Non-clinical Evaluation of Comparative Immunogenicity Risk of Complex Peptide Products

SBIA 2020: Advancing Innovative Science in Generic Drug Development Workshop

Session 1: Complex Active Pharmaceutical Ingredients Including Peptide Products

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CDER | U.S. FDA

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Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's official views or policies.

Learning Objectives

- Discuss current immunogenicity assessment recommendations for generic peptides of synthetic origin referencing a peptide of recombinant origin.[1]
- Examples of in silico and in vitro assays for assessing adaptive immune response
- Examples of the in vitro assessment of innate immune response
- Common deficiencies and issues in the validation and development of in vitro assays

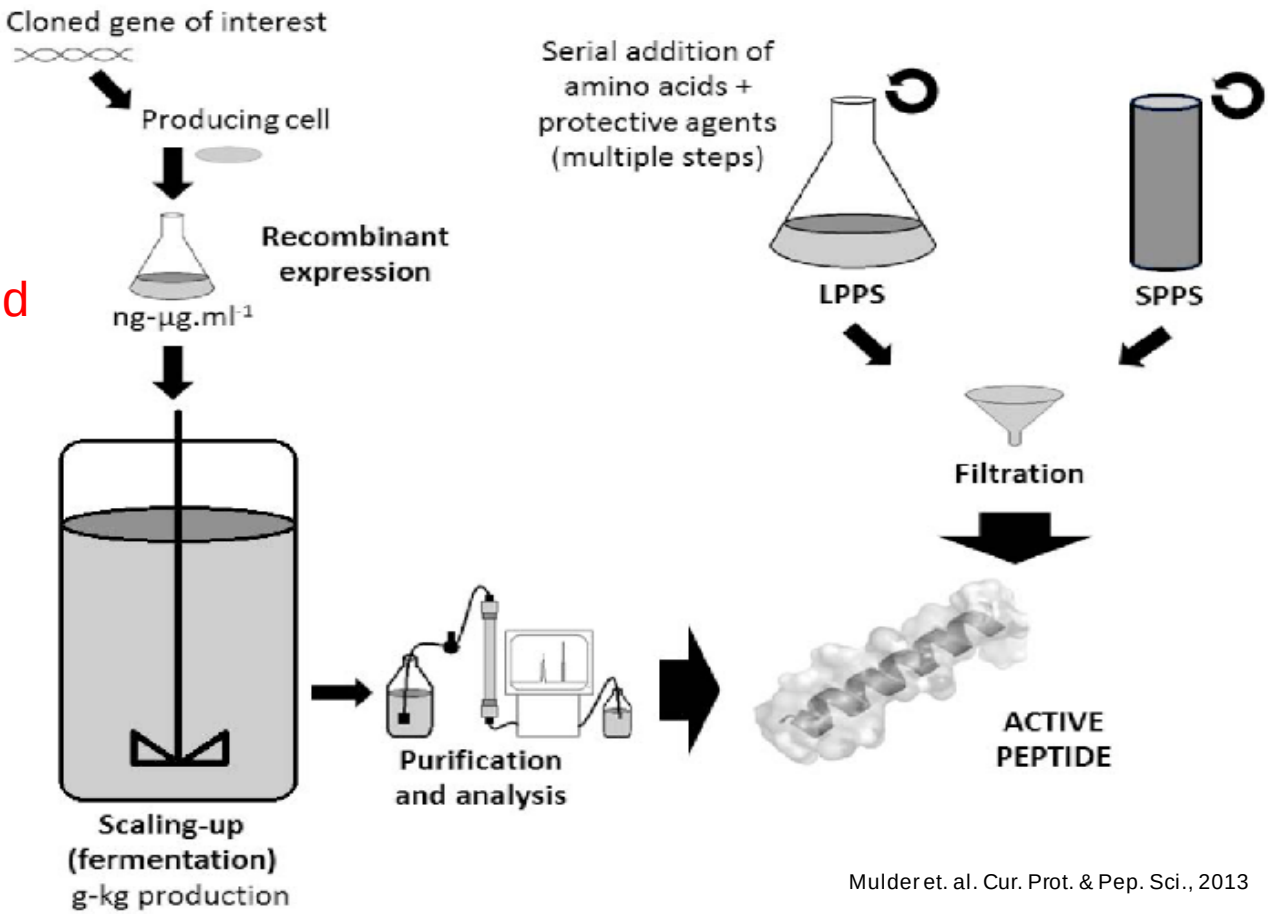
1. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf

Immunogenicity Risk Needs to be Assessed Because It May Impact Safety and Efficacy

- Developing antibodies can affect the pharmacokinetics (PK) by enhancing or delaying clearance
  - Neutralizing antibodies can diminish efficacy
- Anti-drug antibodies can cross-react to endogenous non-redundant proteins, and may cause deficiency syndrome
- Hypersensitivity responses can lead to
  - Cytokine Release Syndrome – rapid release of proinflammatory cytokines
  - Anaphylaxis – serious, acute allergic reactions

