Scale-up and manufacturing comparability of an Sf RVN baculovirus production process from 10 L to 200 L for AAV

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INTRODUCTION

- Robust, scalable manufacturing processes are critical to supporting late-stage clinical development and eventual commercialization of recombinant adeno-associated virus (rAAV) gene therapies[1]
- Traditional transient transfection-based mammalian platforms are often limited by high material costs, low yields, and scalability constraints, particularly for programs requiring high doses or large patient populations[1]
- To address these challenges, Lexeo Therapeutics has developed a scalable Sf9 baculovirus-infected insect cell (BIIC) platform to produce adeno-associated virus serotype rh.10 (AAVrh.10) vectors
- This study evaluates process scale-up and manufacturing comparability of an Sf9 baculovirus production process for rAAV production, assessing whether a 10 L small-scale model is representative and predictive of a 200 L production-scale stirred-tank bioreactor (STR) process

METHODS

Manufacturing Strategy

- Three process development drug substance (DS) batches (two at 10 L scale and one at 200 L scale) and one 200 L scale Good Manufacturing Practice DS batch were manufactured using conserved critical starting materials and aligned process conditions, where feasible

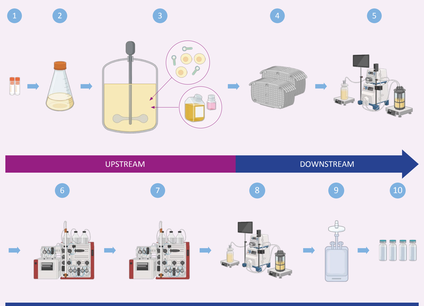
Upstream Process (Figure 1)

- A rhabdovirus-negative *Spodoptera frugiperda* (Sf-RVN) suspension cell line was cultured in chemically defined, animal-component-free medium
- Two GLP recombinant baculovirus (rBV) constructs were utilized: one containing the *Rep* and *Cap* genes for the AAVrh.10 capsid, and the other containing the gene of interest (GOI)
- The Sf9 cell line was infected with rBV to generate AAVrh.10 and GOI BIIC banks
- For rAAV production, the Sf9 master cell bank thawed and expanded in serial shake flasks, followed by inoculation of the STR and infection at low multiplicity of infection with BIICs
- Bioreactor parameters such as agitation and gassing were scaled using power density (P/V) and volumetric gas flow (VVM), respectively

Downstream Process (Figure 1)

- Harvested material underwent chemical lysis and nuclease treatment, followed by depth filtration and tangential flow filtration (TFF)-1
- Purification was performed using affinity (AFF) chromatography and anion exchange (AEX) chromatography
- Final concentration and buffer exchange were completed by TFF-2, followed by sterile filtration to generate bulk drug substance (BDS)

Figure 1: Sf9 baculovirus manufacturing process



Steps indicated in the figure refer to: (1) Sf9-RVN thaw; (2) cell expansion; (3) STR inoculation, infection, and feed addition; (4) harvest and clarification; (5) TFF-1; (6) AFF and (7) AEX chromatography; (8) TFF-2; (9) filtration and BDS fill; and (10) final drug product.

AEX, anion exchange; AFF, affinity; BDS, bulk drug substance; Sf9-RVN, rhabdovirus-negative *Spodoptera frugiperda*; STR, stirred-tank bioreactor; TFF, tangential flow filtration

Comparability Assessment

- Comparability across scales was evaluated using:
  - In-process controls (cell growth, metabolites [data not shown], infection kinetics)
  - Step and overall downstream recoveries
  - Chromatographic performance
  - Critical quality attributes (CQAs), including genome and capsid titer, full/empty capsid distribution, capsid purity, viral protein ratios, and vector genome integrity

