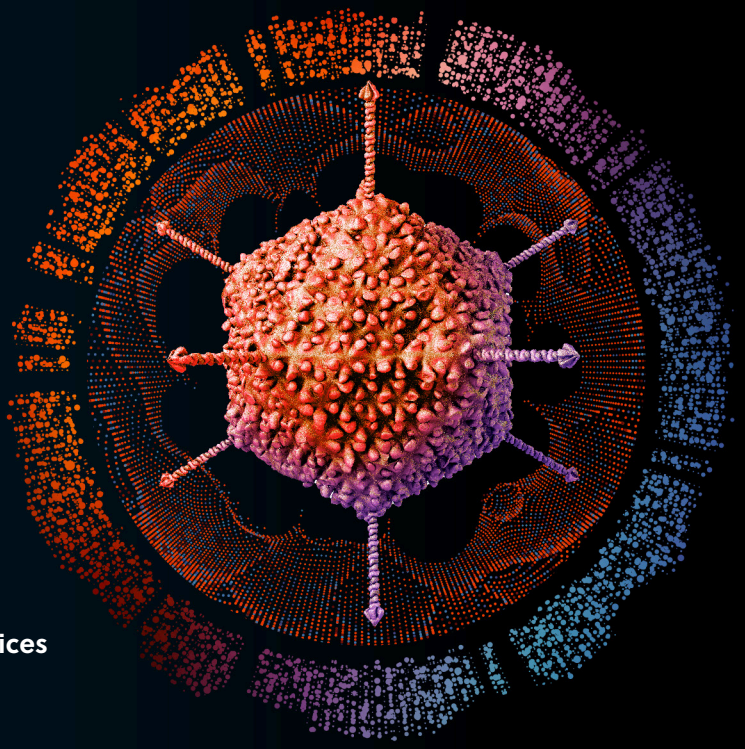
RISK-BASED OPTIMIZATION OF VIRAL VECTOR PROCESSES ADDS REAL VALUE

Elie Hanania, Ph.D., and Tatiana Mello, M.S., Avid Bioservices



Process optimization presents clear efficiency benefits, but because the process of optimization itself requires time and cost, many gene therapy developers opt to postpone optimization to improve titer and yield until they have more funding or a better assessment of their programs. However, in many cases, this approach is misguided. The need to produce additional, non-optimized batches adds time and cost, and delaying optimization activities does not result in cost or time savings over the long term. Optimization should be viewed as an investment, not a cost, because there is potential to add significant value, and finding the appropriate time for this investment is critical.

Lack of Standardization

Over the past few decades, there have been significant scientific developments in the gene therapy sector. Unlike the production of monoclonal antibodies (mAbs), viral vector manufacturing has not yet become standardized across the industry, and the regulatory environment is not yet fully defined.

The large diversity of viral vectors and the myriad applications for which they are used make standardizing production and purification processes difficult. The nature of the viral vector, structure, genetic make-up, protein composition, and overall cargo influence the manufacturing approach. The adeno-associated viruses (AAVs) most often used for gene therapies are small and stable, while lentiviruses (LVs) are much larger and more fragile. In addition, there are many AAV serotypes, which makes targeting specific tissues possible, but different serotypes often require different conditions for purification. The gene of interest (GOI) also has a huge impact on process outcomes.

These variations make the development of standardized or platform processes quite challenging. In addition, not only the vector product itself but the cell culture media, transfection reagent, process conditions, and many other factors all play crucial roles in determining the titer (and associated yield) of vector manufacturing processes.

As a result, biopharma companies use different upstream production systems and recovery strategies, which presents challenges to the quantification and comparison of titer and yield across different programs and, sometimes, even among the same portfolios.

For the downstream process, the approach is more involved and more complex compared with what is used for mAbs. There are different types of filtrations (clarification using depth filters, tangential flow filtration using hollow fibers and cassette filters, as well as sterilizing filters with various membrane composition). When it comes to separating the viral vector from other contaminants (with similar size, charge, or composition), different types and sequential implementation of chromatographic approaches are required. This includes affinity, ion exchange, and/or size exclusion, with many variations within each category. The complexity and multifaceted nature of the purification process have an impact on overall recovery, with inherent losses associated with each step.

