Promiscuous T Cell Binding Epitopes in Two Osteoporosis Medications Lead to Different Immunogenicity Profiles:

A Tale of Two Peptides

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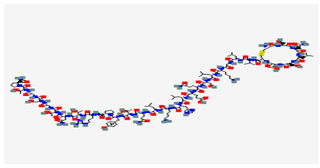
BACKGROUND INFROMATION

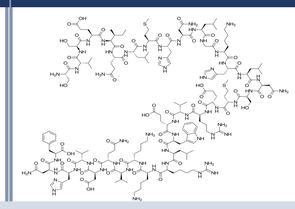
- The peptide drug market has rapidly expanded and is expected to generate in excess of $50 billion for manufacturers. While synthetic peptide synthesis is a cost-effective approach for producing these drugs, regulatory agencies are concerned that impurities resulting from the manufacturing process could introduce an unwanted immune response. Impurities can result from changes in the sequences due to deletions, insertions, substitutions, modifications and other impurities related to the synthetic production.
- Here we provide a case study of two generic peptide drug products, salmon calcitonin and teriparatide, approved for the treatment of post-menopausal osteoporosis. Both peptides contain a promiscuous T cell epitope shown to bind multiple class II HLA DRB1 alleles, while leading to differences in clinical immunogenicity of the peptides themselves along with impurities that arise from the manufacturing process.

Salmon Calcitonin

Salmon Calcitonin (SCT) is an FDA approved treatment for post-menopausal osteoporosis now under development for the generic market. SCT is a foreign peptide sharing only 50% sequence homology with the human homologue. While SCT is therapeutically more potent than human calcitonin, 35-60% of patients develop anti-drug antibodies (ADA), with 45% of those patients developing neutralizing antibodies to SCT impacting the efficacy of the therapeutic.



Teriparatide



Teriparatide (TPT), also used to treat osteoporosis, is derived from the n-terminal 34 amino acids of human parathyroid hormone and as an endogenous peptide is considered to be clinically non-immunogenic. Only 2-4% of patients taking TPT develop anti-teriparatide antibodies.

In silico Analysis

The SCT EpiBar is highly foreign, sharing only 33% sequence homology with the complementary region of human calcitonin. This region is the primary driver of anti-SCT immunogenicity. **Modifications to this region do not impact the immunogenicity of SCT-derived impurities**

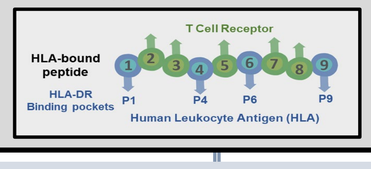
Frame Start	AA Seqeunce	Frame Stop	HLA-DR Supertype Alleles DRB1* 0101	0301	0401	0701	0801	0901	1101	1301	1501	Hits
1	CSNLSTCVL	9										0
2	SNLSTCVLG	10										0
3	NLSTCVLGK	11										0
4	LSTCVLGKL	12										0
5	STCVLGKLS	13										0
6	TCVLGKLSQ	14										0
7	CVLGKLSQE	15										0
8	VLGKLSQEL	16										3
9	LGKLSQELH	17										0
10	GKLSQELHK	18										0
11	KLSQELHKL	19										1
12	LSQELHKLQ	20										1
13	SQELHKLQT	21										0
14	QELHKLQTY	22										0
15	ELHKLQTYP	23										0
16	LHKLQTYPR	24										7
17	HKLQTYPRT	25										0
18	KLQTYPRTN	26										0
19	LQTYPRTNT	27										0
20	QTYPRTNTG	28										0
21	TYPRTNTGS	29										0
22	YPRTNTGSG	30										0
23	PRTNTGSGT	31										0
24	RTNTGSGTP	32										0

JanusMatrix Score: 0.92 ***Highly Foreign***

EpiMatrix identifies T cell epitopes that bind HLA by a well-characterized interaction of amino acid side chains and pockets in the HLA-DR molecule binding groove. Epitopes are predicted for nine supertype alleles that cover >95% of the global population.

EpiBar- a promiscuous T cell epitope predicted to bind 4 or more supertype alleles

JanusMatrix looks for potential human homology by comparing the TCR-facing amino acids with corresponding amino acids in the human proteome.



