Table 6: Best Practices for Predicting, Elucidating and Monitoring Hotspots by MS

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Scope:

Hotspots analysis of protein therapeutics is a critical aspect of Product Characterization packages during early and late stages of development. Hotspot analysis is performed during early process development for molecule assessment to support the selection of the best candidate clone and provide initial product characterization in support of IND's, as well as late stage product development to support MA or BLA. A well characterized product enables robust process development and provides insights into potential clinical success.

Typically, hotspots are assessed/evaluated using a) in-silico tools that are based on literature or prior knowledge, and/or b) experimental analysis using stressed material to magnify hotspots for identification and characterization. MS is fundamental tool in structural elucidation as it confers the advantage of detection and quantitation of the hotspots. This characterization allows assessment of clinical impact and provides avenue for routine monitoring if they become critical quality attributes.

This roundtable discussion will focus on best approaches for prediction of hotspots and current practices to characterize and/or monitor them using mass spectrometry. We will also discuss areas of improvement or harmonization of approaches to provide a consistent data package to regulatory authorities.

Questions for Discussion:

1. What is the current practice of elucidating structure/hotspot analysis in your company?
2. What are the gaps and pain points in current workflows for elucidating, monitoring and analyzing hotspots?
3. What in-silico tools do you currently use, if any? What gaps and pain points do you encounter when using these tools?"
4. What hardware and software are needed to perform these analyses? Is there a preferred hardware/software configuration?"
5. At what stage(s) in development is this assessment performed? Does the scope of the analysis change as process development matures?
6. What approaches do you use to monitor hotspots that are also deemed to be CQA's?

Discussion Notes:

1. What is the current practice of elucidating structure/hotspot analysis in your company?

The process of elucidating hotspots within the structure begins during early molecule assessment as clone selection and process development are beginning to take place. A subset of the most promising clones are purposefully stressed (for example, by heat or high pH) and analyzed by peptide mapping to determine the location of any resides that are prone to modification.

2. What are the gaps and pain points in current workflows for elucidating, monitoring and analyzing hotspots?

Experience is a key bottleneck. The greater your knowledge and experience of potential hotspots, the more quickly you can assess and analyze future molecules. Developing this understanding is key. A potential gap in assessing hotspots is lack of knowledge in how a hotspot forms or grows: identifying its source, its potential impact on potency, and its potential impact in the clinic. In addition, if hotspots are not monitored with enough frequency, it may be difficult to assign a rising hotspot to a specific process change.

3. What in-silico tools do you currently use, if any? What gaps and pain points do you encounter when using these tools?

Though some in-silico options exist for monitoring hotspots that have already been identified in previous analyses, there do not appear to be in-silico options that are able to highlight potential hotspot residues prior to analysis. This was identified as a potential pain point as it necessitates analyzing a dataset with minimal guidance and/or relies more heavily on the experience of the analyst.

4. What hardware and software are needed to perform these analyses? Is there a preferred hardware/software configuration?

Currently, the software used is typically able to assign a potential modification to a residue based on a combination of the MS1 and MS2 data. Ideally, however, the software would be able to predict potential hotspots and minimize false positives by leveraging this predictability.

5. At what stage(s) in development is this assessment performed? Does the scope of the analysis change as process development matures?

The assessment of hotspots is typically performed early in development during clone selection, and again during late stage characterization. Further assessment outside of these cases is largely situational depending on the presence of CQAs or changes in other analytical profiles.

6. What approaches do you use to monitor hotspots that are also deemed to be CQA's?

Once a hotspot is identified, steps are taken in order to determine the potential impact on safety, efficacy, and whether or not the hotspot is located in the active site of the molecule. This often includes MS support to determine levels of the hotspot, but may also include support from other assays, such as potency-related assays. In instances where the potential impact seems critical, the hotspot may be considered a CQA, which may necessitate more frequent and rigorous analysis of that specific site.