Evaluation of a Human Neurovascular Model to Complement a Parallel Non-human Primate Selection for Blood–Brain Barrier Penetrant AAV Capsids

Poster #XXX

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Introduction

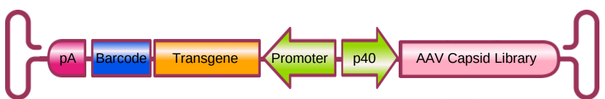
- Delivery of genomic medicine to the central nervous system (CNS) is a major hurdle for clinical applications of gene therapy; the blood–brain barrier (BBB) limits the brain distribution of virtually all intravenously administered macromolecules.
- Several adeno-associated virus (AAV) serotypes, most notably AAV9, distribute to the brain after intravenous (IV) administration but require high doses to achieve limited expression.
- AAV capsid engineering has produced novel variants that are superior to their parental serotypes and have progressed into the clinic for several indications. However, translation of clinical programs from preclinical models to humans remains a challenge for the entire gene therapy field, including capsid engineering efforts.
  - Two factors for a stringent selection campaign have emerged: library designs that incorporate functional cellular transduction pressure, and selection of appropriate in vitro and/or in vivo models.
- In this study, we employed SIFTER™ (Selecting In vivo For Transduction and Expression of RNA) to engineer capsids with improved CNS transduction following IV administration in *Cynomolgus macaque* (non-human primates [NHPs]). This was followed by implementation of an all-human cell model of the BBB that recapitulates many key BBB properties to address discordant capsid performance observed in vitro vs in vivo and between species.

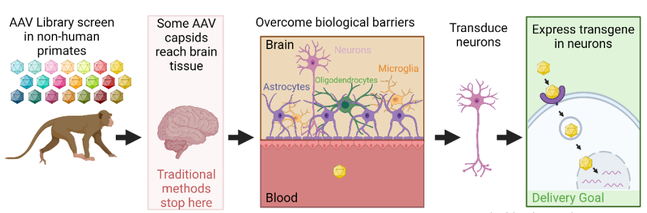
Methods

SIFTER™ platform

We have developed an AAV capsid discovery platform, SIFTER. The wild-type AAV genome is modified by replacing Rep with a bar-coded expression cassette and Cap with the capsid library. During production, Rep is provided in trans to facilitate library packaging. The vector cassette expresses a bar-coded transgene from a promoter of choice. By establishing a link between the bar code and the capsid variant, we are able to determine the performance of a given capsid variant by tracking the bar code via next-generation sequencing (NGS). This allows for functional RNA-based selection in vivo with cell type–specific expression driven by promoter choice.

SIFTER™ library design:





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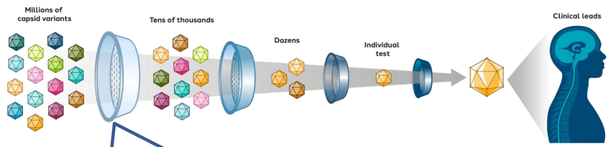
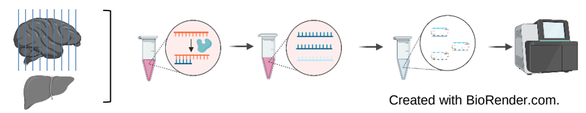
Results

NHP round 1 selection study



Round 1 selection

A total of 3 SIFTER libraries were constructed and injected into 2 animals* per group. Total RNA was harvested from brain tissue samples and converted to cDNA. Bar codes were amplified by polymerase chain reaction (PCR) from cDNA and sequence by NGS. The capsid variant was bioinformatically determined via a predetermined bar code–variant lookup table.



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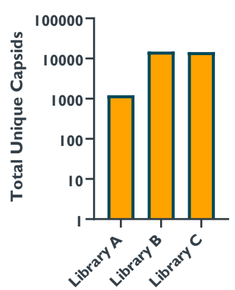
Groups (n=2)	Library	Diversity	RoA	Endpoint analysis
1	Library A	28,764	IV infusion	• Total RNA extraction from coronal brain slices and select peripheral tissue • Reverse transcription of purified RNA • Barcode PCR amplification and NGS
2	Library B	>1e8		
3	Library C	>2.5e7		

*All animals were prescreened and neutralizing antibody (nAb) negative for library parental serotype
RoA, route of administration