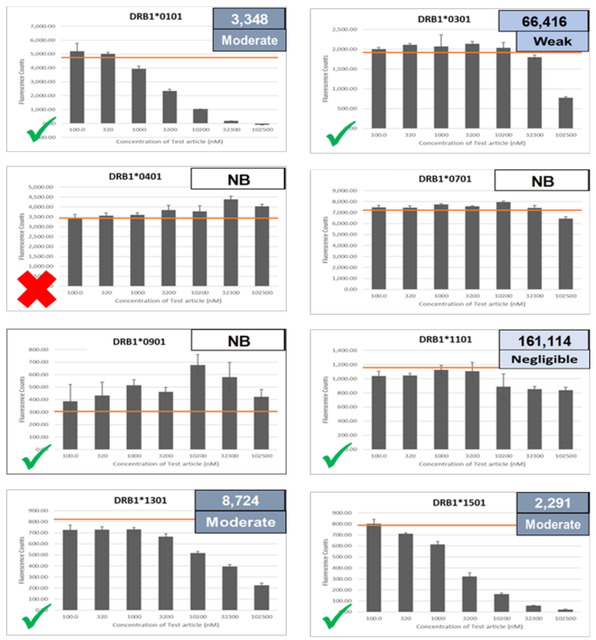
Frame Start	AA Seqeunce	Frame Stop	HLA-DR Supertype Alleles DRB1* 0101	0301	0401	0701	0801	0901	1101	1301	1501	Hits
1	SVSEIQLMH	9										0
2	VSEIQLMHN	10										0
3	SEIQLMHNL	11										0
4	EIQLMHNLG	12										2
5	IQLMHNLGK	13										8
6	QLMHNLGKH	14										0
7	LMHNLGKHL	15										2
8	MHNLGKHLN	16										2
9	HNLGKHLNS	17										0
10	NLGKHLNSM	18										0
11	LGKHLNSME	19										1
12	GKHLNSMER	20										0
13	KHLNSMERV	21										0
14	HLNSMERVE	22										0
15	LNSMERVEW	23										0
16	NSMERVEWL	24										0
17	SMERVEWLR	25										0
18	MERVEWLRK	26										0
19	ERVEWLRKK	27										0
20	RVEWLRKKL	28										0
21	VEWLRKKLQ	29										2
22	EWLRKKLQD	30										0
23	WLRKKLQDV	31										2
24	LRKKLQDVH	32										0
25	RKKLQDVHN	33										0
26	KKLQDVHNF	34										0

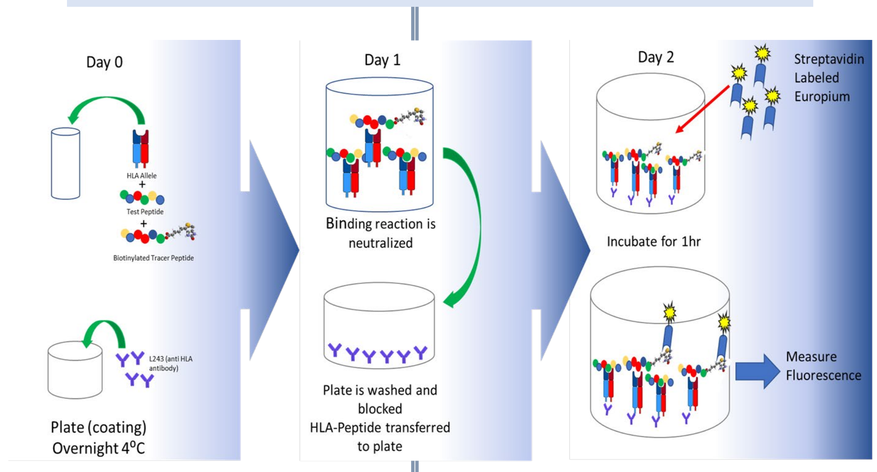
JanusMatrix Score: 4.74 ***Highly Human***

The TPT EpiBar shares significant homology with other endogenous proteins (β-tubulin, a highly ubiquitous protein). Preliminary data shows that this region of TPT acts as a Tregitope and can suppress the immune response. **Modifications to this region that make it more foreign significantly increase the immunogenicity of TPT impurities**

In vitro HLA Binding

In vitro HLA Binding Assays confirmed the presence of a promiscuous class II HLA binding epitope in each peptide.

