Enabling In-Vivo Gene Therapy by Developing Intensified Downstream Processing for the Manufacturing of Lentiviral Vectors

F Leseigneur, R Esposito, S Pasupuleti, V Ferro, M Wachowius, H Martins, N Marquardt, G Ganjam, E Ayuso

[1] Siegfried DINAMIQS AG, Schlieren, 8952, Switzerland

Contact: roberto.esposito@dinamiqs.com

DINAMIQS
a Siegfried company

Introduction

The growing demand of lentiviral vectors (LVVs) for in-vivo applications—such as direct in vivo Chimeric Antigenic Receptor - T cells (CAR-T) generation, for cancer and autoimmune disorders—calls to produce high-quality vectors that meet stringent safety and regulatory standards. DiNAMIQS has established a scalable downstream process, using the lentiviral pseudotype VSV-G, which is applicable for adherent and suspension upstream process (see Fig.1). This strategy enables the manufacturing of high-quality vectors with enhanced titers, addressing both the scalability and affordability challenges.

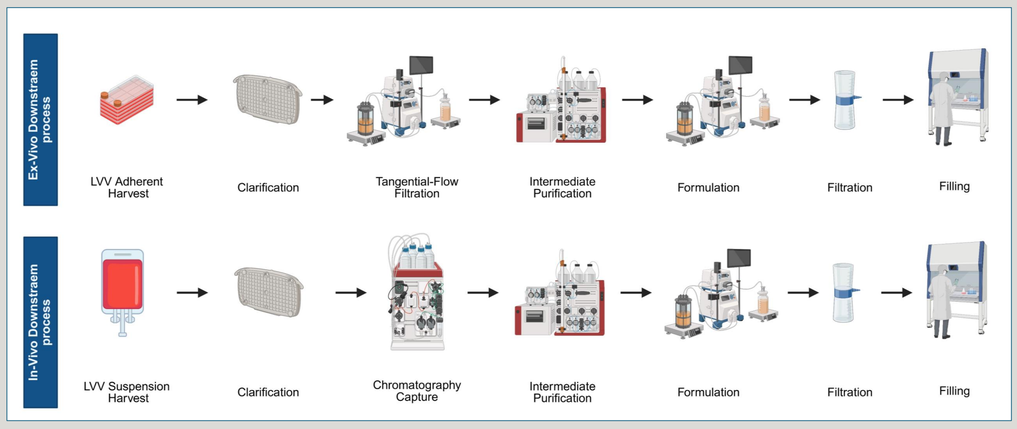

Figure 1: *Process flow diagram of LVVs dowstream manufacturing platforms at DiNAMIQS.*

Results and Discussion

1 Downstream Process Development for Scalable Lentiviral Vector Manufacturing in Ex-Vivo Gene Therapy

DiNAMIQS developed a tangential flow filtration (TFF) unit operation for viral vector purification, comparing hollow-fiber (HF) and flat-sheet cassette (FSC) modules (300/500 kDa, mPES). Despite the theoretical low-shear advantage of hollow fibers, flat-sheet cassettes performed better (see Fig.2) achieving >70% physical recovery (p24 ELISA) and >50% functional recovery (TU Assay). The critical process parameters (CPPs) identified were transmembrane pressure (TMP), permeate flux and loading density (LD).

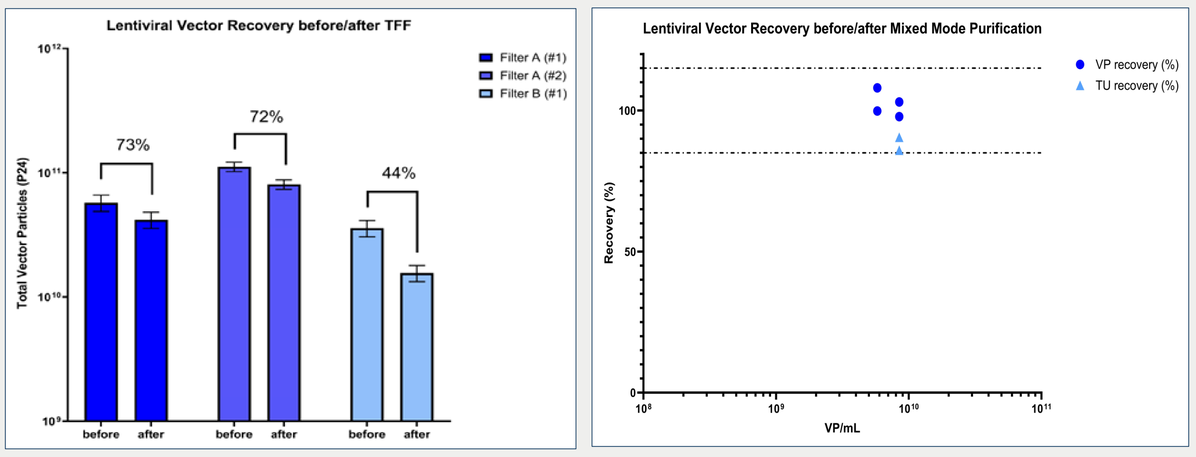

Figure 2: *Bench scale process recovery for TFF (left) demonstrating superior performances with FSC (Filter A) compared to HF (Filter B). Performances of the MMC comparable (VP and TU) manteining LD and at different flow-velocities.*

