# Regulatory Considerations for Complex and Synthetic Peptides – Not Just Another Generic

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# Abbreviations

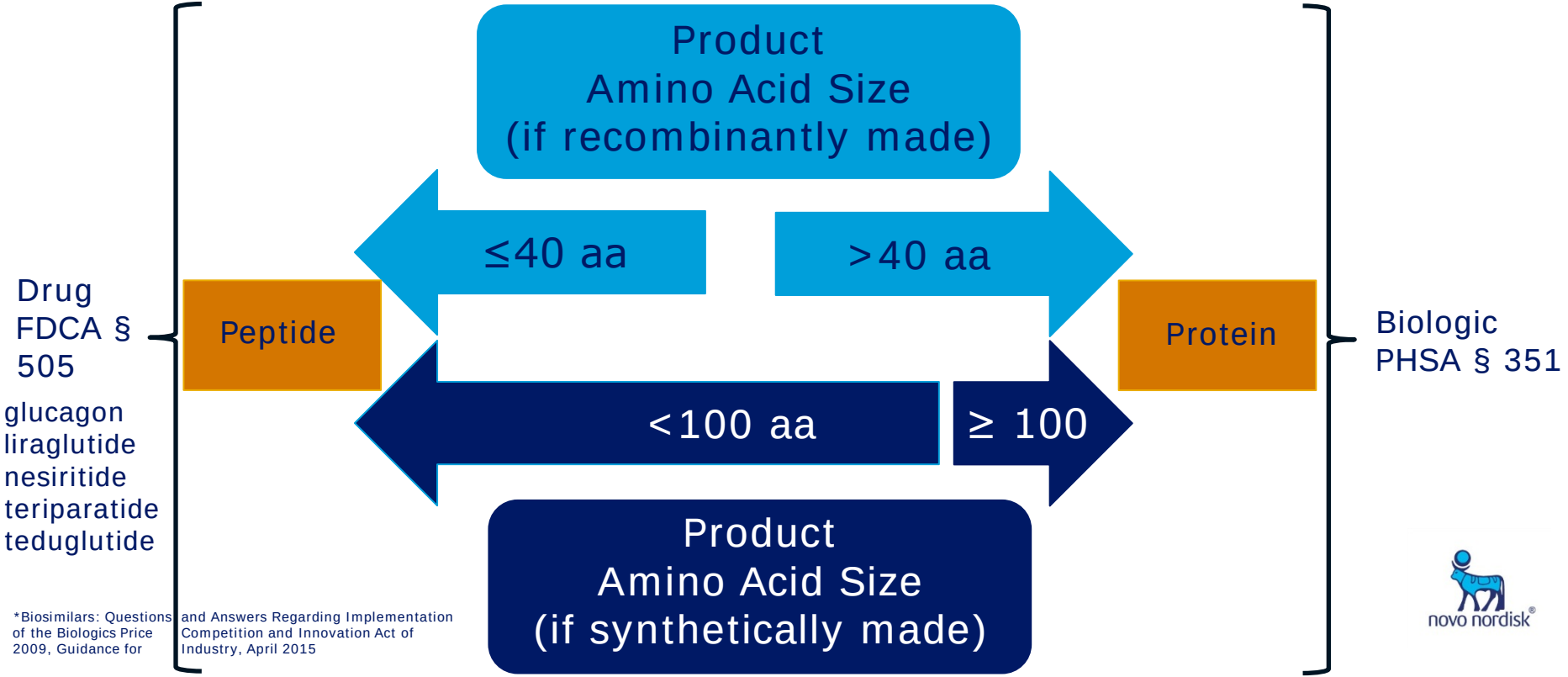
- RLD = Reference Listed Drug
- ANDA = Abbreviated New Drug Application (US FDA)
- ADA = Anti-drug Antibody
- aa = amino acid
- FDCA = Food Drug and Cosmetic Act
- PHSA = Public Health Service Act
- rDNA = recombinant deoxyribonucleic acid
- GLP-1 = Glucagon-like peptide 1
- RP-HPLC = Reversed-phase high-performance liquid chromatography
- LC-MS = Liquid chromatography–mass spectrometry
- MHC II = Major Histocompatibility Complex II

