CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Future-proof your AAV process with a complete producer cell line

Dovile Gruzdyte

Stable producer cell lines helped to make mAb therapies the powerhouses they are today. To deliver on the promise of AAV-based gene therapy, we need similar technology. The challenge is that multiple genetic elements—rep, helper, capsid, and gene of interest—must be present. Ideally, everything needed to produce the required rAAV would be stably integrated into a single cell line. This article will describe an all-inclusive cell line platform that can be customized for a specific gene of interest and capsid.

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Despite a lot of dialog, cell and gene therapy (CGT) is still an emerging field. Since the first approval of a modern cell therapy in 2017, CGTs make up just 7% of all FDA-approved biologics and have a very low commercialization success rate when compared to their small molecule and other biologics counterparts.

Given the low commercialization success rate, the financial risk for a therapy developer is immense. Entering the world of prevalent diseases and increasing patient populations might be one way to reduce that risk, and we expect to see a growing number of CGTs being commercialized in the near future for large patient populations. This brings new challenges, notably the ability to make enough material to cover clinical trials and beyond.

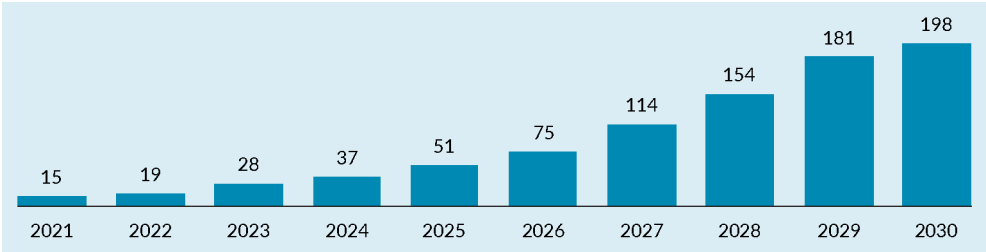
As shown in Figure 1, it was estimated that 19,000 patients would need viral vector material for commercial administration in 2022, and by 2024 that number is predicted to double. Current manufacturing methods and infrastructure are already at capacity and will need to evolve rapidly to keep up with demand.

CURRENT AAV MANUFACTURING METHODS

Selecting a production method as early as possible during development can be a defining moment for a viral vector therapy. If production methods and processes aren't fit for scaling, then the entire project can be put at risk due to the additional time and expense incurred.

FIGURE 1

Global commercial demand for viral vectors and plasmid DNA (in thousands of patients) 2021–2030 [1].



There are four distinct production methods of adeno-associated virus (AAV) that are most widely adopted (Figure 2). Classic triple transfection is the current state. These transient transfection systems allow the greatest flexibility as they don't require prior cell line generation and are therefore commonly used in the early stages of development to rapidly optimize and test lead candidates. However, the scalability of transient transfection processes is limited.

