Partnering for Scalable Suspension Processes Tailored to AAV Gene Therapies

By **Samir Acharya**, Andelyn Biosciences

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Achieving optimized, scalable suspension processes for adeno-associated virus (AAV)-based therapeutics comes with a number of technical and business challenges. Many biotherapeutic companies experience hurdles while transitioning from adherent platforms to suspension, as well as when scaling suspension processes to meet the needs of commercial scale. These realities make it critical to identify the right manufacturing partner that can overcome these hurdles. The ideal partner will have the know-how and experience to optimize unit operations, the expertise to maximize yields without compromising quality, and capabilities that enable phase-appropriate production that is adaptable to changing client needs.

Andelyn Biosciences is a gene therapy contract development and manufacturing organization (CDMO) originally spun out of Nationwide Children's Hospital (NCH) with all of these attributes. Andelyn possesses deep expertise supporting AAV therapies from preclinical to commercial production for both adherent and suspension processes. While having been at the forefront of AAV development and production using an adherent platform for over two decades, Andelyn has also leveraged its know-how for its AAV suspension platform to achieve the highest, most consistent quality and yields possible, from 125 milliliter flasks to 2,000-liter bioreactors and every scale in between.

Andelyn Offers Seamless Scaling Through Comprehensive Optimization and Design of Experiments Approach

The optimization necessary to generate a robust AAV platform that can support scale-up is complex. Everything from the reagents and cell lines used to the purification approach transforms when a product is transferred from adherent to suspension or developed directly in suspension. The ideal CDMO must understand what parameters are necessary to achieve linearity in productivity and quality from flask to bioreactor, what variables are at play, how differing technologies and approaches influence those variables, and how to balance technical considerations with manufacturability.

To address the challenges that accompany AAV manufacturing, Andelyn uses a combination of reductionist and holistic approaches to perform end-to-end optimization for its suspension production platform. Consistency in productivity and quality are used as benchmarks for evaluation of progress at each stage of development and are measured with state-of-the-art technologies. Starting at a small flask scale, several factors are considered that could potentially influence AAV productivity and quality at upstream (production) and downstream (purification) stages. These factors include:

1. cellular factors such as choice of cell line, cell growth passage, cell density at transfection, harvest cell density, and cell viability;
2. media factors such as type of media and media additives;
3. transfection reagent type and amount used, complexation parameters, and ratio of reagent to DNA;
4. DNA factors such as DNA ratios and total DNA per cell;
5. harvest factors to include day of harvest and virus released in harvest media versus cell pellet;
6. filtration factors such as choice of filters for clarification and ultrafiltration/diafiltration; and
7. chromatography factors to include choice of buffers and column chemistries, retention times, and final formulation buffers.

Rather than evaluating these factors independently, which is time-consuming and does not reveal multi-parameter interactions, Andelyn has employed a multifactorial Design of Experiment (DoE) approach. This allows Andelyn to statistically evaluate the relationships between multiple input factors and output responses to generate the best design space of optimized parameters, geared toward maximizing productivity and quality. Andelyn has performed the DoE optimization of its suspension platform at flask scale and has adopted the same approach for scale-up to bioreactors. This strategy has introduced several new variables (scale-dependent and scale-independent), such as the optimal impeller speed inside the bioreactors, the power necessary to avoid shear, the appropriate gassing strategy needed to ensure cell health, and the mixing strategy to ensure high transfection efficiencies.

Finally, Andelyn's optimization protocols have established the harvest, clarification, filtration, and concentration strategies – as well as the chromatography and ultracentrifugation-based approaches – suitable for each AAV serotype. A direct outcome of Andelyn's development and optimization studies is the demonstration of consistency in productivity, purification, and quality across different scales of the suspension process. The end result is a platform comprised of process steps that are modular, with options that can be tailored to specific needs for different AAV serotypes.

Furthermore, Andelyn has prioritized identifying and acquiring technologies best suited to optimizing, closing, and, where possible, automating AAV

production, with a focus on technologies that aid in bridging the gaps between scales as early as possible in development. This early adoption approach is often crucial to meet a client's development timelines. While many gene therapy companies may be reluctant to transition to suspension technologies during clinical investigations, the pitfalls of waiting to transition to suspension can create significant delays and added costs. Partnering with a CDMO like Andelyn facilitates the early de-risking and validation of a suspension process, which streamlines development and offers more seamless and efficient scale-up to commercial production.

Andelyn Leverages Expertise to Achieve Commercial Success

Choosing the appropriate time to transition to suspension hinges on a number of factors, including how much drug substance will be needed at commercial scale, the type of drug delivery method used for the particular disease indication, and whether the gene of interest (GOI) is suitable for suspension platforms. Andelyn performs early feasibility assessments with its clients to determine the most effective and economical path for commercial production and will perform comparative studies as necessary. Andelyn has developed and optimized its suspension platform with a commercially available cell line for a variety of serotypes and has also validated the platform with an internally developed modified cell line and client-procured cell lines as well. The result is that Andelyn can offer processes adapted for common serotypes and bespoke ones. Such a flexible process approach combined with Andelyn's foundational process knowledge serves to facilitate faster development and scale-up for closely related serotypes, as well as adapting processes based on the gene of interest and other unique variables.

Andelyn also offers validation of process development at small scales, which is a cost-effective and crucial component of achieving later manufacturing consistency. In addition, the utilization of the same platform equipment and materials for later manufacturing scales has positioned Andelyn to offer its clients the equivalency necessary to transition to GMP and commercial production with similar high titers and quality.

With over 16 customizable production suites, a configurable suspension-based manufacturing platform with scales ranging from 50L to 2,000L, and more than two decades of experience in viral vector manufacturing, Andelyn is the partner of choice for drug developers seeking to achieve reliability, scalability, and speed-to-market for their innovative AAV therapeutics.

About Andelyn Biosciences

Andelyn Biosciences is a full-service cell and gene therapy CDMO focused on the development, characterization, and production of viral vectors for gene therapy. With more than 20 years of experience, Andelyn's deep scientific expertise has resulted in the production of cGMP material for more than 450 clinical batches and 75 global clinical trials. Andelyn supports customers developing curative cell and gene therapies from concept through plasmid development and manufacturing, process development, and cGMP clinical and commercial manufacturing. Its versatile capabilities include cGMP manufacturing capacity for both adherent and suspension processes up to a 2,000-liter capacity. An advanced digital model, quality system, full regulatory support and supply chain vertical integration help Andelyn accelerate the development and manufacturing of its clients' innovative cell and gene therapies. For more information, visit andelynbio.com.

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