# Factors that can impact safety, efficacy, and potency in peptide products

- Structure
- Impurities
- Immunogenicity
- Manufacturing Process

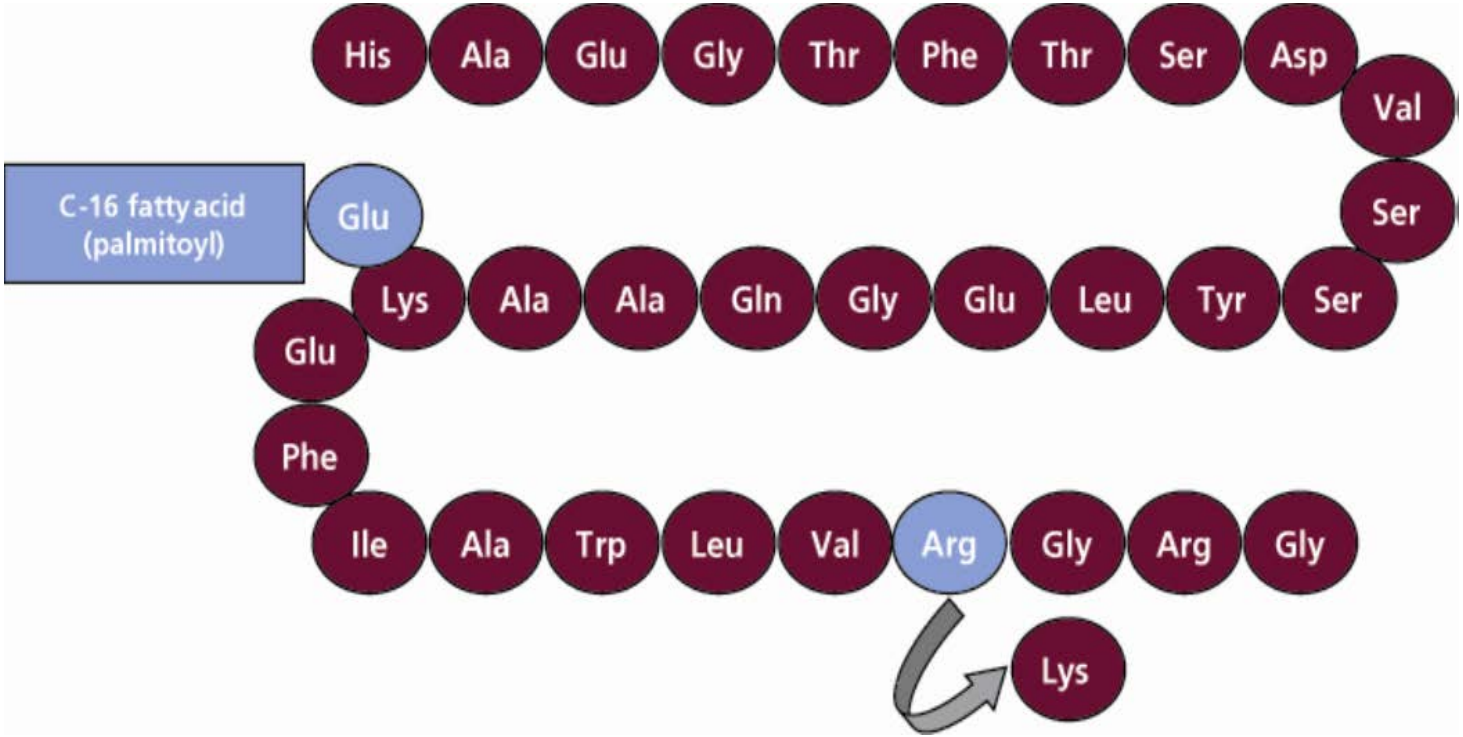
# What is a Peptide vs a Protein?

- The US FDA has defined peptide and protein in FDA guidance*:
- The definition differs from the rest of the world




*Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009, Guidance for Industry, April 2015

# An example: Liraglutide GLP-1 Molecule, 31 aa

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# ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin*

- In the past, analytical methods have not always been capable of adequately characterizing peptide products for submission in an ANDA. However, given the current state of technology for peptide synthesis and characterization, **FDA now believes it is possible for an ANDA applicant to demonstrate that the active ingredient in a proposed generic synthetic peptide is the same as the active ingredient in the RLD** that is of rDNA origin, and demonstrate that such products are pharmaceutical equivalents.