While the optimization of viral vector production processes is complex, it is essential for each unit operation because any slight change in the viral vector, starting material, or processes conditions may impact the outcome. All stakeholders in the field are looking to improve both titers and yields, but to be truly successful the industry must first overcome asset-specific challenges while still optimizing processes.

Real Consequences

The number of doses and the total number of virus particles per dose are directly impacted by titer and yield. Lower titer and yield mean more manufacturing batches to meet production scale needs. Total particle titer is therefore one of the parameters on which to focus when developing and optimizing a process. Titer helps determine productivity and hence estimation of the required production scale. Yield can be calculated by accounting for the volume at each step and is used to assess product recovery or loss throughout the process. It is also important to determine the functional (infectious) titer, especially at the end of a process, because it is the true measure of how much active virus has been generated and the true yield of the production/purification process.

Limited Lessons Learned from mAb Manufacturing

The transient transfection or infection processes for viral vector production are quite different from the cell culture process used to manufacture mAbs. There are four primary unit operations within the upstream process for vectors: (1) plasmid development and production, (2) cell expansion to the desired cell density, (3) plasmid transfection or viral infection, and (4) viral vector production, where transfected or infected cells are then allowed to produce the viral vector.

Only the cell expansion step is shared with other biomanufacturing processes, such as those for mAbs. Previous experience and learnings regarding this unit operation may be directly applicable. Beyond that, extensive specific viral vector production expertise is required to be able to improve titer and yield.

In the early days of mAb production, titers were likely in the range of milligrams per liter, whereas today, they are as much as tens of grams per liter. Much effort focused on cell line development, as well as improvement of the performance of individual unit operations, can be attributed to these advances. With viral vectors, transient transfection or continuous infection dominates, and there is still much work needed to enable the practical use of producer and packaging cell lines. The biggest issue is the fact that many viral vectors are toxic to the cells in which they are produced, which is not an issue for recombinant proteins and mAbs. As a result, there are many more parameters to optimize to realize higher titers and yields. Separately, there is also progress being made with the design of the plasmid DNA used to produce viral vectors. For instance, plasmids are getting smaller through the removal of unneeded sequences, leading to greater cell line productivity.

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Upstream Optimization Opportunities

Optimization efforts can vary significantly from program to program and from company to company. Some are focusing on capsid engineering and payload design, while others are trying to develop more robust and stable producer cell lines. As a CDMO with a focus on the cell and gene therapy market, Avid Bioservices seeks to support clients by optimizing their viral vector production systems as they transition from preclinical and early clinical projects to cGMP clinical and commercial manufacturing.

It is best to focus optimization efforts first on the upstream process, because, if more material is generated upstream, the tolerance can be higher for some losses during downstream purification. In addition to improvements in the plasmids used for vector manufacturing, cell culture media suppliers have also introduced media design specifically to support viral vector manufacturing through higher cell densities, allowing the growth of more cells – and with those cells the production of more virus – in a smaller footprint.

From the upstream perspective, we have seen several-fold improvements when optimizing cell culture parameters, improving transient-transfection platforms and developing better strategies for product harvest and clarification. We are gaining a better understanding of how and when to transfect or infect the cells to produce more virus. The challenge at this point in the process is to understand the physiology and metabolism of the cells and how each environmental or physical parameter will impact cell growth and maintenance.

It is essential to maintain the cells under optimal conditions throughout the upstream process to enable the production of as many viral particles as possible. Optimizing physical and chemical parameters – including the temperature, pH, dissolved oxygen, osmolality, shear stress, seeding viable cell density (VCD), gassing strategies, and nutrient supply – is crucial.

Once a process is designed to achieve desired titer and yield, the focus shifts to ensuring the quality and safety of the vector product, with potency, efficacy, and infectivity of the viral particles being key attributes.

For processes that rely on transient transfection for vector production, the concentration of viable cells at the time of transfection, as well as the total amount of plasmids, transfection reagent selection, DNA-to-transfection reagent ratio, plasmid ratio, and transfection time, are target parameters for titer optimization.

Optimization of productivity and titer for vectors produced via infection focuses on parameters such as the multiplicity of infection (MOI), infection time (TOI), feed supplementation, and harvest time. The quantity of virus seed must be optimized; adding too much can kill the cells due to the toxicity of the virus, while adding too little will not lead to virus production in a practical timeframe.