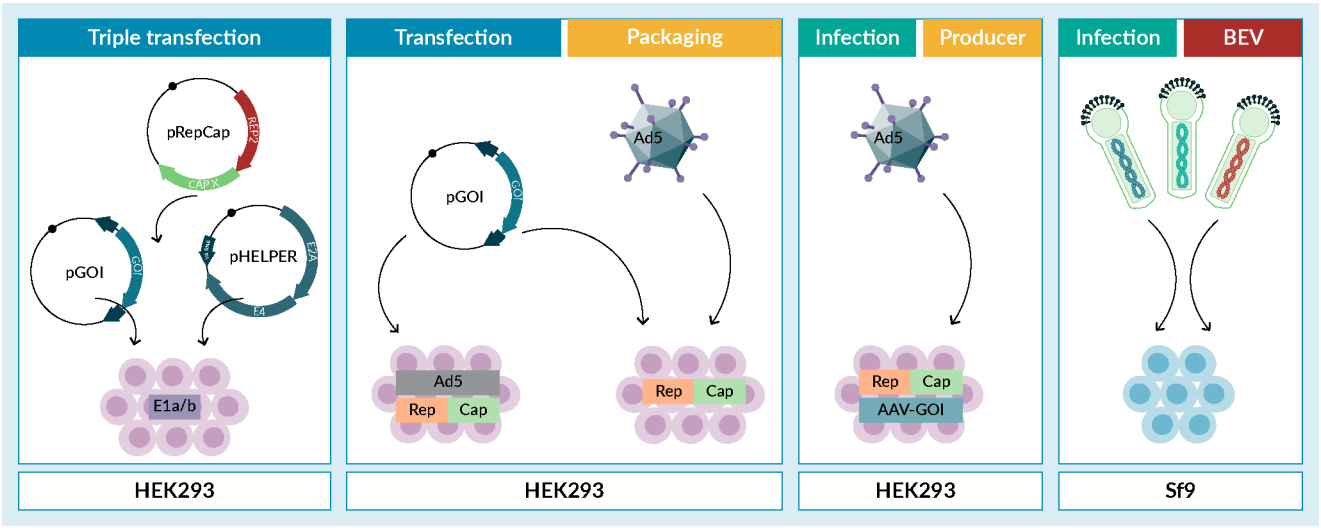
To improve the scalability, packaging or producer cell lines were developed. Scalability is improved due to fewer components needing to be integrated; however, the transfection step and infection step are still required.

An alternative production platform, the baculovirus expression vector system, uses Sf9 insect cells, making it very scalable and cost-effective. However, insect cell systems produce viral vectors with low infectivity due to non-mammalian post-translational modifications, leading to a requirement for higher doses.

All of these systems require manual steps that lead to batch-to-batch variations. It is, therefore, paramount that operators are intimately familiar with the process to maintain as much consistency as possible. When we think about where AAV is manufactured, around 70% is manufactured in contract development and manufacturing

FIGURE 2

AAV production methods.



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organizations. If the production method is highly variable, involves many manual tasks, and is prone to error, it will be harder to carry out tech transfer, resulting in lower performance and variability in productivity of each batch. This makes it hard to plan batches accurately for clinical studies and beyond, and can cause significant delay.

A TRUE PRODUCER CELL LINE FOR AAV PRODUCTION

The scale, accessibility, and cost of mAbs only became manageable when producer cell lines became available. Cytiva now offers the highly similar ELEVECTA™ cell line for AAV production.

All components for AAV production are stably integrated into the genome of the customer's ELEVECTA producer cell line for continuous manufacturing. The cells require no transfection of plasmids or infection with a helper virus at the manufacturing stage, just the addition of an inducer agent. This simple production process allows for minimal batch-to-batch variability, saving valuable time in tech transfer and producing high-quality material.

The ELEVECTA producer cell line is by stably integrating the tailor-made AAV vector components, including the serotype-specific capsid gene and the transgene, into the genome of the Alpha cell line (Figure 3). Using the latest cell line screening technologies, the producer clones are selected, characterized, and cryopreserved as a research cell bank (RCB) ready for handover to the customer.

Following good manufacturing practices (GMP) cell bank creation, the cells can be expanded to the desired scale and cell density. AAV production can then be switched on at an optimal time point by addition of a simple induction agent.

SCALE-UP STUDIES

To illustrate how the technology works in practice, scale-up studies were performed with Cytiva's model ELEVECTA cell line, with runs at 10, 50, and 200-liter scales.

Figure 4A shows how the viable cell concentration, as well as cell viability, increases from 10–200 liters, demonstrating that the performance of the cell line is not compromised when moving to larger-scale production.

FIGURE 3

Generation of an ELEVECTA producer cell line.