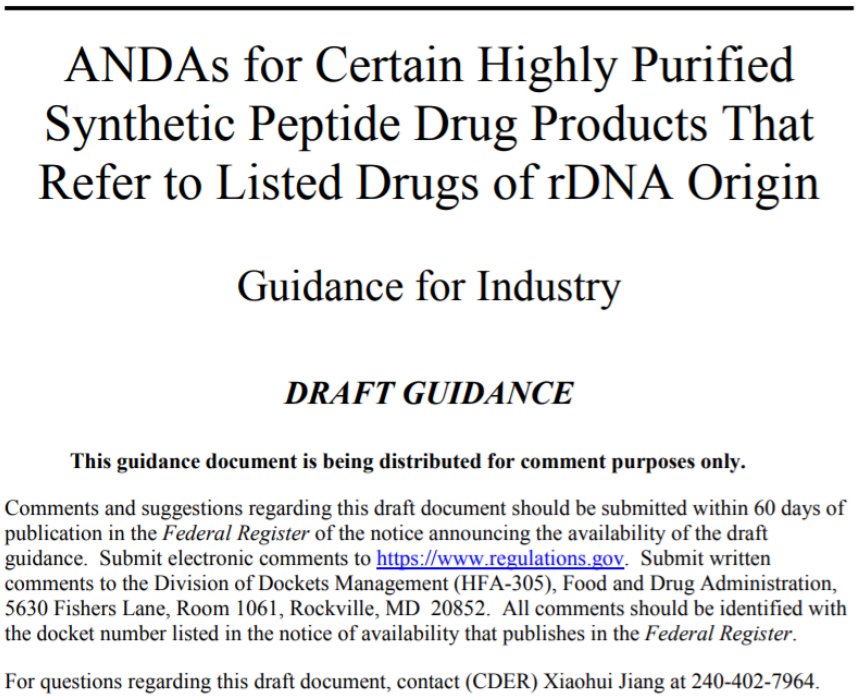
Peptide Made through Recombinant and Synthetic Processes

Differences in process-related impurities



Mulder et. al. Cur. Prot. & Pep. Sci., 2013

FDA Outlined Current Thinking and a Pathway in following Guidance for **Glucagon, Liraglutide, Nesiritide, Teriparatide, and Teduglutide**



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DRAFT GUIDANCE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.

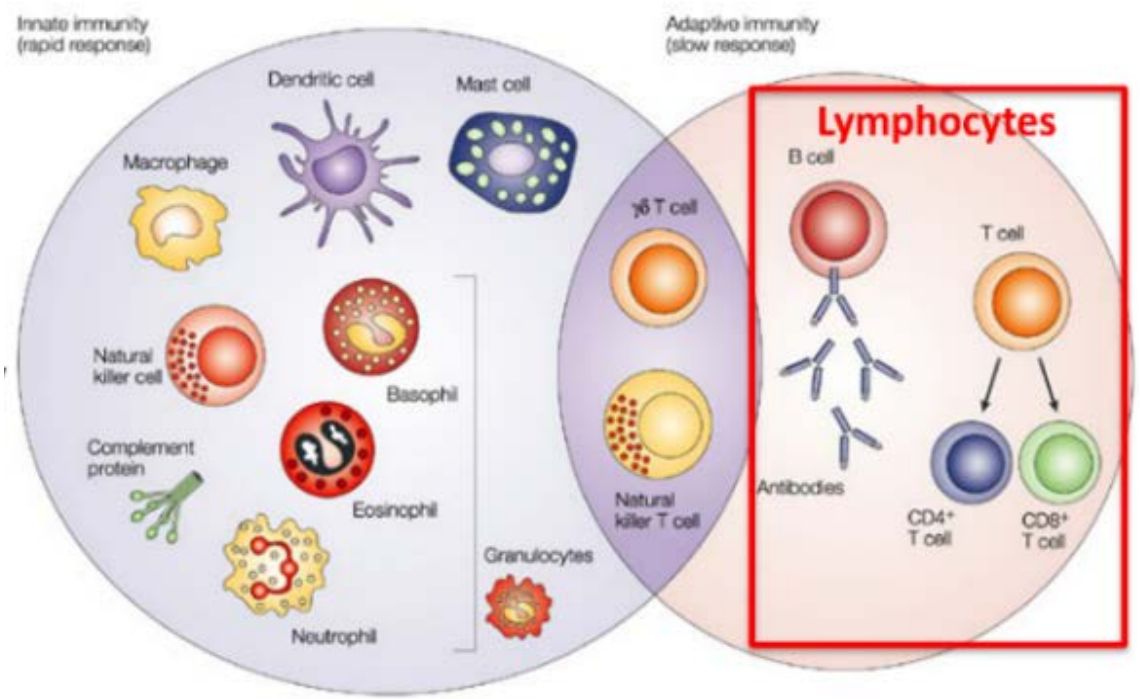
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf

Peptide-related Impurities

- For specified impurities common to proposed generic and reference listed drug (RLD)
  - Level in proposed generic ≤ RLD
- For any new impurities in the proposed generic
  - > 0.5% is not acceptable
  - Impurities at 0.1%- 0.5% identified, characterized and justified for not affecting the safety and efficacy, including comparative immunogenicity risk tests

