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SPECIAL REPORT

Viral-Based Gene Therapy: Key Challenges and Bioprocessing Innovations

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Introduction

In recent years viral vector gene therapies reentered the advanced modality spotlight — notably with the 2017 approval of Spark's Luxturna and the subsequent approval of Novartis/AveXis' Zolgensma. Both of these therapies conveyed transformational potential for patients in high morbidity or mortality indications with significant unmet medical need. The immense promise of the budding adeno-associated virus (AAV) gene therapy field drove a flurry of M&A deal activity, with large biopharma and global contract development and manufacturing organizations participating in many billion-dollar-plus deals.[1]

While the field has experienced several setbacks related to safety and efficacy, the global pipeline continues to grow, reaching hundreds of preclinical assets and more than 100 clinical assets by May 2022,[2] and in November 2022, CSL Behring received Food and Drug Administration (FDA) approval for Hemgenix for hemophilia B.[3]

For the pipeline to translate into a significant number of marketed therapies, the field will need to address eight key challenges inherent in the gene therapy design (i.e., what is made) and the manufacturing process (i.e., how it is made). In this special report, L.E.K. Consulting reviews these challenges and the bioprocessing solutions that could shape the trajectory of the viral-based gene therapy landscape.

Key challenges and bioprocessing innovations

L.E.K. has identified eight challenges related to either gene therapy design or the current manufacturing process:

1. Reach more patients by targeting a broader range of organs
2. Improve safety at higher doses
3. Maximize durability of response
4. Overcome pre- and post-treatment immunity
5. Improve transfection efficiency
6. Increase cell culture scalability
7. Increase purity
8. Improve batch-to-batch inconsistency

Challenges in gene therapy design

Four main challenges require changes in the gene therapy design itself: reach more patients by targeting a broader range of organs, improve safety at higher doses, maximize durability of response, and overcome pre- and post-treatment immunity.

1. **Reach more patients by targeting a broader range of organs:** Today's marketed and pipeline gene therapies have mainly targeted organs that are relatively easy to reach — namely, the eye, the brain and the liver. The eye and the brain have two main advantages — therapies can be administered directly to them, and they are immune-privileged, meaning that foreign tissues can survive for long periods of time without eliciting a significant immune response. As the body's filter, the liver is also thought to be a relatively easy-to-reach organ, since gene therapies administered systemically naturally tend to accumulate there. Gene therapies in development for hemophilia target the liver because it is the primary site of clotting factor synthesis. Two major issues must be addressed to move to other organs. First, the gene therapy must be able to reach the desired tissue when it is not directly injected, and once there, it must be able to survive that organ's immune response if the organ is not immune-privileged. If these are addressed, the range of applications and therefore the overall market for viral-based gene therapies could greatly expand.

2. **Improve safety at higher doses:** Luxturna, locally administered to the eye, has a dose of 1.5 x 10^{11} (150 billion) vector genomes (vg) per eye. Systemically dosed gene therapies, which are necessary for reaching a broader range of organs, typically require dosages of 10^{13}-10^{14} (tens to hundreds of trillions) vg per kilogram of body weight — on the order of thousands of times greater than Luxturna — because not all the vector genomes will reach the desired tissue. And these figures only represent vector genomes. There is an additional (usually undisclosed) number of empty capsids, making the total amount of virus the patient is exposed to even higher. The higher the dose, the more significant a patient's potential immune response to that dose may be — and indeed, serious adverse events such as severe liver damage and sepsis, and ultimately deaths, have been observed in clinical trials of these therapies.[4] This has resulted in some recent clinical holds, including on Pfizer's PF-06939926 for Duchenne muscular dystrophy (since lifted) and Astellas' AT132 for X-linked myotubular myopathy.

   Several high-dose gene therapy pipeline assets should have readouts in the coming 18 months that will be instructive as to the magnitude of this challenge, including in Duchenne muscular dystrophy (see Figure 1).

