Engineering Novel AAV Capsids for Cardiac Gene Delivery

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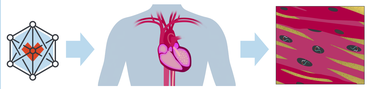
Introduction

Gene therapy is an emerging treatment option for both acquired and inherited cardiac disorders. While certain known adeno-associated virus (AAV) serotypes can achieve moderate transduction of the heart, the requirement of high doses and the substantial viral load to the liver or off-target cell types raise the critical need for novel AAV capsids with improved properties. We have established an in-house AAV capsid engineering platform and successfully screened over 30 diverse, proprietary AAV libraries (rational design, peptide insertion, variable region, chimeric, scanning, etc.) representing more than one billion unique capsids in multiple *in vitro*, *in vivo*, and *in silico* models to discover novel AAV capsids that can target the different types of cells in the heart through different routes of administration. Our gene therapy programs require efficient cardiomyocyte targeting via systemic administration of therapeutic DNA encapsulated in AAV vectors and this poster presents our efforts to identify optimal AAV capsids for this application.



GENE THERAPY:

Deliver healthy copies of genes or other therapeutic payloads to cardiomyocytes through systemic administration.

Figure 1. Tenaya AAV-based gene therapy platform. Tenaya utilizes AAV vectors for delivery of therapeutic genes to the heart in its gene therapy programs. Gene therapy targets patients with defective heart cells (e.g., due to mutations) and restores cell function by using viral vectors to deliver healthy copies of genes or other therapeutic payloads. The ideal AAV capsid for this approach should transduce cardiomyocytes efficiently and have reduced off-target trafficking (e.g., to the liver) following systemic administration.

Targeting Cardiomyocytes by AAV

Targeting cardiomyocytes following systemic delivery is critical to enable gene therapy treatments for many cardiac conditions. Results from clinical trials and preclinical animal models suggest that AAV9 transduces the heart well. AAVrh.10 and AAVrh.74 are muscle tropic serotypes that have been hypothesized to be suited to cardiac gene therapy programs. We set out to compare the cardiomyocyte transduction properties of AAV serotypes AAV9, AAVrh.10, and AAVrh.74 following systemic administration. Our data suggest that AAV9 transduces cardiomyocytes *in vivo* more efficiently than AAVrh.10 and AAVrh.74.

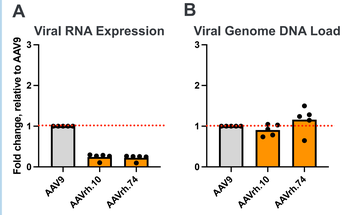


Figure 2. AAV9 transduces cardiomyocytes *in vivo* more efficiently than AAVrh.10 and AAVrh.74. We performed an NGS-based pooled comparison of these serotypes in mouse and quantified their transgene RNA expression levels from a cardiomyocyte-specific reporter cassette and viral genome DNA levels in the heart following systemic administration. Although all three serotypes show similar viral genome DNA load (B) in the heart, AAV9 generates higher level of RNA transcripts (A) than the other two serotypes, suggesting more efficient functional transduction of cardiomyocytes.

To identify novel AAV capsids that transduce cardiomyocytes more efficiently than AAV9, we performed our 1st-generation capsid engineering studies by combining directed evolution and candidate validation in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), mice, and non-human primates (NHPs) (data presented at ASGCT 2020 and 2022 meetings). We identified a proprietary capsid, TNC-CM3, that has superior heart transduction efficiency compared to AAV9. Importantly, TNC-CM3 shows consistent performance across difference species.

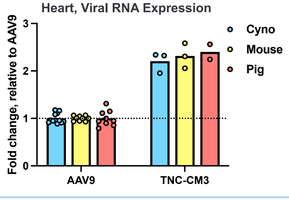


Figure 3. Tenaya's 1st-generation cardiomyocyte-targeting novel capsid shows superior heart transduction efficiency in all three species tested. TNC-CM3 was identified in our 1st-generation capsid engineering efforts. When compared head-to-head against AAV9, TNC-CM3 shows improved heart transduction in Cynomolgus monkey (Cyno) following systemic delivery. More importantly, TNC-CM3 shows consistent performance in all three species tested, suggesting high likelihood of having benefits in humans.

