Table 3: Strategies and Challenges for Setting Specifications for MAM in Relation to Conventional CE Methods

Facilitator: Lipika Basumallick, *Genentech, a Member of the Roche Group, Palo Alto, CA, USA*

Scribe: John Valliere-Douglas, *Seagen Inc., Bothell, WA, USA*

Scope:

CE assays are routinely included as part of product characterization and as an element of the analytical control strategy to ensure product purity, structure, and stability during development and manufacturing.

The emerging technology of Multi-attribute method (MAM), a liquid chromatography-mass spectrometry based method that measures several attributes in a single assay, has the potential to replace multiple conventional methods in the control strategy. HA's are currently requesting that companies wanting to use MAM for commercial release begin implementing MAM early in clinical development and run the MAM alongside the other (conventional) assays. The sponsor would then present the datasets and a suitable justification that the MAM is not missing something that is detected in the conventional assay(s). With adequate justification, the agency is permitting sponsors to sunset charge variant, N-glycan and identity methods with MAM for commercialization. How does a sponsor translate specifications from a current assay to the MAM given that in the aforementioned conventional methods the charge variant regions and peaks have very heterogeneous composition while the MAM assay specifically monitors PTMs at single amino acid and so, presumably, would have specs set on site specific levels of PTMs?

Questions for Discussion:

1. What can the process reliably deliver (from a product quality standpoint)?
2. What can the method reliably deliver (from robustness, intermediate precision standpoint)?
3. What is the level of criticality (impact to patient) associated with the attribute around which the specification is being set?
4. How would one go about translating a CE charge variant spec to a MAM method??
5. Consider other conventional methods that are used on release for size variant monitoring and identity such as CE-SDS and cIEF/icIEF for identity. What are some of the challenges associated with replacing those types of methods with MAM and translating specifications for size variants and identity from the conventional method to MAM?

Discussion Notes:

The primary focus of discussion at this roundtable revolved around the translation specifications associated with CE-based methods to MAM (multi-attribute method; eg MS peptide map) for particular attributes.

In setting up the conversation, it was noted that there has been some success on the part of sponsors replacing legacy release methods such as N-glycosylation (CZE-LIF/HILIC-UPLC), identity and charge variant (CV) methods with MAM. Of these various applications of MAM, direct translation of specs from legacy N-glycan methods to MAM could be supported with sufficient bridging and establishing specs for a MAM identity method would not be philosophically different than a conventional peptide map identity assay. However, translating specifications from classic CV assays such as CEX/cIEf/icIEF to MAM is much more challenging so the bulk of the conversation dealt with that topic. General observations are listed below.

- MAM specs are molecular attribute (PTM) quant based while classic CV assay specs are on peaks which are compositionally heterogeneous. Conventional CV assays excel at capturing changes that are closely associated with process consistency (eg glycation which is evident as an acidic variant) while MAM based approaches may excel at capturing particular local PTMs that are associated with activity such as deamidation of a critical Asn residue.

- One approach suggested for translating classic CV specs to MAM is to preparatively purify the charge variant (such as acidic variant) species around which the spec is set and then spike this material back into a nominal sample at a level such that the resulting comix is near the spec limit. This sample could then be tested by MAM and the levels of PTMs observed in that sample (by MAM) would then correlate back to the CV spec and could be used as an element of the justification around specs for particular attributes quantitated by MAM.

- While sponsors are gaining health authority (HA) acceptance to sunset legacy release assays such as N-glycans, identity and charge variants with MAM, it was noted that this only occurred after extensive bridging and implementation of MAM in QC alongside the legacy assays during clinical development. In the event that HA's did approve implementation of stand-alone MAM for release, approval typically occurred around commercialization. More specific information regarding HA feedback on MAM specifications was not known.

- Regarding the use of MAM for other CE based assays such as size variants; the general consensus was that their would be a considerable amount of work on the part of the sponsor required to show that the method was sufficiently fit for purpose. This skepticism was mainly due to the fact that MAM involves a proteolytic digestion step which may create the same peptidic clips that would occur endogenously in the sample so reliably proving the clips occurred in the sample and not as an artifactual digestion product could be challenging.

- Finally, it was noted that many legacy CE and chromatographic assays used for biotherapeutic release can serve the useful function of capturing a unexpected impurity or contaminant as a new peak. These 'new peaks' may then lead to uncovering unknown manufacturing deviations that otherwise would have gone unnoticed. The general consensus was that MAM would struggle in this area by comparison because new peak detection approaches are challenging to implement and would fail to detect an impurity below or above the m/z range of data collection. Additionally MAM sample prep buffer exchange steps used for desalting eliminate unexpected small molecule impurities thus leading to non-detection of these impurities. It is unclear at present, how MAM can be adapted to address that limitation.