Assessing Immunogenicity Risk of Peptide Drugs: Generic Drug Perspective

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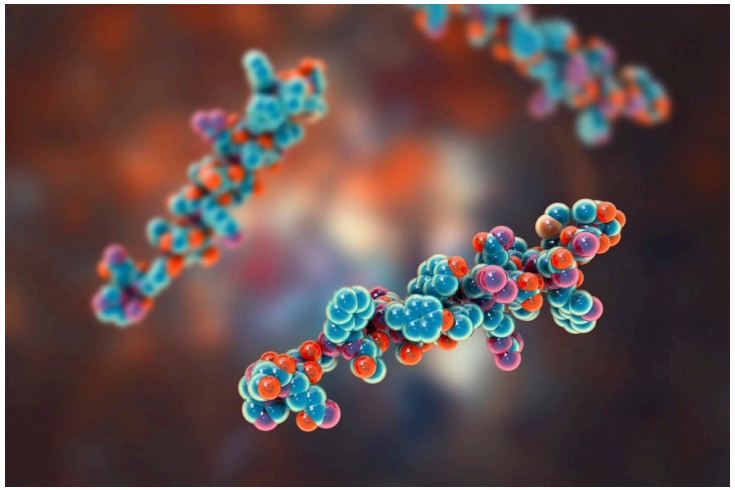
This presentation reflects the views of the presenter and should not be construed to represent FDA's official views or policies.

Any representation of brand name drugs in this talk are for purposes of scientific discussion and specific comparison.

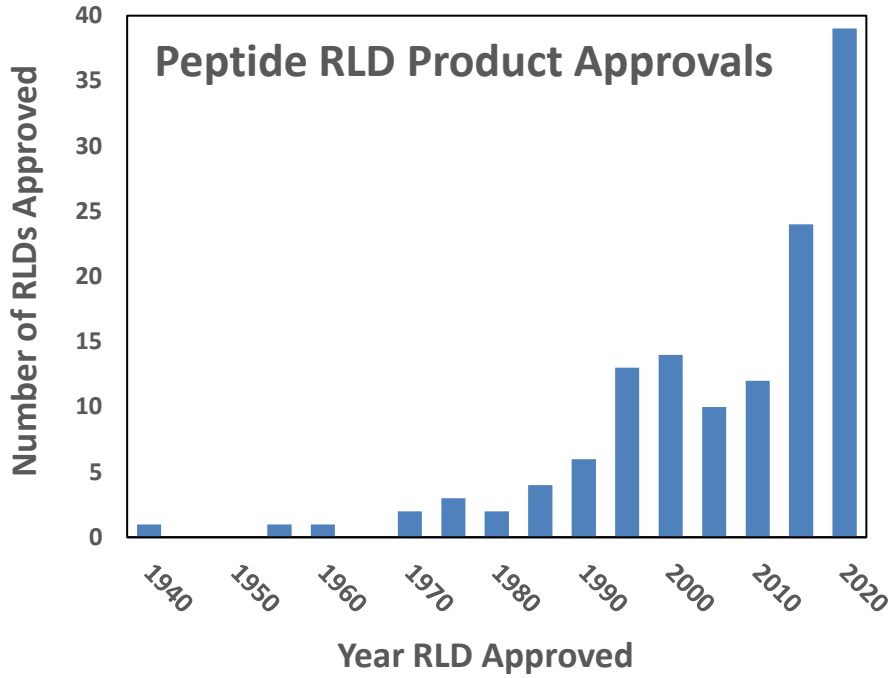


Outline

- Background:
  - Overview of peptide drugs
  - Synthetic peptide guidance
  - Overview of *in vitro in silico* immunogenicity assessment (IVISIA)
- In vitro immunogenicity assays and challenges
- Future development and directions
  - Generic recombinant peptides

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 peptide 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

