Immunogenicity Risk Assessment of Peptide Drugs and their Impurities (using in silico tools)

EpiVax:
Brian Roberts PhD
Christine Boyle, PhD
Aimee Mattei, MS
Sandra Lelias, MS
Mitchell McCallister
Bill Martin

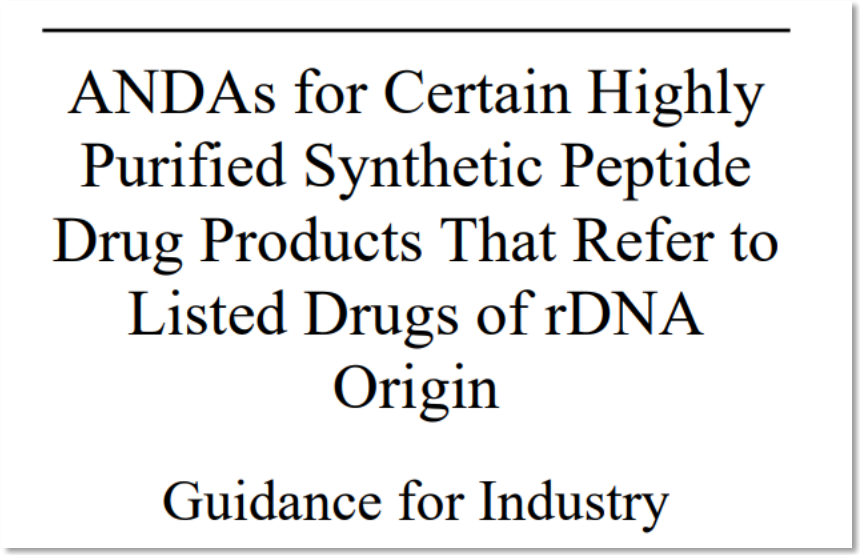
FDA:
Eric Pang, PhD
Daniela Verthelyi, PhD,
Christina Howard,PhD

CUBRC:
Katie Edwards PhD

EpiVax

Anne S. De Groot, MD
Founder and CSO

2021 FDA ANDA Guidance for Generic Peptide Drugs



ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

May 2021
Generics

"…Differences between the peptide-related impurities in a proposed generic synthetic peptide and those in an RLD of rDNA origin could produce different impurity profiles, which could adversely affect the safety or effectiveness of a proposed generic synthetic peptide product, if uncontrolled…"

Specifically, in lieu of clinical trials. sponsors asked to perform **immunogenicity risk assessment** studies on:

- … Impurities that are new in the … generic peptide … **in excess of 0.1% of the API….**
- …impurities … present in both the RLD and generic drug … **if the abundance …exceeds that of the RLD**

"…demonstrate … that the impurity does not contain sequences that have increased affinity for … MHC, known as T cell epitopes"

Our Immunogenicity Risk Assessment Methods – Published 2023

Drug Discovery Today
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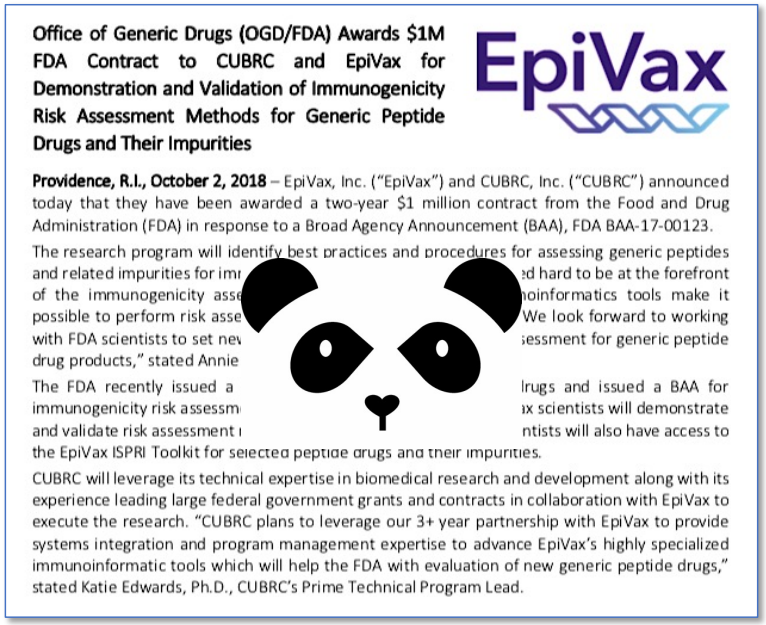
Immunogenicity risk assessment of synthetic peptide drugs and their impurities

Anne S De Groot [1 2], Brian J Roberts [1], Aimee Mattei [1], Sandra Lelias [1], Christine Boyle [1], William D Martin [1]



Office of Generic Drugs (OGD/FDA) Awards $1M FDA Contract to CUBRC and EpiVax for Demonstration and Validation of Immunogenicity Risk Assessment Methods for Generic Peptide Drugs and Their Impurities

Providence, R.I., October 2, 2018 – EpiVax, Inc. ("EpiVax") and CUBRC, Inc. ("CUBRC") announced today that they have been awarded a two-year $1 million contract from the Food and Drug Administration (FDA) in response to a Broad Agency Announcement (BAA), FDA BAA-17-00123.

The research program will identify best practices and procedures for assessing generic peptides and related impurities for imr ed hard to be at the forefront of the immunogenicity asse oinformatics tools make it possible to perform risk asse We look forward to working with FDA scientists to set nev essment for generic peptide drug products," stated Annie

The FDA recently issued a lrugs and issued a BAA for immunogenicity risk assessm ix scientists will demonstrate and validate risk assessment i ntists will also have access to the EpiVax ISPRI Toolkit for selected peptide drugs and their impurities.

CUBRC will leverage its technical expertise in biomedical research and development along with its experience leading large federal government grants and contracts in collaboration with EpiVax to execute the research. "CUBRC plans to leverage our 3+ year partnership with EpiVax to provide systems integration and program management expertise to advance EpiVax's highly specialized immunoinformatic tools which will help the FDA with evaluation of new generic peptide drugs," stated Katie Edwards, Ph.D., CUBRC's Prime Technical Program Lead.

#75F40120C00157 (October 2018)
#HHSF223018186C (October 2020)

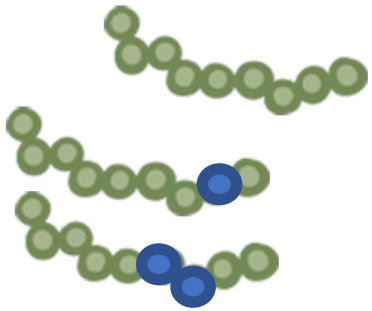
Outline

EpiVax

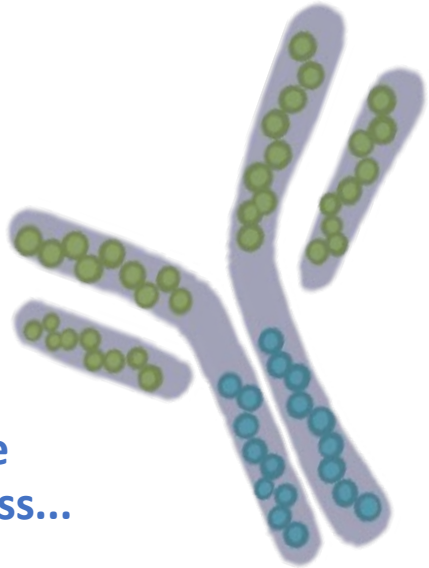
- Immuno informatics Basics
- Orthogonal Approach to identifying T cell Epitopes in synthetic peptides and impurities
  - In Silico Analysis
  - In Vitro Risk Assessment
- Case Study: Teriparatide
- Prospective Identification of Synthetic Peptide Impurities- The What If Machine

Immuno informatics / In Vitro Methods for Immunogenicity Risk Assessment

EpiVax



FDA: Novel / Generic Peptides (and their impurities)… should be assessed for Immunogenicity risk.



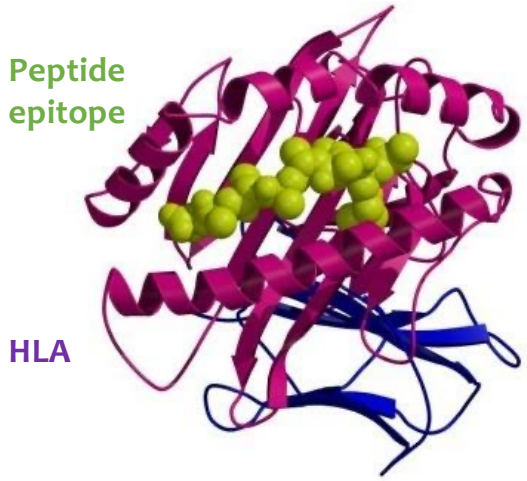
Biologics developers have been using immunoinformatics tools such as those developed by EpiVax for *decades* to address… Immunogenicity Risk !

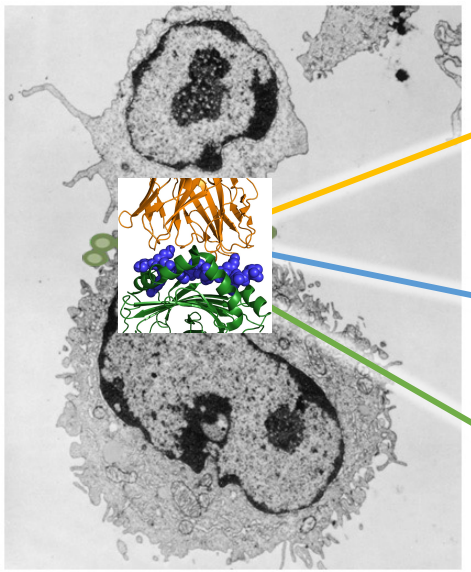
In silico Immunogenicity Assessment for Sequences Containing Unnatural Amino Acids: **Mattei AE**, et al.Front Drug Discov (Lausanne). 2022;2:952326. doi: 10.3389/fddsv.2022.952326.

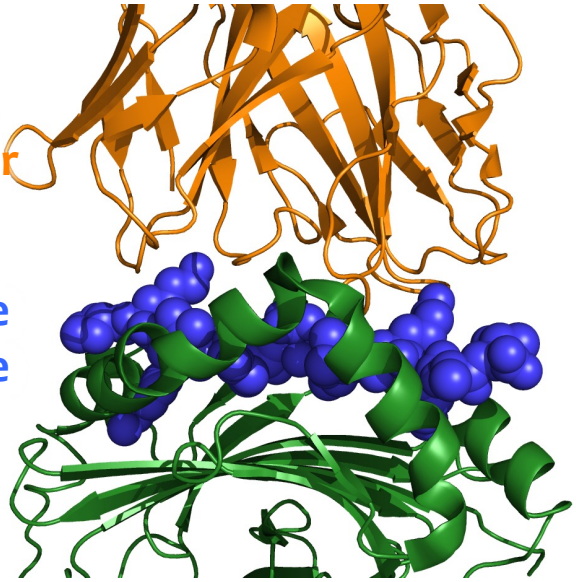
In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies. **Mattei AE**, et al. MAbs. 2024 Jan-Dec;16(1):2333729. doi: 10.1080/19420862.2024.2333729. Epub 2024 Mar 27.PMID: 38536724