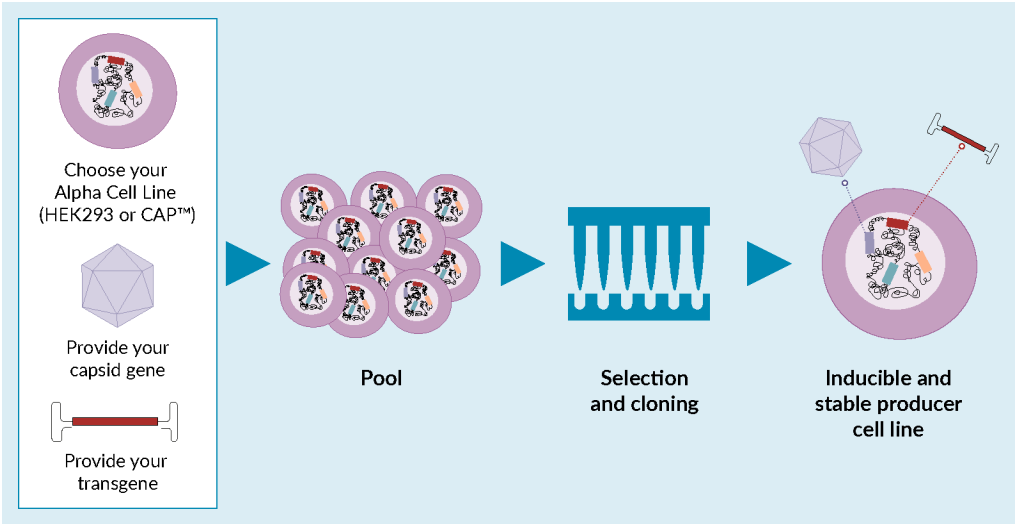


FIGURE 4

Scalable process from lab- to process-scale as demonstrated in 200 L Allegro™ STR bioreactor.

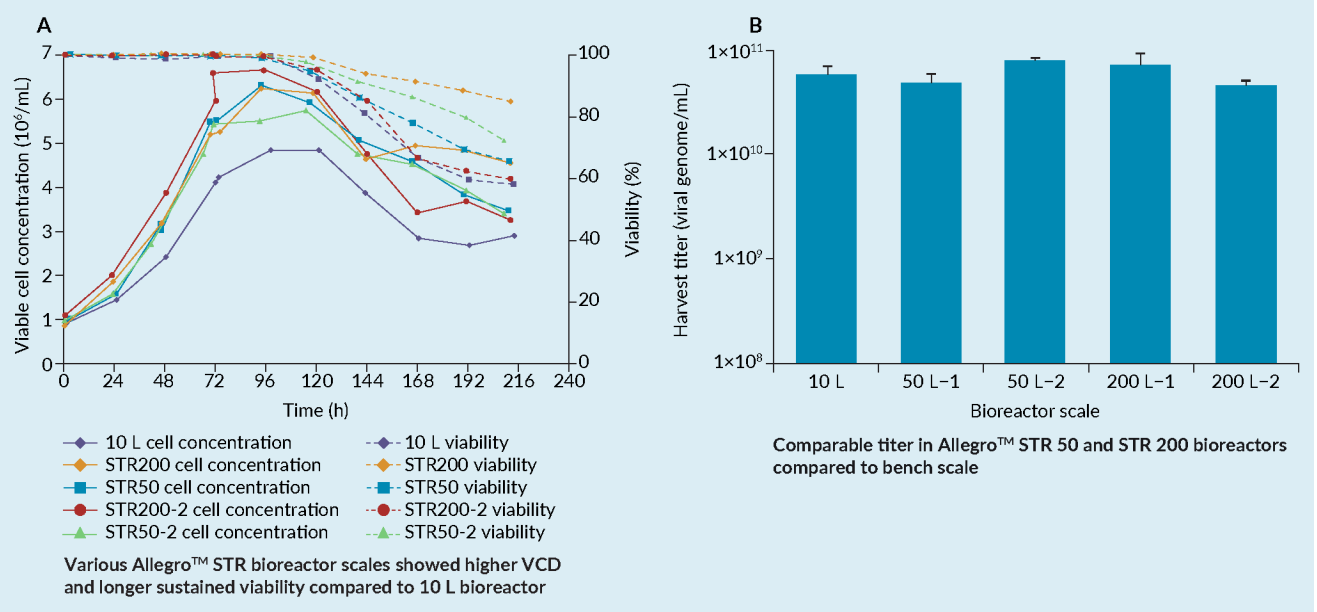


Figure 4B demonstrates that viral titers for different volumes remained consistent across all scales, with minimal batch-to-batch variability across different runs.

