Using recombinant adeno-associated viral vectors for long-term expression of a hyperactive human Factor IX mutant in hemophilic mice and comparison of AAV-LK03 and AAV-KP1 in nonhuman primates

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ABSTRACT

We used CB 2679d-GT – a single-stranded recombinant adeno-associated virus (rAAV) vector with a strong liver-specific promoter to express a next generation human coagulation Factor IX (huFIX) variant, in a hemophilic mouse model and in rhesus macaques. CB 2679d-GT carries the same huFIX sequence as the subcutaneously delivered dalcinonacog alfa, a high potency FIX variant developed using rational design with three amino acid substitutions. Daily subcutaneous delivery raised FIX levels in severe hemophilia B patients into the mild hemophilia range in a Phase 2b clinical study (Mahlangu et al., Haemophilia 2021). A novel chimeric capsid, KP1, was used to package the CB 2679d-GT rAAV construct. KP1 was shown to transduce human and mouse hepatocytes with high efficiency in a chimeric humanized xenograft mouse model (Pekrun et al., JCI Insight 2019).

We injected groups of 3 or 5 hemophilic mice with $1x10^{11}$ or $1x10^{12}$ vector genomes (vg)/kg respectively of an rAAV vector expressing the mouse codon optimized CB 2679d-GT sequence under control of the ApoE-HCR-hAAT promoter. Over 3 months, antigen and activity levels for this construct were considerably higher than in a previous study employing the DJ-8 capsid and a self-complementary expression construct (Nair et al., Blood 2021). Specific huFIX activity in plasma from mice injected with CB 2679d-GT was approximately 10- or 2-fold higher than mice injected with wild-type or Padua huFIX rAAV, respectively. We subsequently infused capsid seronegative juvenile rhesus macaques with $3.5x10^{12}$ vg/kg of a human codon-optimized and CpG depleted version of CB 2679d-GT packaged with either KP1 or LK03 capsid (N=3 per group). LK03 had previously been shown to selectively transduce human hepatocytes when tested in a xenograft mouse model (Lisowski et al., Nature 2014) and successfully delivered a huFVIII expression vector in a clinical study (George et al., NEJM, 2021). Animals injected with KP1-CB 2679d-GT had up to 10-fold lower peak expression levels of the variant huFIX than those injected with LK03-CB 2679d-GT. Two animals from each group showed sustained huFIX expression at 3 months post-administration with antigen levels ranging from 2% to 4% of the normative human range and activity levels between 50% and 80% of the normative human range, consistent with the enhanced potency of this huFIX variant. One animal from each group showed declining circulating huFIX antigen levels beginning after day 28, coinciding with the emergence of high anti-huFIX antibody levels. Transduction as measured by 3-month liver DNA and mRNA levels was approximately 10-fold lower in the KP1 group compared to the LK03 group. However, the data obtained from this rhesus study does not rule out that human liver may be transduced more efficiently using the KP1 capsid, as both capsids provided similar levels of human hepatocyte transduction in the xenograft mouse model.

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INTRODUCTION

Cb 2679-GT is an engineered next-generation coagulation Factor IX using rational protein design with enhanced functionality through triplet substitutions (R318Y, T343R and R338E) that increase catalytic activity, increase resistance to antithrombin inhibition and improve affinity for activated FVIII.

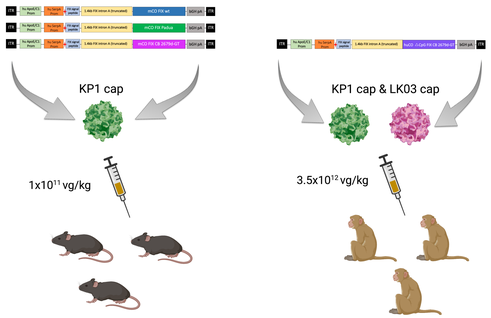
In a Phase 1/2 study these enhancements resulted in a 22-fold improved activity over BeneFIX® which has wild-type (wt) huFIX sequence[1].

When this hyperactive huFIX variant was expressed in hemophilic mice using a self-complementary AAV-DJ8 vector activity of huFIX in the plasma was enhanced considerably over that of the Padua variant and clotting time was reduced[2].

