



From ex vivo to in vivo: Shaping the next generation of viral vector manufacturing

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The shift from ex vivo to in vivo gene delivery is rewriting the rules of viral vector manufacturing, forcing developers to rethink scale, purity, cell lines and analytics as vectors themselves become the drug. AGC Biologics is betting on stable producer lines, next-gen purification and deeper characterization to meet the far stricter demands of in vivo therapies.

Viral vector manufacturing is undergoing a revolution, moving from patient-specific ex vivo production to scalable, in vivo supply. In turn, that transition is redefining process design to analytics and purification.

In this interview, AGC Biologics' Upstream Vector Development Manager Silvia Ungari and Analytical Methods Development Manager Chiara Boroni discussed the evolving landscape and what the future holds for manufacturing process development for lentiviral vector (LVV) and adeno-associated vector (AAV) platforms.

Ex versus in vivo

The core manufacturing process for viral vectors doesn't change dramatically between ex vivo and in vivo applications, Ungari said. What's altered is how the vector is managed, controlled and qualified, as well as the scale at which it needs to be produced.

With ex vivo treatments, patient cells are removed, genetically modified outside the body and reinfused. "The vector is essentially a manufacturing tool," Ungari explained, "and the final drug product is the patient's modified

cells.” Because those cells undergo washing steps before reinfusion, the vector itself is not the final therapeutic entity. Impurities can be mitigated downstream.

In vivo therapies, on the other hand, fall into a different paradigm: the vector is the drug. What comes off the production line is injected directly into the patient, with no opportunity for additional purification. That reality drives far stricter purity requirements and far less regulatory flexibility.

The regulatory landscape reflects this split. In Europe, the EMA typically classifies ex vivo vectors as starting materials. In the U.S., the FDA treats them as drug substances but still allows a risk-based approach because the final product is the engineered cell.

For in vivo vectors, however, regulators across regions concur: purity must be exceptionally high, and the manufacturing process is the only line of defense. As a result, safety methods such as (endotoxin, mycoplasma) and impurities analysis (residual DNA, and host cell proteins) must all meet the stringent standards applied to any injectable biologic.

Simplifying Comparability Validation Strategies

The scale of production is the most striking difference between in vivo and ex vivo vector manufacturing, common to either AAV or LVV, Ungari said.

Ex vivo therapeutic approaches typically require lower vector doses. But in vivo therapies demand orders of magnitude. Bioreactors must grow from 200 liters up to 2,000 liters, and every step of the process must scale with them.

However, process scale-up involves more than simply increasing equipment size; larger volumes require dedicated procedures, particularly for mixing transfection complexes, which is a crucial step for process success.



Silvia Ungari, Upstream Vector Development Manager, AGC Biologics

Downstream scale-up is rarely straightforward, often requiring re-optimization of purification steps because equipment and column/membrane formats do not scale linearly across volumes. And then there is the facility itself.

Large-scale vector manufacturing requires early planning for equipment layout, transfer systems and process flow. Ungari noted that the optimization process “should be considered six months to one year in advance,” a reminder that manufacturing strategy is now inseparable from infrastructure strategy.

The cell line bottleneck

While manufacturing scale and product purity remain central challenges in AAV process development, Ungari highlighted another critical bottleneck: the producer cell line itself.

For decades, the field has relied heavily on HEK293T cells, which express the SV40 large T antigen (LTA) to enhance vector production by promoting the replication of episomal plasmids. This feature has made HEK293T a workhorse for transient transfection-based manufacturing platforms. However, LTA is also a well-characterized oncoprotein that interferes with key tumor suppressor

pathways, including p53 and RB, raising regulatory concerns regarding the potential carryover of LTA-derived contaminants into the final product.

In ex vivo gene therapy applications, these risks can be largely mitigated through robust downstream purification processes.

In contrast, in vivo therapies face a higher level of scrutiny, as even trace amounts of residual LTA DNA or protein may represent a safety concern. As a result, the industry is increasingly pursuing two complementary approaches. The first focuses on further improving downstream purification strategies to maximize the removal of LTA-related impurities. The second aims to eliminate the issue at its source by developing LTA-free producer cell platforms.

Additional emerging solutions for in vivo LVV include a new class of immune-evasive producer lines that incorporate immune-evasion molecules into their envelope, reducing innate and adaptive immune responses upon in vivo administration.

