AAV9 Exhibits Superior Cardiomyocyte Transgene Expression *in vivo* in Murine and Non-Human Primate Models Relative to AAVrh.10 and AAVrh.74 and Mediates Greater Efficacy in a Cardiomyopathy Disease Model

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INTRODUCTION

- We have been focusing on the genetics associated with conditions affecting the heart muscle, also known as cardiomyopathies, that can lead to heart failure, and we prioritize addressing the underlying disease biology using adeno-associated virus (AAV) based methods, including but not restricted to gene therapy, gene editing, and gene silencing. Our AAV-based programs require efficient cardiomyocyte targeting via systemic administration of therapeutic DNA encapsulated in AAV vectors, as superior cardiomyocyte targeting leads to better therapeutic efficacy and/or lower dose requirement.

AAV-Based Cardiac Therapy:

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

Figure 1. Tenaya AAV-based cardiac therapy platform. Tenaya utilizes AAV vectors for delivery of therapeutic genes to the heart in its AAV-based cardiac therapy programs. Our programs target 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.

OBJECTIVE

- Several natural AAV serotypes, including AAV9, AAVrh.10, and AAVrh.74, have been hypothesized to efficiently transduce cardiomyocytes *in vivo* and are being advanced in clinical-stage AAV-based cardiac therapy programs. While transduction and transgene expression studies comparing two or three of these serotypes have been previously reported, ubiquitous reporters were used and expression in cardiomyocytes cannot be distinguished with signal originated from other cell types in the heart. To better guide capsid selection for our AAV-based cardiac therapy programs, we performed a series of studies specifically comparing cardiomyocyte targeting efficiency via systemic administration and efficacy mediated by AAV9, AAVrh.10, and AAVrh.74.

AAV9 Mediates Superior Cardiomyocyte Transgene Expression in Mice Relative to AAVrh.10 and AAVrh.74

- We started with characterizing their heart transduction and cardiomyocyte transgene expression properties in mice using a cardiomyocyte transgene expression reporter. We compared their overall transduction at DNA level in the whole heart with all cell types combined, as well as specifically detected their transgene expression, aka functional transduction, in cardiomyocytes (account for <=50% of all cardiac cells) by measuring RNA and protein products from the cardiomyocyte reporter transgene.

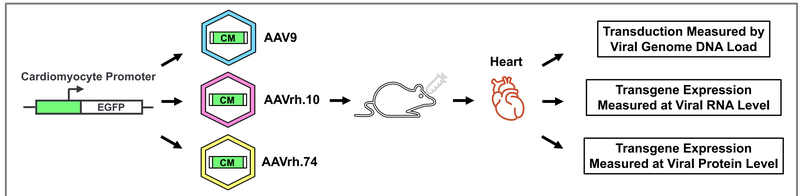

Figure 2. Comparing heart transduction and cardiomyocyte targeting efficiency of AAV9, AAVrh.10, and AAVrh.74. We designed a cardiomyocyte transgene expression reporter ("CM") by coupling a cardiomyocyte-specific promoter with EGFP coding sequence. This cardiomyocyte reporter was packaged in AAV9, AAVrh.10, and AAVrh.74 capsids and administrated to mice via retro-orbital injection. Transduction and transgene expression properties were measured at DNA, RNA, and protein levels.

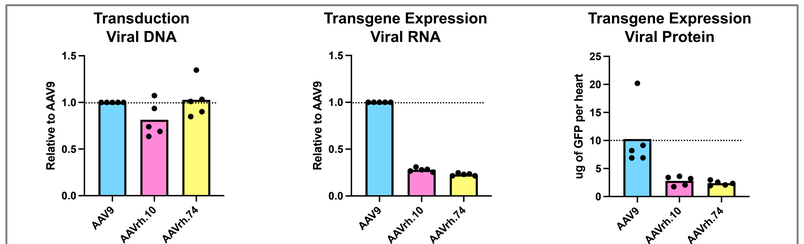

Figure 3. AAV9 mediates superior cardiomyocyte transgene expression in mice. We measured viral DNA, RNA, and protein levels of these three serotypes in mouse heart using cardiomyocyte reporter. All three serotypes show similar transduction to the heart as measured by viral genome DNA load, however, AAV9 generates higher expression level of cardiomyocyte-specific reporter RNA transcripts and protein products than the other two serotypes manufactured in parallel on the same platform. Both barcoded pooled and individual administration were tested and generated similar results. In this figure, DNA and RNA data with relative quantification are from barcoded pooled study, and protein data with absolute quantification are from individually-dosed study. Five animals were enrolled in each study and are presented as individual dots on this figure.

AAV9, AAVrh.10, and AAVrh.74 Exhibit Different Cell Type Tropisms in the Heart Following Systemic Delivery

- The discrimination between DNA level, whole heart transduction and RNA/protein level, cardiomyocyte-specific transgene expression raises the possibility of differential cell type preferences by these serotypes. We performed a similar study with fibroblast reporters being used instead of cardiomyocyte reporter. While AAVrh.10 and AAVrh.74 show lower cardiomyocyte transgene expression levels than AAV9, they mediate transgene expression in cardiac fibroblasts as efficiently as AAV9, demonstrating the difference in cell type tropisms between AAV9 and AAVrh.10/AAVrh.74.