Immunoinformatics tools illuminate immune response to biologics/peptides

EpiVax



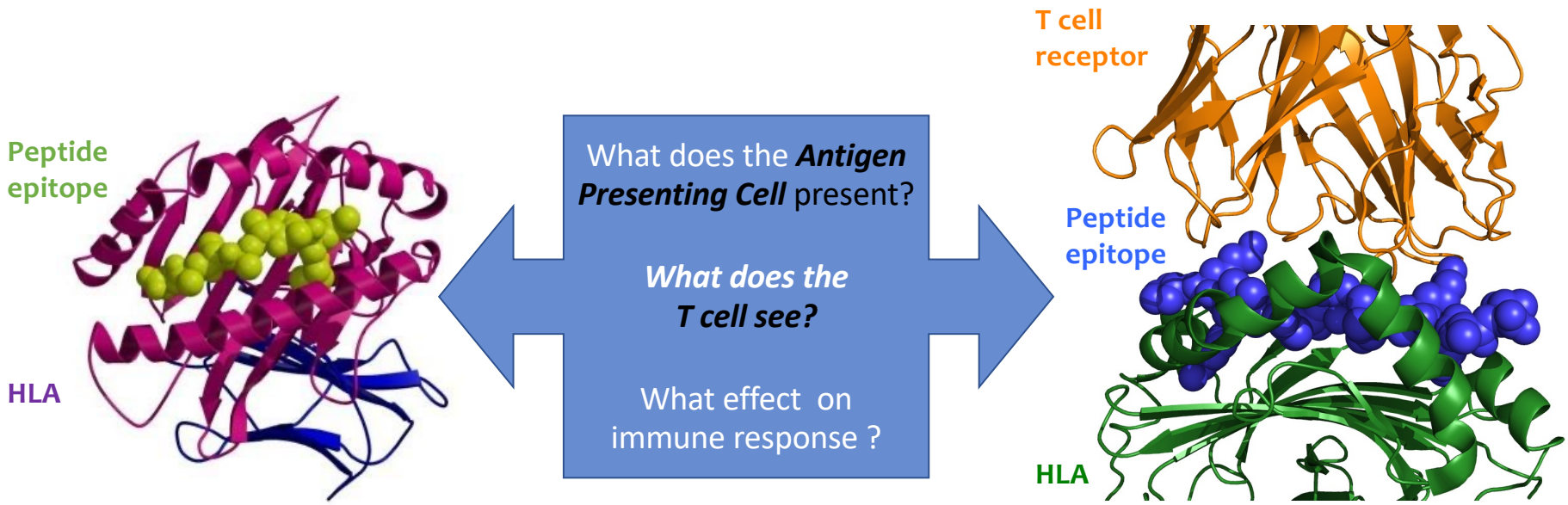




The T cell epitope is linear when bound to the HLA molecule that presents it to the T cell

immunoinformatics/ in vitro tools illuminate immune responses to peptide drugs

EpiVax



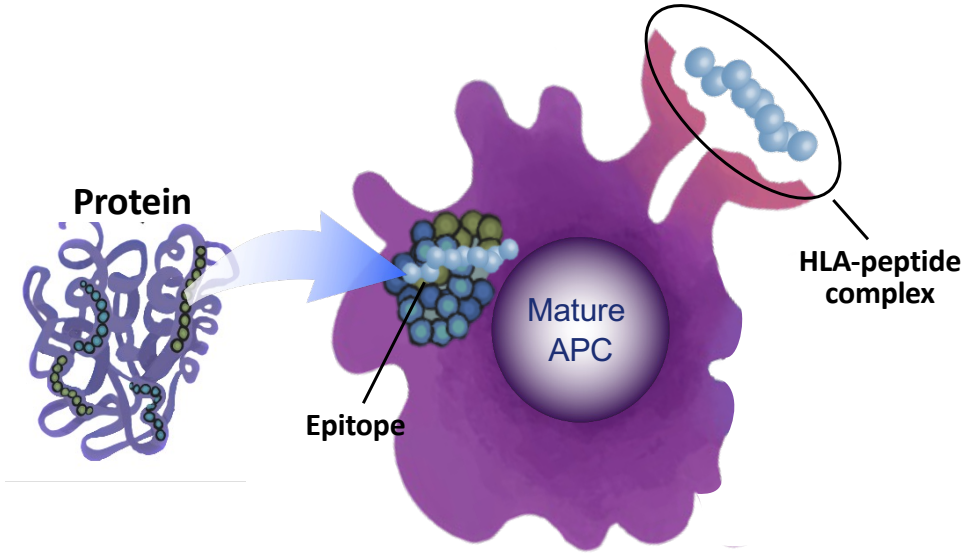
T cell epitope and immunogenicity analysis for peptides and their impurities

How is In Silico Risk Assessment Done?

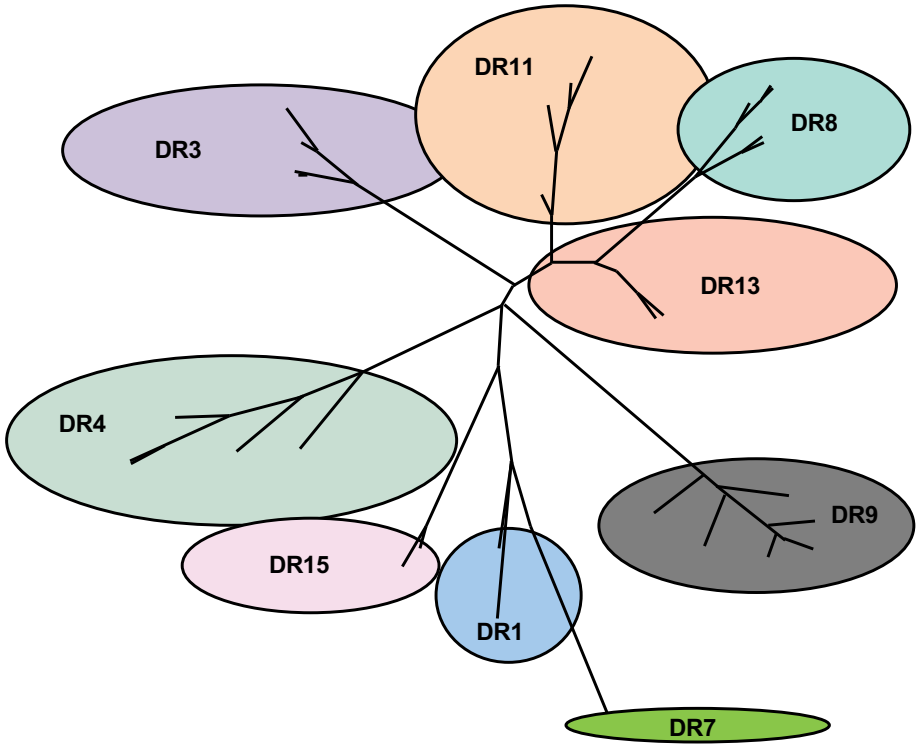
EpiMatrix™ T Cell Epitope Prediction

EpiVax

- EpiVax proprietary algorithm: **EpiMatrix™**
  - Matrix-based algorithm for predicting linear T cell epitopes
- EpiMatrix™ predicts Class II HLA binding potential and "potential" for T cell response



Many HLAs in Human Population
HLA "Supertype" Coverage



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 needing to test each haplotype individually.**

*Lund et al. Definition of Supertypes for HLA Molecules Using Clustering of Specificity Matrices. Immunogenetics. 2004; 55(12):797–810.

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

Summing Epitopes to Assess Risk

More T cell epitopes = Higher immune response

EpiVax

Total T cell epitope content = Predicted immunogenic potential

Immunogenic potential increases with increasing T cell epitope content

De Groot A.S. and L. Moise. Prediction of immunogenicity for therapeutic proteins: State of the art. Current Opinions in Drug Development and Discovery. May 2007. 10(3):332-40.

Analysis of each 9mer frame For probable binding to HLA

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EpiMatrix Report

File: Your File - Sequence: Your Protein

Frame Start	AA Sequence	Frame Stop	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
1	APELLGGPS	9	0.1	-0.88	-0.34	-0.84	-0.65	-0.4	-1.72	-0.17	0
2	PELLGGPSV	10	1.07	-0.62	0.33	0.13	-0.09	0.39	-0.28	0.59	0
3	ELLGGPSVF	11	-0.17	0.45	0.26	0.48	-0.28	-0.21	-0.11	-0.32	0
4	LLGGPSVFL	12	1.78	1.73	1.43	1.87	0.69	0.29	1.24	1.93	4
5	LGGPSVFLF	13	-0.21	0.4	-0.13	0.46	-0.32	0.07	0.99	-0.02	0
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87	KEYKCKVSN	95	-0.68	0.07	-1.29	-0.96	1.31	-0.09	0.52	-0.61	0
88	EYKCKVSNK	96	-0.75	-1.04	0.44	-0.78	0.67	-0.64	-0.97	-1.6	0
89	YKCKVSNKA	97	1.85	1.92	1.94	2.58	2.47	2.41	1.56	1.4	6
90	KCKVSNKAL	98	1.15	0.11	0.44	1.59	0.21	0.52	0.53	1	0
91	CKVSNKALP	99	-0.06	1	0.06	-0.47	0.69	1.47	0.86	-0.18	0
92	KVSNKALPA	100	1.6	1.41	1.92	1.26	1.09	1.86	1.54	1.4	2
93	VSNKALPAP	101	-1.29	0.19	-1	-0.98	1.05	0.66	0.74	-0.28	0
94	SNKALPAPI	102	1.28	1.45	0.8	1.05	0.77	0.55	1.62	0.98	0
95	NKALPAPIE	103	0.62	0.3	0.48	-0.19	1.65	0.76	0.62	0.26	1
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205	HYTQKSLSL	213	1.44	0.63	1.24	1.46	0.52	0.94	1.49	1.46	0
206	YTQKSLSLS	214	0.68	1.68	0.76	0.86	2.46	2.02	2	0.94	4
207	TQKSLSLSP	215	0.8	0.75	1.4	1.54	0.25	1.09	0.56	0.8	0
208	QKSLSLSPG	216	0.68	0.54	0.67	-0.18	1.64	1.42	0.65	0.95	0
209	KSLSLSPGK	217	0.66	0.57	0.94	0.39	0.47	1.02	0.33	0.8	0

Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.18	2.5	2.42	2.63	2.47	2.41	2.84	2.49	--
Sum of Significant Z-scores	20.14	23.2	22.19	26.64	27.15	20.78	21.88	10.08	172.05
Count of Significant Z-Scores	11	12	11	14	13	11	11	5	88
Total Assessments Performed: 1672		Deviation from Expectation: -13.95				Deviation per 1000 AA: -8.34			
Adjusted for Regulatory Epitopes		Deviation from Expectation: -34.27				Deviation per 1000 AA: -20.50			

Individual HLA Binding Assessment

Promiscuous Epitope

EpiMatrix Immunogenicity Score

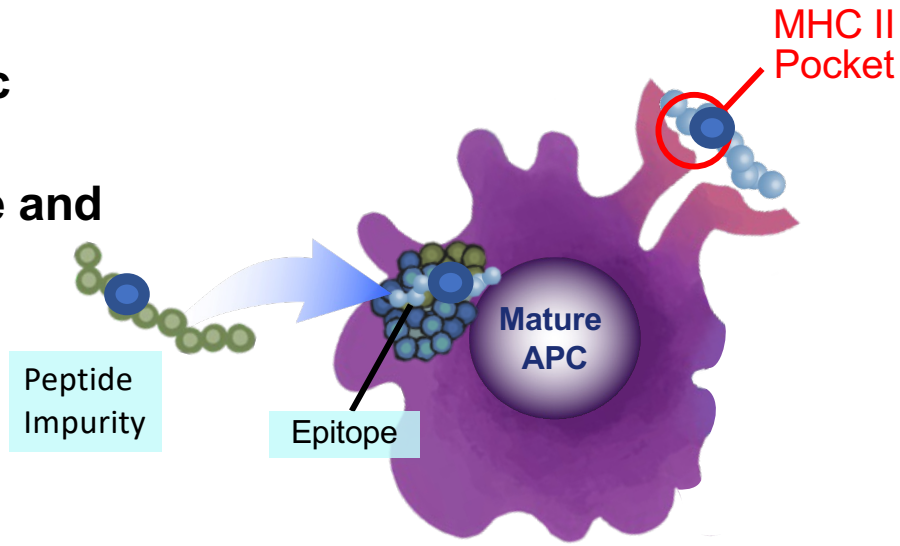
Treg epitope -adjusted Score

Outline

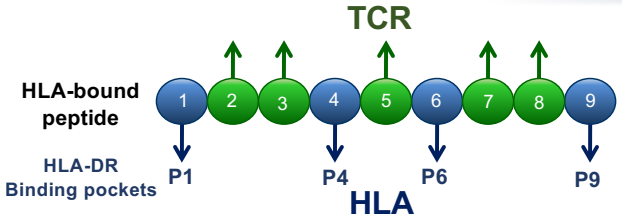
- Immuno informatics Basics
- Orthogonal Approaches for Identifying T cell Epitopes in synthetic peptides and impurities
  - In Silico Analysis
  - In Vitro Risk Assessment
- Case Study: Teriparatide
- Prospective Identification of Synthetic Peptide Impurities- The What If Machine

If the impurity changes HLA binding or TCR face introduces a change to baseline Immunogenicity

- **Many peptides are 'Self Peptides" e.g Teriparatide (PTH), GLP-1RA.**
- **The API is usually non immunogenic because "self" and tolerated.**
- **The impurity changes the sequence and can trigger immune response**
  - **Prediction: in silico**
  - **Test: HLA Binding**
  - **Test: Immune response (in vitro)**

