Engineering Novel AAV Capsids for Cardiac Gene Delivery

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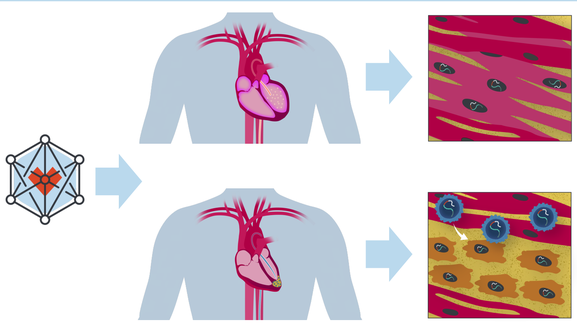
Introduction

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.



GENE THERAPY:

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

CELLULAR REGENERATION:

Deliver proprietary combinations of genes (reprogramming/regeneration factors) to cardiac fibroblasts through direct injection.

Figure 1. Tenaya AAV-based platform approaches. Tenaya utilizes AAV vectors for delivery of therapeutic genes to the heart in its gene therapy and cellular regeneration 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. Cellular regeneration targets patients with permanent heart cell loss (e.g., due to heart attack) and induces the formation of new heart cells *in vivo* by using viral vectors to deliver proprietary combinations of genes. The ideal AAV capsid for direct reprogramming-based approach should efficiently transduce cardiac fibroblasts after direct injection to the myocardium, where most of the surface area and tissue mass are occupied by cardiomyocytes.

We have developed unique insights through our studies across different AAV serotypes and discovered that distinct parental serotypes should be used as the starting point for capsid engineering efforts for these two applications. This poster presents our efforts to target cardiomyocytes via systemic delivery.

Directed Evolution in NHPs

Targeting cardiomyocytes following systemic delivery is critical to enable gene therapy treatments for many cardiac conditions and AAV9 has become the workhorse capsid for this type of gene transfer. In our earlier capsid engineering work (1st-generation, data presented at ASGCT 2020 and 2022 meetings), 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 human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) failed to perform as expected in non-human primates (NHPs). To exclude these confounding factors and identify novel capsids for cardiac gene delivery in humans, we designed our 2nd-generation capsid engineering strategy in which the directed evolution were performed solely in NHPs, the *in vivo* model that is evolutionarily closest to humans.

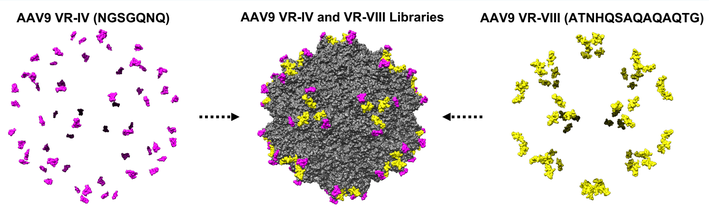


Figure 2. Modifying VR-IV and VR-VIII of AAV9 capsid. Variable regions VR-IV and VR-VIII locate at the three-fold protrusion and have been linked to transduction properties of AAV9 by our internal data, as well as in the literature. We introduced various modifications into these regions, including random 6- or 7-amino-acid mutations, single amino acid substitutions, double-amino-acid insertions, NXN substitutions, and machine learning designed sequences.