Innate and Adaptive Immunities

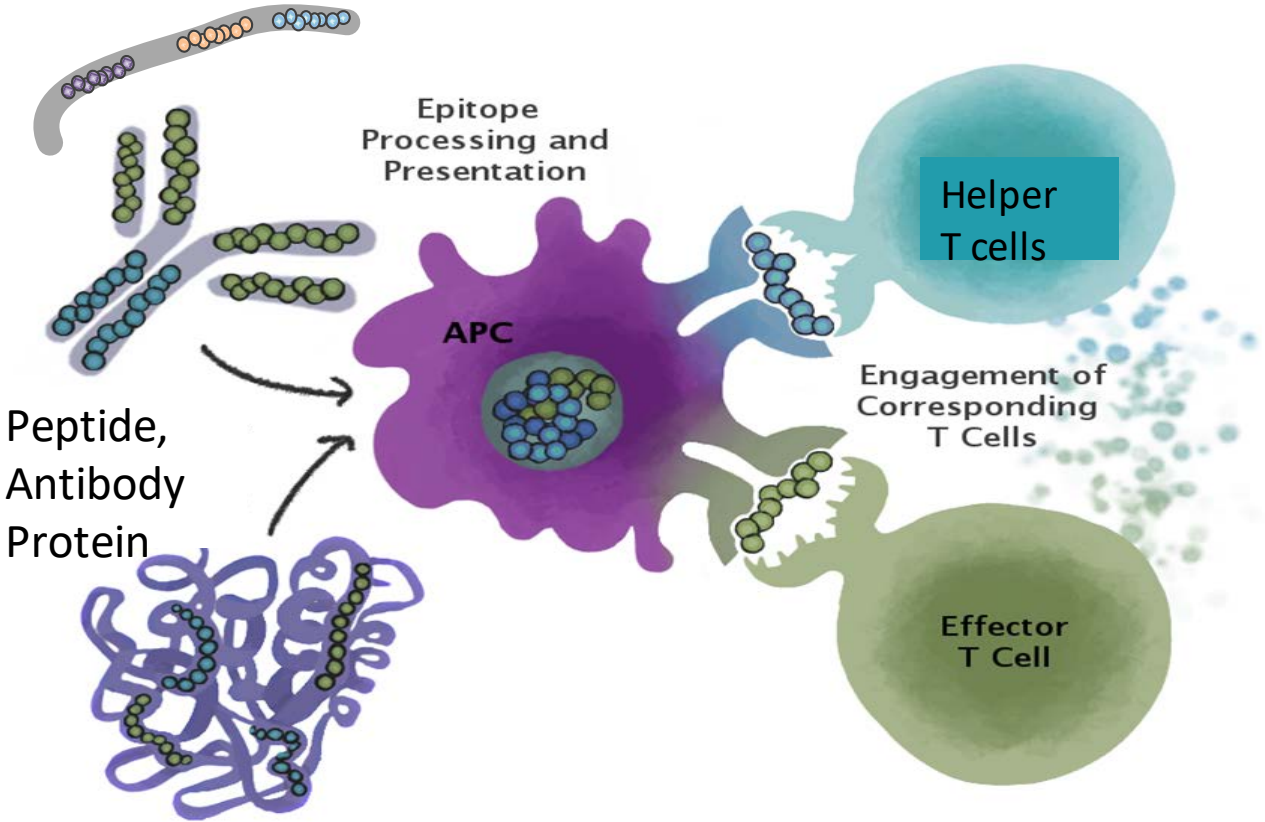
Innate immunity
- All process-related impurities and contaminant
- On the whole product

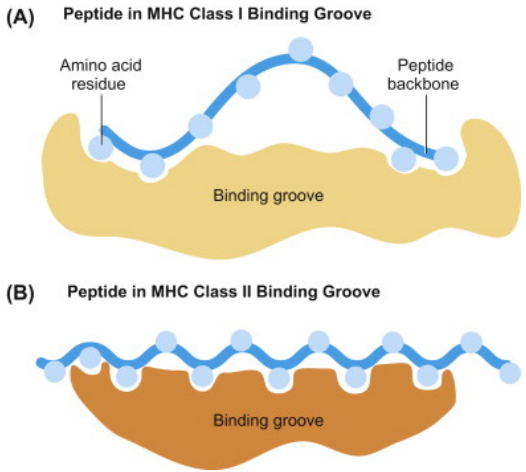


Adaptive immunity
- T-cell epitope in Peptide-related impurities
- New impurities in ANDAs (0.10 – 0.5%)