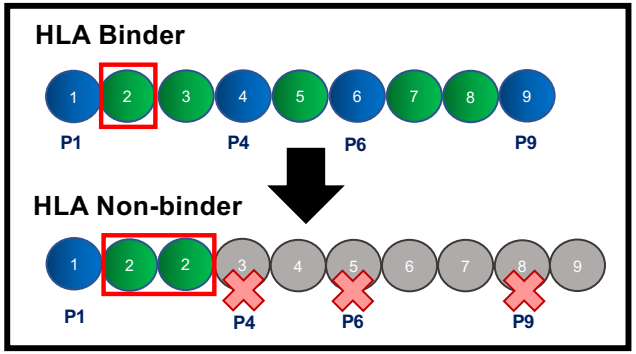


Peptide Impurities & Immunogenicity: Impact of Impurities - Duplications



Example Impurity - Duplication of Amino Acid 2:

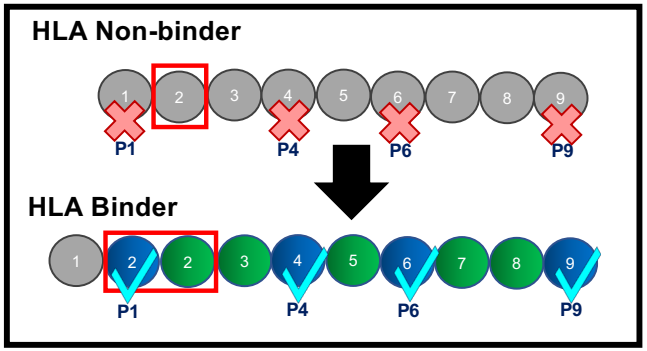
Scenario 1: Binder→ Non-binder



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

Deciphering hypersensitivity to Taspoglutide

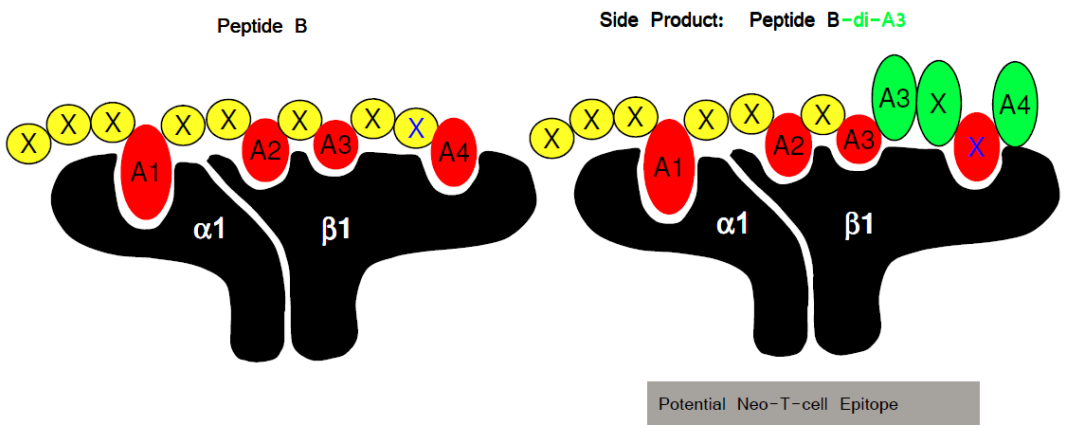
CHI Immunogenicity Summit 2013, courtesy of Dr. Harold Kropshofer

EpiVax

Serious Systemic Hypersensitivity:

Epitope Prediction: Synthesis Side Product

Roche



Changes the T cell Receptor Face

Suspicion: Side Products may give rise to novel T-cell epitopes

PD Dr. Harald Kropshofer 13 November 2013 21

Not all T cell Epitopes are the Same!
Characterizing Putative Tolerizing T cell Epitopes

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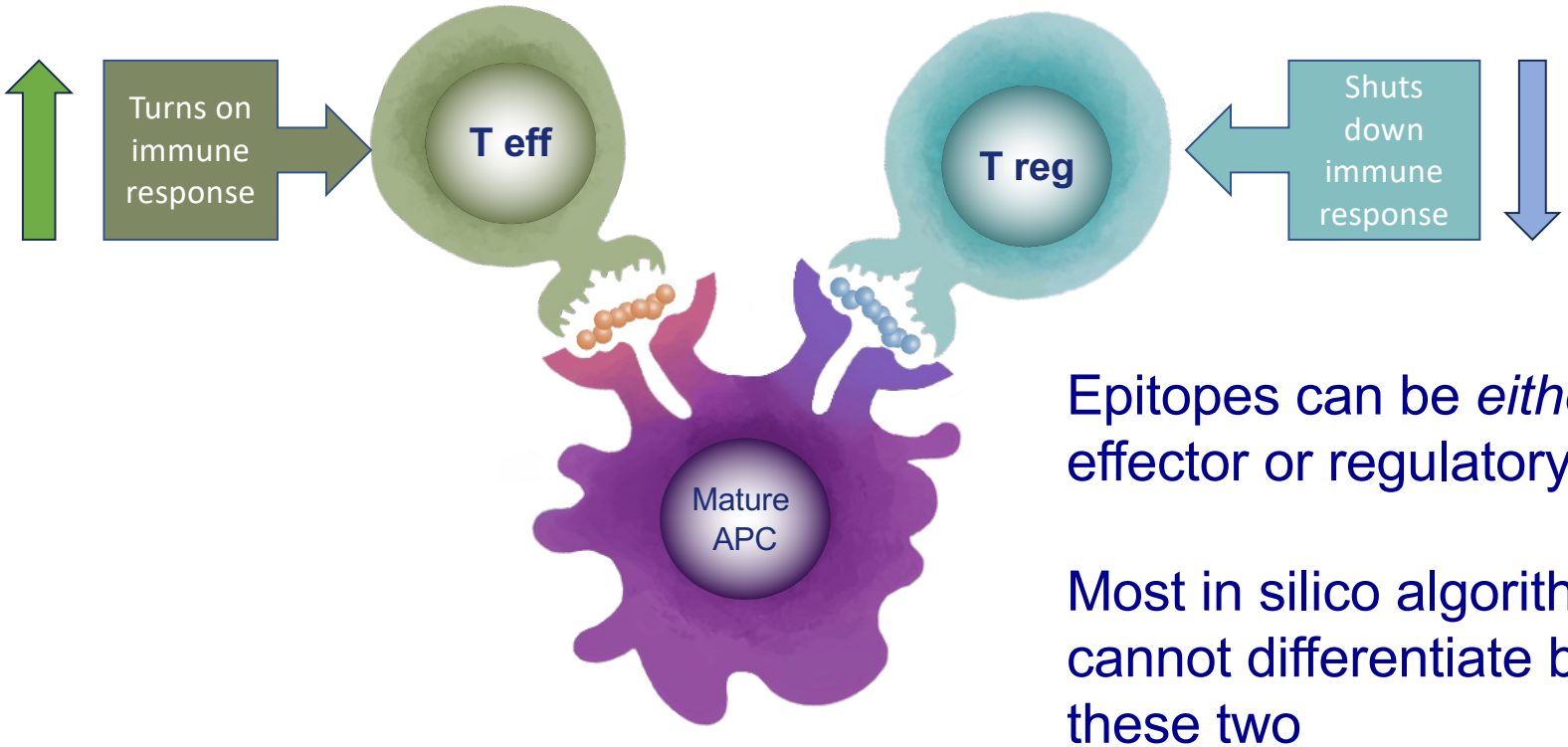
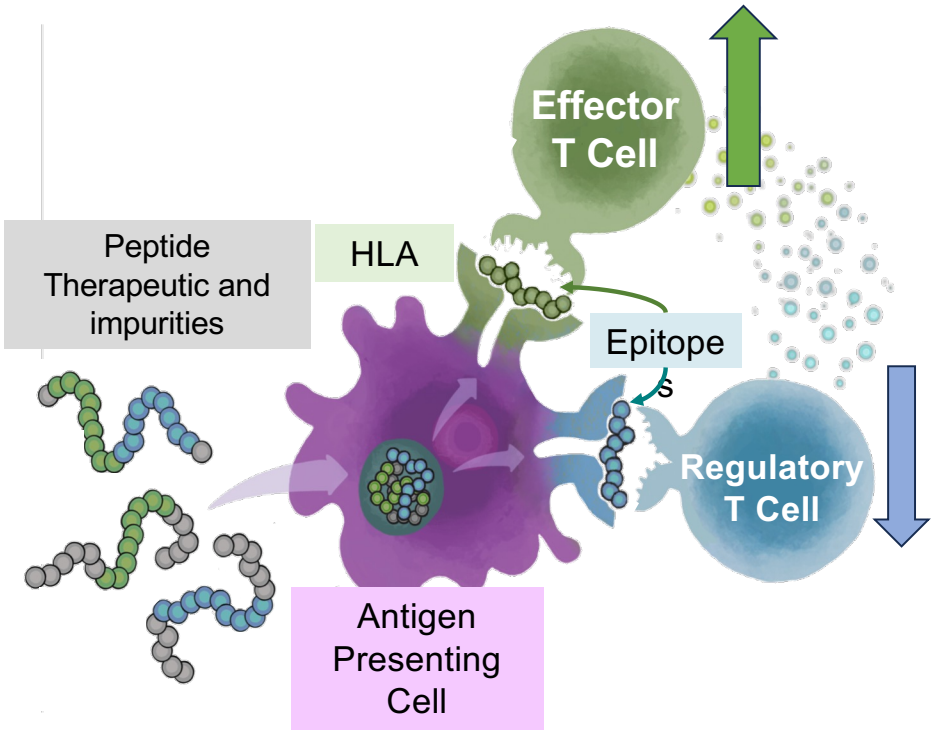


Epitopes can be *either* effector or regulatory

Most in silico algorithms cannot differentiate between these two

Depending on whether Treg or Teffector are engaged Immune response may be repressed or activated



Analyze each peptide and its impurity
For interaction at both faces of the T cell epitope

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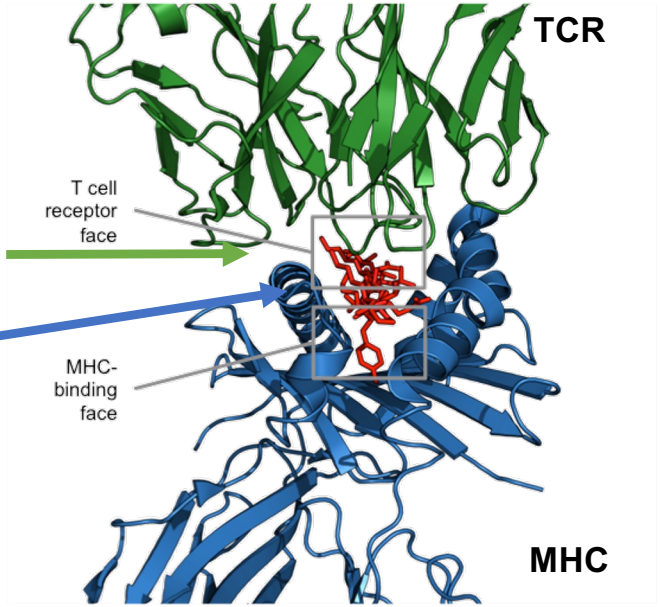
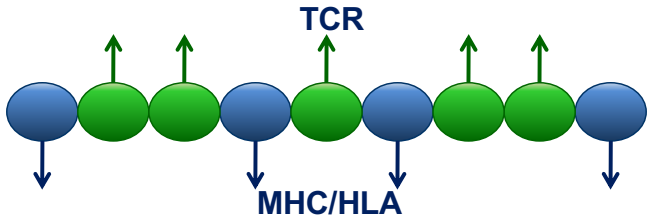
The TCR-interacting face: Epitope