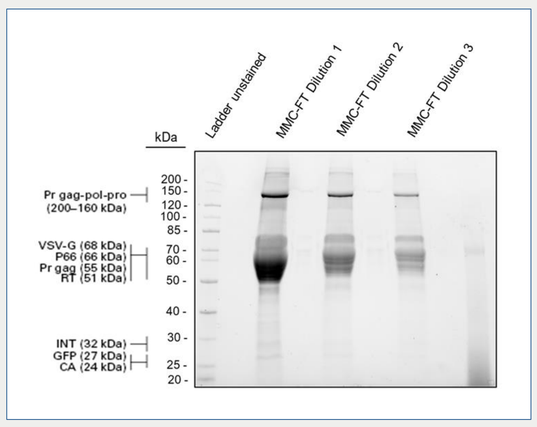

Figure 3 *End-to-end platform showing residual proteins clearance after TFF and MMC.*

Table 1 *End-to-end platform deliver >30% recovery maintaining functional titer over the process.*

Attribute	Test	Value	Unit
VP recovery est.	P24 ELISA	**30**	%
VP/TU Ratio	P24/TU Assay	**5849**	
Polydispersity index	DLS/UV-Vis	**0.35**	
Res. dsDNA	Qubit	**9.9**	µg/mL

A multi-modal chromatography (MMC) in flow-through (FT) mode was implemented as the next unit operation to further reduce contaminant burden, leveraging the mixed-mode properties of the resin Capto™ Core 400. Scale-down studies across both low and high flow velocities demonstrated >85% physical and functional recovery of LVV VSV-G vectors (see Fig.2). The process is scalable, time-efficient, and helps maintain vector stability, while reducing impurities (see Fig.3), removing serum, and lowering volumes to improve cost efficiency in downstream operations.

2 High-quality Lentiviral Vector Manufacturing For In-vivo Gene Therapy

The downstream process for in vivo gene therapy aims at delivering high quality vectors. It relies on harvest clarification, anion exchange (AEX) capture, and mixed-mode polishing. The AEX step uses a macro-porous media to efficiently remove impurities while maintaining >65% recovery of physical and functional lentiviral vector titers.

Bottom-up experiments were conducted on a CIM DEAE monolith column to investigate the adsorption and desorption behavior of LVV-VSV-G. Using linear gradient elution (LGE), elution was observed between 300–600 mM NaCl, consistent with literature reports.

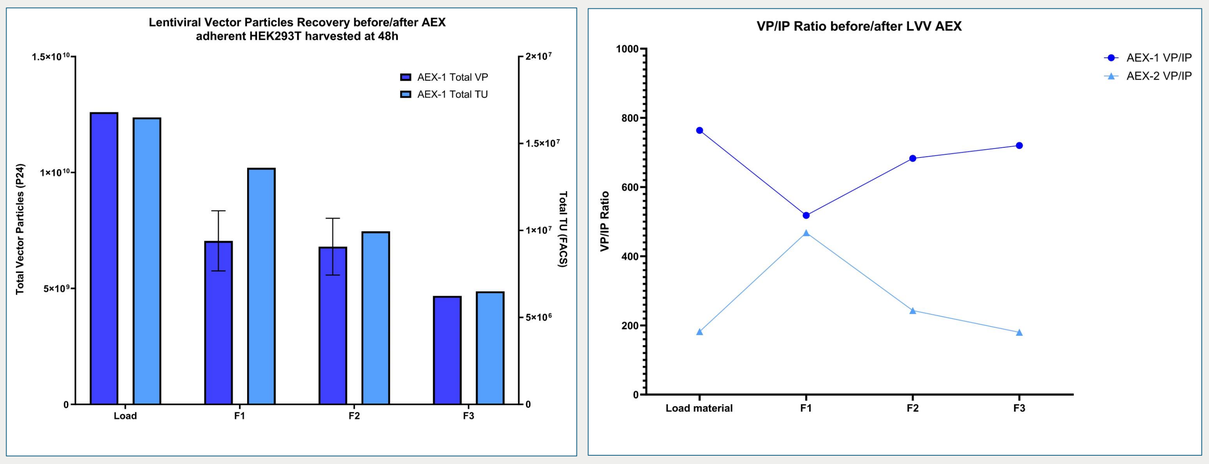

Figure 4: *Step-gradient elution under optimized conditions achieved 75% physical and 77% functional recovery (left). Despite charge heterogeneity between 48h and 84h harvests, VP/TU ratio (right) for purified fraction was in a consistent range for both adherent and suspension process.*

The confirmation run using step-gradient elution under the optimized scale-down conditions (see Fig.4) achieved 75% physical recovery (p24 ELISA) and 77% functional recovery (TU Assay). Interestingly, a shift in negative surface net charge was observed between 48h and 84h harvests, suggesting inter-particle structural heterogeneity. Regardless on the heterogeneity, VP/TU ratio is maintained (see Fig.4).

