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).

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

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

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.