Biodistribution and pharmacokinetics of AAVRh.10-A1AT mediated gene therapy in humanized-liver mice as a predictor of A1AT human expression levels following intravenous delivery

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Background

- Alpha-1 antitrypsin (A1AT) deficiency (A1AD) is an autosomal co-dominant disease caused by mutations in the A1AT gene which lead to impaired liver secretion and low blood concentration of serine protease inhibitor A1AT. A1AD results in impaired neutrophil elastase regulation and a high risk of pulmonary emphysema.
- ADVM-043 (AAVRh.10-CAG-A1AT) was developed as an intravenous gene therapy to treat A1AD, mediated by liver transduction and expression of normal A1AT. When administered to male C57/B6J mice at $1x10^{13}$vg/kg, the A1AT serum levels reached 23±5.6µM (1250µg/mL), close to the normal human level, 22µM (1196µg/mL), and well beyond the therapeutic threshold, 11 µM (597µg/mL).
- However, the evaluation of ADVM-043 in the ADVANCE clinical trial (phase 1/2 - NCT02168686) at the doses between $1x10^{12}$ and $1.5x10^{13}$vg/kg resulted in maximum A1AT blood levels of 0.24 µM. While well-tolerated, this did not meet clinically meaningful levels.

Study Objectives

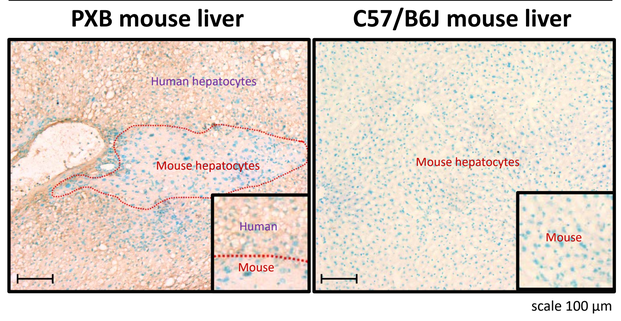
1. To understand the discrepancy between human and mouse A1AT levels, we compared the biodistribution and pharmacokinetics of AAVRh.10-CAG-A1AT.HIS in humanized-liver mice (PXB mice), following the intravenous administration of $1x10^{11}$vg (i.e. ~$5x10^{12}$vg/kg). C57/B6J mice were used as controls.
2. We also evaluated strategies to optimize expression by using AAV capsid variants and expression cassettes.

The PXB humanized-liver mouse model (PhoenixBio Co., Ltd. Japan)

Purified hepatocytes from a single, healthy, adult human donor

Human hepatocytes transplanted into uPA/SCID mice generate fully functional liver

Repopulation of PXB liver with human hepatocytes evaluated by IHC α-human albumin



Study Design and Methods

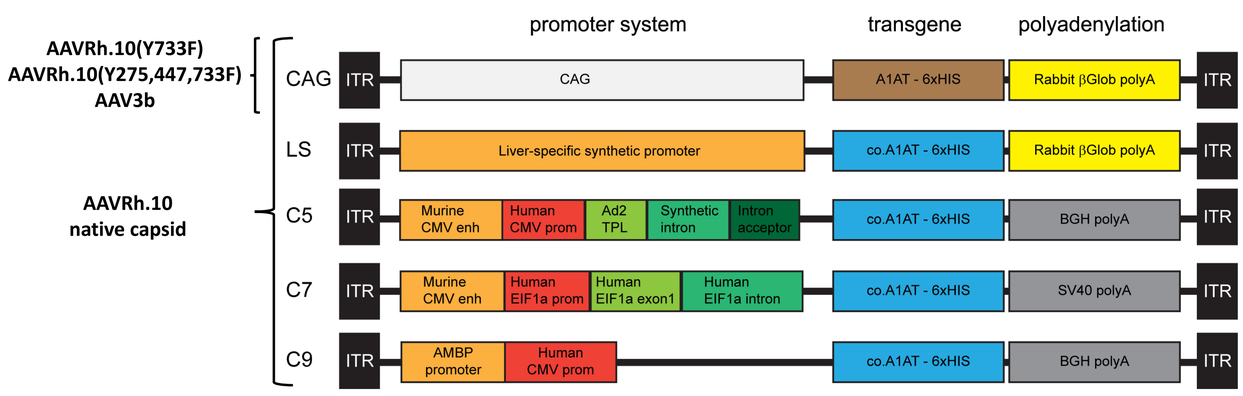
Comparison of serum A1AT level in adult male C57/B6J and PXB mice following tail-vein injection of $1x10^{11}$vg AAVRh.10 vector encoding A1AT.6HIS in 200µL bolus:

