Engineering Novel AAV Capsids for Cardiac Gene Delivery

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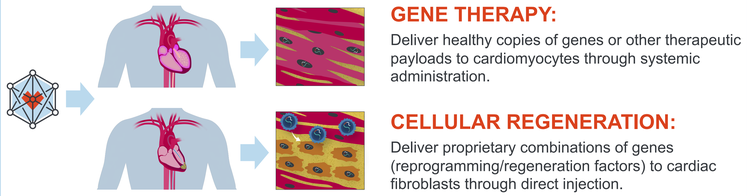
Introduction

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 of 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.

1st-Generation Novel Capsids

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. By combining directed evolution and candidate validation in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), mice, and non-human primates (NHPs), our initial efforts identified a proprietary capsid, TNC-CM3, that has three-fold better heart transduction measured by pooled RNA-based assay in NHPs, as well as 5-fold better heart-to-liver transduction ratio, compared to AAV9.

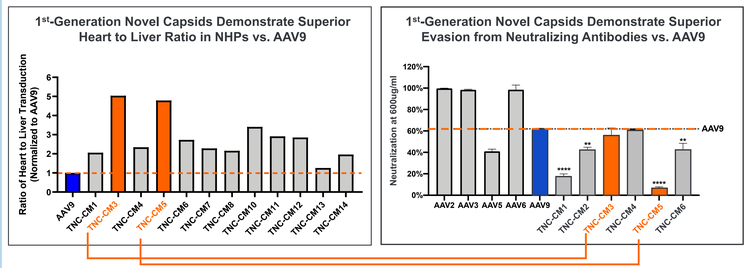


Figure 2. 1st-generation cardiomyocyte-targeting capsids show superior properties. Novel capsids from our 1st-gen capsid engineering efforts were tested head-to-head against AAV9 in NHPs and pooled human IgG. All capsids packaged barcoded RNA reporter cassettes and were pooled at equal VG. Transduction and viral load were measured by NGS-based quantification of barcodes. Left panel shows heart to liver ratios of Tenaya novel capsids compared to AAV9 in NHPs. Right panel shows neutralization results by 600ug/ml pooled human IgG. We have previously presented IgG neutralization, as well as rodent and *in vitro* data of 1st-generation capsids at ASGCT 2020.

2nd-Generation Capsid Engineering Efforts

Although our 1st-generation capsid engineering efforts generated novel capsids with superior heart to liver ratio in NHPs compared to AAV9, 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 human clinics, 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.

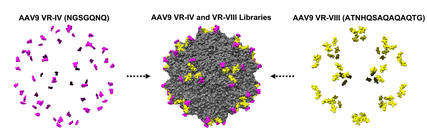


Figure 3. Modifying VR-IV and VR-VIII of AAV9 capsid. Variable regions VR-IV and VR-VIII locate at the three-fold protrusion and have been linked to transduction properties of AAV9 by our internal data, as well as in the literature. We introduced various modifications into these regions, including random 6- or 7-amino-acid mutations, single amino acid substitutions, double-amino-acid insertions, NXN substitutions, and machine learning designed sequences.

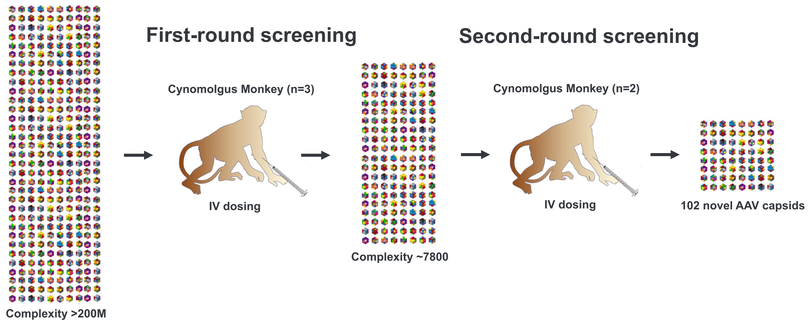


Figure 4. Directed evolution in NHPs. We started with more than 200 million variants in the initial library and performed the 1st-round screen in NHPs. Three Cynomolgus monkeys were dosed via IV and sacrificed 4-week post-injection. Heart transduction of variants was identified by next-generation sequencing (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 Cynomolgus 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.

