How to Resolve Vector-Based Therapeutics Major Manufacturing Pain Points by Innovation-Driven Collaborations

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Introduction

With increasing approvals of viral vector-based gene therapies, the industry is shifting its focus to the expansion of viral vector manufacturing to meet the demands of the growing number of therapies on the market. Collaboration and expertise are crucial for continuous improvements in the efficiency of viral vector production to meet demand and get lifesaving therapies to patients quickly. This must be carefully balanced with consistency and quality of the final product. Multiple applications and technologies to address current manufacturing challenges and solutions to increase overall viral vector yield throughout development will be explored. Collaborative case studies will be presented to demonstrate the power of partnerships that accelerate the progress associated with entire process intensification and optimization of AAV and lentivirus from benchtop development to large-scale commercial manufacturing.

Objective

Demonstrating how collaborations and combined expertise in gene and cell therapy fields are critical for rapid continuous improvements in the efficiency of viral vector production to meet demand and get lifesaving therapies to patients quickly.

Upstream AAV and LV intensification using KrosFlo® TFDF®

AAV/LV Industrial TFDF Platform

Intensify viral vector production by integrating Perfusion and Clarification Platform

KrosFlo TFDF Perfusion System

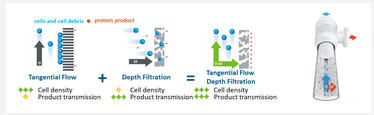
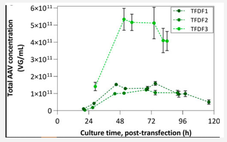


Figure 2: Elongated tubular depth filter with 2-5 um pore size (Polypropylene/PET) operated in tangential flow mode. Combined benefits of tangential flow and depth filtration

Figure 3: KrosFlo TFDF-based perfusion GMP system using single use closed gamma-irradiated flow paths (including single-use pump head, flow and pressure sensors)

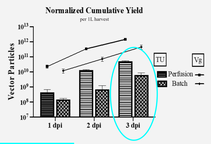
High-yield AAV production: Collaborative TFDF evaluation study iBET and Repligen



8-fold increase of Potent LV per batch

Figure 4: TFDF perfusion process results: Concentration of total AAV generated including intracellular, extracellular and permeate fractions

High-yield potent LV production: Collaborative TFDF evaluation study McGill and Repligen



4.9 x 10^{10} TU/L perfusion
6.4 x 10^{9} TU/L batch

8-fold increase of Potent LV per batch

Figure 5: Cumulative yields normalized per 1 L of harvest (at dpi perfusion runs: 1.2e11 TFP, 3.5 e12 TVP: batch runs: 4.4e9 TFP, 3e11 TVP.

TFP=Total functional particles; TVP= Total vector particles

Game changing agnostic solutions for viral vector manufacturing

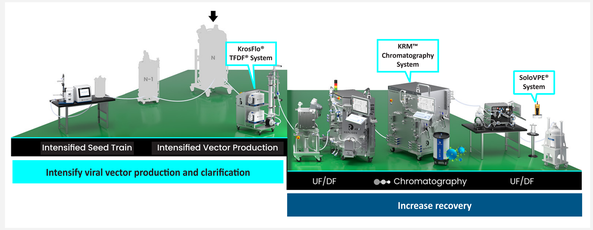


Figure 1: Advanced viral vector end-to-end manufacturing process. Powerful Collaborative efforts with:

1. KrosFlo TFDF perfusion system with iBET and McGill
2. KrosFlo KRM Chromatography System with Forge Biologics
3. SoloVPE with PTC Therapeutics

Downstream AAV intensification using KRM™ Chromatography System

AAV Capture Chromatography Scale-up Platform

AAV Industrial Chromatographic using advanced gentle KRM™ Platform

Case study: Scalability and reproducibility of the AAV9 capture step using KRM™ Chromatography System