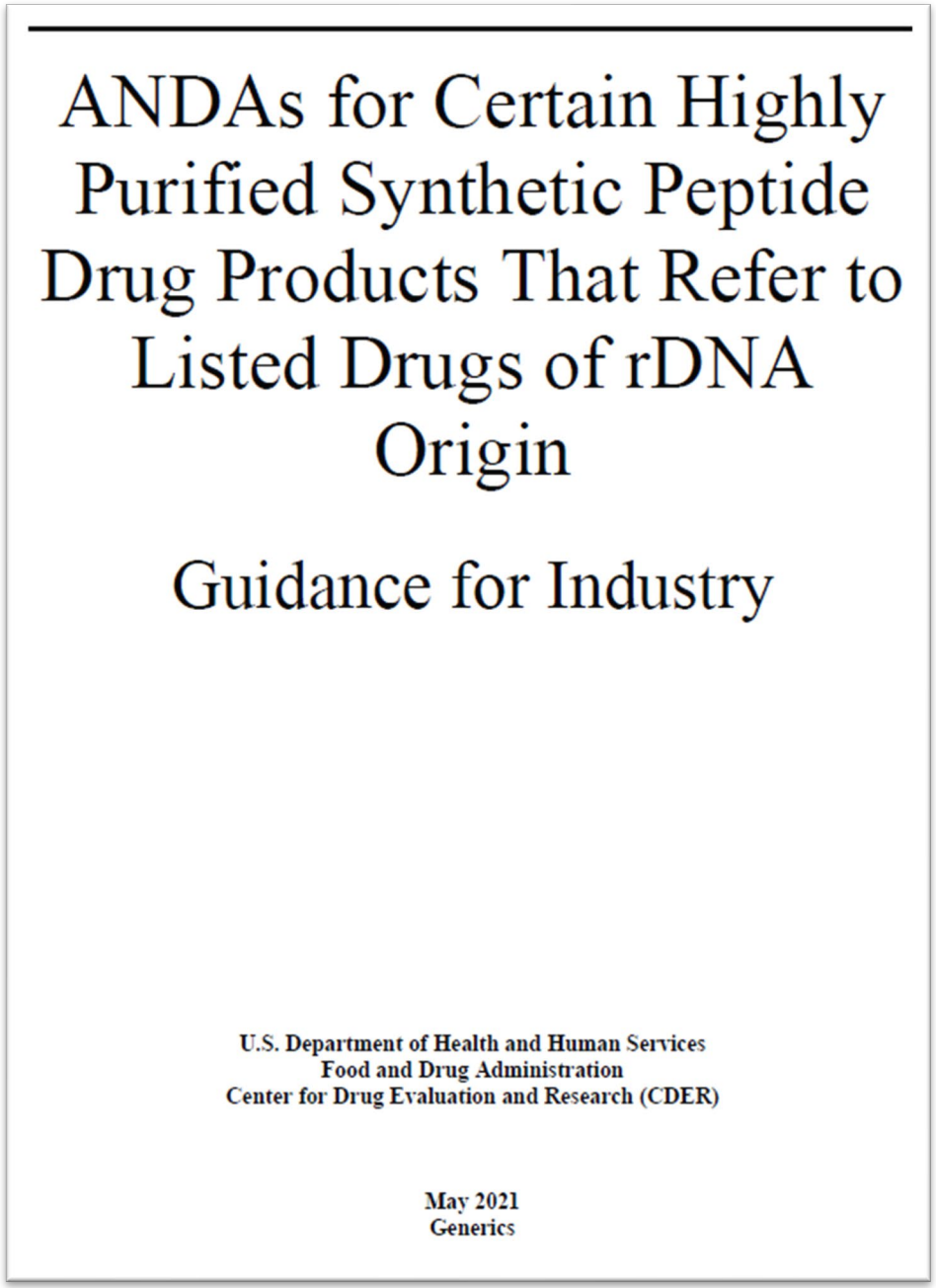
Differences in Manufacturing and Impurities of Peptide Drugs

Manufacturing pathways

- Synthetic - made by chemical synthesis (e.g., step-by-step amino acid synthesis addition)
- Recombinant (rDNA origin) - peptide extracted from recombinantly expressed yeast or bacteria cells
- Extraction from natural sources

Different manufacturing process can result in different impurities, *which may give rise to different safety risks*

- Process-related (host cell proteins, leachable extractables, microbial contaminant, etc.)
- Peptide-related (impurities related to the active peptide, such as deletion, duplication, etc.)



