Poster 507

Multiple generations of capsids engineered in NHPs yield improved performance in the central nervous system

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Abstract

The number of gene therapies in development that target indications in the central nervous system (CNS) has increased dramatically in recent years, driven by proof-of-concept studies established in the clinic. At the same time, the limitations of wildtype (WT) adeno-associated virus (AAV) serotypes to broadly and uniformly transduce cells throughout the CNS, particularly in subcortical and deep-brain structures, have been well documented.[1] Many indications that are amenable to genetic therapies benefit from transduction throughout the brain, which is difficult to achieve through conventional delivery methods. Engineered capsids capable of crossing the blood–brain barrier following intravenous (IV) delivery and broadly and efficiently transducing cells throughout the CNS have been previously reported,[2,3] but these variants have shown poor translatability to non-human primates (NHPs).

As such, we sought to utilize Capsida Biotherapeutics' novel, NHP-driven AAV engineering platform to develop recombinant AAVs capable of crossing the blood–brain barrier following IV administration. Capsida's platform was employed to select AAV9-based vectors with brain enrichment and transduction of cells throughout the CNS. In both a broad and deep search sequence space, variants spanning a range of improvements in CNS selectivity were discovered. As measured by vector genome residence, capsids were found to be greater than 25-fold improved over AAV9 across both juvenile and infant NHPs. Variants were tested additionally on induced pluripotent stem cell-derived neurons and demonstrated improved transduction *in vitro*. These candidates are being advanced with paired cargo into preclinical disease models for clinical development.

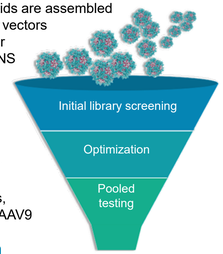
Introduction

- Local delivery to the CNS is an invasive approach
- Capsida has employed its capsid engineering platform to systematically improve AAV9 and AAV2-based vectors, with the objective of generating capsids that broadly transduce the primate CNS following IV administration
- Previous efforts in CNS AAV engineering have poor translatability from murine to primate models
- Capsida has employed multiple generations of engineering efforts in primates achieving improved penetrance with each subsequent generation

Methods

Initial library screening

- Billions of novel capsids are assembled into Capsida's library vectors and screened for their ability to reach the CNS
- 10s–100,000s of variants are put into a second round of screening where enrichment to input virus is measured for each engineered capsid with replicates, benchmarked to WT AAV9



Variant optimization

- Top identified variants have 10s–100,000s of mutations made to optimize their performance within their sequence
- Variants are again benchmarked to WT AAV9 and 10s are carried into pooled study characterization

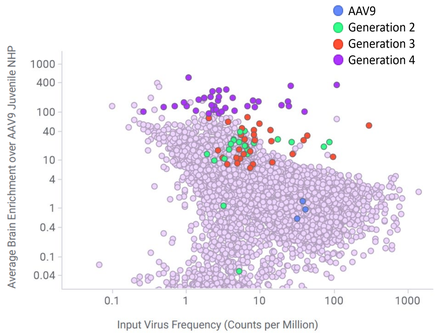
Pooled screening

- 10s of variants were injected, packaging a fluorescent protein (infants) or therapeutic gene of interest (juveniles) tagged with hemagglutinin (HA)
- Variant performance was measured by readouts of next generation sequencing (NGS) barcode replicates on the cargoes at the DNA level
- Pool performance was measured by DNA biodistribution and protein expression across the CNS

Results

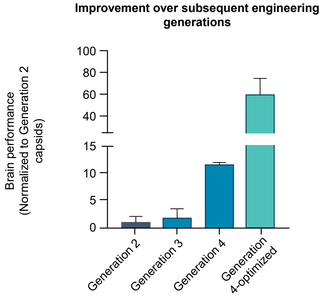
- Libraries screened in NHPs for increased CNS penetrance compared with AAV9 showed generational improvement using Capsida's platform (Figures 1 and 2)
- Improved variants from the latest round of engineering efforts in juveniles showed a >100× improvement in CNS penetrance over AAV9 in small pooled testing (Figure 3)
- Immunofluorescence imaging from the pooled screening study showed increased transduction even in a study with 20 capsids at a low dose (1.2 x 10^{12} vg/kg per capsid) over AAV9 (Figure 4A)
- Results were consistent in juvenile NHPs where a study screening the same capsid family found expression in cortical regions as well as particularly robust expression subcortically and in the cerebellum (Figure 4B)

Figure 1. Infant library data shows increasing CNS penetrance with engineered capsids over AAV9 parent capsid as evolution progresses



