Table 7: Best Practices for Reporting MS Data in Regulatory Filings

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Scope:

Mass spectrometry is an essential and powerful technique for characterizing biopharmaceuticals from demonstrating process comparability, understanding structure-function relationships, characterizing product-related variants, process-related impurities, and more. As biopharmaceuticals increase in complexity, the applications and need for mass spectrometry techniques increases as well. How do you leverage mass spectrometry in product and process characterization? How do you decide what to report in regulatory filings? What best practices do you take when reporting MS data?

Questions for Discussion:

1. How do you demonstrate to the regulatory authority that you have confirmed primary structure (i.e. amino acid sequence)? For example, do you present chromatogram overlays, deconvoluted and raw data? butterfly plots against reference standard, peptide lists
2. How do you decide which post-translational modifications to report?
3. What phase appropriate strategy do you implement for reporting data, if at all?
4. How do you determine quantitative or qualitative acceptance criteria for late phase/commercial comparability studies?
5. How do you report sequence variants and host cell protein (HCP) data, if at all?

Discussion Notes:

In general, colleagues at the roundtable session want to learn how other companies do things and want to learn additional perspectives out of their current understanding. They also want to understand how providers can help from their perspective. With this, here are the discussion notes:

1. **How do you demonstrate to the regulatory authority that you have confirmed primary structure (i.e. amino acid sequence)? For example, do you present chromatogram overlays, deconvoluted and raw data? butterfly plots against reference standard, peptide lists?**

The participants mention that Tables with PTMs & sequences and annotated chromatograms are most commonly seen. The sequence coverage doesn't have to be 100% in regulatory filings, but confirmation of terminals (N-term and C-term) is critical. There are companies with internal

guidelines suggesting 85% or higher sequence coverage for IND filings, and would prefer a higher number for BLAs. The sequence coverage reporting is also complemented by other analytical assays. The MS/MS information for the sequence confirmation is not usually included in the filing but people do usually keep that ready to address any questions that they may receive from agencies.

To confirm the sequence, people usually rely on MS/MS in combination with high resolution MS. To increase sequence coverage, one can perform a digestion with shorter time to allow more miss-cleaved peptides. Alternative enzymes can also be used to generate different peptides.

The team also mentioned that amino acid analysis used to be involved in filing to confirm primary sequence, but was no longer needed owing to the high variability of the method. Edman degradation and monosaccharide analysis are also examples that people no longer report in regulatory filings. To convince teams to exclude assays from filing, people usually provide results from orthogonal techniques and don't include certain assays from IND stage to begin with. It also requires evolvement of techniques by vendors and inputs from agencies.

2. How do you decide which post-translational modifications to report?

The team agree that this usually relies on force degradation studies. Usually people report PTMs that have changes beyond 1% in force degradations. The threshold can vary based upon legacy understanding. Moreover, this also relies on legacy data from studies early on. All in all, team was suggesting a phase appropriate risk-based approach. The team also mentioned that it will be good to have a system tracking details behind each decision behind the scenes.

The team also touched on when do people usually carry out a force degradation study and concluded that this should be performed earlier in the process if possible. There are companies that carry out a force degradation study immediately after they receive the material to generate PQA list.

In terms of PTMs that are not seen in force degradation studies, the team suggest that it is important to assess the risk case by case, and especially careful in the IND stage as those PTMs may not be there any more in the BLA stage owing to process change.

Lastly, the team also touched on whether the strategy will change for non-US or Non-EU filings, and the team mentioned that the countries that require more information are usually filed later, and the specs can be different based on different requirements across different countries.

3. What phase appropriate strategy do you implement for reporting data, if at all?

The team mentioned a few examples. The first one is for deep characterization, colleagues prefer to perform a deep characterization at a later stage if it is a regular antibody program,but will perform it at an earlier stage if it is a new modality.

The second example is SEC-MALS is used in filing regarding on high molecular weight species, but peak collection by SEC followed by mass spectrometry analysis is also commonly seen. If a

peak collection is performed in earlier stage, it may or may not to perform it again as the program moves forward. The agencies have been accepting IND data at BLA stage but with excessive comparability. For unknown peaks seen in these studies, the team suggest involving different analytical assays for comprehensive characterization.

4. **How do you determine quantitative or qualitative acceptance criteria for late phase/commercial comparability studies?**

The key point is to understand historical data. For quantitative acceptance criteria, PPM error is often reported for intact/reduced analysis. For peptide mapping, the team tends not to define a quantitative acceptance criterion but to report "no unknown peaks" and provide chromatogram overlays.

As for either to report total ion chromatograms or UV chromatograms, the team has a preference of reporting UV for comparability studies and reporting both for characterizations. The reason is that TIC is more sensitive to perturbations, there are cases where analysts are seeing differences in the TIC but turns out that they are not related to molecule itself.

5. **How do you report sequence variants and host cell protein (HCP) data, if at all?**

Sequence variant can be reported in impurity section in BLA but is usually less than 0.1%. It usually depends on actual residue and not being reported regularly. The team also suggests being upfront and gather data for root cause analysis if needed. When monitoring feeding strategies, mic-incorporation is a must-have.

For HCP, the team suggest not to report mass spec data unless ELISA is showing a high HCP level and requires mass spec for further justification. It is agreed among the team that the mass spec HCP is not required unless seeing an abnormal ELISA reading.

Other than the five pre-posted questions, the team also touched on the following topics:

1. Do people suggest using a template for regulatory filing?

   The team suggests using template to smooth the filing process.

2. How much details do people include when reporting a method?

   The team suggest keeping the method description at high level, and to platform as much as possible. The team also mentioned to always use platform method at the starting stage of the program, and shift to molecule-specific method if needed at later stages.

3. How are MAM accepted in regulatory filing?

   Not on release panel yet.

4. For HCP reporting, does the agency prefer mass spec or ELISA? In theory, both.