Dranoff, G., Nature Rev. Cancer, 2004

MHC Recognition by T-Cell - Potential for Immunogenicity Response



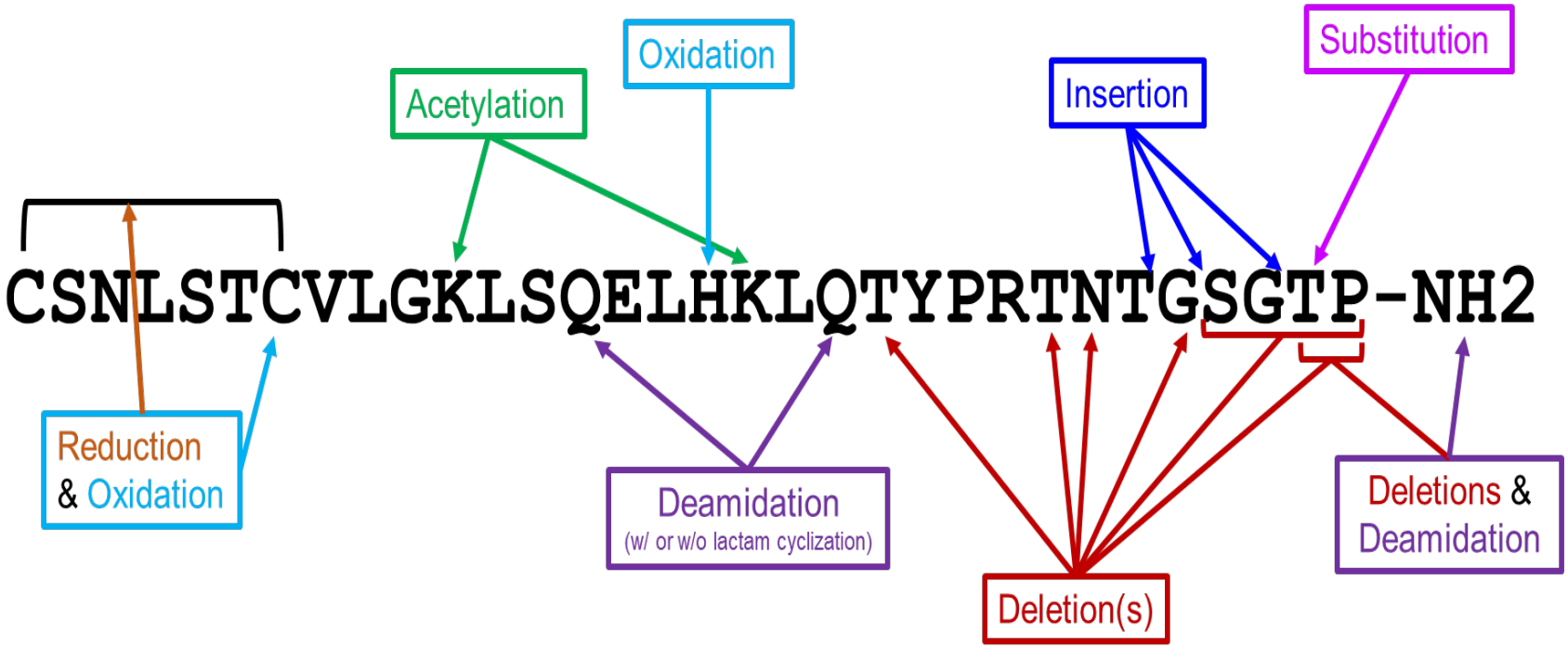


Mak et al. Primer to the immune Response, 2014, Chapter 6

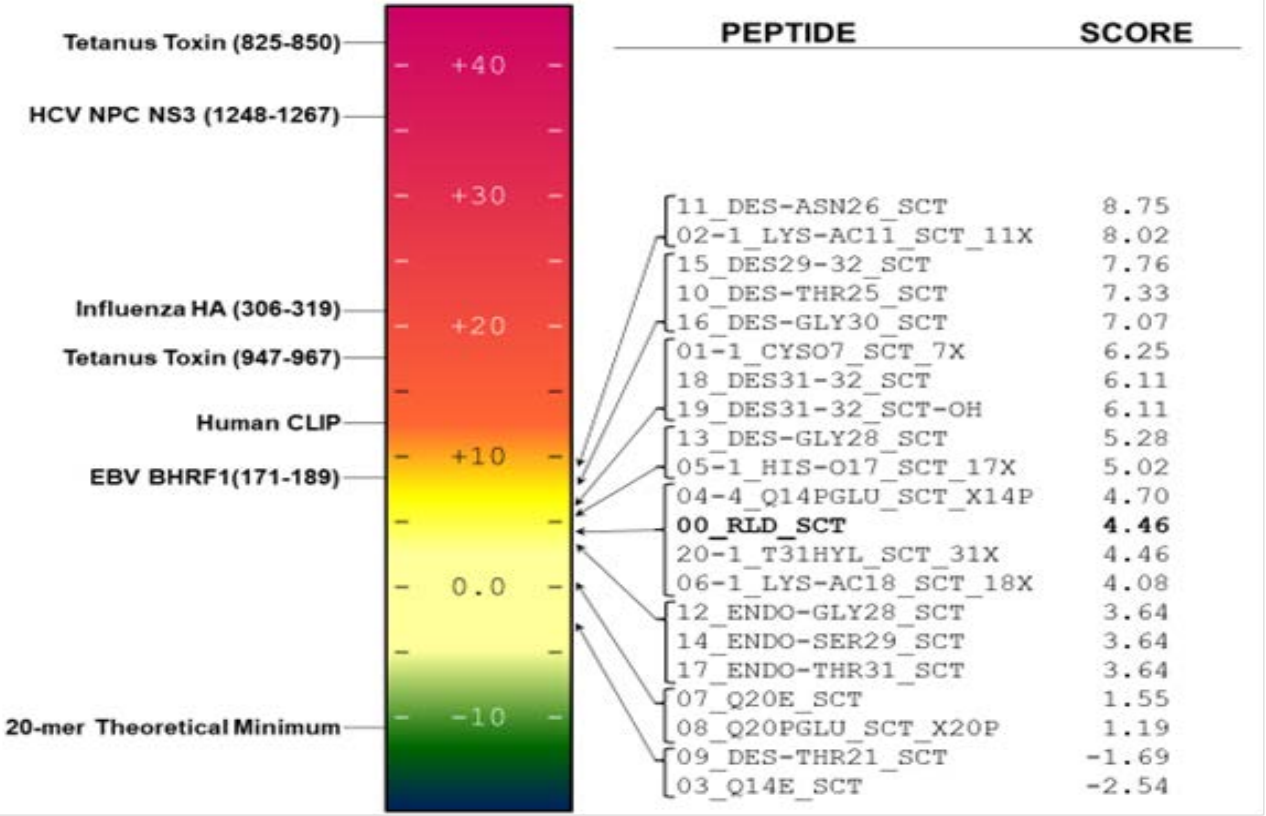
Adapted from EpiVax

MHC = Major Histocompatibility Complex

Some Peptide-related Impurities Found in Synthetic Salmon Calcitonin Products



In Silico Assessment of MHC Binding Can Reveal the Relative Immunogenicity Risk



MHC = Major Histocompatibility Complex

… and Its Risk to Various HLA Allele Populations

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
1	CSNLSTCVL	9	1.22	1.03	-0.85	0.07	1.18	-0.51	0.52	-0.72	0.04	0
2	SNLSTCVLG	10	0.90	0.21	0.99	1.13	-0.20	0.32	0.01	0.64	0.04	0
3	NLSTCVLGK	11	0.56	0.17	-0.58	0.94	0.52	0.32	0.12	0.39	-0.27	0
4	LSTCVLGKL	12	1.37	-0.54	0.41	-0.55	0.13	1.24	-0.23	-0.53	0.65	0
5	STCVLGKLS	13	0.86	1.05	-0.10	0.59	0.53	0.05	0.60	0.32	0.69	0
6	TCVLGKLSQ	14	0.56	-0.18	-0.22	0.12	-0.69	0.82	1.39	0.17	0.15	0
7	CVLGKLSQE	15	0.24	-1.29	0.53	-0.87	-0.96	0.36	-0.61	-0.03	-0.29	0
8	VLGKLSQEL	16	0.39	1.37	1.75	0.60	2.22	1.70	0.79	0.97	1.43	3
9	LGKLSQELH	17	-0.43	0.82	0.49	0.72	0.40	1.49	1.16	0.24	-0.22	0
