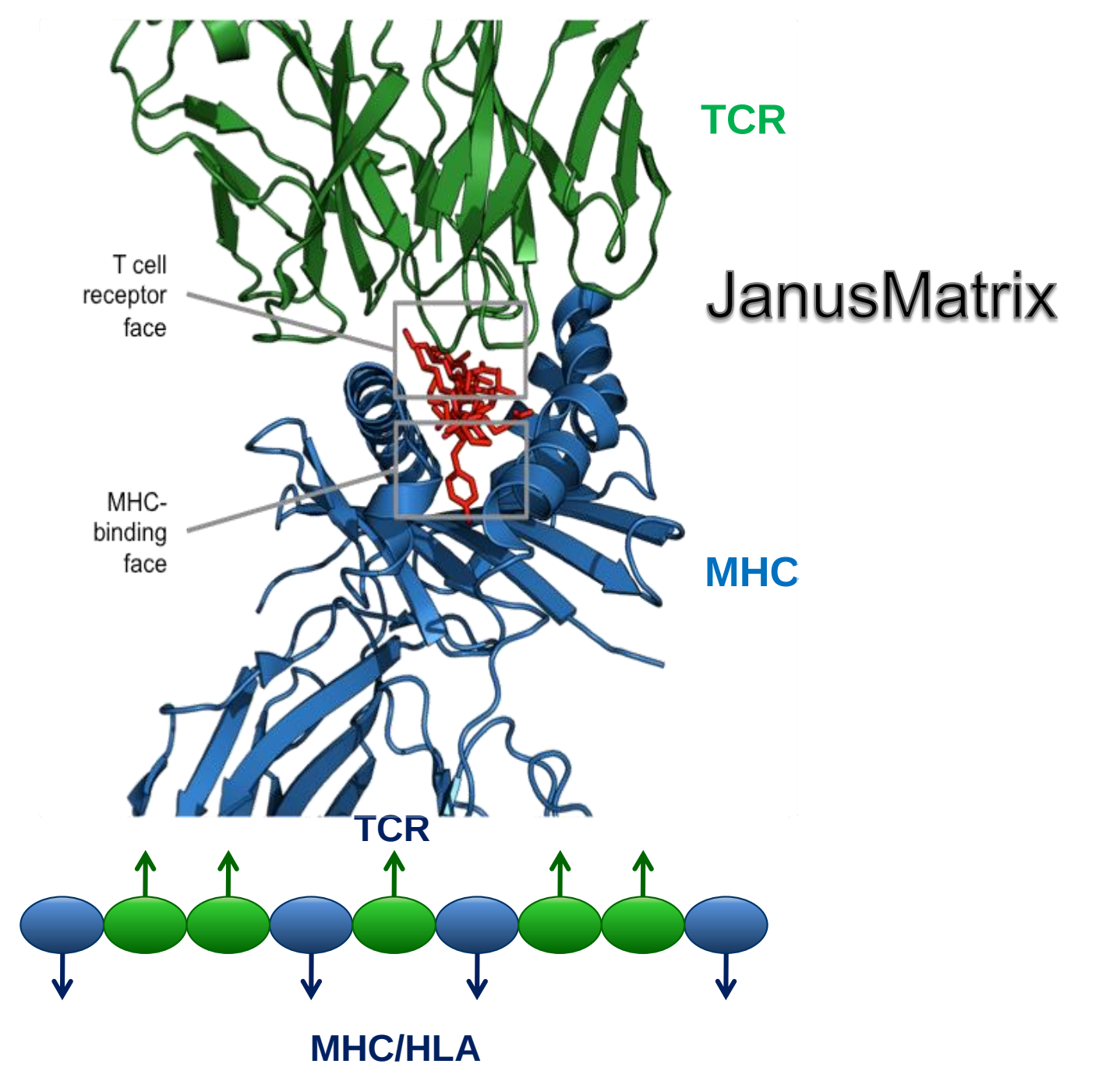


- EpiMatrix excess and shortfall in predicted aggregate immunogenicity relative to a random peptide standard
- EpiMatrix Cluster Scores above ten are comparable to those of known promiscuous Class II epitopes, commonly used as positive controls in T cell assays and included for reference on the above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

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

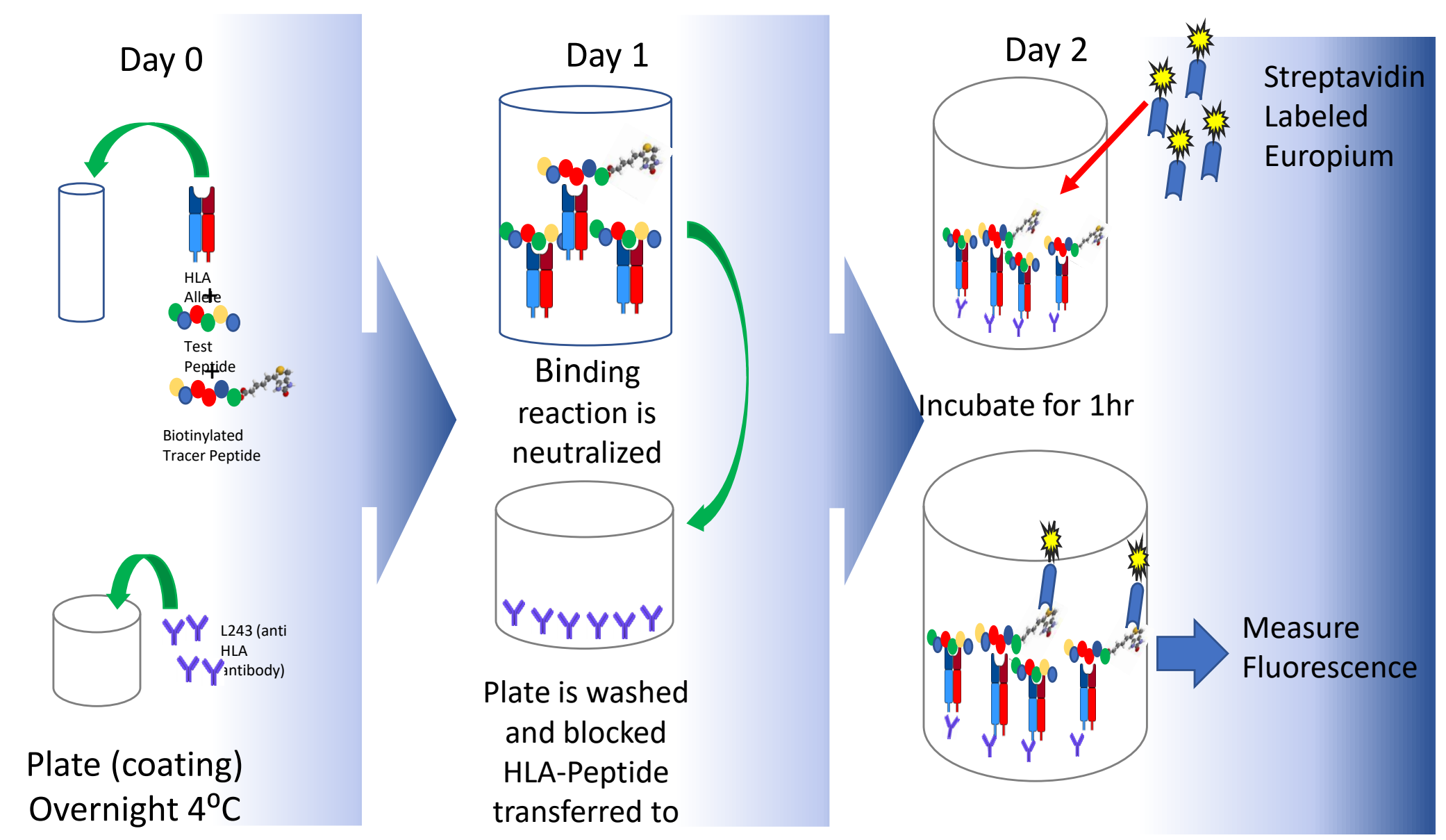




- EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left.
- This allows us to provide results that are representative of >95% of human populations worldwide** without the necessity of testing each haplotype individually.

