Evolution of blood-brain barrier penetrant AAV capsids in non-human primates using a multiplexed transcription dependent capsid engineering platform

Abstract #1203

Matthew Tiffany, Stephanye Frias, Lori Andrews, Ankitha Nanjaraj, Russell Darst, Yuri Bendaña, Alex Ward, Kenneth Kennard, Sarah Meuller, David S. Ojala, Amy M. Pooler

Sangamo Therapeutics, 7000 Marina Blvd, Brisbane, CA

Introduction

- The clinical translation of genomic medicines to treat disorders of the central nervous system (CNS) has been limited by inefficient gene delivery.
- AAV capsids that cross the blood brain barrier (BBB) in rodents exhibit widespread CNS transduction and efficacy, however, capsids that cross the BBB in non-human primates have been challenging to engineer.
- We previously conducted a first-round SIFTER (Selecting In vivo for Transduction and Expression of RNA) library selection that identified ~65,000 AAV variants that exhibited enrichment in CNS tissue following intravenous administration in cynomolgus macaques.
- A second-round library was created that multiplexed four parental AAV serotypes creating five sub-libraries (Library A-E). The barcoded library transcript is expressed under the control of a neuron specific Human synapasin1 promoter or a ubiquitous CMV promoter.
- The library was administered to two cynomolgus macaques and barcodes were recovered from CNS and peripheral tissues to assess biodistribution and expression of the library transcript.
- A total of ~14,000 variants were recovered and 1236 variants that exhibited significant enrichment in CNS have been selected for evaluation in a final in vivo selection.

SIFTER Platform

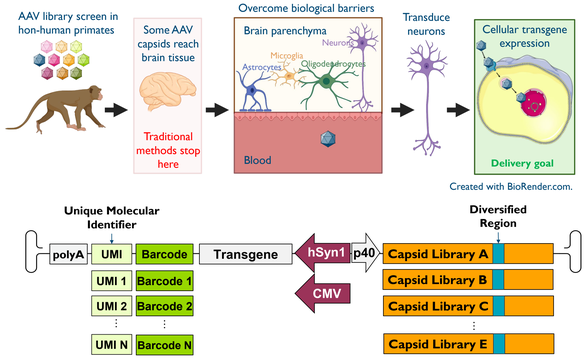
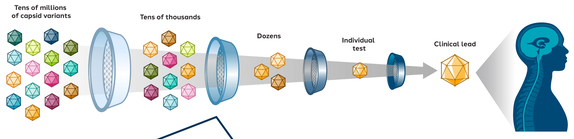


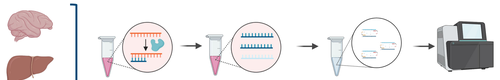
Figure 1. Each capsid is linked to multiple barcodes defined in a look-up table and created by oligo pool synthesis. Different oligo pool designs allow for multiplexing libraries varying parental capsid identity or mutational strategy. Hundreds to thousands of unique molecular identifiers (UMIs) are cloned per barcode enabling detection of individual transduction events. Barcodes and UMIs are expressed under the control of either a neuron specific hSynapsin1 or ubiquitous CMV promoter allowing the read out of capsid performance in multiple cell types.

Methods



Round 2 selection

10 SIFTER libraries were constructed and injected into 2 animals per group. Total RNA was harvested from coronal brain slices and other tissues (e.g., liver) and converted to cDNA. Barcodes were amplified by polymerase chain reaction (PCR) and sequenced. The capsid variant was bioinformatically determined via a predetermined barcode–variant lookup table.



Library	Diversity	Promoter	RoA	Endpoint analysis
Library A	1412	hSyn1 and CMV	IV infusion	• Total RNA extraction from coronal brain slices and select peripheral tissues • Reverse transcription of purified RNA • Barcode PCR amplification and NGS
Library B	1114			
Library C	2888			
Library D	1345			
Library E	62006			

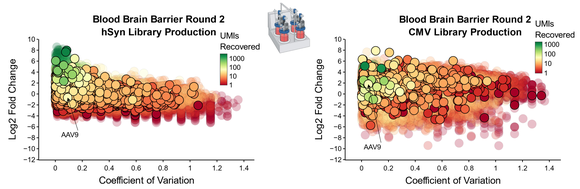


Figure 2. Library production.

