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

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Introduction

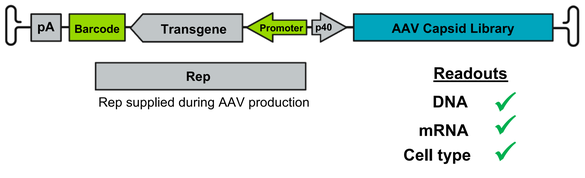
- Delivery of genomic medicine to the central nervous system (CNS) is a major hurdle for clinical applications of gene therapy; the blood–brain barrier (BBB) limits the brain distribution of virtually all intravenously administered macromolecules.
- Several adeno-associated virus (AAV) serotypes, most notably AAV9, distribute to the brain after intravenous (IV) administration but require high doses to achieve limited expression.
- AAV capsid engineering has produced novel capsids that are superior to their parental serotypes and have progressed into the clinic for several indications [1]. However, translation of clinical programs from preclinical models to humans remains a challenge for the entire gene therapy field, including capsid engineering efforts of the CNS.
  - 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*. In addition, we characterized the Emulate Brain-Chip that recapitulates many key BBB properties to address discordant capsid performance observed *in vitro* vs *in vivo* and between species.

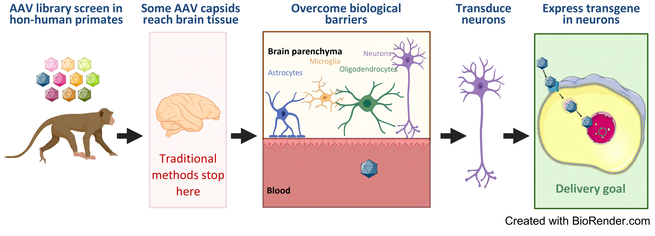
Methods

SIFTER™ platform

We have developed an AAV capsid discovery platform, SIFTER[2]. The wild-type AAV genome is modified by replacing Rep with a barcoded expression cassette and Cap with the capsid library. During production, Rep is provided *in trans* to facilitate library packaging. The vector cassette expresses a barcoded transgene from a promoter of choice. By establishing a link between the barcode and the capsid variant, we are able to determine the performance of a given capsid by tracking the barcode via next-generation sequencing (NGS). This allows for functional RNA-based selection *in vivo* with cell type–specific expression driven by promoter choice.

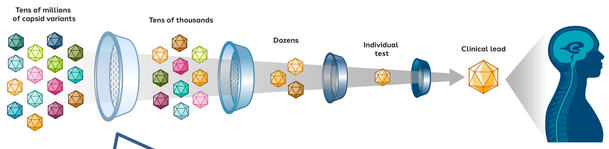
SIFTER™ library design:





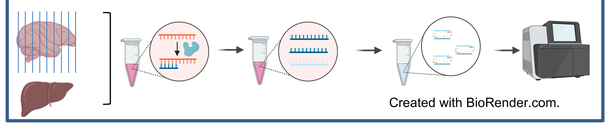
Results

NHP round 1 selection study



Round 1 selection

Three 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 sequence by NGS. The capsid variant was bioinformatically determined via a predetermined barcode–variant lookup table.



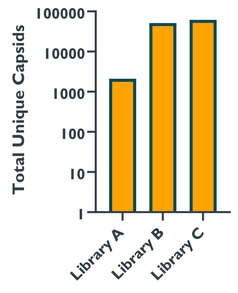
Groups (n=2)	Library	Diversity	RoA	Endpoint analysis
1	Library A	28,764	IV infusion	• Total RNA extraction from coronal brain slices and select peripheral tissues • Reverse transcription of purified RNA • Barcode PCR amplification and NGS
2	Library B	>1e8		
3	Library C	>2.5e7		

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

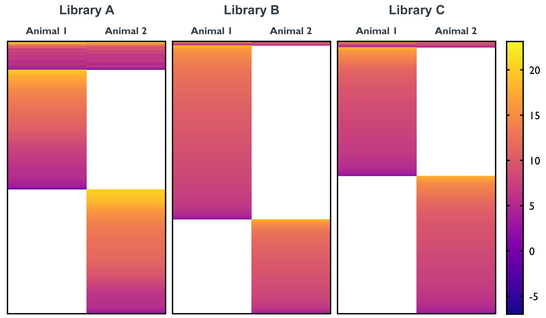
NHP round 1 selection results

In total, we discovered between thousands and tens of thousands of capsids from each library. Recovered capsids are being taken forward into a second round of selection in NHPs.

