Analysis of Gene Expression, Tissue Tropism, and Safety of Novel AAV Variants in Mice Following Intravenous Administration

108

Tawny Neal, Steven Tobia, Alex Bauer, Ming Ni, Mehdi Gasmi, and Annahita Keravala

Adverum Biotechnologies Inc.; 1035 O'Brien Drive, Menlo Park, CA 94025. USA

ADVERUM BIOTECHNOLOGIES

Background

- *In vivo* gene therapy studies provide valuable information regarding vector specificity, therapeutic efficacy, and safety.
- Tropism of naturally occurring AAV serotypes can potentially be altered by chemical modification, peptide insertion into surface-exposed regions, or site-directed/ random mutagenesis.
- AAV.7m8, 2.5T, and ShH10 are variants that were discovered by *in vitro* or *in vivo* directed evolution.
- AAV/7m8 are hybrid variants that were engineered by inserting a 10 amino acid peptide loop (7m8) into the surface-exposed region of the parental capsid.

Purpose

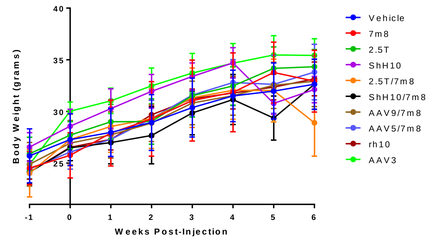
- We are developing AAV variants that can overcome the limitations associated with restricted tropism or immune response.
- Additionally, we are generating capsids that could potentially be used for re-dosing.
- For these purposes we sought to evaluate tissue tropism, efficacy, and safety in mice of some of Adverum's existing variants compared to rh10, candidate vector for two of our lead programs.
  - 3 variants discovered by directed evolution: 7m8 (photoreceptors), 2.5T (airway epithelia), and ShH10 (Muller cells)
  - 4 capsids engineered through rational design: 2.5T/7m8, ShH10/7m8, AAV9/7m8, and AAV5/7m8
  - 1 naturally occurring serotypes: AAV3

Methods

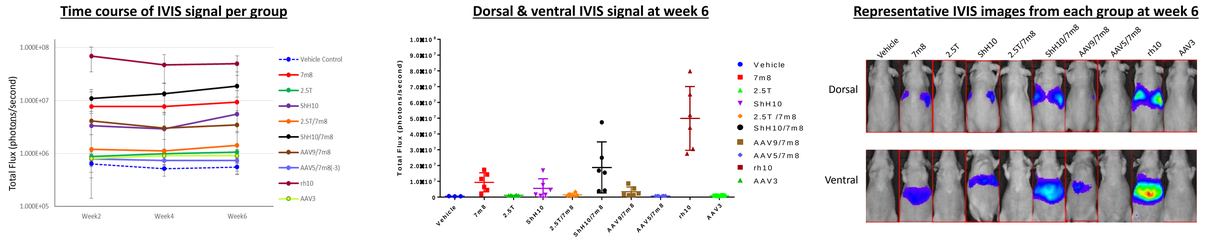
Fig 1. Biodistribution & Safety Study in Mice. (1) 8-10 week old, male SKH-1 hairless mice (immunocompetent) were obtained from Charles River. (2) Bolus tail vein intravenous injections of AAV variants were administered with $1x10^{11}$ vg/mouse in 200µL. The genomes of all vectors were identical; consisting of luciferase (Luc) as transgene driven by the CAG promoter (3) In vivo live imaging using IVIS Spectrum was performed at 2, 4, and 6 weeks post-dose. (4) Mice were sacrificed after the 6 week imaging time point and tissues were collected: blood, brain, heart, liver, lung, kidney, pancreas, spleen, quadriceps, and testes. They were either stored in RNA*later* for mRNA analysis or flash frozen for protein assessment (5) Tissues were homogenized and (6) luciferase (Luc) mRNA was extracted using Qiagen's RNeasy Kit. (7) RT-PCR was performed to obtain Luc cDNA, (8) which was assessed by running it out on the gel or quantitating it by using qPCR with Taqman primer/probe to luciferase transgene.

