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Case Study: Comparability between HEK293 and Sf9-Baculovirus AAV manufacturing processes

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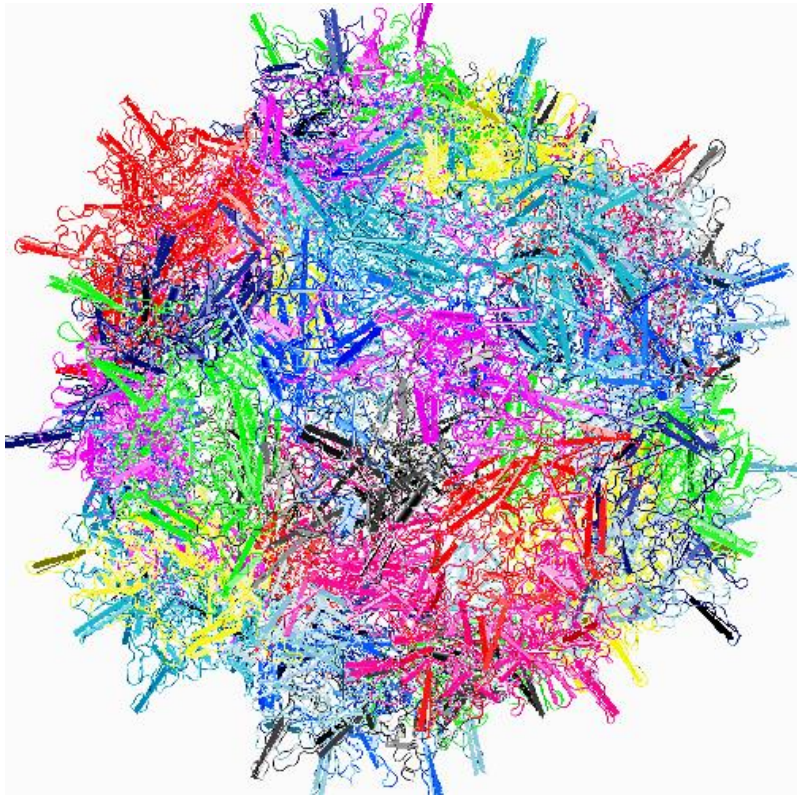
Prevail Therapeutics, a wholly owned subsidiary of Elil Lilly and Company

Adeno-associated virus (AAV) capsid is a variable multi-subunit vector



https://www.ncbi.nlm.nih.gov/Structure/pdb/1LP3

AAV virions are composed of 60 ***capsid subunits:*** *VP1:VP2:VP3 at a 1:1:10 ratio*

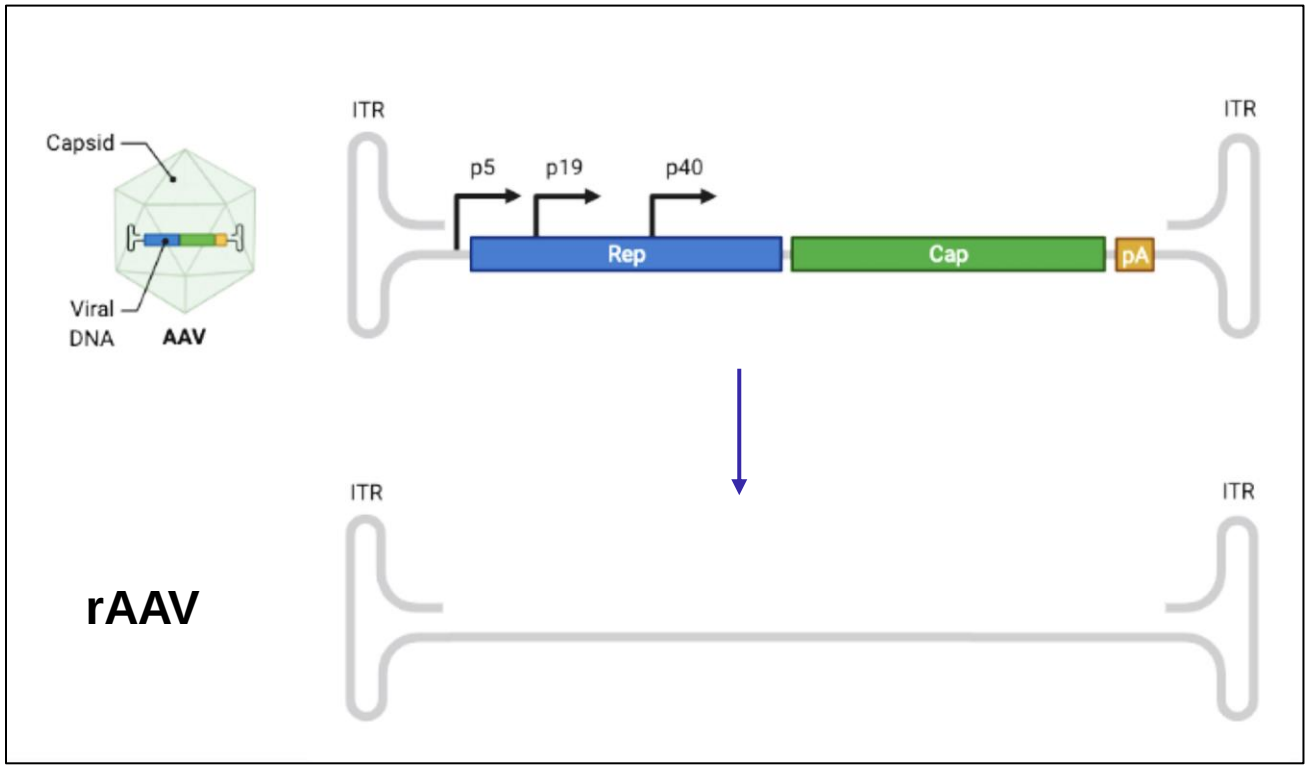
Each subunit has nine **variable regions** on the virion surface that determine the primary **tropism** and intracellular **trafficking**

