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

Matthew Tiffany, Stephanie Fras, Lori Andrews, Aniktha 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 expression in the CNS, but expression is transitory.
- We previously conducted a first-round SIFTER (Selecting In vivo for Transduction and Evolution of AAV) library selection that identified ~61,000 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 a sub-libraries (library A-E). The barcoded library transcript is expressed under the control of a neuron specific hSynapsin1 or ubiquitous CMV promoter.

Methods

- The library was administered to two cynomolgus macaques and barcodes were recovered from CNS tissue 48 to 72 hours post-injection.
- A total of ~14,000 variants were recovered and 1236 variants that exhibited significant enrichment in CNS tissue were selected for evaluation in a final selection.

Results

- In vitro library characterization in human iPSC-derived neurons
  - Library barcodes were recovered from cDNA reverse transcribed from total RNA extracted from CNS tissue.
  - Library diversity ranged from 1039 to 6728 variants from the hSyn1 library, and 4005 variants from the CMV library.
  - 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.

- In vivo library recovery from cynomolgus macaque CNS
  - 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.

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 CNS tissue following IV infusion.
- The library transcript.

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