Session 2 - Table 14: Advanced Mass Spectrometry Methods in Biologics Characterization and Regulatory Filing

Facilitator: Roger Liu, Vertex Pharmaceuticals
Scribe: Doug Richardson, Merck & Co., Inc.

Abstract

Over the past two decades, we have witnessed an increasing number of Biological License Application (BLA) approvals. The modalities have evolved from protein replacement therapy to monoclonal antibodies, multi-specifics, fusion proteins, antibody-drug conjugates, and many others. The increasing complexity in the design of the molecules posts novel challenges in the characterization of these potential biotherapeutics.

To address these challenges, there has been numerous advancements of the analytical tools, among which mass spectrometry (MS) is a critical member. With increasing capabilities of MS instrumentation, elevated sensitivity allows the analysis in greater depth. The attributes that MS monitors progressed from amino acid sequence confirmation to complex quality attributes such as oxidation, deamidation, higher order structures, and host cell proteins (HCP).

As the capabilities expand, we also see more and more overlaps between MS-based approaches versus conventional approaches. Some examples are multi-attribute method for the replacement of multiple conventional HPLC & CE-based release methods, and HCP proteomics for the replacement of HCP ELISA. What are the general strategies for implementing MS-based methods and what are the gaps of replacing the conventional approaches with MS-based assays is a hot topic in the community.

Accompanied with the increasing capabilities is the data complexity. How to summarize and present the data to regulatory agencies in a concise, informative, and unbiased manner becomes critical, especially for complicated workflows such as HCP proteomics. How to effectively determine factors such as limit of detection/quantitation, reproducibility, and assay variability is also of crucial importance, as these greatly determines the potential of expanding these assays into the quality space for release testing in the future.

MS-based approaches have taken an increasing role in biologics characterization. The great potential of MS is yet to be fully utilized, during which the community is determined to address any challenges along the way.

Session Objectives & Questions

In this session, we will be discussing the use of MS in biologics characterization, particularly on the aspect of how scientists leverage MS in product and process characterization; how do scientists report the MS data in regulatory filings; and what are the new methods for deeper understanding of different attributes. In general, colleagues at the roundtable session would learn the current practice of the community and gain additional perspectives out of their current understanding.

Discussion Notes

1. Leveraging MS-based approaches in biologics characterization

a. What are the attributes that you are monitoring with MS-based approaches?

-PTMs (oxidation, deamidation, glycans, N/C terminal modifications)
-HCP (ID, coverage, quantitation)
-Sequence coverage for protein→ Sequence Variants
-Disulfide bonds, free thiol, disulfide shuffling→ NR pep for disulfide bond & disulfide shuffling, differential alkylation (NEM/D_5-NEM) for site-specific free thiol
-Aggregate characterization (native MS, SEC-MS, Intact Mass)
-Intact Mass for Charge Variants
-Cell culture performance (proteomics, metabolomics, Multi-omics)
-Potency→ mRNA in vitro translation
-HOS→ FPOP, HDX, Epitope mapping
-Identity→ both peptide mapping and intact mass

b. Are the MS-based methods actively replacing any conventional approaches to deepen the understand of biologics molecules?

-Free thiol vs Ellman's
-HCP for monitoring process, characterization, comparability, alternative hosts, MRM is quantitative is accurate, proteomics relative quantitative can be inaccurate
    -If these don't impact safety, quality, efficacy why switch to mass spectrometry?
    -Problematic HCPs are still a challenge to quantify at low abundances
-Replacing Edmans for sequencing
    -Do you need all fragment ions (i.e. y/z) for all peptides with MS based sequencing?
        No singe answer for this question
    -Have others has success with BLA without Edman's?--> Yes success in BLA

c. What are the efforts of determining the limit of detection/quantitation for MS-based approaches?

-Determination of LOD/LOQ is challenging for relative quantitation assays
    -Don't report number if not accurate is good practice
-How do you ensure precision and accuracy for quantitation?
    -Labeled material
    -Purchase commercial standards to benchmark method performance
        -Helps understand cutoff/risks for HCP proteomics assays
-General agreement that these is not much true quantitation in "Biologics"
-Limited dynamic range with electrospray is a limitation for quantitation
    -4-5 orders of magnitude with significant debate on this topic
- Question- How does MS sensitivity compare to ELISA for coverage? (i.e. worse, equal, better)
    -Depends on the location in process - Upstream = better, downstream is worse
    -Driven by dynamic range
    -Enrichment is key tool for increasing sensitivity for either workflow
        -5-10 HCPs without enrichment
        -50 HCPs with enrichment

d. Are there any initiatives for bringing the MS-based assays into QC space?

-Question Skipped

2. Reporting & utilizing MS data in regulatory filing

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

- Elucidation of structure section→ your report based on QbD
    - Only if it's a CQA→ What if you don't know?
        - More forced deg
        - Characterization
        - Product related impurities or process related impurities
- Working closely with functional colleagues→ Understanding where structure function relationship breaks
-Glycans - Location and abundance but too much detail
    - Cut off <1%?--> many agreed→ Depends on the criticality and impact on function and phase of development
    - Small molecule cutoff is <0.5%?
-Limiting purity in early development can narrow design space/tox qualification

b. How to determine quantitative/qualitative acceptance criteria for late phase/commercial comparability studies?

- Question skipped

c. How to report data from complex MS assays, for example, sequence variant and HCP proteomics?

-Consensus was no from group
-Discussion on different expectation from CDER vs CBER
-Collect coverage data on most concentration sample (samples with most possible HCPs, i.e., protein A eluate)
    - Maximize coverage of antibodies
-Do companies include TIC in submission?
    - One example for each assay (Intact, pep map, etc.)
    - Helpful to include visual examples is important

d. Do people suggest using a template for regulatory filing? Is so, how is the template built?

- Question skipped

3. New methods for deeper understanding of attributes

a. What new approaches are the mass spec community actively developing? What value can they bring for deeper understanding of the molecule?

-CDMS (Charge Detection)
-I2MS (Individual Ion-Comparison to SEC-MALS has been done)
-Native MS→ Many companies Investing
-SEC-MS
-CEX-MS→ Many companies investing
-cIEF-MS
-CZE-MS (ZipChip)
-Mass Photometry

b. What are the approaches for characterizing antibody-drug conjugates, especially for cysteine conjugates where the heavy chain and the light chain may not be covalently attached?

-Question skipped

c. Are there any mass spec applications for higher order structure determination approaches (e.g., hydrogen deuterium exchange) for the support of regulatory filing?

-Question skipped

4. Summary

To summarize, the team had an engaging and candid discussion on the topics listed above. Topics expand from MS in characterization, regulatory filing, and emerging technologies. In particular, team spent time discussing some common challenges such as using MS-based approaches to provide additional information on top of conventional technologies, MS-based methods for host cell protein characterization and quantification, the nature of relative quantification of MS-based approaches in the biologics space (in contrast of many absolute quantification methods supporting release), and what types of new technologies are being developed to deal with the increasing complicated new modalities. Importantly, the closing remark also focused on the need of another roundtable on Mass Spectrometry Software & data presentation (opportunity for CASSS MS 2024).