Selecting the Best AAV Capsid for Human Studies

To the editor:

The best approach for selecting a recombinant adeno-associated virus (rAAV) capsid that is most likely to be highly effective in AAV-mediated human gene therapy applications remains a complex issue with no definitive solution to date. Numerous factors are known to be important. For example, the presence of large amounts of preexisting neutralizing antibodies will inhibit transduction. Similarly, capsid-induced T cell–mediated responses can eliminate transduced hepatocytes in humans. Although this response has yet to be fully understood or recapitulated in animal models, it can be reduced by a short course of oral steroids at its onset. However, even in the absence of these complex variables, we still lack the ability to determine which capsid provides the most robust vector transduction efficiency for a particular organ in humans. Even though there are only limited clinical data, rAAV serotypes 2 and 8 (rAAV2, rAAV8) vectors containing a human factor IX expression cassette administered systemically into humans resulted in similar peak levels of plasma factor IX in hemophilia B patients.[1–3] While the rAAV2 dose response in various animal studies correlates with the human data, similar animal studies using rAAV8 vectors predict human transgene expression levels much higher than what has been observed.

We recently proposed that a mouse ($Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-}$) partially repopulated with primary human hepatocytes may be more predictive than other animal models including nonhuman primates when screening rAAV vectors for their intrinsic transduction potential of human liver before consideration for human trials.[4] The chimeric liver contains a mixture of mouse and primary human hepatocytes. We used this model to compare relative transduction efficiencies of rAAV2 and rAAV8 vectors in human and mouse hepatocytes, and our results parallel the human clinical data to date. We demonstrated that by using transgene expression as the readout, rAAV2 transduced mouse and human cells equivalently, yet rAAV8 transduced mouse hepatocytes at least 10 times better than human hepatocytes.[4]

In a study reported in *Molecular Therapy*, Wang *et al*.[5] used the same humanized mouse model and showed that rAAV8 transduction in human hepatocytes was higher than what our laboratory reported.[4] The authors used a single human donor, whereas our experiments were consistent between independent experiments performed with two unrelated hepatocyte donors (Figures 1 and 4 in ref. 4) performed in two different laboratories. Strikingly, the results of the study by Wang *et al.* are not consistent with their earlier collaborative work in which rAAV8 transduction in humanized mice was similar to what we observed (Supplementary Figure 4 in ref. 6). In addition, we waited no more than 14 days (and in some studies measured transgene expression every 2 days), whereas Wang *et al.*[5] waited 3 weeks before measuring transduction. We also maintained our animals on low-dose NTBC, a drug required to minimize continued mouse hepatocellular turnover and human hepatocellular regeneration, which would affect final estimates of transduction in both the mouse and human hepatocytes. We purified our vectors by CsCl centrifugation, whereas Wang *et al*. purified most of their vectors (except AAV2) by iodixanol gradient fractionation, which results in a higher proportion of empty capsids. Finally, we found that transduction measured by transgene expression and vector genomes do not necessarily correlate and can vary between species.[4] Therefore, quantifying vector genomes may not reflect the true transduction efficiency and should not be used as the primary type of analysis. These factors underscore the importance of testing promising rAAV vectors in different models and by independent laboratories.

In addition to testing the relative transduction efficiency of parental vectors, we passaged a shuffled AAV capsid library constructed from 10 multispecies parental variants[4] through four rounds of selection in humanized mice. The most abundant selected capsids were used to make vectors. We found a chimeric capsid (LK03) that after vectorization (rAAVLK03) was 10 to 20 times more efficient at transducing human hepatocytes *in vivo* compared to rAAV8 with the same expression cassette.[4] Although the LK03 capsid most closely resembles AAV3B, based on total amino acid sequence, this capsid is derived from at least four different parental serotypes. This suggests that important AAV capsid domains were selected for in our screen. Moreover, a single amino acid change in LK03, resulted in a capsid (LK19) with altered transduction parameters.[4] Interestingly, Wang *et al*.[5] found that rAAV8 was superior to rAAVLK03 in transducing nonhuman primates, whereas AAV3B was more robust than AAV8. Li *et al*.[7] found that rAAV3B and a 3B variant were substantially better than rAAV8 at transducing nonhuman primate liver. Moreover, we are aware of additional studies in which rAAVLK03 expressing human factor IX performed at least as well, if not better than rAAV8 in nonhuman primates (A. Nathwani and A. Davidoff *et al*., personal communication). Regardless of these findings, there are not as yet enough data to establish a definitive generalizable correlation between nonhuman primates and human studies.

No model organism will substitute for a human, but the question that remains is how well the human xenotransplant models can predict human transduction outcomes. In the case of the liver model, Wang *et al.* propose that differences in blood vasculature supply to the human hepatocytes and/or the lack of nonparenchymal human cells are important parameters that could affect the predictive reliability of this model. Yet, such variables are not consistent with the similar rAAV2 transduction results observed in both mouse and human hepatocytes.[4] We believe the variables more likely to influence transduction outcomes include (i) human-to-human variation between hepatocyte donors, (ii) the relative ratio of human to mouse hepatocytes in individual mice, (iii) conditions in which the chimeric mice are maintained before measuring gene transfer/transduction, and (iv) method used to quantify vec-

tor transduction. Until more alternate serotypes such as LK03 are studied in humans, we will not be able to directly establish the value of such studies. However, xenotransplantation models are not restricted to the liver, and perhaps gene transfer predictions might be made using other humanized organ systems (e.g., skeletal muscle, heart, or brain). At this time, our study comparing rAAV2 and rAAV8 transduction in humanized liver mice best recapitulates human clinical trial data to date. The value of these and other related models in predicting human outcome is an important area of continued investigation.

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DISCLOSURE

MAK is a scientific founder of Voyager Therapeutics and Logic Biotherapeutics. MAK is an inventor on patents regarding AAV-LK03 held by Stamford University.

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