Using preclinical risk assessment tools to identify and mitigate risks for therapeutic proteins and peptides

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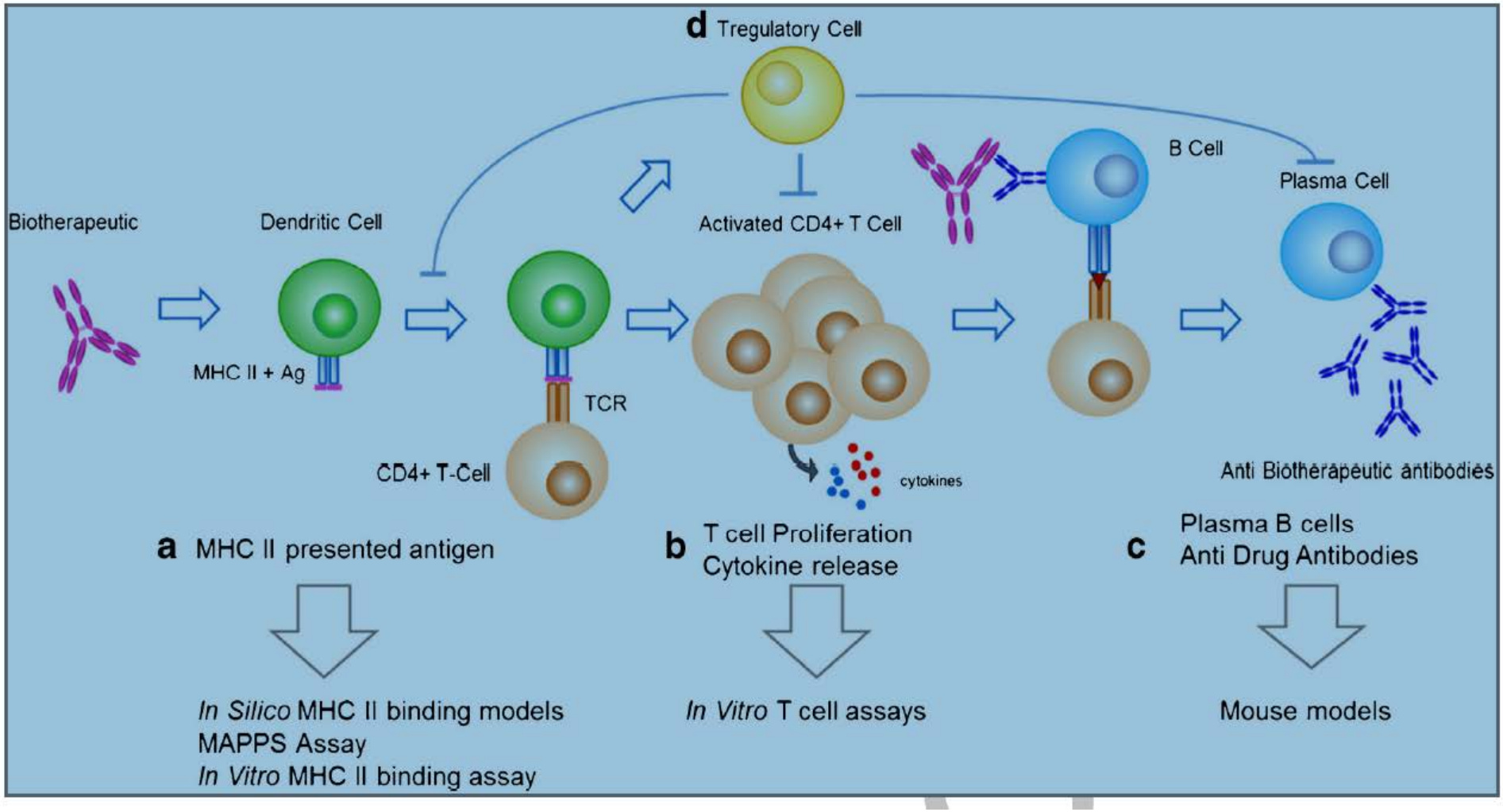
Outline

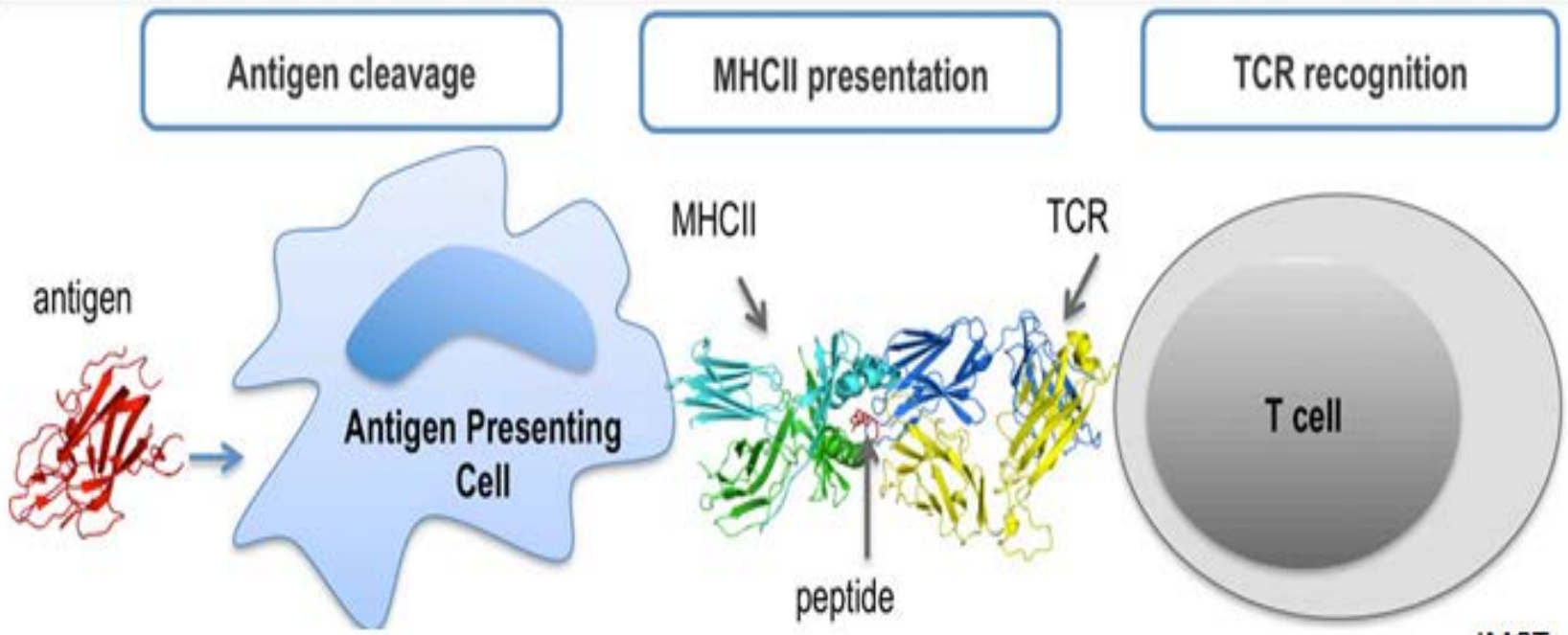
- Tools
  - In Silico
  - In Vitro
- Case Studies- Proteins
  - Whole Proteins and Immunodominant peptides associated with proteins
  - Pcsk9 Ab comparison
- Case Studies - Peptides
- Cell Based Assays for Risk Assessment
  - Considerations for Development and Qualification