There are 13 naturally occurring serotypes, each with variations of the capsid protein sequence

Case study Serotype:

- **AAV9**

AAV genome can be replaced with a therapeutic payload for delivery



modified from: https://www.dynotx.com/introduction-to-aav-as-a-gene-therapy-vector-part-1/

Wang et. al 2019

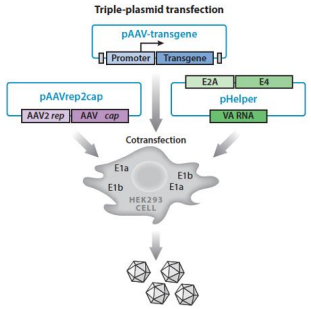
*Only the **145 bp AAV ITRs** are **necessary** for AAV propagation. They induce transgene expression, play roles in vector production and ensure persistent transduction.*

*~96% of the AAV genome can be removed to accommodate **therapeutic transgenes** for gene therapy up to ~4.7kb.*

CMC Strategy and Capabilities: Transition from HEK → Sf9 platform

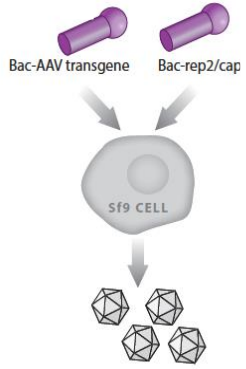
HEK293 Process

- Partnership with established CDMO with platform process
- Robust adherent HEK293 process used to maximize speed to the clinic for early Prevail programs

