Process and Formulation Development for a Novel Blood-Brain Barrier Penetrant AAV Capsid

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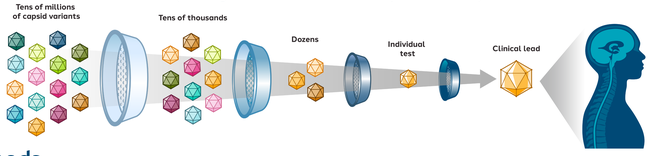
Poster #1052

Introduction

- Application of conventional AAV serotypes to central nervous system (CNS) diseases has been limited transport through the blood-brain barrier (BBB) to the brain.
- Capsid engineering approaches improve AAV delivery and tissue specificity including the potential to cross the BBB and deliver therapeutic cargo for CNS indications. However, these changes also necessitate optimization of the manufacturing process and formulation stabilization.
- Design and optimization of manufacturing processes that enable large-scale production and purification of AAV vectors is challenging, but critical to enable clinical studies and eventual commercialization.
- In this study, we report the development of a scalable process for the production and purification of a recently identified engineered AAV capsid with an exceptional ability to cross the BBB in nonhuman primates (known as Sangamo Therapeutic AAV Capsid – Blood Brain Barrier, or STAC-BBB). Furthermore, a stable formulation was developed to enable optimal shelf-life and DP presentation aligned with the target product profile for CNS indications.
- To learn more about other STAC-BBB related content, please visit our abstracts # 117 and # 1616.

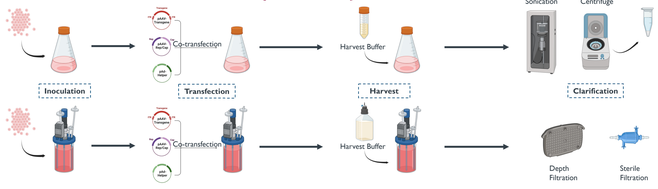
SIFTER Platform for Identification of Novel AAV Capsids

(Selecting In vivo for Transduction and Expression of RNA)



Methods

AAV Production in HEK293 Cell Suspension System



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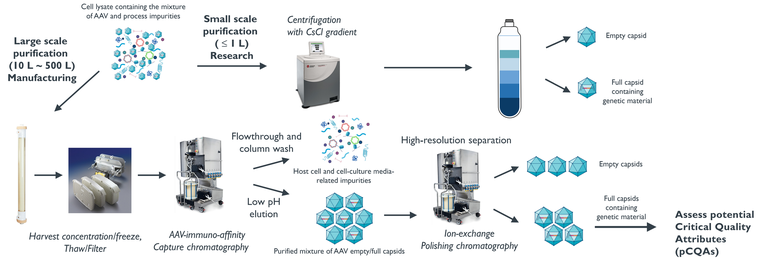
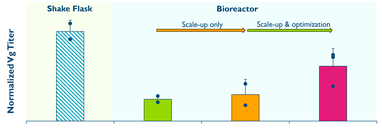
AAV Downstream Purification



Results

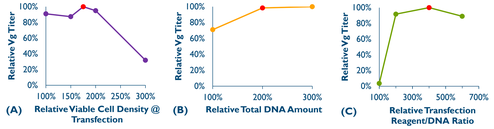
Productions at Various Scales



Comparison of average clarified harvest vg titer from productions at various scales: 40 mL, 3 L, 40 L and 50 L for engineered AAV capsid STAC-BBB.

- STAC-BBB production in shake flask vs. bioreactor have different productivity performance.
- A scalable production process in bioreactors was optimized for novel AAV capsid STAC-BBB with improved yields during scale-up.

Upstream Process Optimization



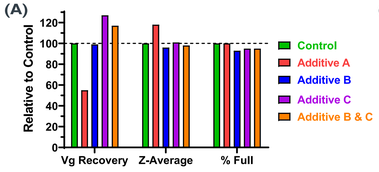
The impact on production yield by varying in-process parameters **(A)** viable cell density at transfection **(B)** total DNA plasmid amount and **(C)** ratio of transfection reagent over total DNA amount at 40 mL scale in shake flask production.