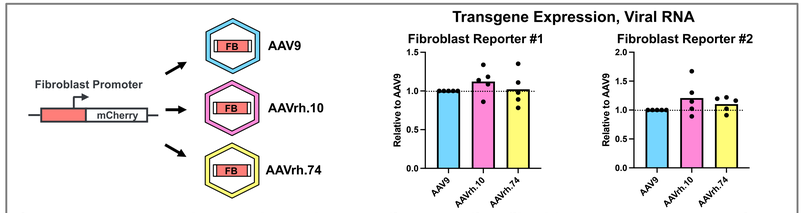

Figure 4. AAV9, AAVrh.10, and AAVrh.74 mediates cardiac fibroblast transgene expression at similar levels. We designed fibroblast transgene expression reporters ("FB") by coupling fibroblast-specific promoters with mCherry coding sequence. Due to the heterogeneity of cardiac fibroblasts, we constructed two fibroblast reporters by varying the promoter to increase the coverage of cardiac fibroblast populations. Five animals were enrolled in this study and are presented as individual dots on this figure.

AAV9 Mediates Greater Efficacy in a Cardiomyopathy Disease Model

- We further investigated the relationship between cell-type-specific transgene expression level and cardiac efficacy by treating a cardiomyocyte-autonomous cardiomyopathy mouse model with the same gene editing based therapeutic cassette delivered by AAV9, AAVrh.10, and AAVrh.74 at the same dose. The degree of efficacy, which was highest with AAV9, correlated with *in vivo* cardiomyocyte transgene expression level and not with viral genome DNA load in the heart.

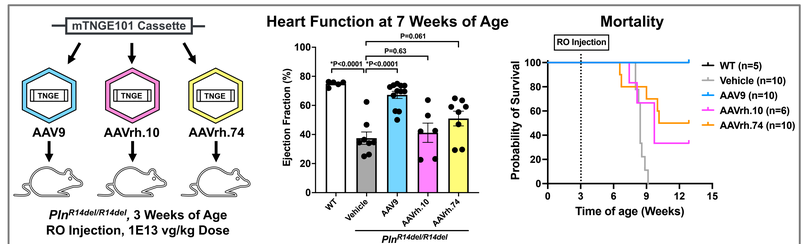

Figure 5. AAV9 mediates superior gene editing efficacy in *Pln-R14del* mouse model. We delivered mTNGE101, an all-in-one gene editing cassette targeting mouse *Pln-R14del* disease-causing allele, by AAV9, AAVrh.10, and AAVrh.74 via retro-orbital administration at 1E13 vg/kg dose to 3-week-old $Pln^{R14del/R14del}$ homozygous mice. We measured heart function parameters (ejection fraction is shown here) at 7 weeks of age (4 weeks post-injection) and AAV9:mTNGE101 treated group showed the best maintenance of heart function. Additionally, all AAV9:mTNGE101 treated animals were alive at 13 weeks of age, while AAVrh.10:mTNGE101 and AAVrh.74:mTNGE101 treated animals started to show mortality at around 7 weeks of age.

AAV9 Mediates Superior Cardiomyocyte Transgene Expression in NHPs Relative to AAVrh.10 and AAVrh.74

- Finally, we measured cardiomyocyte transgene expression of all three serotypes in non-human primates (NHPs) and observed similar trends as those achieved in mice.

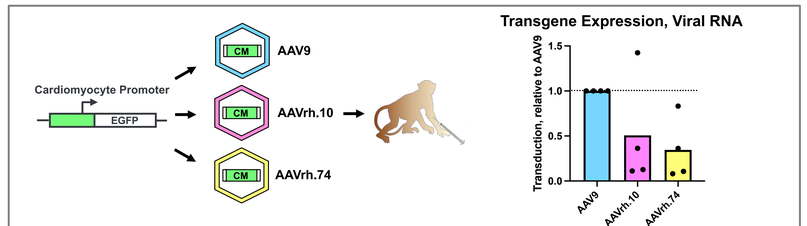

Figure 6. AAV9 mediates superior cardiomyocyte transgene expression in three out of four NHP animals tested. AAV9, AAVrh.10, and AAVrh.74 capsids carrying barcoded cardiomyocyte reporter transgene were pooled and administrated to Cynomolgus monkeys systemically. Transgene expression was measured at 4-week post-injection at RNA level. Data from benchmark capsids and other variants in the same pooled study imply compatibility issues between the outlier animal and AAV9 VR-VIII sequence, as all capsids carrying wildtype AAV9 VR-VIII sequence underperformed in this animal compared to in the other three animals.

CONCLUSIONS

- **AAV9 more efficiently expresses transgene in cardiomyocytes following systemic administration and mediates greater efficacy in *Pln-R14del* cardiomyopathy model compared to AAVrh.10 and AAVrh.74 on our hand .**
- AAV9, AAVrh.10, and AAVrh.74 have different cell type tropisms in the heart.
- It is crucial to use cell type specific reporters while performing comparison studies to support capsid selection for cardiac and other categories of AAV-based programs, as failing to do so could lead to the risk of misguided selection.
- By combining wet lab screening and *in silico* modeling and designing, we have identified and are iteratively engineering novel AAV variants that have enhanced *in vivo* cardiomyocyte transgene expression efficiency and superior heart-to-liver ratio.