Key processes involved in the development of a humoral, MHC class II-mediated anti-biotherapeutic IgG response and corresponding predictive immunogenicity tools



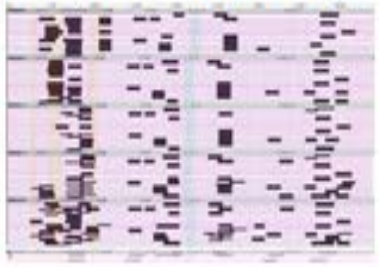
Gokemeijer J, Jawa V and Mitra-Kaushik S. The AAPS Journal (# 2017)DOI: 10.1208/s12248-017-0143-z

In silico Risk Assessment Tools



APPL

Antibody Processed Peptide Library



EpiMatrix
IEDB consensus
nn_align
Net_MHCIIpan

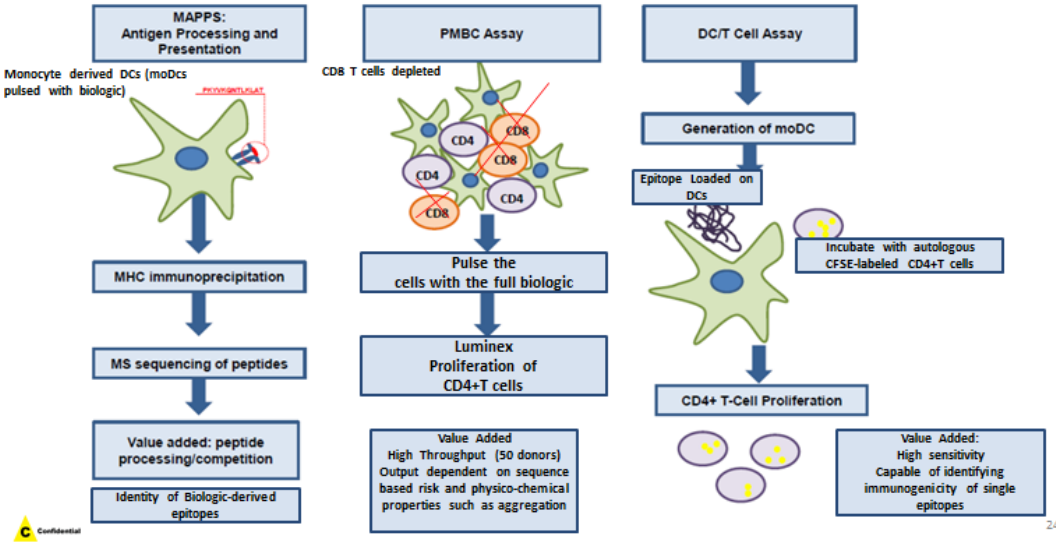
JanusMatrix

IMGT
SwissProt
IEDB

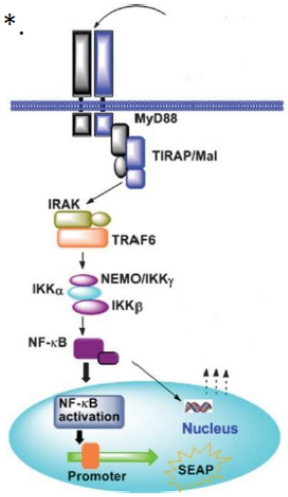
MHCII
Immunopeptidome

LTA
Library Therapeutic Antibodies

Immunogenicity *Ex Vivo* Assays



2**.



Reporter Cell Lines:

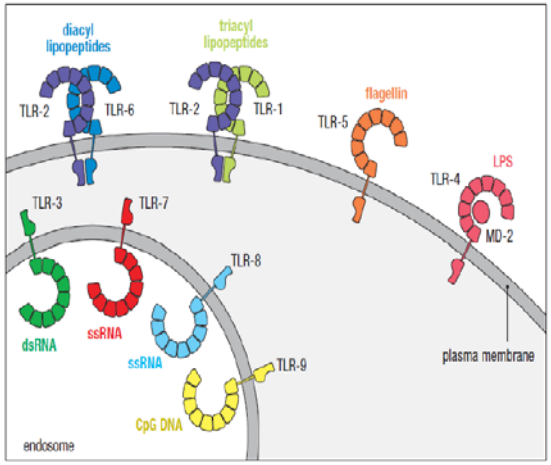
- Raw-blue cell lines (TLRs*: 2, 6, 7, 8 & 9)
- THP-1-Xblue cell lines (TLRs*: 1, 2, 3, 4, 5, Dectin 1, NOD 2)

(* **TLR** : Toll-like Receptor)

Reporter cell line assays to detect innate immune response for product comparison

2. Reporter Cell line-based Assays for Detecting Innate Immune Response Modulating Impurities (IIRMs) in Peptide Product

- TLR (Toll-like receptors) agonists are major innate immune response modulating impurities (IIRMs).
- Innate immune responses leads to adaptive immune responses