Total Unique Capsids Recovered From Brain Samples



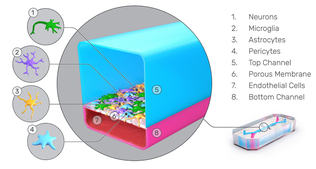
Capsids were mostly recovered from independent animals in the round 1 selection; however, between ~1% to ~20% of all capsids discovered were recovered from both animals per group. Below is the fold change (log2) of recovered capsids:



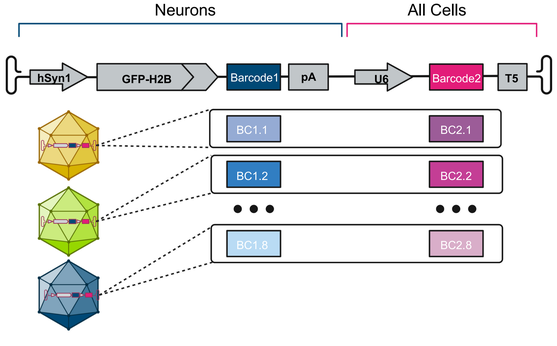
Overall, the level of recovery from the brain samples agrees with our understood stringency of the BBB. Based on input library diversity, the capsids recovered are ~0.1%-4% depending on the library. In addition, the biological variability between animals also agrees with our expectations.

In vitro BBB pooled library evaluation

For the *in vitro* assessment, we used the Emulate Brain-Chip, the most comprehensive *in vitro* model of the human neurovascular unit for preclinical research. This microfluidic Organ-on-a-Chip model contains 5 human cell types in a dynamic and tunable microenvironment.

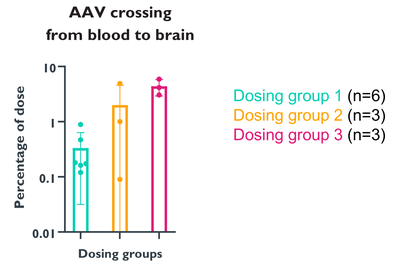


We created a pooled library composed of 8 parental AAV serotypes vectorized with a dual-expression cassette[2]. One barcode is expressed from the neuronal specific hSyn1 promoter, and a second barcode is expressed from the ubiquitous U6 promoter. Each parental serotype was manufactured with a unique combination of four barcode pairs.

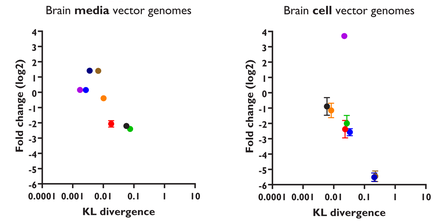


Pooled evaluation of *in vitro* BBB model

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.



We observed similar results, independent of dosing group, for the relative abundance of the parental serotypes included in the pool in the brain side channel. There were significant changes in the relative abundance of vector genomes recovered from both the media and cells. Below is the fold-change of each parental (colored circles) plotted against KL divergence, a measure of how consistent the fold enrichment is across each of the four barcode pairs.



Conclusions

- We optimized the Emulate Brain-Chip dosing conditions 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%-1% of injected dose). However, at higher doses there is a substantial increase in the amount of transcytosis.
- 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

1. Kuzmin, Dmitry A et al. The clinical landscape for AAV gene therapies. *Nature reviews. Drug discovery* vol. 20,3 (2021): 173-174. doi:10.1038/d41573-021-00017-7
2. US Patent Application Publication No. 2020/0370137

Acknowledgments

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Author disclosures

Sauveur Jeanty is an employee of Emulate Bio. All other authors are or were employees of Sangamo Therapeutics.

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