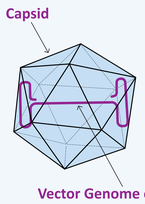
Improving VP1 Ratios Impact on CQAs in rh10 AAV Manufactured Through Sf9 Platform

Elena Bianchetti, Alexandro Berumen, Aileen Rottinger, Eric Tompkins, Ambryice Riggs, Noah Miller-Medzon, Eric Lin, Christina Pelella, Marina Goldfeld*, David Lin*, Tim Fenn

*Former employee of Lexeo Therapeutics

INTRODUCTION

Recombinant Adeno-associated Virus (rAAV) vectors are comprised of a DNA sequence encoding a gene of interest (GOI) encapsulated within a protein capsid [1]. The rAAV protein capsid is comprised of three main proteins: Viral Protein (VP) 1, 2 and 3 [2], which assemble into a near-spherical shell of 60 subunits [2]. The correct ratio and stoichiometry of each VP are essential for delivery of cargo to the target cells and for the overall efficiency and safety of AAV-based therapies [3]. AAVrh.10 is an adeno-associated virus (AAV) serotype derived from rhesus macaques and exhibits high tropism for both central nervous system (CNS) and cardiac tissues in preclinical models [4;5]. Studies have compared rh10 AAV with other AAV serotypes (e.g., AAV9, AAV8) and found that rh10 AAV often exhibits superior transgene expression, particularly in muscle tissue, including the heart [5].



STUDY-AT-A-GLANCE

Our goal was to optimize Lexeo's Sf9-baculovirus manufacturing process for rh10 AAV to ensure comparability to our first-in-human HEK293 based process. As the process was developed in parallel with early clinical stage development, and further optimized to meet commercial robustness and demand, comparability was evaluated in terms of Critical Quality Attributes (CQAs), with an emphasis on potency. We found that VP ratios correlated to the resultant potency of the AAV material. As a result, we used the VP ratio as a proxy during process development for potency, in addition to analyzing the impact of process changes through a variety of analytical methods.

METHODS

Extensive process and analytical development led to the creation of a novel Sf9-Baculovirus process for manufacturing rh10 AAV with improved productivity, purity, and potency expression. We have utilized this platform process to successfully produce rAAV across multiple GOI at scales ranging from shake flasks to 200L stirred tank bioreactors. DOE (Design of Experiment) studies were performed across a variety of upstream and downstream conditions to yield the batches presented below.
For a detailed description the Lexeo manufacturing process, please refer to the QR code in **More Information**.

On every lot manufactured, we performed the following analytical characterization:

- Capsid purity and VP ratios by CE-SDS (Figure 1). The VP ratios shown are normalized to a total VP3 of 10.
- Vector genome (VG) titer by ddPCR.
- Full capsid percentage by Mass Photometry (MP).
- GOI potency was evaluated at either the mRNA or protein level, depending on the program.

Figure 1. CQAs tested across lots covering multiple rAAV constructs at varied scale.

a. CE-SDS analysis (VP Ratio and Capsid Purity). Manufacturing process optimization led to an increase in VP1 levels across all three rAAV constructs.

	Lot #	Scale	Capsid purity	VP1	VP2	VP3
rAAV-A	AAVL08	200L PD	98.4	2.3	1.3	10
	AAVL07	10L PD	98.2	2.0	1.3	10
	AAVL06	<1L PD	98.3	1.5	1.1	10
	AAVL05	<1L PD	98.0	1.5	1.4	10
	AAVL04	<1L PD	98.4	1.3	1.2	10
	AAVL03	2.5L PD	97.7	1.1	1.3	10
	AAVL02	<1L PD	96.9	1.0	1.3	10
	AAVL01	2L PD	98.6	0.6	1.0	10
		RS Lot (HEK293)	98.8	2.8	4.4	10

	Lot #	Scale	Capsid purity	VP1	VP2	VP3
rAAV-B	AAVL16	200L GMP	97.0	2.0	1.4	10
	AAVL12	10L PD	98.7	2.0	1.2	10
	AAVL11	10L PD	98.0	1.8	1.2	10
	AAVL10	200L PD	97.2	1.1	1.1	10
	AAVL09	200L GMP	97.2	0.9	0.9	10
		RS Lot (Sf-9)	98.1	0.8	0.8	10