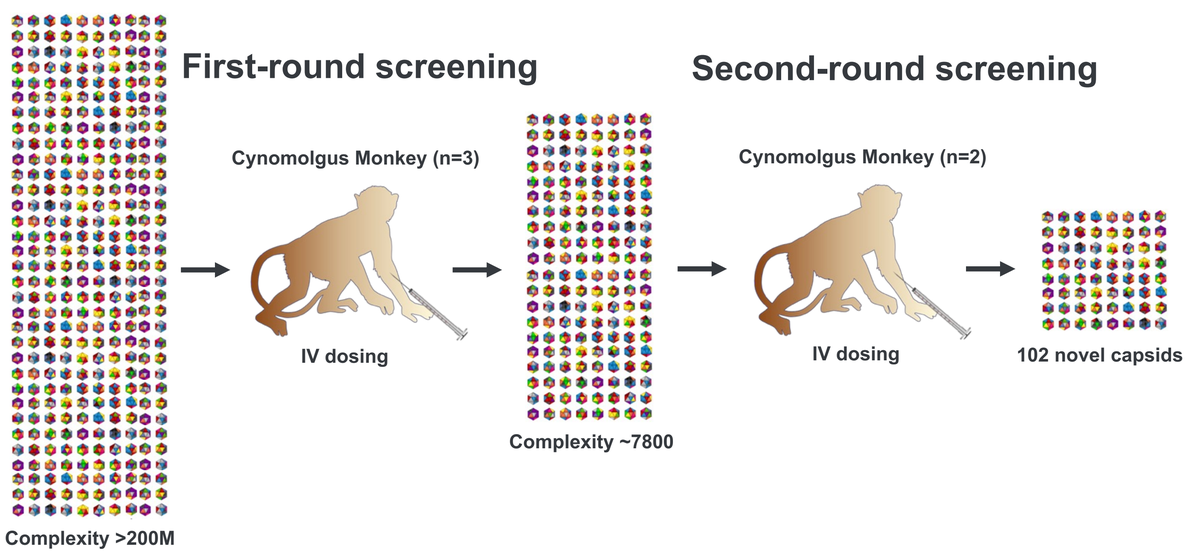


Figure 3. Directed evolution in NHPs. We started with more than 200 million variants in the initial library and performed the 1st-round screen in NHPs. Three Cynomolgus (Cyno) monkeys were dosed via IV and sacrificed 4-week post-injection. Heart transduction of variants was identified by next-generation sequencing (NGS) of the RNA isolated from the hearts. Around 7800 variants were selected for the 2nd-round screen. These variants were re-cloned with two or more synonymous codon replicates and pooled together to generate the viral library. Two Cyno monkeys were dosed via IV and after 4-week incubation, animals were sacrificed and heart and liver were sampled. Heart transduction and liver viral load of each variant were measured.

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.

Novel Capsids for Cardiac Delivery

To better compare 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.

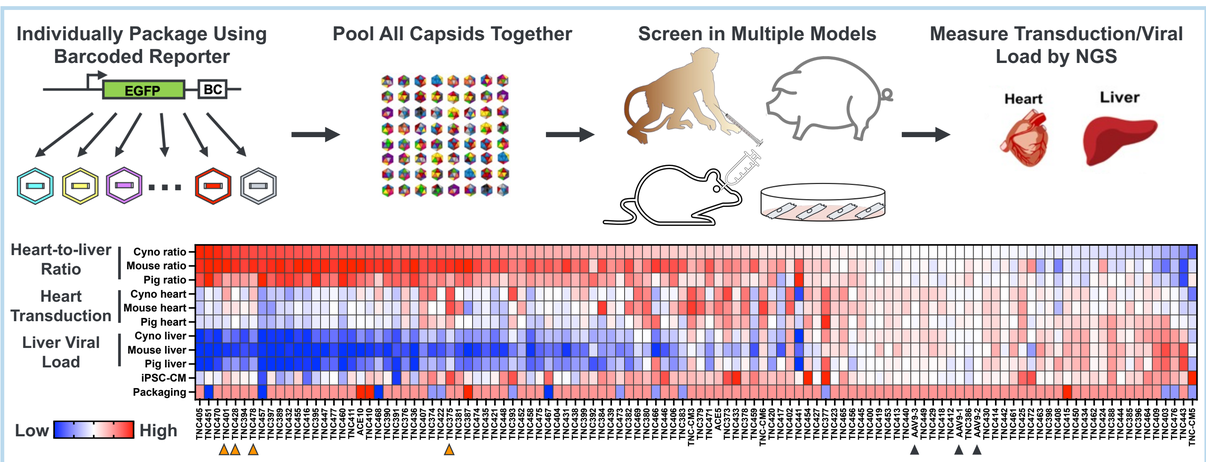


Figure 4. Comparing novel capsids in multiple models. Novel capsid candidates and control capsids were individually packaged using barcoded transgene cassette, pooled together, and screened *in vivo* in Cyno monkeys, mice, and pigs, as well as *in vitro* on hiPSC-CMs. In order to use a better surrogate of the risk associated with liver viral load and increase the stringency of our liver de-targeting criteria, we utilized viral DNA-based detection method for the liver, in contrast to the RNA-based detection for functional transduction in the heart. Heart-to-liver ratio was calculated by dividing heart transduction level by liver viral load. On the heatmap, the capsids were ranked from left to right by the highest heart-to-liver ratio in Cyno to the lowest.

Results from clinical trials and preclinical animal models suggest that AAV9 transduces the heart well, however, a large amount of AAV9 viruses infect the liver after systemic administration, leading to dose-limiting toxicities. We specifically looked for novel capsids that have significantly reduced liver tropism, while maintaining at least comparable heart transduction to AAV9. This analysis resulted in four top candidates.