Downstream Improvements Possible

During downstream processing, optimization is achieved by carefully matching the chemistry of the specific viral vector product with the conditions of the purification and recovery steps, such as pressure, buffer composition, pH, and conductivity. Fit-for-purpose solutions can be developed for each unit operation depending on the nature of the vector and the goal of the specific purification step, enabling optimization of both titer and yield. Importantly, advances in resin technologies are providing new and different approaches to purification that help minimize losses while increasing purity.

Early Optimization is Essential

Process optimization is initially performed at small scale, but always with the ultimate commercial-scale needs in mind. It is essential to understand which process gaps need to be managed and how much the product titer needs to be increased to attain the program's needs. Starting as early as possible in the production cycle is crucial, because improving productivity while maintaining good process control and product quality attributes is a challenge. It is also extremely difficult to make process changes following product approval; it is best to have the process optimized before regulatory filing.

Design-of-experiment (DoE) studies performed in Erlenmeyer shake flasks or benchtop reactors make optimization efficient, because multiple DoE runs can be performed at the same time with lower cost and operational time, allowing multiple parameters to be evaluated simultaneously.

It is important that, within a DoE study, the same vessel type be used for all runs so as not to introduce additional variables (e.g., different geometries) that can impact pH and DO control, the gas transfer strategy, agitation, and so on. The best approach is to define a single upstream system that will be used to run the optimization study and vary only the selected parameters without adding inherent variations and associated errors. Once the cell culture optimization is complete, the outcome can be evaluated/verified in other types of vessels (scale-up models).

The initial focus is improving the titer – the quantity-per-volume of virus, because ultimately the functional titer will determine the number of doses that must be manufactured. Once a process is designed to achieve desired titer and yield, the focus shifts to ensuring the quality and safety of the vector product, with potency, efficacy, and infectivity of the viral particles being key attributes. For AAV, maximizing the ratio of full to empty capsids is also important.

Be Aware of Vector Differences

Although there are similarities in the general production scheme for viral vectors, there are still many differences related to the nature/origin of the vectors. Physical characteristics (e.g., enveloped vs. naked; size; shape; arrangement of proteins and nucleic acid), genetic composition (e.g., RNA, DNA, SS, DS), and size (20 to ~ 400 nm) are among the major factors that contribute to such differences. As such, applying a standard or plug-and-play production purification approach may result in varied outcomes.

However, it is possible to apply a similar strategy to process optimization independent of the type of vector. All vectors are produced via cell culture and using transient transfection or infection processes, and the goal for upstream processes regardless of the virus type involved is to maintain healthy cells, use optimal starting materials, and establish optimal process conditions that are conducive for efficient transfection or infection to thus enable production of the largest quantity of functional viral particles possible. For downstream processes, the vector size and chemistry will impact the choice of chromatography and filtration materials, but the overall approach to optimization of each unit operation remains the same: maximize the purity of functional viral particles while minimizing losses.

Step-by-Step Approach Recommended

Optimization of viral vector processes is best performed using a multistep approach. The first step is to evaluate the overall process flow diagram and determine product titers for each unit operation. The goal is to identify which process steps require improvement and which need to be prioritized (while considering product quality, cost, and time).

Next, the critical parameters must be defined, and their potential impact on the overall process must be assessed. A common practice is to perform a DoE study to evaluate these critical parameters. Unlike one-factor-at-a-time (OFAT)–based optimization, a DoE-driven approach allows evaluation of the impact of multiple interdependent factors on a given output.

Once the optimal titer has been achieved, the overall yield can be estimated and used to determine the production scale required to meet client requirements (i.e., number of doses based on amount of virus per dose). The mass balance for the process is then generated in order to calculate the yield for each of the successive production steps. Product recovery and loss are assessed to identify unit operations that may require further optimization to manage excessive loss.

Product quality should always be factored in while optimization is being performed. All residuals – including host-cell DNA, host-cell proteins, plasmids (if used), empty capsids, and any other reagents and materials introduced during production – are accounted for, measured, and eliminated. A good downstream purification approach is essential for eliminating such contaminants.

Optimization is an Investment

Three key parameters typically impact products: time, cost, and quality. Con-

trolling one or two of these factors is manageable, but it is extremely challenging to focus on all three simultaneously.

Time is often the most critical factor for gene therapy developers, because it has a direct impact on regulatory filing and delivery of clinical/commercial products to patients. Quality (and associated safety) cannot be compromised for any reason. Process shortcuts or speed cannot be justified if the outcome is impacted negatively. Accordingly, focusing on the "time" and "quality" parameters will require that tasks be done in parallel, supply chain orders be expedited, and testing and characterization be prioritized. Hence, cost will be high!