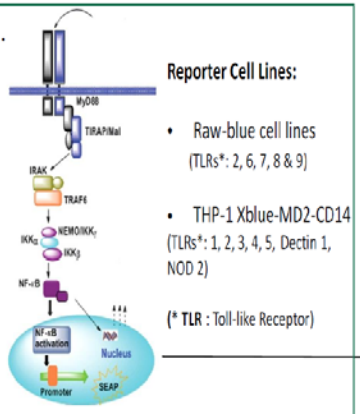
Cell lines*	Agonists	Corresponding receptor
RAW-Blue cell	FSL-1	TLR2/6
	Imiquimod	TLR7
	CpG	TLR9
	CL075	TLR7/8
THP-1-XBlue-MD2-CD14	Pam3CSK4	TLR1/2
	Poly I:C	TLR3
	Endotoxin	TLR4
	Flagellin	TLR5
	Zymosan	TLR2/Dectin 1
	MDP	NOD2

Janeway's immunobiology book 9th Edition page 89

*Detection of Innate Immune Response Modulating Impurities in Therapeutic Proteins, Plos One 10(4), 2015

2. Reporter Cell Line Assays to Detect Innate Immune Response for New Impurities Present in Peptide Product

2.



Reporter Cell Lines:

- Raw-blue cell lines (TLRs*: 2, 6, 7, 8 & 9)
- THP-1 Xblue-MD2-CD14 (TLRs*: 1, 2, 3, 4, 5, Dectin 1, NOD 2)

(* **TLR** : Toll-like Receptor)

Reporter cell line assays to detect innate immune response for product comparison

The reporter cell line based assessment devoid of donor to donor variability and provide information of the type of IIRMs present.

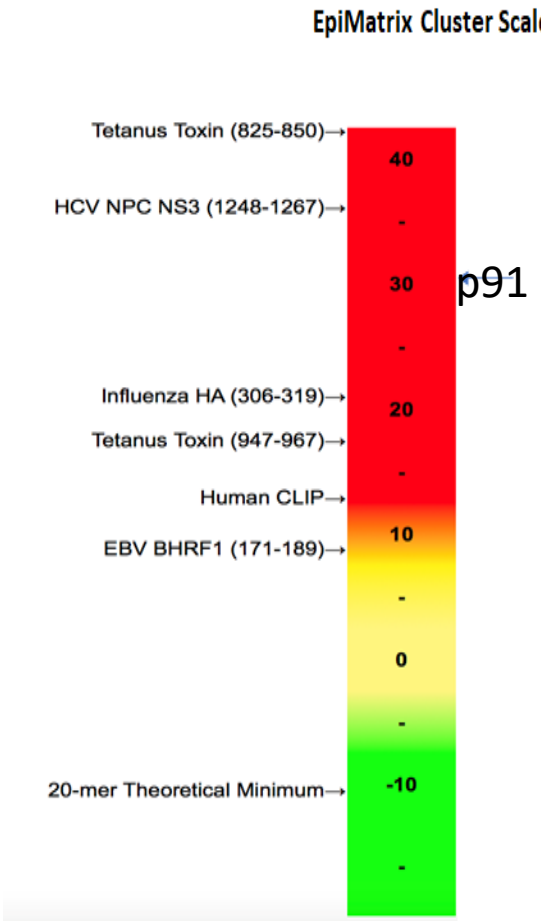
Take supernatant, add Quanti-blue and develop, read at OD650

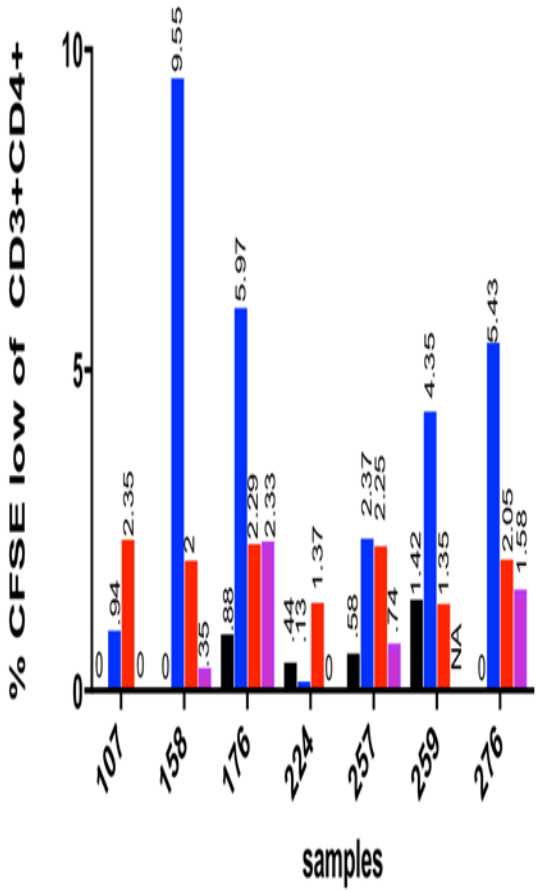
Figure adapted from Science Advances 10 Apr 2015: Vol. 1, no. 3

Case studies

- Risk Assessment
  - Whole Proteins/Clusters within Proteins
    - Pcsk9 Antibodies; Comparison
    - Humira and an epitope cluster
    - Herceptin/Trastuzumab : Oxidation and Deamidation related impurities

