Roundtable Session 1 – Table 5 – Expectations for the Development and Application of Extended Characterization Methods

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Abstract:

Increased abundance and complexity of protein-based large molecule therapeutics and vaccines such as monoclonal antibodies, antibody-drug conjugates, and fusion proteins require robust characterization packages that supplement release criteria. Extended Characterization (EC) methods provide deeper understanding of biological products by appropriate techniques, including the determination of physicochemical properties, biological activity, immunochemical properties (if any), purity, impurities, contaminants, and quantity. EC methods are used for the purposes of product knowledge, site and/or process comparability, reference standard (RS) characterization, and investigations associated with manufactured batches. The methods may not have acceptance criteria as they are intended to provide additional information that typically complement Critical Quality Attribute (CQA) testing, but they do often require data interpretation beyond numerical assessment.

This round table will serve as an interactive forum to discuss current issues and perspectives across industry and regulators.

Discussion Questions:

1. How are EC methods established in a phase appropriate manner?

   a. Are platform methods or program-specific methods most commonly used? Does this change over the course of development?

   b. Which EC methods require qualification and what are the qualification approaches and timing (phase of development) for these EC methods?

2. Do EC method panels for comparability studies start broad or narrow during early stages of development? How does this change as the program progresses?

3. Which EC methods are typically assigned Numeric Assessment Criteria (NAC) during comparability or reference standard qualification, and which EC methods are limited to qualitative, spectra-based comparison?

   a. Has specific feedback been received from a Health Authority to assign NAC to EC methods?

   b. How are data sets that fall outside of the NAC justified?

4. How are EC methods deployed in product variant characterization (size and charge variants)?

a. Which EC methods are deployed for characterization of charge/size variants?

b. When during development are charge and size variant characterizations performed?

c. Are these datasets typically used for internal knowledge building only or are they shared in Health Authority submissions?

Notes:

1. How are EC methods established in a phase appropriate manner?

For proteins participants used similar technologies for all the proteins therapeutics, but methods may differ slightly for different projects. Project specific methods are needed for biological activity (i.e., ELISA).

Comparability methods include biophysical and mass spectrometry structure analysis and binding assays. Participants use platform methods or if outsourced use method from CDMO in early stage after some method assessment/feasibility and as we move on through development.

Not going to qualify characterization method unless needed and then done in late stage (i.e., PPQ or later). Some methods like binding ELISA might be qualified earlier, cell-based may stay as characterization but be qualified. Might also qualify other methods specific to MOA. Other mass spectrometry or biophysical methods may not be qualified. Experience shared by participant that in preparation for BLA of a product where glycosylation impacted activity with and do limited qualification on characterization methods that might be leveraged to understand the activity. Participant shared they performed HDX epitope mapping to demonstrate and explain the correlation of the structure function studies with change in glycosylation. In general participants agreed that level of qualification would be decided by how the method is used, for example methods that are the primary measure of that attribute may get more qualification than those that are supplemental.

Extent of qualification might depend on platform. Historical data is enough to tell you about your method and whether it is suitable for new molecules. For newer modalities, they might not be good fit for platform so some additional suitability data may bee needed to ensure platform fits. Qualification will be leaner than those for release tests (for example, might perform intermediate precision with 3 samples instead of 6). It is common amongst participants to spike in forced degradation material for some types of assays for qualification. Participants noted some queries from health authorities to provide qualification for characterization methods, specifically for biosimilars with EMA. Other agencies have not requested qualifications of extended characterization methods. Qualification may be performed as needed or on the platform method with some verification of suitability for new molecules.

2. Do EC method panels for comparability studies start broad or narrow during early stages of development? How does this change as the program progresses?

Participants generally used a trimmed down panel of characterization tests for first IND and increase as you invest resources in a Phase dependent manor. Client-facing organizations (i.e., CROs) rely on what the client asks for but can recommend options to clients. Some participants do more characterization at early stage because they do not understand the molecule/process as much and want to gain further understanding. This would include primarily mass spectrometry and biophysical methods, while cell-based bioassays and other functional methods may not be fully developed at this stage and therefore are not used.

For comparability between Phases, some participants expressed doing a preliminary comparison of pilot materials prior to GMP manufacturing to remove any risk (including intact, glycan, CD, DLS) and do a full panel of characterization tests in the final comparability of the GMP materials.