- Specifically, this guidance covers the following five 20 peptide drug products: **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, October 2017 (Draft — Not for Implementation), https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf

# Peptide Structure Matters

❖ FDA has described a “peptide” as having “less three-dimensional structure” than a “protein,” and, therefore, “generally characterized more easily than proteins.”*

- While peptides may be shorter amino acid sequences they can, and often do, have 2° and 3° structure
- Improper folding or receptor interaction can impact safety, efficacy and potency
  - Liraglutide adopts both well-defined secondary (alpha-helix) and tertiary structure (oligomerisation) and correct folding is critical for receptor interaction and potency of the molecule
  - Protein aggregates where the monomer is structurally perturbed may lead to unfavorable properties and loss of potency

*FDA, *Guidance for Industry: Biosimilars - Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009* (April 2015), at 15, available at https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM444661.pdf.

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# Threshold levels are not scientifically justified & immunogenicity can't properly be determined without clinical immunogenicity studies in humans

- FDA Draft Guidance* recommends follow-on ANDA manufacturers:
  1) Identify each peptide-related impurity that is 0.10 percent of the drug substance or greater, and states generally new specified peptide-related impurity levels of no more than 0.5 percent of the drug substance **do not raise immunogenicity concerns**
  2) For each new specified peptide-related impurity that is not more than 0.5 percent of the drug substance, the ANDA **applicant should characterize the** impurity and provide justification for why such impurity does not affect the safety
  3) Demonstrate for each new impurity that the impurity **does not contain sequences that have an increased affinity** for major histocompatibility complex (MHC), known as T-cell epitopes
  4) Demonstrate that the proposed generic synthetic peptide **does not increase the aggregation propensity or the quality of the aggregates formed**, especially under stress conditions, and does not contain impurities or contaminants that produce a greater or distinct stimulation of innate immune activity as compared to the RLD

*ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin, Guidance for Industry, Draft Guidance, October 2017 (Draft — Not for Implementation), https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf

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# Product Homology Immunogenicity Considerations

- A lower homology to native peptide may lead to higher ADA formation
- However, a higher homology increases the likelihood that ADAs will be cross reactive to native peptide

**Example:** GLP-1 peptide agonist product homology to native human GLP-1

Liraglutide: 97% homology
Taspoglutide: 93% homology
Exenatide: 53% homology

However, ADA formation between taspoglutide and liraglutide, that both have a >90% homology to native GLP-1, differ greatly, suggesting other factors are involved

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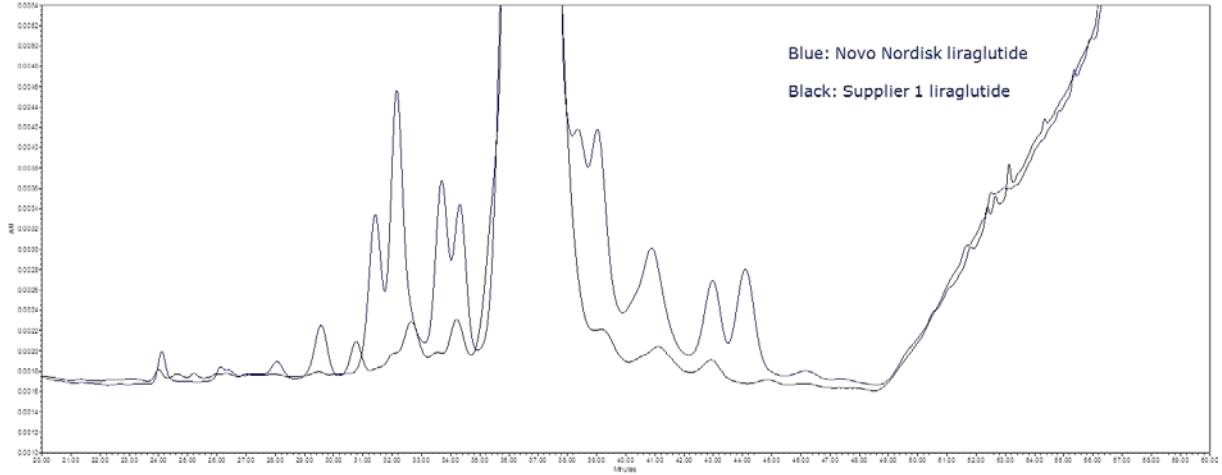
# Recombinant vs Synthetic Manufacturing Results in Different and Untested New Impurities

- Tested liraglutide drug substance batches, Supplier 1




***Overlay plot of RP-HPLC purity analysis 044-SM-7004 of Novo Nordisk's liraglutide drug substance and liraglutide drug substance from Supplier 1***

