Roundtable Session 2 – Table 4 – High Order Structure Comparability: Acceptance Criteria, Analytical Comparisons, and Drawing Meaningful Conclusions

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Abstract:
Ensuring the comparability of Higher Order Structures (HOS) is fundamental to the development and regulatory approval of biopharmaceutical products. This roundtable session will focus on the methodologies, acceptance criteria, and regulatory perspectives surrounding HOS comparability across different batches, manufacturing processes, or biosimilar products. The discussion will center on the latest advancements in analytical techniques used for HOS comparison, best practices for establishing phase-appropriate comparability, and the regulatory landscape's evolving expectations. Participants will explore how to interpret HOS data effectively, ensuring product consistency, safety, and efficacy through robust conclusions.

Discussion Questions:

1. How is Higher Order Structure (HOS) defined in the context of biotherapeutics, and does every class of biopharmaceuticals require HOS characterization?

   General agreement that HOS consists of secondary through tertiary structural characterization, largely based on the canonical structure/function paradigm and variability in bioassay readouts. Scope of HOS characterization in protein biologics is fairly well established, although new technologies are always being advanced. Gene therapy product (LNP/RNA/AAV) approaches are still evolving.

2. What are the most effective analytical techniques for comparing HOS across different batches or processes, and what factors influence the selection of these techniques?

   Phase appropriate deployment seems to be the standard. CD, FTIR, DSC generally used. HDX- and oxidative footprinting-MS approaches are sometimes used in protein biologics as well, but perhaps not as widely as spectroscopic and thermodynamic methods. AAV gene therapies may require TEM, AUC, DSF. NMR is being introduced at some companies, already has a role in biosimilar development. Raman, FTIR, and fluorescence may be valuable for ADC characterization. Agreement that orthogonal methods should be employed, particularly in later phases of development.

3. What are the key acceptance criteria for establishing HOS comparability in biopharmaceuticals, and how do they differ across product types and phases of development?

   Acceptance criteria may vary depending on the amount of historical knowledge that can be applied to the program and the phase of program development. Initial IND may only have a tox and a clinical lot with an aim to demonstrate lot equivalence. In that case, spectral overlay may be sufficient. Later in development with process scale up or other major changes the emphasis may shift to demonstrating equivalent process capability, so multiple lots from each process may be evaluated. In that case, spectral similarity, comparison of deconvoluted or regularized data, or other statistical approaches may be employed.

4. How do regulatory agencies assess the significance of HOS comparability data, and how is it weighted relative to other types of data (e.g., primary sequence, functional assays)?

   No regulatory presence at the table, however structure/function relationship was discussed and consensus was that HOS quality attributes must be controlled to ensure product quality. It was noted

that functional assays often have high variability, and in those cases there is value in building assurance with orthogonal structural techniques.

5. What are the common challenges encountered when establishing HOS comparability, and what strategies can be employed to address these issues effectively?

   Introducing new technologies is difficult. Not all companies have access to all technologies.

6. How can companies ensure that the conclusions drawn from HOS comparisons are scientifically robust, reproducible, and sufficient for regulatory approval?

   Companies can leverage historical data to generate platform methods for early development. Later in development space product specific methods can be developed. There was general consensus that fit-for-purpose method development is appropriate for extended characterization HOS methods, and that these development reports can help ensure method acceptability.

7. What role does risk-based assessment play in defining HOS comparability acceptance criteria, especially when bridging between different manufacturing sites or processes

   When applying a risk-based approach to development the product innovator would compile all product quality attributes and define which are critical and which are not. If HOS characteristics are assessed to be low risk in the product, an argument can be made to decrease study orthogonality relating to those attributes, and can also be used to justify more general acceptance criteria. The method sensitivity would also be considered.

8. How can advancements in digital and analytical technologies further enhance the precision and accuracy of HOS comparisons

   Efforts have been made to advance spectral similarity scoring and principal component analysis to improve the sensitivity, precision, and accuracy of HOS comparisons.

9. How does the comparability of HOS between innovator biologics and biosimilars evolve with product lifecycle, and what are the best practices for maintaining consistency across the lifecycle?

   See 3.

Additional notes:

There was discussion about buffer conditions for various HOS methods. Some methods have significant buffer interference problems, and if the product is in that buffer system exchange is required. General feedback was that changing the buffer system for a particular method is okay as long as all samples are treated the same for that method.

General commentary about the interplay between business, science, and regulatory. Businesses often want to decrease package content for strategic or financial reasons, scientists want to maximize orthogonality to ensure maximal product understanding, and regulatory has been known to ask for specific types of data on a case-by-case basis. Many groups collect a lot of data, but only provide a subset to regulators in the filing and then may or may not be asked for supporting data sets through IRs (reviewer dependent?).

There is sometimes business inertia that slows down introduction and adoption of new technologies. Without regulatory encouragement to innovate, business sees no need to invest and take the risk of introducing novel techniques. No-one wants to risk being first.

General interest in harmonizing regulatory requirements.

NIST-mAb is highly thought of, good start to help standardize practices.

NMR for biologic characterization is gathering support, but there are concerns about technology availability for smaller companies. Some companies are including NMR data in IND/BLA, but slow to integrate for a number of reasons including concerns about risk to filing package and lack of business justification as agencies seem to be satisfied with typical methods (CD, FTIR, DSC, etc). Companies who are integrating NMR are adding it as additional characterization in addition to their usual practices in anticipation of gathering regulatory feedback.