Figure 1
Duchenne muscular dystrophy gene therapy pipeline assets compared to Luxturna and Zolgensma

Company	Asset	Disease	Stage	Dose (vg/kg)	Next inflection point
Spark Therapeutics	Luxturna	Retinal dystrophy	Marketed	1.5 x 10^{11} (150 billion) per eye	N/A
Novartis	Zolgensma	Spinal muscular atrophy	Marketed	1.1 x 10^{14} (110 trillion)	N/A
Sarepta	SRP-9001	Duchenne muscular dystrophy	Phase 3	1.33 x 10^{14} (133 trillion)	BLA submitted for accelerated approval Oct. 2023: Phase 3 primary completion date
Pfizer	PF-06939926	Duchenne muscular dystrophy	Phase 3	1-3 x 10^{14} (100 to 300 trillion)	Jan. 2024: Phase 3 primary completion date
Solid Biosciences	SGT-001	Duchenne muscular dystrophy	Phase 1/2	2 x 10^{14} (200 trillion) (high-dose cohort)	Early 2023: Long-term data from phase 1/2

Note: BLA=Biologics License Application
Source: Clinicaltrials.gov, company websites and press releases, U.S. Food and Drug Administration, NeurologyLive, product labels, L.E.K. research and analysis

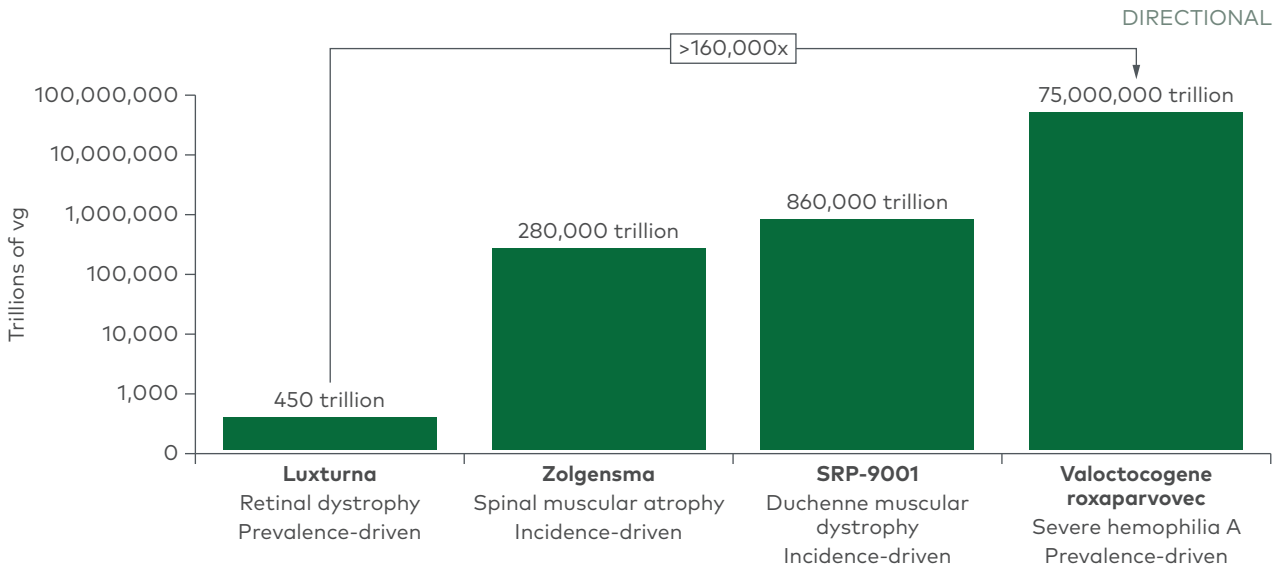
3. **Maximize durability of response:** The once broadly disseminated hypothesis that gene therapies would be curative one-time treatments has come into question. AAV gene therapies do not integrate into the patient's DNA, meaning that their expression could wane as cells divide without them being copied in each new cell. There have been gene therapy trials that demonstrated five-year durability in efficacy, but concerns persist. For example, BioMarin received a Complete Response Letter from the FDA in 2020 for its submission of valoctocogene roxaparvovec for treatment of severe hemophilia A because Factor VIII expression waned significantly over the course of a few years.[5] The company recently generated new phase 3 data showing two years of therapeutic benefit, released phase 1/2 data showing five years of therapeutic benefit and refiled this September. Ultimately, more time and more data will tell whether gene therapies can be curative one-time therapies.

