Optimizing Yields, Tech Transfer, and the Supply Chain for Viral Vector Manufacturing

By **Andrew Moreo** and **Lenore Giannunzio,** Andelyn Biosciences

Gene therapies offer an unprecedented ability to treat — and even cure — genetic diseases, and their potential spanning diverse therapeutic areas, including oncology, cardiology, and infectious diseases, continues to expand. While innovations in the development and manufacturing of viral vectors for gene therapies proceed at a rapid pace, the industry remains immature, with considerable work needed to standardize production platforms, improve yields, and reduce costs to advance the sector to a place analogous to more mature modalities, like monoclonal antibodies. Success in producing viral vectors with the appropriate yields, purity, efficacy, and quality requires a development and manufacturing partner with deep process and product understanding, the ability to develop built-for-purpose production and purification platforms, and the expertise to navigate supply chain and regulatory hurdles.

Driven to Cure Patients

Formed to develop gene therapies for as many patients as possible with a wide variety of rare diseases that often otherwise have no hope for treatment, Andelyn Biosciences has worked with numerous adeno-associated virus (AAV) serotypes and genes of interest and learned to overcome many hurdles to production, testing, formulation, and regulatory approval. The scientific team at Andelyn has observed how the size of an insert, the specific promoter used, or the addition of introns to key areas of plasmids can influence the production capacity, purity, and other critical quality attributes (CQAs) of AAV vectors.

This extensive and broad experience has placed Andelyn in a position to formulate responses to these many different challenges. We are able to select the appropriate components of our production, purification, and testing platform, choosing the right approach to manufacture each vector that also takes into consideration the required dosage, expected target, and required timeline. With our extensive process and product understanding, we apply the principles of quality by design (QbD) to ensure the highest possible yields, purity, efficacy, and quality for each product.

Andelyn's deep knowledge in viral vector development and manufacturing is also being leveraged for the design of new development and manufacturing sites. We continue to further build capabilities to even better respond to known and as yet unidentified hurdles.

Inherent Flexibility in a Tried-and-True Platform

Andelyn began platform development with an eye toward managing the complexity of possible programs, including considering multiple serotypes and multiple transgenes. Our team determined that we had to build a platform that was flexible from all perspectives, including both adherent and suspension processes. Using these platforms, Andelyn has produced clinical-grade material and supported approximately 70 investigational new drug (IND) applications. In essence, we have applied an established platform that was designed to adapt to each unique project while leveraging the learnings obtained during previous projects. We even used our extensive knowledge in adherent cell culture to develop our suspension cell culture platform, which is performed in single-use (SU) bioreactors.

Key to our success is our experienced team, which we bring in very early at the proof-of-concept stage to discuss all aspects of a project with the client. By collecting all relevant information from clients at the beginning of a project, we can perform a yield assessment, typically using a full design-of-experiment (DoE) approach, to evaluate how best to match the unique characteristics of each program with our platforms.

Importantly, Andelyn Biosciences was formed from a unique perspective focused on the science and patients and drawing on our roots in Nationwide Children's Hospital. With this internal experience in clinical research from multiple points of view, we have a wealth of insights that allow us to address the manufacturing, quality, and other aspects of viral vector development and commercialization. That foundation in science and our extensive experience in viral vector projects – including supporting a wide range of AAV serotypes – enables us to anticipate potential issues and consider the deepest details when working with our clients.