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, …"

Assessing Immunogenicity Risk for Peptide Products

Immunogenicity May Impact Safety and Efficacy

- Developing antibodies can affect the pharmacokinetics (PK) by enhancing or delaying clearance
- Neutralizing antibodies can diminish efficacy, and there is concern of cross-reactivity with endogenous non-redundant proteins

Generic peptides approval relies on the finding of safety and efficacy of the RLD and should have no greater risk than RLD

- Generic drug is essentially copies of the approved RLD

But…. what about the differences between proposed generic and RLD?

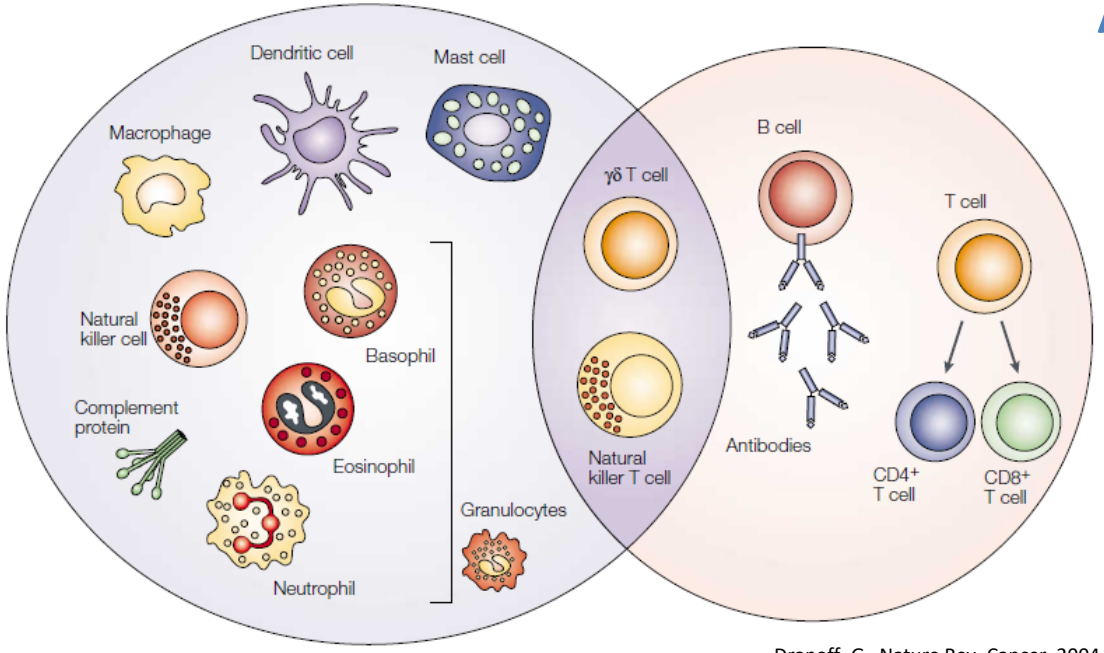
Potential for Impurity-related Immunogenicity Risk: Innate and Adaptive Immunities

Innate immunity

All **process-related impurities**
(host cell impurities, contaminants)

⇩

Testing on whole product



Dranoff, G., Nature Rev. Cancer, 2004

Adaptive immunity

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

⇩

Testing on individual impurity: T-cell epitope in peptide-related impurities

Innate immune response modulating impurities (IIRMI) assays

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

In silico assays

In vitro cell-based assays to identify responsive T cells

In Vitro In Silico Immunogenicity Assessment (IVISIA)

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* studies MHC binding and T-cell response

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

MHC = Major Histocompatibility Complex

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In Vitro Assays to Assess MHC Binding

In vitro MHC binding

❑ **Focus on binding**, broad spectrum of MHCs, high throughput.