Immunogenicity: the invisible constraint

Immunogenicity is one of the most critical challenges for in vivo gene therapy, Ungari said. It is worth distinguishing two phenomena: immunogenicity, the ability of the vector to induce a new immune response, and pre-existing immunity. Innate immunity is often triggered by impurities such as residual plasmid DNA rich in CpG motifs, host cell proteins or complement activation. Adaptive immunity — neutralizing antibodies and T-cell responses — can lead to loss of efficacy or, at high doses, severe hepatotoxicity.

Mitigation strategies span both design and manufacturing. For LVV, envelope proteins must be engineered to target specific cells and avoid unwanted immune responses. For AAV, capsid engineering through directed evolution or AI-driven design is becoming standard.

On the manufacturing side, developers are turning to plasmid-free DNA systems such as doggybone

DNA and minicircles, which eliminate bacterial backbones and CpG motifs. Ultimately, Ungari noted, the most definitive solution is the transition to stable producer lines, which remove plasmid DNA from the process entirely.

Stable producer cell line push

One of the most strategically important directions for both LLV and AAV is the move towards stable producer cell lines, which would eliminate the transient plasmid transfection entirely, Boroni said. The elimination of this step reduces both the costs associated with larger-scale GMP plasmid manufacturing and the complexity related to transfection and larger volumes.

Stable producer cell lines offer improved batch-to-batch consistency, a cleaner impurity profile and a more scalable and cost-effective manufacturing process.

“The transition to stable cell lines provides analytical benefits but also poses challenges in developing and validating new analytical methods,” Boroni said. “Specifically, genomic integration site analysis, vector genome integrity assessment and genetic stability testing become essential to confirm that integrated sequences remain intact and functional throughout production.”



*Chiara Boroni, Analytical Methods Development Manager,
AGC Biologics*

Purification: where LVV and AAV face distinct challenges

Downstream purification is where the gap between ex vivo and in vivo requirements is felt most acutely. For LVV, the standard ex vivo purification train — benzonase treatment, anion-exchange chromatography and tangential-flow filtration — performs well. But in vivo demands significantly higher purity, and the vector's fragile envelope constrains the chromatographic conditions that can be applied.

For AAV, the same stringent requirements on residual impurities apply, but an additional and defining challenge layers on top: the separation of full from empty capsids. Empty capsids can represent up to 90% of total particles depending on the serotype — fully immunogenic yet therapeutically inert — making their removal both a purification imperative and a critical process design consideration.

Convective flow-through chromatography on monolithic supports is emerging as one of the most promising solutions across both platforms, offering superior impurity clearance and strong potential for full/empty capsid resolution.

New perspectives for the analytical platform

The evolution of analytical technologies for in vivo viral vector quality control is one of the most exciting areas in the analytical world. Dedicated analytical support is necessary at every step of in vivo development, as it helps guide changes in the manufacturing process, Boroni said.

For both LVV and AAV platforms, developers are pushing toward greater sensitivity, faster turnaround, higher specificity and more sustainable methods.

On the safety testing front, several established methods are being challenged or supplemented by more modern alternatives. Impurity characterization is also evolving by the introduction of mass

spectrometry, ddPCR and specific DNA fragment size distribution assays.

AAV programs are driving the most advanced structural analytics. A suite of complementary technologies are enabling increasingly detailed analysis of capsid populations, distinguishing full, empty and aberrant particles with growing speed and accessibility.

While these technologies are not standard, laboratories are adopting them to meet rising expectations from the EMA and the FDA for deeper product characterization and real-time monitoring during manufacturing.

How AGC Biologics is solving these new hurdles

To address today's manufacturing challenges, AGC Biologics leverages decades of commercial experience in viral vector manufacturing and drug products with multiple approved products on the market.

The company is investing in proprietary cell line development, new packaging approaches and stable producer cell line programs. Upstream, AGC Biologics is scaling its lentiviral and AAV platforms toward the 1,000-litre volumes required for in vivo therapies, with a focus on robustness and cost of goods. Downstream, it is evaluating next-generation purification technologies to meet the far higher purity thresholds of in vivo products.

AGC is also focused on analytical depth. With more than 160 in-house assays, the company can support programs from early vector development through fill-finish release.

"AGC Biologics is committed to being at the forefront of this transition," Boroni said. "Our manufacturing expertise, analytical capabilities, and regulatory track record make us the right partner for developers bringing in vivo therapies to patients."

AGC Biologics is addressing these challenges and proposing solutions in an upcoming whitepaper.