Impact of neutralizing antibodies against AAV is a key consideration in gene transfer to nonhuman primates

To the Editor: Adeno-associated virus (AAV) vectors have been widely used as tools for gene delivery in animal studies as well as gene therapy–based medicines. Recently, Hongliang Li and colleagues[1–4] published four studies in *Nature Medicine* applying AAV serotype 8 vectors (AAV8) in nonhuman primate (NHP) models of nonalcoholic steatohepatitis. In each study, genes delivered to the liver via AAV vectors demonstrated transduction in these large animal models, and therapeutic effects were achieved.

Although AAV vectors are a good choice for the purpose of these studies, it is unclear from the methods reported whether the issue of neutralizing antibodies (NABs) was considered. It is well regarded in the gene therapy field that pre-existing immunity in the form of NABs against the viral capsids of AAV vectors is a major hurdle for successful AAV-based gene transfer in both NHPs and in humans[5]. Typically, 20–40% of patients are excluded from enrollment in liver-directed gene therapy with a specific AAV serotype because of pre-existing NABs[6]. The same concern applies to NHP studies, as AAV8 is an AAV serotype of NHP origin, and it is estimated that ~70–90% of monkeys have pre-existing NABs against AAV8 (refs [7,8]). In an unpublished study, we found that 4% of 120 monkeys were seronegative for the AAV8 NAB (data not shown). It should also be noted that the probability of NAB occurrence should actually be higher in older animals, such as those used in these studies (aged 8–9 years)[1–4], as they are more likely than younger animals to have been exposed to AAV8 and other AAV serotypes in their lifetime. Furthermore, in animals or humans lacking pre-existing NABs, the vector cannot be readministered (unless a different viral capsid is chosen) owing to potent NAB responses that occur within days after the initial exposure to vector, which can persist for months. The inability to readminister the vector because of potent NAB formation upon vector administration is a serious problem in the gene therapy field and has only partially been solved thus far by using alternate capsids, immune suppression and decoy capsid approaches.

However, the overall experimental design for the cited studies[1–4,9] in this correspondence challenges the conventional doctrine of previous findings on pre-existing NABs against AAV virus. For example, in the above studies in question, it was not stated whether any of the monkeys were screened for AAV8 NABs. And although a total of 2×10^{13} vector genomes of AAV8 vectors were injected into the portal vein and it may be possible that such a high dose administered via this route could overcome a modest level of pre-existing NABs, resulting in some level of gene transfer in some of the treated NHPs, it seems unlikely that such an approach would be effective in all the NHPs tested. Furthermore, in a correction to their CFLAR paper[1,9], the authors indicated that the same AAV8 vector was injected into a peripheral vein at week 7 to "ensure stable expression". However, the high-titer NABs against AAV8 that should have developed from the first injection would have prevented any successful gene transduction by the second administration, particularly via an intravenous route[10,11]. A similar readministration experiment that did not consider the NAB issue was carried out in the CYLD study[4]. Also, we should note that even minor differences in NAB titers should result in wide interanimal variability, even in small animals. But high transduction efficiency and low variability were reported in these studies, in sharp contrast to the experience of many others.

Finally, we should also note that the authors used GFP to assess the efficacy of gene transfer to the liver. They show transduction of nearly 100% of hepatocytes, and expression was maintained for 30 weeks. This result is rather surprising, considering that no one else in the field has been able to achieve uniform liver gene transfer even if all monkeys were prescreened for NABs, because AAV8 strongly prefers hepatocytes near the portal vein structures in NHPs[12,13]. In addition, no one has been able to achieve long-term expression of GFP after AAV administration to the liver of NHPs[14]. Although hepatic gene transfer can promote immune tolerance to transgene products, GFP is highly immunogenic in NHPs, resulting in potent $CD8^+$ T cell responses that eliminate transduced hepatocytes. Thus, it is unclear how long-term GFP gene transfer to nearly all hepatocytes was accomplished.

Data availability. All data supporting this article are available from the authors upon reasonable request. ❐

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Published online: 4 June 2018
https://doi.org/10.1038/s41591-018-0062-2

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Competing interests

The authors declare no competing interests.