However:

- ❖ Affinity of displaced peptides plays a critical role
- ❖ MHC supertypes, usually HLA-DR

MAPPs (MHC associated Peptide Proteomics) Assay

❑ **Native Ag** processing & presentation.

However:

- ❖ Low throughput and complex
- ❖ Sufficient donors to cover presentable regions
- ❖ Most MAPPs studies are restricted to using HLA-DR

- ➢ Justify method used (size, MHC, target population)
- ➢ Cell selection, culture conditions, loading, maturation, and monocyte derived dendritic cell (moDC) lysis, peptide elution & immunoprecipitation conditions
- ➢ LC-MS/MS settings
- ➢ Data analysis and algorithms

Adopted from slides presented at CRCG 2024, Mohanraj Manangeeswaran

In Vitro Assays to Assess T Cell Responses

- Multiple formats:
  - T cell proliferation assays
  - Cytokine ELISPOT (Interferon Gamma)
  - DC:T cell assays

- Low throughput and complex
- High donor-donor variability
- Low frequency of naïve T cells
- Drug formulation interference
- **No standardized protocols**
  - APC prep
  - Restimulation & clone expansion
  - Readout

- **Provide clear experimental design and culture conditions**
  - Justify study design (size, MHC, target population, readouts)
  - Culture conditions (DC:T cell ratio) & product concentration
  - Few responding T cells (0.1-10 specific T cells/M)- screen at least 2 M/condition.
  - Readout selection (proliferation, cytokines, cell markers)
  - Suitability controls that confirm sensitivity for naïve T cell responses

Adopted from slides presented at CRCG 2024, Mohanraj Manangeeswaran

Typical Deficiency: Positive Control for Adaptive Immune Response Assays

- To confirm that a test peptide is not immunogenic, we need to make sure that the assay system used has the sensitivity and specificity needed to capture a potential novel (naïve) T cell response.
- A good suitability control peptide will confirm your assay, under the conditions used, can detect naïve T cell responses to peptide epitopes that are similar to the test material (i.e., positive control peptide with known immunogenicity in the clinic or in-house impurity peptides known to elicit a naïve T cell response).
- LPS, PHA, KLH, PADRE, SEB, CEFT and PPD can be used as assay controls to confirm the presence of live and responsive APC in the culture, but do not inform on the sensitivity or specificity of your assay to capture naïve T cell responses.
- Establish that your positive control does not have innate immune response modulating or other impurities that can trigger an immune response or act as adjuvants as this can distort/misrepresent the sensitivity of the assay.

Typical Deficiency: Concentration of Peptide Impurities Used in the Assay

To allow for interpretable data, the immunogenicity risk of the peptide impurities should be tested under conditions that ensure adequate presentation by Antigen Presenting Cells (APCs). Test the impurities at the same concentration as the suitability control.

Note that the comparative assessment is used to determine whether the impurity poses an immunogenicity risk which then informs the degree of control needed to mitigate the risk. The concentration of the impurity tested in the assay does not directly determine the acceptable limits for that impurity. That determination is made using a totality of evidence approach. The acceptability of the specifications will be a review issue.

Test article	Concentration tested in the assay
Product related impurity 1	*0.15 µg/mL*
Product related impurity 2	*0.075 µg/mL*
API	*10 µg/mL*
Suitability control peptide 1	*20 µg/mL*

Adopted from slides presented at CRCG 2024, Mohanraj Manangeeswaran

Typical Deficiency: Monitoring antigen specific T cell response

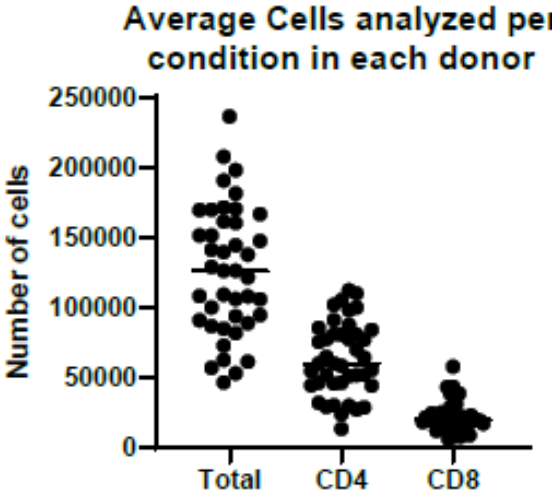
Number of T cells screened is too low to detect responder naïve T cells:

