Immunogenicity Assessments in Peptides: Progress and Remaining Challenges

Session 1: Complex Active Ingredients (Peptides & Oligonucleotides) & Immunogenicity

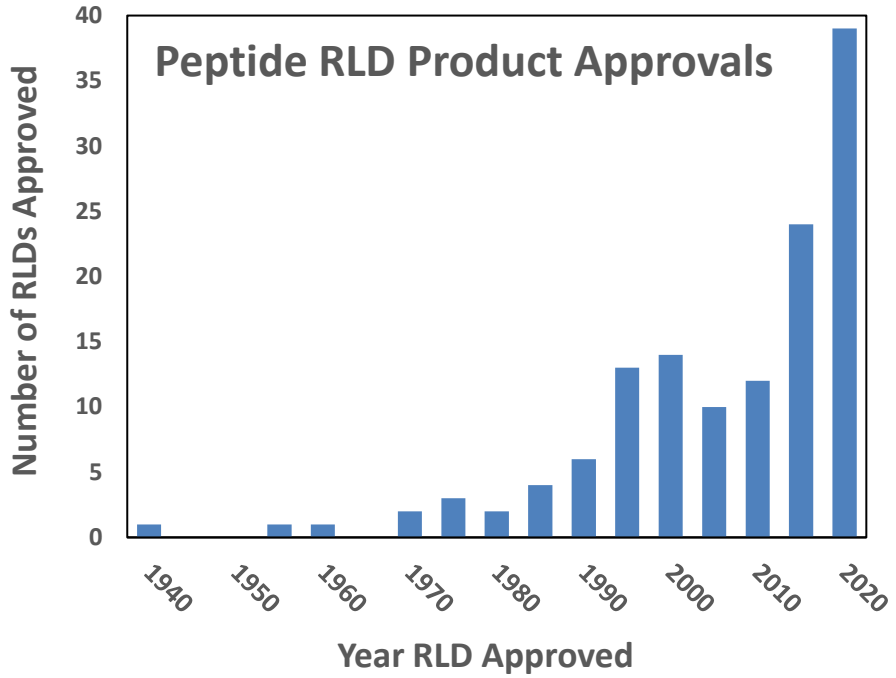
Eric Pang, PhD.
Senior Chemist
CDER/OGD/ORS/DTP1

Outline

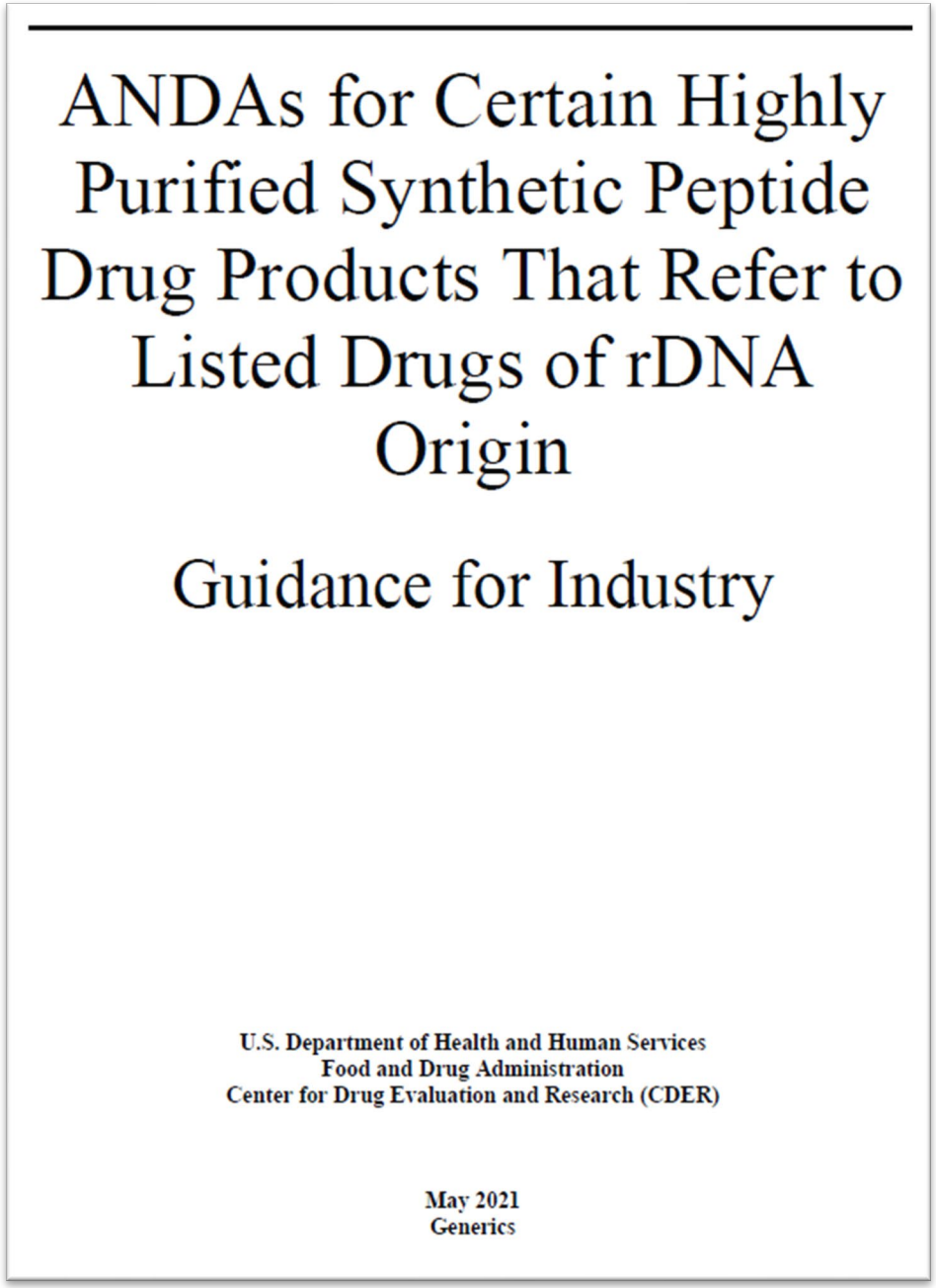
- Overview of peptides and synthetic peptide guidance
- Introduce in vitro in silico immunogenicity assessment (IVISIA)
- Current understanding and challenges with
  - In silico assessment
  - In vitro assessment
- Future research directions

