Addressing Immunogenicity of Observed and Theoretical Impurities for Peptide Abbreviated New Drug Applications

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The PANDA Platform

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

Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Xiaohui Jiang at 240-402-7964.

- In 2017, the FDA released a draft guidance that requires generic peptide drug manufacturers to provide evidence that synthesis-related impurities found in their drug substance do not increase the immunogenicity of the drug product.
- Peptide drugs can be associated with impurities that result from changes in the sequences due to failures in the manufacturing process leading to deletions, insertions, integration of incorrect amino-acids, side-chain modifications and other modifications.
- We have used both immunoinformatics-driven analysis and in vitro validation assays to perform immunogenicity risk assessment of peptide generics. This combination of in silico and in vitro tools is referred to as the PANDA process can be used to support generic peptide drug equivalency in an ANDA application.

PANDA: Immunogenicity Risk Assessment for Synthetic **P**eptide **A**bbreviated **N**ew **D**rug **A**pplication Using Computational and Analytical Methods

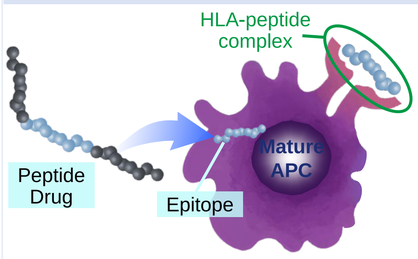
- in silico — Epitope Prediction
- in vitro — HLA Binding
- in vitro — T cell Activity
- ex libris — Literature Review

Statement of Immunogenicity

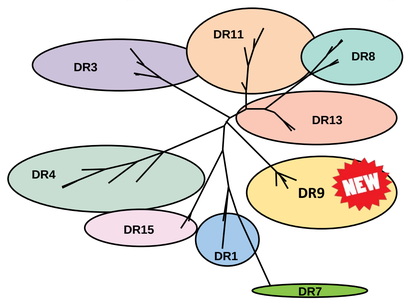
EpiVax's expert opinion on the T cell mediated immune response to RLD vs. synthetic peptide generic

In Silico Evaluation of Immunogenicity

Searching for T Cell Epitopes with EpiMatrix



- EpiMatrix predicts T cell epitopes
- HLA binding is a prerequisite for immunogenicity



- EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"[3] shown above.
- These are representative of >95% of human populations worldwide.[2]

EpiMatrix Detail Report: Teriparatide RLD

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**

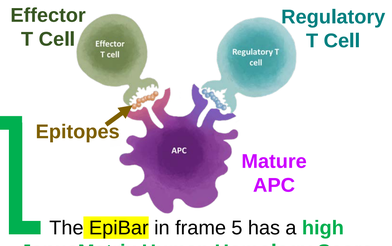
***7 frames contain putative T cell epitopes** (Z-scores ≥ 1.64, medium and dark blue shading)

EpiBar = promiscuous binding motif

Teriparatide has a total of 19 EpiMatrix Hits

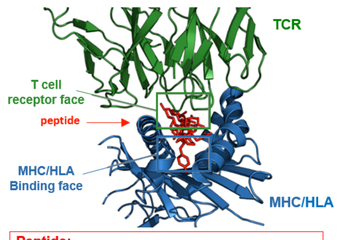
High EpiMatrix Score (scores above 10 indicate significant immunogenic potential)

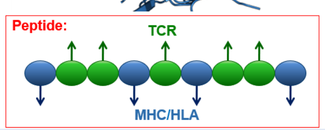
Searching for Human-like epitopes with JanusMatrix



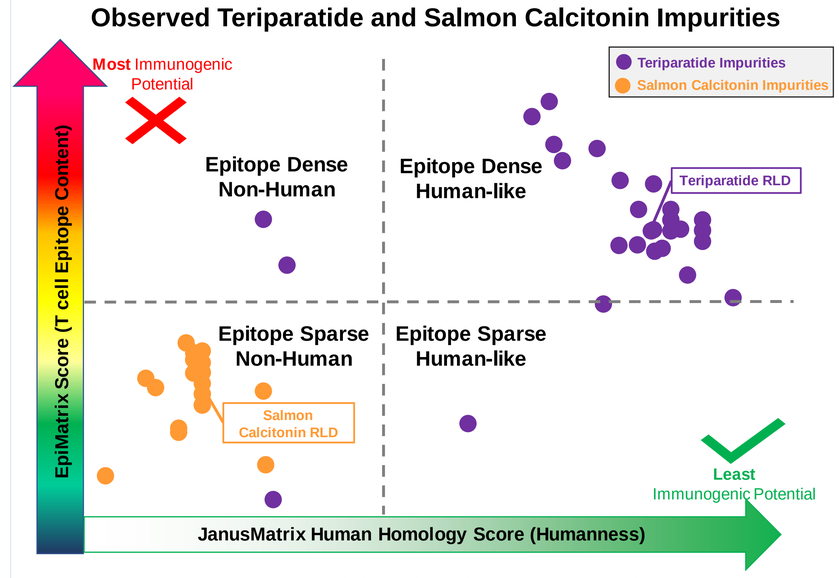
The EpiBar in frame 5 has a **high JanusMatrix Human Homology Score** suggesting it is a potential regulatory epitope (Tregitope) and will be tolerated or even actively tolerogenic*