The MHC-binding face: Agretope

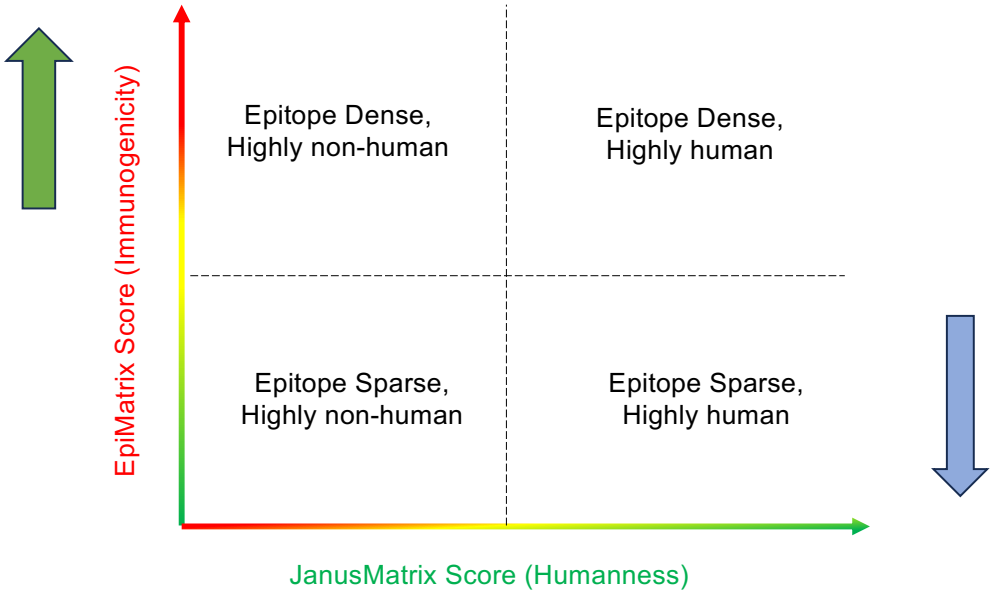


JanusMatrix
EpiMatrix

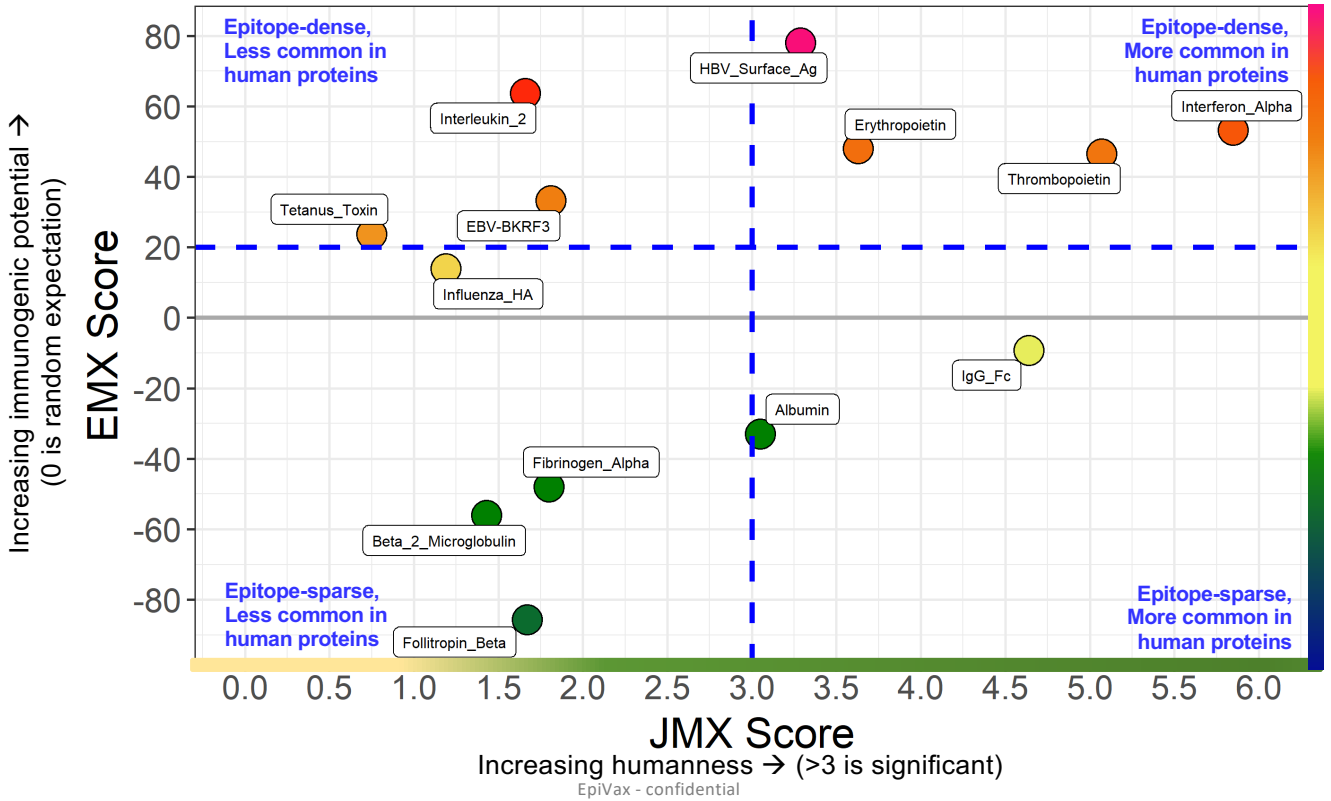


Evaluation of Immunogenicity: Immunogenicity vs Humanness

- Impurities that are predicted to be immunogenic in silico have **high EpiMatrix scores** and **low JanusMatrix scores**.
- Based on these two parameters, impurities can be divided into **four quadrants:**



How to read: Immunogenicity Quadrant Plot
Standards: biologics and vaccine antigens



Putting it all together:
Application of Quadrant Plots to Generic Drugs / Impurities
Original API may have low risk but impurities move higher

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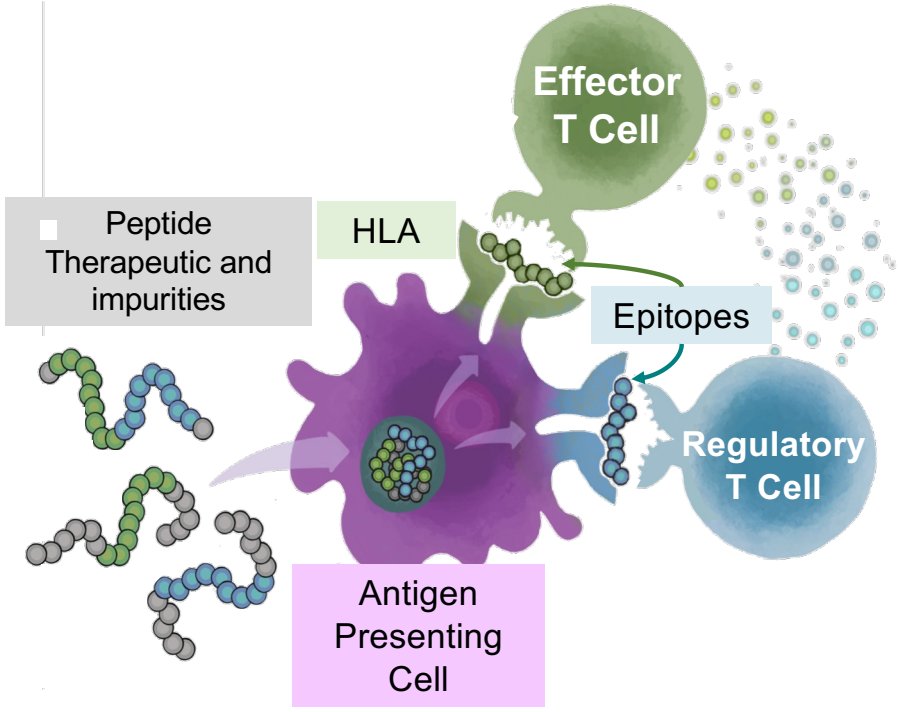
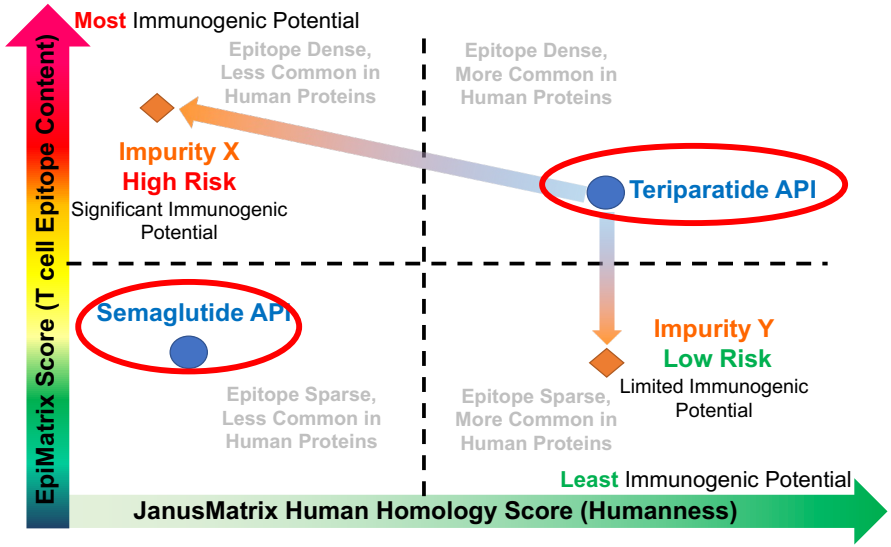
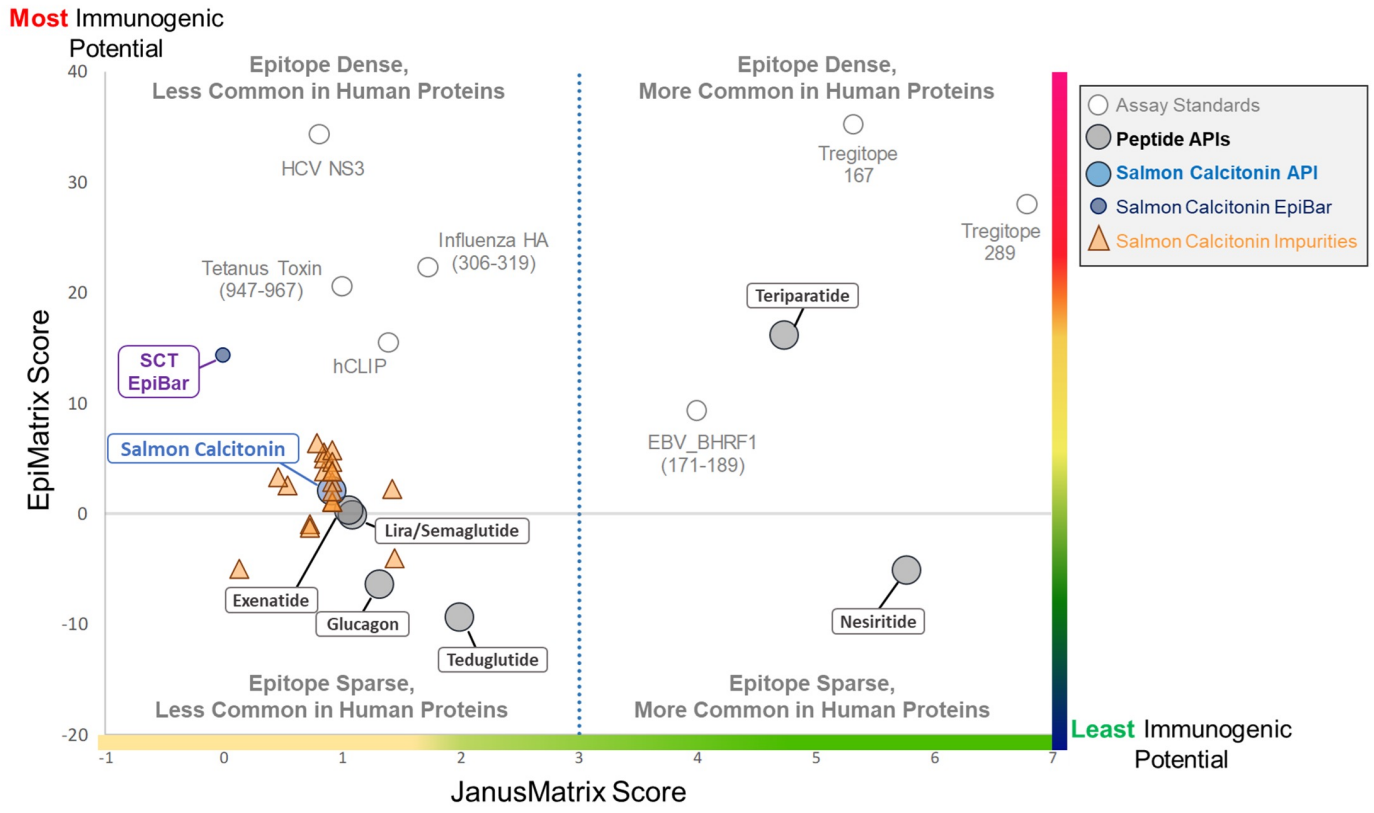


EpiMatrix
predicts "what the HLA will present"
identifies putative T cell epitopes

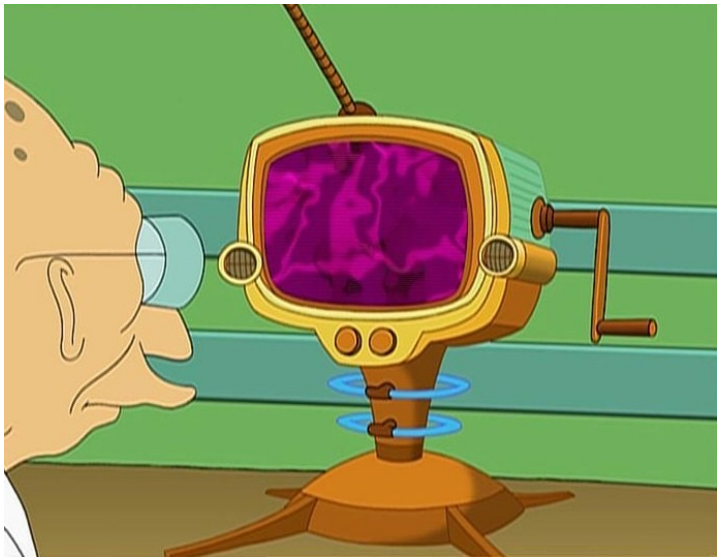
JanusMatrix
predicts "what the T cell will see"
Human (tolerated) vs. Foreign (immunogenic)



ANDA Peptide Drugs and their Impurities



Do we know the immunogenic risk of all possible impurities? The "What If Machine"



Prof. Farnsworth contemplates what could be using the What if Machine (in "Futurama")

Image attributed to *"Futurama,"* 20th Century Fox Broadcasting

EpiVax has a **"What If " Machine** for peptide impurities.