- **Male C57/B6J mouse:** 9 weeks of age, 15-25g body weight.
- **Male PXB humanized-liver mouse:** 12 weeks of age, 21-26g body weight, >80% human hepatocytes.
- **Biodistribution and Expression Analyses:** Vector biodistribution in liver by qPCR quantification, longitudinal serum A1AT levels by ELISA, and A1AT.HIS protein level in liver tissue by Western blot.

Comparison of liver transduction efficiency in C57/B6J and PXB mice, mediated by AAVRh.10 variants and AAV3b vectors:

- AAV capsid mutations that substitute surface exposed tyrosine (Y) residues for phenylalanine (F) result in reduced virus degradation, increased nuclear trafficking and transgene expression (Zhong *et al*, 2008). AAVRh.10 tyrosine capsid mutants AAVRh.10(Y733F) and AAVRh.10(Y275,447,733F) were engineered based on the corresponding residue locations on the AAV8(Y733F) and AAV2(Y272,444,730F) variants.
- The AAV3b capsid vector has been previously shown to efficiently and selectively transduce human liver cells (Wang *et al* 2015).

Optimized expression cassettes evaluated in C57/B6J and PXB mice



Results

20-50-fold lower transgene secretion in humanized liver mouse, with no improvements with variant capsids, or AAV3b