Novel Capsids with Reduced Liver Tropism

In our 1st-generation capsid engineering work, we noticed significant species-to-species differences in heart and liver transduction, as well as *in vivo* versus *in vitro* differences. Many novel capsid candidates identified in mice and hiPSC-CMs failed to perform as expected in NHPs. To exclude these confounding factors and identify additional novel capsids for cardiac gene delivery in humans, we designed our 2nd-generation capsid engineering strategy in which the directed evolution was performed solely in NHPs, the *in vivo* model that is evolutionarily closest to humans.

Results from clinical trials and preclinical animal models suggest that AAV9 transduces the heart well, however, a large amount of AAV9 viral particles infect the liver after systemic administration, potentially being the cause of dose-limiting toxicities. We specifically looked for novel capsids that have significantly reduced liver tropism, while maintaining at least comparable heart transduction to AAV9.

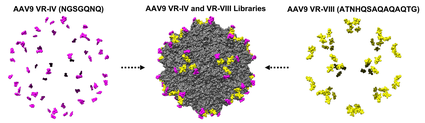


Figure 4. Modifying VR-IV and VR-VIII of AAV9 capsid. Variable regions VR-IV and VR-VIII locate at the three-fold protrusion and have been linked to transduction properties of AAV9 by our internal data, as well as in the literature. We introduced various modifications into these regions.

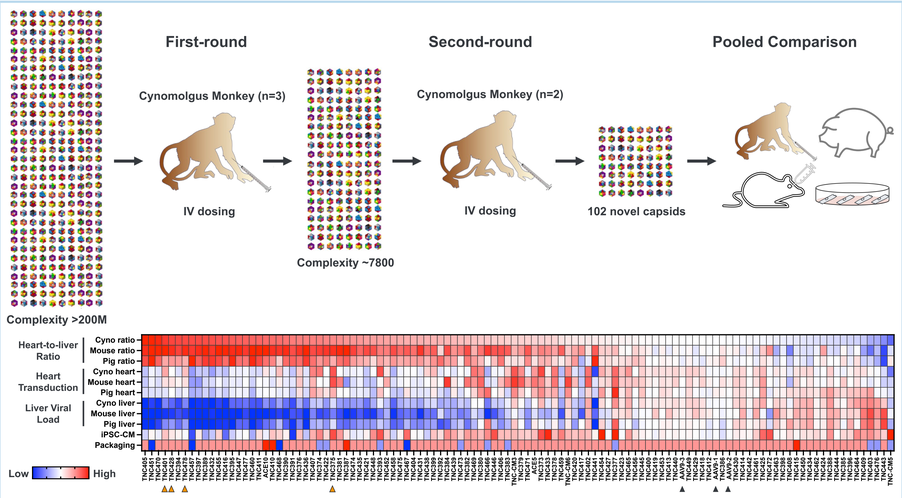


Figure 5. Directed evolution in NHPs and candidate comparison in multiple models. We started with more than 200 million variants in the initial library and performed two rounds of directed evolution in NHPs. We identified 102 novel AAV capsids that outperformed AAV9 in our 2nd-round NHP screen, based on heart transduction, liver viral load, and consistency between synonymous codon replicates. To better characterize the performance of our novel capsids and identify the best candidates to move forward, we performed a pooled capsid comparison study in which over 100 capsids were tested in parallel in multiple models enabled by NGS-based barcoding.