The AEX capture step was also applied to the suspension production platform, harvested at 48h. Step-gradient elution on a CIM DEAE yielded >60% physical and functional recovery. Residual host cell impurities analysis (see Fig.5) showed dsDNA levels <1 µg/mL in the drug substance, corresponding to 1–1.5 log reduction value (LRV). Total protein clearance of 0.7 LRV, which could be potentially improved with a final TFF step (300 kDa cut-off).

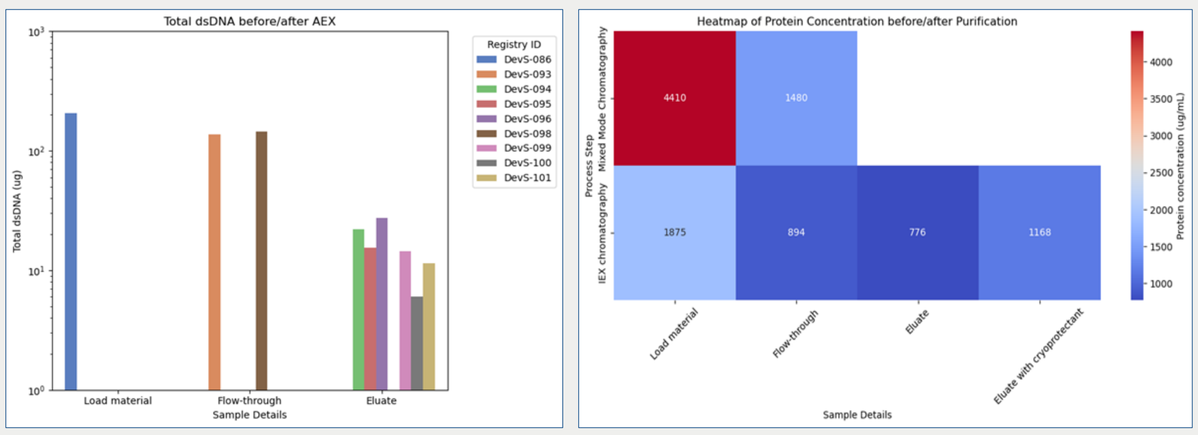

Figure 5: *AEX in-process control for dsDNA is shown on the left. When coupled with MMC, AEX achieved a >5-fold residual proteins, as illustrated in the process heat map (right).*

Conclusions

- **Lentiviral vector pseudotype agnostic platform for ex-vivo gene therapy** achieving ~30% physical and ~50% functional recovery of LVV VSV-g vectors, with a scalable process preserving vector shelf-life, maximizing speed, throughput, and manufacturing flexibility.
- **Effective Contaminant Reduction.** Implemented multi-modal chromatography, achieving >85% functional vector particles recovery and significantly lowering contaminant burden while maintaining low processing time.
- **Streamlined In-vivo Process based on Quality by Design (QbD) approach**. AEX chromatography captured LVVs with >65% physical and functional titer, effectively removing major impurities for high-quality gene therapy vectors.

References

- Pamenter G, Davies L, Lamont C, Rahim D, Knevelman C, Miskin J, Mitrophanous K, Dikicioglu D, Bracewell DG. Lentiviral vector determinants of anion-exchange chromatography elution heterogeneity. Biotechnol Bioeng. 2024 Sep;121(9):2936-2951. doi: 10.1002/bit.28766. Epub 2024 Jun 14. PMID: 38874319.
- Moreira AS, Cavaco DG, Faria TQ, Alves PM, Carrondo MJT, Peixoto C. Advances in Lentivirus Purification. Biotechnol J. 2021 Jan;16(1):e2000019. doi: 10.1002/biot.202000019. Epub 2020 Dec 8. PMID: 33089626.
- Valkama AJ, Oruetxebarria I, Lipponen EM, Leinonen HM, Käyhty P, Hynynen H, Turkki V, Malinen J, Miinalainen T, Heikura T, Parker NR, Ylä-Herttuala S, Lesch HP. Development of Large-Scale Downstream Processing for Lentiviral Vectors. Mol Ther Methods Clin Dev. 2020 Mar 30;17:717-730. doi: 10.1016/j.omtm.2020.03.025. PMID: 32346549; PMCID: PMC7177191.
- Labisch JJ, Evangelopoulou M, Schleuß T, Pickl A. Investigating Ultrafiltration Membranes and Operation Modes for Improved Lentiviral Vector Processing. Eng Life Sci. 2025 Jan 3;25(1):e202400057. doi: 10.1002/elsc.202400057. PMID: 39801560; PMCID: PMC11717145.