Limitations of Marmosets as an Animal Model for AAV Mediated Liver Gene Transfer

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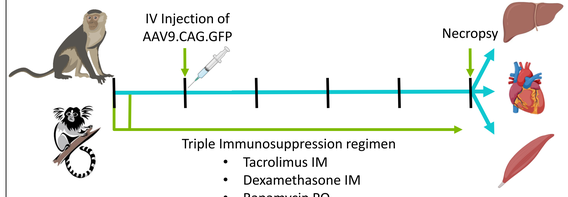
Introduction

Affinia Therapeutics is pioneering a shift to a new class of rationally designed gene therapies via our proprietary Affinia Rationally designed Therapies (ART) platform. One focus is developing novel AAV capsids that enhance tissue specificity, immunological profile, and manufacturability and we have recently characterized multiple novel capsids with these attributes for skeletal muscle, heart and CNS diseases.

To properly address enhancements of tissue specificity while detargeting liver, AAV vectors need to be tested in the appropriate animal model. Nonhuman primates (NHPs) are commonly used for this purpose. Cynomolgus macaques (*Macaca fascicularis*), an old-world monkey, and marmosets (*Callithrix jacchus*), a new-world monkey, have been used extensively in preclinical studies. There are notable differences between these two species, especially in body weight and absence of CMAH (Cytidine monophosphate-N-acetylneuraminic acid hydroxylase) which has an important effect on the tropism of AAVs using sialic acid as the primary receptor. Marmosets are significantly lighter animals, making this species a desirable model especially for systemic administration of AAV due to reduced manufacturing cost. Here we compare the biodistribution profile of an AAV9.CAG.GFP vector intravenously (IV) administered in cynomolgus and marmoset monkeys.

Methods and Materials

- NHPs were pre-screened using an in-vitro neutralizing antibody (NAb) assay[a] and selected from a pool of animals to ensure they were free of NAbs against AAV9 prior to study initiation
- Immunosuppression was initiated 5-7 days prior to vector administration to limit immune response to AAV capsid and GFP transgene
- All NHPs were dosed at 1E14vg/kg
- Doses were delivered IV via the saphenous vein in either 10mLs (Cyno) or 0.75mL (Marmoset)



- 28 days post-injection NHPs were euthanized, PBS perfused and tissues harvested with samples collected for IHC, RTddPCR and ddPCR
- Samples for IHC were scored by a pathologist for percentage of cells expressing GFP

Results

Figure 1. IHC GFP Analysis

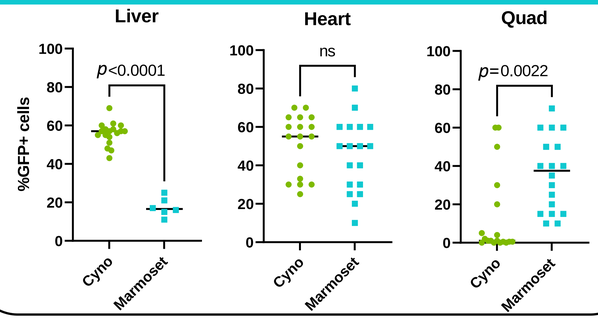


Figure 2. RT-ddPCR Analysis

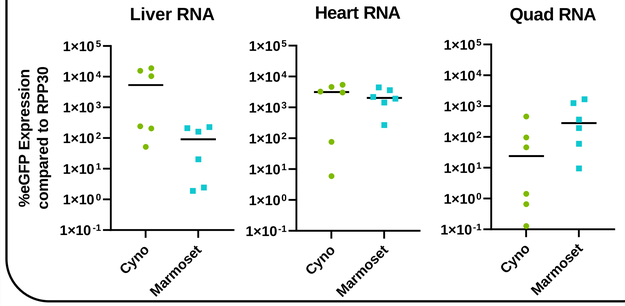


Figure 3. ddPCR Analysis

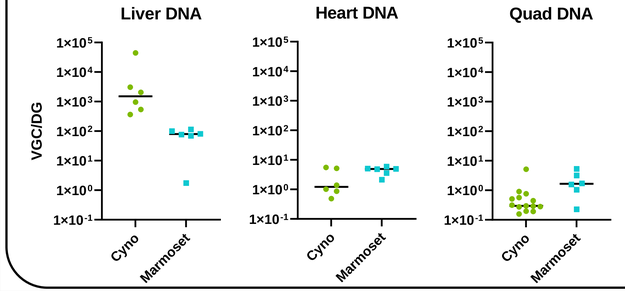


Figure 4. IHC GFP Representative Images

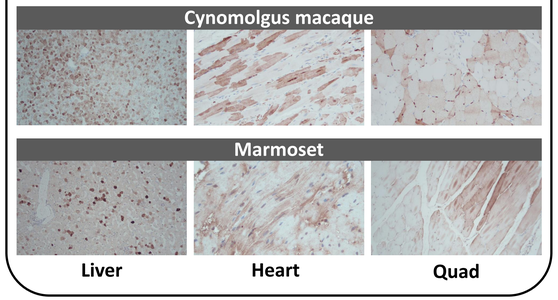
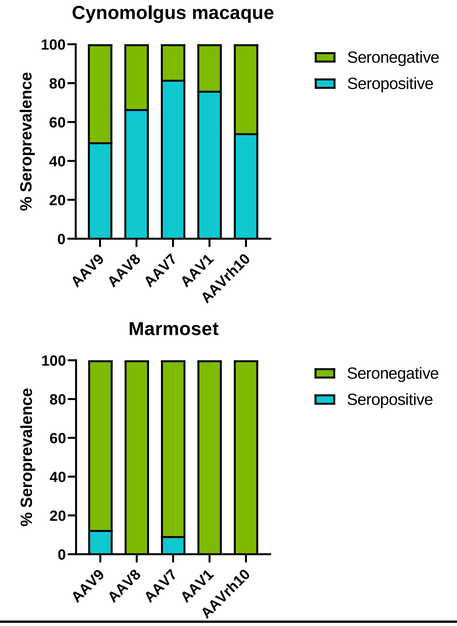


Figure 5. AAV NAb Seroprevalence



Results Summary

- Liver biodistribution data demonstrated a lower gene transfer and gene expression in marmosets compared to cynomolgus macaques as assessed by IHC (Fig. 1), RT-ddPCR (Fig. 2) and ddPCR analysis (Fig. 3)
- Cardiac muscle (heart) biodistribution and levels of gene expression were comparable between species
- Skeletal muscle (quadriceps) biodistribution data showed a higher gene transfer and gene expression in marmosets compared to cynomolgus macaques
- Seroprevalence analysis of marmoset and cynomolgus macaque colonies showed marmosets have a significantly lower level of NAbs across multiple serotypes (AAV9, 8, 7, 1 & rh10) compared to cynomolgus macaques

Conclusions

Although our study included a small number of animals (n=6) and a single vector dose (1e14 vg/Kg), our data indicate that marmosets may not be an ideal translational animal model to evaluate liver gene transfer and safety with administration of AAV vectors. AAV seroprevalence and liver transduction are significantly higher in cynomolgus macaques. The low levels of liver transduction observed in marmosets may not be predictive of adverse events observed in humans, especially with a high dose systemic AAV administration. Therefore, macaques may be a more representative translational model to evaluate liver transduction for systemically administered AAV.

References: [a]Assessment of Humoral, Innate, and T-Cell Immune Responses to Adeno-Associated Virus Vectors. Calcedo R et.al. Hum Gene Ther Methods. 2018 Apr;29(2):86-95.
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