The keys to achieve success with commercial AAV manufacturing scale-up

Once you have developed the optimal gene sequence and plasmids for a therapeutic and selected your HEK293 cell line for adeno-associated virus (AAV) transient transfection, achieving robust and consistent high-quality productivity becomes the next hurdle to successful commercial manufacturing. The keys to accomplish this depend on clearly defining your criteria for successful scale-up. Additionally, using a fully optimized cell culture medium, and identifying it early in the development process, can avoid time-consuming and costly redevelopment at scale-up.

Defining success

Establishing the criteria for success in your gene therapy process is a critical first step in scale-up process development. By carefully defining the criteria, you can identify and optimize your medium more carefully in preparation for commercial production. This requires determining and evaluating the key factors associated with successful commercial manufacture, including upstream factors for robust and consistent cell growth, viability, and vector productivity. It is also important to appreciate the impact of these factors on downstream purity and efficiency. However, most crucial to success is the delivery of a high-quality product.

Product quality

The top priority is the establishment of critical-to-quality (CTQ) product attributes and parameter requirements. These attributes and requirements are usually defined well before commercial scale-up, since they are essential in establishing that the product is viable for clinical trials, regulatory approvals, and commercialization. Once established, the CTQ characteristics must be achieved throughout the commercial manufacturing process.

For AAV manufacturing processes, the particle attributes considered most important include those associated with safety, identity, potency, and purity. This includes maintaining a high reproducible level of full capsids that contain the correct target gene sequences and demonstrate comparable infectivity and transduction in a disease-relevant cell line. Measuring these characteristics may require a variety of methods depending on the specific quality attribute. For example, AAV physical titer is measured using an ELISA, and genomic titer can be measured with quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) analytical methods. However, the keys to scale-up success are to first make sure all CTQ attributes are well-defined and then to assess the long-term feasibility and sustainability of the process as production scale increases.

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Scalability

Additional closely related factors for successful scale-up involve the early consideration of long-term maximum production scale and timing. Accurate maximum scale forecasting and timing considerations—depending on the estimated dose, indication, target population, and regulatory approval path—can help make assessments regarding the ease of manufacturability and long-term sustainability of the process. Such considerations can help you make more accurate and informed decisions about whether a process will successfully take you through to clinical and commercial manufacturing, without requiring time consuming and costly redevelopment.

Most AAV manufacturers understand that suspension cell culture methods are important for successful commercial scale-up, especially for therapeutics targeting high dosages or large patient populations. However, some may not readily consider key upstream factors such as medium format (i.e., a liquid or dry format) and whether their format will be feasible and sustainable at maximum scale. When selecting a medium format, prototyping can be a valuable tool to understand the manufacturability of your formulation. Small-scale media prototyping allows for performance evaluation with your cells and process before costly scale-up to larger commercial volumes. For example, if you find a dry format is best for higher-scale production and need a medium converted from liquid to dry format, prototyping may be essential, as all liquids do not readily convert to dry format. Further, utilizing a well-established supplier with strong quality standards can help maintain the sustainability of your process.

Downstream considerations

Achieving effective downstream scale-up requires maintaining consistent, adequate product purity levels. Downstream purification success is often closely related to successful upstream production and midstream lysis and clarification steps. For example, maintaining an effective and efficient downstream process relies on high-quality capsid production and minimized contamination upstream. To address these issues, many AAV manufacturers understand the importance of using animal origin–free (AOF) and chemically defined products that can help reduce unexpected components or potential contaminants, and in turn, help sustain more consistent downstream purity and efficiency.

Identifying and selecting the optimal media and process

Once you have considered what success looks like in your process, it is then time to select the optimal media for commercial scale-up. To identify a medium, many AAV manufacturers will typically conduct screenings of multiple catalog media, which, due to a lack of exact formulation knowledge, may limit the selection of a diverse range of components and concentrations. More often than not, this approach does not effectively identify an optimal formulation for their cells. To achieve the best results, an improved approach is to begin screening with a range of media options with known diverse component concentrations that are specifically designed for your cell type and application.