Hypothetical example:

Each well of a T cell activation assay is seeded with 5x10^4 PBMCs. Ten replicate wells are used for each test condition for a total of 500,000 cells/condition. The cells are harvested after stimulation with the test peptide for a week to measure test-article specific IFN γ production in the assay. The flow assay collects 300,000 cells/condition.

Is this adequate to identify a naïve T cell response?

- As the frequency of naïve T cell responses responding to a specific epitope is in the range of 0.2 to 10 cells per million naïve CD4+ T cells, we recommend the screening of at least 2 million T cells per test condition.
- Short assays allows for no or minimal expansion of T cell clones hindering detection. As the frequency of the antigen specific T cells is low, expansion of the responding clones will increase the chance of capturing the T cell response.
- While IFN γ production is a good marker for T cell activation. Consider the use of additional markers of Th2 or Th17 responses such as IL4, IL5, IL13 or IL17 to generate a more complete assessment.



	Total	CD4	CD8
Mean	126351	63175	21699

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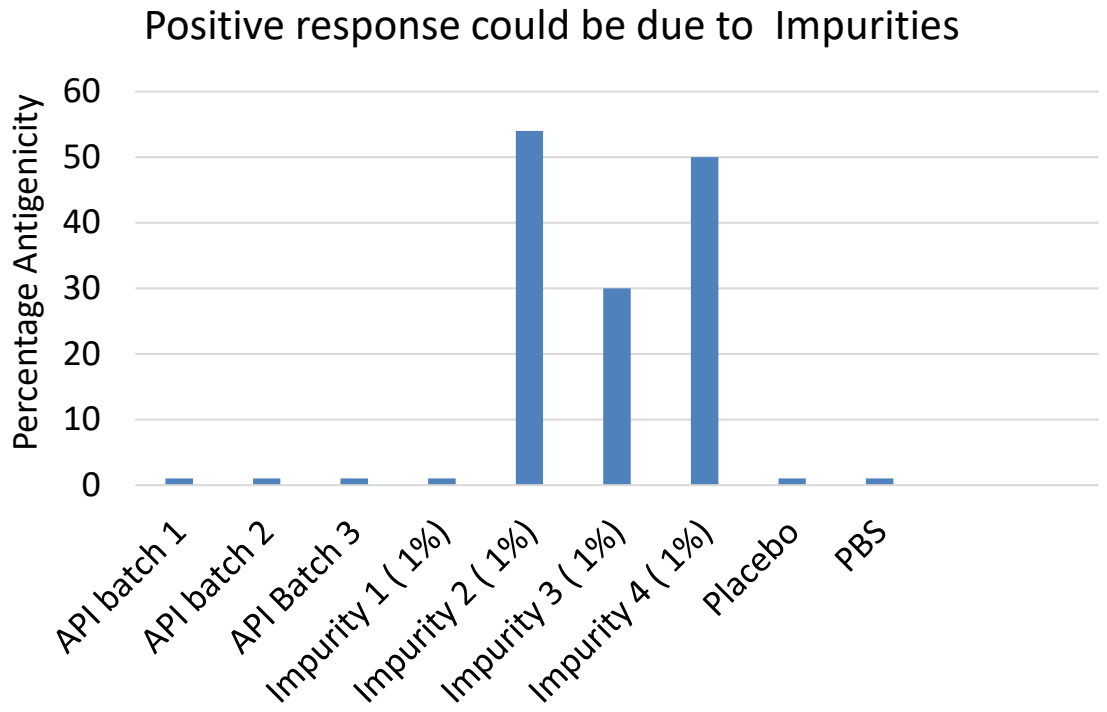
Typical Deficiency: Purity of impurities

Control the quality of your test materials (API, impurities, positive controls) for the adaptive immune response assay.

