Table 5: Structural MS – Best Practices for Predicting, Elucidating, and Monitoring Physiochemical Hotspots and Biotransformation by MS

Facilitator: Mellisa Ly, *Pfizer, Inc., Andover MA USA*
Scribe: John Tran, *Genentech, a Member of the Roche Group South San Francisco, CA USA*

Scope:
With increasingly challenging biological targets, the number of therapeutic protein modalities and delivery mechanisms are expanding. As a result, it is important to characterize stability for modalities from manufacturing, storage, and biotransformation(alteration of drug post-in vivo administration) for both drug discovery and development. A comprehensive understanding of hotspots, which includes amino acid modifications (i.e. oxidation and deamidation), truncation, aggregation by LC-MS serves as the basis for well-designed process and product quality attribute(PQA) monitoring of biotherapeutics. Major hotspots are typically engineered out in the molecular design phase, but many hotspots are monitored during process development, long term storage, and pharmacokinetic studies. In this roundtable session, we will discuss the opportunities and best practices for predicting, elucidating, and monitoring hotspots for insights into structure-function-relationships and PQA assessments. In addition, we will discuss strategies for characterizing and predicting biotransformation for different types of modalities in the context of healthy and diseased samples from discovery research.

Questions for Discussion:
1. Prediction of Hotspots: Discuss how physiochemical hotspots are predicted from primary structure. What scientific approaches and characterization experiments are utilized?
2. Elucidation and Monitoring: How is elucidation vs monitoring being performed? How were orthogonal methods used for hotspot analysis? Discuss challenges in building monitoring tools during process development. Was hotspot monitoring used to support control strategies?
3. Physiochemical Hotspots of Interest: Discuss specific examples of notable modifications. Are there any modifications in certain regions linked to structure/function relationship or potency changes?
4. Impact of Biotransformation in Drug Discovery: How biotransformation can impact potency, PK parameters and safety?
5. Biotransformation/In Vitro Degradation Translatability: What types of biotransformation are predictable from in-vitro stress used in process development?
6. Biotransformation Species/Species and Healthy/Diseased Translatability: What understanding do we have in biotransformation amongst different species and the potential differences present in diseased samples?

Discussion Notes:

1. **Prediction of Hotspots:** Discuss how physiochemical hotspots are predicted from primary structure. What scientific approaches and product characterization experiments are utilized?
   - Can predict hotspots by forcing degradation under various conditions, not all currently performing *in vivo* studies.

2. **Elucidation and Monitoring:** How is elucidation vs monitoring being performed? How were orthogonal methods used for hotspot analysis? Discuss challenges in building monitoring tools during process development. How was hotspot monitoring used to support control strategies?

   a. Generally, we are monitoring for liabilities that are predicted.
   b. Risks with liabilities are mainly associated with manufacturing and storage conditions.
   c. Hotspots monitored are studied earlier in development.
   d. Prioritize research projects that are moving forward in development.
   e. Hotspots observed with low artifact peptide mapping may be followed up with orthogonal analyses such as intact analysis or subunit analysis.

3. **Physiochemical Hotspots of Interest:** Discuss specific examples of notable modifications. Are there any modifications in certain regions linked to structure/function relationship or potency changes?

   a. Some key product quality attributes include proteoforms such as oxidation, deamidation, aggregation. We are most concerned if these PTMs these occur in CDRs or binding regions.
4. **Impact of Biotransformation in Drug Discovery:** How biotransformation can impact potency, PK parameters and safety?
5. **Biotransformation/In Vitro Degradation Translatability:** What types of biotransformation are predictable from in Vitro Stress used in process development.
   a. Deamidation translates quite well from *in vitro* to *in vivo* samples.
   b. More research will be needed for understanding models to predict for *in vivo* oxidation.

6. **Biotransformation Species/Species and Healthy/Diseased Translatability:** What understanding do we have in biotransformation amongst different species and the potential differences present in diseased samples.
   a. ADC's biotransformation can vary dramatically from different animal species.
   b. Clipping of protein therapeutics can be very different from diseased vs healthy animals because the levels of proteases might be different.
7. Other discussions:
   - Some vendor sources are better at desolvating vs other sources.