Results

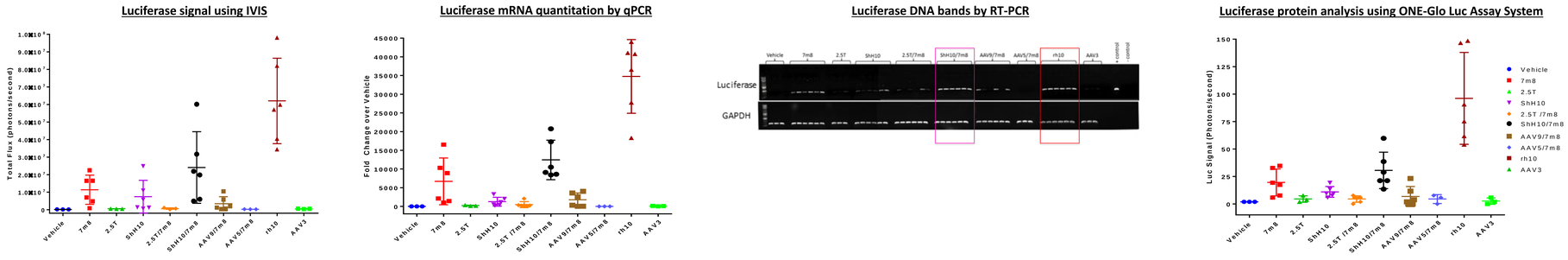
Body weights for mice in all groups followed the expected increasing trend



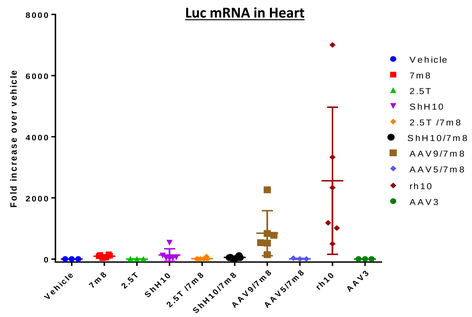
In Vivo Live Imaging System (IVIS) shows variable levels of luciferase expression mediated by novel AAV capsids in mouse organs over time



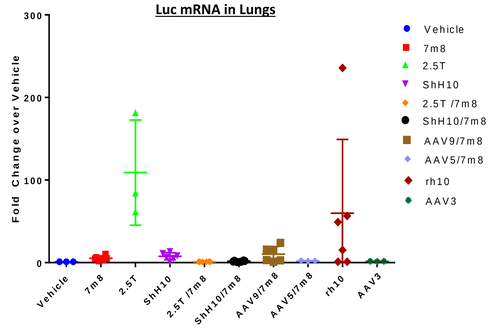
ShH10/7m8, and rh10 mediate high levels of luciferase expression in mouse liver. This data is consistent across different methods used to analyze luciferase signal by live imaging, mRNA levels, and protein expression.



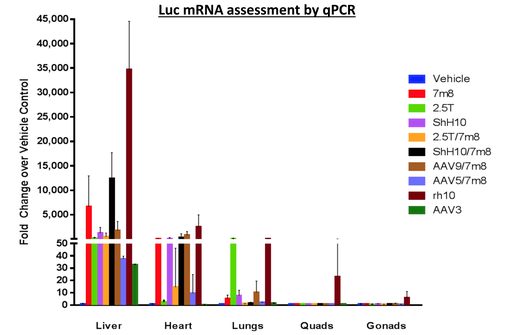
AAV9/7m8 and rh10 mediate high levels of Luc expression in heart



2.5T and rh10 mediate higher levels of Luc expression compared to other variants in lung



AAV variants mediated Luc expression was highest in mouse liver compared to other organs after IV injection



Summary of Results

- ✓ All novel capsids tested in this study were safe and well tolerated by the mice with no observed adverse events.
- ✓ Although in this mouse study rh10 mediated the highest amount of transduction overall, there were a few other variants from this pool that were promising and exhibited varying tissue tropism (analyzed by transgene expression) in mice after systemic, IV injection:
  - ✓ ShH10/7m8 specifically transduced the liver efficiently.
  - ✓ 2.5T showed good transduction of lungs compared to other variants.
  - ✓ AAV.7m8, a variant that was discovered by DE for photoreceptors, transduced liver.
  - ✓ AAV9/7m8 was trophic to the heart.
  - ✓ Even though transduction was observed in tissues other than the liver, levels of Luc expression in heart and lungs were 10-100 fold lower than in liver.
  - ✓ Most of these vectors were unable to efficiently transduce the mouse brain.

Conclusion and Next Steps

- ➢ This initial mouse study proved useful in examining tissue tropism, gene expression, and safety after systemic delivery of the novel AAV variants that were originally designed for other purposes, compared to rh10.
- ➢ It provided insight into the ability of a few novel variants that could be optimized further for specifically targeting liver, lungs, or heart.
- ➢ Next steps would involve testing some potential candidates in a larger species such as NHPs or specifically for liver targeting, in humanized mice that have 90% of human liver tissue.
- ➢ In order to develop these variants for re-dosing, an *in vivo* study will be performed to assess immune cross-reactivity between these novel variants and rh10.

Disclosures

AK, MG, MN, AB, and ST are employed by Adverum Biotechnologies Inc.

Contact

annahita@adverum.com