Considerations For Addressing Analytical Differences for Biosimilars: Immunogenicity

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Addressing Analytical Differences

- Charge variants
- Glycosylation variants
- Process related impurities (different because they are not designed or expected to match)

Biosimilar/biosimilarity

- "…the biological product is highly similar to the reference product not withstanding minor differences in clinically inactive components"
- "…there are no clinically meaningful differences between the biological product and the reference product and the reference product in terms of the safety, purity, and potency of the product"

How to address analytical differences

- Modify the manufacturing process to eliminate the difference
- Assess whether analytical differences may be clinically meaningful
  - Identify the root of the difference
  - Evaluate the potential for that root to have clinical impact

Example: Charge Variants

- Identify variants
  - C-terminal lysine differences
    - Generally supported by a risk assessment based on prior knowledge
  - Oxidized or deamidated species
    - Identify variants
    - Evaluate for impact on other quality attributes as indicated, e.g.:
      - » Potency
      - » Aggregates

Example: Charge Variants

- Risk assessment for potential impact on immunogenicity, e.g. and as indicated:
  - Impact on quality attributes associated with increased or decreased immunogenicity, e.g. aggregates
  - In silico analyses to identify changes to T cell epitopes
  - In vitro analyses to evaluate whether changes have biological impact
  - Prior knowledge
- Discuss with FDA how to address cases where risk assessment indicates increased risk of immunogeincity

Example: Glycosylation Variants

- Afucosylation differences
  - Afucosylation is known to impact effector function
  - Differences in effector function may impact product safety and efficacy

Example: Glycosylation Variants

- To evaluate the impact of afucosylation differences on IgG1
  - Use orthogonal methods to assess the likelihood of clinical impact, e.g.:
    - Compare effector function in appropriate cell-based assays
    - Compare binding to FCγRIIIa (for IgG1, particularly 158F allotype, or both 158F and 158V allotypes)
    - Compare binding of C3
- Discuss with FDA how to address cases where afucosylation differences impact effector function

Example: Glycosylation Variants

- Murine to CHO cell line change for monoclonal antibody products:
  - Generally, see increased sialylation, NGNA, and alfa-gal linkage with murine cell lines
  - Differences in sialylation may impact PK
  - Increased NGNA and alfa-gal are associated with increased immunogenicity
  - PK studies can address the potential impact of sialylation
  - Risk assessment for impact of NGNA and alfa-gal is needed

Process Related Impurities

- Biosimilar products are not expected to match impurities
- Process related impurities may impact immunogenicity and safety, e.g.:
  - High levels of HCP were associated with increased injection site reactions to recombinant hGH[1]
  - High level of undetected HCP resulted in increased immunogenicity of Omnitrope during clinical development[1]
  - Undetected CHO derived chemokine MCP1 resulted in histamine release related adverse events during a clinical trial[1]
- Process related impurities must be appropriately controlled
- Host cell derived impurities are most difficult to control because of their heterogeneity

1Vanderlaan M et al. Experience with Host Cell Protein Impurities in Biopharmaceuticals. AICE Biotechnol. Prog. 2018 34:828 – 837.

Process Related Impurities

- Controlling host cell related materials
  - 50 – 97% of protein in harvest material are host cell related
  - Critical to reduce HCP during purification
    - Characterize critical HCP reduction steps
    - Evaluate the consistency of HCP reduction and factors that impact HCP reduction
    - Quantify and Identify HCP
      - Current practice is a combination of HCP ELISA and MS
      - New methods and technologies are emerging
  - Test HCP at release or validate removal

Process Related Impurities

- Perform a risk assessment for identified proteins[2,3,4,5]
  - Potential to impact immunogenicity
  - Potential for bioactivity
  - Use product knowledge and literature
    - In silico and in vitro testing may support the safety of HCP

[2] BioPhorum maintains a searchable multi-company collaborative view dataset on high-risk host cell proteins
[3] Panikulam S, et al. Host cell protein-mediated adjuvanticity an immunogenicity risks of biotherapeutics. Biotechnology Advances. 2025. 81:108575
[4] Jawa V, et al. T-Cell dependent immunogenicity of protein therapeutics pre-clinical assessment and mitigation-updated consensus and review 2020. Frontiers in Immunology. 2020. 11:article 1201.
[5] de Zafra, et al. Host cell proteins in biotechnology derived products: A risk assessment framework. 2015. Biotechnology and Bioengineering. 2014. 112(11):2284 – 2291.

Process Related Impurities

- There is no single HCP assay that identifies and quantifies all HCP
- HCP ELISA
  - HCP are detected using a pool of anti-HCP antibodies usually purified from HCP immunized animals
  - HCP that did not elicit antibodies are not detected using the assay

Process Related Impurities

- HCP ELISA
  - Quantitation using HCP ELISA is inaccurate
    - Calculated using curves derived from an HCP standard
    - Pool of proteins in the standard may be different from the pool of proteins in the test article
    - Not all test article proteins are detected by the antibodies used in the ELISA
  - Critical to use orthogonal methods to characterize the process and the HCP assay

Process Related Impurities

- HCP ELISA
  - HCP detection coverage should be verified by orthogonal method: e.g. 1D and 2D gels with sensitive protein stains
  - Detected and undetected proteins should be identified
  - Using risk assessment evaluate the risk from proteins – particularly proteins not detected by the ELISA
  - Determine whether HCP ELISA needs to be supplemented with a specific protein ELISA (uncommon)

Process Related Impurities

- Immune system activation may be induced by non-proteinaceous host cell materials and by synergistic combination of low levels of impurities[6]
- Non-clinical assays may be used to assess the risk from such impurities

[6]Haile LA, et al. Cell based assay identifies TLR2 and TLR4 stimulating impurities in interferon beta. 2017. Sci. Rep. 7(1)10490.

Summary

- Any analytical differences identified are assessed to understand the impact on the biosimilar product's clinical performance relative to the reference product
- Orthogonal analytical assays, including bioassays, product knowledge, and publicly available information can provide information about the potential for clinical impact
- In some cases, manufacturing processes may need to be modified to address uncertainty

Summary

- Process related impurities are not expected to be compared between a reference and biosimilar product
- Process related impurities can increase the risk of immunogenicity of a product
- Therefore, process related impurities should be:

Summary

- Therefore, process related impurities should be:
  - Minimized
  - Characterized
    - Quantity
    - Identity
    - Clinical risk
  - Controlled

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