If the response of your tests assessing T cell responses to an impurity is positive, data establishing the purity of the tested peptides is critical to understand whether the response is due to the peptide impurity or to a non-specific impurity in the peptide preparation tested.

Test peptide preparation for innate immune response modulating and other impurities that could act as adjuvants or elicit a T cell response



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Work in Progress…

- Recommend 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

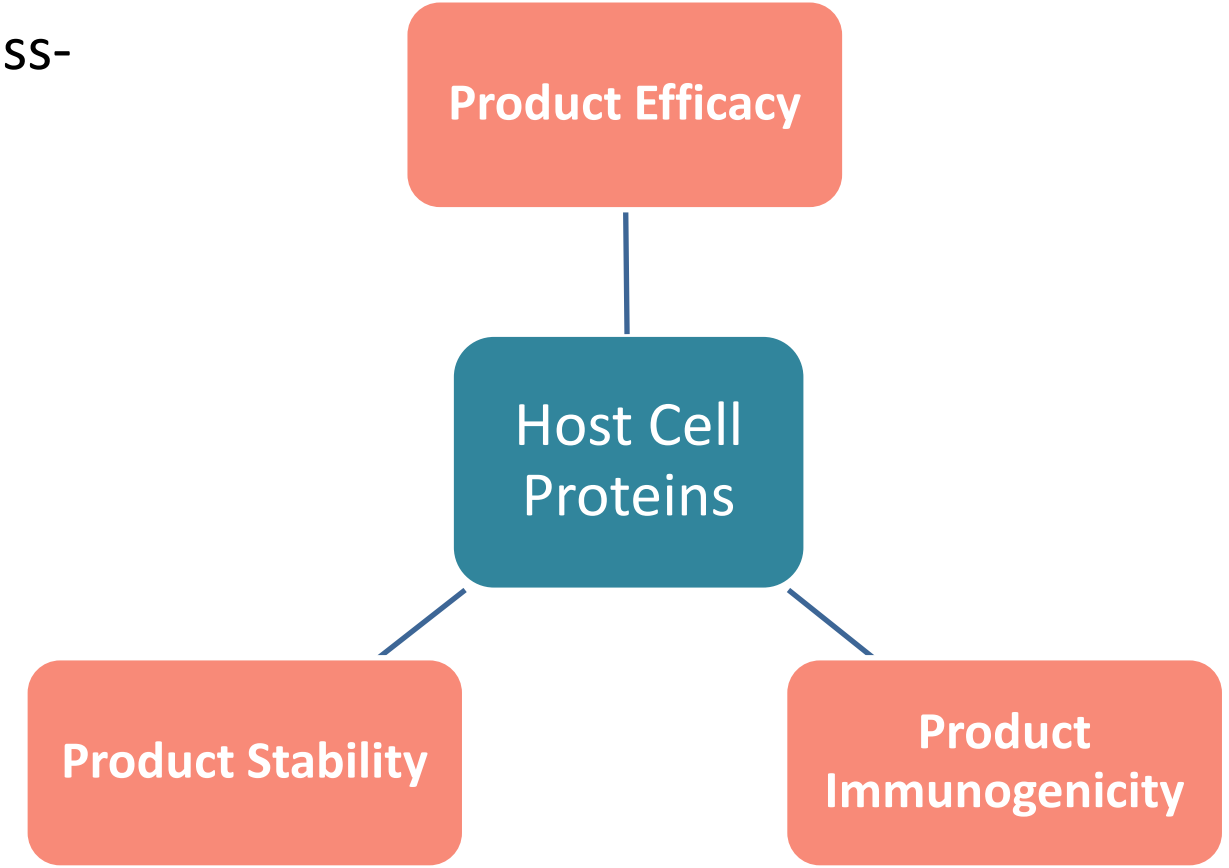
We fund GDUFA-related research!

https://www.fda.gov/drugs/generic-drugs/generic-drug-research-collaboration-opportunities

