Roundtable Session 1 – Table 4 – Mass Spec in Cell and Gene Therapy

Facilitator: Christopher Sauer and Andy Mahan, *Johnson&Johnson Innovative Medicine*

Scribe: Mason Chilmonczyk, *Andson Biotech*

Abstract:

Cell and gene therapies are revolutionizing the landscape of personalized medicine, offering unprecedented opportunities to treat previously incurable genetic disorders, cancers, and degenerative diseases. These therapies harness the body's own cells or modify genetic material to restore or enhance biological function, presenting both immense promise and complex engineering challenges. With the rapid pace of innovation, there is a critical need for rigorous analytical techniques to ensure the safety, potency, purity, and consistency of these advanced biological products.

Mass spectrometry has emerged as an indispensable tool in the field of cell and gene therapy, providing molecular characterization at the proteomic, genomic, and metabolomic levels. Its high sensitivity, specificity, and versatility has enabled researchers to identify, quantify, and validate critical quality attributes of therapeutic products—ranging from detailed molecular profiles of gene editing outcomes to the detection of impurities and product variants. Furthermore, mass spectrometry facilitates the monitoring of therapeutic stability, the assessment of biological activity, and the validation of manufacturing processes, thereby supporting regulatory compliance and accelerating product development.

This roundtable will delve into the transformative impact of mass spectrometry on the advancement of cell and gene therapies. Participants will explore recent innovations, emerging applications, and best practices that are leveraging mass spectrometry to overcome key analytical challenges.

Discussion Questions:

1. How are recent MS advancements enhancing the ability to characterize structural variants, post-translational modifications, and impurities in complex cell and gene therapy vectors?
2. How has your lab implemented higher-order structure methods, such as native mass spectrometry, CDMS, and ion mobility MS to improve characterization?
3. How has your lab incorporated automation and other high throughput methods to improve screening?
4. What are the major bottlenecks still facing MS in cell and gene therapy? Sample preparation? Instrumentation and data acquisition? Data analysis?
5. What roles do bioinformatics, AI, and advanced computational methods play in MS analyses of cell and gene therapy products.
6. What else is needed?

Notes:

1. How are recent MS advancements enhancing the ability to characterize structural variants, post-translational modifications, and impurities in complex cell and gene therapy vectors?

“Vectors” – could be anything in this space; Nonviral – lipids, LNPs

What is a cell and gene therapy (CGT)? We’re probably talking more about characterizing the vector than the CGT itself in this case. Easier to characterize.

Aside – is it more convenient to go towards the cell or gene therapy? It really depends. The FDA is really leaning towards autologous it seems.

One key advancement for the space is CDMS

AUC vs CDMS: A lot of benefit to AUC – it’s first principal, so is CDMS. AUC: cannot measure harvest. Volume size is half a mil minimum, but concentration has to also be a certain level. Can make it high throughput.

AUC – has multiple wavelengths – can look at just one wavelength. However, it can be on more than one type of protein

CDMS – The advantage is the low end -> 200 kDa. It’s gotten much better. UHMR is much worse at the “middle range”. Charge allows you to detect charge, which gives you structural differences. AUC also gives you some information on conformation.

“What about mass photometry” – not based on first principal, based on wave scattering. Turn around, high throughput. Mass photometry.

“What about light scattering?” – if you run something on a DLS, you can be misled. It’s not first principal, so it weights by numbers.

2. How has your lab implemented higher-order structure methods, such as native mass spectrometry, CDMS, and ion mobility MS to improve characterization?

How do you get your CDMS Data? “MegaDalton”

“UHMR/DMT” – looking at top down work. You could potentially do top down on the UHMR system – plus you can top down. The charge resolution on the linear ion trap is even better.

3. How has your lab incorporated automation and other high throughput methods to improve screening?

Automate everything is hard – especially for CGT. Automating for AAV is only working SP3 – single pot, solid phase extraction. Very useful for complex AAV analysis

Multi attribute method – Regulatory is open to it, but the knowledge isn’t there yet. Trying to incorporate all three into one. We are limited for materials.

What else is needed?

Even higher analysis of high molecules. LNP analysis

LNPS: The mass spec is not the problem – it’s the sample cleanup process.

VIRUSES: Sensitivity would be a lot better

Getting your system to be BSL-2 compatible is very important – you have to make sure that MS is compatible with BSL-2 and GMP

What throughput do you need for AAV analysis – not that high yet, but it’s going there. Avoid digest completely, do everything top-down in one system. You do need to know where the defects are not just that they are there. “Some sight resolution” but if they’re spaced far apart.

4. What are the major bottlenecks still facing MS in cell and gene therapy? Sample preparation? Instrumentation and data acquisition? Data analysis?

Sample prep & data analytics are one in the same. Trying to do filters to validate findings. Try to find flags that help to have somebody check something more carefully.

Human intervention still critical for a lot of these workflows.

5. What roles do bioinformatics, AI, and advanced computational methods play in MS analyses of cell and gene therapy products.

Not discussed at length