

# Prevalence and affinity/avidity assessment of pre-existing NABs against AAV2, 5 and 8 analyzed in the serum of 300 healthy donors

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## BACKGROUND

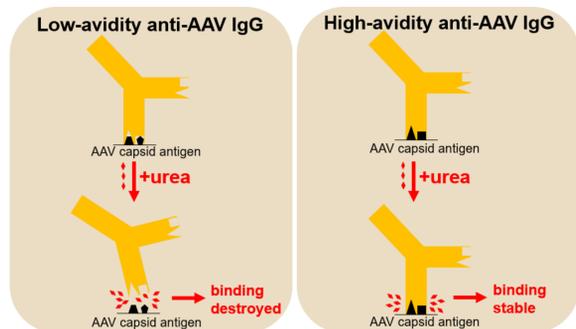
- Even low levels of anti-AAV2 or anti-AAV8 NABs have been related to a decrease or total impairment of AAV liver transduction in NHPs and humans when delivered intravenously [1, 2].
- We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector [3].
- Those results suggest that differences in the neutralization ability of antibodies might exist between AAV serotypes.

## OBJECTIVES

The aim of the present study was to assess the binding characteristics of the pre-existing anti-AAV NABs, found in the serum samples of healthy donors, to AAV antigen for AAV2, AAV5 and AAV8 serotypes. Prior to avidity analysis, the prevalence of NABs against AAV serotypes 1, 2, 5 and 8 in those serum samples was established.

## METHODS

Serum samples from 300 healthy human donors (100 American males, 100 European males and 100 American females) were purchased from BioreclamationIVT (West Sussex, UK). Samples were analyzed for the presence of anti-AAV1, anti-AAV2, anti-AAV5 and anti-AAV8 NABs with the use of anti-AAV NABs luciferase-based bio-assays. Serum samples of donors that returned anti-AAV NABs titers above 50 were analyzed for anti-AAV IgGs avidity (overall strength of antibody-antigen complexes). The Avidity index (AI) assesses effect of chaotrope agent on the antibody-AAV binding. AI is the serum dilution factor for half-maximal binding after urea exposure divided by the corresponding value in the absence of urea, expressed in percentage (Figure 1).



Avidity index (AI)/residual binding

- <40% Low: weak antibody-antigen complex binding
- 40-55% Intermediate
- >55% High: strong antibody-antigen complex binding

Figure 1. General principle and classification of avidity index assessment of anti-AAV IgGs present in serum samples.

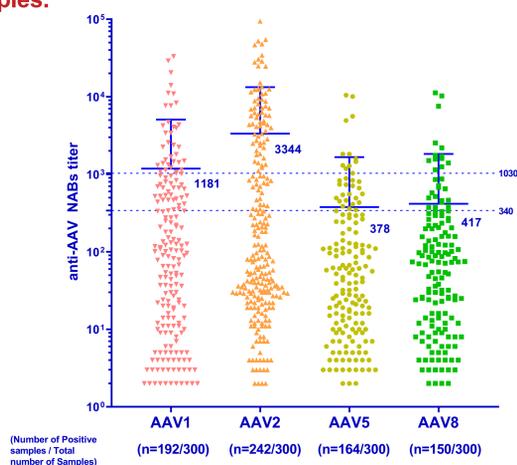


Figure 2. Pre-existing anti-AAV NAB prevalence in serum of healthy human donors. Plotted individual titers and mean with SD.

## RESULTS

### Anti-AAV5 and anti-AAV8 NABs are the least prevalent in the healthy donor population

- Serum samples from 300 healthy donors were screened for pre-existing NABs against AAV1, 2, 5 and 8. Thirty-nine donors (13%) had no detectable anti-AAV NABs against any of the serotypes analyzed (anti-AAV NABs titers were below 2). The highest prevalence of NABs in healthy donor serum was against AAV2 serotype as 81% (n=242 out of 300) of all donors had titers above 2. Less prevalent were NABs against AAV1 serotype, with 64% (n=192 out of 300) of donors being positive for anti-AAV1 NABs. Furthermore, prevalence of NABs against AAV5 and AAV8 in healthy donor serum samples were 55% (n=164 out of 300) and 50% (n=150 out of 300) respectively (Figure 2).

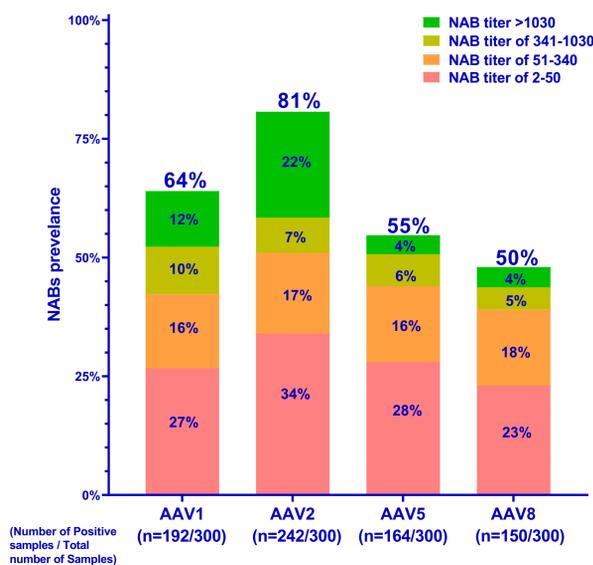


Figure 3. Prevalence and titer ranges of pre-existing NABs against AAV serotypes 1, 2, 5 and 8 in serum of 300 healthy human donors.

