USP Virtual Roundtable on AAV
7 - 9 December 2021

Executive Summary: Adeno-associated virus (AAV) is fast becoming the dominant delivery system for gene therapies. The FDA has already approved two AAV products, and the number is poised to grow significantly with over 100 therapies in clinical trials. However, there are still challenges in manufacturing AAVs caused by a lack of consensus on best practices for quality control.

In December 2021, USP convened a roundtable between manufacturers and regulators to discuss AAV manufacturing challenges. Over two days, 18 panelists representing gene therapy manufacturers and regulators joined with over 50 attendees to discuss best practices and propose and prioritize standards and tools that support the quality of AAV delivery systems for gene therapy products. The panel discussion clarified that new approaches are needed, especially for addressing comparability following changes to the manufacturing process.

The development of biologically relevant tests for potency and phase-appropriate use was a significant topic of discussion. Most participants used multiple orthogonal methods for assessing the product's infectivity, gene expression, and functional activity. They noted several areas where additional guidance was needed to address challenges and limitations. For example, assays for infectivity have low resolution and often use cell lines that are not biologically relevant for the gene therapy product. Furthermore, *in vivo* studies used for potency testing in early development are often unsuitable for the QC environment due to variability related to animal models. Unknown mechanisms of action (MOA) also complicate testing for some products. Participants agreed that better alignment on best practices and appropriate approaches to addressing potency measurement is needed.

One of the most significant impacts on the potency of AAV gene therapies is caused by empty capsids, which lack the necessary genetic information required for desired therapeutic effect. Various analytical methods are currently used to analyze the ratio of empty and full capsids in the drug substance. Participants agreed that consensus was needed on which methods are most appropriate for use, an issue USP, NIST, and NIIMBL are addressing through an interlaboratory study. Appropriate usage and validation of next-generation sequencing (NGS) for assessing gene integrity were also discussed. The panelists agreed that more regulatory guidance on NGS was needed to establish performance characteristics. Other challenges include the lack of fit-for-purpose and regulatory-compliant ancillary and raw materials that enable commercialization.

In summary, manufacturers need consensus on best practices for characterizing AAV at each step of the manufacturing process to ensure they produce a quality product. They also need access to physical reference standards for method development, bridging across methods, and establishing system suitability. USP is addressing this challenge by facilitating dialogue between all relevant stakeholders and by establishing the AAV Gene Therapy Expert Panel to draft a general chapter on best practices for AAV-based products.

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