Roundtable Session 2 – Table 2 – Toolboxes for Novel Modalities: What do we use and what are we missing?

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Abstract:

In recent years, there have been rapid advancements in novel modalities such as antibody-drug conjugates (ADCs), cell and gene therapies, and oligonucleotide-based therapeutics. However, the unique characteristics of these therapies pose significant challenges in their characterization. This roundtable discussion aims to explore the current state of the art in analytical tools, identify critical gaps, and foster collaborative discussions to accelerate the development of next-generation therapeutics. The session will provide a platform to share insights and best practices, as well as to discuss emerging analytical technologies.

Discussion Questions:

Antibody-Drug Conjugates:

How to effectively characterize the heterogeneity of ADCs, including variations in drug-to-antibody ratio (DAR), drug loading, and antibody glycosylation?

How to accurately assess linker stability and potential degradation products?

How to measure and quantify the release of the drug from the ADC under different conditions?

How to develop sensitive and specific methods for identifying and quantifying impurities, including aggregates, free drug, and antibody variants?

Cell Therapies:

How to reliably assess cell viability, potency, and function, considering the complexity of cell-based products?

How to ensure accurate characterization of cell identity and purity, including detection of potential contaminants?

How to evaluate the impact of cryopreservation on cell viability, potency, and product quality?

RNA-based Therapeutics

How to ensure the accurate characterization of RNA sequence integrity and purity?

How to characterize the secondary and tertiary structure of RNA therapeutics? Is it even useful/necessary? How critical is such characterization to improving stability and efficacy?

How to identify and quantify impurities, such as truncated RNA, double-stranded RNA, and host cell proteins?

How to assess the stability of RNA-based therapeutics under different storage conditions and identify degradation products?

Discussion notes:

What is considered as a novel modality? How does one define it?

- Novel modalities are those for which biophysical characterization and/or comparability study protocols are not well established. Examples include protein conjugates such as antibody-drug conjugates (ADCs), co-formulations, oligonucleotides, and cell and gene therapies.

What additional tools to use for ADCs for elucidation of structures?

- In one example ADC, a chiral linker is used, and far-UV and near-UV circular dichroism (CD) cannot be used. Instead, one can use infrared (IR) or Raman spectroscopy to obtain information about the secondary and tertiary structures, respectively.

How does one determine ADC content / concentration?

- With the added complexity of a linker and a payload, determining the UV extinction coefficient at 280 nm often becomes challenging. One suggestion was to use the UV extinction coefficient of the unconjugated antibody at 280 nm for ADCs. Although the absolute content values may be off, the content is supported/validated through clinical studies and remains fixed over the lifecycle of the product.
- Another suggested approach relies on the fact that the infrared (IR) responses of the mAb peptide bond remain unchanged before and after conjugation. Therefore, the IR signal from the unconjugated mAb can be used to determine the content of the ADC. Then, by obtaining the UV280 absorbance of the ADC and dividing it by the ADC content determined by IR, we can determine the UV extinction coefficient at 280 nm.

What additional tools can we use to assess ADC self-associations?

- Mass photometry was discussed as the primary novel tool to look at self-association behavior. Several advantages (e.g., sample volume, speed) and limitations (e.g., limited range of concentrations) were pointed out.

What are the challenges with co-formulation products?

- The primary challenge was set to be around the content and purity determination. In one product example, 25 monoclonal antibodies (mAbs) are co-formulated. Sometimes, the co-formulation includes mixed modalities. The discussion group noted that we currently don't have answers to these questions, and they still need to be worked out.

What new tools/applications caught participant’s attention at the 2024 CASSS HOS meeting?

- A notable increase in the use of NMR for characterizing new modalities was noted. For instance, P31-NMR is being used in mRNA analysis to determine attributes like the P to S ratio.
- Small angle X-ray scattering (SAXS), small angle neutron scattering (SANS) and electron density topography (EDT) were used to study molecular dynamics of biologics at high concentrations in their native state.

For lipid nanoparticles, are there multi-attribute characterization tools available, similar to MS multi-attribute methods (MAM)? What about the use of AF4-MALS (asymmetric-flow field flow fractionation-multiangle light scattering) for LNPs?

- No MAM-like tools could be named.
- AF4-MALS is considered orthogonal to SEC-MALS and can be run in formulation buffer. Waters has developed a new column specifically for LNPs. Nanoparticle tracking analysis instrument (NTA) was mentioned to be considered superior for determining LNP particle size and size distribution compared to dynamic light scattering (DLS), yet this statement was subject to debate.