The production of the libraries is relatively uniform with most variants found ± 32-fold change from the cloned library. As expected, variants that produce well are associated with more UMIs recovered. Bubbles circled in black outlines are variants that were selected for a third-round evaluation from NHP tissue samples. The position of AAV9 in the library is highlighted.

Results

In vitro library characterization in human iPSC-derived neurons

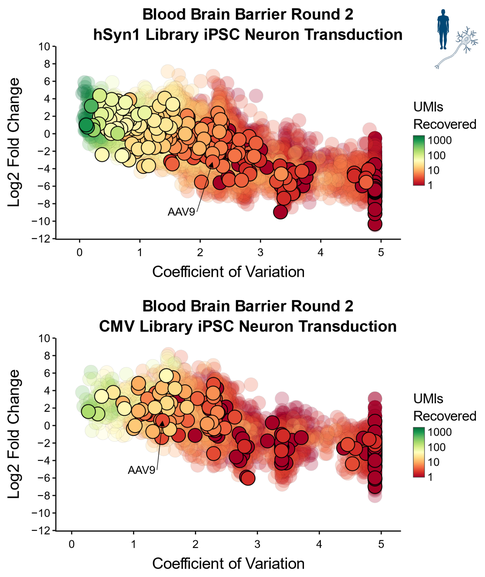


Figure 3. In vitro iPS neuron transduction.

Overall, the performance of the library in vitro is not predictive of the performance in vivo as many recovered variants from the NHP samples are less enriched in cultured neurons.

In vivo library recovery from cynomolgus macaque CNS

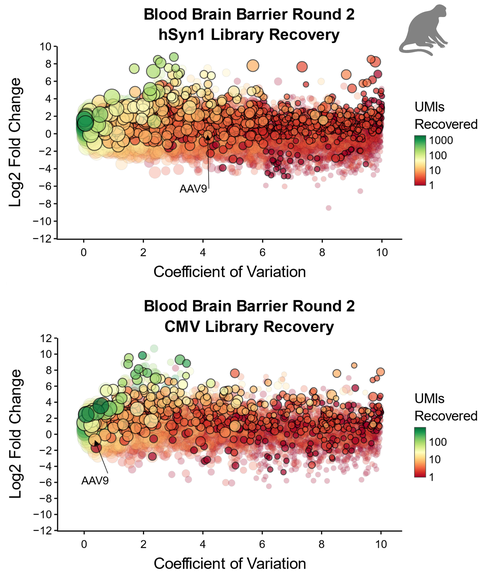


Figure 4. Library recovery from NHP samples.

After filtering out variants that were poorly recovered, we identified a total of 6728 variants from the hSyn1 library, and 4005 variants from the CMV library.

Based upon consistency in recovery across animals and fold enrichment we selected 810 variants from the hSyn1 library and 426 variants from the CMV library for a final evaluation in NHP.

Bubble plot legend

The graphs show the fold-change in variant enrichment normalized to the administered viral library on the y-axis and the coefficient of variation in the detection on the x-axis.

The bubble size corresponds to the fraction of replicates the variant was recovered from.

Lastly, the color scale indicates the number of unique molecular identifiers (UMI) associated with each variant.

The **top** graph is the variants' performance under the control of the neuron specific hSyn1 promoter and the **bottom** graph is under the control of the ubiquitous CMV promoter.

Bubbles circled in black outlines are variants that were selected for a third-round evaluation from NHP tissue samples.

Conclusion

- A library of ~65,000 variants each under the control of neuron specific hSyn1 promoter or a ubiquitous CMV promoter was administered to two NHPs.
- Library barcodes were recovered from cDNA reverse transcribed from total RNA extracted from CNS tissue.
- ~14,000 variants from a first-round SIFTER library were recovered in this second-round selection.
- A total of 1236 variants were selected for evaluation in a final selection to nominate lead capsids.
- Importantly, several of the variants exhibit substantial improvement over AAV9 in CNS transduction.

Presented at the ASGCT 26th Annual Meeting, 2023