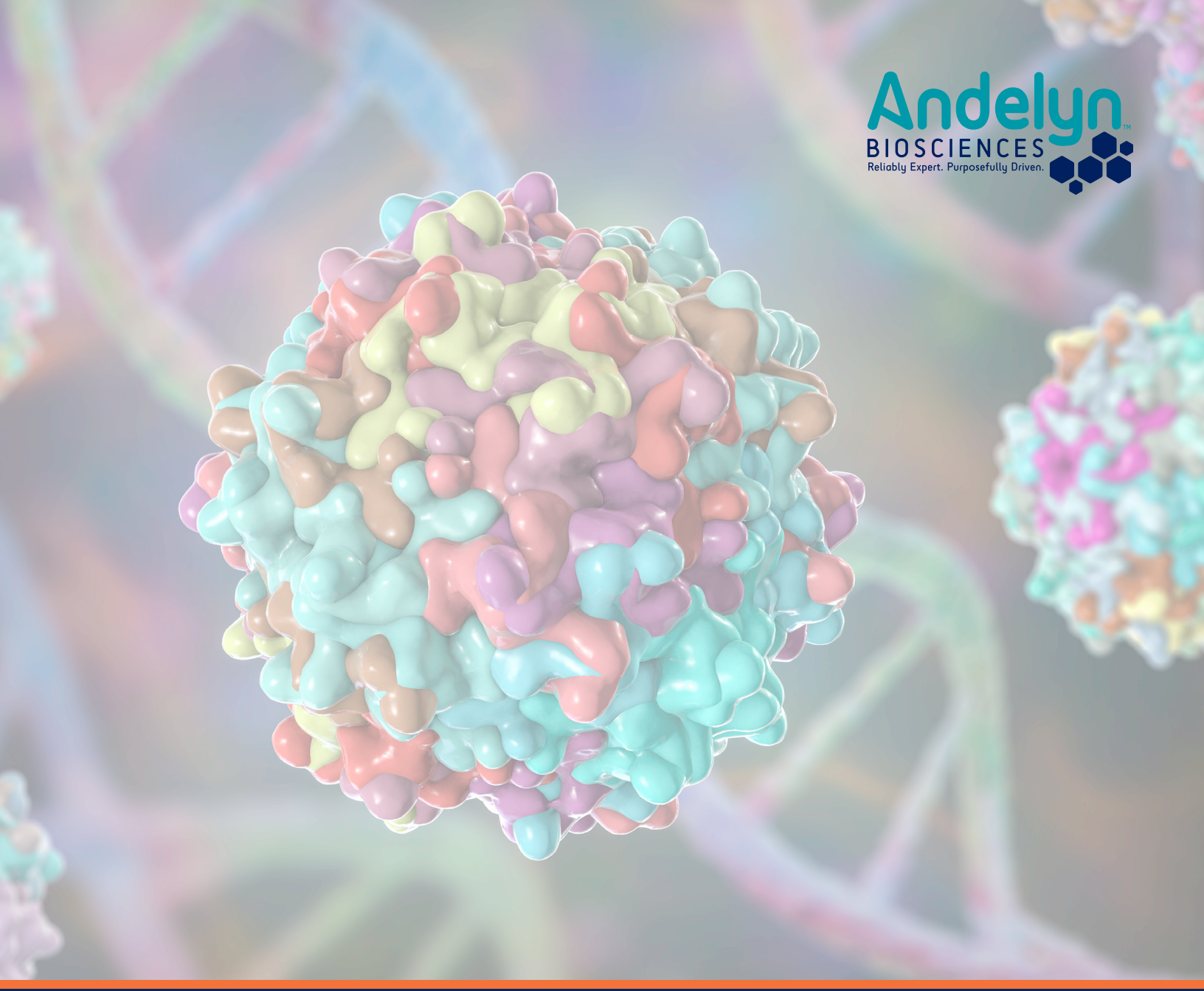

The Future of Viral Vector Manufacturing: Yield Optimization

By **Andy Moreo**, Head of Process Development, Plasmid and Viral Vector Core Facilities, and **Lenore Giannunzio**, Principal Research Scientist, Andelyn BioSciences

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Viral vectors are more than a vehicle for a payload – their ability to target specific cells has made them one of the most promising treatment modalities to emerge in recent years. But the specificity enabled by this tropism has created complexities impacting their scale-up.

Ensuring optimal yield for engineered viral vectors is crucial to ensuring their eventual commercial viability. Despite this, it can be easy to overlook the factors that impact yield performance early enough, resulting in suboptimal yields, rework, and the potential scrapping of an otherwise promising therapeutic. Frequently, issues related to yield are not uncovered until toxicology testing has already commenced, and considerable time and cost investment has already been leveraged toward a transgene that, while effective, is production-inefficient.