4. **Overcome pre- and post-treatment immunity:** Once patients are exposed to an AAV gene therapy, they may develop neutralizing antibodies against it. The percentage of individuals with neutralizing antibodies varies greatly across studies and AAV serotypes (from as few as 3% to the majority of patients).[6,7] Neutralizing antibodies mobilize a patient's immune system to neutralize the AAV gene therapy before it can have a meaningful therapeutic effect. As a result, a significant proportion of patients otherwise eligible for gene therapy cannot receive it, and re-dosing with the same AAV serotype is not possible because patients develop neutralizing antibodies from the first dose. If re-dosing was required to prolong therapeutic effects, gene therapy developers would need to determine how a re-dosed therapy could evade the immune system, likely by further engineering the viral capsids used to deliver it and/or modulating the immune system response.

Challenges in the manufacturing process

The above gene therapy design challenges must be solved in order for the growing gene therapy pipeline to remain viable. And if they are solved, more assets in the pipeline, larger target patient populations, higher doses per asset and the potential need for re-dosing will cause an exponential increase in market demand for viral-based gene therapy. BioMarin's Valrox alone would require over 100,000 times more bioprocessing output than Luxturna, based on dosing and prevalent population differences (see Figure 2). In order to meet this demand, there will need to be a large increase in manufacturing yield. Manufacturing costs will also need to decrease because therapies addressing broader patient populations and requiring re-dosing will not be able to command the ultraorphan pricing models of today's therapies.

Figure 2
Relative addressable US vector genome (vg) demand for different gene therapies*



*Values represent dose per eye or per kg, multiplied by relevant multiplier (e.g., average kg for patient of age to receive therapy), multiplied by U.S. disease prevalence or incidence. Does not account for patients ineligible due to preexisting neutralizing antibodies.
Source: Company websites and press releases, U.S. Food and Drug Administration, hemophilia.org, Journal of Neuromuscular Diseases, Orphanet Journal of Rare Diseases, Centers for Disease Control and Prevention, *Hemophilia*, product labels, L.E.K. research and analysis

Several additional limitations across today's AAV bioproduction process drive overall cost and limit total yields. They challenge the industry to:

5. **Improve transfection efficiency:** The first step of gene therapy manufacturing is transfection, whereby DNA containing the genes required for virus production is introduced into a cell line to produce genetically modified cells. For today's AAV therapies, this is typically accomplished through transient triple transfection, whereby three plasmids containing genes required for AAV production are simultaneously introduced to cells to produce AAV. However, these plasmids are not integrated into the cell's genome, and thus the process must be repeated for every new batch of gene therapy produced. This method was adopted because it was the historical approach to producing AAV and was perceived as the fastest and lowest-risk path to market given uncertainties about the feasibility of establishing stable producer AAV cell lines. However, repeating this process for each batch is very inefficient, is difficult to scale and drives high batch costs (e.g., each batch requires significant good manufacturing practice [GMP] plasmid input and labor costs) and batch-to-batch variability.

6. **Increase cell culture scalability:** After genetic modification comes cell expansion, in which transfected cells are cultured to scale in a bioreactor. Many of today's gene therapies are produced using adherent cell culture (where cells are cultured in a monolayer on an artificial surface) instead of suspension cell culture (where cells are cultured in a three-dimensional liquid volume). Adherent culture is common for AAV gene therapy manufacturing because commonly used adherent HEK293 cell lines are available "off the shelf" and the process can be quickly and easily developed at lab scale with less bioengineering expertise required.[8] However, adherent culture is limited in scale by the surface area available for cell growth. Adherent culture may not be sufficient to produce the amount of virus required for high-dose gene therapies, or to produce doses for large addressable populations.

7. **Increase purity:** The final steps are purification and polishing, in which viral vector products are separated from process impurities as well as from low-quality AAV products. One key measure of purity from this process is the ratio of full AAV capsids (i.e., properly packaged capsids containing the therapeutic gene of interest) to empty capsids (i.e., AAV capsids that are improperly packaged and lack the gene of interest). With today's purification methods, a significant portion of empty capsids remains in most batches. This means that significantly more AAV capsids need to be produced (and delivered into the patient) to achieve an effective gene therapy dose, which strains capacity, increases costs and likely increases the rate of adverse events.