When generic drug impurities are **unknown**, modifications at each amino acid position in the peptide can be performed in silico, their immunogenicity risk predicted and they can be assigned an **impurity risk score**.

The "**What if Machine", performs all possible changes to the natural amino acid sequence of the drug substance** and measures their impact on the epitope content of the peptide.

This includes: **Amino acid modifications, duplications, insertions, deletions and truncations on the epitope content of the peptide drug substance AT EVERY SITE and COMBINATION of sites** in the peptide.

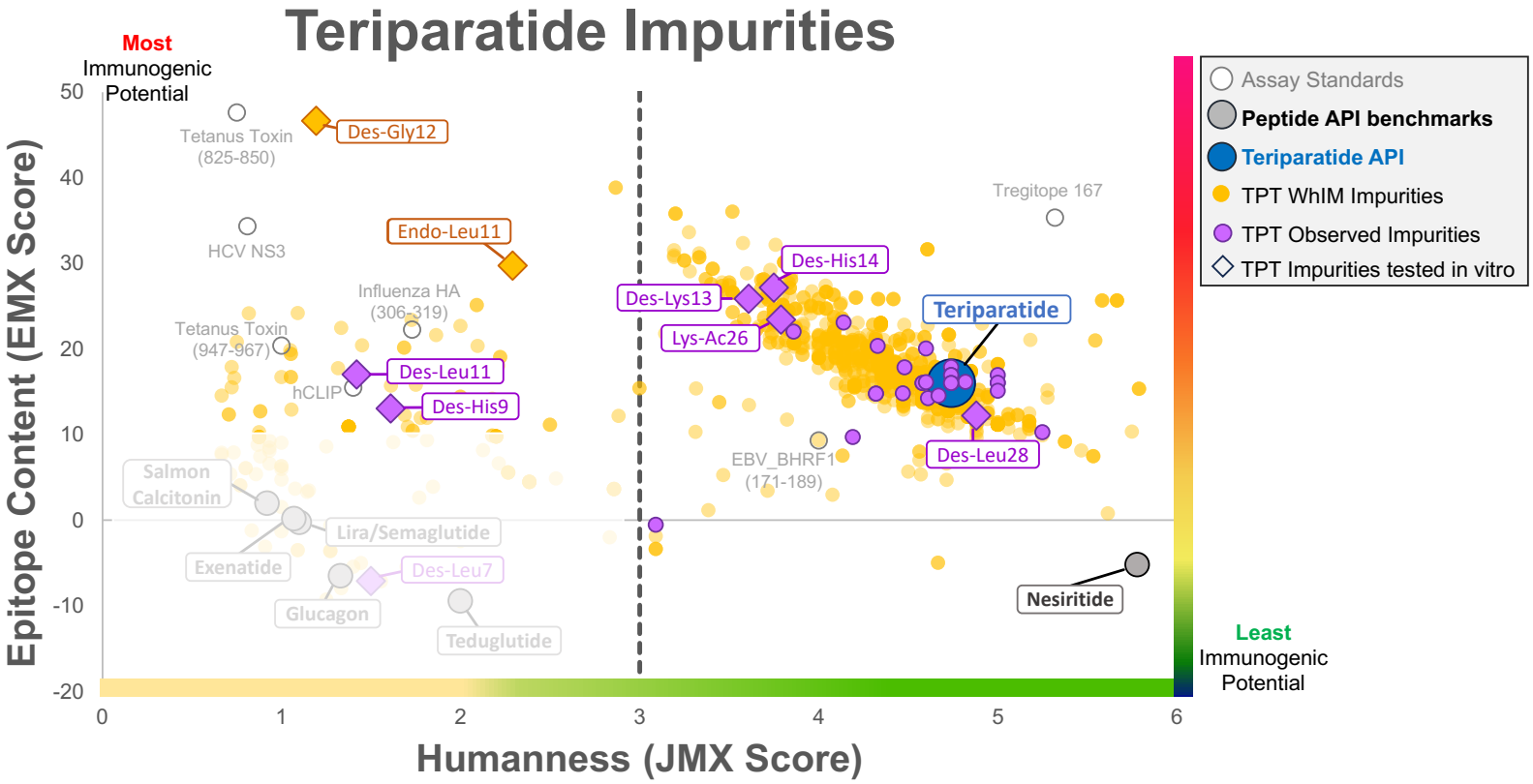
WhIM: The What If Machine – Examples

EpiVax

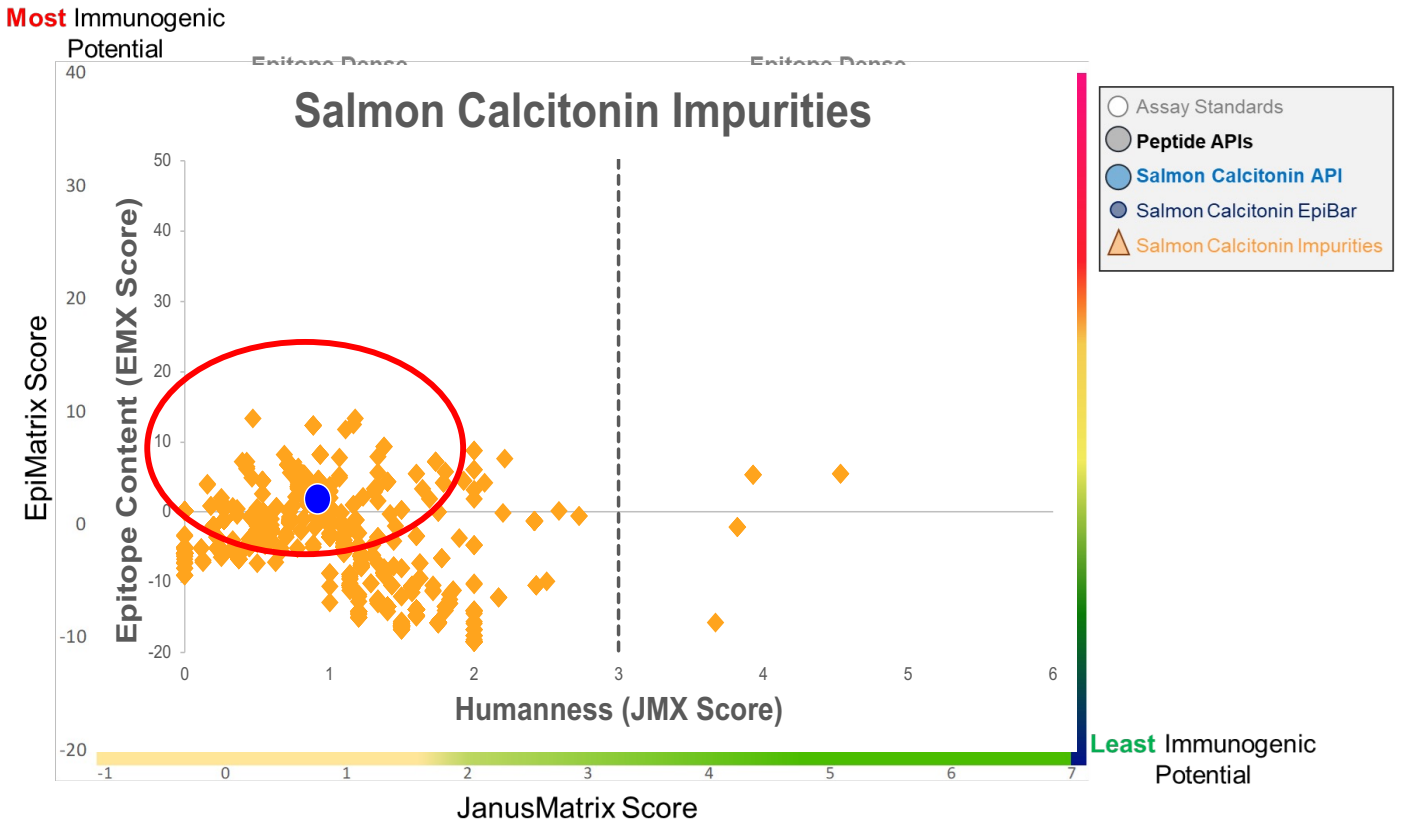
Here we will show WhIM analysis examples for several generic peptide drugs (Salmon Calcitonin, Teriparatide, others) and several Novel peptides (that may be of concern).

High-risk impurities identified by the what-if-machine could be identified flagged and communicated to drug manufactures at early stages in the drug development process, saving resources in the effort to ensure the development of safe and effective novel or generic peptide therapeutics.

Teriparatide – Known and Unknown



Salmon Calcitonin and Impurities by "WHiM"



Readout from WhIM: Teriparatide

EpiVax

TERIPARATIDE

Teriparatide API

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

Promiscuous T cell epitope that has shared TCR-face with epitopes in the human proteome

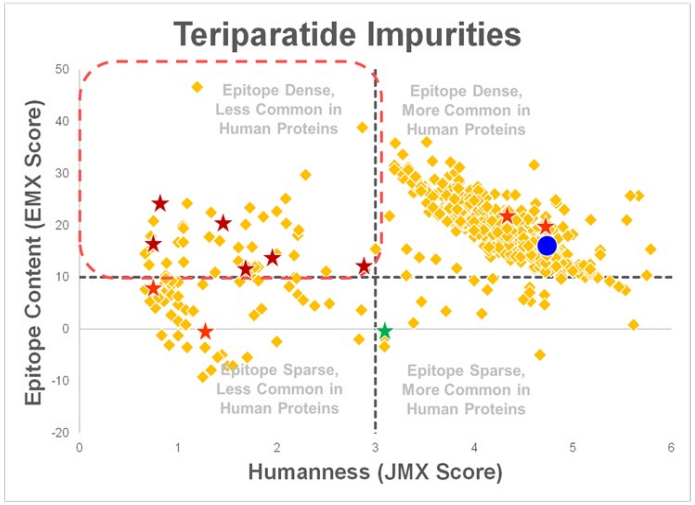
EpiMatrix	16.03	**High** Score indicates significant epitope content
JanusMatrix	4.74	**High** Score indicates high potential for tolerance

WhIM Impurities

Most impurities have significant epitope content (EMX>10) and decreased human cross-conservation relative to the API.

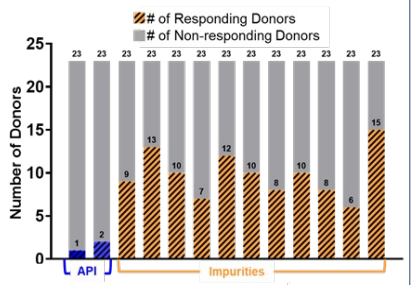
Synthetic teriparatide carries a **high risk** for generating immunogenic impurities, especially modifications within the tolerogenic promiscuous epitope region.