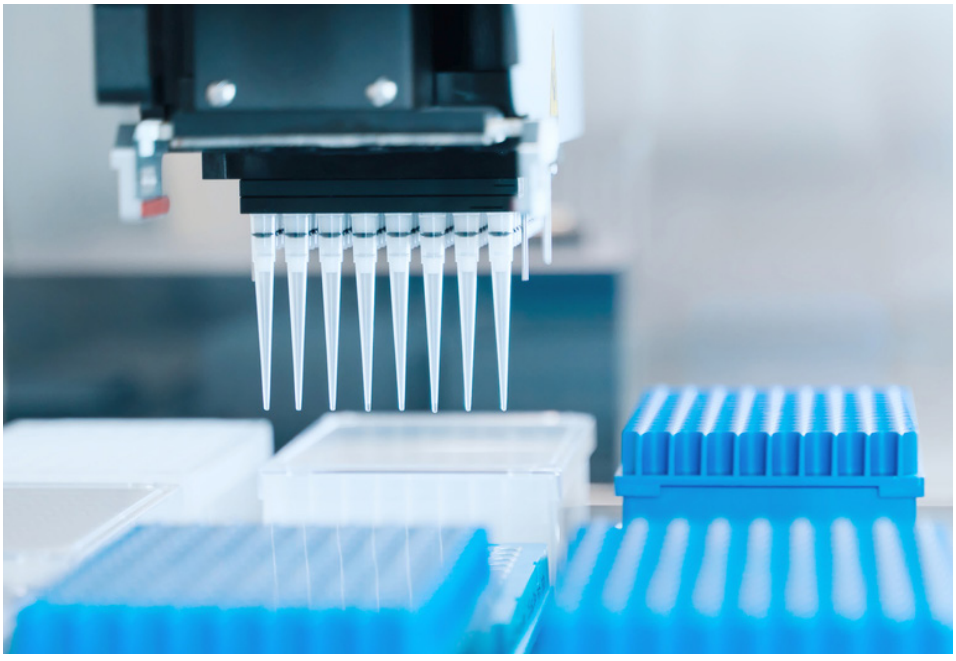
Understanding Serotype Behaviors

Adherent cell culture approaches continue to dominate upstream processes for cell and gene therapies, especially during early development, but there has been increased innovation and application of suspension-based platforms. Suspension-based processes offer a range of appealing benefits, including easier scale-up, reduced costs owing to the utilization of less consumables, simplified supply chain compliance, and reduced contamination risks.

The adherent approach is labor-intensive, and scale-up does not reduce the labor required, but adherent AAV production remains a strong option for several primary reasons. First, many indications, such as those with low required dosages (eye, scalp, mouth) or low disease prevalence, simply do not require enormous yields. For these products, sufficient amounts of vector can be quickly and efficiently produced in Corning® HYPERStack® vessels. Through our collaboration with BIA Separations, we have developed a process that affords a much cleaner harvested feed stream from our HYPERStack® platform than is obtainable using other production routes. Second, for certain membrane-associated serotypes, the adherent route is optimal for avoiding additional process-related impurities.

Additionally, adherent cell culture presents a simpler solution for lentiviral (LV) vector production. With LV vectors, time and temperature are critical production factors. HYPERStack® vessels afford high yields in concentrated volumes, and cooling of the harvest can occur quickly or can be bypassed altogether, allowing immediate purification.

In the absence of a definitive advantage of one of the manufacturing systems over the other, it is especially critical for gene therapy developers to partner with service providers that have extensive experience in commercial viral vector manufacturing. Without the benefit of the experience that comes from executing multiple programs spanning different serotypes, developers may gravitate toward one production platform without even realizing that, for their specific serotype, another platform might be more suitable.



Supporting Regulatory Advances

As a pioneer in viral vector production and the first to manufacture for clinical use, we have a wealth of unique practical experience, which we leverage not only to continuously improve our platforms and processes but to provide feedback to the U.S. Food and Drug Administration (FDA).

Andelyn Biosciences has been working with the FDA to set expected purity levels for AAV products and has been developing methods for achieving them. We have consistently been able to deliver very high yields. The real challenge at this point is the quality of the raw materials and the purification. The question we have been aiming to answer since we started communicating about this issue with the FDA 15 years ago is what amount of DNA impu-

rities are acceptable in the raw materials and final product.

At this point, we have determined the required purity levels for our raw materials, as well as the critical process parameters to monitor and the critical quality attributes to meet for our products.

Developing a Customized Single-Use Purification Solution

One interesting problem that has resulted from working to minimize DNA impurities while maximizing yields is the issue of using SU tubing sets during purification. Optimally, SU items should be used for both plasmid and virus manufacture to remove concerns about DNA cross-contamination. The presence of even minute levels of DNA from other products can impact safety and quality. With SU tubing, however, there is a greater potential for product loss due to retention of product solution in the tubing.

