Vector Biodistribution and Transgene Expression in the Heart Following Gene Transfer of AAVrh.10 vs. AAV9 Capsids

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BACKGROUND

- As heart disease accounts for 1 out of every 3 deaths in the US, there is an **unmet need** for therapies with improved outcomes.[1]
- Pre-clinical studies have revealed the potential for **myocardial gene transfer** to treat cardiac pathologies.[1,2]
- AAV9 is a robust and **widely used** gene therapy vector due to its enhanced transduction efficiency and transgene expression.[3,4]
  - However, its biodistribution and functional effects relative to other cardiotropic AAV vectors have not been fully elucidated.
- AAVrh.10 has been shown to have **comparable or better** infectivity and efficiency than AAV9.[5-10]
  - AAVrh.10 demonstrated **2-fold greater cardiac biodistribution** within the first 72 hours post-intravenous administration to non-human primates compared to AAV9.[10]
  - Thus, AAVrh.10 may be **better suited** for targeting gene therapies to the heart in the treatment of cardiac diseases.

OBJECTIVES

The objectives of this study were to compare the:

1. **Vector biodistribution** and eGFP **transgene expression** in pigs (anatomically similar to humans) administered AAVrh.10 or AAV9.
2. **Efficacy** of AAVrh.10hPKP2 and AAV9hPKP2 in **improving cardiac function** in severe mouse models of arrhythmogenic right ventricular cardiomyopathy (ARVC) harboring a mutation in the *plakophilin-2* (*PKP2*) gene, as proof-of-principle.

METHODS

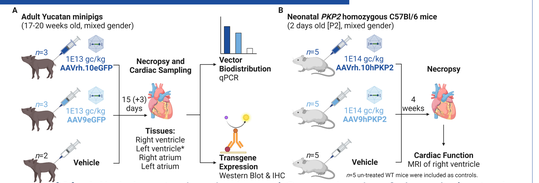

Figure 1. **(A)** Minipig study design. *Comprised of basal (anterior, posterior, lateral) walls, mid (anterior, posterior, lateral) walls, apex, and basal/mid septum. **(B)** ARVC mouse model study design. All vectors were manufactured under the same conditions. Statistical significance was not assessed due to limited sample sizes.

RESULTS

1. AAVrh.10 exhibited heightened vector biodistribution across the porcine heart compared to AAV9.

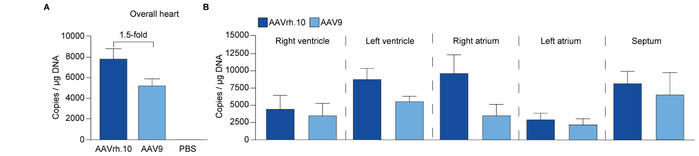

Figure 2. Quantitative polymerase chain reaction (qPCR) analysis of vector copy number across **(A)** the overall heart or **(B)** different regions of the heart of Yucatan minipigs administered a high dose of AAVrh.10eGFP, AAV9eGFP, or a PBS control (PBS + 0.005% Pluronic F-68). Left ventricle data includes an averaged analysis of basal (anterior, posterior, lateral) walls, mid (anterior, posterior, lateral) walls, and apex samples. Septum data includes an averaged analysis of basal and mid septum samples. Data represent averages ± SEM. *n*=3 pigs for each AAV group or 2 for the PBS group.

2. AAVrh.10 exhibited heightened eGFP transgene expression in the overall porcine heart compared to AAV9.

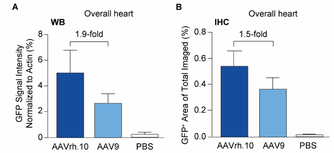

Figure 3. Analysis of eGFP transgene expression in the heart of Yucatan minipigs administered a high dose of AAVrh.10eGFP, AAV9eGFP, or a PBS control (PBS + 0.005% Pluronic F-68) by **(A)** western blot (WB) or **(B)** immunohistochemistry (IHC). WB data includes an averaged analysis of right ventricle, left ventricle, right atrium, and left atrium samples. IHC data includes an averaged analysis of samples from 12 separate heart regions. Data represent averages ± SEM. *n*=3 pigs for each AAV group or 2 for the PBS group.