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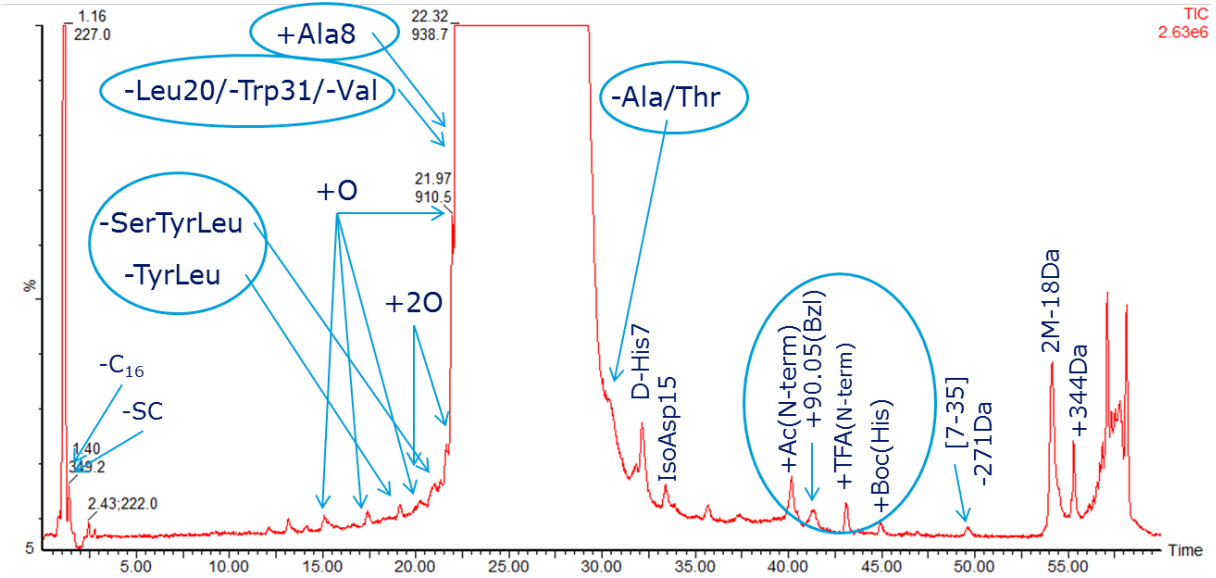


***LC-MS analysis of Supplier 1 Drug Substance. New and clinically unqualified impurities compared to Novo Nordisk's liraglutide are marked with blue circles.***

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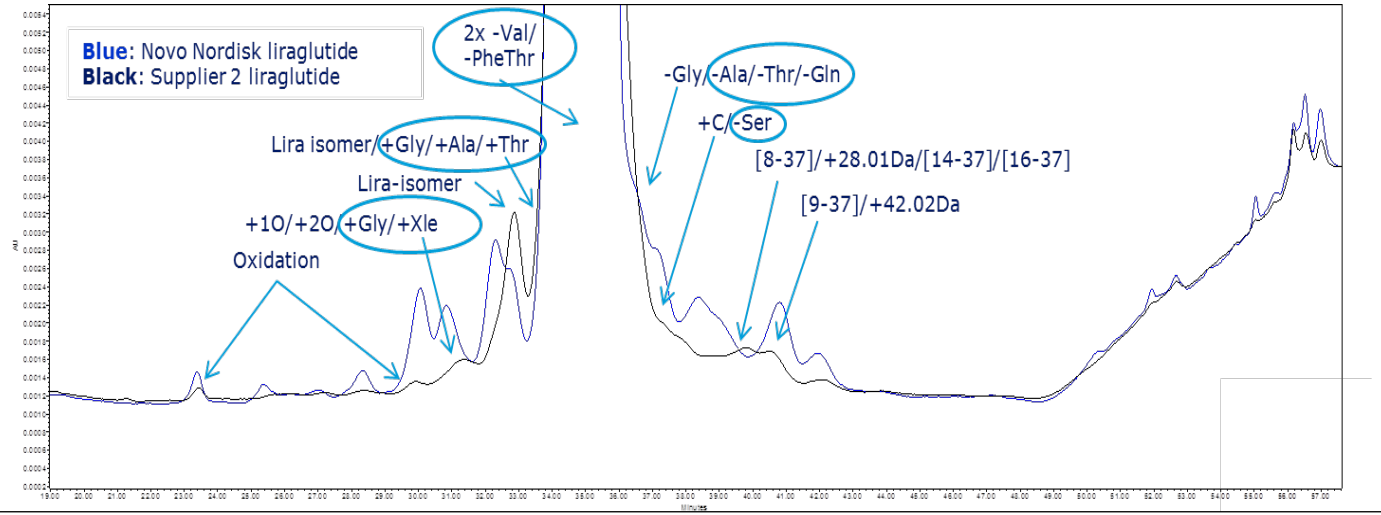
# Recombinant vs Synthetic Manufacturing Results in Different and Untested New Impurities