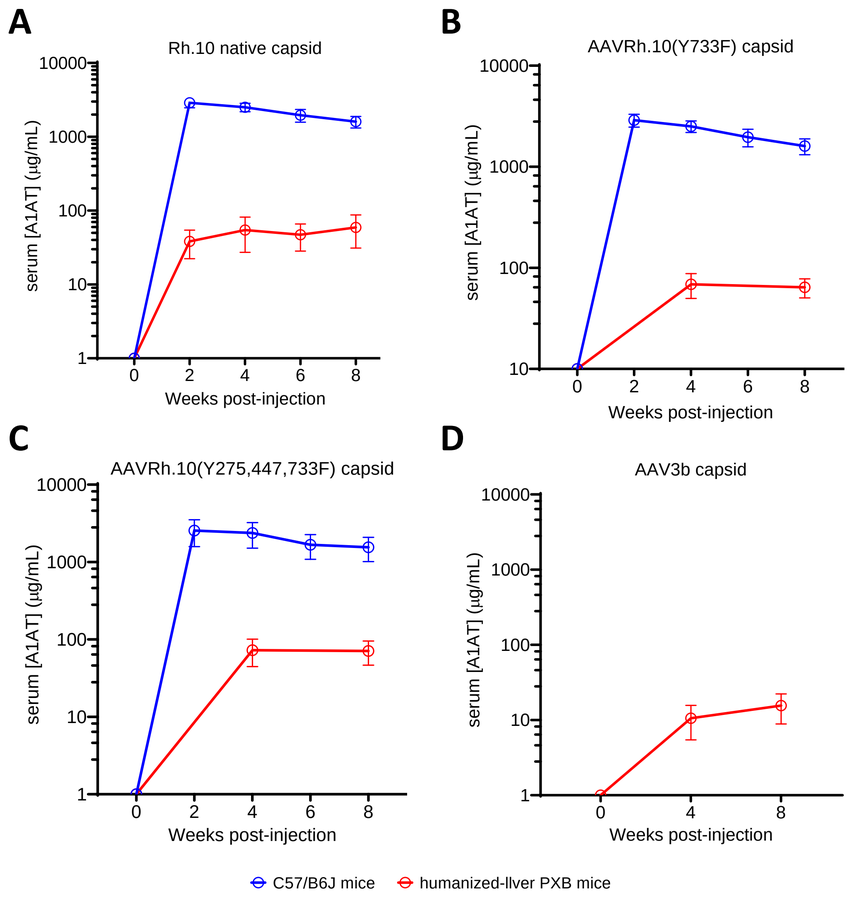


Figure 1. Comparison of the serum concentration of A1AT in C57/B6J and PXB mice following intravenous administration of $1x10^{11}$vg of different AAVRh.10 vector capsid variants or AAV3b vector. All vectors packaged the same expression cassette encoding A1AT.HIS cDNA under the control of the CAG promoter and the rabbit β-globin polyA. Serum levels of A1AT.HIS were measured by ELISA assay. **(A)** AAVRh.10 native capsid. C57/B6J n=7, PXB n=6-11. **(B)** Rh.10(Y733F) capsid variant. C57/B6J n=7, PXB n=5. **(C)** Rh.10(Y275,447,733F) capsid variant. C57/B6J n=7, PXB n=5. **(D)** AAV3b capsid vector. C57/B6J n=0, PXB n=4-5. Y-axis: Log10 scale.

Lower transgene expression in humanized-liver, across all expression cassettes and regulatory elements evaluated

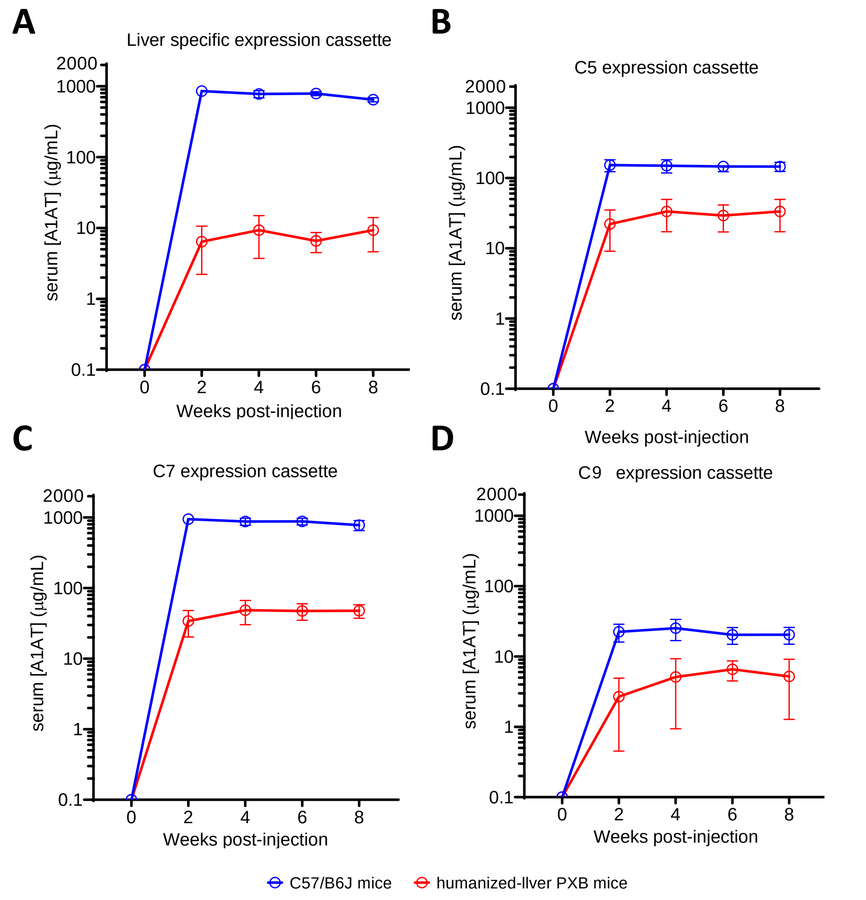


Figure 2. Comparison of the serum concentration of A1AT in C57/B6J and PXB mice, following intravenous administration of $1x10^{11}$vg of AAVRh.10 vector encoding different A1AT.6HIS expression cassettes. All vectors are packaged in AAVRh.10 native capsid and encode a codon optimized cDNA of A1AT. Each vector group: C57/B6J n=7, PXB n=6. Serum levels of A1AT-HIS measured by ELISA assay. **(A)** Liver specific (LS) expression cassette driven by a synthetic liver specific promoter and the rabbit β-globin polyA. **(B)** C5 expression cassette **(C)** C7 expression cassette **(D)** C13 expression cassette. Y-axis: Log10 scale.