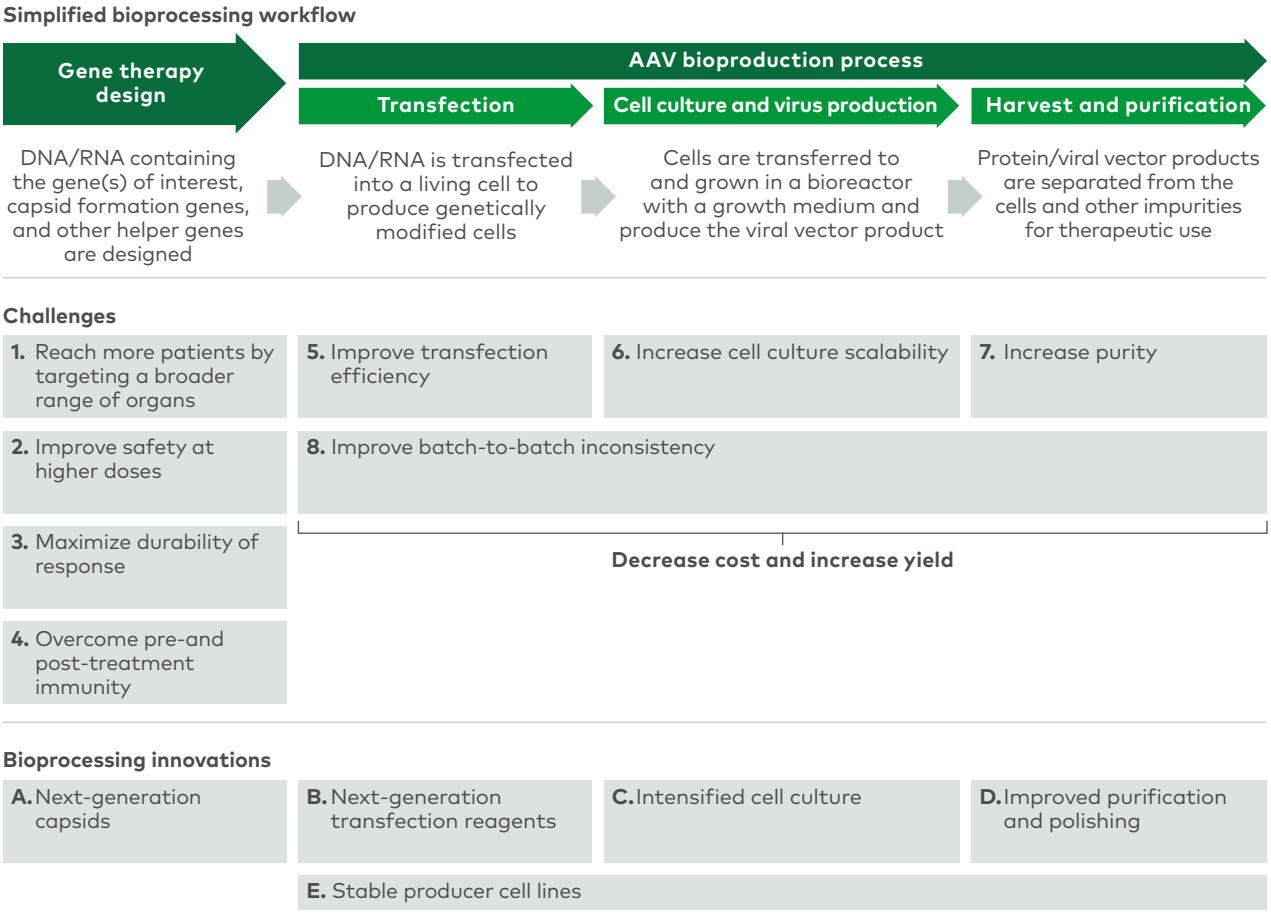
8. **Improve batch-to-batch inconsistency:** Across the AAV bioproduction process, the complexity and high degree of variability lead to inconsistency in yields and high rates of batch failures. This in turn strains capacity and increases cost, because manufacturers must plan to make even more virus to account for these inefficiencies.