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

HLA Class II Binding Assay

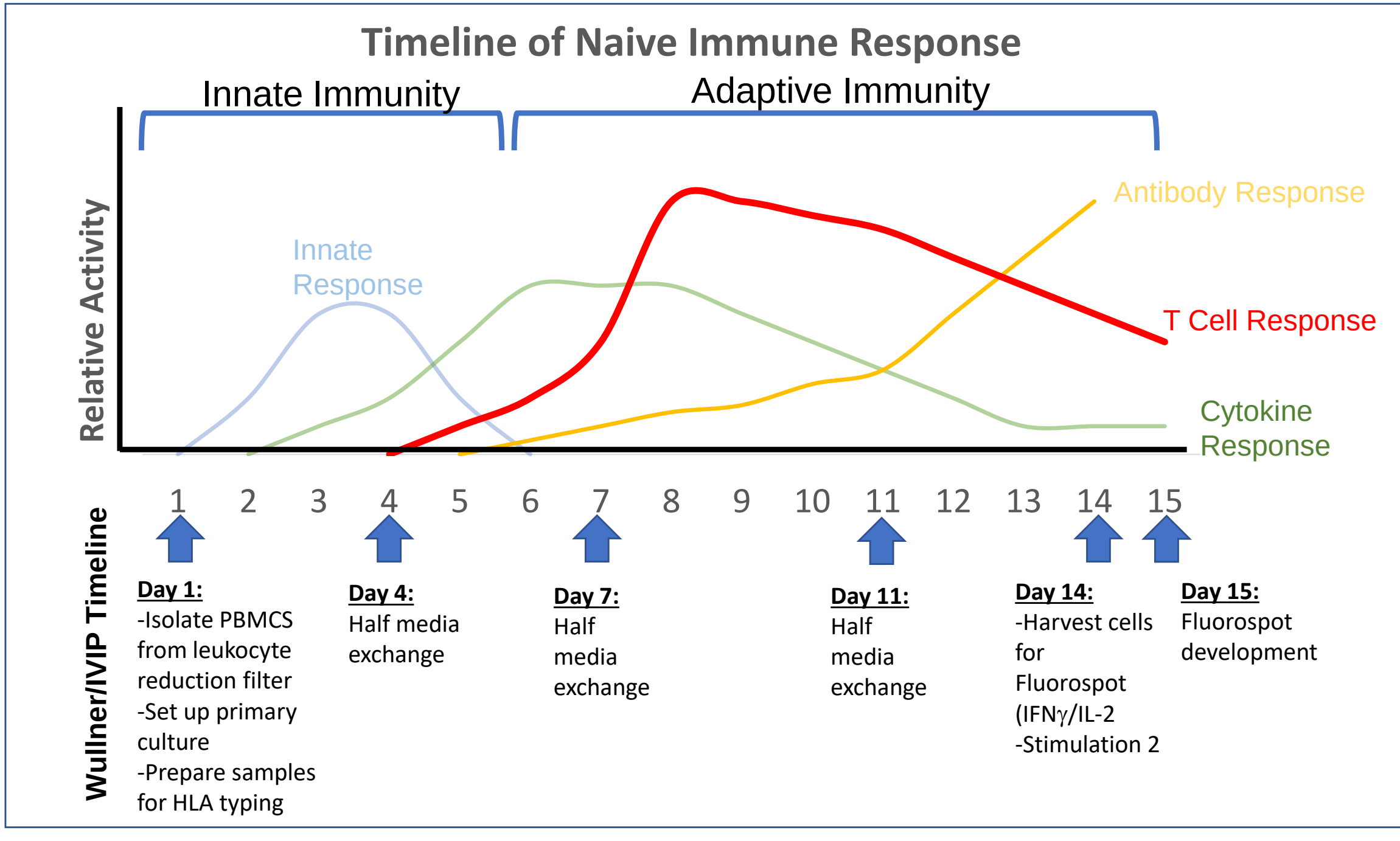


Adapted from Steere et al, 2006

HLA-Class II Binding Assay

- Peptides that are predicted to bind HLA are synthesized and assayed over a range of 7 concentrations, allowing for the generation of an IC50 value which provides information about the relative binding affinity of the peptide.
- 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.

*I*n *V*itro *I*mmunogenicity *P*rotocol (IVIP)



Day 1: -Isolate PBMCS from leukocyte reduction filter -Set up primary culture -Prepare samples for HLA typing

