Table 1: Analytical Challenges and Solutions of Therapeutics Beyond mAbs

Facilitator: Andras Guttman, *Horvath Csaba Laboratory of Bioseparation Sciences, University of Debrecen*

Scope:
New modalities represent the next generation of biopharmaceuticals. While monoclonal antibodies still dominate the field, especially in the biosimilar side, cellular and gene therapies (CGT) are getting more and more attention. New modalities include mAbs variants such as bispecifics/trispecifics,antibody drug conjugates (ADC), IgG derivatives like FC-fusion proteins and nanobodies and Viral Like Particles (VLPs). Gene therapy approaches comprise DNA/RNA molecules, viral and lipid nanoparticle (LNP) delivery vehicles as well as CRISPR mediated techniques. Routine analytical techniques for these new generation drugs include LC and CE as well as their hyphenation with mass spectrometry and bioassays. The level of characterization is typically dependent on the phase of the program and the criticality of the product attribute that should be continuously monitored. This roundtable aims to discuss both the technical and strategic challenges associated with the characterization of new modalities in the biopharmaceutical industry.

Questions for Discussion:
1. What are the biggest analytical challenges for non MAB therapeutic proteins?
2. What are the biggest analytical challenges for CGT products?
3. Are there common themes between the analytical setup of MAB based biopharmaceuticals and CGT products?
4. What does it take to transform a traditional analytical lab for MABs and therapeutic proteins into a CGT testing lab?

Discussion Notes:

1. What are the biggest analytical challenges for CGT products?

- Main challenges is the bottleneck of live production because of the limited amount mAb based assays can be converted to bio assaying gene therapy products, e.g., sizes are different. Main QA is the ratio of full and empty viral particles and confidently measuring them is a challenge. Can be addressed using analytical ultracentrifugation but only with low resolution and a lot of calculation is necessary. Native MS with extended mass range also suffers low resolution for different mass, composition, and +/- DNA. CE or using a nanoflow source can be promising. The size changes are not apparent when the DNA is inside. Other option is to destroy the capside and see DP1, DP2 and DP3, check in the intact virus level. Super-molecular assembly related issues also exist. DNA sequence should be checked too, but before inserted into the virus. MS analysis of oligonucleotides in the kilodalton range is also challenging, especially for a mixed DNA composition. Cell specificity of AAV2 and AAV5 is also important.
- Other delivery vehicles such as single layer structures like liposomes or lipid nanoparticles did not work well. Exosomas can be an option, especially for targeted regenerative therapy. Exosomes require MAM including DNA, RNA and protein analysis.
- Quantities for therapy: gene number per ml should be in the range of 10^11.
- Gene therapy was already investigated in the late 90s, a lot of companies started those days e.g., Novartis and GSK.

- Definition of new modality is vague, can range from cells to peptides, interfering RNA therapies, etc.
- Early solutions to enter the market: big pharma companies did not want to be too early but not too late either, let's see what happens in 5 years.
- Problems with bispecifics (Immuno-oncology) is the dose that is cc 40 ug, clinical phase is 100 ug/mL per vial, most analytical methods are just not applicable for such low concentrations. Analytical techniques for bispecific derived from IgG, 125 kDa. Sensitivity issue and excipients with equal mass such as polysorbate.
- A lot of action is in the field of potency assays. Need ug quantities for that. Another issue is to find out the real mode of action. Cell based assays are usually used.

2. *Are there common themes between the analytical setup of MAB based biopharmaceuticals and CGT products?*

- Certain methods are considered, but MS is not a release method for this except ID testing based on mass. Sequence coverage is another issue and validation is challenging. UV based is methods would be the way to go and different labs with same tests as it provides direct readout to validate as it is more dedicated for QC. Problem for MS companies is that the easy to operate MS systems are low resolution.
- Trend within MS companies to produce more advanced QDA, only a mass detection rather than a MS. TOF up to 10K, from Waters is small. Implementation to MS, having a UV or FL method. Mindset change is needed to verify the validity of the results in MS. A lot of companies presented unique workflows and software sources.

4. What does it take to transform a traditional analytical lab for MABs and therapeutic proteins into a CGT testing lab?

- Different expertise is needed. In cell therapy the hurdle is that it is done in the clinic (sampling and treatment both) with analytical in between that should be close. Methods used: next gen sequencing and immunological methods, rather than chemical methods. Current status is based on centralized laboratories rather than benchtop systems in every single lab.
- Gene therapy: from the established mAb based analytical methods for 140 kDa up to millions represents a big step. While immunoaffinity based techniques are coming in place, the current platforms cannot fulfill the analytical requirement. Orthogonal techniques are crucial. Change from small molecules to proteins were the task in the 80s, now to cells, etc.
- Peptide mapping: CQA some do not come out after digestion. Conventional bottom up analytical techniques, rather middle or intact level.
- CAR-T: cell from patient > manipulation > back to patient. Becoming popular, more and more companies are using CAR-T technology.
- The situation is the same as for mAbs at the 80s, i.e., not yet known what to look for and how. Whoever leads the market defines the things and everyone will follow, even the regulatory agencies.
- Development of mAbs used centralized approaches but gene therapies are not mentioned in the chart. Analytical companies should produce special analytical tools.
- For mAb it was easy to develop a method but not for viruses. CROs have to wait to get samples, and stds and probably need molecular biologists for the upcoming work.
- New Thermo MS checked for viruses UHMR MS to support.

- Subvisible particles ranging down to the low nanometer range can possible differentiate between full and empty capsids. Nanoparticle laser tracking for hydrodynamic radius measurement is one the options. Resonant mass measurement, particle density is the base of the measurements, polysorbate, etc.