Highest risk zone (EMX >10, JMX < 3)



Confirmatory T Cell Assays

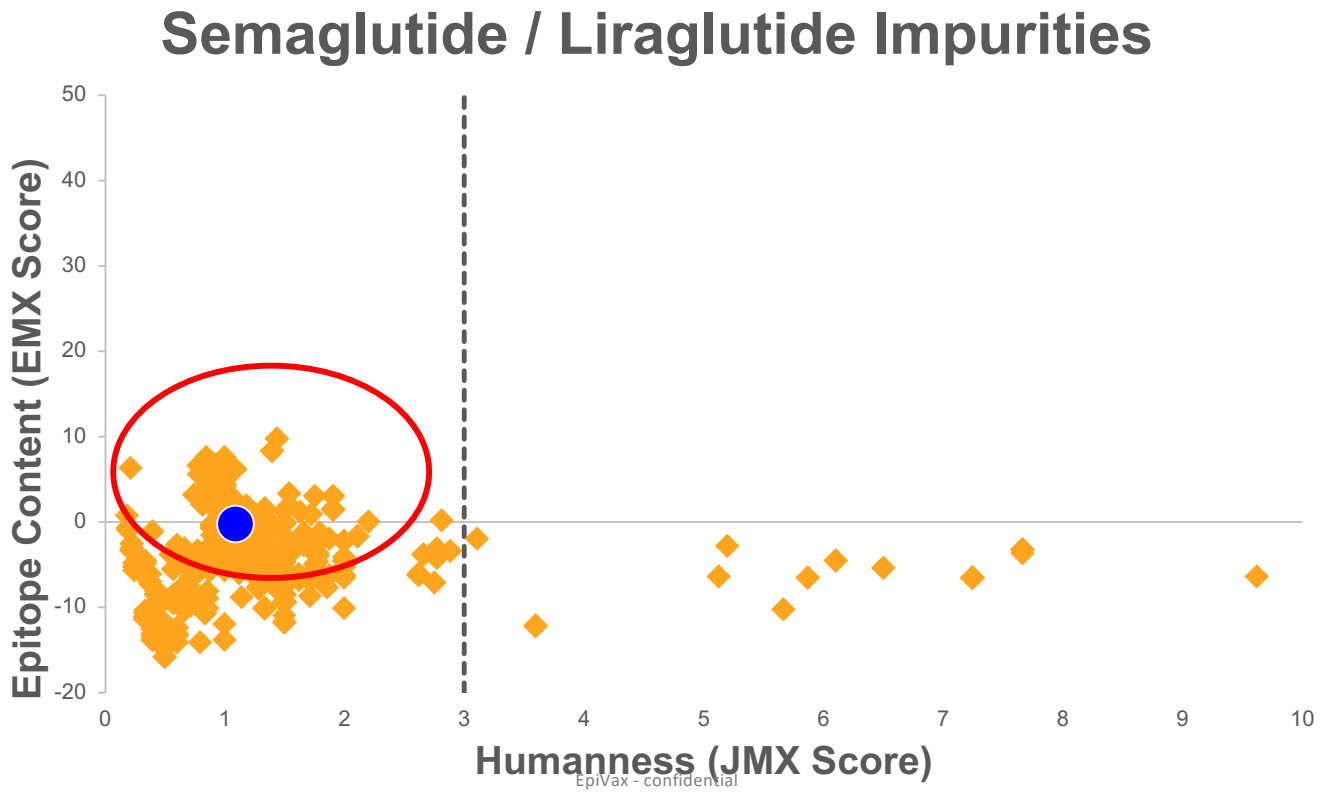
The **teriparatide API** peptide was *tolerated* in most donors, as predicted in silico.



Selected teriparatide impurities (★) impacting the tolerogenic promiscuous epitope region and/or introducing predicted new epitope content were more immunogenic than the API at equal concentrations, as predicted in silico.

WhIM accurately predicted that synthetic teriparatide carries a high risk for generating immunogenic impurities. Due to the presence of a tregitope in the n-terminus, modifications that ablate this feature result in peptide impurities that are significantly more immunogenic when compared to the teriparatide API peptide.

Semaglutide / Liraglutide impurities



Example Summary Readout from WhIM: Semaglutide

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SEMAGLUTIDE

Semaglutide API

HAEGTFTSDVSSYLEGQAAZEFIAWLVRGRG

Indicates promiscuous T cell epitope

A = Alanine substituted for Aib
Z = low affinity placeholder for K(OEG-OEG-γGlu-C18diacid)

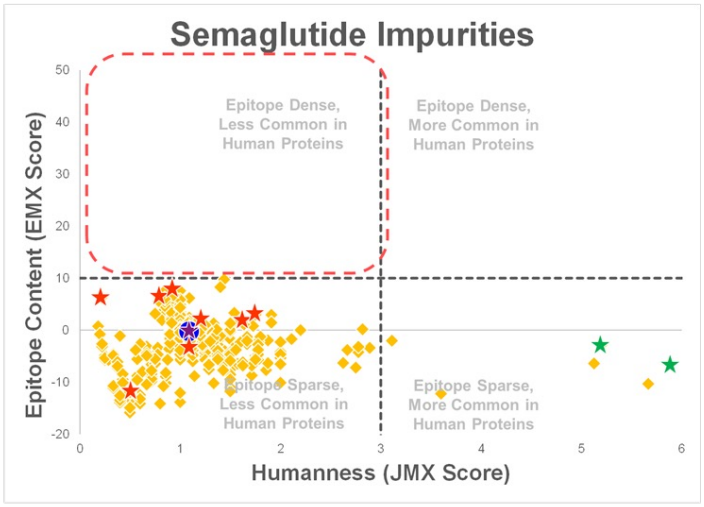
EpiMatrix	-0.25	**Neutral** Score indicates average epitope content
JanusMatrix	1.10	**Low** Score indicates low potential for tolerance

WhIM Impurities

Most impurities have decreased epitope content relative to the API. No impurities fall in the high-risk zone.

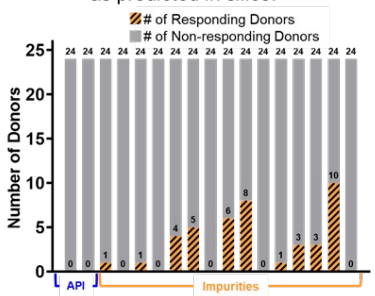
Synthetic semaglutide carries a **low risk** for generating immunogenic impurities.

Highest risk zone (EMX >10, JMX < 3)



Confirmatory T Cell Assays

The **semaglutide API** peptide was *non-immunogenic* in all donors tested, as predicted in silico.



Selected semaglutide **impurities** (★) with a range of predicted immunogenicity were equally non-immunogenic or had increased immunogenicity relative to the API at equal concentrations, as predicted in silico.

WhIM accurately predicted that synthetic semaglutide has a low risk for generating immunogenic impurities

WhIM: The What If Machine – Examples

EpiVax

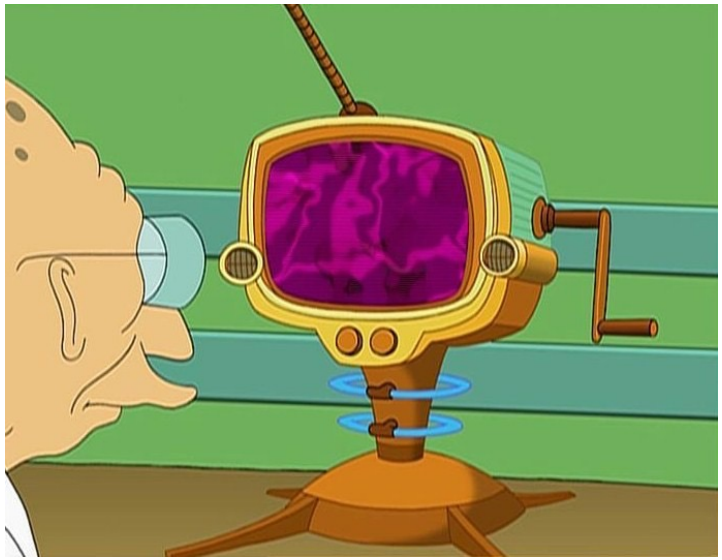
Here we will show WhIM analysis examples for several generic peptide drugs (Salmon Calcitonin, Teriparatide, others) and several Novel peptides (that may be of concern).

High-risk impurities identified by the what-if-machine could be identified flagged and communicated to drug manufactures at early stages in the drug development process, saving resources in the effort to ensure the development of safe and effective novel or generic peptide therapeutics.

The algorithm could be used by regulators (to assess novel impurities listed by manufacturers) or by sponsors, who wish to identify impurities that they should be careful to exclude in the synthesis and purification process due to their potential for immunogenicity.

It is recommended that WhIM be used in conjunction with in vitro HLA binding and T cell assays, which serve to validate the predicted immunogenic sequences if they are in fact identified in the drug product, during the course of generic drug development.

Potential synergies for reducing risk:

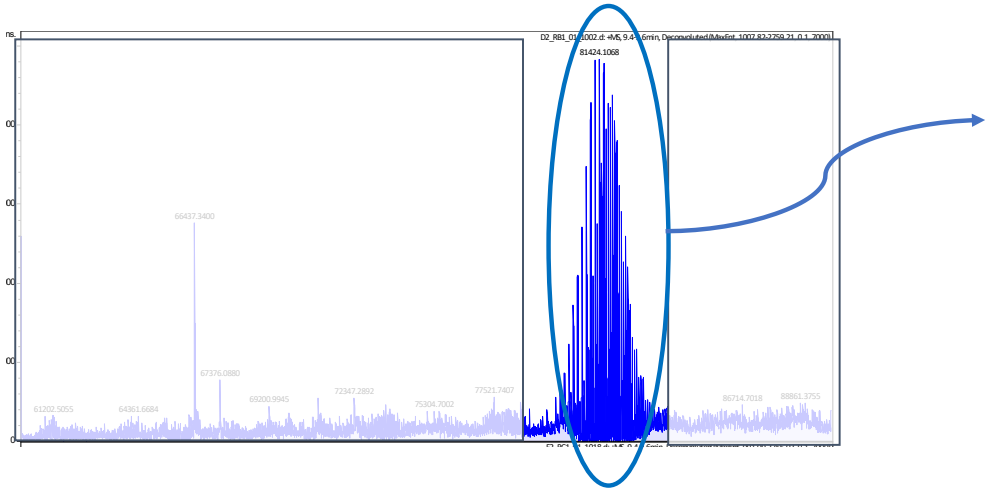


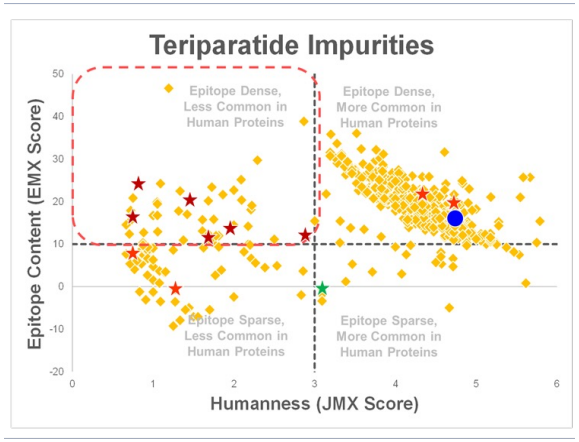
Prof. Farnsworth contemplates what could be using the What if Machine (in “Futurama”)

The **What-if Machine (WhIM)** is an algorithm that, for a given input peptide sequence, **models** (in silico) nearly all **impurities** that may occur during peptide manufacturing and storage.

Use with LC/MS? Combine knowledge of Co-eluting impurities with in silico risk assessment to identify risk.

Match LCMS with WhIM in ANDA process?



