Identification of high-performing ocular AAV capsids through directed engineering across intravitreal and suprachoroidal delivery routes

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Introduction

Efficient and broad distribution of adeno-associated virus (AAV) vectors to the retina is constrained by biological barriers, including the inner limiting membrane, blood retina barrier, and route-dependent biodistribution. These challenges affect both intravitreal (IVT) and suprachoroidal (SCS) administration and contribute to variable retinal gene expression in translational and clinical contexts. In addition, current ocular gene therapies may require high vector doses to overcome these barriers, increasing exposure to viral DNA and the risk of inflammatory responses. Capsid optimization is therefore critical to improve retinal transduction while potentially reducing vector dose and associated safety risks. Capsida's engineering platform was applied to generate and evaluate novel AAV variants designed to enhance tissue penetration, distribution, and expression across ocular compartments.

Methods and Materials

Capsid engineering was initiated from two parental serotypes, AAV2 and AAV8, and performed across IVT and SCS routes of administration. Diverse capsid libraries composed of several million variants were evaluated across two rounds of in vivo selection in non-human primates. Variant performance at the library stage was assessed by determining RNA enrichment across multiple ocular tissues, animals, and codon replicates. Top-ranked variants were advanced to pooled testing, where DNA and RNA enrichment were compared with wild-type serotypes and published engineered capsids.

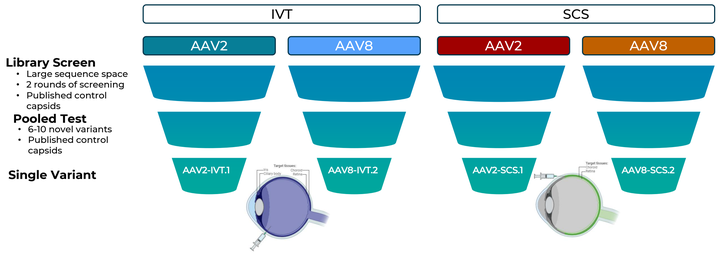
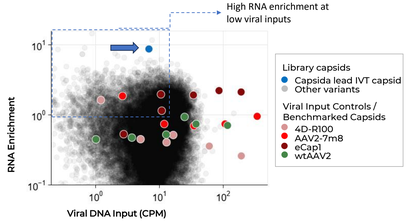


Figure 1. Four parallel paths to identify top performing novel capsids through directed evolution.



Variant selection is optimized to lower efficacious dose

- AAV2 library dosed IVT in NHPs (retina tissue)
- Platform selects for variants with high RNA enrichment at low viral inputs to decrease viral load
- **Lead IVT capsid is ~10x more enriched than clinical capsids in low range of viral DNA input**

Figure 2. Engineering strategy is designed to enable robust expression at lower doses by selecting variants with high RNA enrichment at lower viral inputs, optimizing safety and efficacy.

Results and Discussion

Following a single round of engineering via IVT administration, a novel capsid was identified with best-in-class performance, exceeding that of clinical and published control capsids in target retinal tissues. Capsids engineered through SCS administration also demonstrated improved performance relative to their wild-type serotype control. Pool-stage testing validated library-stage selection, with novel variants showing superior enrichment compared with all published capsids evaluated. Lead capsids demonstrated robust RNA expression in the retinal pigment epithelium/choroid and retina (>10^6 WPRE copies/µg RNA) across all evaluated conditions. The lead IVT capsid exhibited retinal RNA enrichment of up to 100-fold relative to AAV2 and previously reported engineered capsids. Novel capsids also displayed elevated RNA:DNA ratios, suggesting improved transcriptional efficiency with the potential for improved safety profiles compared to published capsids.

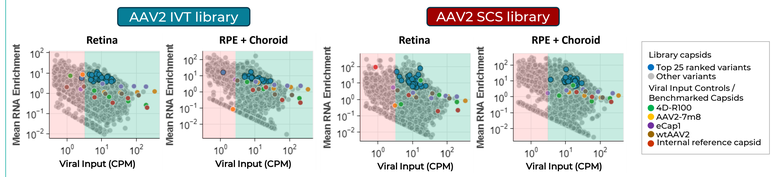


Figure 3. Proprietary ranking algorithm identifies highly enriched variants with reliable performance from library screening. Variability is assessed based on linearity of control capsids spiked in at multiple concentrations. Low variability regions shaded green; High variability regions shaded red.

