Engineering Novel rAAV Vectors with Enhanced Cardiac Tropism

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Background and Purpose

Gene therapy is an emerging therapeutic option for both acquired and inherited cardiac disorders. Particularly, recombinant adeno-associated virus (rAAV) serotype 9 has become the workhorse vector for gene transfer to cardiomyocytes due to its ability to transduce the heart following systemic delivery. While AAV9 can achieve moderate transduction of the heart, the majority of vector trafficks to the liver. Moreover, in order to achieve therapeutic levels of transduction in the heart, high systemic doses are required, potentially leading to systemic inflammation and in turn, toxicity.

Here, we employed our proprietary capsid engineering platform to identify novel AAV variants with improved cardiomyocyte tropism. Tenaya has generated over 100 million AAV variants through the modification of naturally occurring serotypes via alteration of surface-exposed regions, shuffling of capsid genes, random peptide insertion and combinations of all three capsid modification techniques.

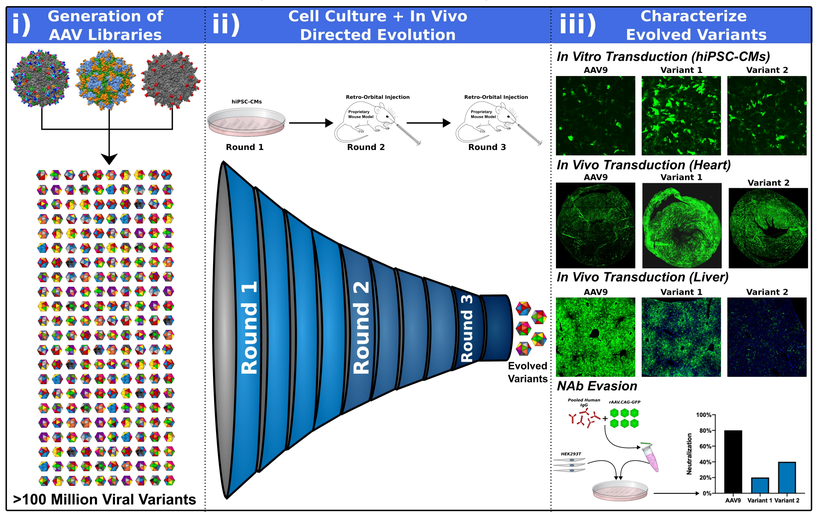

Figure 1 - Schematic depicting library generation, directed evolution and variant validation. (**i**) Production of multiple AAV libraries through a combination of capsid modifications. (**ii**) Directed evolution strategy consisting of one round of in vitro screening and two rounds of in vivo screening. (**iii**) Evolved variants were screened for transduction efficiency *in vitro* and *in vivo*. NAb evasion of variants was also assessed.

Methods

Directed Evolution

Three rounds of directed evolution were applied to Tenaya Therapeutics AAV libraries, with the first round occurring in human iPSC-derived cardiomyocytes (hiPSC-CMs) and two subsequent rounds performed in a rodent model. Nine candidate vectors were identified for further characterization.

hiPSC-CM Transduction Assay

Ventricular hiPSC-CMs were treated with either a parental AAV serotype (AAV2-6 and AAV8-9) or a novel AAV variant (TN1-9) packaging a ubiquitously expressing GFP construct (CAG-EGFP) at an MOI of 50,000 for 24 hours. Three days following transduction, cells were harvested and relative transduction was quantified by flow cytometry.

Human Neutralizing Antibody Evasion Assay

Naturally occurring AAV serotypes or evolved capsid variants packaging CAG-EGFP were incubated at 37°C for 1hr in the absence or presence of 600µg/mL of pooled human IgG isolated from over 2500 patients. Following the incubation, HEK293T cells were infected at a MOI of 100,000 and GFP expression was quantified 48 hours later via flow cytometry. Samples incubated with IgG were normalized to the no IgG control.