Screening cells within your process with these types of media can more effectively and efficiently narrow down the field and identify the better media candidates. Screening of a well-designed panel of media can provide key information about which component groups are important for further optimization. The recent emergence of HEK293 AAV–specific media panels has aided this process by providing a nutritionally diverse range of preformulated media options that can help identify the best base formulation and reveal the potential component groups crucial to further improving productivity.

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A media panel process is designed so that once the better-performing base medium and critical component groups are identified, the component levels can be fine-tuned as well as identifying if other components or supplements could maximize productivity. The optimization process for enhancing the base medium could use a number of different analytical and statistical approaches but should involve design of experiment (DOE)–based growth and productivity assays. For high-density and stable processes, spent media analysis may be used to identify how a range of media components—such as amino acids, vitamins, or lipids—change over time. More recently, additional modeling using multi-omics or omics and DOE approaches, including proteomics and metabolomics, are coming into greater use and help provide in-depth insight into cell metabolism pathways and provide potential for even greater optimization.

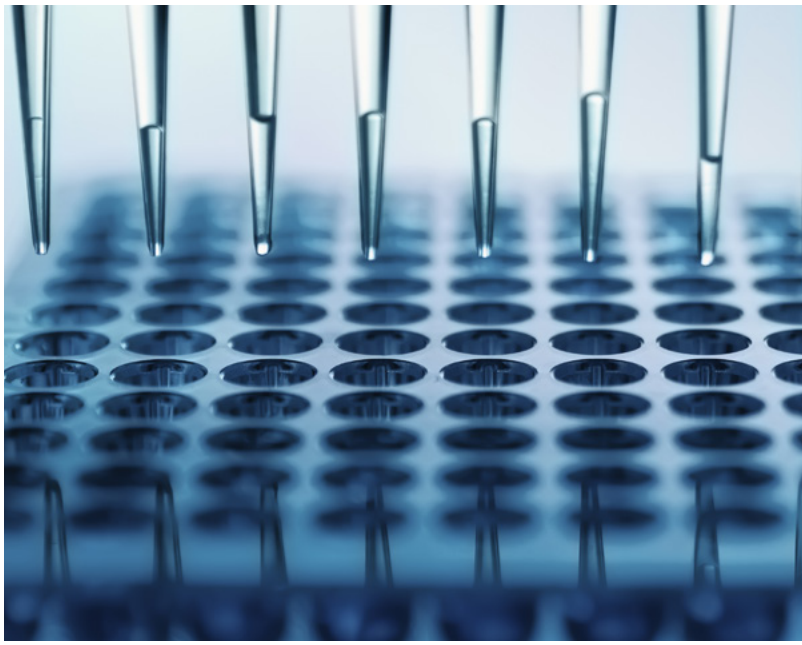
Furthermore, optimization with any of these approaches can be extended to identify whether component mixtures or feeding-process strategies could be utilized to maximize cell growth and productivity. The effective use of these technologies for complex medium and process optimization requires a combination of extensive knowledge and experience in cell biology, cell culture, and media formulation chemistry and methods. Additionally, adequate labor, analytical capabilities, and data processing power are required to correctly identify the factors that will lead to a fully optimized medium and process. Manufacturers should strongly consider whether this type of optimization could be conducted best with current in-house capabilities or whether collaborating with an experienced supplier would be a more effective strategy.

In addition, an optimization plan should be customizable and based on your desired outcomes. For example, the optimization process could be tailored to help identify a platform-based medium for multiple molecules or a more specialized, fine-tuned medium for a specific molecule. Either way, the degree and focus of optimization should be individualized and customized to meet your specific needs.

To optimize or not?

While a manufacturer may be able to bring a biotherapeutic to market without optimization, the longer-term consequences may result in product delays and substantially higher costs down the line. Although it would be attractive for product demand to increase, an un-optimized medium or process may not allow manufacturing to meet output requirements, and redesign may be needed. These types of changes could require extended development work, risk assessments, additional regulatory submission, and bridging studies. Consequently, this work may impact product availability and require additional resources and costs.

However, with the appropriate consideration of key factors and effective planning, AAV-based gene therapy manufacturers can potentially lower production costs by increasing yield and production capacity. With this approach, the long-term sustainability of your process is improved, and the potential risks and costs associated with redesign are minimized.



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