In addition, certain downstream processing steps required to achieve the necessary purity levels for viral vectors have not yet been supported by SU technologies. For example, iodixanol ultracentrifugation is commonly used to achieve the removal of partial and empty capsids to the extent required for gene therapies. This process is very complex, however, and chromatography is preferable. Achieving the same impurity profile when moving from the technique-heavy iodixanol ultracentrifugation to chromatography has been a monumental challenge, including the need to demonstrate the fundamental equivalence of the methods.

For 15 years, the scale of most available chromatography equipment has not been appropriate for AAV purification, which requires a gradient approach to separate full capsids from empty and partially full capsids and partially assembled capsids. Since, for a systemically delivered AAV therapy, the total volume of 25 adult doses of an AAV product once purified may only measure 200 mL, the ability to achieve a highly pure and highly concentrated product with the SU systems on the market has been limited.

Therefore, approximately six years ago, Andelyn began collaborating with Verdot IPS to customize its VERDOT Ips^2 FlexiPro benchtop chromatography system. The new FlexiPro solution has four different tubing systems customized specifically for the purification of gene therapy products, ranging in flow rates from 10 mL/min up to 10 L/min. In addition, monitoring of UV, conductivity, pH, and other parameters is possible while maintaining the SU capability. Andelyn now has three of these customized FlexiPro systems on site that can be used for purification at multiple scales. It should also be noted that the system is currently being optimized to achieve all-column purification at a large scale.

Improving Overall Yields

The question of yields is intuitive; the lower the yield of viral vector per cell, the higher the level of contaminating proteins, DNA, and other materials. A low-yielding process, even if it produces sufficient vector for a client, is not necessarily worth completing, because downstream purification results in too much product loss and the isolation of low-quality vector that will not meet release criteria. The harvest of a low-yielding product results in high rates of loss owing to non-specific binding and the selection of only highly pure chromatography fractions.

It is thus essential to push the envelope on yield for every product. At Andelyn, we employ a QbD approach, completing upfront development work to ensure the development of high-yielding processes. This additional work on the front end directly impacts the design of the manufacturing process and, in particular, downstream purification, saving a considerable amount of time and effort during scale-up and commercialization.

That upfront development includes evaluating all aspects of the process – from the original plasmids to all parameters – to ensure that we can develop an optimal solution. Plasmids have a tremendous impact on the performance of vector production processes. Replication of intact virus is a concern with all viruses but is generally minimized with AAV. With generation 1 plasmids, however, it is possible to get spurious packaging of *Rep* and *Cap* genes, which can result in the formation of replication-competent AAV (rcAAV). Generation 2 and 3 plasmids have been engineered to reduce rcAAV, which can have a significant impact on purification and the overall success of the project.

Andelyn can also help with vector plasmid design to ensure higher expression levels, because we have worked with such a diversity of vectors and can apply that knowledge to each new project. For instance, we can help control ITR (inverted terminal repeats) deletions, which can change the yield profile of a vector in very significant ways.

At Andelyn, we employ a QbD approach, completing upfront development work to ensure the development of high-yielding processes. This additional work on the front end directly impacts the design of the manufacturing process and, in particular, downstream purification, saving a considerable amount of time and effort during scale-up and commercialization.

Managing Supply Chain Vulnerabilities

In addition to the challenges posed by the COVID-19 pandemic, maturation of the gene therapy field has created supply chain issues. In some cases, advanced, higher-performance reagent-grade materials have been developed that present considerable advantages but are offered by younger companies that are not able to offer large-scale quantities of GMP-grade materials. As a result, while it is possible to develop efficient processes with these new materials, they cannot always be easily scaled for commercial production.

For our critical raw materials, we continue to evaluate new options from new suppliers and leverage new strategic partnerships to find the best solutions for managing current supply chain issues. Multiple, redundant sourcing is necessary to confirm that, if both our primary and first backup supplier for a given medium or consumable become unavailable, we will have another alternative that will allow our clients' programs to move forward.

Remaining Regulatory Hurdles

Dialogue with the FDA continues regarding aspects of viral vector manufacturing that remain unaddressed. Two further key issues that continue to be discussed include identifying sub-visible particles in release assays and reducing contaminating capsid proteins during the production process.