In Vivo Evaluation of AAV Variants

8 week-old CD1 mice (n=5/group) were injected with $1x10^{11}$ vg (~$3.33x10^{12}$ vg/kg) of AAV9 or a capsid variant packaging CAG-EGFP via the retro-orbital venous sinus. 15 days following injection, animals were sacrificed and the heart and liver were collected either processed for histology or homogenized. GFP expression was quantified using a GFP ELISA (abcam: ab171581).

Transduction Efficiency of Novel AAV Variants in Human Cardiomyocytes

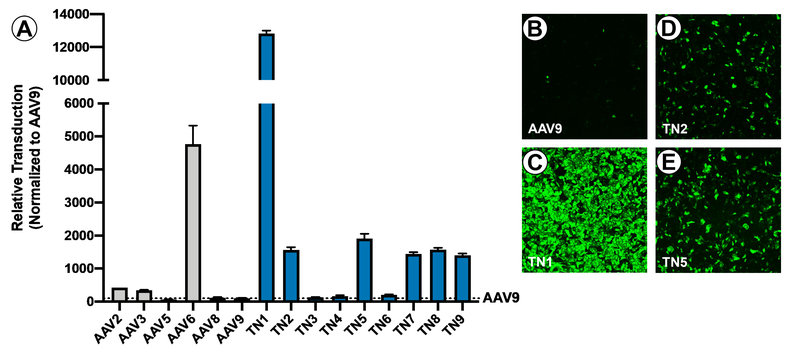

Figure 2 - TN1 is vastly superior to parental serotypes at transducing human iPS-derived cardiomyocytes. (**A**) Flow cytometry quantification of relative transduction (relative transduction = GFP-positive cells x intensity of GFP-positive cells) 3 days post-infection following incubation of parental AAV serotypes or novel capsid variants (n= 3 wells/group). Representative fluorescent images of (**B**) AAV9, (**C**) TN1, (**D**) TN2 and (**E**) TN5 three days post-infection. Green = transient GFP expression

Evaluation of Human Neutralizing Antibody Evasion

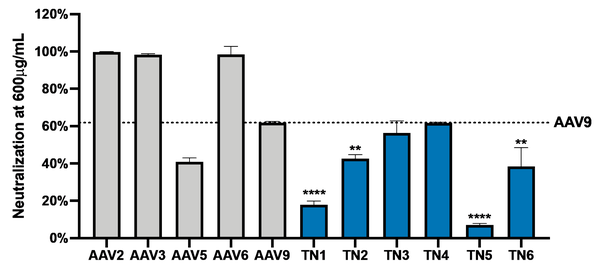

Figure 3 - TN1 and TN5 display improved NAb evasion compared to naturally occurring AAV serotypes. Measurement of AAV neutralization following incubation with pooled human IgG. N = 3/group; ** = p<0.01, **** = p<0.0001, One-way ANOVA; Dunnett's multiple comparison test.

Schematic of Experimental Design to Evaluate AAV Variants *In Vivo*

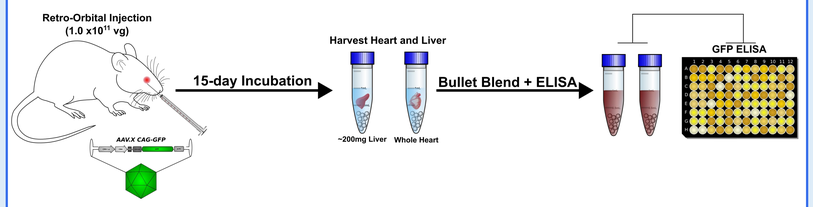

Figure 4 – In vivo experimental design. Age-matched wildtype CD1 mice were injected with various capsids packaging CAG-EGFP via retro-orbital venous sinus. 15 days post-injection, heart and liver tissue was collected and homogenized using a bullet blender. GFP expression was quantified via ELISA.

