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ASGCT 2026 ANNUAL MEETING ABSTRACTS

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Methods

To address this challenge, we employed directed evolution technology to generate AAV9 capsid variants with Nab resistance. Diverse AAV9 capsid libraries were constructed by replacing the amino acids in the surface-exposed variable regions that are critical for antibody recognition. The libraries were subjected to *in vitro* selection in the presence of intravenous immunoglobulin (IVIG), followed by next-generation sequencing (NGS) to identify enriched variants. Top candidates from each library were selected for individual AAV package with GFP reporter gene under the control of ubiquitous promoter. The mutations from different variable regions were also combined for potential syngeneic effects on resisting Nab. The selected capsid variants were subjected to *in vitro* and *in vivo* validation with IVIG.

Results

In vitro transduction in HEK293T and C2C12 cells demonstrated that some lead variants exhibited significant resistance to neutralization by IVIG. The combinations further improved the resistance. Notably, some variants showed enhanced transduction efficiency compared to the parental AAV9. In the presence of IVIG, the lead Nab-resistant candidates exhibited efficient muscle and liver transduction, while AAV9 showed no transduction in the mice after intravenous administration. Structural analysis revealed that the key amino acid mutations in the capsid variants altered the antigenic epitopes. The mutated epitopes could be adapted to our CNS-targeting RtAAV capsids or other AAV9 variants.

Conclusion

Collectively, our study provides a robust strategy for engineering Nab-resistant AAV9 vectors via directed evolution, which holds great potential for expanding the applicability of AAV9-based gene therapy to patients with preexisting anti-AAV neutralizing antibodies.

2025 An automated, high-throughput viral mRNA capture platform for in vivo AAV capsid-directed evolution

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Introduction Recombinant adeno-associated virus (rAAV) is a widely used gene therapy vector, and directed evolution has emerged as a powerful strategy to engineer capsid variants with enhanced tissue targeting. Accurate, quantitative assessment of capsid performance in vivo is essential for identifying superior variants. While viral mRNA–based screening provides a functionally integrated readout of transduction efficiency, its utility is limited by the low abundance of viral transcripts, especially in refractory organs and large-animal models. Most current approaches rely on oligo(dT)-based enrichment to capture viral transcripts, but these methods offer only modest sensitivity and limited scalability for high-throughput in vivo screening. To overcome these limitations, we developed an automated, scalable viral mRNA capture platform that enables sensitive detection of low-abundance viral transcripts through sequence-specific enrichment in a single-tube workflow, facilitating in vivo AAV capsid evolution.

Methods Total RNA was extracted from tissues following in vivo administration of AAV libraries and subjected to solution-phase hybridization with biotinylated DNA probes targeting conserved regions of AAV-derived transcripts. Probe–RNA complexes were captured using Dynabeads MyOne Streptavidin C1 (Thermo Fisher Scientific), enabling poly(A)-independent, sequence-specific enrichment under carefully optimized hybridization conditions. The workflow was fully automated on a KingFisher Flex platform (Thermo Fisher Scientific), allowing parallel processing of up to 96 samples and concentrating RNA from milligram- to gram-scale tissue inputs into a single reverse-transcription reaction, regardless of input scale.

Results

In mouse models screened with a peptide-inserted capsid library, this strategy achieved an average ~80-fold enrichment of viral mRNA in skeletal muscle and heart, as quantified by qPCR. In tissues with low transduction efficiency, such as femur and lung, the number of detectable viral sequences increased by >70-fold following enrichment, as determined by NGS. This dramatically improved the sensitivity for detecting low-abundance signals. Using a barcode library in mice, the enrichment method increased the number of detectable barcodes across all tested tissues (e.g., from 81 to 105 in kidney) and reduced inter-sample variability (mean coefficient of variation decreased from 42.6% to 33.5%). Capsid variant abundance profiles were highly correlated between enriched and non-enriched samples (Pearson $R > 0.95$), indicating that target signals were amplified while preserving biological fidelity. In non-human primates, the platform performed robustly on large tissue inputs (1–2 g), achieving several hundred- to over 1,000-fold enrichment of viral sequences in brain, lung, and bone. Finally, multiplexed enrichment using probes targeting the capsid, barcode, and epitope-tag libraries allowed for the simultaneous resolution of all three libraries, yielding sequencing read distributions that closely matched the predefined input ratios.

Conclusion We present an automated, sequence-specific viral mRNA capture platform that overcomes sensitivity and throughput limitations in in vivo AAV capsid screening. It enables detection of low-abundance viral transcripts across species and accelerates directed

evolution of therapeutic AAV capsids.

2026 Engineered AAV capsid achieves robust transduction in non-human primate central nervous system after low dose systemic administration

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Introduction

Adeno-associated viral (AAV) vectors have transformative potential for treating neurological disorders. However, limitations in effective coverage of the central nervous system (CNS) coupled with safety concerns due to high systemic exposure have hindered clinical translation. We previously reported discovery of a novel AAV capsid ATC-134 that achieves widespread CNS coverage and durable expression after intravenous (IV) dosing in adult cynomolgus macaque non-human primates (NHPs).

Methods

ATC-134 vectors packaging a miRNA (miR) cargo against Atxn2 gene (CBh-miR), or hemagglutinin-tagged histone payload (CBh-H2B-HA), both driven by a ubiquitous chicken-beta hybrid (CBh) promoter were administered IV to adult cynomolgus macaque NHPs at 2e13 vg/kg, 3e13 vg/kg, or 6.7e13 vg/kg dose-levels. At 28- or 60-days post IV administration, NHPs were sacrificed for pharmacological and histological evaluations performed across multiple CNS regions including cortical, deep-brain and spinal cord anatomical locations.

Results

In NHPs treated with ATC-134-CBh-miR, a dose-dependent increase in vector genome (vg) biodistribution, RNA expression, Atxn2 RNA knockdown (KD), and neuronal transduction detected by in situ hybridization (ISH) was observed throughout CNS. Compared to historical data with AAV9 treated at similar doses, 50-fold increase in CNS transgene expression, with minimal transduction observed in liver and dorsal root ganglia was achieved with ATC-134. Overall, ATC-134 packaging a miR cargo demonstrated widespread CNS coverage and potent activity as measured by >50% neuronal transduction, and >50% Atxn2 KD respectively, at the dose of 6.7e13 vg/kg. Following successful validation with miR cargo, the transduction