The bioprocessing innovations

When reviewing the challenges facing the gene therapy field, there is no doubt that bioprocessing limitations are part of the problem and that bioprocessing must also be a part of the solution. Enabling technology and bioproduction players across the value chain are working toward a range of potential innovations to help overcome the above challenges (see Figure 3).

A. **Next-generation capsids to address gene therapy design challenges:** Addressing the challenges in gene therapy design can involve changing the payload (i.e., the gene of interest or other promoter genes) or the delivery vehicle (i.e., the AAV capsid or a different viral or nonviral delivery vehicle). While changes to the payload are core competencies of biopharma companies, bioprocessing companies are working on

Figure 3
Overview of gene therapy bioprocessing workflow, challenges and bioprocessing innovations



Note: AAV=adeno-associated virus
Source: L.E.K. research and analysis

next-generation AAV capsids. Companies like 4D Molecular Therapeutics, AskBio, Dyno Therapeutics, Oxgene and others are using directed evolution and/or artificial intelligence-informed rational design to develop capsids that more effectively target desired tissue types (even when dosed systemically), reduce immune responses, do not have the prevalent pool of existing neutralizing antibodies in patients and/or transduce a greater proportion of target cells. These developments could increase the margin of safety for gene therapy drugs and potentially enable re-dosing of patients in some indications. Other potential approaches to next-generation vectors include non-AAV vectors (e.g., herpes virus), nonviral vectors (e.g., lipid nanoparticles) that could circumnavigate barriers to re-dosing, and co-dosing of immunomodulators (e.g., Selecta Biosciences' ImmTOR platform).

Next-generation capsid engineering, though, will not solve the challenges that contribute to high cost and low yields. Some solutions that are actively being invested in include:

B. Next-generation transfection reagents to increase yield: Reagent providers such as Polyplus, MiRus and others have developed next-generation GMP transfection reagents that enhance transfection efficiency, increase the proportion of cells in culture that contain all three plasmids required for AAV production, and afford a higher functional titer of virus produced. Though material, these improvements alone are unlikely to be sufficient to overcome the significant scale-up cost challenges that limit the field's potential.

C. Intensified cell culture to increase yield, increase speed and decrease human labor requirements: Given that AAV has historically been produced using adherent cell culture, high-dose programs such as Novartis' Zolgensma took advantage of fixed-bed bioreactors (e.g., Pall's iCELLis), in which fibrous layers are tightly assembled to allow adherent cells to attach while utilizing more of the available volume of the media and reactor. Going forward, manufacturers are continuing to look for ways to intensify the cell culture processes and increase scale and yield; suspension culture, which is commonly used for commercial-scale bioproduction of other biological drugs (e.g., monoclonal antibodies), is viewed as a way to do this.

Compared to adherent culture, it is operationally simpler, can be performed at a larger scale, requires less employee hands-on time and likely results in higher batch yields. To change to suspension culture, manufacturers can switch to suspension-adapted cell lines such as Sf9 and HeLa (accepting potential drawbacks these production systems might present compared with HEK293 cells), or they can adapt and engineer HEK293 cells for suspension culture. Improvements in suspension HEK293 cell lines are allowing manufacturers to increasingly pursue this approach. Suspension culture also opens the possibility of moving from fed-batch culture to perfusion culture, in which the cell culture is continuously fed. This can further enable higher-yielding and more cost-effective production processes, especially in conjunction with stable producer cell lines (see section E below).

D. Improved purification and polishing to increase yields, improve full-to-empty ratio and therefore improve safety: Purification and polishing are two downstream steps in the manufacturing process that are accomplished through chromatography.

In purification, a capture ligand or resin is used to extract the AAV capsids. Purification players are investing in next-generation resins and capture ligands to increase the amount of AAV recovered, as well as optimized buffers to improve recovery from anion exchange chromatography, which would increase yield per batch. In polishing, high-quality AAV is separated from low-quality AAV (such as empty capsids). Suppliers are also investing in improvements at this step to enable the delivery of the same therapeutic benefit (number of full capsids) with a lower overall dose of AAV capsids, potentially reducing immune response and thus improving safety, of particular concern for high-dose gene therapies.