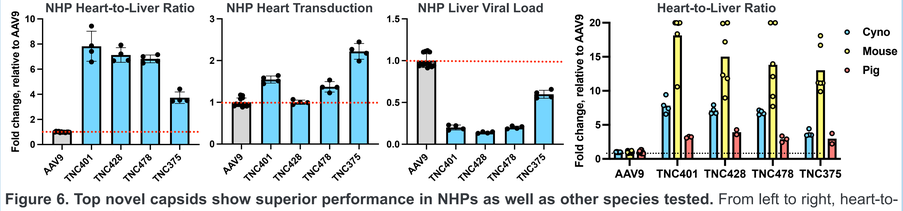


Figure 6. Top novel capsids show superior performance in NHPs as well as other species tested. From left to right, heart-to-liver ratio, heart transduction, and liver viral load data measured in Cyno are shown for the top four novel capsids as well as wildtype AAV9 control. The right panel plots heart-to-liver ratios measured in Cyno, mouse, and pig.

Validation of Liver-Detargeting in NHPs

To evaluate the potential clinical advantages of our novel capsids, we scaled up the production of our top novel capsid candidates with a clinically relevant manufacturing platform and administrated them individually to NHPs at a clinically relevant dose.

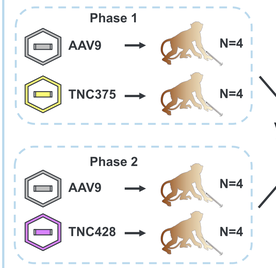


Figure 7. Validating liver-detargeting novel capsids in NHPs. TNC375, TNC428, and AAV9 encapsulating cardiomyocyte-specific reporter cassette were systemically administrated to Cyno Monkeys (4 animals per group) at 5.4E+13 vg/kg dose. At 4-week post-injection, animals were sacrificed and tissue samples were collected. Viral transgene RNA expression and viral genome DNA load in heart and liver were measured by RT-qPCR and qPCR.

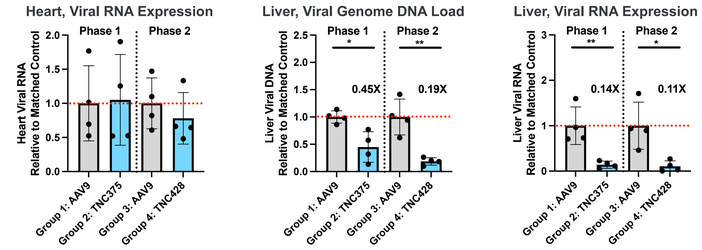


Figure 8. TNC375 and TNC428 show reduced liver tropism while efficiently transduce the heart in NHPs. From left to right, viral RNA transgene expression levels in the heart, viral genome DNA levels in the liver, and viral RNA transgene expression levels in the liver are shown. Each individual dot represent one animal. The measurements of novel capsid groups were normalized to matched AAV9 control groups. Both TNC375 and TNC428 show significantly reduced viral load and transgene expression in the liver, while maintaining comparable heart transduction efficiency to AAV9.

Conclusions and Future Directions

Through directed evolution and capsid comparison studies in multiple models, we identified several novel AAV capsids with superior properties to the industry standard, AAV9, for cardiomyocyte-targeted gene delivery with systemic administration. These novel capsids have the following advantages:

- Superior cardiomyocyte transduction efficiency, which may enable more efficacious therapy and/or allows lowering the dosage without compromising the efficacy.
- Superior heart-to-liver ratio, which potentially improves the safety profile of gene therapy drugs and reduces adverse events in treated patients.

We are performing a next-generation capsid engineering study which includes more diverse starting serotypes/variants, utilizes the insights from the first two generations of efforts, and makes extensive use of robust external machine learning capabilities to help us generate new variants and prioritize our hits at different stages in the screening process.

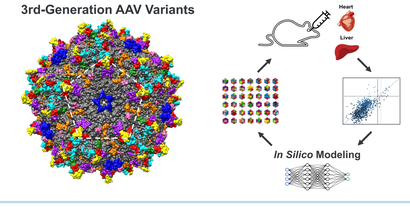


Figure 9. Tenaya 3rd-generation capsid engineering efforts. We are exploring additional regions on the parental capsids and introducing larger numbers of modified amino acids compared to previous efforts. To overcome the limitations of transitional random library approach, we are applying *in silico* modeling to capsid engineering for iterative generation of new candidates that potentially carry desired properties.