3. AAVrh.10 was associated with heightened eGFP transgene expression in various regions of the porcine heart compared to AAV9.

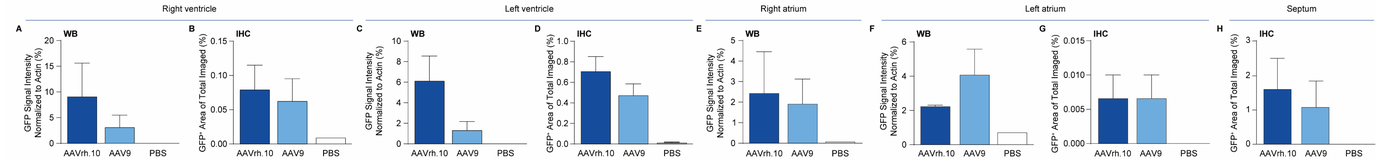

Figure 4. Analysis of eGFP transgene expression in the **(A-B)** right ventricle, **(C-D)** left ventricle, **(E)** right atrium*, **(F-G)** left atrium, and **(H)** basal septum** of Yucatan minipigs administered a high dose of AAVrh.10eGFP, AAV9eGFP, or a PBS control (PBS + 0.005% Pluronic F-68) by WB (A,C,E,F) or IHC (B,D,G,H). For the left ventricle, IHC data includes an averaged analysis of basal (anterior, posterior, lateral) walls, mid (anterior, posterior, lateral) walls, and apex samples, while WB data includes the left ventricle analyzed as a whole (no septum). All data represent averages ± SEM. *n*=3 pigs for each AAV group or 1 PBS pig. *eGFP undetected by IHC (data not shown). **sample not collected for analysis by WB.

4. The cardiac functional rescue achieved in an ARVC mouse model trended higher with a *PKP2* transgene delivered by AAVrh.10 compared to AAV9.

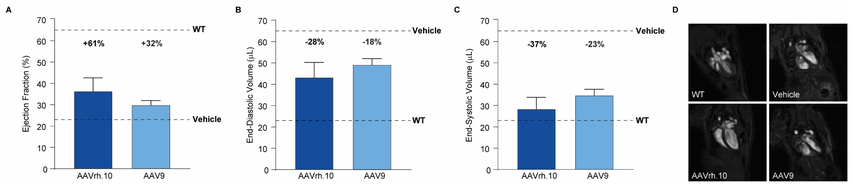

Figure 5. Cardiac function in the right ventricle of neonatal *PKP2* homozygous (Hom) mice administered 1E14 gc/kg of AAVrh.10hPKP2 or AAV9hPKP2 or vehicle, or un-injected wild-type (WT) mice as evaluated through magnetic resonance imaging (MRI)-based measurements of **(A)** ejection fraction, **(B)** end-diastolic volume, and **(C)** end-systolic volume. Data represent averages (± SEM). *n*=5 mice per group. **(D)** Representative MRIs of WT and *PKP2* Hom mice.

CONCLUSIONS

1. Consistent with previous findings[10], our results demonstrated the preferential use of AAVrh.10 over AAV9 for targeting gene therapies to various regions of the heart in the treatment of cardiac diseases with a genetic component.
   a) AAVrh.10 was associated with trends of **enhanced vector biodistribution** and **transgene expression** in Yucatan minipigs as compared to AAV9.
   b) Gene delivery via AAVrh.10 also led to trends of **improved functional rescue** of cardiac function in a mouse model of ARVC as compared to AAV9.
2. It will be important to consider **species-specific factors** (e.g., immune response) and tropism when translating these findings to human cardiac diseases.

FUTURE DIRECTIONS

- Statistical significance was not assessed due the limited sample sizes.
- As such, the results of this study, which confirmed previous findings, may be considered as preliminary assessments that will inform power analyses for **larger studies** in the future.

MORE INFORMATION

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This poster was created by Lauren Todd from sixsense strategy group, Inc.

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