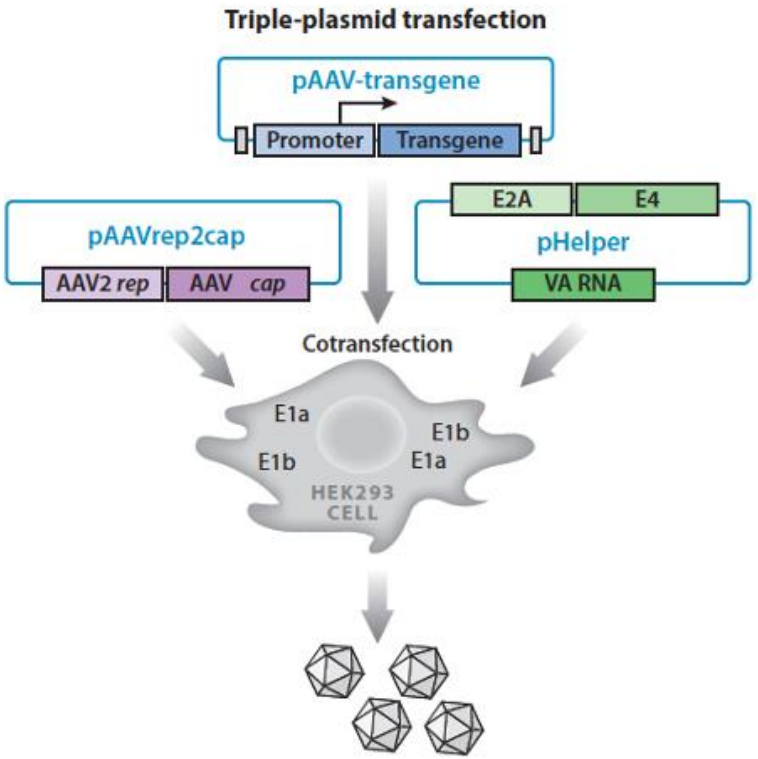


Sf9 Baculovirus Process

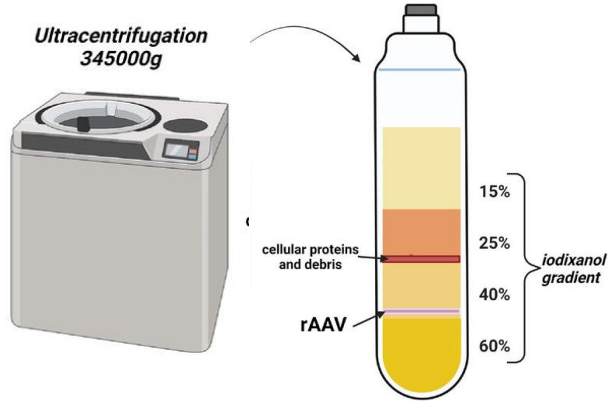
- Transitioned to baculovirus platform to establish high-yield scalable process for future preclinical, clinical and commercial material and future pipeline
- Process and analytical development established at Prevail
- Process transferred to CDMO for GMP manufacturing for clinical supply
- Significant reduction of cost per dose



Process v1.0: HEK293 platform manufacturing using ultracentrifugation



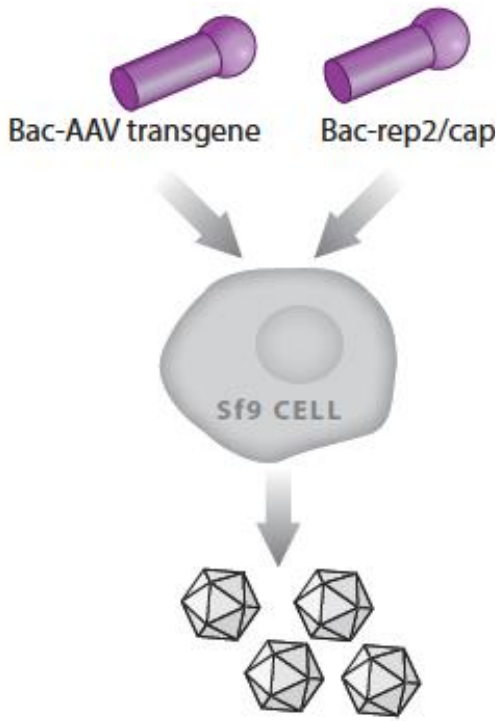
Adherent culture



Ultracentrifugation

- Pros:
  - Established platform, more experience
- Cons:
  - Low productivity (pool upstream lots)
  - Very labor intensive and hard to scale (scale out vs. scale up)
  - Ultracentrifugation: MFG challenges