- Tested liraglutide drug substance batches, Supplier 2




***Overlay plot of RP-HPLC purity analysis 044-SM-7004 of Novo Nordisk's liraglutide drug substance and liraglutide drug substance from Supplier 2. New and clinically unqualified impurities compared to Novo Nordisk's liraglutide drug substance are marked with blue circles***

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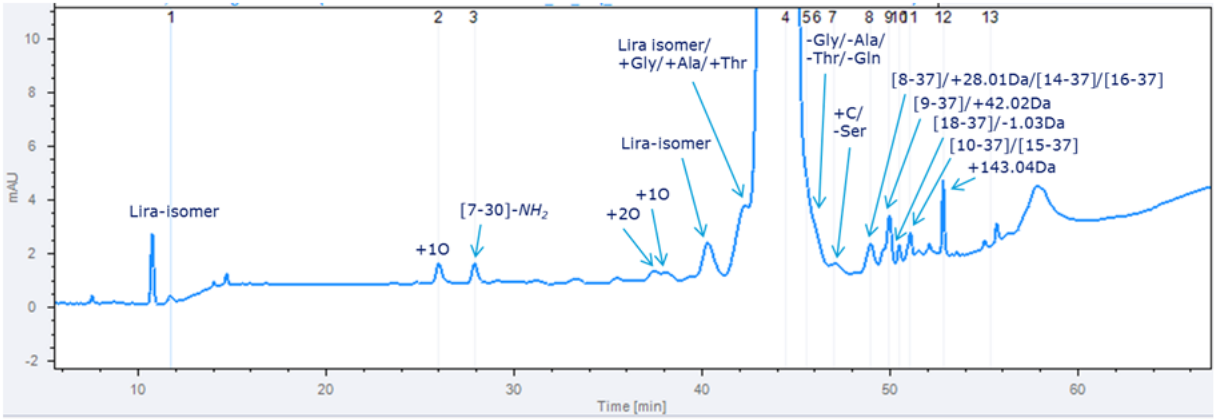
# Recombinant vs Synthetic Manufacturing Results in Different and Untested New Impurities

- Tested liraglutide drug substance batches, Supplier 2




***2D-LC-MS ID of liraglutide impurities of Supplier 2 Drug Substance***

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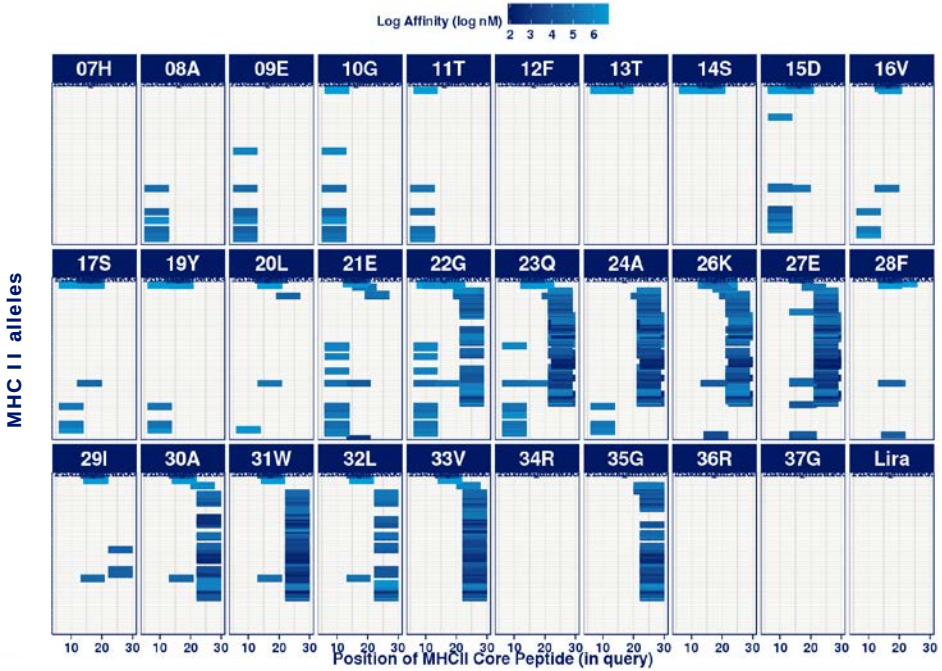
# Peptide-related impurities from synthetic liraglutide contains potential T-cell epitopes