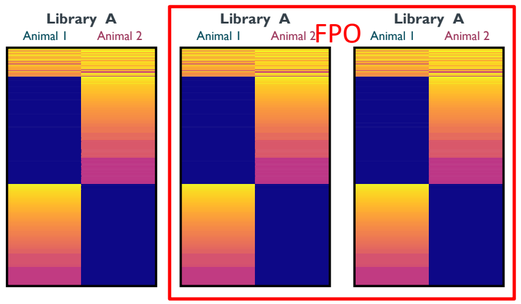
NHP round 1 selection results

In total, we discovered between thousands and tens of thousands of variants from each library. All variants will be recloned and taken forward into a second round of selection in NHPs.

Total Unique Capsids Recovered From Brain Samples



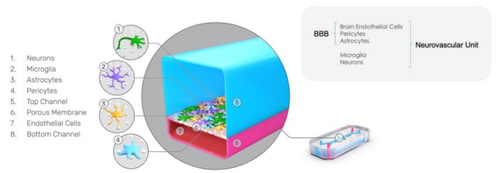
Capsid variants were mostly recovered from independent animals in the round 1 selection; however, between ~10% to 20% of all capsids discovered were independently recovered from both animals per group.



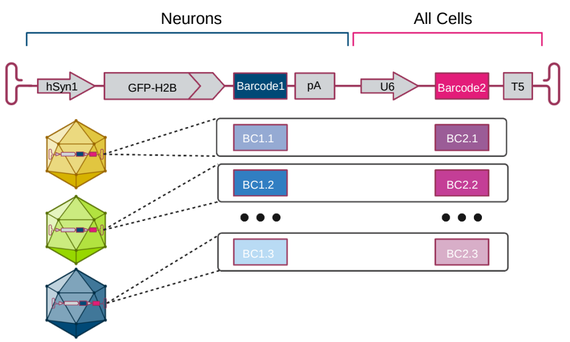
Overall, the level of recovery from the brain samples agrees with our understood stringency of the BBB. Based on input library diversity, the capsid variants recovered are 0.06%-4% depending on the library. In addition, the biological variability between animals also agrees with our expectations.

In vitro BBB pooled library evaluation

The model uses a microfluidic chip that is the most comprehensive in vitro model of the human neurovascular unit for preclinical research. There are 5 all-human cell types in a dynamic and tunable microenvironment.

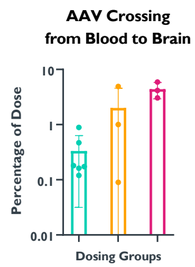


We created a pooled library composed of 8 parental AAV serotypes vectorized with a dual-expression cassette. Each parental received a unique combination of bar-coded transcripts.



Pooled evaluation of in vitro BBB model

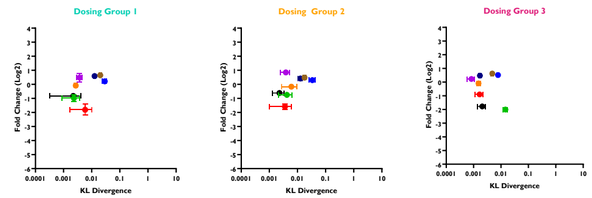
We detected between 0.5% and 4.4% of injected dose crossing the in vitro endothelial cell monolayer depending on dosing conditions. These data were replicated in 2 independent experiments for dosing group 1.



Dosing group 1 (n=6)
- Replicates from 2 independent experiments

Dosing group 2 (n=3)
Dosing group 3 (n=3)

We observed similar results, independent of dosing group, for the relative abundance of the parental serotypes included in the pool. There was no significant enrichment in any condition for an individual serotype, but there was selection against certain parental AAV serotypes. However, there was a strong selection for AAV parentals that were expressed in brain side cells (data not shown).



Conclusions

- We have optimized this system to assess distribution of AAV vectors and found that the levels of transcytosis across the endothelial monolayer are consistent with macromolecule distribution in NHPs (0.1%-0.2% of injected dose).
- Capsids arising from both in vitro and in vivo selection campaigns have the potential to provide intravenously administered CNS delivery solutions for a broad range of therapeutic indications.

References

SGMO US Patent 2020/0370137.

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Author disclosures

All authors are or were employees of Sangamo Therapeutics.

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