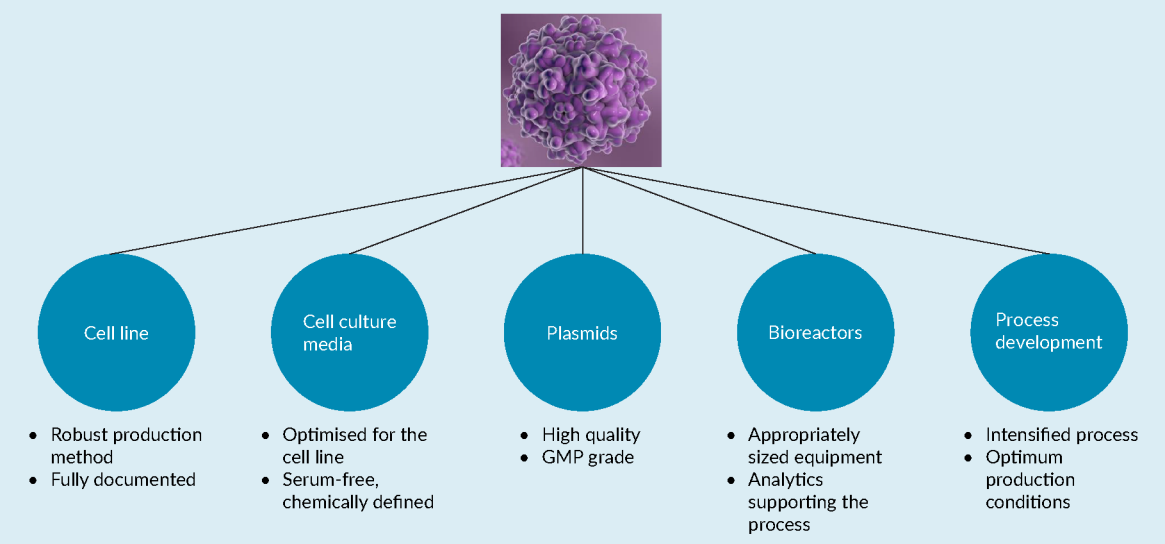
Upon handover of the customer-specific producer cell line, a product license agreement is set up before any GMP batches commence. The ELEVECTA producer cell line can then be used for multiple batches with no further cell line development needs.

A HOLISTIC APPROACH TO UPSTREAM PROCESSING

Upstream processing does not start with a bioreactor. It starts long before, in the cell culture

FIGURE 5

Holistic approach to upstream processing.



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lab, where the host cell lines are at the heart of the process and define the manufacturing path.

Figure 5 shows the many factors that contribute to success in upstream bioprocessing. The ELEVECTA producer cell line provides a firm foundation for large-scale GMP-grade manufacturing of AAV-based therapies.

REFERENCE

1. Research Report: Viral Vectors, Non-Viral Vectors and Gene Therapy Manufacturing Market (4th Edition), 2021–2030. (2021). Roots Analysis Private Limited.

ASK THE AUTHOR

Dovile Gruzdyte, Global Product Manager for Cell Line Development, Cytiva answers your questions on AAV production with the ELEVECTA cell line.

Q Does ELEVECTA work with any AAV serotype and gene of interest?

DG: So far, we have tested AAV 2, 5, 8, and 9, as well as some of the new capsid formats, and we believe that the technology works with all serotypes. As for genes of interest, as long as the packaging capacity is respected for AAV there should be no problem.

Q What allows for minimal batch-to-batch variability for the ELEVECTA cell line?

DG: The production process being so simple ensures that the production is robust. Since it's a monoclonal cell, there is minimal variability in production, and that's what makes this an excellent platform.

Q What scale-up studies did you perform during the development project?

DG: First, we created a stable polyclonal producer pool in roughly 50-milliliter volumes. After the single-cell cloning, we screened the top-performing clones in a miniaturized bioreactor system with volumes of 15 milliliters, then further tested the best-performing

clones in 3–10-liter benchtop stirred tank bioreactors, before performing process optimization to choose the best process conditions at this scale.