Day 4: Half media exchange

Day 7: Half media exchange

Day 11: Half media exchange

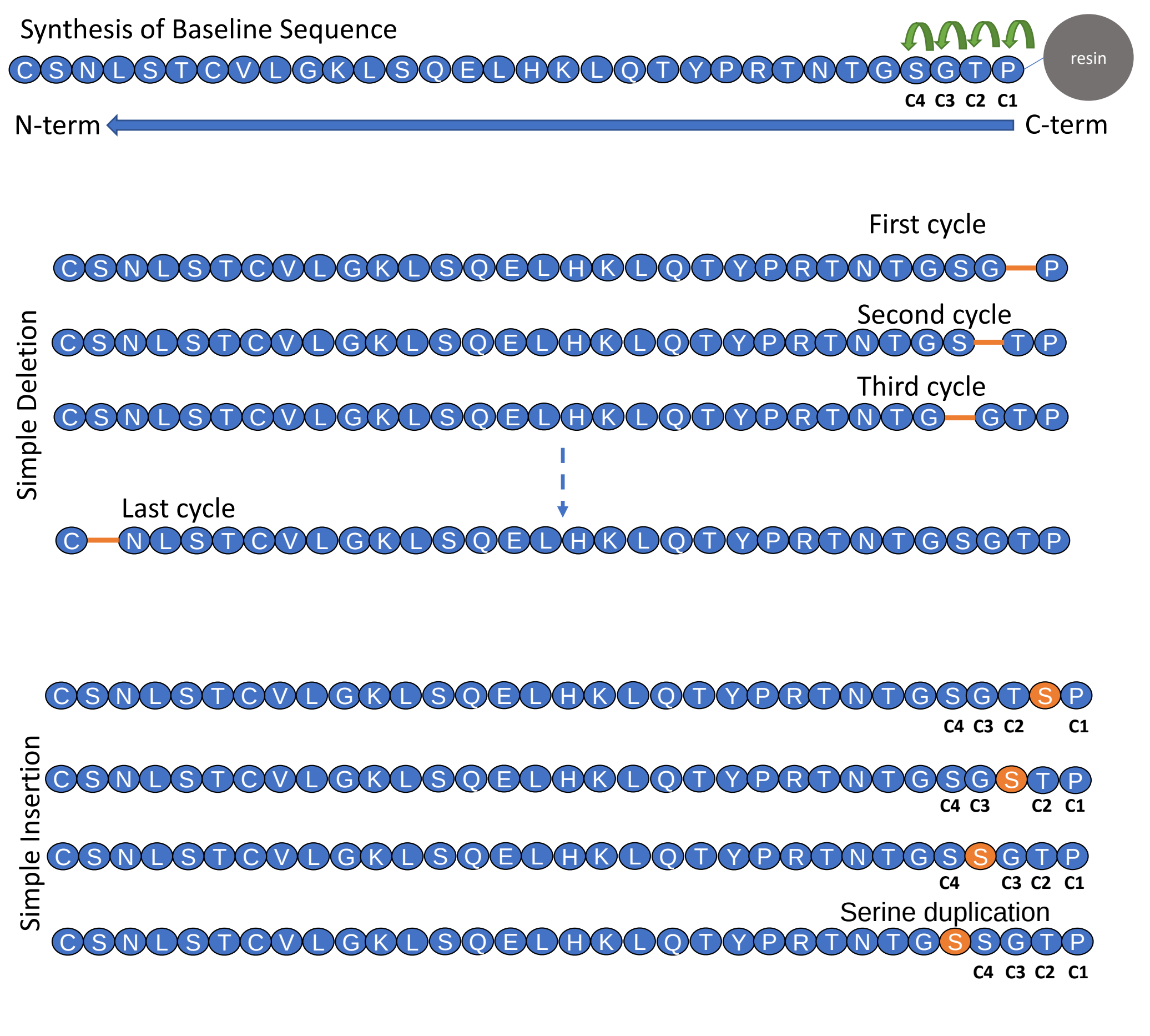
Day 14: -Harvest cells for Fluorospot (IFNγ/IL-2 -Stimulation 2

Day 15: Fluorospot development

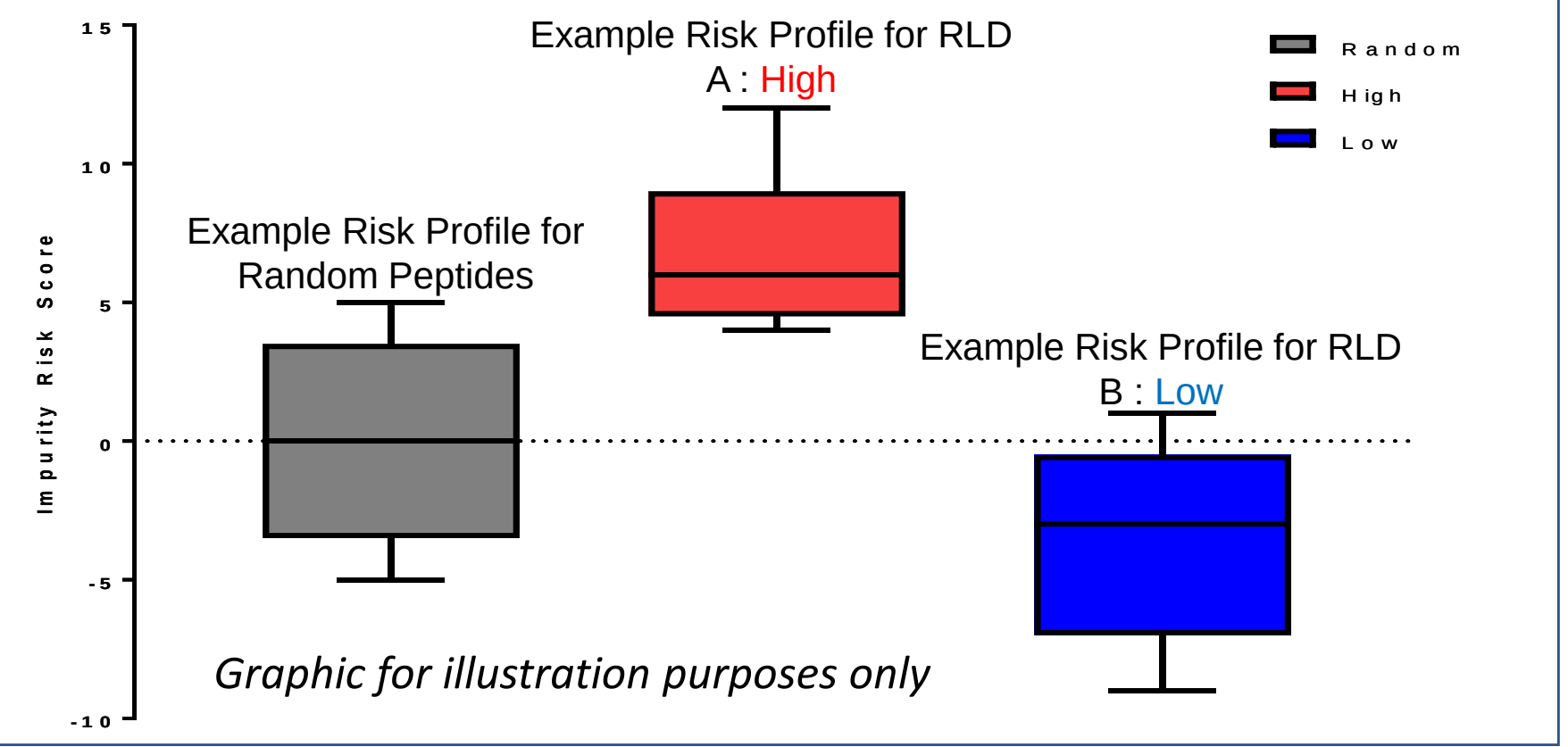
IVIP Assay

- 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.

What if Machine (WhIM)