- Neoepitopes resulting from the sequence modifications in the impurities were identified by subtracting MHC II binding epitopes of the liraglutide backbone sequence




***Predicted MHC II binding neoepitopes (T-cell epitopes) for the backbone sequence of impurities with a single amino acid deletion at the position indicated***

*Graphic representation of the predicted MHC II-binding neoepitopes and their binding affinity to the tested MHC II alleles. Each bar represents a distinct peptide with its position in the sequence (X axis) and its binding affinity to a given MHC II allele (Y axis). Per definition, no MHC class II binding neoepitopes are identified for the reference (liraglutide (Lira) backbone sequence).*

* algorithm NetMHCIIpan (3.1) was used to predict binding to HLA-DR and HLA-DP/DQ isotypes

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# Content of residual solvents, salts and metal ions can differ from synthetic vs recombinant manufacturing

| | Supplier 1 [ppm] | Supplier 2 [ppm] | Novo Nordisk [ppm] |
|---|---|---|---|
| Ethanol | 417 | 300 | <40 |
| Methanol | <20 | 843 | <20 |
| 2-propanol | 24 | 402 | <20 |
| Acetonitrile | 68 | 353 | <20 |
| Acetate | 28,000 | 500 | ≤300 |
| $K^+$ | 53 | ≤2.5 | 8.3 |
| $Cu^{+/2+}$ | 2.5 | 0.93 | ≤0.5 |
| $Zn^{2+}$ | 6.2 | 7.8 | ≤0.5 |

***Residual solvents, salts and metal ions in Supplier 1 Drug Substance, Supplier 2 Drug Substance, and Novo Nordisk liraglutide drug substance.***

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# Physical stability of some peptides is dependent on scale and production equipment

- Some peptides are known to have higher fibrillation/aggregation tendency
  - Glucagon
  - Liraglutide
- Some peptides are sensitive to manufacturing stress and have a tendency to become less physically stable with scale up
- For some peptide products, losing their higher order structure is directly linked to increase in the risk of fibrillation, thus increasing the molecules immunogenicity potential

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# Fibrillation of Glucagon: An extreme case

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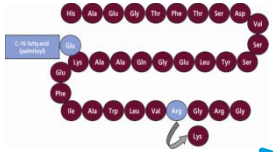
# Immunogenicity can be impacted at different stages of manufacturing



Active ingredient
(and peptide/non peptide related impurities)

Production methods impact

Container/closure

Impurities
(peptide/non peptide related)

Fibrillation/ Aggregation

Overall impact on immuno-genicity

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# Conclusion

- Analyses conducted by Novo Nordisk on two purported copies of liraglutide drug substance reveal that the two purported copies contain multiple impurities not present in Novo Nordisk's liraglutide drug substance and that could potentially result in immunogenicity reactions or other adverse health consequences.
- Differences from the RLD drug substance and a fundamental variation in the source or process can result in significant changes in impurity profile which may impact immunogenicity, and thereby safety and efficacy
- Unlike generics, many peptide sequences have secondary and tertiary folding critical to potency of the molecule
- A completely different manufacturing process will result in **new** peptide related and unrelated impurities (including leachables), many of which are clinically unqualified

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# Conclusion

- Currently available analytical methods are insufficient to establish the clinical “sameness” of a different manufacturer’s purported synthetic follow-on and properly assess immunogenicity for new impurities
  - Can’t assess whether purported generic is “identical” to RLD, or
  - Whether a purported follow-on can be expected to have the same therapeutic effect

  Both are statutorily required for approval under the ANDA pathway
- Analytical assessment:
  - Doesn’t assess B cell activation & maturation into antibody-producing plasma cells
  - Won’t address patient-related factors and impact of repetitive injections
  - Were developed and optimized for therapeutic proteins and monoclonal antibodies for purposes of initial determination of immunogenicity potential, not as a substitute for clinical immunogenicity studies
- Animal models are not a suitable alternative to evaluate immunogenicity risk of any therapeutic protein or peptide due to the lack of predictability for human immune responses
- At a minimum clinical immunogenicity studies that compare the follow-on product with its RLD are required to determine that a follow-on liraglutide product is as safe and effective as the RLD.

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