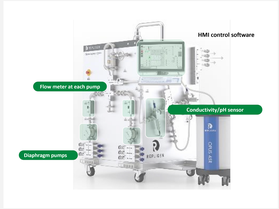
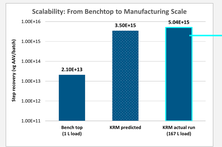


Figure 6: Advanced KRM chromatography system. No hose barbs at critical junctions, injection molded tubing and multi-valve arrays reducing hold-up volume, minimize cross-contamination and ultimately enhance recovery

Run #	Bench Top				KRM 10		
	1	2	3	4	1	2	3
Load volume [L]	1			0.6	50	167	
Flow rate [mL/min]	2.09				214		
Peak volume [L]	0.009	0.009	0.01	0.008	170	198	190
Peak area ratio	2.1				7.3	8.6	8.3

Table 1: Scalability from 1 L bench top system to KRM 10 system and reproducibility performance of the scale-up system.



Total viral Vector increased by 240 X fold

Figure 7: The scalability of the AAV capture step from the benchtop to the manufacturing scale using KRM Chromatography Systems was analyzed by comparing process performance and product quality at two scales. There was no increase of endotoxins, particle aggregations, or residual non-product related impurities such as HCP.

Accelerating AAV manufacturing: Using SoloVPE® analytical technology

Streamlining AAV titer determination using variable pathlength technology

Figure 8: SoloVPE , At-line variable pathlength technology, development analytical tool enabling in line process analytics for samples post chrom1, chrom2, TFF2 and DS

Correlation between SoloVPE and qPCR/ELISA: Testing of in-process samples

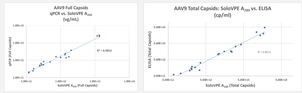


Figure 9: SoloVPE & FlowVPX, At-line and in-line variable pathlength technology

Excellent agreement with qPCR and Capsid ELISA data with SoloVPE (260/280) data

Conclusion

AAV and LV upstream and downstream intensification using advanced innovative technologies through successful collaborative efforts

KrosFlo TFDF-based perfusion viral vector upstream intensification:

- The AAV8 titers and clarification yields obtained with TFDF demonstrated the capabilities of the TFDF perfusion device for continuous integrated production, harvest and clarification of AAV
- Perfusion mode using the TFDF perfusion device resulted in 9X fold LV doses compared to batch mode enabling large scale demand
- Continuous perfusion is critical to intensified AAVs and LVs production
- No limitation for virus production from nutrient deprivation and/or accumulation of inhibitory metabolites

KRM™ chromatography system enable downstream step intensification during scalability of an AAV capture purification step to the manufacturing scale

- By maintaining the quality attributes such as purity and even improving process recovery while maintaining process parameters
- High recover yield (136%) of AAV9 was achieved at large scale due to low hold up volume and gentle flow
- In the downstream collaborative study, Forge Biologics and Repligen successfully developed and advanced scalable capture chromatography step

AAV rapid titer determination using at line using variable Variable pathlength technology (VPT)

- It has been demonstrated how at-line process controls, using VPT, can offer quick and direct total viral vector analysis during development to enhance throughput and improve decision- making and accelerate moving from development to clinical
- The implementation of SoloVPE technology during development provides a new method for AAV titer determination using real-time monitoring, which eliminates the dependency on off-line testing and associated variability caused by sample manipulation
- The SoloVPE, an at-line analytical tool, enables a seamless transition into an automated sterile and single use in-line analysis using the FlowVPX.

Acknowledgments

Thanks to all industrial collaborators that we worked along to generate the data for this poster, McGill, iBET, Forge Biologics and PTC Therapeutics.

References

1. Mendes et al, Frontier in Bioengineering and Biotechnology , 2022 (10.3389/fbioe.2022.1020174)
2. Yen Tran et al, Frontier in Bioengineering and Biotechnology , 2022 (10.3389/fbioe.2022.887716)
3. Ferraiolo & Chen, Cell & Gene Therapy Insights 2022; 8(9), 1099–1111. DOI: 10.18609/cgti.2022.164