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White Paper

Scaling Up the Learning Curve for Large-Scale AAV Manufacturing

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This article is the first in a four-part series exploring the challenges associated with high-demand AAV manufacturing from early development to commercial production.

Manufacturing challenges, especially at larger scales, remain some of the biggest hurdles when commercializing adeno-associated virus (AAV) therapies. Demand for AAV vector production is greater than ever, driven by clinical pipeline growth, a proliferation of therapies targeting broader patient populations, and an increasing number of therapies requiring higher dosages. According to the FDA's Peter Marks[1], 2024 is likely to be a "breakout year" for gene therapy approvals. However, significant manufacturing challenges remain both for treatments targeting very small patient populations (<100 per year) as well as for treatments targeting large patient populations (>10,000 per year). For many current AAV therapies, the scale of manufacturing is in a "sweet spot" where some economies of scale reduce manufacturing costs, but the manufacturing batch sizes are small enough to effectively utilize existing technologies and avoid the challenges associated with scaling to larger batch sizes[1].

The complexity of manufacturing AAV products and the importance of managing costs underscore the importance of improving the efficiency and scalability of these processes. The price of these therapies has been a major focus for industry and news coverage since their introduction. Since their introduction in the U.S., the handful of approved AAV drugs on the market range in price from just under $1 million per patient to more than $3 million[2,3], limiting access for some patients. Additionally, when therapies target larger patient populations, pricing pressures on manufacturers from payers increase. One way these challenges are being addressed has been to focus on lowering the cost of goods for these therapies, underscoring the need to develop optimized, scalable, cost-effective AAV production processes.

For companies looking to manufacture AAV therapies, there may be different time pressures and regulator pathways for therapies targeting small patient populations versus larger populations. For example, for an ultrarare disease indication for which effective therapies may not exist, manufacturers may benefit from expedited regulatory approval. In contrast, a larger-scale program targeting a more common disease with approved treatment options is less likely to be afforded an expedited approval pathway and instead may face other considerations, such as establishing sufficient manufacturing capacity.

While the size of a drug's patient population directly affects manufacturing needs, dosing requirements also play a key role in determining commercial scale. For instance, consider the first two AAV therapies to achieve FDA approval: Luxturna®, an ocular medication treating an inherited retinal disease, and Zolgensma®, a drug used to treat spinal muscular atrophy. The dosing difference between Luxturna®, which is administered directly to the eye, and Zolgensma®, which must achieve more systemic circulation, is more than 1,000-fold[4,5]. This large difference in dosing has significant implications for the manufacturing demand and scale. For high-dose AAV therapies, especially those targeting large patient populations, scaling up manufacturing while optimizing productivity is critical to lowering the cost of goods.

Yet, even companies with experience with monoclonal antibodies at large scale or AAV at small scale face a steep learning curve when moving to large-scale AAV production, where experience is limited. Moving to large-scale production requires manufacturers to decide whether to scale up or scale out AAV production. From a technical standpoint, transitioning to larger process scales is more challenging than adding more manufacturing lines. However, more manufacturing lines means higher capital costs, more personnel and dedicated space, increasing the cost of goods. Consequently, manufacturers are highly incentivized to scale up and optimize their AAV process when demand is high.

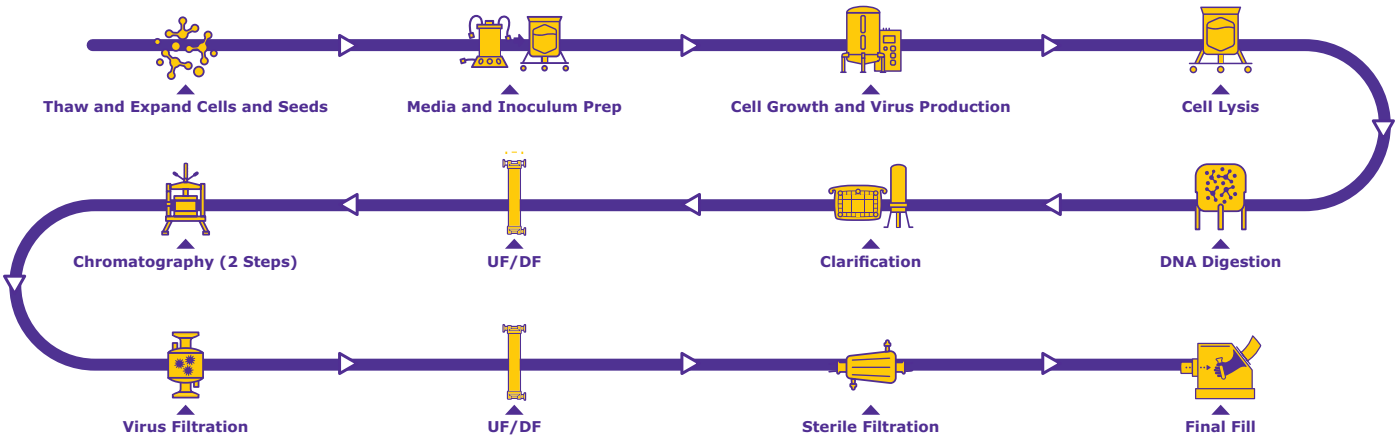

Figure 1:

Typical AAV process unit operations

Upstream And Downstream: Common Bioprocessing Challenges Remain

Many times, one of the first challenges to scaling up production of AAV is to convert the upstream steps from an adherent platform, which relies on two-dimensional scale-up (surface area), to a suspension platform that enables scale-up in three dimensions (bioreactor volume). In addition to reducing footprint in the manufacturing space, suspension platforms typically require less equipment and less manual intervention than adherent platforms. However, this shift, from adherent to suspension platform, requires changing the production cell line, adding both time and risk to the development process. Likewise, other aspects of the upstream process including the cell culture medium, transfection process, and bioreactor equipment and control will also change considerably. While these risks may not be considered worthwhile for lower demand therapies, for higher demand AAV therapies the adoption of a suspension upstream platform is usually an essential step to reaching cost targets.

Processes that have successfully transitioned to suspension cell culture platforms still face various issues impacting their cost effectiveness, many linked to scalability and process productivity. One common challenge is achieving optimal plasmid transfection at larger process scales. Without careful optimization of the transfection step at the larger scale, titers may be considerably lower at the 2000 L scale compared to the 200 L scale, where developers might already have experience. While some processes may reduce or eliminate the challenge of large-scale transfection by using alternative approaches such as packaging cell lines, helper viruses, or producer cell lines, most processes currently rely on a triple-plasmid transfection step for AAV production.

Optimizing media, feed, cell line development, plasmid engineering, bioreactor conditions, and transfection efficiency concurrently can improve titers, resulting in smaller-scale upstream processes or fewer batches. While there are opportunities in each area to improve productivity, it is important to recognize the interplay between these activities. For example, the media and feed are optimized for a specific cell line, but they should also be compatible with the transfection reagents.

During cell harvest, the number of variables impacting ultimate success makes optimization a challenge. Cell lysis often uses detergents to release the viral vector from the cells, along with other cellular debris and impurities. DNA digestion is commonly accomplished using endonuclease, and these steps are typically followed by clarification using depth filtration and then, optionally, tangential flow filtration (TFF) to concentrate the AAV and exchange buffer. Many of the unit operations within the harvest step are the targets of potential improvement – for DNA digestion, for example, there is the challenge associated with determining the optimal amount of endonuclease to use. Sufficient enzyme should be used to ensure robust DNA digestions with an appropriate safety factor to accommodate potential process variation, while at the same time the amount of enzyme should be minimized to reduce costs. The lysis step also presents opportunities to improve process yield – for example, high-salt lysis has been shown to improve AAV recoveries[6]. Additionally, the selection of the right endonuclease can serve to optimize this unit operation by making certain the endonuclease has high activity under the relevant process conditions such as salt concentration.

Downstream AAV purification typically uses both capture and polishing chromatography steps, which also face challenges related to costs as well as product purity. Affinity resins can be effectively used for AAV capture but their reuse is often limited, resulting in higher manufacturing costs. During polishing chromatography, achieving an appropriate full/empty capsid ratio is critical, as too many empty capsids in a product can impact the drug's safety, limiting the potential dosing range and putting clinical success at risk. Developing the polishing chromatography process requires identifying conditions that balance the need for high recovery with purity.

Forging The Future of Gene Therapy

The AAV therapies of the future will require the AAV manufacturing processes of the future. As the industry sees pipelines with more AAV programs targeting high-dose indications and/or larger patient populations, demands will continue to grow for processes that can manufacture more material and reduce the cost of goods. These demands can be met with a concurrent advancement in the process technology to improve productivity and drive down costs.

Opportunities exist throughout each unit operation in these processes to increase upstream titers, improve downstream recovery, reduce batch failures, and more efficiently utilize materials and equipment.

This article, the first in a four-part series exploring the challenges associated with large-scale AAV manufacturing, looks at the challenges facing these modalities broadly. The subsequent articles will look more closely at some of the specific technical challenges for these processes and potential solutions.

References:

1. Marks, P., 2024, "Expediting the development of cell and gene therapy", State of the Industry Briefing, Alliance for Regenerative Medicine, January 8, 2024, San Francisco
2. Wilson, R., Carroll, D., The daunting economics of therapeutic genome editing. CRISPR Journal 2019, 2 (5), 280-284
3. Naddaf, M., Researchers welcome $3.5-million haemophilia gene therapy – but questions remain. Nature 2022, 612, 388-389
4. Luxturna® [package insert]. Spark Therapeutics, 2022
5. Zolgensma® [package insert]. Novartis Gene Therapies, Inc., 2023
6. Yu, C., Trivedi, P., Chaudhuri, P., Bhake, R., Johnson, E., Caton, T., Potter, M., Byrne, B., Clement, N., NaCl and KCl mediate log increase in AAV vector particles and infectious titers in a specific/timely manner with the HSV platform. Molecular Therapy Methods and Clinical Development 2021, 21, 1-13

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07/2024