*confirmed with unpublished in vitro studies





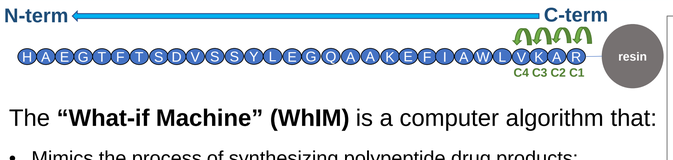
Immunogenicity Quadrant Plot



Salmon Calcitonin and its impurities fall into the Epitope Sparse, Non-human quadrant. Observed immunogenicity to SCT can be attributed to foreign epitopes within the sequence.

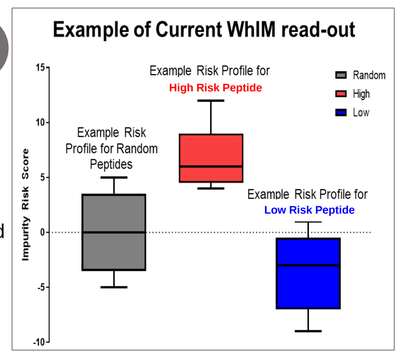
Teriparatide and many impurities fall into the Epitope Dense, Human-Like quadrant. Two observed impurities create non-human epitopes and are predicted to be immunogenic.

Evaluating Risk of (nearly) all possible peptide-related impurities with the What-If-Machine (WhIM)

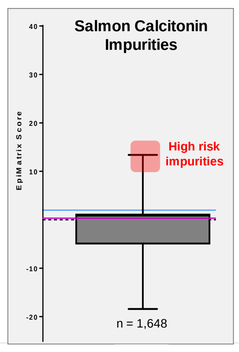


The **"What-if Machine" (WhIM)** is a computer algorithm that:

- Mimics the process of synthesizing polypeptide drug products;
- Records all possible product impurities created through known failures in the synthesis process[4];
- Scores each potential impurity for T cell epitope content (EpiMatrix) and human cross-reactive potential (JanusMatrix);
- Weights each impurity based on an assumed probability of occurrence;
- Summarizes the scores of all potential impurities in order to calculate an impurity risk profile.

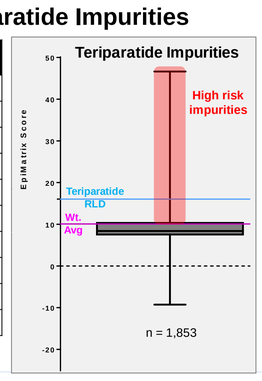


Risk Profile for Salmon Calcitonin & Teriparatide Impurities



Measure	Salmon Calcitonin	Teriparatide
RLD	1.99	16.03
Max	13.39	46.63
Upper Q	1.06	10.33
Median	0.87	8.38
Lower Q	-4.90	7.54
Min	-18.42	-9.26
Weighted Avg	0.32	10.07
Total	1648	1853
High Scoring (Count)	9	488
High Scoring (percent)	0.55%	26.34%

WhIM-generated impurity sequences with EpiMatrix Scores above our standard threshold for immunogenicity (>10)