Immunogenicity Risk in Follow-on Recombinant Peptides: Product- and Process-Related Impurities

- Most of the comparative immunogenicity risk evaluation is focused on product- and process-related impurities

- Main differences between a follow-on recombinant peptide product and the product it references, are in a subset of process-related impurities: **host cell remnants/proteins**



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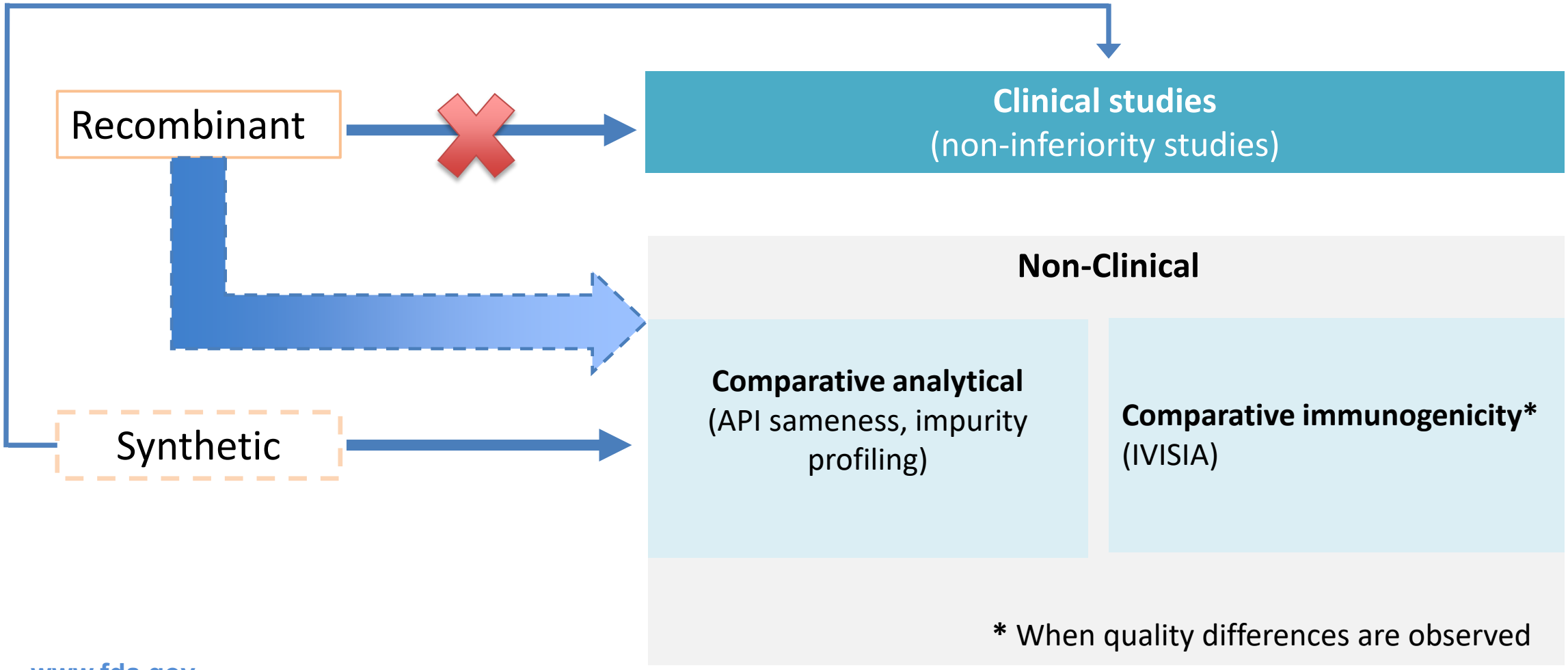
Immunogenicity Assessment of Generic Peptides & Role of *In Vitro and In Silico* Immunogenicity Assessment