E. Stable producer cell lines to increase scale and decrease cost: Among all solutions, developing stable producer cell lines, in which some or all of the necessary genes required to produce the vector are fully integrated into the cell line's genome, may be the most impactful. Switching from transient triple transfection to a stable producer cell line would be a significant step forward in improving scalability and reducing cost of manufacturing. It could reduce batch costs by >30%, driven by reduction in GMP plasmids, transfection agents and labor/time costs.[9] These cell lines can also be optimized for output (functional titer) and quality (full-to-empty ratio), which could further reduce costs by producing a higher concentration of more efficient drug from a smaller-scale bioreactor.[10] Stable producer cell lines are used today to manufacture monoclonal antibodies, but they are much more challenging to engineer for viral-based gene therapies. For viral-based gene therapies, the cell line would need to have more than 10 different genes stably integrated into its genome, compared with only one or two for most antibodies.

Innovators such as Ultragenyx have demonstrated scaled manufacturing with their proprietary Pinnacle PCL platform, including 2,000-liter batch production for clinical trials. Similarly, CEVEC Pharma's HEK293-based ELEVECTA platform allows efficient stable producer cell line generation for AAV, and ELEVECTA cells have been successfully scaled up using both batch and perfusion processes.[11] CEVEC has licensed its technology to several large pharma players, including Biogen and Roche/Spark, and the company was recently acquired by Cytiva. Other partnerships (e.g., Thermo Fisher and Berkeley Lights) are exploring new methods of screening and identifying stable clones for application as producer cell lines, a critical bottleneck in the process of developing a stable producer cell line.

It may take several years for producer cell lines to gain traction in the gene therapy pipeline. Developing a producer cell line can take significant time (driven by clone screening). Also, this additional development may be perceived as slowing time to

clinic or driving additional execution risk. Ultimately, switching to a stable producer cell line will be driven by a risk/reward assessment for each potential AAV pipeline program. Factors that would potentially drive switching to a stable producer cell line include (a) higher dose required, (b) larger addressable patient population, (c) less in-class competition and pressure of speed to market and (d) incidence-driven indication, where a significant portion of the patient pool is replenished each year. Producer cell lines could play a critical role in the future industrialization of AAV-based gene therapy for programs that fit one or more of the above archetypes.

Conclusion

The growth of advanced therapeutic modalities like viral-based gene therapies is triggering a step change in bioprocessing complexity and variability and is placing increased and evolving demands on bioprocessing manufacturers. This is a market that is fundamentally supply constrained — there is a large gap in overall capacity and many manufacturing limitations to address. The bioprocessing ecosystem is actively exploring innovations to solve the key challenges and write the next chapters of the gene therapy market. As of 2021, gene therapy represented 0.5% of global bioprocessing capacity in liters.[12] Advances in bioprocessing will play a critical role in determining what that figure will be a decade from now. L.E.K. has significant experience advising pharmaceutical manufacturers, contract manufacturers, and bioprocessing equipment and consumables manufacturers on how to best participate in the growing gene therapy market and maximize value in the face of the significant uncertainty in the industry.

For more information, please contact lifesciences@lek.com.

Endnotes

[1]Examples include Novartis/AveXis, Roche/Spark, Astellas/Audentes, Pfizer/Bamboo, Thermo Fisher/Brammer Bio, Catalent/Paragon.

[2]L.E.K. analysis of Citeline's PharmaProjects.

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[9]L.E.K. research and analysis.

[10]Companies have used baculovirus-based cell lines to generate scale, but this approach has led to a relatively poor full-to-empty ratio compared with virus produced using transient triple transfection.

[11]Company presentations at BioProcess International Conference, Boston, Massachusetts, October 2022.

[12]BioProcess International, "Total Global Capacity Finally Shows Improved Productivity." https://bioprocessintl.com/business/economics/total-global-biopharmaceutical-manufacturing-capacity-finally-shows-improved-productivity/#:~:text=Since%202018%2C%20global%20bioprocessing%20capacity,toward%20greater%20productivity%20and%20efficiency

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