USP Biologics Open Forum:
Update on USP's Work Supporting Multi-Attribute Methods for Biologics

Event Summary

In early 2020, USP held a Stakeholder Forum focused on the application of multi-attribute methods (MAM) to biologics testing. The consensus from stakeholders was that both documentary and physical standards to support MAM would be helpful. Since then, USP has formed an Expert Panel to convene experts in MAM with the goal of developing a general chapter that describes best practices for peptide-based MAM workflows. On April 28, 2021, USP Biologics held a follow-up Open Forum to provide stakeholders with updates on the Expert Panel's progress and to share data from recent analyses using MAM to evaluate post-translational modifications on USP's monoclonal antibody (mAb) Reference Standards.

Dr. Ed Chess, Biologics Monographs 3–Complex Biologics Expert Committee member and Chair of the MAM Expert Panel, reviewed the outcomes from the 2020 Stakeholder Forum, the Expert Panel's membership, and a tentative general chapter. The plan is for the chapter to focus on the peptide-based mass spectrometry workflow and discuss best practices and considerations for robust application of MAM to biotherapeutics.

Dr. Diane McCarthy, USP's Director of Biologics Pipeline Development, reviewed data on the impact of sample preparation on observed post-translational modifications. During initial characterization of three new USP Monoclonal Antibody Reference Standards, USP noted that the percentage of deamidation varied significantly between labs and methods. Other modifications, including pyroglutamate and C-terminal clipping, showed little variation. To investigate the cause of the variation and further optimize MAM methods, a study was designed, with input from MAM experts, to evaluate the impact of sample preparation conditions on deamidation. The first phase of the study evaluated reduction/alkylation conditions and showed that deamidation increased with increased temperature and pH. However, changes in deamidation were highly peptide specific, emphasizing the need for thorough characterization of individual products to identify sites that are most susceptible to deamidation.

The second phase evaluated the digestion conditions, with variables including the trypsin source, enzyme: substrate ratio, and digestion time. Digestion time had the greatest impact on deamidation, whereas trypsin source and enzyme: substrate ratio had little impact.

What's Next?

These data provided more detailed characterization of the USP mAb Reference Standards and provide the foundation for developing pre-digested mAb Reference Standards. USP's goal is to provide matched-set mAb standards that could enable users to assess both the instrumentation and assay itself, including, impacts from sample preparation, for peptide mapping and MAM applications. USP Biologics is planning to test this method in another laboratory. In addition, the USP MAM Expert Panel's first draft of the chapter is expected to be published in the *Pharmacopeial Forum* in 2022. Stakeholders are encouraged to submit additional recommendations related to MAM methods for biologics or regarding other areas related to quality of biologics to USPBiologics@USP.org. USP Biologics' next Stakeholder Forum will be held August 10th and 12th, 2021 from 10 am to 1 pm EDT each day. The topic of the next Forum will be on development of quality standards to support in-process analysis of biologics that enables near or real-time

decision making during biomanufacturing. The Forum will include expert presentations each day followed by breakout discussion groups to identify challenges and possible solutions. The agenda and registration for the event will be posted at www.usp.org in July 2021.