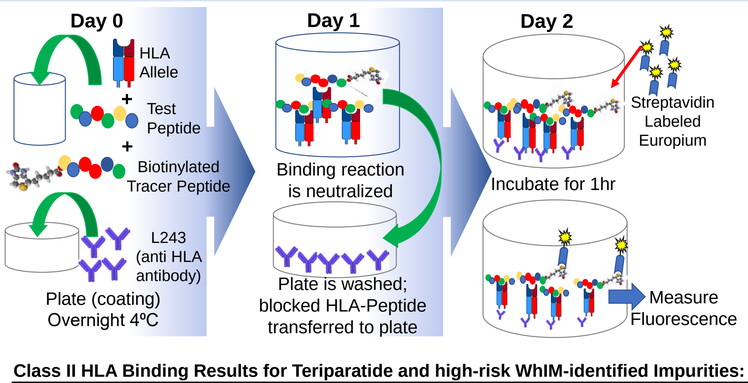


Highest-Scoring WhIM-generated Impurities: Teriparatide

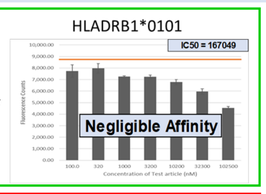
Peptide Sequence	IMPURITY_TYPE	PROBABILITY	EMX SCORE	JMX SCORE	WEIGHT DELTA
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF	*RLD*	*n/a*	*16.03*	*4.74*	*N/A*
SVSEIQLMHNL-KHLNSMERVEWLRKKLQDVHNF	DELETION	1.0E-04	46.63	1.19	1.39%
SVSEIQLMHNLLGKHLNSMERVEWLRKKLQDVHNF	DUPLICATE_AA	5.0E-05	29.72	2.29	-2.76%
SVSEIQLMHNLLGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	2.5E-06	29.72	2.29	-2.76%
SVSEIQLMHNLLGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	5.0E-06	29.72	2.29	-2.76%
SVSEIQLMHNLLGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	2.5E-06	29.72	2.29	-2.76%
SVSEIQLMHNLNGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	2.5E-06	24.21	1.09	-2.78%
SVSEIQLMHNLSGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	5.0E-06	25.13	2.09	-2.12%
SVSEIQLMHNLSGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	5.0E-06	25.13	2.09	-2.12%
SVSEIQLMHILNGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	2.5E-06	23.39	1.73	-2.76%
SVSEIQLMHNLHGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	2.5E-06	24.17	0.82	-3.34%
SVSEIQLMHVNLGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	5.0E-06	22.70	2.00	-2.41%
SVSEIQLMHNVLGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	5.0E-06	22.46	1.33	-2.41%
SVSEIQLMHGLNLGKHLNSMERVEWLRKKLQDVHNF	INSERTION_BETA_AA	5.0E-07	21.64	1.86	-4.15%
SVSEIQLMHNLIGKHLNSMERVEWLRKKLQDVHNF	INSERTION_AA	5.0E-06	20.82	0.75	-2.76%

In Vitro Confirmation Assays

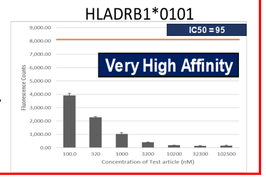
In Vitro Class II HLA Binding Assays



Teriparatide RLD
Binds with Negligible affinity to HLA-DRB1*0101



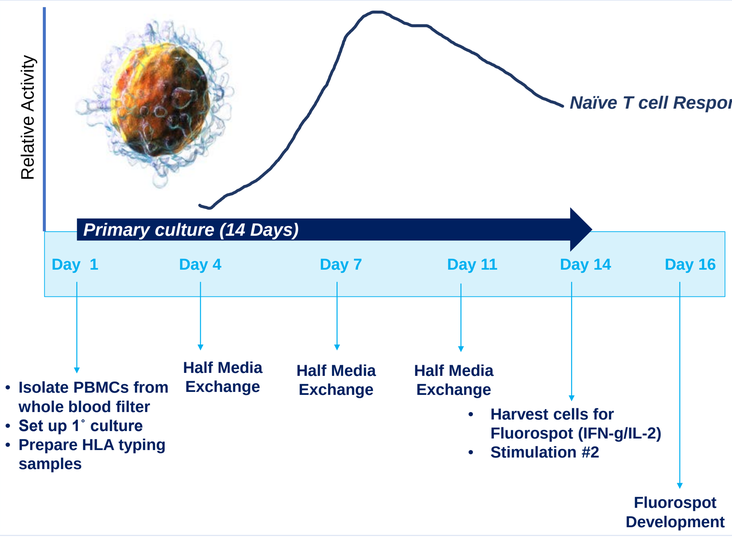
WhIM-identified High-risk Impurity
Binds with Very High affinity to HLA-DRB1*0101