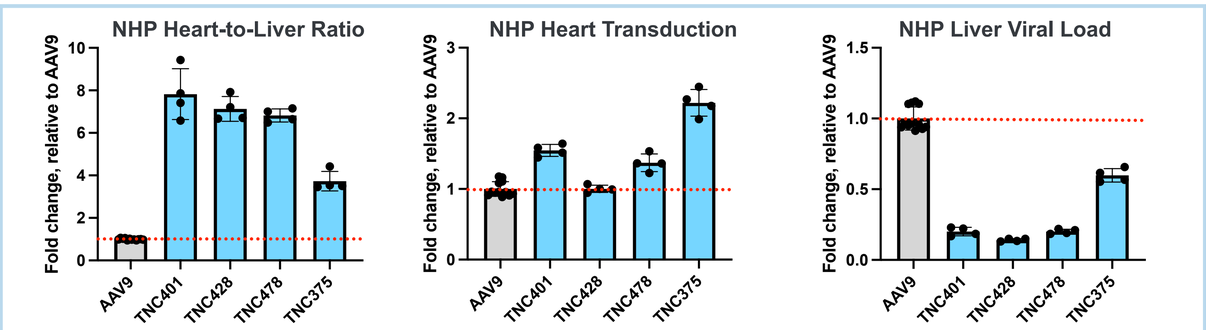


Figure 5. Top novel capsids show superior performance in NHPs. From left to right, heart-to-liver ratio, heart transduction, and liver viral load data measured in Cyno are shown for the top four novel capsids as well as wildtype AAV9 control. Values are fold change relative to AAV9. Four animals were enrolled in this study and are shown as individual dots. Three AAV9 replicates per animal were used.

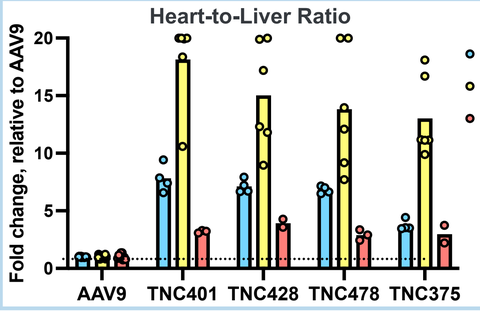


Figure 6. Top novel capsids show improved heart-to-liver ratio in all three species tested. Heart-to-liver ratio measurements of the top four novel capsids and AAV9 control in Cyno, mouse, and pig are shown here. Values are fold change relative to AAV9. Four Cyno monkeys, six mice, and three pigs were enrolled and shown as individual dots. The top four novel capsids demonstrate species consistency in terms of improving heart-to-liver ratio relative to AAV9.

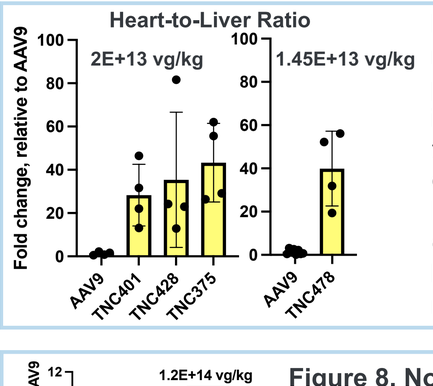


Figure 7. Top novel capsids show improved heart-to-liver ratio when injected individually in mice. To study whether the pooled capsid comparison results can predict performance in individual animal injection (one test article per animal), we tested the top four novel capsids and AAV9 in CD-1 mice with retro-orbital injection. Animals were sacrificed at 18-day post injection. Heart-to-liver ratio measurements are shown here. Values are fold change relative to AAV9. All four novel capsids show improved heart-to-liver ratio in this individual test, consistent with pooled test results.

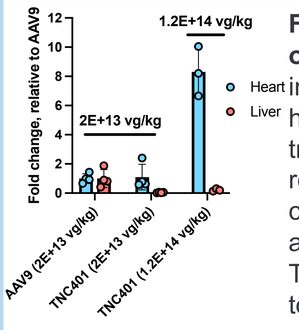


Figure 8. Novel capsid TNC401 can achieve 8X heart transduction without overloading the liver compared to AAV9 in mice. While novel capsids with improved heart-to-liver ratio may reduce liver burden without compromising heart transduction, they may also enable higher safe dosage at which heart transduction is improved and liver viral load is still lower compared to AAV9 at regular dosage. To test these two applications, we performed a proof-of-concept study in CD-1 mice. TNC401 shows comparable heart transduction and 4% liver load relative to AAV9 both tested at 2E+13 vg/kg. Moreover, TNC401 at 1.2E+14 vg/kg dose shows 8X heart transduction level compared to AAV9 at 2E+13 vg/kg, while having just 21% of liver load.

Conclusions and Future Directions

Through directed evolution in NHPs and capsid comparison studies in multiple models, we identified four novel AAV capsids with superior properties to the industry standard, AAV9, for heart-targeted gene delivery with systemic administration. Their superior heart-to-liver ratio may improve the efficacy-to-safety profile of gene therapy drugs and benefit patients by reducing liver-related adverse events, improving efficacy within the safety limit, or both.

We are currently performing a complete NHP biodistribution study to comprehensively profile the tropism of our novel capsids and evaluate their potential clinical advantages.

We are planning 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.