10	GKLSQELHK	18	-1.29	-0.40	0.36	0.19	0.34	-0.19	0.59	0.18	0.06	0
11	KLSQELHKL	19	-0.82	0.68	0.99	0.30	0.71	0.35	0.82	1.72	0.66	1
12	LSQELHKLQ	20	-0.78	1.17	1.00	1.57	1.32	0.90	1.65	0.98	0.70	1
13	SQELHKLQT	21	-1.28	0.47	0.01	0.11	-0.13	0.85	1.38	0.38	1.19	0
14	QELHKLQTY	22	-1.33	-1.27	-0.10	-1.12	-0.47	-1.30	-1.08	0.22	-0.61	0
15	ELHKLQTYR	23	-1.44	-0.59	0.02	-1.42	-0.55	0.47	-1.25	0.47	-1.73	0
16	LHKLQTYRT	24	-1.13	2.52	0.93	2.71	2.20	2.20	2.50	1.00	2.38	6
17	HKLQTYRTN	25	-1.94	0.98	-0.75	-0.12	0.15	0.15	0.59	-0.48	0.13	0
18	KLQTYRTNT	26	-1.67	0.48	-0.65	-0.52	0.57	0.39	-0.61	0.18	0.45	0
19	LQTYRTNTG	27	-1.28	0.84	1.02	1.61	0.75	2.12	1.41	1.55	1.77	2
20	QTYRTNTGS	28	-1.79	0.61	-0.64	0.28	-0.47	0.50	-0.31	0.43	0.07	0
21	TYRTNTGSG	29	-1.44	0.57	-0.13	0.77	0.31	0.98	0.05	-0.40	0.45	0
22	YRTNTGSGT	30	-1.44	2.14	0.32	1.70	1.70	1.12	1.34	0.23	1.15	3
23	RTNTGSGTP	31	-1.48	-0.52	-0.51	-0.36	0.50	-0.45	-0.96	-0.77	0.38	0

Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.52	1.75	2.71	2.22	2.20	2.50	1.72	2.38	--
Sum of Significant Z-scores	4.66	1.75	4.41	6.12	6.02	4.15	1.72	4.15	32.98
Count of Significant Z-scores	2	1	2	3	3	2	1	2	16