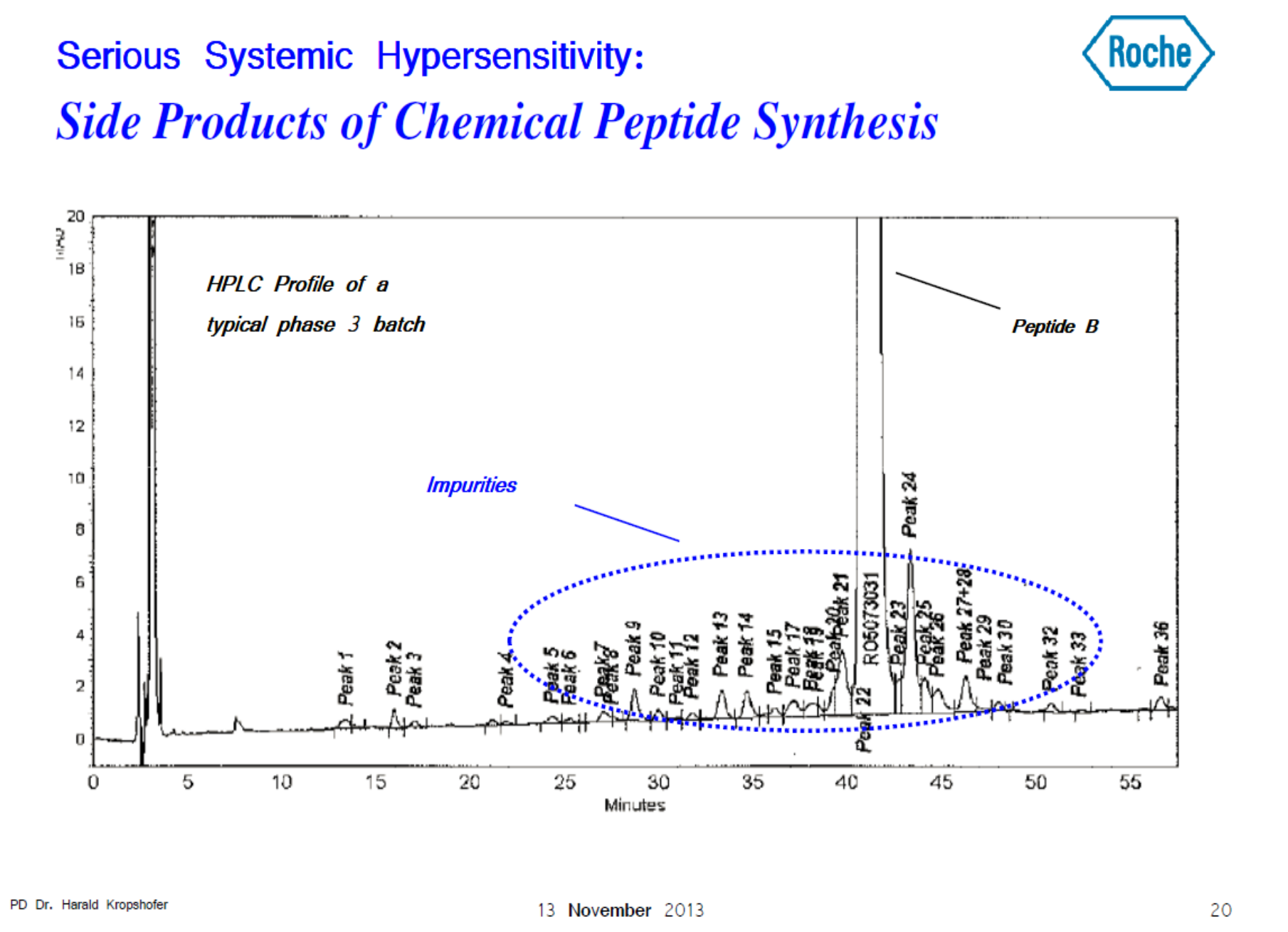


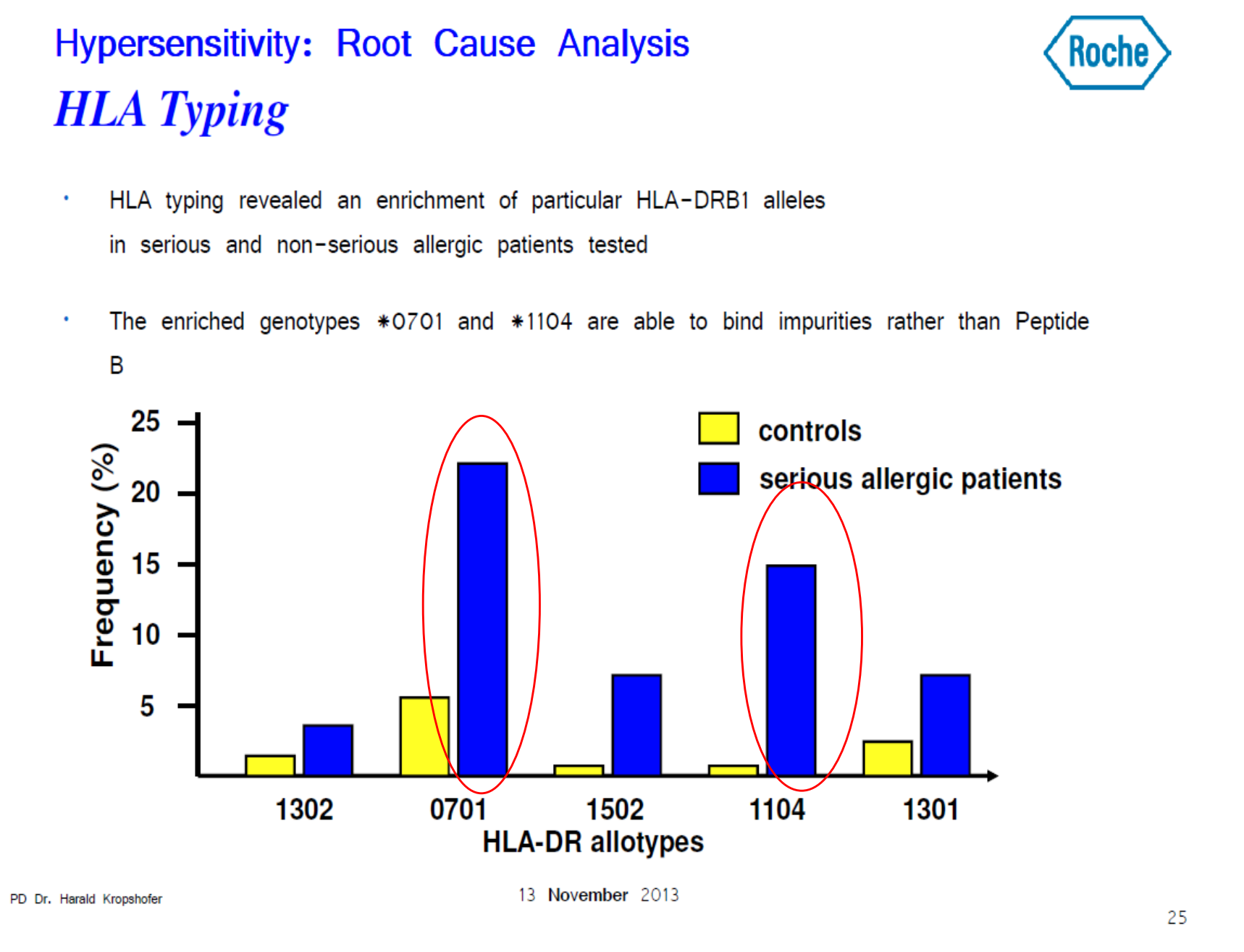
- The What if Machine (WhIM) that mimics the process of synthesizing polypeptide drug products and records all possible product impurities created through known failures in the synthesis process.
- Each identified impurity is scored for putative T cell epitope content (EpiMatrix) and cross conservation with the human proteome (JanusMatrix).
- Impurities are weighted based on assumed probability of occurrence.
- The WhIM currently models simple deletions, amino acid insertions (shown here), β-Alanine insertions and β-Alanine + amino acid insertions



