Roundtable Session 1 - Table 2 - Best Practices for Elucidating Antibody Drug Conjugate Molecules by MS

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Abstract:

Antibody-drug conjugates (ADC) are therapeutic modalities composed of a target-specific monoclonal antibody (mAb) connected via a chemical linker to a small molecule drug (payload) that has cell-killing (cytotoxic) activity. Heightened characterization of antibody drug conjugates (ADCs) presents significant challenges for analytical method development given the complexity of ADCs where cytotoxic drug payloads are conjugated to already diverse antibody proteoforms. As these novel complex modalities of ADCs surfaced, various analytical tools and approaches have been developed to characterize them including native MS of cysteine-conjugated ADCs with and without enzymatic cleavage of the linker, 2D-LC/MS analyses based on hydrophobic interaction chromatography (HIC) coupled to reversed-phase liquid chromatography, ion-mobility mass spectrometric analysis, etc.

LC/MS analysis of most intact ADCs remains challenging due to extensive conjugation with either hydrophobic or very hydrophilic linker/payloads and/or chemically unstable linkers. As well, following partial reduction, ADCs with hinge region cysteine conjugation and non-covalent subunits require native ESI conditions to properly detect the 4-chain proteoforms. The linker chemistry, designated as “cleavable” and “non-cleavable”, can present unique MS challenges, in addition to the site of conjugation, whether lysine or cysteine. Surface accessible linker/payloads pose additional challenges during ADC introduction into the mass spectrometer due to their fragility and amenability to in-source fragmentation. Essentially, ADC chemistry and properties have a significant effect on the analytical strategy utilized for MS characterization, and significant method development is required to establish robust, reliable and informative methods.

Discussion Questions:

- What are the best approaches to obtain high quality intact MS data for ADCs with chemically unstable linkers and non-covalent subunits?
- Do you employ MS for drug to antibody ratio (DAR) determination? If yes, what MS approaches to you implement?
- What are the best MS approaches to characterize hydrophobic interaction chromatography (HIC) peaks for cysteine-conjugated ADCs? HIC peak collection for offline MS characterization, 2D-LC/MS, 1D-HIC coupled online with MS?
- Do you use MS methods for DAR determination beyond characterization, for example for release and stability testing in QC?
- What are best approaches to characterize ADCs structural isomers with the same DAR?

- Importance of MS for conjugation site(s) identification and quantitation? What peptide mapping methods by LC/MS are used?
- What approaches are recommended for monitoring free (unconjugated) payload in ADC samples?
- What factors should be considered for ADC modalities with novel payloads?
- What are other challenges in MS characterization of ADCs?

Notes:

Best approaches for high quality intact MS?

Best practices are dependent upon on ADC conjugation type. Cys-linked ADCs are more challenging than fully covalent ADCs (e.g., Lys-linked ADCs). Ammonium acetate native MS buffers can present challenges with low sensitivity and require optimization Excessively high gas temperature, gas pressure, or ESI voltage can promote in-source fragmentation of Cys-linked ADCs. Optimized methods must balance efficient desolvation with maintaining structural integrity. Can use 10-20% IPA w/ AmAce to prevent sticking to SEC column

Using MS for DAR?

Can use intact RP for quick DAR estimate. This approach requires reduction, will not capture odd DAR for Cys-linked ADCs. Is it possible to use MS as DAR release method? HIC may be misleading when peaks are mixed species or include impurities. Lots of comparison with MS and HIC are needed.

Best approaches to characterize HIC peaks for Cys-linked ADCs?

HIC-fraction collection seems to be a popular approach, but HIC fractions can be tricky if fractions are lost to desalting membranes. Newer native RP method may be very useful. 2DLC (HIC-SEC heartcut method) requires many sample loops, difficult to operate as a platform method. HIC-MS methods are very complicated and not very sensitive.

Best approaches to characterize ADC structural isomers with the same DAR?

HIC can resolve structural isomers but does not necessarily indicate which peak is which isomers – this approach requires that HIC peak is fraction collected. Structural isomers can give different cytotoxicity in vivo (Cyno) studies. IEX and icIEF might be possible, but could yield complex results.

Conjugation site mapping

Non-reduced peptide mapping can be used to identify conjugation sites as an inverse read-out. Conjugated peptides signal will be impacted greatly, but reduction in signal could be used as a diagnostic. Random Lys-linked ADCs are very challenging for conjugation site peptide mapping.

Engineered Cysteine-linked ADC

Cysteines are capped with glutathione or cysteine during expression and thus require reduction prior to conjugation.

Other considerations

Tri-sulfides can be problematic for conjugation during scale-up and may require that reduction is re-optimized, typically involving more TCEP to adjust for slightly increased S-S molar ratio.

Free Drug monitoring

Intact ADC must be removed. Triple quad MS instruments can work well. High concentrations of free drug is present if free drug peak is readily observed in RP method for DAR calculation. Very hydrophilic linker-payloads can be analyzed with HILIC-MS.