Ex Vivo Confirmation of Immunogenic Epitopes





- ANTI PCSK9 MABS:
- EVOLOCOMAB, ALIROCUMAB, AND BOCOCIZUMAB

  - In silico HLA binding potential
  - Observed subject ADA incidence

Anti PCSK9 mAbs: Comparison of In Silico Predictions and Observed Incidence

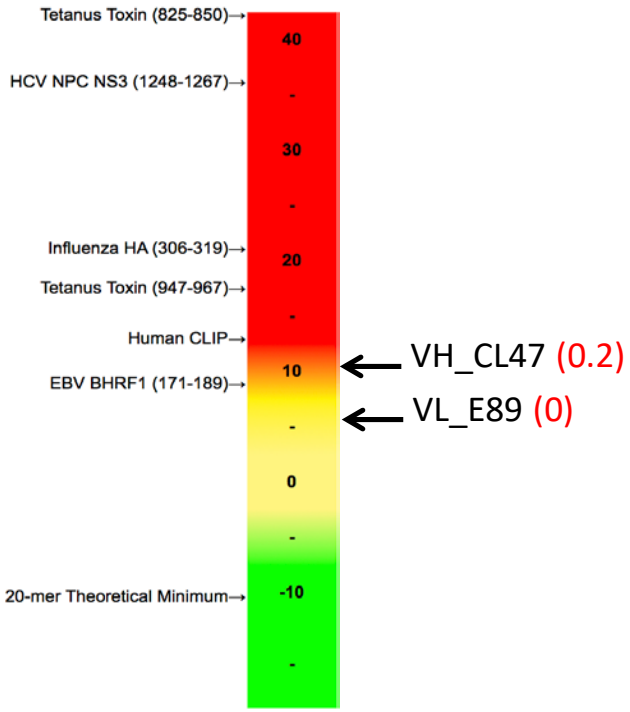
Drug	Structure	In Silico Prediction	In Vitro Prediction	Observe
Evolocumab Repatha	human IgG2	low	NA	0.3%[a]
alirocumab (REGN727)	human IgG1	low-moderate	NA	4.8%[b]
bococizumab (RN-316)	humanized IgG2	high	High*	48%[c]

a: US label; b: US label; c: Ph3 Ridker et al., 2017

*Unpublished results

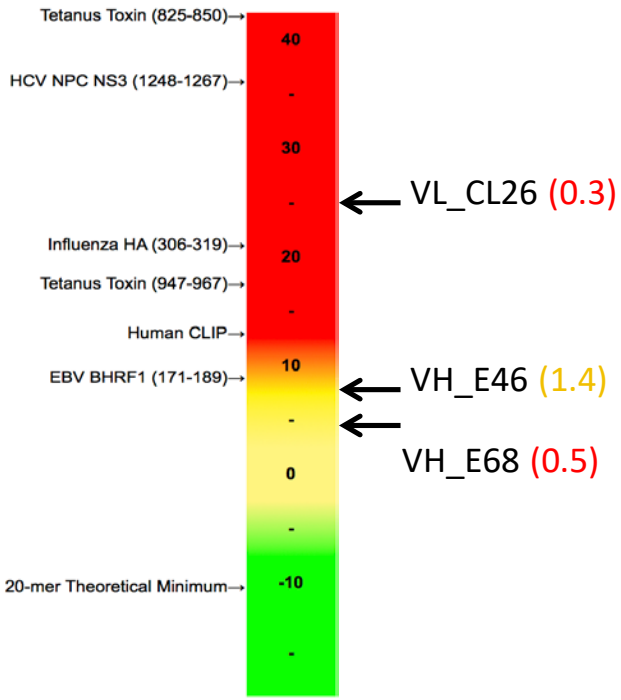
Comparison of anti- pcsk9 antibodies

evolocumab (REPATHA)



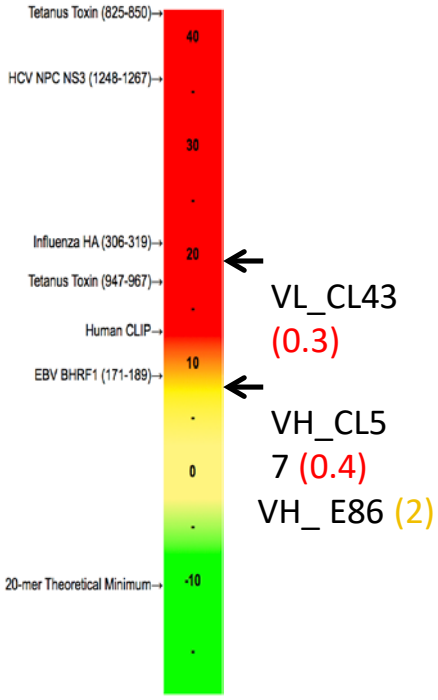
Immunogenicity risk
CL47 : low/intermediate
E89: low
Evolocumab: low
(0.3% observed)

alirocumab (PRALUENT)



Immunogenicity risk
CL26 : if processed High
E46/68: low
Alirocumab: Intermediate
(4.8% observed)

bococizumab



Immunogenicity risk
CL43 : High
CL57: Intermediate
Bococizumab: High
(48% observed)

FDA guidance on Immunogenicity Risk Assessment of Peptides and Process related Impurities