Graphic for illustration purposes only

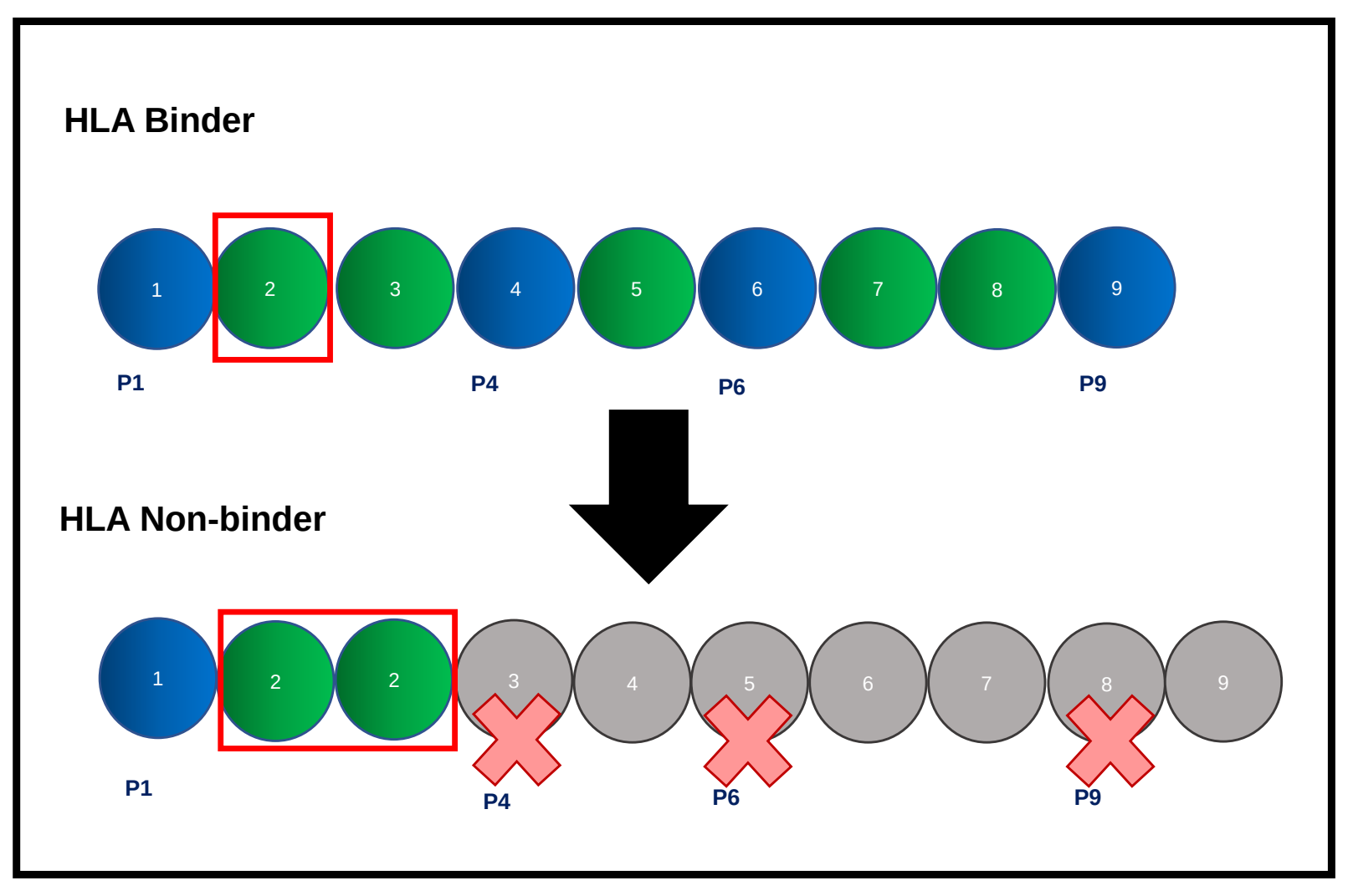
Taspoglutide Case Study





- HLA typing revealed an enrichment of particular HLA-DRB1 alleles in serious and non-serious allergic patients tested
- The enriched genotypes *0701 and *1104 are able to bind impurities rather than Peptide B

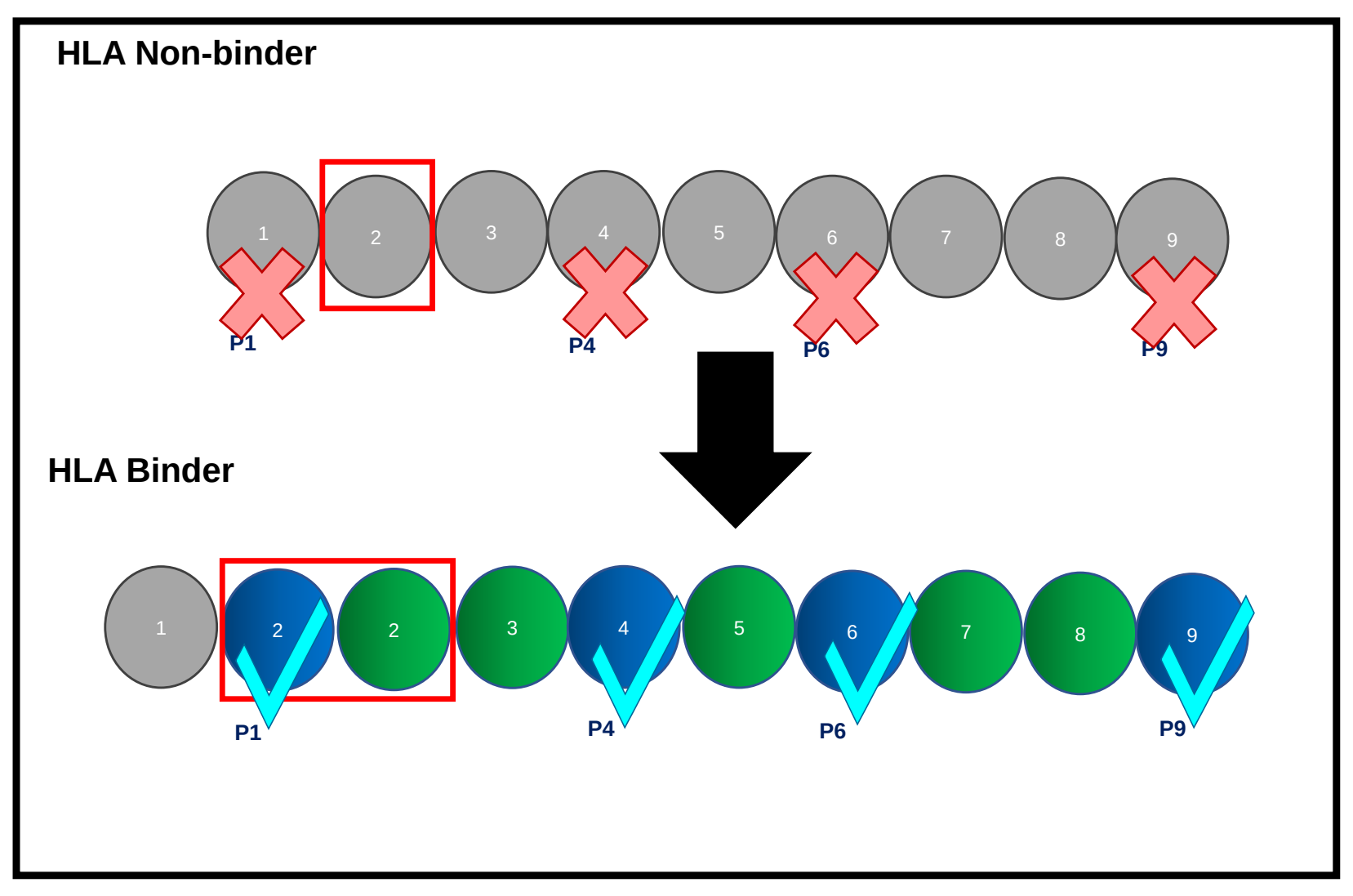
Scenario 1: Binder→ Non-binder



results in a peptide that will no longer bind HLA by shifting subsequent amino acids out of phase

