GENE THERAPY OF HEMOPHILIA B: IDENTIFICATION OF THE MOST EFFICIENT AAV SEROTYPE VECTOR FOR TRANSDUCTION OF HUMAN HEPATIC CELLS

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Key Points:

1. A number of gene therapy trials for hemophilia B have been performed with different AAV serotype vectors (AAV2, AAV5, AAV8, AAVrh10), none of which are truly human liver-tropic.
2. Two of the serotypes, AAV5 (Hemgenix) and AAVrh74 (Beqvez) were approved by the FDA in 2022 and 2023, respectively, both priced at $3.M/dose. Beqvez was discontinued in 2024.
3. AAV3 serotype vectors transduce both a human hepatocyte cell line and primary human hepatocytes most efficiently, and therefore, should prove to be safe and efficacious in the potential gene therapy of hemophilia B.

INTRODUCTION

In 2022, the United States Food and Drug Administration (USFDA) approved Hemgenix, an AAV5-based vector from uniQure for gene therapy of hemophilia B. A relative high dose of $2x10^{13}$ vgs/kg of this vector leads to up to ~12% expression of the human clotting factor IX (hFIX). In 2024, the USFDA also approved Beqvez, an AAVrh74-based vector from Pfizer for gene therapy of hemophilia B, which leads to up to 21% expression of hFIX at a dose of $5x10^{11}$ vgs/kg. Both Hemgenix and Beqvez are priced at $3.5M/dose. However, neither AAV5 nor AAVrh74 serotype vectors possess selective tropism for human liver. More than a decade and a half ago, we observed that of the 10 most commonly used AAV vectors, AAV3 is the most efficient in transducing primary human hepatocytes *in vitro* (*Molecular Genetics & Metabolism*, 98: 289-299, 2009). Subsequently, we reported AAV3 vectors to be 82x more efficient than AAV5 vectors in transducing primary human hepatocytes in humanized mice *in vivo* (*Molecular Therapy*, 24: 1042-1049, 2016). In our current studies, we compared the transduction efficiencies of AAV5, AAVrh74, and AAV3 vectors in human hepatic cells under identical conditions. The results indicate AAVrh74 vectors are ~6x more efficient than AAV5 vectors, whereas AAV3 vectors are ~1,100x more efficient than AAV5 vectors. AAV3 vectors are also ~6x more efficient than AAVrh74 vectors. We have previously reported the remarkable tropism of AAV3 vectors to be mediated by the human hepatocyte growth factor receptor (huHGFR), which AAV3 utilizes as a cellular co-receptor to gain entry into human hepatic cells (*Human Gene Therapy*, 21: 1741-1747, 2010). We have also reported that AAV3 vectors mediate therapeutic levels of hFIX expression in humanized mice (*Human Gene Therapy*, 31: 1114-1123, 2020) as well as in non-human primates (*Molecular Therapy Methods & Clinical Development*, 23: 98-107, 2021). All of these proof-of-concept studies make a compelling case for the use of AAV3 vectors with lower immunogenicity, improved safety, ensuring translation to the clinic with higher probability of success for gene therapy of hemophilia B. Furthermore, the reduced vector production costs as well as lower cost per patient should allow the eligible patient population worldwide to benefit from AAV3 vector-mediated gene therapy of hemophilia B in particular, and human liver diseases in general, in which other AAV serotype vectors have proven to be less than optimal.

RESULTS

First generation of AAV3 vectors are significantly more efficient than first generation of AAV5 (Hemgenix) and AAVrh74 (Beqvez) vectors in transducing human hepatic cells

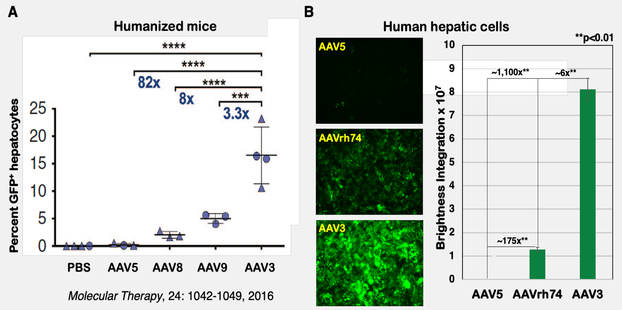

Figure 1: (A) Transduction efficiencies of AAV5, AAV8, AAV9, and AAV3 vectors in human hepatocytes in humanized mice *in vivo*. **(B)** Transduction efficiencies of AAV5, AAVrh74, and AAV3 vectors in human hepatic cell line, Huh7. Cells were transduced in triplicates with $3x10^3$ vgs/cell with each of the vectors expressing the EGFP reporter gene. Transgene expression was visualized and quantitated 72 hrs post-transduction using a Keyence microscope.

AAV3 vectors are also more efficient than AAV5 in transducing primary human hepatocytes, and capsid-modified NextGen AAV3 vectors mediate therapeutic levels expression of hFIX in "humanized" mice and in non-human primates

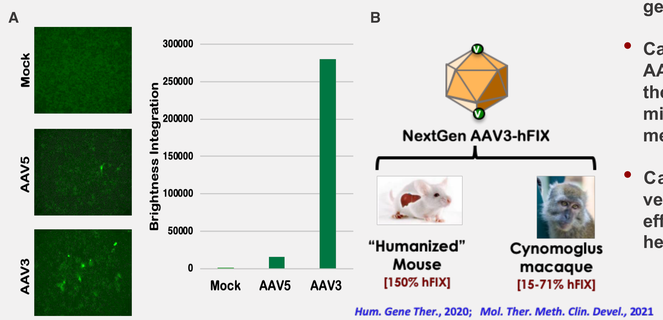

Figure 2: (A) Transduction efficiencies of first generation of AAV5 and AAV3 vectors in primary human hepatocytes *in vitro*. Cells were transduced with $3x10^4$ vgs/cell with each of the vectors expressing the EGFP reporter gene and transgene expression was visualized and quantitated 72 hrs post-transduction using a Keyence microscope. **(B)** Schematic representation of the capsid-modified NextGen AAV3 vector and therapeutic levels expression of hFIX in "humanized" mice and in non-human primates.

CONCLUSIONS

- First generation of AAV3 serotype vectors are significantly more efficient than AAV5 (Hemgenix) and AAVrh74 (Beqvez) serotype vectors that have been used in gene therapy of hemophilia B.
- Capsid-modified S663V+T492V NextGen AAV3 vectors also mediate expression of therapeutic levels of hFIX in "humanized" mice and in non-human primates at low to medium doses.
- Capsid-modified NextGen AAV3-hFIX vectors should prove to be safe and effective for long-term gene therapy of hemophilia B.

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