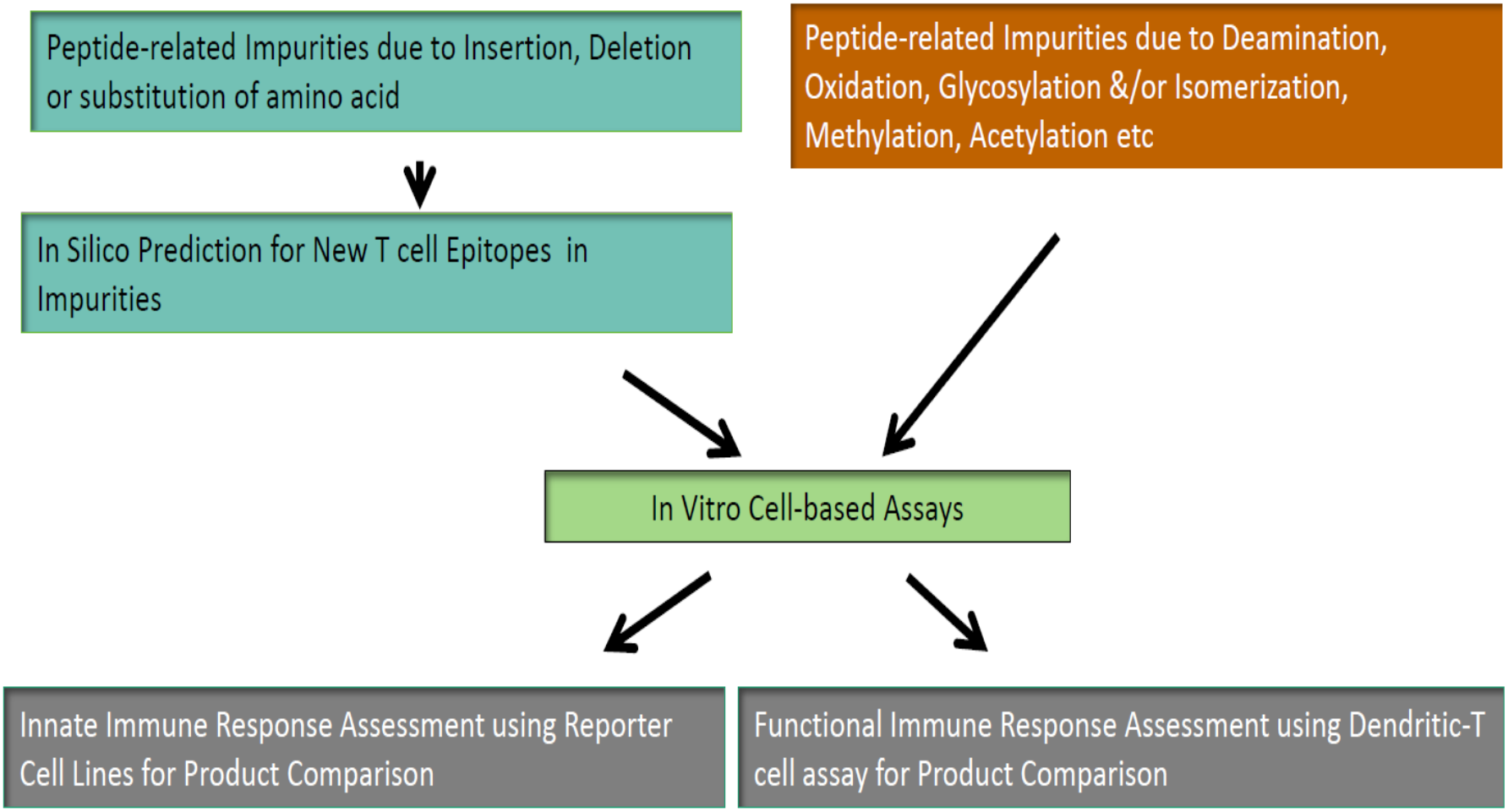
1. That each new impurity does not contains T cell epitopes that have an increased affinity for Major Histocompatibility Complex (MHC), and

2. That the proposed synthetic peptide does not alter the innate immune activity.

3. Additionally, consider performing functional assays (in vivo or in vitro) to support the absence of increased risk of immunogenicity potential of the drug product formulated with drug substance and the RLD.

1. In Silico Approach: For Identification of New T-cell Epitopes

Not All Impurities Can be Assessed for New T cell Epitopes

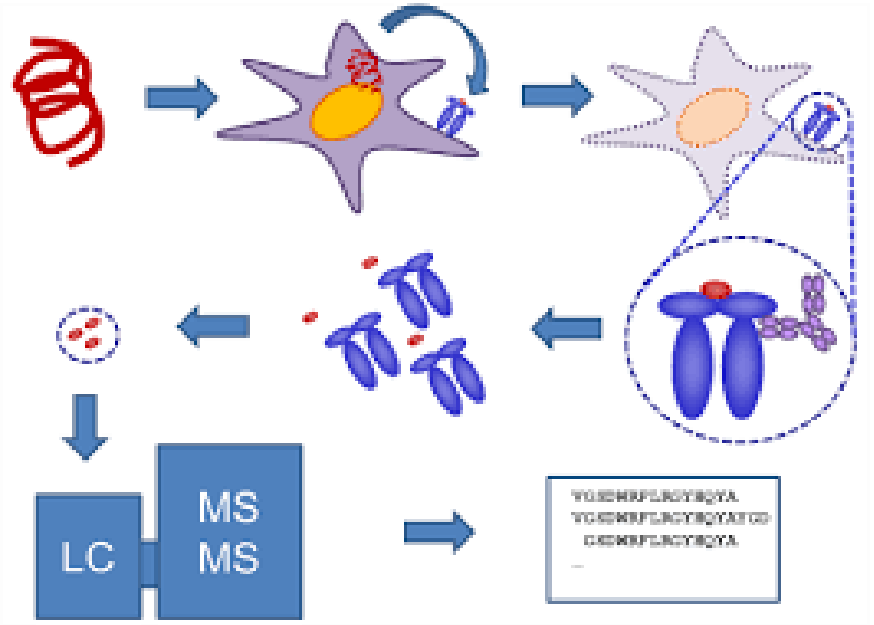


Case studies

- Risk Assessment
  - Whole Proteins/Clusters within Proteins
    - Pcsk9 Antibodies; Comparison
    - Humira and an epitope cluster
    - **Herceptin/Trastuzumab : Oxidation and Deamidation related impurities**
    - **Deimmunized vs wt peptide**
    - **Cyclic peptide**

Therapeutic Antibody Case

- Trastuzumab
  - Stir-stressed for 24 hrs at room temperature and then stored at -70°C
  - Unstressed: stored at -70°C
- MAPPS assay
  - Dendritic cells were loaded with stressed or unstressed Trastuzumab and matured
    - Repeated for 5 donors
  - Cell lysates were generated
  - HLA-DR:peptide complexes were isolated
  - Peptides were isolated
  - Peptides identified via mass spec



Stressed Trastuzumab Treatment Resulted in More mAb-derived Peptides Detected

	Treatment		
Donor	Media	Trastuzumab	Stressed Trastuzumab
1	0	6	97
2	0	4	49
3	0	6	113
4	0	1	80
5	0	16	154

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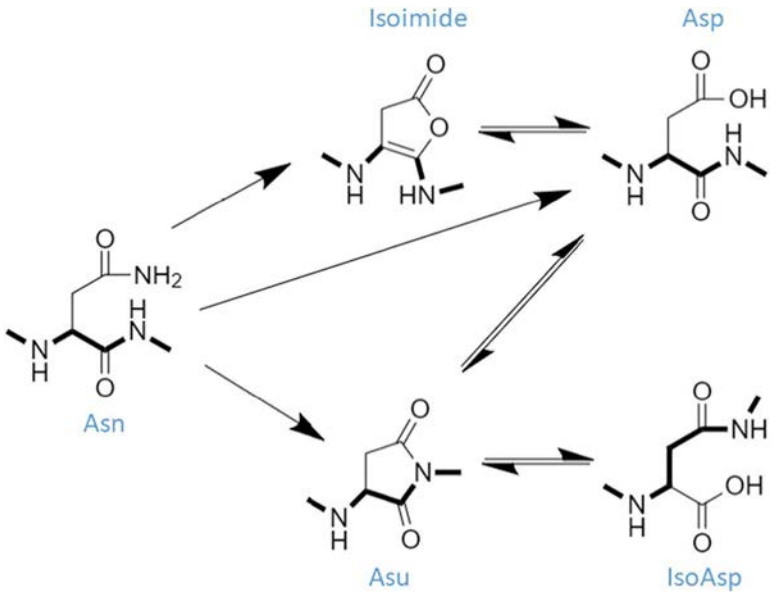
Deamidation

