# Circulating anti-AAV5 neutralizing antibodies titers up to 1:1031 do not affect liver transduction efficacy of AAV5 vectors in non-human primates

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## Introduction

Currently, patients who present levels of anti-AAV neutralizing antibodies (NAB) considered as low are excluded from gene therapy trials. Indeed, low levels of pre-existing anti-AAV NAB have been reported to impair liver transduction by AAV serotype 8 (reported titers of 1:5) in non-human primates (NHPs) (Jiang et al., Blood 2006) and by AAV serotype 2 (reported titers from 1:3.3 and 1:10) in an in vivo mouse model of passive immunity (Scallan et al., Blood, 2006).

## Goal / Experimental Design

Considering that NAB titers are relevant for the initial transgene expression, we sought to assess the impact of circulating anti-AAV5 NAB levels on the liver transduction efficacy of an AAV5-based vector delivered systematically.

The sera of 14 NHPs were assessed for the levels of pre-existing anti-AAV5 NAB before intravenous administration of an AAV5 vector (AAV5-hFIX) at a dose of 5e11 gc/kg (n=3), 5e12 gc/kg (n=5), 2.5e13 gc/kg (n=3) or 9.3e13 gc/kg (n=3). Anti-AAV5 NAB titers were assessed using a specific bioassay sensitive enough to detect NAB at titers as low as 1:1. Transduction efficiency was assessed by measuring transgene proteins levels in plasma 7 days after vector infusion, and vector DNA in the liver 6 months after vector infusion (post mortem).

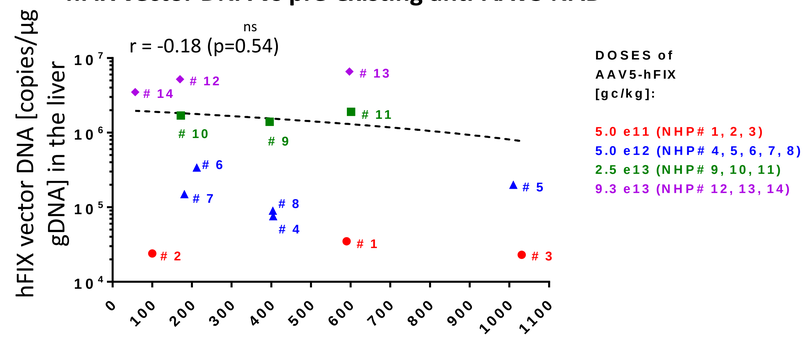
| Groups | IV injection of AAV5-hFIX [gc/kg] |
|---|---|
| 1. (n=3) | 5.0 e11 |
| 2. (n=5) | 5.0 e12 |
| 3. (n=3) | 2.5 e13 |
| 4. (n=3) | 9 e13 |

## Results

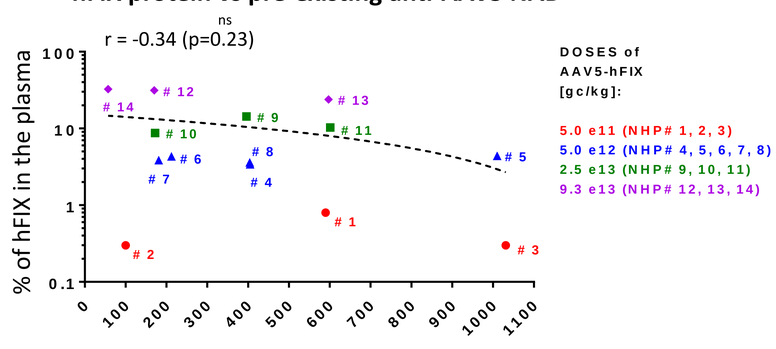
All animals displayed pre-existing anti-AAV5 NAB, at titers ranging from 1:57 to 1:1031. Within each dose group, successful and comparable transduction was achieved, independently of the level of pre-existing anti-AAV5 NAB (**1 a**). At sacrifice, the amounts of AAV5 vector DNA, transgene mRNA in the liver (**2 a**) and hFIX protein in the plasma (**2 b**) were directly proportional to the injected dose of AAV5 and found similar within animal groups tested regardless of the level of anti-AAV5 NAB at pre-administration.

### 1. No correlation between pre-existing anti-AAV5 NAB levels and AAV5 liver transduction efficacy or level of hFIX in plasma of AAV5-hFIX treated animals was observed

**a**

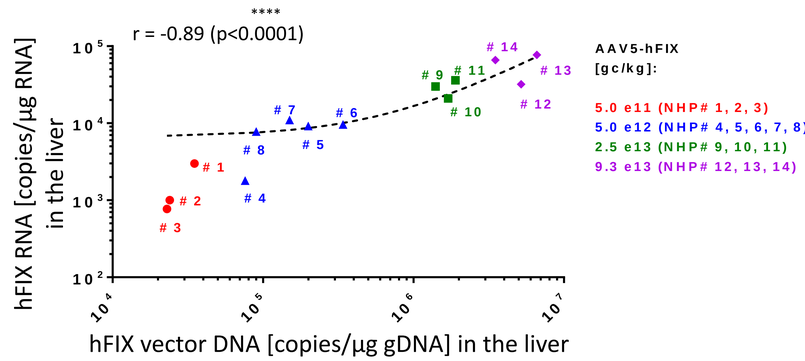



**b**

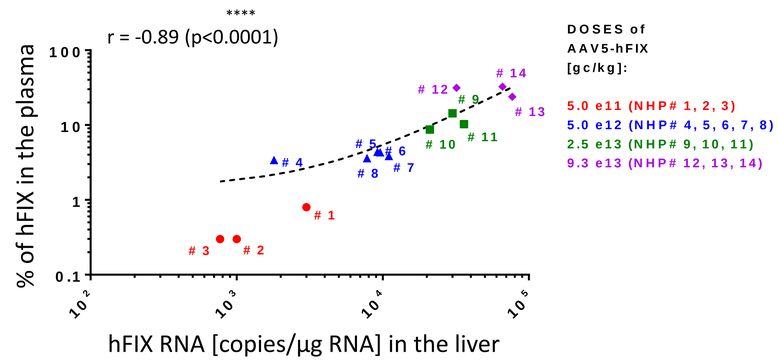



### 2. Liver transduction with AAV5-hFIX (DNA and RNA levels) (a) and hFIX levels in the plasma (b) are proportional to the dose of AAV5-hFIX injected

**a**




**b**




## Conclusion

Our data demonstrate that anti-AAV5 NAB titers up to at least 1:1031 do not impact transduction of the NHP liver by AAV5. This suggests that potentially even patients with pre-existing anti-AAV5 could benefit from AAV5-based gene therapy.

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