The same rationale can be applied to process optimization. When cost is not an primary concern, a large number of optimization studies can be performed on all the factors and steps for production and purification of viral vectors. Studies are typically run in parallel with expedited testing. From a practical consideration, focusing on the key steps and important factors that have the highest impact on process outcome and product quality can save time and cost without compromising quality.

Because optimization has time and cost implications, many gene therapy developers opt to postpone optimization to improve titer and yield until they have more funding or a better assessment of their programs. Going with the notion of "good enough" seems to be common practice, particularly when timing (especially for early programs) is critical. This approach can sometimes be compensated for by producing larger batch sizes or multiple batches to facilitate the delivery of the total number of needed doses.

However, in many cases, such an approach is misguided. The need to produce more batches adds time and cost, and companies will not save time or money in the long term. Optimization should be viewed as an investment, not a cost. There is potential to add significant value by pursuing even basic optimization activities.

A risk assessment of the investment-to-outcome ratio must be performed first. While it would not be worthwhile to spend six months to achieve a 5% increase in titer, doing so to realize a 5- or even 3-fold increase would provide a considerable overall return. In addition, using a standardized approach to optimization that relies on DoE studies can minimize both the time and cost of optimization.

Quality, Technical Skills, and Customer-Centric Approach Equal Success at Avid

Avid is a client-centric CDMO that always considers client needs and timelines. Our strengths as a CDMO include our established quality infrastructure and systems, as well as the technical skills of our team members, who have been in the business for decades and thus have deep knowledge of manufacturing large biological molecules.

Having dealt with different types of viral vectors for gene therapy applications and vaccines, Avid can support all clients with their needs when it comes to more common AAV and LV, as well as less common or unique candidates. In addition, we have expertise in emerging modalities, such as exosomes, which are similar to viral vectors in regard to production and purification. Once such processes are in place, we can ensure they are robust, scalable, and ready for cGMP manufacturing of safe and efficacious products.

Furthermore, at Avid we adopt a holistic approach to each process brought to us by our clients and identify optimization opportunities for measurable improvement. Parallel approaches can be adopted if warranted to rapidly produce material for immediate needs and to optimize titer and yield for future production runs. Our scientific strategy, which leverages DoE studies, our strong analytical team, and our internally developed platform assays, accelerates the optimization of titer and yield to add real value for our clients.

ABOUT THE AUTHORS

Elie Hanania, Ph.D

Vice President Process Development – Viral Vector Technologies

Elie Hanania, Ph.D., Vice President, Process Development–Viral Vector technologies at Avid Bioservices (Costa Mesa, CA) obtained his Ph.D. in cell and molecular biology from the University of Texas Medical Branch (Galveston, Texas), and he did his postdoctoral training at the M.D. Anderson Cancer Center (Houston, TX). Elie led the process development teams at different cell and gene therapy CDMOs, including Progenitor Cell Therapy – a Hitachi Company (Mountain View, CA), Millipore Sigma (Carlsbad, CA), and Fujifilm Diosynth Biotechnologies (College Station, TX) Elie's expertise spans academic, clinical, and industry locales, with diverse experience in molecular and cell biology, virology, cell line development, primary and stem cells, device validation, process development, technology transfer, process characterization, animal models, and assay development with implementation of empowering technologies for the advancement of cell and gene therapy.

LinkedIn: https://www.linkedin.com/in/elie-hanania-653408/

Tatiana Mello, M.S.

Senior Scientist Upstream Process Development – Viral Vector Technologies

Tatiana Mello, Sr. Scientist, Upstream PD, at Avid Bioservices is an experienced scientist with a demonstrated history of working in the biopharma and bioprocess engineering industry. With 5+ years working in the biopharma industry, she specializes in the upstream viral vector process development area, with deep knowledge in bioreactor scale-up, cell culture, exosomes production, and recovery technologies. She has worked on multiple different projects, including tech transfer, feasibility, optimization, and process characterization. Her education background includes two bachelor's degrees, one in biology and one in mechanical engineering, which were combined and intensified during her post-graduate studies in the biochemical engineering area.

LinkedIn: https://www.linkedin.com/in/tatiana-t-s-mello/