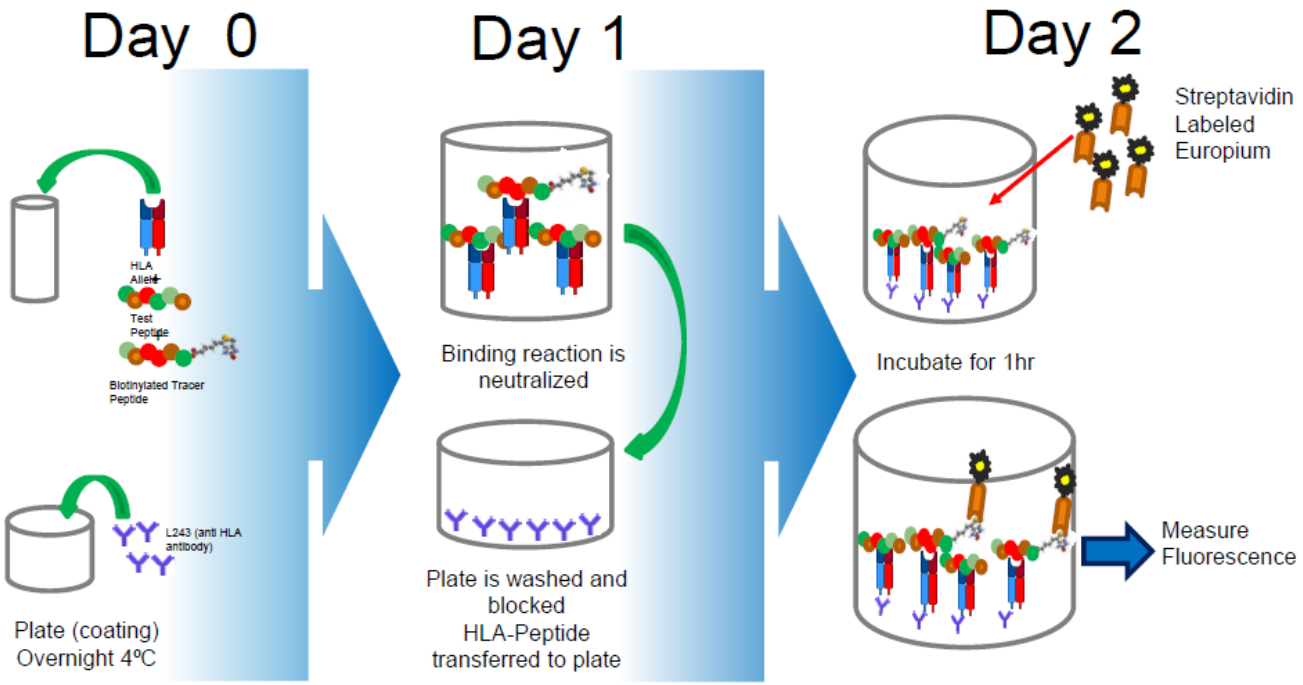
HLA = Human Leukocyte Antigen

In Vitro Assessment of MHC Binding

- In silico assessment alone may not be sufficient
  - In vitro assessment of individual impurities can validate and verify the results from in silico assessment
- Examples of in vitro assessment
  - MHA-Peptide-Binding Assay
  - In Vitro T-cell Assay

Impurity	In silico risk	In vitro risk validation
LYS-AC11	Low risk	Low Risk
DES-THR21	Significant but limited	Low Risk
DES-ASN26	Low Risk	Low Risk
HIS-O17	Significant	Not available
Lys-AC18	Significant	Significant
Q20E	Significant	Significant
Endo-gly28	Low Risk	Low Risk
End-Thr31	Low Risk	Low Risk

In Vitro MHC Binding Assay



Jawa et al. Frontiers in Immunology, 2020

In Vitro T-cell Assay (PBMC)