	Lot #	Scale	Capsid purity	VP1	VP2	VP3
rAAV-C	AAVL15	2.5L PD	97.0	1.8	1.3	10
	AAVL14	2.5L PD	98.8	1.3	0.8	10
	AAVL13	3L PD	99.0	0.7	1.0	10
		RS Lot (HEK293)	99.4	3.0	3.7	10

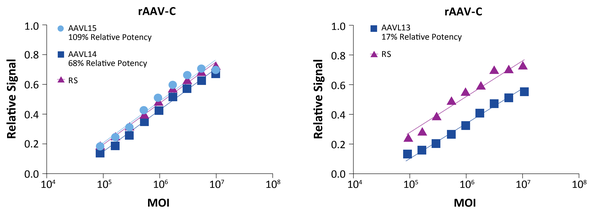
b. VG titer by ddPCR and full capsid % by MP. The process development performed internally at Lexeo resulted in a significant harvest titer improvement. Notably, in conjunction with downstream development, the vector purity climbed as well, as shown by full capsid percentage in the Bulk Drug Substance (BDS) after process optimization.

	Lot #	Scale	Upstream titer by ddPCR (vg/mL)	BDS full capsid by MP (%)
rAAV-A	AAVL08	200L PD	1.74e12	69.1
	AAVL06	1L PD	1.81e12	89.4
	AAVL01	2L PD	1.20e12	69.1
		RS Lot (HEK293)	N/A	33.1

	Lot #	Scale	Upstream titer by ddPCR (vg/mL)	BDS full capsid by MP (%)
rAAV-B	AAVL12	10L PD	1.3e12	65.3
	AAVL09	200L GMP	3.5e11	55.6
		RS Lot (Sf-9)	1.7e11	46.2

	Lot #	Scale	Upstream titer by ddPCR (vg/mL)	BDS full capsid by MP (%)
rAAV-C	AAVL15	2.5L PD	1.34e12	78.1
	AAVL13	3L PD	2.43e11	60.2
		RS Lot (HEK293)	N/A	65.2

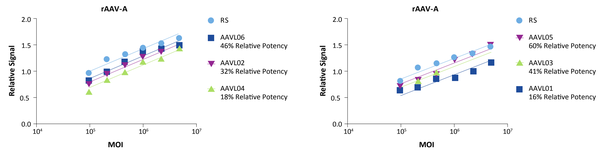
Figure 2. GOI potency was evaluated for rAAV-C at the protein level.



Potency expression assay based on an In-Cell Western method demonstrated a linear correlation between VP1 content and GOI potency. The 3L-scale PD lot with a normalized VP1 of 0.7 showed 17% relative potency compared to the reference standard (RS), whereas the vector lot generated through subsequent process optimization, 2.5L-scale PD (1), showed a relative potency of 109% compared to the same RS.

Figure 3. GOI potency evaluated for rAAV-A at the protein level.

a. The data collected through In-Cell Western again demonstrate a linear correlation between normalized VP1 and GOI potency at a protein level from rAAV-A produced using an Sf9-baculovirus process. Comparability experiments suggest that, after manufacturing process optimization, the Sf9 rAAV-A construct and the HEK293T AAV reference standard are not statistically different in terms of potency and capsid purity.



b. In-Cell Western data for two Sf9-baculovirus batches produced at the 10L and the 200L scale. Even when produced at higher scales, the Sf9 rAAV-A construct and the HEK293T AAV reference standard are not statistically different in terms of potency and capsid purity.