Brief History of Peptide Drugs

- The *Biologics Price Competition and Innovation Act* created a regulatory definition and separate pathways for peptide drugs and protein biologics.*
  - Over 130 FDA approved peptide drug products are designated as a reference listed drug (RLD).
  - Advances in synthetic and recombinant manufacturing have given rise to increased development and approval of peptide drug products.



* https://www.federalregister.gov/documents/2020/02/21/2020-03505/definition-of-the-term-biological-product



for synthetic **Glucagon, Liraglutide, Nesiritide, Teriparatide, and Teduglutide** referencing recombinant RLDs

"ANDA applicant should provide justification for why the presence of such impurity would not be expected to affect the safety of the proposed generic synthetic peptide or its effectiveness as compared to that of the RLD, including with respect to the risk of **immunogenicity related to peptide-related impurities…**

…such data should demonstrate that each new impurity does not contain sequences that have an **increased affinity for MHC**, known as T-cell epitopes, and that the proposed generic synthetic peptide **does not alter the innate immune activity**."

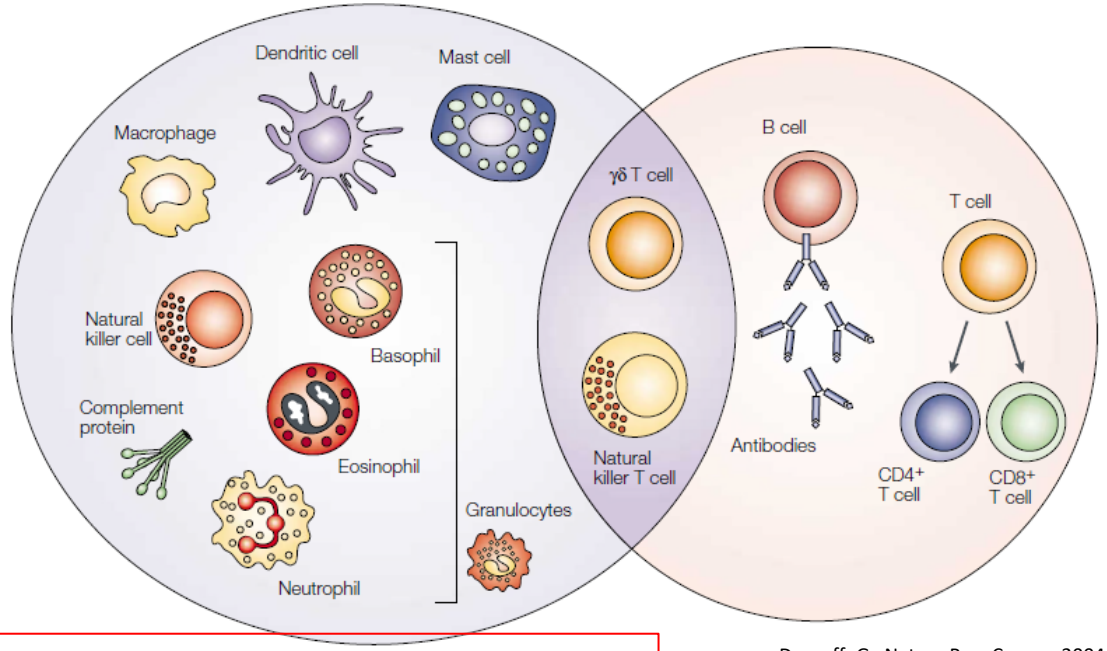
Impurity-related Immunogenicity Risk: Innate and Adaptive Immunities