- Anti-AAV5 and anti-AAV8 NABs also presented the lowest seroprevalence (4%) of titers above 1030 among positive donors. On the other hand, NABs against AAV2 had the highest prevalence in a higher titer range (22%) (Figure 3).

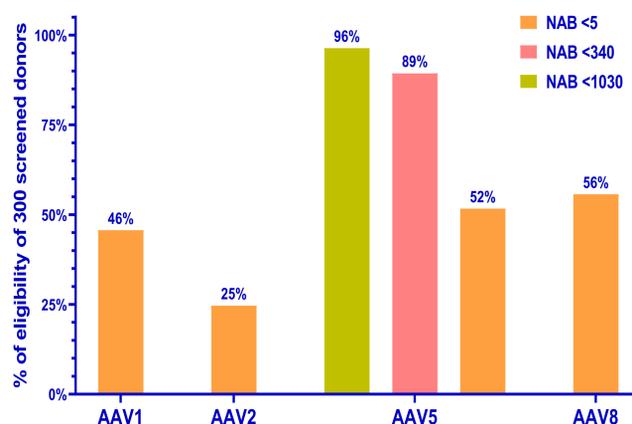


Figure 4. Theoretical systemic AAV-based treatment eligibility of 300 healthy donors.

- Our previously reported data in non-human primates indicate that anti-AAV5 titers up to 1030 and in humans up to 340 are compatible with efficient systemic AAV5-based gene delivery. 89% of 300 donors had anti-AAV5 NAB titers <340, while 96% of donors presented NABs titer <1030 (Figure 4).

### Anti-AAV5 antibodies found in healthy donor serum have weaker avidity to AAV5 antigen than anti-AAV2 or anti-AAV8 antibodies have to AAV2 or AAV8 antigens respectively

- Avidity of pre-existing AAV-specific IgG antibodies in healthy human population is significantly different between AAV serotypes. Pre-existing anti-AAV2 or anti-AAV8 IgGs form stronger antibody-antigen complexes with AAV2 and AAV8 antigens than pre-existing anti-AAV5 IgGs with the AAV5 antigen. Overall, these data suggest why pre-existing anti-AAV5 NABs measured *in vitro* do not interfere with the AAV5-based *in vivo* transduction as much as pre-existing anti-AAV NABs against other serotypes do. Furthermore, no significant differences between gender or ethnic origin on the prevalence or avidity of pre-existing anti-AAV antibodies was observed in the analyzed healthy human population (Figure 5).

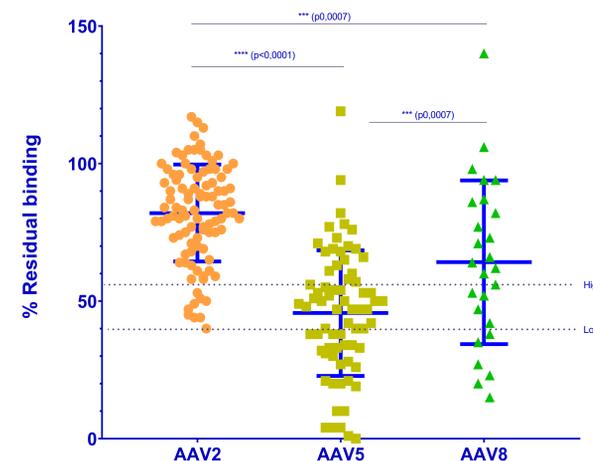


Figure 5. Avidity of AAV-specific IgG antibodies in healthy human population. Significant difference was observed between the different serotypes. One-Way ANOVA followed by Tukey's correction for multiple comparisons test analysis was performed.

## CONCLUSION

- We have previously reported that anti-AAV5 NABs titers up to 340 in humans and as high as 1030 in primates did not interfere with the therapeutic efficacy of intravenously administered AAV5 vector [3].
- The results obtained demonstrate that the avidity of pre-existing AAV-specific IgG antibodies in healthy human population is significantly different between AAV serotypes.
- Pre-existing anti-AAV5 IgGs create the weakest antibody-antigen complexes with the AAV5 antigen when compared to anti-AAV2 or anti-AAV8 IgGs. Indeed, pre-existing anti-AAV5 NABs measured *in vitro* do not interfere with the AAV5-based *in vivo* transduction as much as pre-existing anti-AAV NABs against other serotypes do.

## REFERENCES

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