Sub-visible particles in viral vector manufacturing are frequently aggregates of the vector product or of unassembled capsids. Unlike with protein-based biologic drugs, these impurities are not necessarily undesirable or detrimental to final gene therapy products. More information is needed to make such a determination. With respect to capsid protein impurities, the typical purification scheme for AAV vectors results in the presence of large amounts of these contaminants in the product. More discussion around this area is also needed. Andelyn is adding additional analytical capabilities so that we can understand what effects scale, capsid serotype, concentration, and formulation have on the AAV product.

There is also a need for additional harmonization and standardization going forward. Working with different requirements from product to product is not sustainable. Andelyn is very much active in that conversation and as a leader in the field is positioned to play an important role in helping to establish consistent standards.

Achieving that harmony in the short term will be difficult, because many players in the field are still working on platform development to achieve the scales that are needed to meet some of the evolving indication demands. Baculovirus, suspension HEK293, and adherent HEK293 platforms have different impurity profiles with respect to host-cell proteins (HCPs) and various other process- and product-related impurities.

Therefore, some base-level decisions will need to be made as an industry regarding what is and is not acceptable. The FDA is making progress in this regard, but these standards have not yet been well defined. Many questions remain. For instance, what should the base guidance be for HCPs? If a specific purity level is set, what would that purity mean? What can the contaminants be? How many and which ones must be defined?

In addition, is the full-versus-empty capsid ratio important? In certain indications, absolutely, but perhaps not for others. In an intrathecal route, an eye indication, or certain other indications that require a high viral vector concentration, empty capsid impurities would reduce the concentration of the product. At the same time, there are other aspects that would make this issue less significant in those same indications. It's a balancing act.

Indeed, concentration and formulation are further complex issues with gene therapies, both from an impurity perspective and with respect to stability. Unlike recombinant proteins and mAbs, viral vectors are biological organisms – not necessarily alive, but "live" viruses that have specific requirements. Formulation is very important in the drive to achieve higher titers, an omnipresent concern for developers. For instance, stability is different at different buffer ionic strengths, and higher vector concentrations typically result in higher concentrations of other viral proteins, such as HCPs and other contaminants.

These uncertainties make it difficult to develop platform processes; the QbD approach is only optimal when based on sufficient basic guidance. On the other hand, it is difficult to set a base guidance without being able to refer to platform processes and the products they generate.

Facilitating Growth of the Gene Therapy Industry

The gene therapy field is moving beyond rare and terminal diseases to diseases that affect much larger patient populations. The goal is to be able to treat something like male pattern baldness, which affects a larger percentage of the adult male population. The industry will have truly arrived when gene therapies for quality-of-life indications are available at a large scale.

That time has yet to come. From a big-picture perspective, the key issue is cost, which of course relates to the current manufacturing limitations. There are also concerns about increased use of SU technologies and the environmental impact that they will have if gene therapies move to very large-scale production. In addition, leveraging only SU systems is also one of the largest drivers of cost, outside of the unavoidable costs associated with drug development in general.

Once again, flexibility in manufacturing is absolutely critical. Andelyn remains focused on building flexible platform solutions that can serve the entire gamut of potential projects, from gene therapies that treat a dozen patients per year to those that will, in the future, help thousands to millions of people annually to improve their lives.

ABOUT THE AUTHORS

Lenore Giannunzio
Principal Research Scientist

Lenore is a Principal Research Scientist at Andelyn Biosciences responsible for scale-up and bioprocessing of suspension and fixed-bed bioreactors. Previously, she worked at Nationwide Children's Hospital as the process development manager and was responsible for development and tech transfer of AAV production and purification processes that are now being used at Andelyn. Lenore has also worked in fermentation, dry chemistry testing, and analytical testing. Lenore has a BS in biochemistry from Alma College in Alma, Michigan.

LinkedIn: www.linkedin.com/in/lenore-giannunzio-100a8188/

Andrew Moreo
Head of Process Development

Andrew Moreo currently serves as the Head of Process Development, Plasmid, and Viral Vector Core Facilities at Andelyn Biosciences, where he leads a diverse organization of interdisciplinary scientists specializing in process development of new vector production platforms, as well as offering solutions to clients for accelerating gene therapy products to the patients who need them. Andrew has a B.S. in biology from Purdue University and two decades of experience in genetic and molecular research, with the last 15 years at Nationwide Children's Hospital and Andelyn focusing on AAV production and development.

LinkedIn: www.linkedin.com/in/andrew-moreo-647b03123/