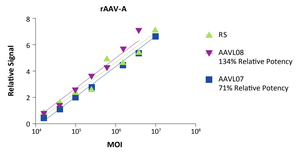
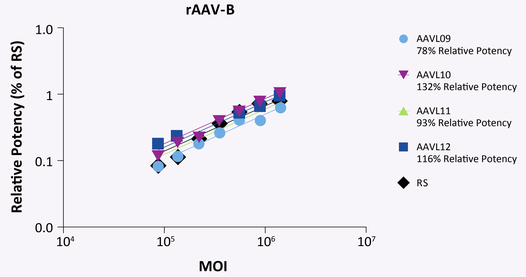
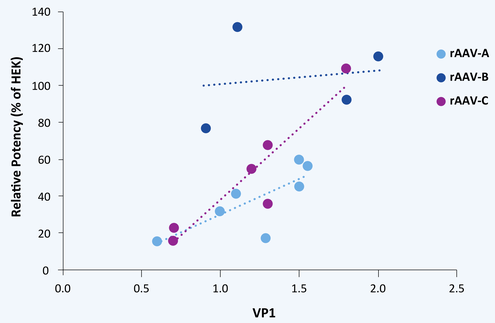


Figure 4. GOI potency was evaluated for rAAV-B at the mRNA level.



A potency expression assay based on RT-ddPCR demonstrated a linear correlation between VP1 content and GOI potency. Potency expression was also impacted, with a 200L-scale PD lot showing 132% relative potency compared to the RS.

Figure 5. Impact of VP1 levels on AAV potency expression across multiple GOI.



Across all 3 rAAV constructs, linear regression analysis of relative potency data compared to normalized VP1 levels demonstrates and predicts that potency increases as VP1 levels increase.

KEY TAKEAWAYS

1. Optimization of the Lexeo Sf9-baculovirus platform process highlights the direct relationship between VP1 and CQAs, including potency. The data indicate that increasing the VP1 ratio within the viral capsid significantly enhances potency. This agrees with prior literature using HEK293 based manufacturing processes [8].
2. Our data support the impact of improving VP1 levels is GOI-specific, indicating that process development is required for each program to optimize manufacturing conditions and vector quality effectively.
3. Our findings suggest that the overall likelihood of comparability study success is substantially increased by the impact of Sf9-baculovirus process development due to its resultant effect on VP ratio and potency.
4. Our data may partially explain discrepancies reported in prior studies [6;7] with respect to the Sf9-baculovirus platform's AAV manufacturing capabilities. We demonstrate that platform success is contingent on the extent of process development, with a particular emphasis on VP ratio.

MORE INFORMATION

To **learn more**, visit: **www.lexeotx.com**

For more insight into the Lexeo platform manufacturing process, visit our **Publications** page

ACKNOWLEDGMENTS

We acknowledge the technical support and feedback provided by the Lexeo Therapeutics CMC team, the process support provided by our CMC Process Development team, and poster support from Nilomi Shah and Theoria Creative. We also acknowledge our colleagues at Millipore for the technical support provided, and our manufacturing partners and collaborators at the Belfer Gene Therapy Core Facility (BGTCF) at Weill Cornell Medicine, at Virovek and Fujifilm.

REFERENCES

1. "Characterization of Recombinant Adeno-Associated Viruses (rAAVs) for Gene Therapy Using Orthogonal Techniques." Cole L. et al, 2021. Pharmaceutics. 2. "Adeno-associated virus vector as a platform for gene therapy delivery." Wang D. et al, 2019. Nature Reviews Drug Discovery. 3. "Characterization of Adeno-Associated Virus Capsid Proteins with Two Types of VP3-Related Components by Capillary Gel Electrophoresis and Mass Spectrometry." Oyama H. et al, 2021. Human Gene Therapy. 4. "The adeno-associated virus rh10 vector is an effective gene transfer system for chronic spinal cord injury." Hoshino Y. et al, 2019. Scientific Reports. 5. "RH10 provides superior transgene expression in mice when compared with natural AAV serotypes for neonatal gene therapy." Hu C. et al, 2011. The Journal of Gene Medicine. 6. "Methods Matter: Standard Production Platforms for Recombinant AAV Produce Chemically and Functionally Distinct Vectors." Rumachik N.G. et al, 2020. Molecular Therapy. 7. "Systematic comparison of rAAV vectors manufactured using large-scale suspension cultures of Sf9 and HEK293 cells." Liu S. et al, 2024. Molecular Therapy. 8. "Characterization of AAV vectors: A review of analytical techniques and critical quality attributes." Kontogiannis T et al., 2024. Methods & Clinical Development.