Process v2.0: Sf9 platform manufacturing using scaleable chromatography

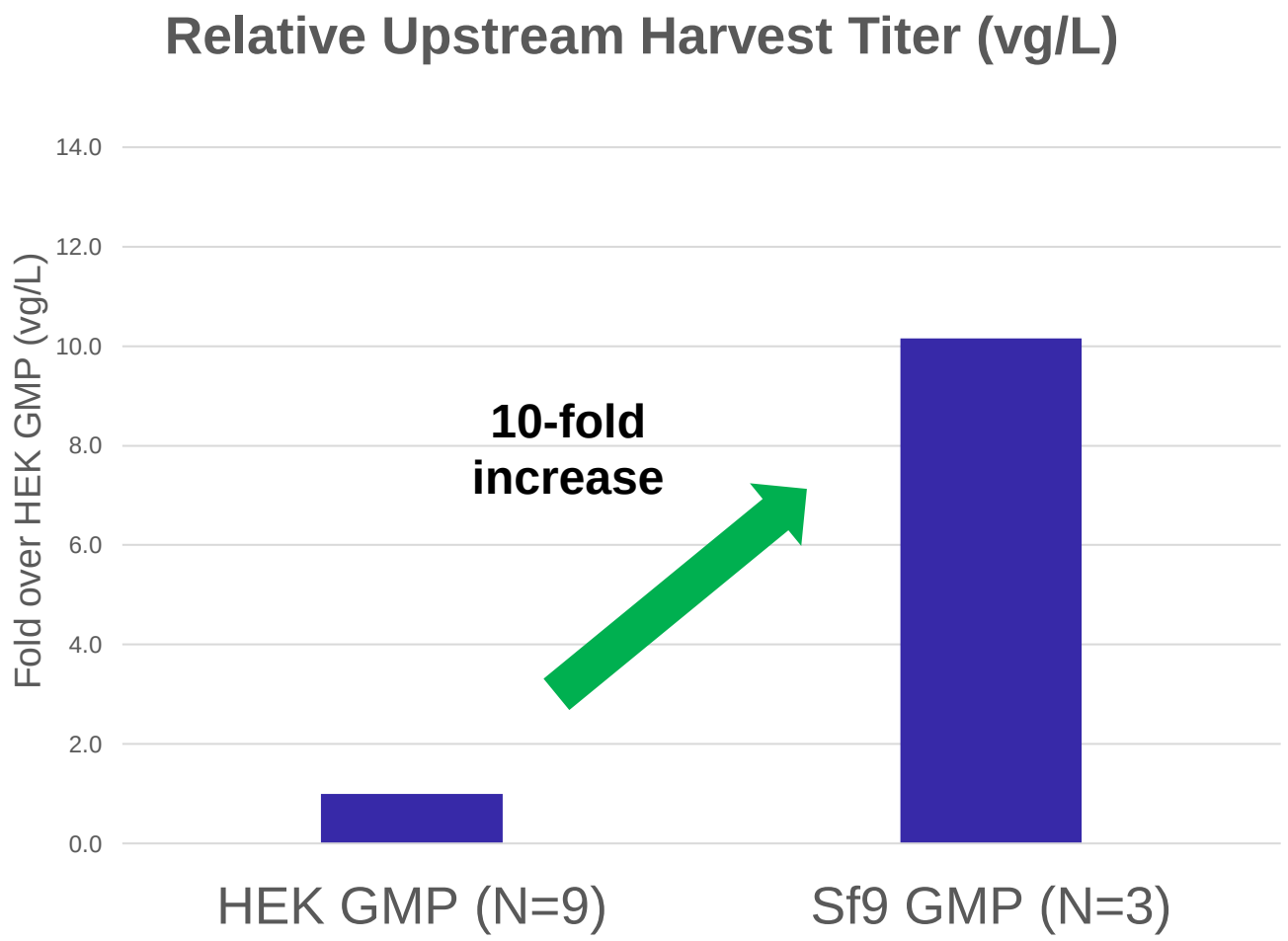


Suspension culture

Chromatography:
Affinity + Ion Exchange

- Pros:
  - Can be manufactured in bioreactors and scaled up >250L scale
  - Significantly higher productivity than HEK transfection systems lower long-term costs
  - IEX can be a robust viral clearance step
  - Equipment used similar to non-gene therapy manufacturing
- Cons:
  - Longer initial development time and fewer CDMOs with experience
  - Overall higher upfront costs and development time