RESULTS

Upstream Performance

- Characteristics such as cell growth, viability, and cell diameter were comparable across scales
- Infection kinetics showed consistent asynchronous infection behavior at 10 L and 200 L scales (**Figure 2**)
- Genome titers (ddPCR) and capsid titers (ELISA) measured in crude viral lysate demonstrated consistent productivity and packaging efficiency during scale-up from 10 L to 200 L bioreactor scales (**Figure 3**)

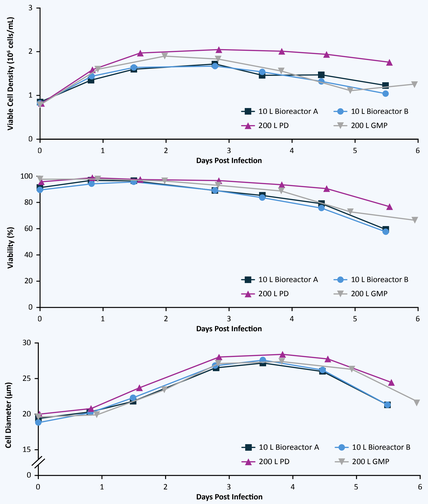
Downstream Performance

- Depth filtration, TFF, AFF and AEX chromatography all performed reproducibly at 10 L and 200 L scales
- AFF and AEX chromatograms demonstrated comparable peak shapes and elution profiles across scales
- AEX chromatography achieved consistent enrichment of full capsids, with ≤20% empty capsids in BDS (**Figure 4**)

Yield and Recovery

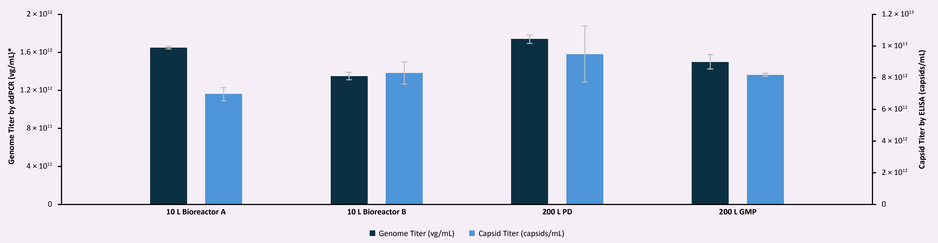
- Comparable step recoveries were observed across process scales, indicating robust scale-up of downstream AAV unit operations (**Figure 5**)
- Overall downstream recovery was ≥50% at both 10 L and 200 L scales, with higher recoveries observed at 200 L, consistent with improved manufacturing efficiency at larger processing volumes
- Purified yields reached >1 × 10^{15} vg/L at the 200 L scale, supporting high-dose and large-population clinical supply

Figure 2: Cell culture during AAV production at 10 L and 200 L bioreactor scales



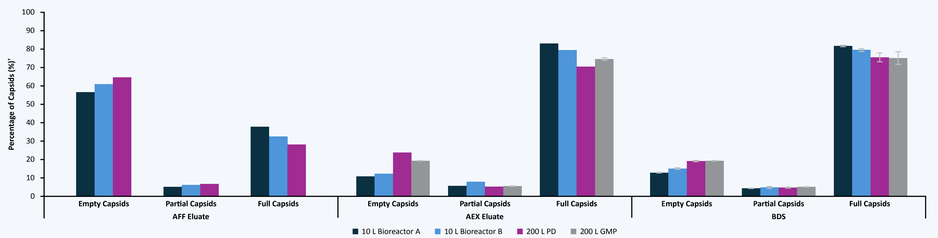
AAV, adeno-associated virus; GMP, good manufacturing practice; PD, process development

Figure 3: Crude lysate genome and capsid titers at 10 L and 200 L bioreactor scales