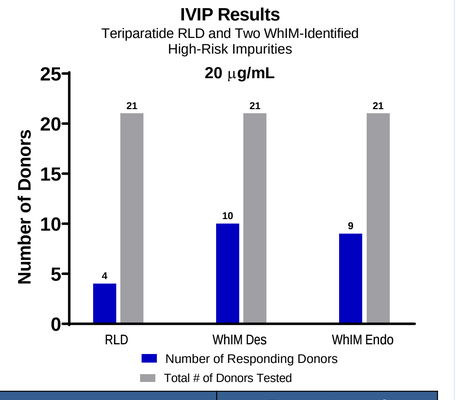


Class II HLA Binding Results for Teriparatide and high-risk WhIM-identified Impurities:

Peptide	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0901	DRB1*1101	DRB1*1301	DRB1*1501
Teripratide_RLD N-term (1-18)	Negligible	Negligible	Low	Non-Binder	Negligible	High	Moderate	Moderate
Teriparatide_RLD C-term (18-34)	Non-Binder	Non-Binder	Non-Binder	Non-Binder	Non-Binder	High	Moderate	Negligible
WhIM_DES-GLY12_Teriparatide (1-21)	Moderate	Low	Moderate	Non-Binder	Non-Binder	High	Moderate	High
WhIM_ENDO-LEU11_Teriparatide (1-21)	Very High	Negligible	High	Negligible	Non-Binder	High	Moderate	Very High

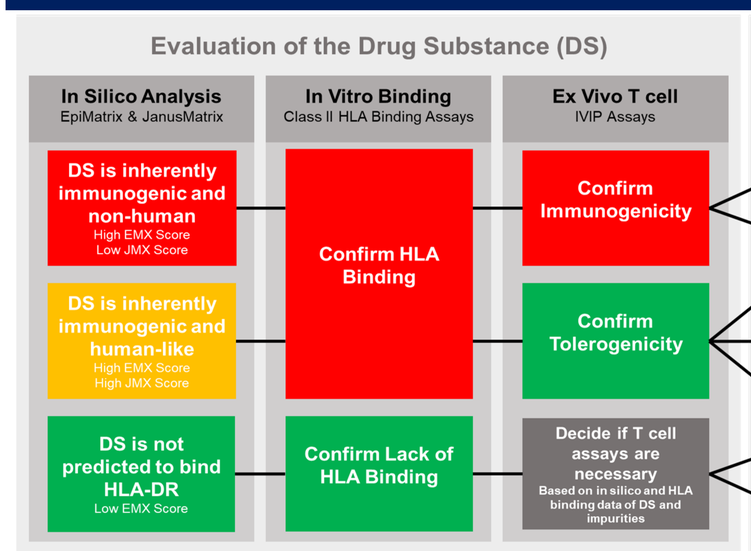
In Vitro T cell Assays – In Vitro Immunization Protocol (IVIP)





Peptide	Percentage of Responding Donors	
	0.2µg/mL	20µg/mL
Teriparatide	n/a	20%
WhIM_DES-GLY12_Teriparatide	10%	50%
WhIM_ENDO_LEU11_Teriparatide	10%	45%

Conclusions



- **It is important to assess the potential immunogenicity of not only peptide drug candidates, but also their synthesis-related impurities in early stages of drug development.**
- **In the recent FDA guidance, peptide drug manufacturers must provide proof that synthesis-related impurities do not increase the immunogenicity of the drug substance.**
- **In silico tools such as EpiMatrix and JanusMatrix can provide a quick and cost-effective method to screen peptides for immunogenicity.**
- **When impurities are unknown, the What-if-Machine can quickly screen all plausible peptide-related impurity sequences and identify potentially immunogenic impurities.**
- **Combining these in silico tools with in vitro HLA binding and T cell assays is referred to as the PANDA process can be used to support generic peptide drug equivalency in an ANDA application or in the immunogenicity screening of novel peptide therapeutics.**

References

1. ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin Guidance for Industry; Draft Guidelines issued by Office of Generic Drugs (OGD) in the Center for Drug Evaluation and Research (CDER) Food and Drug Administration Federal Drug Agency. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/andas-certain-highly-purified-synthetic-peptide-drug-products-refer-listed-drugs-rdna-origin.
2. Lund et al. Definition of Supertypes for HLA Molecules Using Clustering of Specificity Matrices. Immunogenetics. 2004; 55(12):797–810.
3. Southwood et al. Several Common HLA-DR Types Share Largely Overlapping Peptide Binding Repertoires. J Immunol. 1998; 160(7):3363–73.
4. D'Hondt M., Bracke N., Tavernier L. Related impurities in peptide medicines. J. Pharm. Biomed. Anal. 2014;101:2–30

For questions regarding in silico antigen screening and vaccine design, please contact: Katie Porter at 401-272-2123, ext. 115; or at info@epivax.com

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