EMA queries for early state project (Ph1/2) comparability were received by one participant with a question about iCIEF characterization and a request to characterize the charge variants. Participant reported providing overlays of iCIEF electropherograms to address the query with discussion that the same profile and no new peaks were observed. Other participants also received similar queries regarding characterization and provide overlays to show profile comparison and ensure no new peaks/species observed.

For complex systems where you might need additional characterization to address a change in the profile. If a change in acidic or basic are observed how does that impact your comparability? Participants suggest that if differences are observed in iCIEF, additional characterization methods (mass spectrometry, CEX-MS, or analysis of fractions) can be used to understand these differences.

3. Which EC methods are typically assigned Numeric Assessment Criteria (NAC) during comparability or reference standard qualification and which EC methods are limited to qualitative, spectra-based comparison?

Acceptance criteria are generally qualitative, for example report results and any differences observed between pre and post change materials will be discussed and justified or comparable profiles and observed masses agree with theoretical masses. Participants may also include numerical acceptance criteria in certain instances. Generally participants do not have criteria of simply “comparable” but have some additional language around what comparable means for that characterization method. Most participants are not typically applying numerical criteria to most characterization assays (i.e., mass spectrometry and biophysical) but there may be some assays and instances where these methods have criteria applied for that specific program. Generally participants had mass accuracy criteria for ID that was applied based on instrument capabilities.

Some instances where feedback from agencies was given to provide numerical criteria for acceptance criteria (for DLS or DSC). Participants indicated they would in that case define criteria by review (including ranges) around historical data or variation from the reference standard to provide numerical acceptance criteria for the data.

Weighted-spectral difference was discussed for spectra-based methods. Generally participants agreed that using statistical tools for spectral based techniques, similarity score and statistics, need some qualification using the same test article to determine variability, include a forced degraded sample to detect difference. Not necessarily include in the regulatory filings but may performed for internal reports.

Table discussed that modern software for certain instruments (MMS, CD) may provide some statistical spectral analysis. Table participants said that MMS has some statistical analysis that they are incorporating. Some vendors now incorporate the statistical analysis in the software. Participants mention that for some methods (MMS/MMIR and far UV-CD) spectra may not be significantly different across many mAbs in portfolio so need to understand if method can differentiate different between molecules. To determine what is a true difference with these outputs you may do some forced degradation spiking or compare two different mAbs and give us a more realistic view of what would be considered comparable for this molecule. Participants expressed that you may need more replicates and method understanding to drive comparability criteria and goes back to how much qualification is require for these methods. Even some slight changes in spectral profiles could be significant to impact function.

Table discussed that for certain modalities (i.e., ADC) you might get interference in your spectral methods. Challenging for ADC changes that impact drug load. May use changes observed based on mass spectrometry characterization of the at the drug conjugate level, these might impact spectral methods making them hard to interpret the differences.

Table discussed that SV-AUC is very cumbersome but used widely for characterization. See same amount of high molecular weight species by SV-AUC as SEC commonly. Some participants use SV-AUC for comparability, others only for characterization of reference material in S.3.1, while other participants said they do not use SV-AUC for any of their programs or are moving to not using AUC. DLS and SEC-MALS can characterize the HMMS, but AUC can ensure no column matrix interference. One participant noted that SV-AUC was instrumental in informing them or issue with SEC method capability to recover HMMS, participant ran forced degradation realize that the column is absorbing HMMS so it was not observed in SEC profile. So participant noted re-developing the SEC method to ensure all HMMS were observed and SV-AUC and SEC data were comparable.

4. How are EC methods deployed in product variant characterization (size and charge variants)?

Participants noted mostly using fraction collection to characterize charge variants. The release test is iCIEF, but develop CEX method to separate each charge variants and collect fractions for characterization.

Question for table was how separated do you get in the fractionation? Some participants separate full acidic and basic species together, still mixture of all acidic and basic not fully separated and perform studies on the overall basic and acidic. Fractionation can be challenging so best to look at larger fractions. Some participants have additional fractionation of acidic or basic species but noted getting enough for potency or characterization testing is challenging.

For fraction testing, some participants mostly focused on mass spectrometry and potency of fractions, others do full panel of product quality assays including SEC, iCE, CGE.

Studies of charge and size variants are done late stage but may be done earlier to address change in profile or agency query. One option for earlier study is to perform online (CEX-MS, SEC-MS) methods if you have the capability to perform those methods.

Generally, some level of the characterization is shared with the health authorities for structure characterization. Less commonly performed for comparability.