Innate immunity

All **process-related impurities**
(contaminants, leachables)

⇩

Testing on whole product (independent of presence of new impurities)



Dranoff, G., Nature Rev. Cancer, 2004

Innate immune response modulating impurities (IIRMI) assays

Detect innate immunogenic potential of low levels of process and product-related impurities

Adaptive immunity

Peptide-related impurities
(e.g., deletions, insertions…)

⇩

Testing on each isolated impurity:

- T-cell epitope in peptide-related impurities
- New impurities in proposed generic (0.1%-0.5%)

In silico assays

In vitro cell-based assays to identify responsive T cells

Evaluating the Risk of Immunogenicity through IVISIA

- Adaptive immunogenicity assessment T-cell activation potential of peptide-related impurities
  - *in silico* studies MHC binding,
  - *in vitro* binding and functional assays

- Innate immune activity comparison between proposed generic and RLD products
  - *in vitro* cell-based assays

MHC = Major Histocompatibility Complex

In Silico Adaptive Immune Assessment

In silico immunogenicity risk analysis on peptide related impurities may be able to identify risk of impurities*

- Orthogonal approaches using in silico and in vitro may be valuable
- Developed in silico immunogenicity assessment tool to identify and predict the worst-case scenario in peptide impurities

Current challenges

- Accurately predict peptides with unnatural or modified amino acids
- Validation of in silico model
  - Performance of different in silico models from different sources
  - Assessing in silico model as part of the model master file

* Roberts et al. Frontiers in Pharmacology, 2024. https://doi.org/10.3389/fphar.2024.1363139

In Vitro Assay for Adaptive Immune Assessment

In vitro assays assess adaptive immune risk of peptide-related impurities through T-cell activation, proliferation which in turn potential to induce adaptive immune response

- Including MHC binding assay, MHC-associated peptide proteomics (MAAPs), T-cell proliferation, ELISpot assay, and dendritic T-cell assay
- In vitro T cell assays are often used orthogonally to in silico assessment

Current Challenges

- Significant variability in assay format and protocols making it difficult to assess assay performance and sensitivity
  - Developing working standards that can be used across various in vitro assays
- Understand the amount of impurity (concentration and dose of the drug) in relation to clinical impact

Innate Immune Response Assays

Innate immune response assays, once optimized and validated, can be used to detect certain process-related impurities

- In order to ensure that the assay is fit for purpose, key parameters including sensitivity, specificity, precision, impurity coverage, and the impact on cell viability need to be controlled*

Remaining challenges

- Effect of formulation buffer can negatively affect the assay readout**
- Improving and standardizing in vitro immunogenicity assays by funding research projects via collaborations with industry and academia to refine and align these assays.

*Holley et al. Molecules, 2021. https://doi.org/10.3390/molecules26247461

**Thacker et al. Front Immunol, 2022. https://doi.org/10.3389/fimmu.2022.970499

Research Directly Contribute to Regulatory Actions

Immunogenicity risk assessment and understanding evolve due to research effort and internal knowledge gained

- Risk of a generic peptide product moved away from RLD's immunogenicity risk to risks associated with product-specific differences
- Revised and new product specific guidances

Notable approvals

- Dec 2021, Vasopressin injection - Small peptide widely used as a potent vasopressor that FDA outlined a stepwise application specific approach for assessing immunogenicity risk.
- Nov 2023, Teriparatide injection - One of the first approved generic peptides outlined in the guidance ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin.
- Dec 2024, liraglutide injection - First generic GLP-1 peptide approved

Future Directions

- Continue the support for developing standards for IVISIA
  - Facilitate the development of working standards for validating the performance of innate and adaptive immunogenicity assays on peptide products
  - Fund research and publish on the best practices for conducting in vitro studies
- Additional research programs into immunogenicity risk assessment for recombinant peptides and oligonucleotides will enable the development and assessment of these complex generics
  - Assessment of host cell proteins in recombinant peptides
  - Immunogenicity risk of oligonucleotide impurities

Acknowledgement

Internal and External Researchers and Collaborators

OGD/ORS

OPQ/OPQA and OPQR


FDA
U.S. FOOD & DRUG
ADMINISTRATION