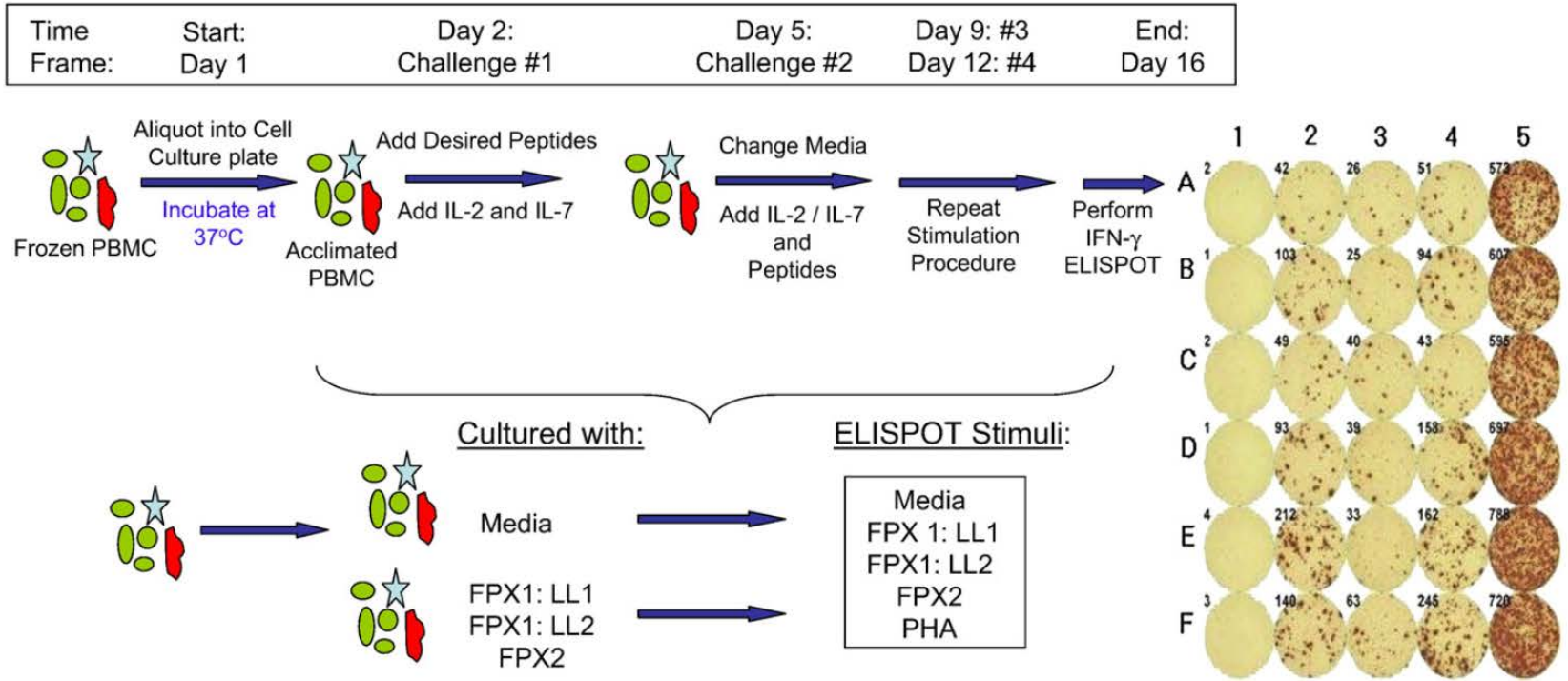


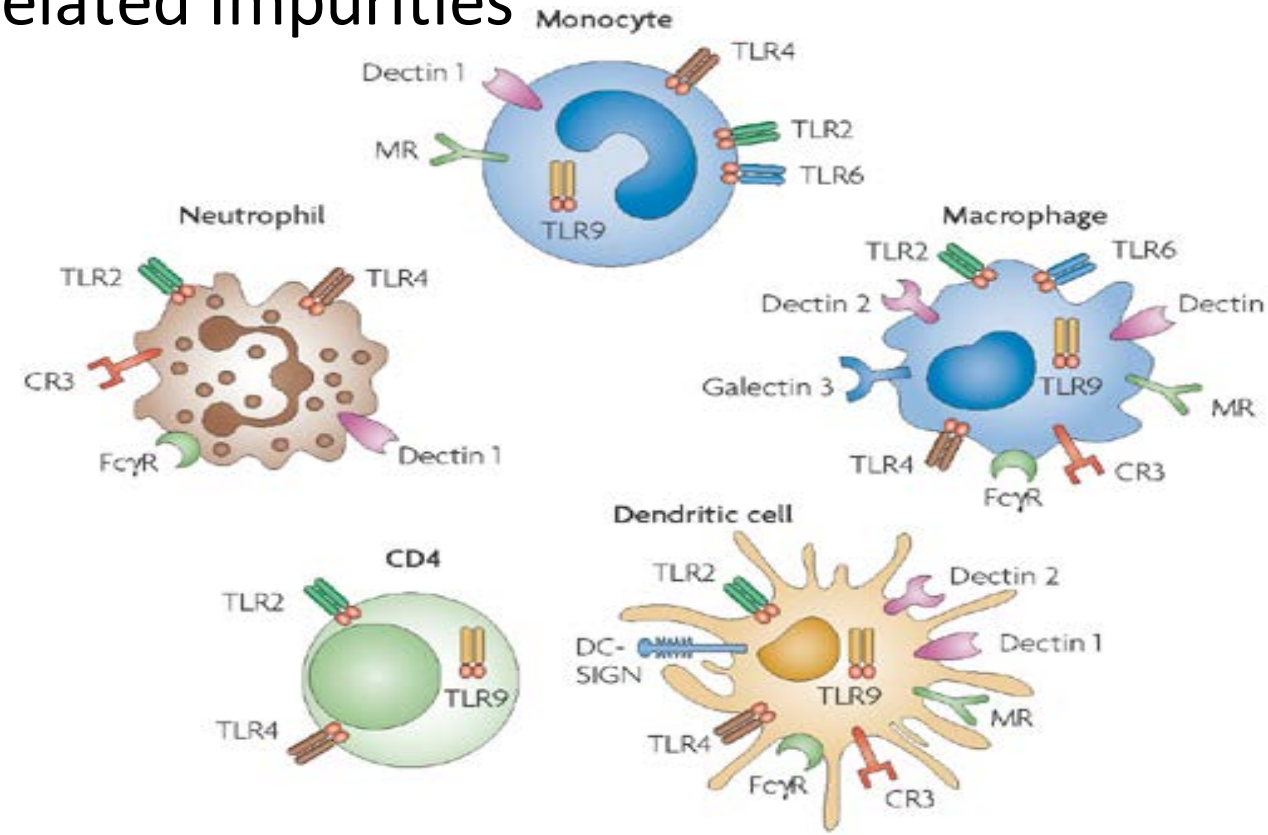
Figure 1 In vitro sensitization of PBMC assay format.