Manufacturing Improvements:
Sf9 platform significantly increases productivity

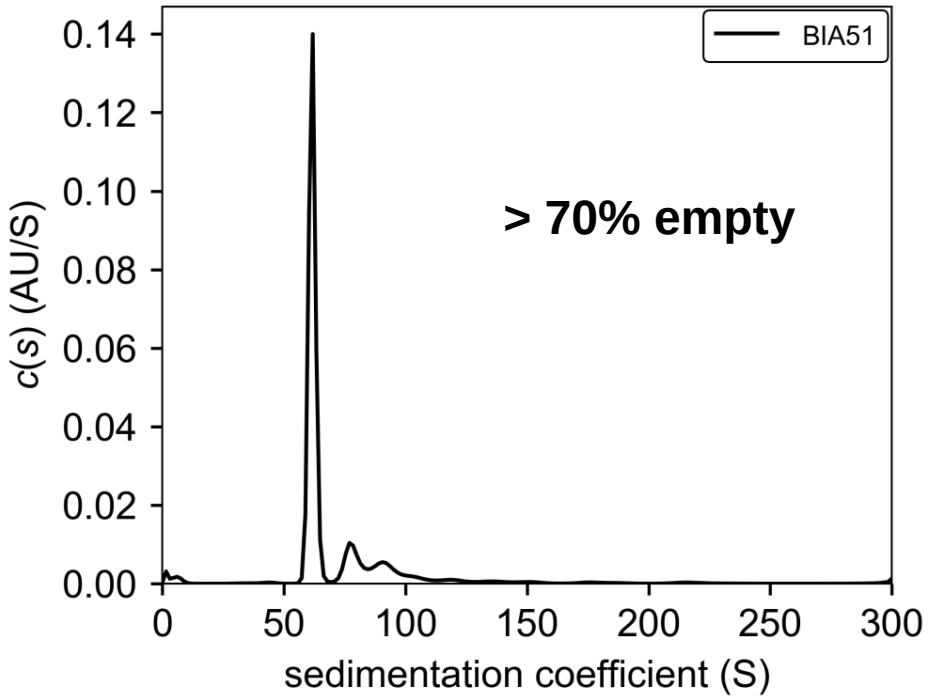


- Significant COG reductions >10-fold
- More doses per batch
- Increased batch size supports characterization, release, and stability testing
- No need to pool upstream batches

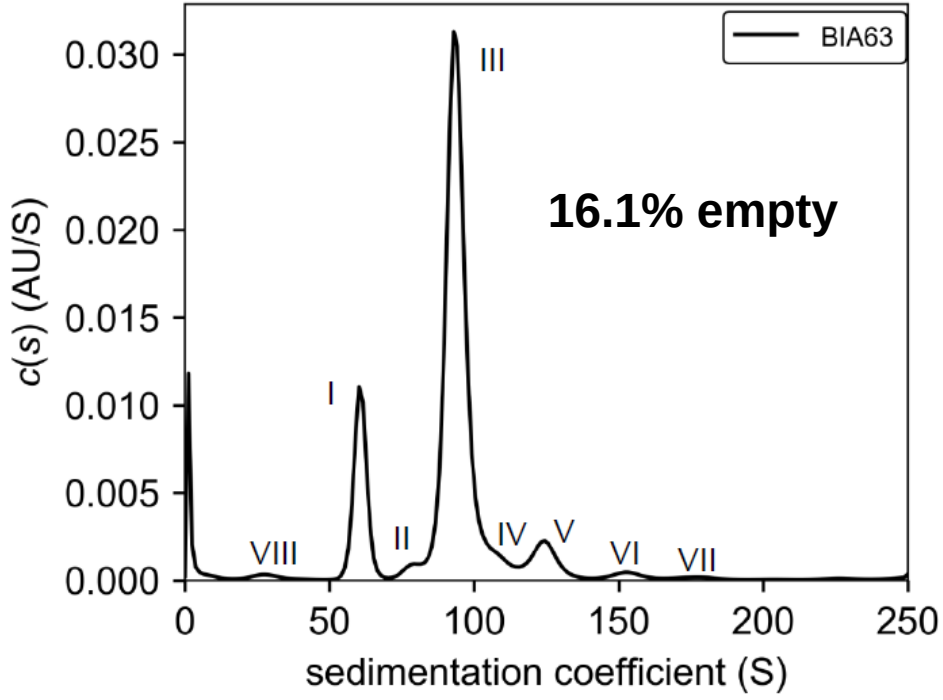
Manufacturing Improvements:
Sf9 platform significantly increases packaging efficiency

Pre – Empty Capsid Clearance: Analytical Ultracentrifugation

Upstream HEK293



Upstream Sf9



12.0-19.9% Empties (250L Sf9 scale N = 5)

ICH Q5E, comparability of biotechnological/biological products subject to changes in their manufacturing process

*"**The goal of the comparability exercise is to ensure the quality, safety, and efficacy of drug product produced by a changed manufacturing process** through collection and evaluation of the relevant data to determine whether there might be any adverse impact on the drug product due to the manufacturing process changes."*

*"The demonstration of comparability **does not necessarily** mean that the quality attributes of **the pre-change and post-change product are identical, but that they are highly similar** and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have **no adverse impact upon safety or efficacy of the drug product**."*

- **European Medicines Agency. Questions and answers on comparability considerations for advanced therapy medicinal products (ATMP) - Scientific guideline (2019).**

How to Show Comparability ?