Low-Risk Impurity*

Scenario 2: Non-binder→ Binder

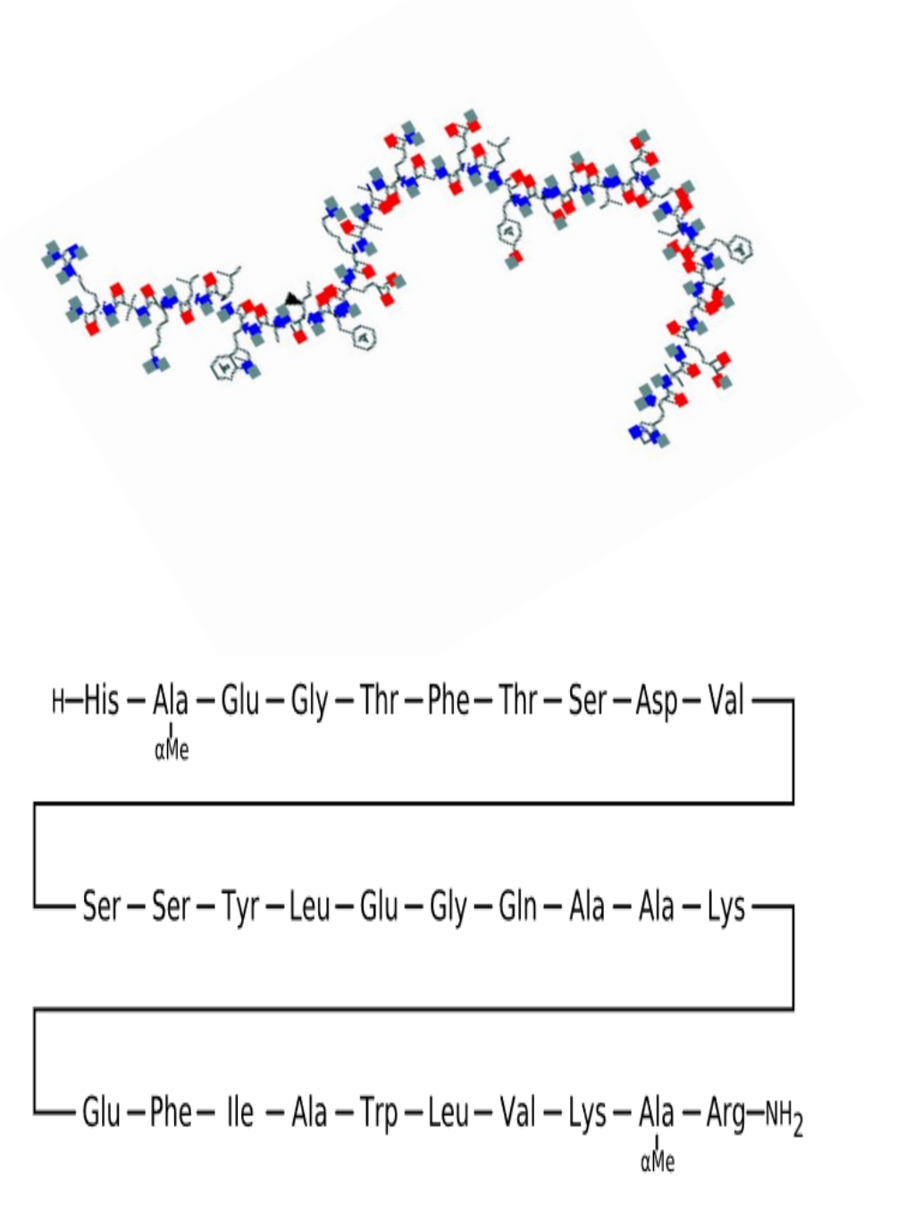


results in a peptide that will now bind HLA by shifting subsequent amino acids into phase