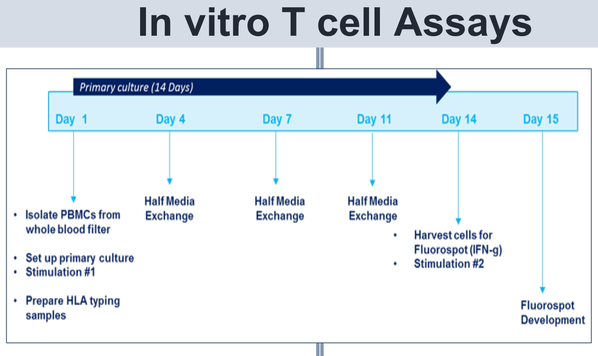




In vitro T cell Assays

Salmon Calcitonin is immunogenic due to appearing highly foreign to the immune system. Synthesis related impurities of an immunogenic peptide will be immunogenic but are unlikely to impact the immunogenicity of the drug product if present at the allowable limits

Donor	API	Impurity			
	SCT	LYS-AC18_SCT	Q20E_SCT	ENDO-GLY28_SCT	ENDO-THR31_SCT
EV0279	-	+	+	-	+
EV0320	-	-	-	-	-
EV0321	+	-	-	-	-
EV0322	+	+	+	+	+
EV0323	+	+	+	+	+
EV0324	+	+	+	-	+
EV0325	-	+	+	+	+
EV0326	-	-	-	+	+
EV0327	-	+	+	+	+
EV0328	-	-	+	+	+
EV0331	-	-	-	+	-
EV0334	-	-	-	-	-
EV0335	+	-	-	+	+
EV0337	-	+	-	-	-
EV0340	+	+	+	+	+
EV0342	+	+	+	+	+
	7 /16	9/16	9/16	10/16	11/16



Teriparatide Impurities That Are Less Human Increase Immunogenicity

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%

JanusMatrix Score ↓ Immunogenicity ↑

Donor ID	API	Impurity								
	Teriparatide	LYS-AC26	DES-LEU7	DES-HIS9	DES-LEU11	DES-LYS13	DES-HIS14	DES-LEU28	DES-GLY12	ENDO-LEU11
EV0360	-	-	+	-	-	-	+	-	+	-
EV0362	-	+	+	+	+	+	+	+	+	-
EV0363	-	-	-	-	-	-	-	-	-	-
EV0365	-	-	-	-	-	-	-	-	-	-
EV0366	+	+	+	+	+	+	+	+	+	+
EV0367	+	+	-	-	-	-	-	-	-	-
EV0368	-	-	-	+	+	-	-	-	-	+
EV0370	+	+	+	+	+	+	-	+	+	+
EV0371	-	-	-	-	-	-	-	-	-	-
EV0372	-	+	+	+	+	+	+	+	+	+
EV0373	-	+	+	+	+	+	+	-	+	-
EV0374	-	+	+	+	+	+	+	+	+	+
EV0375	+	-	-	-	-	-	-	-	+	+
EV0376	-	-	-	-	+	+	-	-	-	-
EV0377	-	+	+	-	-	+	+	-	-	+
EV0381	-	+	+	+	+	+	-	-	+	+
EV0382	-	-	-	-	-	-	-	-	-	+
EV0383	-	-	-	+	-	-	+	-	-	-
EV0384	-	-	-	-	-	-	-	-	-	-
EV0385	-	-	-	-	-	+	-	-	-	-
EV0386	-	-	-	+	-	-	-	-	-	-
	4/21	9/21	9/21	10/21	9/21	10/21	8/21	5/21	9/21	9/21

Teriparatide is non-immunogenic due to its human origin and the Tregitope found within the N-terminus. When the Tregitope is disrupted and made to be less human, impurities can increase the immunogenicity of the product even when present at the allowable limits.

TAKE HOME MESSAGE

- Understanding the immunogenic or tolerogenic properties of a synthetic peptide drug is crucial to understanding the impact of impurities on the immunogenicity of the final drug product.
- Peptide impurities derived from immunogenic therapeutic peptides are unlikely to raise the immunogenicity profile of the drug product, while peptide impurities derived from non-immunogenic peptides have a greater chance of enhancing the immunogenicity to the drug product.
- In silico evaluation followed by complementary in vitro studies provide a critical understanding of the immunogenic nature of both the API peptide and its impurities.

FUNDING & REFERENCES

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