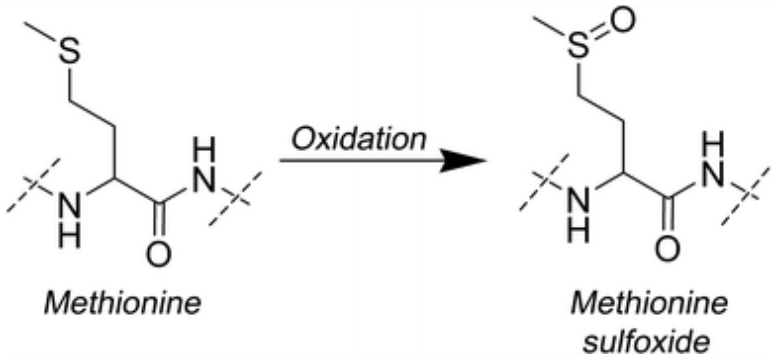
Influenced by neighboring amino acid, temperature, buffer composition and pH



Oxidation

Methionine, Tryptophan

Reactive oxygen sources could be light, transition metals, solvent exposure



https://doi.org/10.1080/19420862.2018.1548233

Stressed Trastuzumab Sample Was Enriched for Deamidated and Oxidized Peptides Eluted from HLA-DR

Peptides Enriched in Stressed Sample	Donors	Location
KNTAYLQMNSLRAEDTA	1,2,3,4,5	HC 76
KNTAYLQM(+15.99)NSLRAEDTA	1,2,3,4,5	HC 76
KNTAYLQMN(+.98)SLRAEDTA	1, 3, 5	HC 76
KN(+.98)TAYLQMNSLRAEDTA	2,3,5	HC 76
KNTAYLQM(+15.99)NSLRAEDT	1,2,3,4,5	HC 76
NTAYLQM(+15.99)NSLRAEDT	1,2,3,5	HC 77
RTPEVTCVVVDVSHEDPE	1	HC 259
KDSTYSLSSTLTLSK	5	LC 169
EQDSKDSTYSLSSTLTLSKA	5	LC 165
DSKDSTYSLSSTLTLSKA	5	LC 168
KNTAYLQMNSLRAED	5	HC 76
DTSKNTAYLQMNSLRAEDTA	5	HC 73

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Stressed Trastuzumab Samples Had Unique Peptides Eluted From HLA-DR

Peptides Unique to Stressed Sample	Donors	Location
KNTAYLQM(+15.99)NSLRAEDT	1,2,3,4	HC 76
KNTAYLQM(+15.99)NSLRAEDTA	1,3,4	HC 76
NTAYLQM(+15.99)NSLRAEDT	2,3	HC 77
EKHKVYACEVTHQGLSSPV	1,2,5	LC 187
KHKVYACEVTHQGLSSPV	1,5	LC 188
EAKVQWKVDNALQSGNS	1,2	LC 143
KDSTYSLSSTLTLSK	1,4	LC 169
DTSKNTAYLQM(+15.99)NSLRAEDTA	1,2,4,5	HC 73
EQDSKDSTYSLSSTLTLSKA	1,2	LC 165
DSKDSTYSLSSTLTLSKA	1,3,4	LC 168
KNTAYLQMNSLRAED	1,2,3,4	HC 76
DTSKNTAYLQMNSLRAEDTA	1,2,3,4	HC 73
NTAYLQM(+15.99)NSLRAED	1,2,3,4,5	HC 77
DTSKNTAYLQM(+15.99)NSLRAEDT	1,3,5	HC 73

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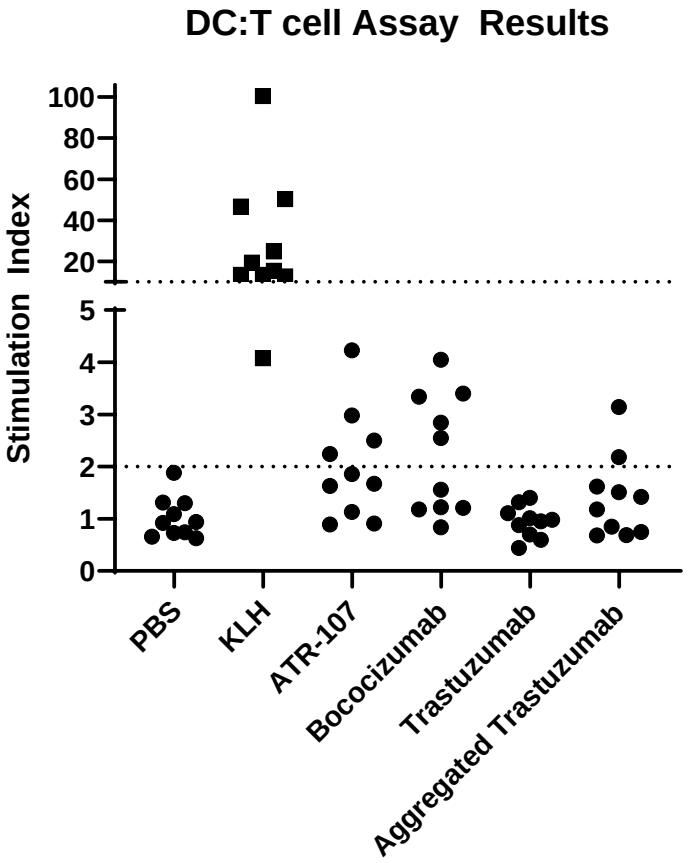
Donors 3 and 5 Responded to Aggregated Trastuzumab in a DC:T cell Assay