The heavy lifting inherent to development in cell and gene therapy contributes to the high costs of production for these therapeutics, and the commensurately high cost for a patient. Zolgensma, a gene therapy for spinal muscular atrophy, is the most expensive drug on the planet, averaging more than $2 million for a single dose. It is critical to drive down the cost of viral vector development to make these life-changing therapies more accessible, facilitating more targeted treatments for a greater range of diseases and quality-of-life improvements.

The Variables that Impact Yield Optimization for Viral Vectors

Scientists have greatly advanced the field of engineered serotypes by fine-tuning their epitopes and proteins to improve tropism, transduction, and specificity. In contrast, "wild serotypes," such as rh74 or AAV9, have had over a billion years to optimize their packaging efficiency, resulting in a superior yield profile in a production setting.

Another core focus of this research has been transgene development, with a particular emphasis on expression. Early in a product's life cycle, the driving question centers on how to design a transgene that achieves optimal expression with an already limited payload capacity. This is a complex endeavor in itself, as a portion of that capacity is earmarked for inverted terminal repeats (ITRs), promotors, poly(A) signals, and other features that must be present to meet regulatory requirements. Therefore, the molecular development needed to produce a functional coding sequence, with a high expression level, able to be packaged in a tailored capsid is already a difficult task; working concomitantly to ensure yield may fall to the wayside as a result of this complexity.

Optimizing a yield profile early, and subsequently scaling with yield performance in mind, can help companies more easily achieve the productivity demands required for the whole product lifecycle. Downstream purification losses average between 50 and 70 percent for non-optimized processes. Ultra-centrifugation is one of the most notorious points that spur product loss, as well as one of the most common workhorse steps to purification; transitioning entirely to column purification would be one potential solution to minimizing that loss. But purification losses are typically compounding; while a discrete purification step may boast an average 20 percent product loss, previous and subsequent purification steps with the same average loss will contribute to the majority of a product lost to purification when evaluated holistically. Some level of static, adsorptive loss is unavoidable at each step, but improving up-front yield can serve to mitigate losses and improve downstream purification.

Mitigating Costs Through Standardized Platform Technology

It is important for biopharmas to understand that yield profiles of a product can vary greatly based on serotype and DNA sequence. Therefore, researchers must go through a thorough screening process of transgenes, backbones, and serotype configurations to establish critical production parameters prior to selecting the best candidate for production and clinical performance. To achieve this, AAV candidates can be sent to a platform development lab with standardized processes, equipment, materials, and expertise to evaluate their performance through a standardized Design of Experiment (DoE) study in adherent or suspension cultures. By balancing customization and standardization through flexible approaches, companies can gain important insights on both upstream yield and downstream purification.

While prioritizing tropism and transgene expression is integral, focusing on other variables, such as cell density, packaging efficiency, incubation, and hold time duration, stability and impurity profiles are critical to mitigating costs and creating efficiencies. Enabling the interchangeability of materials and working toward process standardization across as many serotypes as possible is key to the future of the cell and gene therapy space. At present, the

proliferation of custom-built platforms for specific transgenes has created a flood of siloed, individualized processes, impacting cost, time to market, and manufacturing efficiency.

Optimizing viral vector yield also means right-sizing processes. Accurate lot sizing is critical for this optimization; the ability to operate at multiple scales, considerations such as sub-lot production size to minimize pooling, or other considerations can reduce waste and lower cost of production. This standardization can be elusive for some therapeutics, but for those whose projects can accommodate it, it can serve as an important tool for optimizing yield at a reduced long-term cost. Many of the nuances in AAV production and scale-up can only be identified and addressed over multiple production runs. Experience and standardization on the part of a manufacturer can prove critical in avoiding unnecessary delays.

Future Trends in Standardizing Yields for Viral Vectors

The current AAV research landscape is still far from achieving the standardization of other parts of the biopharmaceutical market, because of the inherent variability of the biological process. The sheer amount of data needed to enable predictive analytics is still a long way off for many developers, most of whom are working with highly proprietary, engineered serotypes in a highly competitive market. But achieving product equivalency will be critical to maturing the market in the coming years, and the maturation of the analytics behind that market will be key to achieving that equivalency.

The variability between vectors is immense, owing to both the selected serotype and transgene, as well as other factors like size of the genetic payload and sequence, which can affect its packaging, density, buoyancy, or affinity. The intersecting variables that inform an AAV's development, coupled with the competition and evolving regulatory standards that attend these therapies, has made developing a standard approach difficult. This, along with interchangeability and a push for harmonization, represents the future of AAV therapies that are affordable and accessible.

Ultimately, partnering with a contract development and manufacturing organization (CDMO) with the expertise and platform technologies to help balance a project's customization and standardization can help companies avoid the more common pitfalls of AAV development. Ensuring optimal yield for these therapeutics is a complex endeavor; it is also a crucial one, as many projects with immense therapeutic potential have been waylaid by poor yield. In a market prohibited by costs, both on the part of the developer and the end user, pursuing long-range advancements in process standardization, data collection, and yield optimization will serve to stabilize the space and advance more targeted therapies.