

## Roundtable Session 2 – Table 3 – Novel Applications of Molecular and Structural Biology in Characterization of Well Characterized Biologics

Facilitator: Kavita Aiyer, *Teva Pharmaceuticals*

Scribe: John Amery, *Pfizer, Inc.*

### **Abstract:**

ATMPs, including gene therapies, cell therapies, and engineered tissue products, present unique characterization challenges due to their inherent complexity, heterogeneity and dynamic biological behavior. Conventional analytical paradigms established for traditional biologics are often insufficient to fully define critical quality attributes or to establish clear links between molecular structure, function, and clinical performance. Advancements in molecular and structural biology are enabling a shift shifting from traditional, low-resolution methods to high-resolution, integrative, and often AI-driven approaches.

This roundtable will explore emerging molecular and structural biology approaches—such as high-resolution cryo-electron microscopy, advanced sequencing and multi-omics technologies, single-cell analytics, structural mass spectrometry, and integrative computational modeling—and their application to ATMP characterization. Discussion will focus on how these tools can enhance understanding of mechanisms of action, product heterogeneity, potency, and stability, and how they may be applied to support comparability and lifecycle management.

From a regulatory perspective, the session will examine how advanced molecular and structural data can be incorporated into science- and risk-based control strategies and aligned with global regulatory expectations. From a regulatory perspective, the session will examine how advanced molecular and structural data can be incorporated into science- and risk-based control strategies and aligned with global regulatory expectations.

### **Notes:**

Characterization - Historically this has been done with proteins. Molecular biology techniques for characterizing DNA containing products is more recent. Characterization is assays that don't go on the release specification.

Gene therapy, cell therapy, and vaccine projects may benefit from molecular biological characterization.

NIST – Mission to advance measurement science and standards to support innovation and industrial competitiveness. Industry, biopharma. What are the needs? What are the emerging analytical technologies. ddPCR / qPCR / NGS for nucleotides. Some work done in the past on harmonizing methods for measuring empty / full lenti viral and adeno viral vectors.

Lenti viral vectors – Need to understand attributes predictive of function in modifying T cells. Using reporter T cells. Why is one batch better than another. Virus like particles. What structural and chemical differences have an impact on infecting T cells. Structural predictability. When we change the vector or the process we need to know what we are making and if it's still of good quality. We can make the vector with modified envelope proteins, and then look for how good infectivity / titer. This is reactive and not predictive. This is current state.

What is the full compliment of MS / molecular biology / biophysical tools we can use? ddPCR / qPCR. Charge detection mass spectrometry (CDMS) you can look at intact capsid structure / charge population. Help understand how protein capsid changes impact the structure. Full / empty ratio is important.

MS / peptide mapping is a well known tool to probe the protein capsid space.

Changing a site or process scale. If you don't know what you're making and what's important then it's hard to argue you don't need a new clinical trial. Need to understand the critical molecular attributes of the vector. Using established technologies. What do we need to do to develop the CQA understanding? Are there post translational modifications that impact the function? If you alter the process are you changing any attributes that impact the quality?

Understanding the aggregation state of the viral vectors. Presumably monomeric vector is best for getting into the cells. What process steps impact this? Is it just titer that's important, or are there other attributes that impact quality?

Historically for mAb's we can degrade the mAb and measure the impact to potency. Understand what degradation products impact potency. Can we do this for viral vectors?

Opportunity to partner with academia? Partner with vendors?

Every modality goes through this journey. Mabs have. AAV has. Commercialization takes its own time to understand the attributes critical to quality.