Potentially Immunogenic Impurity*

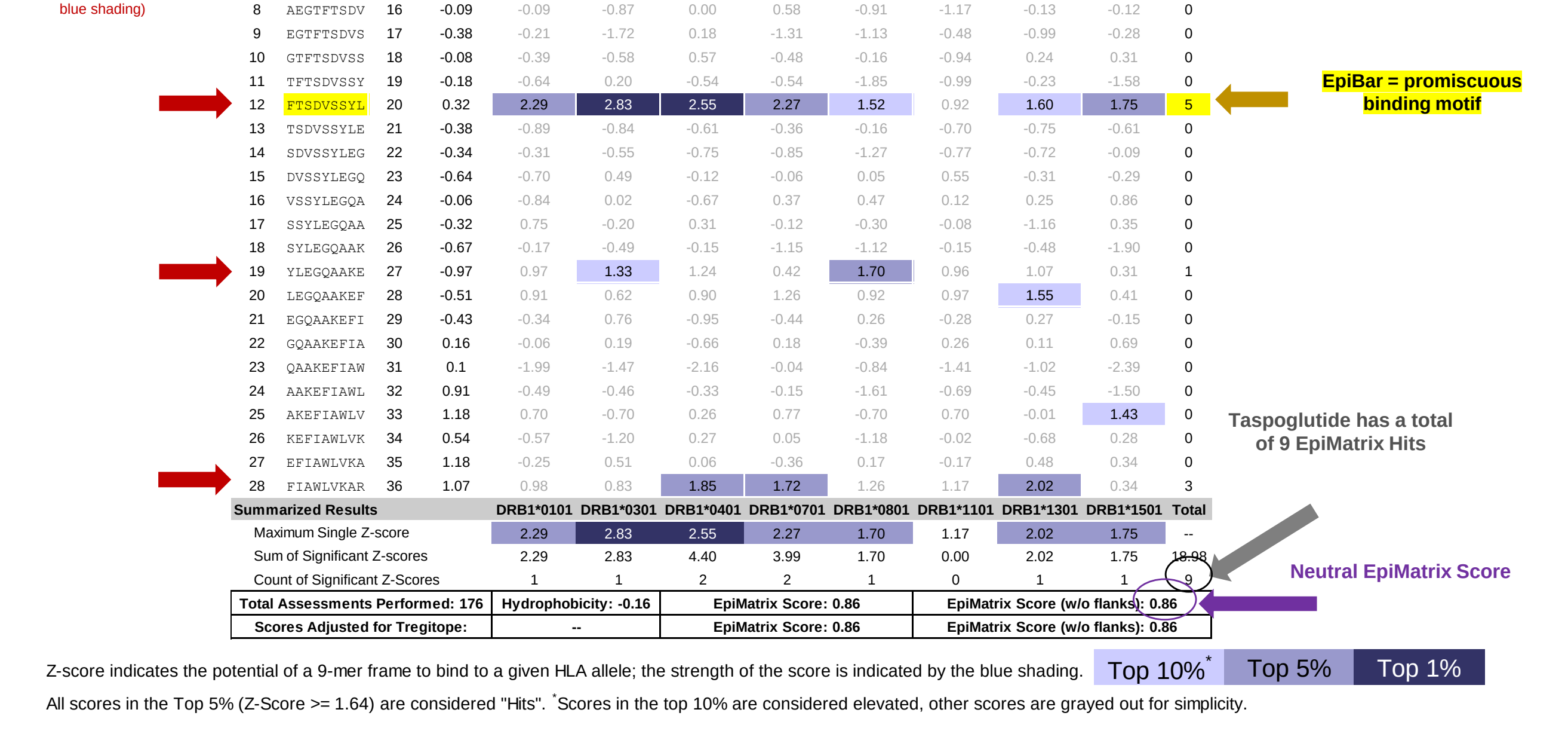
*Based on T cell epitope content alone

Taspoglutide



EpiMatrix Cluster Detail Report

File: TASPOGLUTIDE Sequence: 00_HGLP-1 Cluster: 7



3 frames contain putative T cell epitopes (Z-scores ≥ 1.64, medium and dark blue shading)

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
7	HAEGTFTSD	15	-0.91	-1.99	-1.54	-1.97	-1.57	-1.08	-2.78	-1.96	-1.97	0
8	AEGTFTSDV	16	-0.09	-0.09	-0.87	0.00	0.58	-0.91	-1.17	-0.13	-0.12	0
9	EGTFTSDVS	17	-0.38	-0.21	-1.72	0.18	-1.31	-1.13	-0.48	-0.99	-0.28	0
10	GTFTSDVSS	18	-0.08	-0.39	-0.58	0.57	-0.48	-0.16	-0.94	0.24	0.31	0
11	TFTSDVSSY	19	-0.18	-0.64	0.20	-0.54	-0.54	-1.85	-0.99	-0.23	-1.58	0
12	FTSDVSSYL	20	0.32	2.29	2.83	2.55	2.27	1.52	0.92	1.60	1.75	5
13	TSDVSSYLE	21	-0.38	-0.89	-0.84	-0.61	-0.36	-0.16	-0.70	-0.75	-0.61	0
14	SDVSSYLEG	22	-0.34	-0.31	-0.55	-0.75	-0.85	-1.27	-0.77	-0.72	-0.09	0
15	DVSSYLEGQ	23	-0.64	-0.70	0.49	-0.12	-0.06	0.05	0.55	-0.31	-0.29	0
16	VSSYLEGQA	24	-0.06	-0.84	0.02	-0.67	0.37	0.47	0.12	0.25	0.86	0
17	SSYLEGQAA	25	-0.32	0.75	-0.20	0.31	-0.12	-0.30	-0.08	-1.16	0.35	0
18	SYLEGQAAK	26	-0.67	-0.17	-0.49	-0.15	-1.15	-1.12	-0.15	-0.48	-1.90	0
19	YLEGQAAKE	27	-0.97	0.97	1.33	1.24	0.42	1.70	0.96	1.07	0.31	1
20	LEGQAAKEF	28	-0.51	0.91	0.62	0.90	1.26	0.92	0.97	1.55	0.41	0
21	EGQAAKEFI	29	-0.43	-0.34	0.76	-0.95	-0.44	0.26	-0.28	0.27	-0.15	0
22	GQAAKEFIA	30	0.16	-0.06	0.19	-0.66	0.18	-0.39	0.26	0.11	0.69	0
23	QAAKEFIAW	31	0.1	-1.99	-1.47	-2.16	-0.04	-0.84	-1.41	-1.02	-2.39	0
24	AAKEFIAWL	32	0.91	-0.49	-0.46	-0.33	-0.15	-1.61	-0.69	-0.45	-1.50	0
25	AKEFIAWLV	33	1.18	0.70	-0.70	0.26	0.77	-0.70	0.70	-0.01	1.43	0
26	KEFIAWLVK	34	0.54	-0.57	-1.20	0.27	0.05	-1.18	-0.02	-0.68	0.28	0
27	EFIAWLVKA	35	1.18	-0.25	0.51	0.06	-0.36	0.17	-0.17	0.48	0.34	0
28	FIAWLVKAR	36	1.07	0.98	0.83	1.85	1.72	1.26	1.17	2.02	0.34	3
Summarized Results				DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score				2.29	2.83	2.55	2.27	1.70	1.17	2.02	1.75	--
Sum of Significant Z-scores				2.29	2.83	4.40	3.99	1.70	0.00	2.02	1.75	18.98
Count of Significant Z-Scores				1	1	2	2	1	0	1	1	9

Total Assessments Performed: 176	Hydrophobicity: -0.16	EpiMatrix Score: 0.86	EpiMatrix Score (w/o flanks): 0.86
Scores Adjusted for Tregitope:	--	EpiMatrix Score: 0.86	EpiMatrix Score (w/o flanks): 0.86

EpiBar = promiscuous binding motif

Taspoglutide has a total of 9 EpiMatrix Hits

Neutral EpiMatrix Score

Z-score indicates the potential of a 9-mer frame to bind to a given HLA allele; the strength of the score is indicated by the blue shading. Top 10%* Top 5% Top 1%

All scores in the Top 5% (Z-Score >= 1.64) are considered "Hits". *Scores in the top 10% are considered elevated, other scores are grayed out for simplicity.