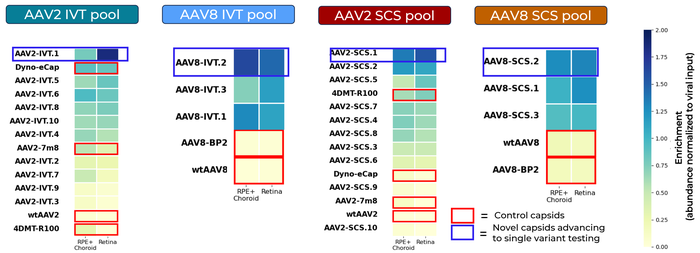
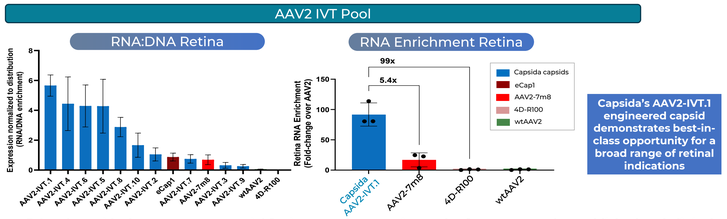


Figure 4. All novel capsids selected for single variant testing outrank clinical/published controls based on RNA enrichment in the pool.



Capsida's AAV2-IVT.1 engineered capsid demonstrates best-in-class opportunity for a broad range of retinal indications

Figure 5. Lead capsids demonstrate potential for improved expression and safety compared to published and clinical capsids.

Results and Discussion

Pool
Doses: IVT 1E11 vg/eye; SCS 3E11 vg/eye
3 week in-life

Single Variant
Doses: IVT 3E10 vg/eye; SCS 1E11 vg/eye
4 week in-life

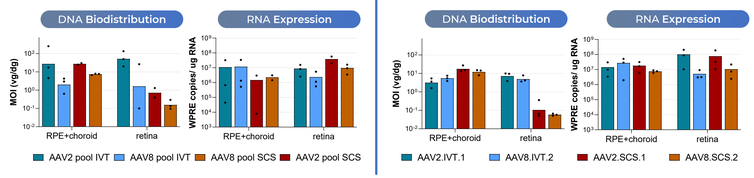


Figure 6. Lead capsids have robust biodistribution to all target tissues in single variant study dosed at a half log lower than the pool.

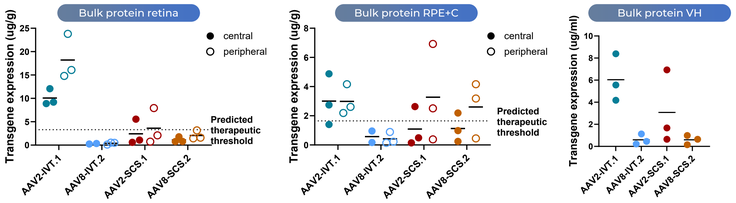


Figure 7. Lead IVT capsid achieves pan-retinal secreted protein expression levels that exceed predicted therapeutic threshold based on pre-clinical model in all target tissues.

Conclusions

Directed capsid engineering across IVT and SCS delivery routes enabled the identification of novel ocular AAV capsids with substantially improved retinal distribution and expression compared with existing serotypes and published/clinical capsids. These data support the translational potential of engineered capsids to overcome biological barriers to ocular gene delivery and enable more efficient and broadly applicable retinal gene therapy approaches.

Best-in-class novel variants enable development in large ocular indications and inherited retinal diseases

Capsida Engineered Capsids		Disease Opportunity	Development Path
Retina	AAV2-IVT.1	Biofactory for a broad range of retinal diseases	Best-in-Class capsid ready for Development Candidate study
		Glaucoma, Diabetic Retinopathy	Lead optimization study to characterize cell-type specificity
RPE Choroid	AAV2-SCS.2 AAV8-SCS.2 AAV2-IVT.4 AAV2-IVT.6	Geographic Atrophy, Retinitis Pigmentosa, Leber Congenital Amaurosis	Best-in-class opportunity following single round of re-diversification library engineering study

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