Peptides Unique to Aggregated Sample	Donors	Location
KNTAYLQM(+15.99)NSLRAEDT	1,2,3,4	HC 76
KNTAYLQM(+15.99)NSLRAEDTA	1,3,4	HC 76
NTAYLQM(+15.99)NSLRAEDT	2,3	HC 77
EKHKVYACEVTHQGLSSPV	1,2,5	LC 187
KHKVYACEVTHQGLSSPV	1,5	LC 188
EAKVQWKVDNALQSGNS	1,2	LC 143
KDSTYSLSSTLTLSK	1,4	LC 169
DTSKNTAYLQM(+15.99)NSLRAEDTA	1,2,4,5	HC 73
EQDSKDSTYSLSSTLTLSKA	1,2	LC 165
DSKDSTYSLSSTLTLSKA	1,3,4	LC 168
KNTAYLQMNSLRAED	1,2,3,4	HC 76
DTSKNTAYLQMNSLRAEDTA	1,2,3,4	HC 73
NTAYLQM(+15.99)NSLRAED	1,2,3,4,5	HC 77
DTSKNTAYLQM(+15.99)NSLRAEDT	1,3,5	HC 73



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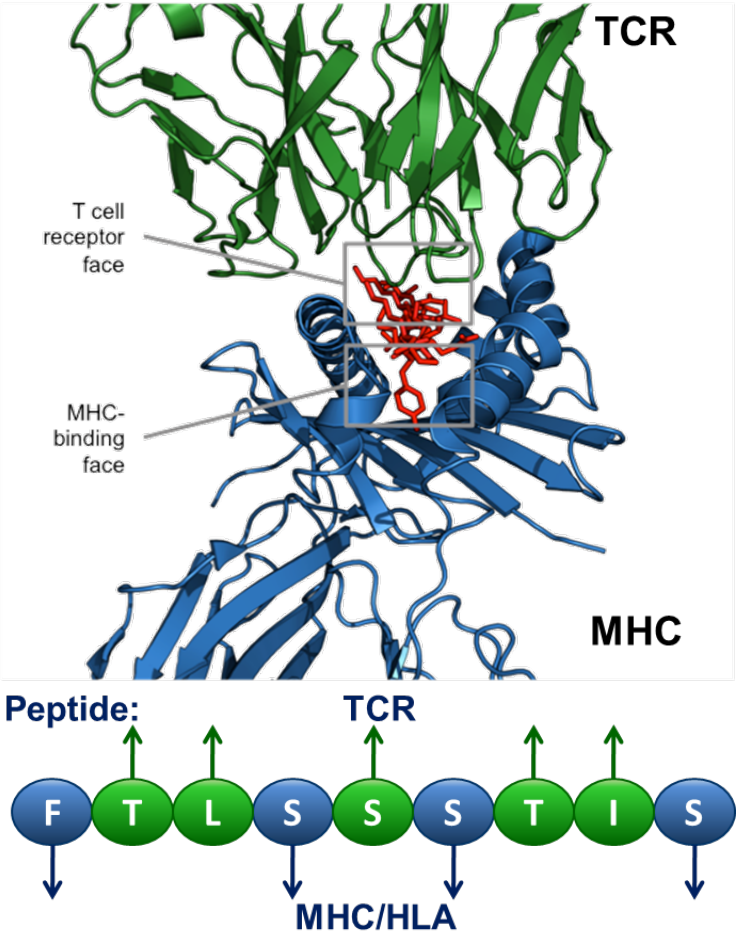
Deamidated and Oxidized Amino Acids are Predicted to Presented to CD4+ T cells

IEDB Net MHC pan 3.2:
K<u>N</u>T*AYLQ**M**<u>N</u>SLRA*EDTA

Italics= possible core amino acids
Underline= deamidation site
Bold = oxidation Site



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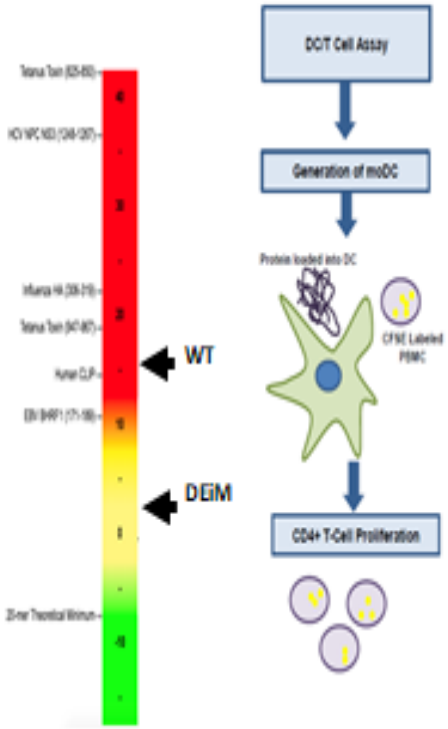
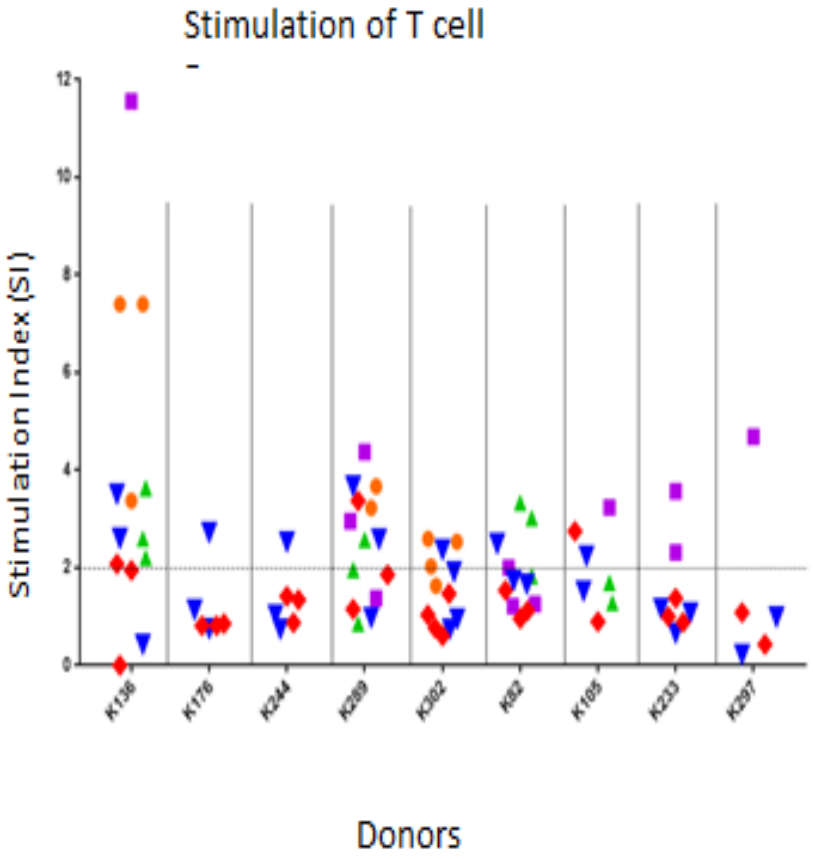
Ex Vivo Confirmation of De-Immunization: Epitope