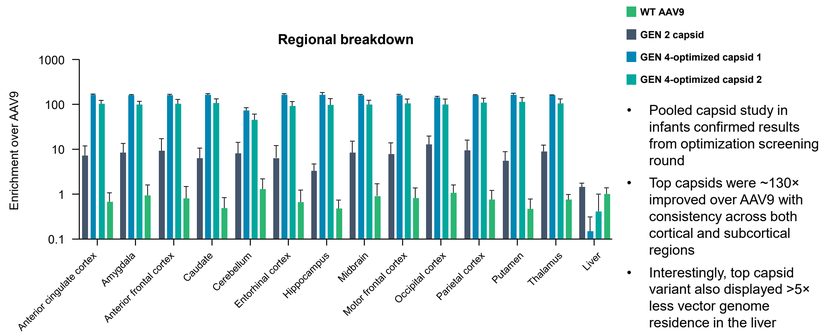
- Each subsequent generation of capsid engineering resulted in capsid families with improved peak enrichment over parent capsid (WT AAV9)
- Top variants identified in this initial library screening show potential for greater than 100× improvement over AAV9
- Two capsids representative of the generation 4 capsid family and sharing a single amino acid were carried forward for optimization

Figure 2. Optimization of generation 4 capsids resulted in continued improvement of capsids over previous capsid generations in juvenile NHPs



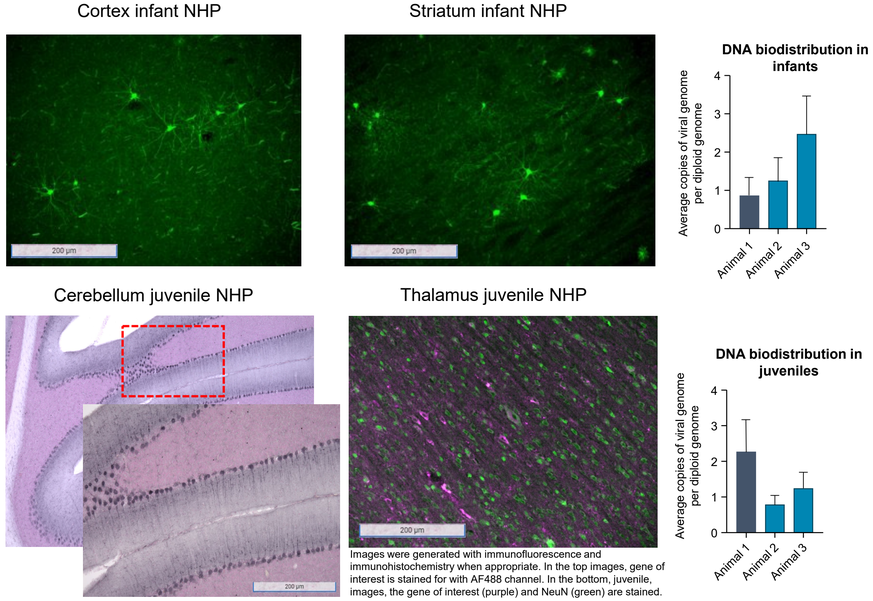
- Further modifications on top of generation 4 resulted in a >3× additional improvement of capsid penetrance in the CNS
- Top improved variants shared similar positional modifications across the generation 4 capsid family
- Improvements indicated a potential >40× improvement over Capsida generation 2 capsids

Figure 3. Infant pooled DNA NGS data shows potential >100× improvement in CNS penetrance over AAV9 parent capsid across cortical and subcortical regions



- Pooled capsid study in infants confirmed results from optimization screening round
- Top capsids were ~130× improved over AAV9 with consistency across both cortical and subcortical regions
- Interestingly, top capsid variant also displayed >5× less vector genome residence in the liver

Figure 4. Pooled study data indicates that variants identified in Capsida's platform show high DNA penetrance and broad expression in both infants and juvenile NHPs



Images were generated with immunofluorescence and immunohistochemistry when appropriate. In the top images, gene of interest is stained for with AF488 channel. In the bottom, juvenile, images, the gene of interest (purple) and NeuN (green) are stained.

Conclusions

- Capsida Biotherapeutics' platform is able to deliver robust improvements in DNA penetrance with IV administration of viral vectors to the CNS
- High penetrance shows corresponding improvements and widespread transduction of both infant and juvenile CNS, with corresponding protein expression
- Further validation of top variants will be used to achieve therapeutic targets for current unmet needs

Poster PDF

Video

Please scan this quick response (QR) code with your smartphone camera or app to obtain a copy of these materials. Alternatively, please click here to download the video.

References

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2. Deverman BE *et al. Nat Biotechnol* 2016;34:204–9
3. Chan KY *et al. Nat Neurosci* 2017;20:1172–79