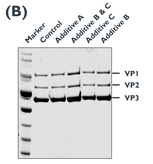
- Critical process parameters (CPP) optimized: viable cell density at transfection, total DNA amount, and ratio of transfection reagent over total DNA amount.
- Data shown here denotes the relative value of each parameter vs. the relative average Vg titer (N=2).
- The CPPs marked as RED were selected in upstream to produce materials for further downstream optimization.

Capture Chromatography Optimization

Clarified Harvest	Affinity Resin	Wash	Elution
STAC-BBB	POROS CaptureSelect	Tris, NaCl, pH 8.0	Buffer Control (pH 3)
			+ Additive A
			+ Additive B
			+ Additive C
			+ Additive B & C

Elution Condition	VP1:VP2:VP3 (by SDS-PAGE)	Purity (by SDS-PAGE)	PDI/Aggregation (by DLS)
Control	1: 1.1 : 11.8	100%	0.13 / No
+ Additive A	1: 1.1 : 10.5	100%	0.33 / Yes
+ Additive B	1: 1.1 : 9.3	100%	0.10 / No
+ Additive C	1: 1.1 : 10.3	100%	0.11 / No
+ Additive B & C	1: 1.1 : 9.2	100%	0.09 / No





Quality attributes measured by various assays.

Vector genome (vg) recovery by qPCR, Z-average by DLS, Full capsid % by SEC-MALS **(A)** and Inter-viral protein ratio and purity by SDS-PAGE **(B)** for the capture eluates are shown.

Polish Chromatography Optimization

Column Screening

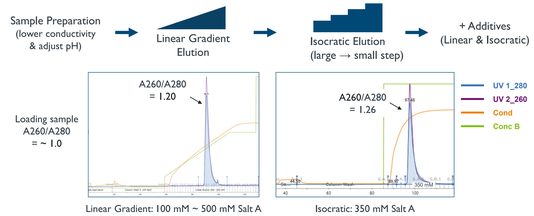
Columns	Binding	Elution	Full capsid Enrichment (A260/A280)
POROS 50 HQ	OK	OK	No
POROS XS	OK	OK	No
CIMmultus QA	OK	OK	Yes
CIMmultus SO3	OK	OK	No

Downstream Process Optimization

Optimization Parameters

- Type of Salt: Salt A, Salt B
- Conductivity for E/F separation
- pH: 7.0 ~ 10.0
- Additives: Stabilizers, Divalent ions

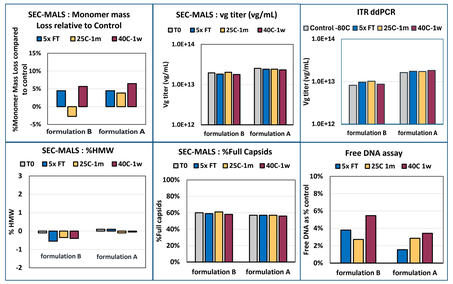
→ Full capsid enrichment monitored by A260/A280 and SEC-MALS



Potential Critical Quality Attributes

CQAs	Assay	STAC-BBB
Vg Recovery	ddPCR	Capture: > 70%
		Polish: > 70%
Purity	SDS-PAGE	VP1:VP2:VP3 1 : 1.1 : 10.3
		Purity: 100%
	SEC	< 1% aggregation
	DLS (PDI/Aggregation)	0.12 / No
Empty/Full	SEC-MAL	> 70% Full capsid
Potency	ZFR Expression and Target Gene Repression	≥ Shake flask material

Formulation Development



Conclusion

- We have successfully developed a robust and scalable production process (up to 50 L) for a novel Blood-Brain Barrier penetrant AAV capsid (STAC-BBB).
- Process and formulation parameters were evaluated and optimized for high product yield, E/F ration and DP stability.
- The column chromatography purification method optimized is scalable to the target manufacturing scale.
- The purified AAV capsids showed acceptable yield, purity, and infectivity.
- Formulation optimization enhanced the stability profile of STAC-BBB capsid. Two lead formulations were identified and demonstrated to be stable over multiple Freeze/thaw, accelerated and stressed stability.

Acknowledgments

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Disclosures

*These authors contributed equally

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