Liver Transduction in a Rodent Model Following Systemic Delivery

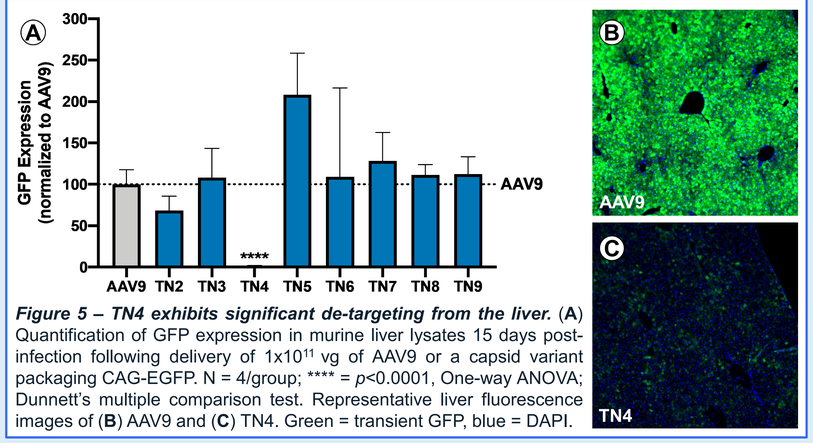

Figure 5 – TN4 exhibits significant de-targeting from the liver. (**A**) Quantification of GFP expression in murine liver lysates 15 days post-infection following delivery of $1x10^{11}$ vg of AAV9 or a capsid variant packaging CAG-EGFP. N = 4/group; **** = p<0.0001, One-way ANOVA; Dunnett's multiple comparison test. Representative liver fluorescence images of (**B**) AAV9 and (**C**) TN4. Green = transient GFP, blue = DAPI.

Heart Transduction in a Rodent Model Following Systemic Delivery

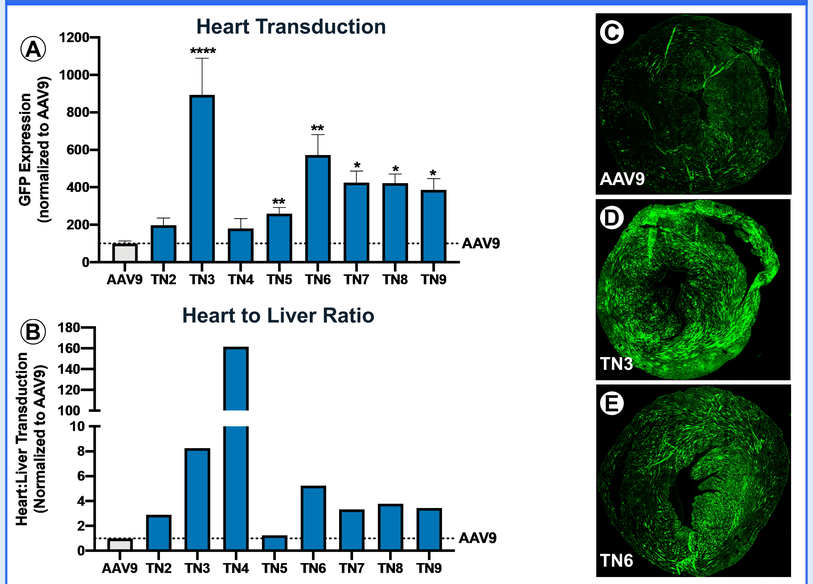

Figure 6 – TN3 and TN6 have significantly improved cardiac tropism compared to AAV9. (**A**) Quantification of GFP expression in murine heart lysates 15 days post-infection following delivery of $1x10^{11}$ vg of AAV9 or a capsid variant packaging CAG-EGFP. N = 4/group; * = p<0.05, ** = p<0.01, **** = p<0.0001, One-way ANOVA; Dunnett's multiple comparison test. (**B**) The ratio of GFP expression in the heart (Figure 6A) and liver (Figure 5A) was calculated and normalized to AAV9. Representative cross-sections of the heart displaying GFP transduction in animals injected with (**C**) AAV9, (**D**) TN3 and (**E**) TN6.

Next Steps – Validation in a NHP

In Progress: head-to-head comparison of AAV9 vs novel capsid variants in cynomolgus monkeys