AAVRh.10 vector biodistribution does not predict protein expression

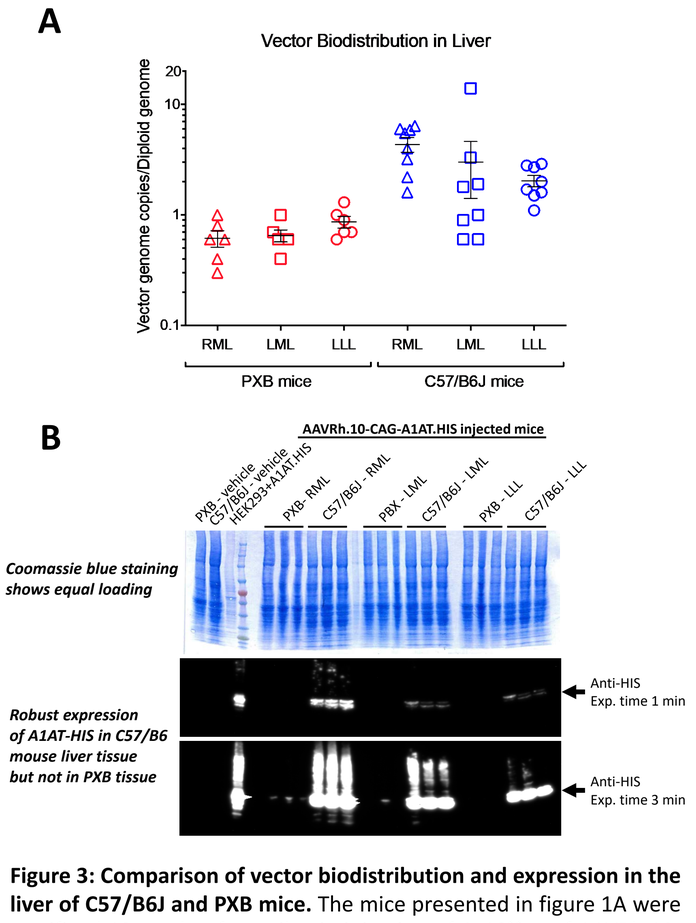


Figure 3: Comparison of vector biodistribution and expression in the liver of C57/B6J and PXB mice. The mice presented in figure 1A were sacrificed 12 weeks after tail-vein injection of $1x10^{11}$vg of AAVRh.10-CAG-A1AT.HIS vector. DNA and total protein were extracted from half of each right medial lobe (RML), left medial lobe (LML) and left lateral lobe (LLL) from three representative mice. **(A)** qPCR analysis of vector copy number per diploid genome. Y-axis: Log10 scale. **(B)** Western blot analysis of protein expression. Upper panel, Coomassie blue staining for assessment of equal loading. Middle and lower panels, anti-HIS at different exposure times. 50µg of total protein per well.

Conclusions

- Our data show that AAVRh.10-mediated liver gene transfer is 25-fold less efficient in humanized-liver mouse than in C57/B6J mice, at 8 weeks after vector administration. This is in line with the discrepancies observed between mouse preclinical studies and the ADVANCE clinical trial. Moreover, this phenomenon was replicated with different expression cassettes and AAV variants.
- Surprisingly, AAV3b vector expresses poorly in humanized-liver mice when compared to AAVRh.10 vectors. This is in contrast with previous histological and biodistribution studies conducted in NHP and humanized-liver mouse models.
- These data illustrate the lack of translatability of mouse studies for AAVRh.10-mediated liver gene therapy and supports the PXB humanized-liver mouse model as a valuable preclinical platform for a successful transition to the clinic.