Table 14: Challenges for Bi-specifics/Tri-specifics

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Scope:
The interest in bispecific and trispecific antibodies is increasing for therapeutic applications, and there is a growing list of molecules currently in clinical studies. These multispecific molecules are capable of binding more than one antigen, and as a class of molecules comprise a large number (more than 50) of different molecular formats. For example, some molecules resemble typical IgG structure while others lack an Fc region. In general, bi- and tri-specific antibodies are assembled from antigen-binding and dimerization building blocks to form the intended functional therapeutic. However, these building blocks can be combined in a number of ways, resulting in closely related but ultimately undesired end-product molecules. These impurities may be very different in mass (e.g. half antibodies) or very close in mass (e.g. light-chain scramble). Consequently, the analysis of bi- and trispecific antibodies using mass spectrometry (MS) presents unique and significant challenges. In this roundtable discussion, we will take a deep dive into the use of MS for bi- and trispecific antibody analysis, seeking to build consensus by identifying trends from participants’ success stories, as well as identifying opportunities from participants’ ongoing challenges.

Questions for Discussion:
1. What additional challenges do bi- and trispecific antibodies impart on traditional analytical workflows used for mAb analysis?
2. How are you using denaturing and/or native MS to support bi- and trispecific antibody characterization?
3. What are the challenges in quantification of populations within bi- and trispecific antibody samples? What level of purity can be confirmed using MS? Accurate quantitation of low level homodimer impurities poses significant analytical challenges. What techniques/methods have you employed to tackle this issue?
4. What are the best practices for sample preparation and/or separation for MS analysis of bi- and trispecific antibodies?
5. What are the challenges in developing platform MS methods for bi- and trispecific antibodies?
6. What hurdles do you encounter when developing robust, high-throughput methods for quantitation of low level IgG impurities?

Discussion Notes:
- Bispecific/tri-specific antibodies have generated considerable interest in pharma development companies because of their ability to extend the therapeutic potential of antibodies drug therapies. In this roundtable, we discussed the analytical challenges of controlling bispecific/tri-specific antibody production in various development phases.
- Advancements in technology have reduced undesired chain mispairing.
  - Many of the roundtable participants are using the knob-into-hole technology where either a “knob” or a “hole” is built into the heavy chain of a half mab. However, the two halves of a bispecific are still engineered to have similar physical and
chemical properties, posing significant challenges for separating and detecting low-level impurities.

- While purification development can clear most impurities, improvements in antibody engineering can further reduce levels of unwanted side products. From across the broad, all participants agreed that improvements in front-end separation techniques will work in synergy with increased MS sensitivity to detect and understand closely related IgG impurities, such as light chain mispairing.

- Another significant analytical challenge is the lack of clinical exposure of bispecific therapies for setting acceptable impurities level.
  - One participant noted it may be appropriate to use a broad acceptance criterion rather than full characterization so long as the impurities level is below safety/tox limits.

- And lastly, these participants did not work on tri-specific so it was not discussed.