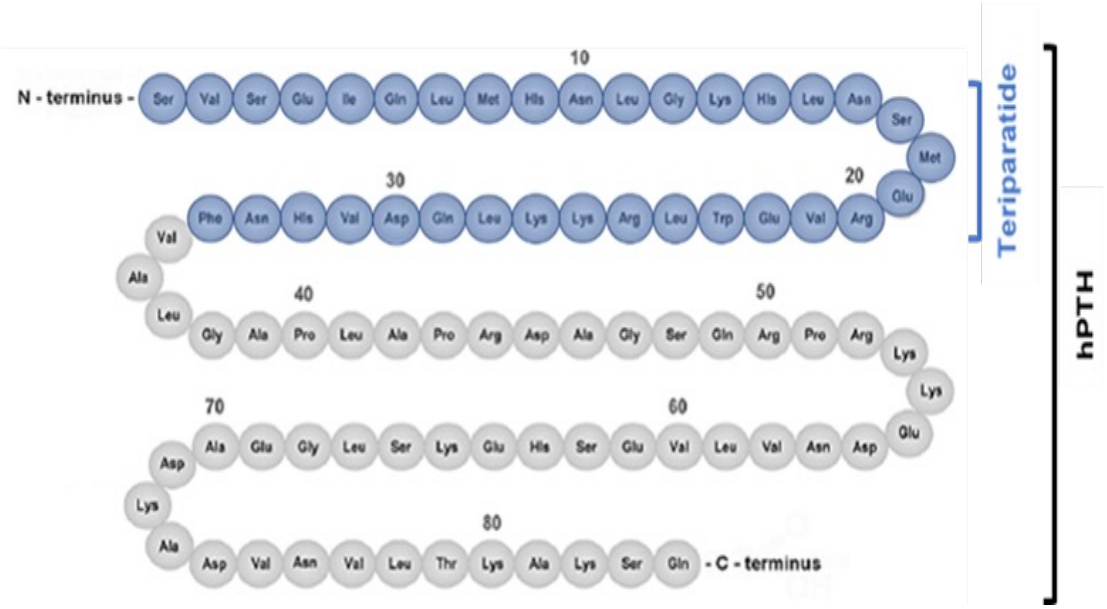


Outline

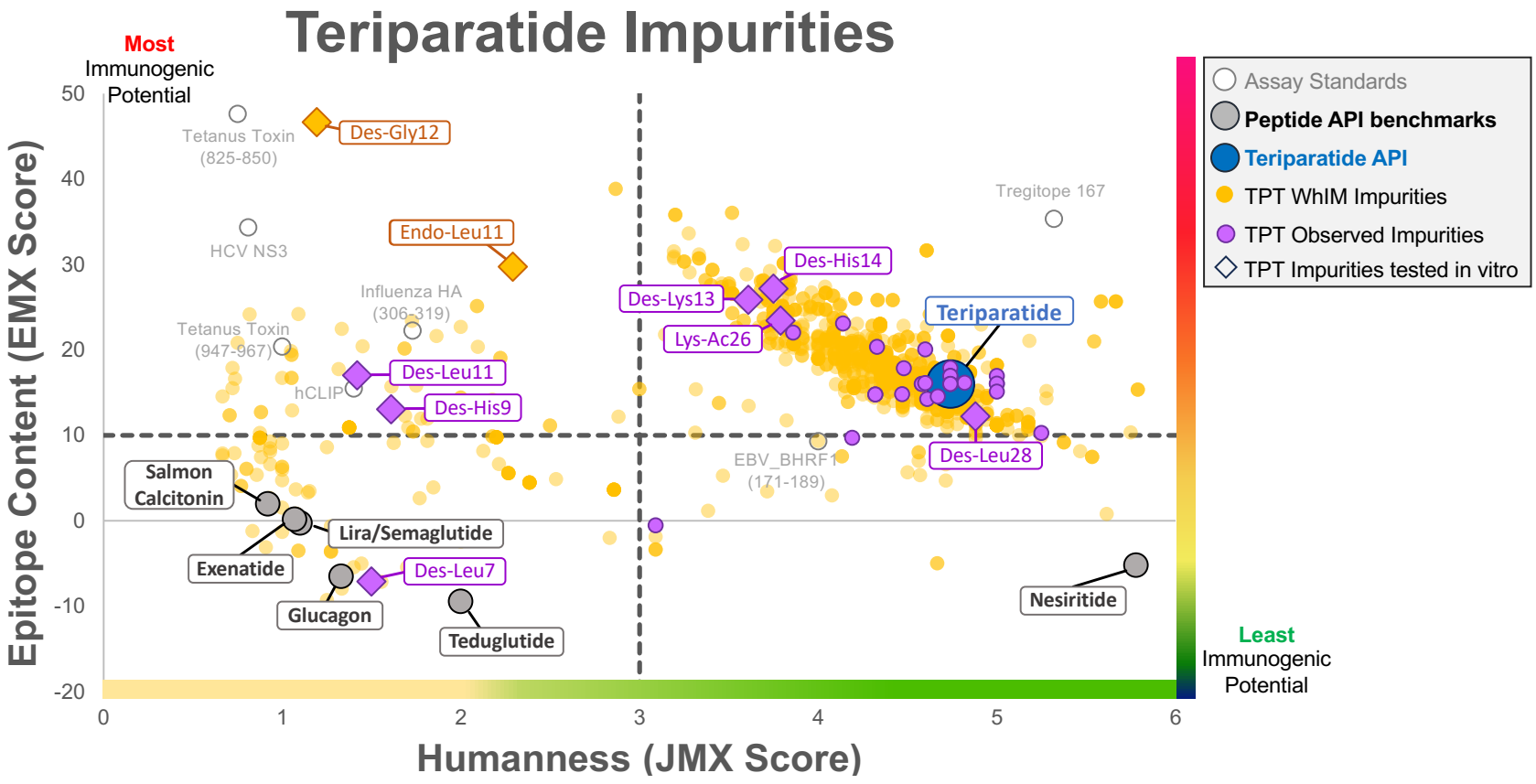
- Immuno informatics Basics
- Orthogonal Approaches to Immunogenicity Risk Assessment
  - Identifying T cell Epitopes in synthetic peptides and impurities
  - In Silico Analysis
  - In Vitro Risk Assessment
- Case Study: Teriparatide
- Prospective Identification of Synthetic Peptide Impurities- The What If Machine

Case Study: Teriparatide

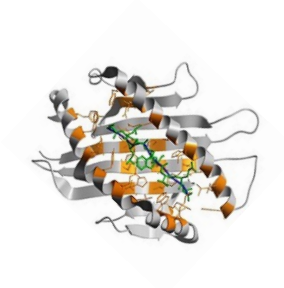
- Teriparatide is a generic drug based off the reference drug product Forteo®
- The teriparatide API peptide is derived from, 34 amino acid of human parathyroid hormone (PTH) (the biologically active region of the hormone)
- PTH is the primary regulator of calcium and phosphate metabolism in the bone and kidney
- The drug is approved by the FDA for the treatment of Osteoporosis in men and women who are at a high risk for bone fracture
- In clinical studies, 2.8% of treated patients develop anti-Teriparatide antibodies after 12 months of treatment



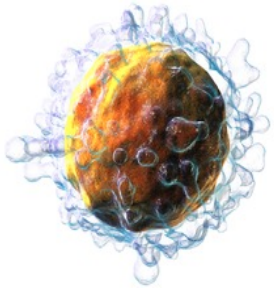
WhiM (What if?) Plot of Impurities for Teriparatide



Following Assessment …Validation -



In Vitro Class II HLA Binding Assays: Class II HLA binding assays may be used to measure the relative binding potential of putative epitopes to multiple HLA alleles. EpiVax employs an adapted competition-based HLA binding assay that utilizes highly-purified Class II HLA molecules of “supertype” alleles. Non-linear regression analysis is performed to produce a curve from which an IC50 value is calculated and used to assess binding strength. This assay format is superior in sensitivity and specificity compared to cell-based binding assay formats.



Naïve Donor T Cell Assay – In Vitro Immunogenicity Protocol (IVIP): EpiVax has adapted an in vitro assay to test the immunogenicity of novel vaccines and therapeutics with human lymphocytes. This assay utilizes blood from HLA-typed healthy donors in order to closely mimic a natural human immune response.

In this assay naïve PBMCs are cultured with the test article and relevant controls. In parallel, PBMCs from the same donor are cultured without test peptide. After 14 days, the cells are stimulated, as a challenge or first exposure, with the appropriate test article or control. The resulting immunoinflammatory and/or immunosuppressive response is measured via Fluorospot assay.

Review of Teriparatide: In silico "EpiBar" is highly conserved with prevalent human protein

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Potential Treg epitope*

Teriparatide:

- EMX Score: **16.03**-***elevated***
- JMX Score: **4.74**- ***potential for tolerance***
- Total Epitope Count: **19**
- 8 hit EpiBar in frame 5
- *Significant* hits for each DRB1 Supertype allele except for DRB1*0901

From this analysis, ***we expect that Teriparatide will have high epitope content*** due to the elevated EpiMatrix score, but ***low immunogenicity due*** to the high JanusMatrix Score

EpiMatrix Detail Report

File: FDA_YR2_TERIPARATIDE Sequence: 00_TERIPARATIDE_RLD : 1

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*0901 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
1	SVSEIQLMH	9	0.29	0.21	0.28	0.57	-0.15	-0.27	-0.16	0.39	0.21	-0.87	0
2	VSEIQLMHN	10	-0.01	-0.37	-0.41	-0.04	-0.65	0.22	0.10	0.82	-0.99	1.11	0
3	SEIQLMHNL	11	-0.06	-0.02	-0.24	-0.41	-0.14	-1.10	-0.83	-0.60	0.52	-0.67	0
4	EIQLMHNLG	12	-0.01	1.00	0.83	1.15	0.28	1.77	0.72	1.78	0.27	1.31	2
5	IQLMHNLGK	13	-0.06	2.47	1.71	2.88	1.67	2.01	1.62	2.89	1.69	2.42	8
6	QLMHNLGKH	14	-0.91	-1.16	-0.46	-0.44	0.20	0.37	0.12	0.01	-0.02	-0.29	0
7	LMHNLGKHL	15	-0.1	2.27	1.06	1.26	2.17	1.17	1.44	1.18	1.26	1.41	2
8	MHNLGKHLN	16	-0.91	1.41	1.26	0.84	0.64	1.84	0.95	1.93	1.49	1.21	2
9	HNLGKHLNS	17	-1.21	0.38	1.07	1.11	-0.04	0.55	-0.10	1.17	0.75	1.45	0
10	NLGKHLNSM	18	-0.64	-0.85	0.93	-1.12	0.03	0.21	0.35	0.28	0.59	-0.24	0
11	LGKHLNSME	19	-0.64	0.06	0.67	0.66	1.09	0.71	0.12	-0.32	2.08	0.30	1
12	GKHLNSMER	20	-1.57	1.00	0.78	1.05	0.33	1.38	0.36	1.06	0.06	1.30	0
13	KHLNSMERV	21	-1.06	0.28	0.34	0.16	0.47	-0.05	0.00	0.25	-0.12	-0.34	0
14	HLNSMERVE	22	-1.01	-1.07	0.26	-1.12	-0.23	-0.12	0.26	-0.13	-0.53	-1.38	0
15	LNSMERVEW	23	-0.76	1.38	1.33	0.20	1.54	0.91	0.80	1.09	1.16	0.91	0
16	NSMERVEWL	24	-0.76	0.35	-0.03	0.31	0.41	-1.17	-0.73	-0.61	-0.70	-1.75	0
17	SMERVEWLR	25	-0.87	-1.07	-0.90	-2.16	-0.92	-0.79	-1.56	-0.55	-0.36	-0.58	0
18	MERVEWLRK	26	-1.21	0.00	0.13	0.68	0.90	-0.03	-0.43	0.71	0.49	1.27	0
19	ERVEWLRKK	27	-1.86	-0.55	-0.29	-0.25	-1.04	-0.77	-0.95	0.55	-0.96	-1.27	0
20	RVEWLRKKL	28	-1.04	-0.05	0.10	-0.47	0.98	-0.22	-0.05	0.23	1.30	0.67	0
21	VEWLRKKLQ	29	-0.93	1.23	1.09	0.96	0.86	2.34	0.23	2.51	1.51	1.38	2
22	EWLRKKLQD	30	-1.79	-0.64	-0.68	-1.47	-0.92	1.47	-0.88	0.09	0.54	-0.07	0
23	WLRKKLQDV	31	-0.93	0.71	1.03	0.16	1.65	2.04	0.88	1.42	0.27	0.48	2
24	LRKKLQDVH	32	-1.19	0.19	0.39	-0.25	-0.14	1.05	0.40	0.61	0.32	-1.21	0
25	RKKLQDVHN	33	-2	0.29	-0.02	0.82	-0.04	0.62	-0.44	-0.07	0.20	1.15	0
26	KKLQDVHNF	34	-1.19	0.19	0.46	0.84	0.60	-0.13	-0.10	0.35	1.20	-1.30	0
Summarized Results				**DRB1*0101**	**DRB1*0301**	**DRB1*0401**	**DRB1*0701**	**DRB1*0801**	**DRB1*0901**	**DRB1*1101**	**DRB1*1301**	**DRB1*1501**	**Total**
Maximum Single Z-score				2.47	1.71	2.88	2.17	2.34	1.62	2.89	2.08	2.42	--
Sum of Significant Z-scores				4.74	1.71	2.88	5.49	10.00	0.00	9.11	3.77	2.42	40.12
Count of Significant Z-Scores				2	1	1	3	5	0	4	2	1	19
Total Assessments Performed: 234				**Hydrophobicity: -0.67**		**EpiMatrix Score: 16.03**				**EpiMatrix Score (w/o flanks): 16.03**			
Scores Adjusted for Tregitope:				**--**		**EpiMatrix Score: 16.03**				**EpiMatrix Score (w/o flanks): 16.03**			