Products need be ”*highly similar*” with “*no adverse impact*” in

Quality | Safety | Efficacy

Prevail Strategy to Show Comparability:

Analytical Comparability

Quality, Safety, Efficacy

In-vivo NHP Toxicology

Safety

Head-to-Head Mouse efficacy study

In-vivo efficacy

Challenges and approach

Challenges

Material
(Few lots, limited availability)

Methods
(Not all Methods Qualified)

Approach

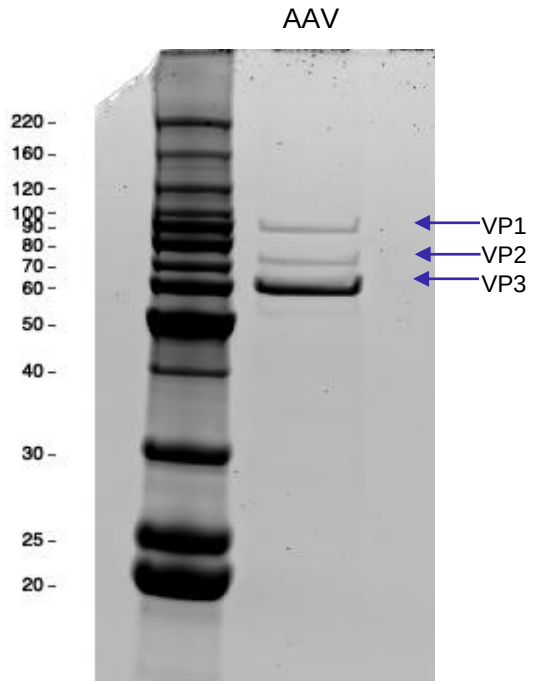
Include non-GMP lots (PD)
Prioritize testing

Test samples side-by-side
Confirm w/ orthogonal method

Panel	Testing performed
Release testing	Full release panels including: Titer, Potency, Aggregation, Empty capsids, Residuals
Characterization	DNA impurities, Mass spec, TEM, VP ratio, Genome: Capsid ratio
Stability	Side by side accelerated stability – Product quality

Quality:
AAV capsid proteins are identical between platforms

SDS-PAGE



Intact Mass Spec

	Lot	VP1 Observed average mass (Da)	VP2 Observed average mass (Da)	VP3 Observed average mass (Da)
HEK293	Lot 1	81288	66206	59729
HEK293	Lot 2	81288	66210	59731
SF9	Lot 3	81289	66205	59729

1 Dalton or less difference

Identical capsid protein MW

Quality: Capsid protein post-translational modifications are highly similar

Post-translation modification by mass spectrometry

PTM	HEK293 Platform		Sf9 Platform
	Lot 1	Lot 2	Lot 3
VP1 N-term	100% N-acetylated	100% N-acetylated	100% N-acetylated
VP2 N-term	Non-acetylated	Non-acetylated	Non-acetylated
VP3 N-term	100% acetylated	100% acetylated	100% acetylated
Deamidation of N314	1.1%	1.1%	1.1%
Deamidation of N329	2.2%	2.3%	7.2%
Deamidation of N409/N410	4.0%	5.4%	3.9%
Oxidation of N452	14.4%	12.7%	15.3%

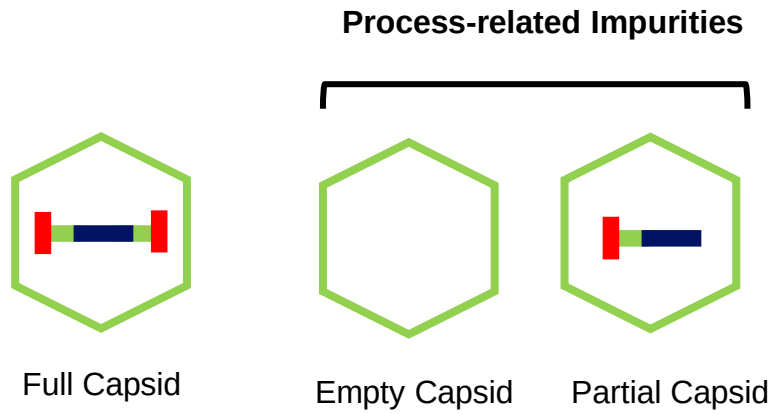
Additionally, no glycosylation were detected