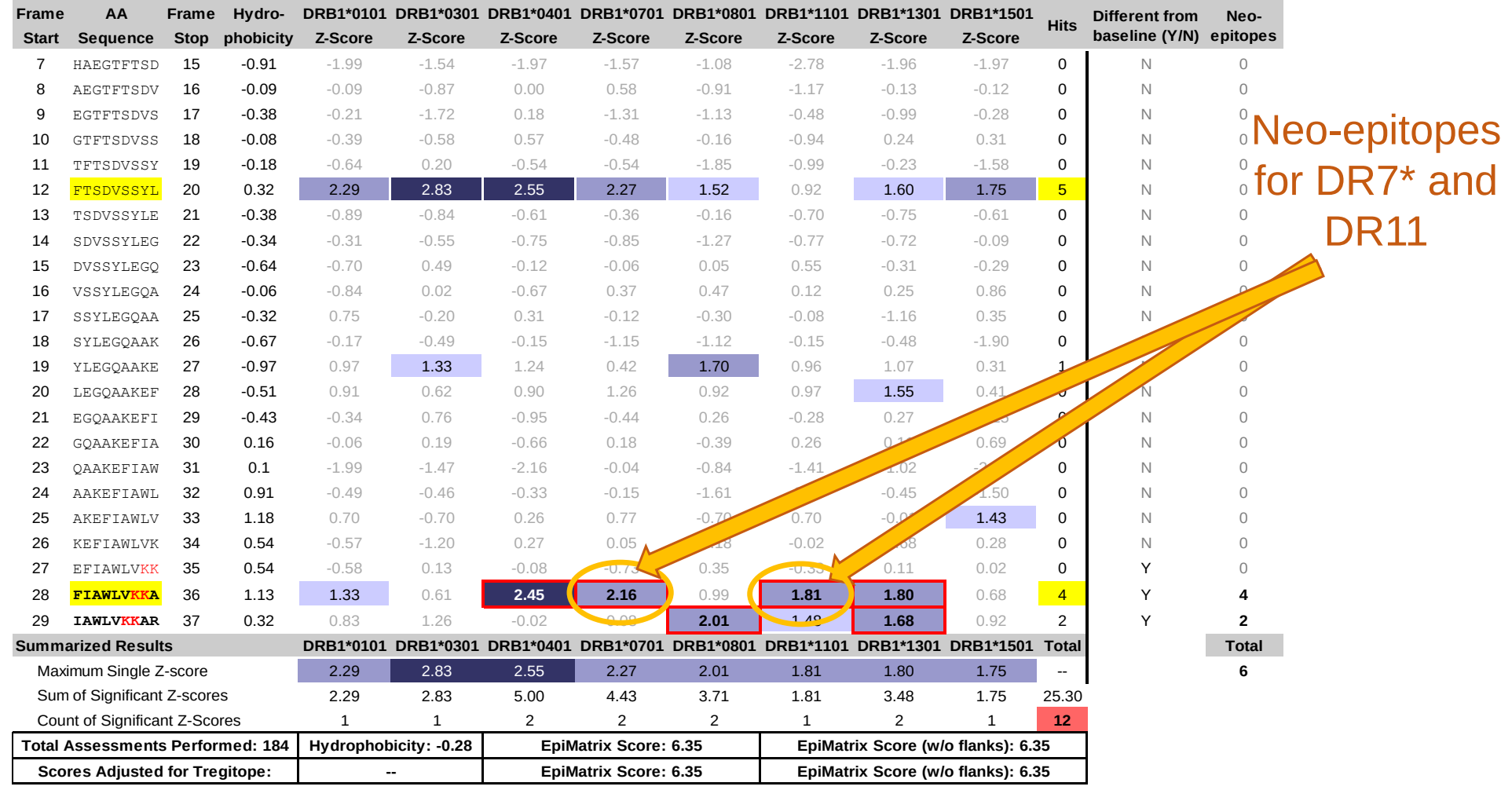
Frames containing four or more alleles scoring above 1.64 are referred to as EpiBars and are highlighted in yellow. These frames have an increased likelihood of binding to HLA.

Hydrophobic amino acid sequences scoring above 2.0 can be difficult to synthesize as peptides.

Lysine34 Duplication

EpiMatrix Cluster Detail Report

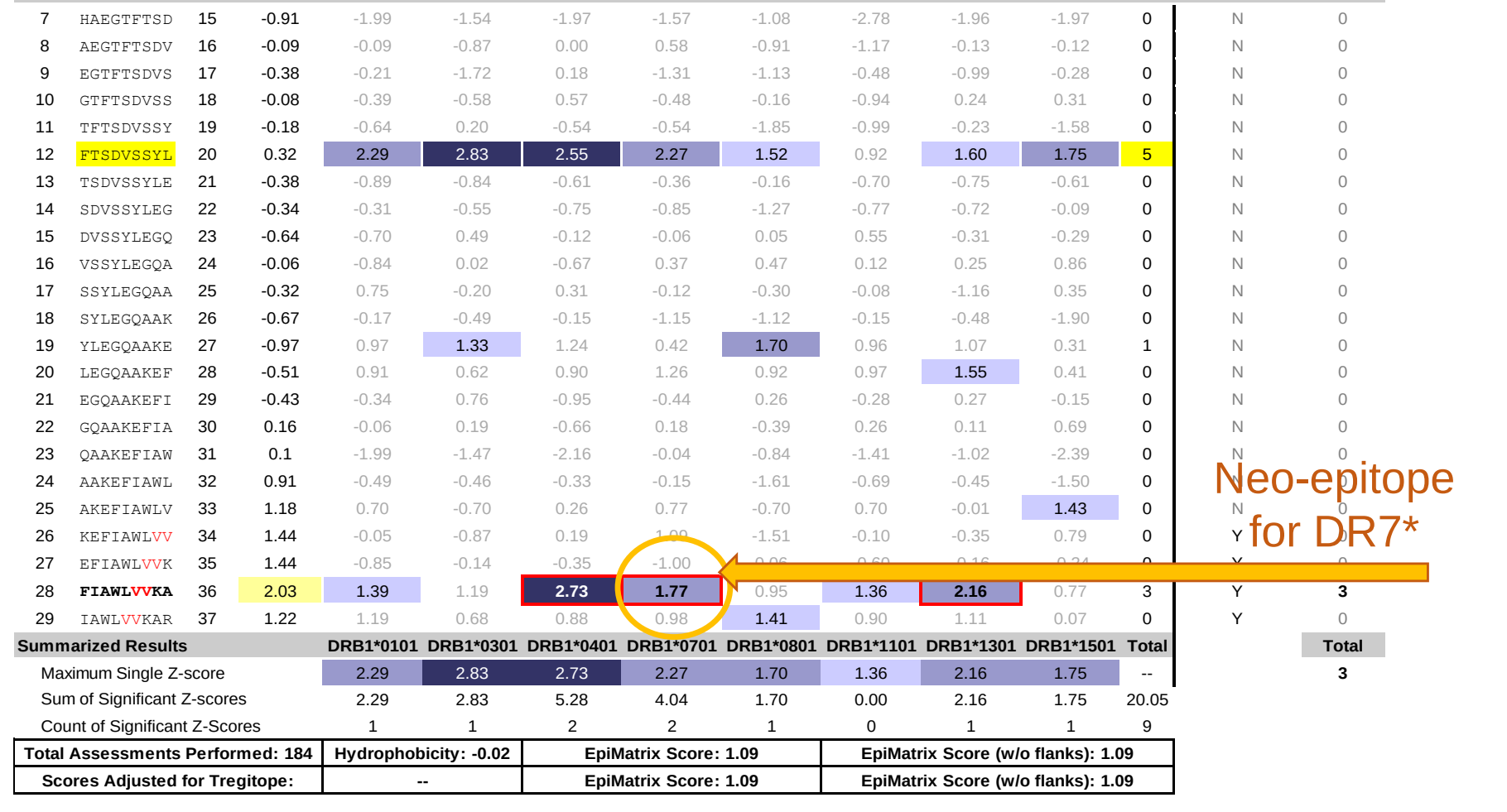
File: TASPOGLUTIDE Sequence: 26_ENDO-LYS34_HGLP-1 Cluster: 7



Valine33 Duplication

EpiMatrix Cluster Detail Report

File: TASPOGLUTIDE Sequence: 25_ENDO-VAL33_HGLP-1 Cluster: 7



EpiMatrix analysis of several Taspoglutide amino acid duplication impurities showed that 10 duplication impurities contained more putative T cell epitopes compare to the baseline sequence. 5 of those impurities contained neoepitopes that are predicted to bind HLA DR7 and DR11. One ore more of these duplication impurities could be contributing to the observed hypersensitivity seen in subjects with DRB1*0701 and DRB1*1104 (two representative impurities are shown here)

References

- Thank you to Dr. Harald Kropshofer for providing the peptide impurity and HLA typing graphs.

- Steere AC, Klitz W, Drouin EE, et al. Antibiotic-refractory Lyme arthritis is associated with HLA-DR molecules that bind a Borrelia burgdorferi peptide . *J Exp Med* 2006; 203: 961–971.
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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.