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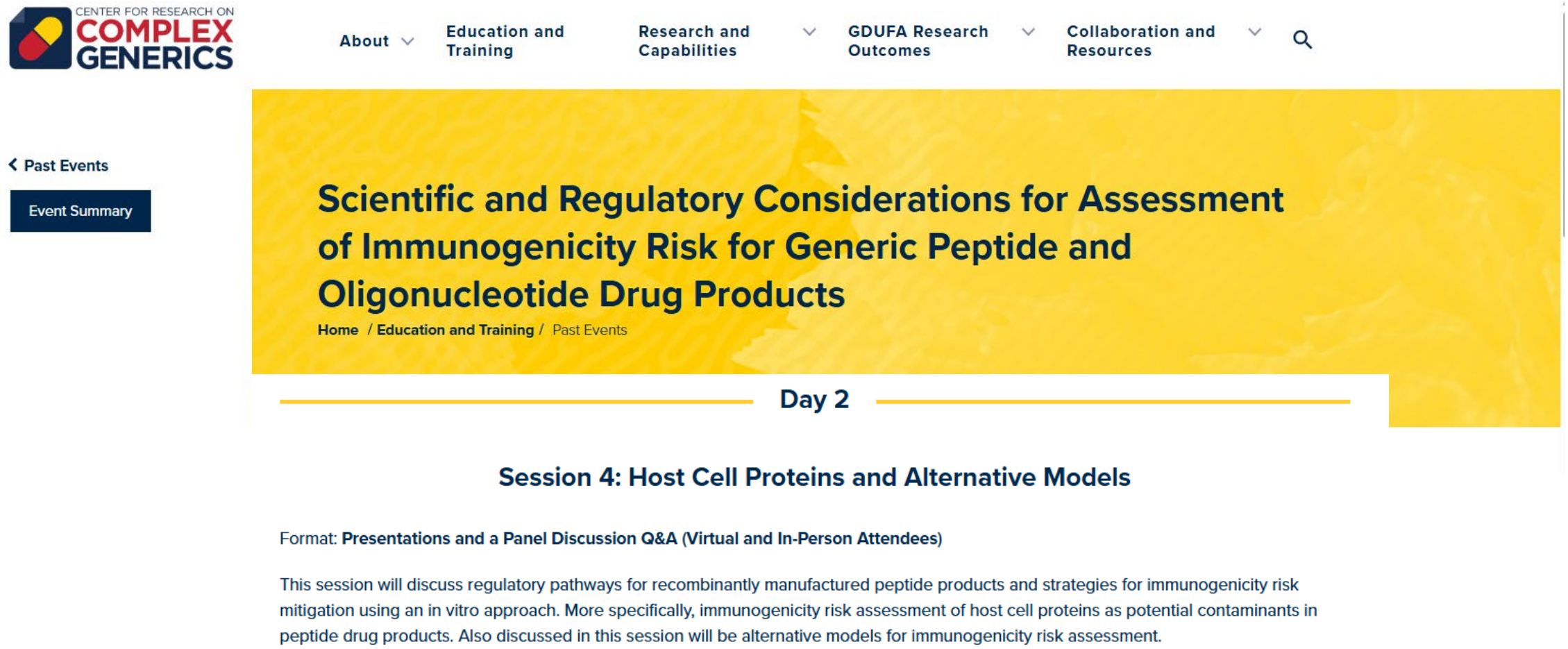
Federal Register Notice for Immunogenicity Risk Assessment of Follow-on Recombinant Peptides

Comments received regarding :

1) Methods currently used for characterization, identification and quantification of Host Cell Proteins (HCPs) in recombinant peptide products.
2) Routinely achievable levels of total HCPs in well controlled recombinant peptide manufacturing process
3) Generally achievable percent coverage of the HCP spectrum for HCP quantification assay
4) Characteristics of HCPs associated with higher likelihood of adverse clinical sequelae
5) Functional assays to assess immunogenicity risk of follow-on products with different HCP profiles

CRCG Conference on Immunogenicity Risk Assessment for Peptides



https://www.complexgenerics.org/education-training/scientific-and-regulatory-considerations-for-assessment-of-immunogenicity-risk-for-generic-peptide-and-oligonucleotide-drug-products/

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