"*Highly similar*" post translation modification on the capsid proteins

Quality: AAV Particle distribution
Sf9 platform shows fewer partial capsids

Analytical Ultracentrifugation

Process	Batch	Empty (%)	Partial (%)	Full (%)
HEK	Lot 1	3.9	47.7	39.9
	Lot 2	12.5	38.6	34.4
SF9	Lot 1	6.6	3.0	76.5
	Lot 2	8.6	3.5	80.9
	Lot 3	5.5	5.2	82.8
	Lot 4	3.1	9.2	84.3

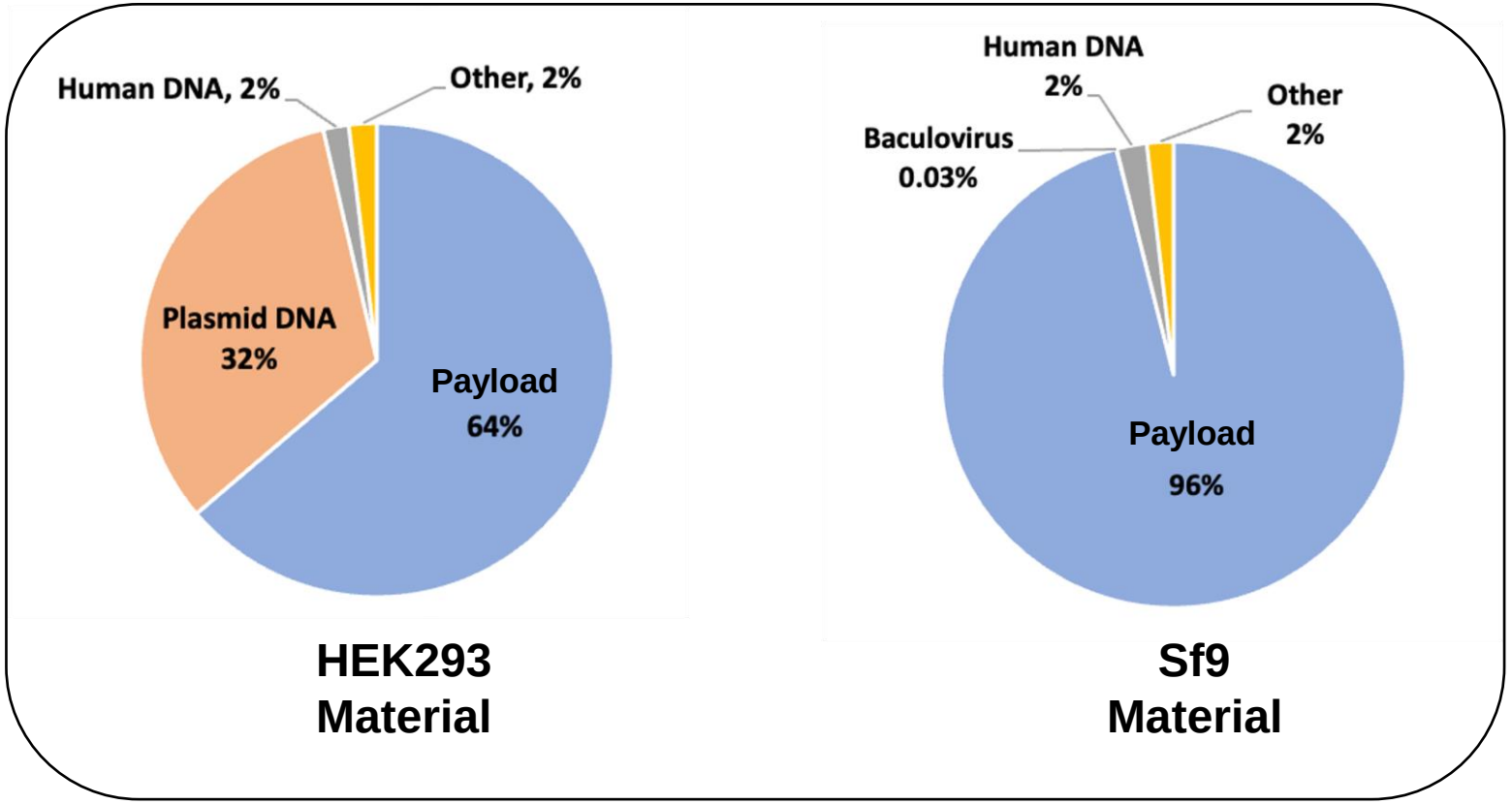


New platform: higher % Full, fewer Partials and Empty Capsids

***"No adverse impact"* on quality**

Quality:
Sf9 platform shows reduced residual packaged DNA

Next Generation Sequencing



Sf9 platform: Fewer DNA residual

***"No adverse impact"* on quality**

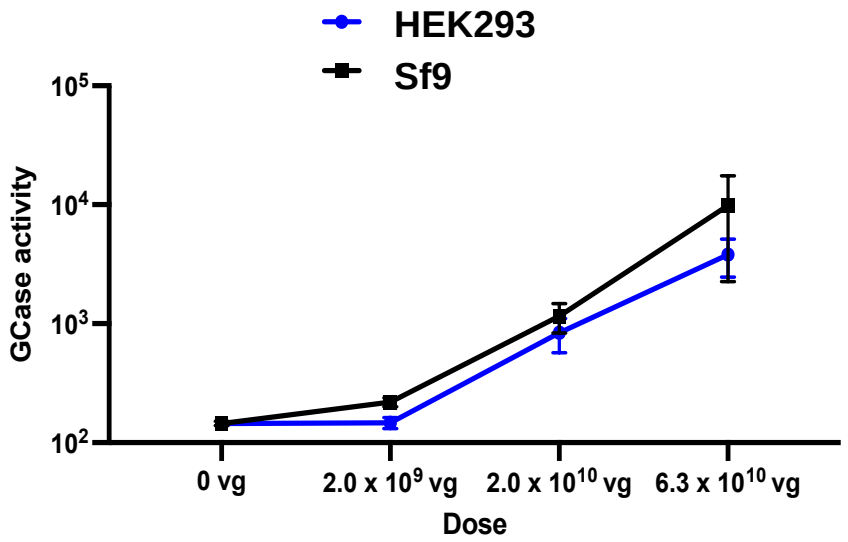
Efficacy:
Sf9 platform shows comparable efficacy

***In-vitro* Analytical Potency Assay**

Platform	Batch	Relative Potency
HEK293	Lot 1	153%
	Lot 2	143%
Sf9	Lot 3	142%
	Lot 4	93%
	Lot 5	113%

Assay variability 30% CV

***In-vivo* Cerebral Cortex GCase activity in the CBE Mouse Model**



No statistically difference between lots, highly similar in-vivo efficacy

Comparable efficacy

Safety:
Sf9 platform shows similar safety profile

CMC Analytics

Test	HEK Process	Sf9 Process
