

Table 9: Characterization and Monitoring of N- and O- Linked Glycans

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Scope:

Protein glycosylation is a common post-translational modification that is critical for a wide range of biological processes. For biopharmaceuticals, glycosylation is generally considered a critical quality attribute (CQA) for monoclonal antibodies and other therapeutic proteins. The complex heterogeneity of glycosylated proteins can lead to challenges in the characterization and control of both N- and O-linked glycans of complex pharmaceutical products. A variety of analytical techniques exist for identification and quantification of glycan structures. These techniques may be used at different stages in drug development process. This roundtable aims to discuss where gaps currently exist and identify potential solutions to allow for improved characterization and monitoring.

Questions for Discussion:

1. Approaches for glycan analysis:
 - a. What approaches do you use for glycan analysis? Do you primarily use MS, or do you primarily use orthogonal methods? Do you use multiple MS-based methods?
 - b. Are different approaches used at different stages?
 - c. How are O-glycans analyzed?
 - d. What challenges currently exist for glycan characterization and monitoring therapeutics?
 - e. What's the status of Multi-Attribute-Method (MAM) for glycan analysis? How is application of MAM for glycan analysis different from other analytical methods?
2. New developments in glycan analysis:
 - a. New software?
 - b. New enzymes/reagents?
 - c. New instrumentation/techniques?
3. What gaps are present around new modality analysis?

Discussion Notes:

The table was well attended with active discussions. Most attendees are from biopharmaceutical companies, along with a few others from vendor companies. There was also a graduate student.

- The approaches for glycan analysis highly depend on the stage of the project and the purpose of the study. Most approaches involve enzymatic digestion, and MS analysis. One attendee gave an example of using endoglycosidase to help identify glycosylation sites by LC-MS in early stage work.
- O-glycan analysis remains challenging. Only 4 attendees have experience with O-glycan analysis. Attendees suggested that ETD may help with O-glycan analysis, if high enough charge states could be obtained. Lack of efficient enzyme to digest O-linked glycoprotein is still a big challenge. Genovis has a new O-glycan-specific endoprotease called "OpeRATOR".
- A challenge currently exist for glycan characterization is the lack of a good standard to confirm MS2 data, especially for isomers with different linkages, for example, 1-3 vs. 1-6 linkage isomers.

- Common MS software is used for glycan analysis, such as Chromeleon, Protein Metrics, Bruker's data analysis, and Biopharma Finder. One attendee mentioned that Protein Metrics was good for data trending and peak annotation.
- Two attendees have experience with Genovis and recommended several of their new endoglycosidases.