Wullner et al. Clinic. Immun. 2010

Some of the Common Deficiencies for In Vitro T-Cell and MHC Binding Assays

- Not demonstrating peripheral blood mononuclear cells (PBMC) population used is representative
  - HLA classes diversity
  - Inadequate subject population size
- Not enough experimental details provided (such as duration of the assay, number of cells per well, concentration of the product used, inadequate suitability controls, etc.)
- The sensitivity is not demonstrated through peptide concentration curve
- Not providing sufficient information about the statistical model and acceptance criteria

Innate Immune Receptors Can Recognize Process-related Impurities



Impurities (inorganic, microbial or mammalian) that are recognized by innate immune system receptors can:

- Activate the innate immune system
- Lead to local inflammation
- Facilitate antigen-specific immune response to exogenous proteins
- Help break tolerance to endogenous peptides/proteins

Netea et. al. Nature Rev. Micro. 2008

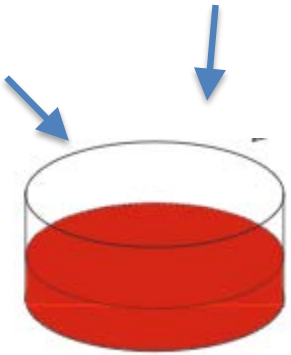
In Vitro Assays for Innate Immune Responses

Cell line	Origin	Commercial Availability
RAW-BLUE	Mouse macrophages	Yes
Macrophage-like-MonoMac6 (MM6)	Human monocytic cell	Yes
THP-1	Human monocyte	Yes
PBMC	Human macrophages, dendritic cells, monocytes and lymphocytes	Yes

PBMC Assays

PBMC or Whole blood

Test Product



Incubate

Collect plasma

And

Measure cytokines (ex. IL-6, IL-12, TNF-α, etc.) with multiplex cytokine kits

- Clinically relevant
- Donor to donor variability
- Complexity in obtaining, preparing and storing
- Key cells underrepresented (macrophages, dendritic cells, polymorphonuclear cells)

Combined Sensitivity of Monocyte/Macrophage Cell Lines to Impurities

- High reproducibility
- Easier to validate and transfer
- Good sensitivity

Receptor	Ligand	RAW-BLUE	MM6	THP-1
TLR4	LPS	100 pg/mL	10 pg/mL	10 pg/mL
TLR2	Pam_3CSK_4	500 pg/mL	500pg/mL	100 pg/mL
TLR3	PolyI:C	negative	negative	2.5 ug/mL
TLR5	Flagellin	negative	negative	100 ng/mL
TLR6	FSL-1	100 pg/mL	500pg/mL	100 pg/mL
TLR7	Imiquimod	50 ng/mL	negative	negative
TLR8	CLO75	50 ng/mL	Negative	negative
TLR9	CPG	12.5 nM (60 ng/mL)	negative	negative
TLR2	Zymosan	10 ug/mL	10ng/mL	10 ng/mL
NOD2	MDP	negative	10ug/mL	negative

Haile et al. PLoS. 2015

Some Common Deficiencies for Innate Immune Assays

- Not investigating innate immune response when there are no new impurities found
- Not providing rationale for the selected cytokine signal readouts
- Not sufficiently demonstrating sensitivity
- Using positive controls that would over-trigger immune response
- Not providing sufficient detail on the methodology

Additional Issues to Consider During Validation and Method Development

- Excipient's effect on cell cultures
- Sample handling that may contribute to assay variability
  - Use of fresh blood vs. frozen blood for PBMC assays
  - The handling of blood samples (obtaining, preparation and storage)
  - The age of the test products
- What threshold to use for demonstrating sameness/differences between the products
- What test peptide concentration to use in the assays
- Quality/purity of the impurities used for testing in the assays

Conclusion

- The immunogenicity risk of generic synthetic peptide products (i.e., the five peptides listed in the guidance) need to be comparatively assessed to the recombinant RLD product
  - Innate immune response and adaptive immune
- In silico assessment can enhance the overall immunogenicity assessment of the new impurities by testing against a wide range of population
- In vitro assessment can be used to validate and verify the in silico assessment
- Method justification and validation need to be provided with submission

Workshop Announcement

Non-clinical Immunogenicity Assessment of Generic Peptide Products: Development, Validation, and Sampling

To communicate current regulatory thinking and considerations on non-clinical assays for comparative immunogenicity risk assessment for generic peptide products.

Jan 26, 2021

Virtual only

Website: https://www.fda.gov/drugs/news-events-human-drugs/non-clinical-immunogenicity-assessment-generic-peptide-products-development-validation-and-sampling

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- Anne De Groot (EpiVax)

Challenge Question #1

If a proposed generic peptide product contains no new peptide-related impurity comparing to the RLD, what non-clinical immunogenicity assessment assay is needed?

A. In silico assessment on existing impurities

B. In vitro MHC binding assay on existing impurities

C. Innate immune response on the whole product

D. No immunogenicity study is recommended

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If a proposed generic peptide product contains no new peptide-related impurity comparing to the RLD, what non-clinical immunogenicity assessment assay is needed?

A. In silico assessment on existing impurities

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C. Innate immune response on the whole product

D. No immunogenicity study is recommended

Challenge Question #2

Which of the following is NOT true?

A. A proposed generic peptide product must use the same manufacturing process as the RLD's

B. Differences in manufacturing process could result in differences in impurities

C. Differences in impurities may affect safety and efficacy of a peptide product

D. Comparative risk of immunogenicity in generic peptide products may be assessed through non-clinical assays

Challenge Question #2

Which of the following is NOT true?

A. A proposed generic peptide product must use the same manufacturing process as the RLD's

B. Differences in manufacturing process could result in differences in impurities

C. Differences in impurities may affect safety and efficacy of a peptide product

D. Comparative risk of immunogenicity in generic peptide products may be assessed through non-clinical assays


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