Sterility	No Growth	No Growth
Endotoxin	≤ 0.5 EU/mL	≤ 0.5 EU/mL
Mycoplasma	Not detected	Not Detected
In- vitro Adventitious virus	Not Detected	Not Detected
In-vivo Viral contaminants	NT	Not Detected
rcAAV (Replicative competent AAV)	Not Detected	Not Detected

Toxicology Study in NHPs

"No in-life or clinical or anatomic pathology findings related to the gene product were observed. Therefore, the dose levels were well-tolerated by male and female monkeys dosed via intracisternal injection to the cisterna magna."

Summary

- Both Sf9 and HEK293 platforms produced identical capsid proteins with comparable post-translational modifications, biological activity, and strength

- Sf9 platform presented fewer AAV partial viral particles and total DNA residual compared to HEK293 process

- Successfully transitioned clinical program from HEK -> Sf9 platforms

The baculovirus/Sf9 process delivers greater product yield with comparable efficacy and greater purity

Acknowledgments

- Jorge Haller
- Mary Ng
- Stuart Nelson
- Shital Kakkar
- Adnan Arnaout
- Ilan McNamara
- Kaavya Maganti
- David Litwack
- Mansuo Lu Shannon
- Franz Hefti
- Jingmin Zhou, Liz Higgins, Yong Dai, Tim Fenn