Cytiva also offers process development services, and those teams work closely with our cell line development teams. We encourage customers to opt for larger-scale cell line development so that the processes can be transferred to them at 50-liter scale or beyond.

Q What material needs to be provided to Cytiva to kick off the ELEVECTA project?

DG: We will need plasmids for the capsids the customer is looking at and the gene of interest. We will clone those into our proprietary backbone for stable integration into the host cell.

As we produce the material in the pool format, around 4 months into the project, we typically provide material to the customer for internal validation of downstream protocols, analytical methods, and infectivity assays.

BIOGRAPHY

DOVILE GRUZDYTE has held various engineering and management positions in the biotechnology sector. She has spent most of her career developing large-scale manufacturing enterprise solutions for biotechnology customers globally, for monoclonal antibodies and gene therapy production. She has a chemical engineering degree from Newcastle University and Delft University of Technology.

AFFILIATION

Dovile Gruzdyte
Global Product Manager for Cell Line Development,
Cytiva

cytiva

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AUTHORSHIP & CONFLICT OF INTEREST

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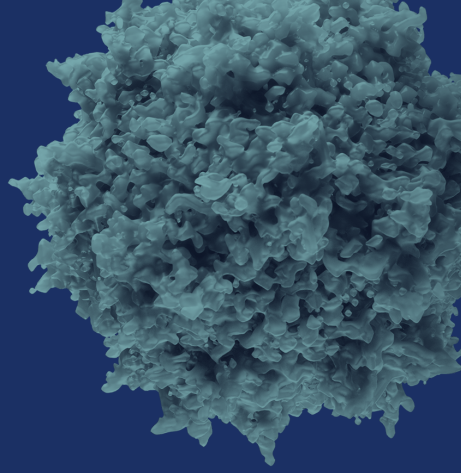

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No transfections
No helper virus

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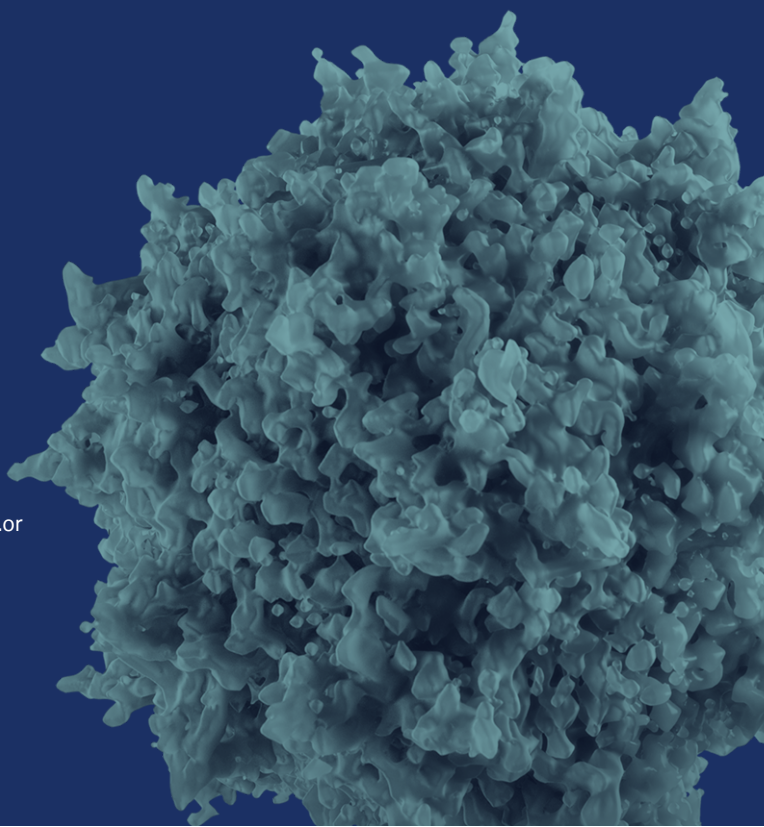

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