N-Term

C-Term

Why is Teriparatide potentially a Treg epitope?
Extensive cross-conservation with self epitopes

EpiVax

Overview of Class II JanusMatrix Results

TERIPARATIDE RLD

Current Database: HUMAN

Protein ID	Protein Description	Start Position	Sequence	Cluster Score	Number Of HUMAN Matches*	Janus HMLGY Score**	DRB1 *0101	DRB1 *0301	DRB1 *0401	DRB1 *0701	DRB1 *0801	DRB1 *1101	DRB1 *1301	DRB1 *1501
TERIPARATIDE		1 - 34	SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF	18.71	22	4.74	DB Ver: June 05, 2016 | EpiMatrix Ver: 1.2 | JMX Hit Threshold: 1.64							
							DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501
		1	SVSEIQLMH		0		0.21	0.28	0.57	-0.15	-0.27	0.39	0.21	-0.87
		2	VSEIQLMHN		0		-0.37	-0.41	-0.04	-0.65	0.22	0.82	-0.99	1.11
		3	SEIQLMHNL		0		-0.02	-0.24	-0.41	-0.14	-1.1	-0.6	0.52	-0.67
		4	EIQLMHNLG		1		1	0.83	1.15	0.28	1.77	1.78	0.27	1.31
sp|P01270|PTHY_HUMAN	Parathyroid hormone	35	EIQLMHNLG				1	0.83	1.15	0.28	1.77	1.78	0.27	1.31
		5	IQLMHNLGK		12		2.47	1.71	2.88	1.67	2.01	2.89	1.69	2.42
sp|P01270|PTHY_HUMAN	Parathyroid hormone	36	IQLMHNLGK				2.47	1.71	2.88	1.67	2.01	2.89	1.69	2.42
sp|P07437|TBB5_HUMAN	Tubulin beta chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|P04350|TBB4A_HUMAN	Tubulin beta-4A chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|A6NNZ2|TBB8L_HUMAN	Tubulin beta-8 chain-like protein L...	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|Q9BVA1|TBB2B_HUMAN	Tubulin beta-2B chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|Q3ZCM7|TBB8_HUMAN	Tubulin beta-8 chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|P68371|TBB4B_HUMAN	Tubulin beta-4B chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|Q13509|TBB3_HUMAN	Tubulin beta-3 chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|Q13885|TBB2A_HUMAN	Tubulin beta-2A chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|Q9BUF5|TBB6_HUMAN	Tubulin beta-6 chain	133	FQLTHSLGG				2.47	1.48	2.72	2.22	2.52	1.96	2.42	2.7
sp|Q9H0H0|INT2_HUMAN	Integrator complex subunit 2	103	QQLRHKLGG				0.39	0.22	-0.19	-0.9	1.87	1.45	1.42	0.8
sp|P14616|INSRR_HUMAN	Insulin receptor-related protein	366	PQLQHSLGL				1.62	0.93	1.41	1.64	0.71	1.13	1.79	1.74
		6	QLMHNLGKH		0		-1.16	-0.46	-0.44	0.2	0.37	0.01	-0.02	-0.29
		7	LMHNLGKHL		1		2.27	1.06	1.26	2.17	1.17	1.18	1.26	1.41
sp|P01270|PTHY_HUMAN	Parathyroid hormone	38	LMHNLGKHL				2.27	1.06	1.26	2.17	1.17	1.18	1.26	1.41
		8	MHNLGKHLN		1		1.41	1.26	0.84	0.64	1.84	1.93	1.49	1.21

Teriparatide "EpiBar" has promiscuous binding

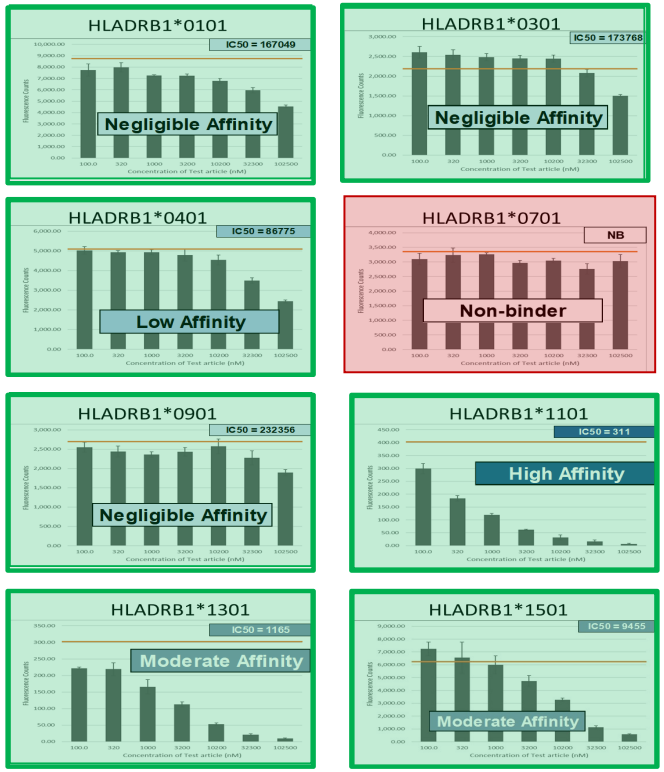
Class II HLA Binding to multiple HLA DR alleles as predicted

EpiVax

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*0901 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
1	SVSEIQLMH	9	0.29	0.21	0.28	0.37	0.15	-0.27	-0.16	0.39	0.21	-0.87	0
2	VSEIQLMHN	10	-0.01	-0.37	-0.41	-0.04	-0.65	0.22	0.10	0.82	-0.99	1.11	0
3	SEIQLMHNL	11	-0.06	-0.02	-0.24	-0.41	-0.14	-1.10	-0.83	-0.60	0.52	-0.67	0
4*	EIQLMHNLG	12	-0.01	1.00	0.83	1.15	0.28	1.77	0.72	1.78	0.27	1.31	2
5*	IQLMHNLGK	13	-0.06	2.47	1.71	2.88	1.67	2.01	1.62	2.89	1.69	2.42	8
6	QLMHNLGKH	14	-0.91	-1.16	-0.46	-0.44	0.20	0.37	0.12	0.01	-0.02	-0.29	0
7*	LMHNLGKHL	15	-0.1	2.27	1.06	1.26	2.17	1.17	1.44	1.18	1.26	1.41	2
8*	MHNLGKHLN	16	-0.91	1.41	1.26	0.84	0.64	1.84	0.95	1.93	1.49	1.21	2
9	HNLGKHLNS	17	-1.21	0.38	1.07	1.11	-0.04	0.55	-0.10	1.17	0.75	1.45	0
10	NLGKHLNSM	18	-0.64	-0.85	0.93	-1.12	0.03	0.21	0.35	0.28	0.59	-0.24	0
11*	LGKHLNSME	19	-0.64	0.06	0.67	0.66	1.09	0.71	0.12	-0.32	2.08	0.30	1
12	GKHLNSMER	20	-1.57	1.00	0.78	1.05	0.33	1.38	0.36	1.06	0.06	1.30	0
13	KHLNSMERV	21	-1.06	0.28	0.34	0.16	0.47	-0.05	0.00	0.25	-0.12	-0.34	0
14	HLNSMERVE	22	-1.01	-1.07	0.26	-1.12	-0.23	-0.12	0.26	-0.13	-0.53	-1.38	0
15	LNSMERVEW	23	-0.76	1.38	1.33	0.20	1.54	0.91	0.80	1.09	1.16	0.91	0
16	NSMERVEWL	24	-0.76	0.35	-0.03	0.31	0.41	-1.17	-0.73	-0.61	-0.70	-1.75	0
17	SMERVEWLR	25	-0.87	-1.07	-0.90	-2.16	-0.92	-0.79	-1.56	-0.55	-0.36	-0.58	0
18	MERVEWLRK	26	-1.21	0.00	0.13	0.68	0.90	-0.03	-0.43	0.71	0.49	1.27	0
19	ERVEWLRKK	27	-1.86	-0.55	-0.29	-0.25	-1.04	-0.77	-0.95	0.55	-0.96	-1.27	0
20	RVEWLRKKL	28	-1.04	-0.05	0.10	-0.47	0.98	-0.22	-0.05	0.23	1.30	0.67	0
21*	VEWLRKKLQ	29	-0.93	1.23	1.09	0.96	0.86	2.34	0.23	2.51	1.51	1.38	2
22	EWLRKKLQD	30	-1.79	-0.64	-0.68	-1.47	-0.92	1.47	-0.88	0.09	0.54	-0.07	0
23*	WLRKKLQDV	31	-0.93	0.71	1.03	0.16	1.65	2.04	0.88	1.42	0.27	0.48	2
24	LRKKLQDVH	32	-1.19	0.19	0.39	-0.25	-0.14	1.05	0.40	0.61	0.32	-1.21	0
25	RKKLQDVHN	33	-2	0.29	-0.02	0.82	-0.04	0.62	-0.44	-0.07	0.20	1.15	0
26	KKLQDVHNF	34	-1.19	0.19	0.46	0.84	0.60	-0.13	-0.10	0.35	1.20	-1.30	0
Summarized Results				**DRB1*0101**	**DRB1*0301**	**DRB1*0401**	**DRB1*0701**	**DRB1*0801**	**DRB1*0901**	**DRB1*1101**	**DRB1*1301**	**DRB1*1501**	**Total**
Maximum Single Z-score				2.47	1.71	2.88	2.17	2.34	1.62	2.89	2.08	2.42	--
Sum of Significant Z-scores				4.74	1.71	2.88	5.49	10.00	0.00	9.11	3.77	2.42	40.12
Count of Significant Z-Scores				2	1	1	3	5	0	4	2	1	19
Total Assessments Performed: 234				**Hydrophobicity: -0.67**		**EpiMatrix Score: 16.03**				**EpiMatrix Score (w/o flanks): 16.03**			
Scores Adjusted for Tregitope:				**--**		**EpiMatrix Score: 16.03**				**EpiMatrix Score (w/o flanks): 16.03**			

HLA Binding

7/8 alleles showed binding affinity.

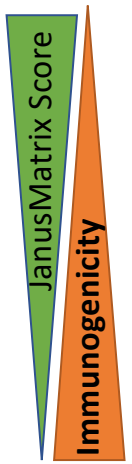


Teriparatide Impurities RESULTS

Loss of "humanness" increases immunogenicity

EpiVax

Test Article	EMX Score	JMX Score	Percent of Responding Donors
Forteo®	***16.03***	***4.74***	***20%***
DES-LEU28	12.23	4.88	**25%**
LYS-AC26	23.44	3.79	**45%**
DES-HIS14	27.16	3.75	**40%**
DES-LYS13	25.85	3.61	**45%**
WhIM_ENDO-LEU11	36.03	3.52	**45%**
DES-HIS9	13.07	1.61	**50%**
DES-LEU7	-7.1	1.50	**45%**
DES-LEU11	17.02	1.42	**40%**
WhIM_DES-GLY12	46.63	1.19	**45%**



When an impurity becomes less human, the immunogenicity increases

Summary thus far:

- Immuno informatics can help assess Immunogenicity Risk
- Consideration of “human-ness” (Tolerance) is important
- In vitro assays for orthogonal evaluation
  - In Vitro HLA binding
  - In Vitro T cell Assays Assessment
- Not discussed here: Appropriate Controls / Innate Immune Responses / Aggregation
- Approach described here is valid for
  - Novel peptides
  - Host Cell proteins
  - Biologics

JUST FOR FUN

EpiVax

- Immuno informatics Basics
- Orthogonal Approaches to Immunogenicity Risk Assessment
  - Identifying T cell Epitopes in synthetic peptides and impurities
  - In Silico Analysis
  - In Vitro Risk Assessment
- Case Study: Teriparatide
- Other Synthetic Peptide Impurities- The What If Machine

Comparison (Control Negative) Bivalirudin

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