2nd-Generation Novel Capsids

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.

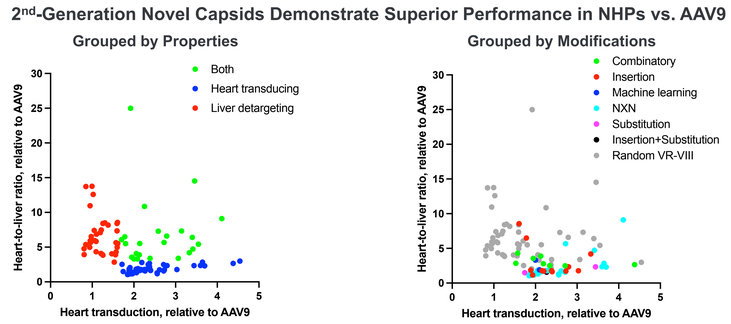


Figure 5. 2nd-generation novel capsids show superior properties in NHPs. This figure shows the performance of our 2nd-generation novel capsids in NHPs. For each capsid, heart transduction was measured by its normalized NGS read count in heart RNA normalized to its normalized NGS read count in the input virus library. Liver viral load was measured by its normalized NGS read count in liver DNA normalized to its normalized NGS read count in the input virus library. Heart-to-liver ratio was calculated by dividing the heart transduction level by the liver viral load. For each capsid, only one synonymous codon replicate (the one picked for further use) are shown.

Novel Capsids with Reduced Liver Tropism

Results from clinical trials and pre-clinical 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. In our 2nd-generation efforts, we specifically looked for variants that have significantly reduced liver tropism, while maintaining at least comparable heart transduction to AAV9. 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.

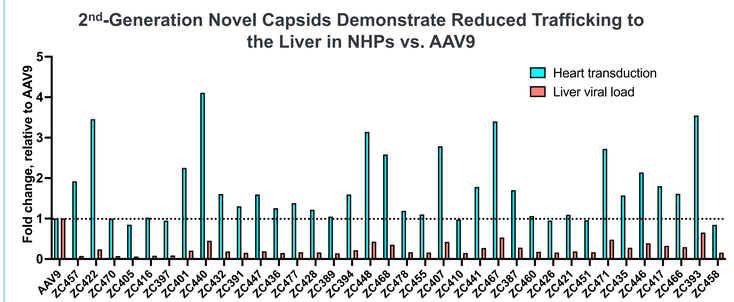


Figure 6. 2nd-generation novel capsids with reduced liver tropism. Novel capsids with greater than 5-fold heart-to-liver ratio in NHPs are shown here. For each capsid, heart transduction was measured by its normalized NGS read count in heart RNA normalized to its normalized NGS read count in the input virus library. Liver viral load was measured by its normalized NGS read count in liver DNA normalized to its normalized NGS read count in the input virus library.

Conclusions and Future Directions

Through two generations of capsid engineering efforts, we identified multiple novel AAV capsids with superior properties to the industrial standard, AAV9, for heart-targeted gene delivery with systemic administration. These novel capsids have the following advantages:

- Superior heart transduction efficiency, which enables 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.
- Superior neutralizing antibody evasion, which may allow us to treat patients who carry pre-existing NAb against AAV9.

We are currently performing a pooled characterization study for our 2nd-generation novel capsids to validate their performance in multiple species, antigenicity, and manufacturability. We aim at selecting 8 or less top performing capsids from this characterization study and will perform a complete NHP biodistribution study to comprehensively profile the tropism of our novel capsids and evaluate their potential clinical advantages.

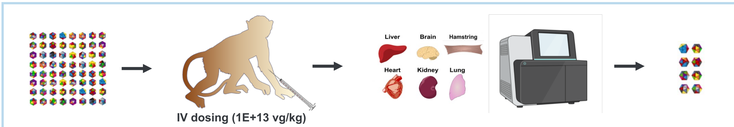


Figure 7. The design of NHP validation study for 2nd-generation novel capsids.

We are planning a 3rd-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 both help us generate new variants and prioritize our hits at different stages in the screening process.