T cell proliferation
VH1 : 7/9
VH1Q3M: 3/9

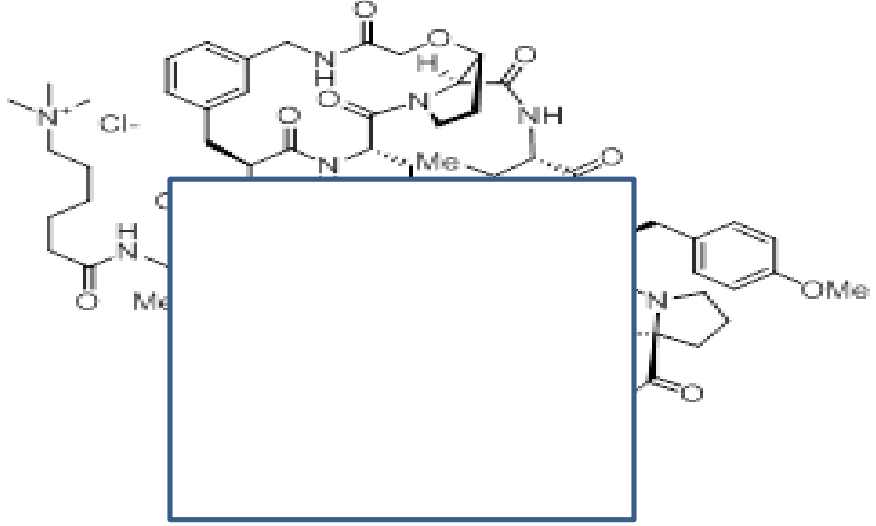
$$SI = \frac{\text{Proliferation}}{\text{Average of Proliferation of Mock}}$$

Proliferation: %CFSE low of CD3+CD4+

OMO: + control (peptide)
P24:+ control (peptide)
KLH:+ control (protein)

Jawa V Mastering Immunity 2019

Immunogenicity Risk Assessment for a cyclic peptide



Structure ; cyclic peptides with unnatural amino acids;
Risk Uncertain
Target and MOA: Non-immune modulatory ; inhibition of the soluble target;
Risk Low
Dose/Route of Administration : Oral ; tolerance/induction of immune suppression/ low risk of anaphylaxis / hypersensitivity/short half life ; Risk Low

Immunogenicity of Peptides: Key Considerations

- Peptides as Foreign Antigens
  - Peptides of non-human origin, designer peptides, modified human sequences vs. Self derived peptides like human peptide hormones and peptide fragments
- MHC Binding Ability
  - Class I and Class II
  - Complexity : To increase stability and/or receptor binding; non-natural amino acid moieties or modified backbones in peptide drug leads.
  - These synthetic modifications decrease rather than increase MHC-binding potential by eliminating either the proper side-chain topo-chemistry or the required hydrogen bonding pattern
- T-cell support requirement
  - short peptides are poor immunogens, unable to elicit antibody production without the support of T helper cells.
  - Although peptidomimetic backbones, side-chains, and stereochemical replacements do render peptides increasingly immunogenic, the effect is still insufficient to induce humoral immune response
- Product and Process related factors
  - Formulation
  - Degraded peptides

Method Development , Optimization and Qualification ; Regulatory Considerations

- Development Data for each assay platform
- Ensure presentation of the protein
  - (MAPPS assay; DC activation markers)
- Optimization ; Dose Response of the protein/peptides; relevant to expected exposure or positive control
- Positive control selection: peptide(s) like CEFT, neoantigen like KLH, LPS, PHA
- Lower limit of Ag specific T cell detection; which method is suitable?
  - Elispot, Tetramer, linear dilution of DC: CD4T cells
- Impact of formulation/matrix on cell quality and integrity
- Understand which specific impurity poses a risk by additional spiking experiments

In Vitro Cell Based Assays: Qualification

- Cell Based Assays
  - Intra Assay, Inter Assay, Limit of Detection, LLOQ
  - Acceptance criterion LPC and HPC
  - Reagent Qualification
  - Stability
  - Donor selection based on
    - HLA genotypes (driven by in silico algorithms)
    - Patient/ Disease specific
    - Demographics
    - Previous Disease/Immunization History
    - Baseline Immune Responsiveness
  - Number of Donors

Conclusions/Future Directions

- Nonclinical Immunogenicity Risk Assessment of Proteins and Peptides can be performed using In silico and in vitro/ex vivo assays
- FDA guidance on peptides and related impurities is recommending use of such assays to perform a robust risk assessment during non- clinical development
- There is a correlation of presence of T cell epitopes with observed immunogenicity in clinic
- Proteins and peptides are associated with process related impurities caused due to stress and associated with post translational modifications like oxidation and deamidation
  - Stir stress causes an increase in the number of peptides displayed via MHC class II (One potential explanation is enhanced uptake of aggregates)
  - MAPPS data suggests that some deamidated/oxidized amino acids can be presented via MHC class II
  - Modifications lie in a region expected to be presented to CD4+ T cells
- It is possible to deimmunize peptides by removing potential epitopes and confirm using in vitro assays
- Further validation of this approach can be performed by epitope mapping of cells from dosed subjects and associating with the HLA genotype

Acknowledgements

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