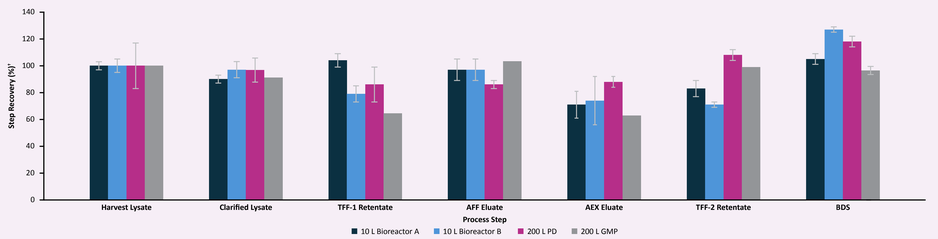
*Error bars represent %CV from assay technical replicates
%CV, percent coefficient of variation; ddPCR, droplet digital polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; GMP, good manufacturing practice; PD, process development

Figure 4: Enrichment of full capsids across the downstream purification unit operations*



*All AFF eluates, and AEX eluates from both 10 L bioreactors and the 200 L PD, were analyzed by MP; the 200 L GMP AEX eluate and all BDS samples were analyzed by SV-AUC. †Where indicated, error bars represent standard deviations from assay technical replicates
%CV, percent coefficient of variation; AEX, anion exchange; AFF, affinity; BDS, bulk drug substance; GMP, good manufacturing practice; MP, mass photometry; PD, process development; SV-AUC, sedimentation velocity analytical ultracentrifugation

Figure 5: Step recoveries by ddPCR at 10 L and 200 L bioreactor scales*



*BDS sample for all scales was tested at different site than in-process samples. †Error bars represent %CV from assay technical replicates
%CV, percent coefficient of variation; AEX, anion exchange; AFF, affinity; BDS, bulk drug substance; ddPCR, droplet digital polymerase chain reaction; GMP, good manufacturing practice; PD, process development; TFF, tangential flow filtration

PRODUCT QUALITY

- Final DS from 10 L and 200 L scale processes showed comparable CQAs (**Table 1**):
  - Capsid purity of ~97–98%
  - Consistent VP1:VP2:VP3 ratios
  - Comparable full/empty capsid distributions by mass photometry and sedimentation velocity analytical ultracentrifugation
  - Expected vector genome length by long-read sequencing

Table 1. Product quality characteristics from runs at 10 L and 200 L bioreactor scales

	Capsid Purity	VP1:VP2:VP3	Empty Capsids (by SV-AUC)	Genome Sequence Length/Expected
10 L Bioreactor A	98.3%	2.0:1.3:10	12.9%	Pending
10 L Bioreactor A	98.4%	2.3:1.3:10	15.1%	99.9%
200 L PD	98.2%	2.0:1.2:10	19.2%	99.9%
200 L GMP	97.5%	1.9:1.5:10	19.4%	Pending

GMP, good manufacturing practice; PD, process development; SV-AUC, sedimentation velocity analytical ultracentrifugation; VP, viral protein

CONCLUSION

- The successful scale-up of Lexeo Therapeutics' Sf9 baculovirus rAAV production process from 10 L to 200 L scale was demonstrated
- The 10 L small-scale model is representative and predictive of the 200 L production-scale STR process
- Comparable productivity, downstream performance, and product quality were maintained across scales
- This scalable platform supports process development, validation strategy planning, and clinical and commercial manufacturing of AAVrh.10 gene therapies

KEY TAKEAWAYS

1 Lexeo Therapeutics' Sf9 baculovirus platform overcomes key limitations of plasmid-based rAAV manufacturing by minimizing the use of critical starting materials

2 High purified yields (>10^{15} vg/L), strong overall recoveries (≥50%), and favorable empty capsid profiles (≤20%) were achieved at the 200 L production scale

3 Demonstrated scalability positions this process for robust commercialization and improved patient access to gene therapies

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REFERENCE

1. Fu Q, et al. *Biotechnol Bioeng.* 2023;120(9):2601-2621.

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DISCLOSURES

All authors are employees of Lexeo Therapeutics, Inc., New York, NY, United States.