The LK03 capsid was derived from a shuffled capsid library screen for transduction of human hepatocytes in the humanized liver mouse model[3] and has shown promising results in a Phase II clinical trial for Hemophilia A[4]. The KP1 capsid was selected after screening a barcoded capsid shuffled AAV library for transduction of human islet cells[5]. When tested in the humanized liver mouse model KP1 was found to transduce mouse and human liver cells with high efficiency[5].

In the current study we used the KP1 capsid to package a single-stranded rAAV genome with a mouse codon-optimized huFIX sequence and compared the performance of the novel huFIX variant with the huFIX wild-type as well as the Padua sequences in hemophilic mice. In a subsequent nonhuman primate study KP1 as well as LK03 capsids were used to package a CpG depleted human codon optimized version of Cb 2679-GT. Juvenile rhesus macaques were injected with those vectors and expression of huFIX was monitored over a course of 14 weeks.

METHODS



RESULTS

After injection of the three different vectors into hemophilic mice, antigen levels, vector copies, and transcript levels were comparable between all groups while specific huFIX activity in the CB 2679d-GT group was around 10-fold higher than in the wild-type and 2- to 3-fold higher than in the Padua group (Fig. 1). Antigen and activity levels were approximately 10-fold higher using this improved expression cassette packaged with KP1 capsid as compared to a previous study that had employed a self-complementary rAAV Cb 2679-GT construct packaged with DJ-8 capsid[2].

We infused rhesus macaques intravenously that had tested negative for pre-existing α-capsid neutralizing antibodies with LK03 and KP1 packaged human codon-optimized and CpG depleted CB 2679d-GT rAAV vector. Human FIX plasma levels spiked shortly after administration and then dropped to low, but persistent levels (Fig. 2A and B). Two animals in the LK03 group showed transient elevations in liver enzymes (not shown). Two animals from each group lost huFIX antigen expression several weeks post injection, coinciding with the emergence of high levels of α-huFIX antibodies (Fig. 2C). Human FIX transcript levels in liver samples harvested at the end of the study were dramatically reduced in those two animals when compared to the other animals from the same group (Fig. 3B). However, loss of expression was not found to be correlated with a loss of vector containing hepatocytes as determined by comparable vector copy numbers (Fig. 3A). This finding suggests that transcription from episomal rAAV may have been silenced in these two animals.

Vector copy numbers as well as huFIX transcript levels were significantly higher in the livers of the LK03 group animals as compared to the KP1 group animals as shown by qPCR (Fig. 3A and B) and confirmed by RNAScope *in situ* hybridization (Fig. 4) and Southern Blot analysis (Fig. 5A). High-molecular weight forms of rAAV episomes could only be detected in the LK03 group animals due to higher abundance of vector (Fig. 5B).

Both vectors showed limited off-target transduction with the highest vector copy numbers detected in spleen (Fig. 3C). However, due to the liver specific promoter huFIX transcripts were non-detectable in those tissues (data not shown).

Recombinant AAV vectors were largely cleared from the plasma within two weeks post infusion (Fig. 2D). Neutralizing α-capsid antibodies were detected as early as 3 days post injection and titers remained at high levels starting at 1 week post infusion (data not shown).

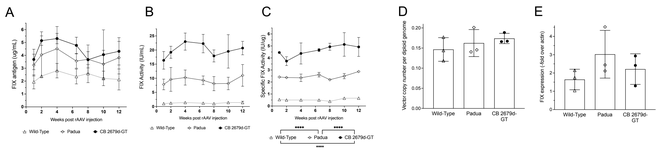


Figure 1. Hemophilic mice were injected with $1x10^{11}$ vg/kg of the huFIX wild-type, Padua, or Cb 2679-GT KP1 packaged rAAV constructs (n=3). Antigen levels (A), activity levels (B) and specific activity levels (C) were determined in plasma samples over a course of 3 months. After 3 months mice were euthanized and vector copy numbers (D) and huFIX transcript levels (E) were determined in mouse liver using qPCR. Two-way ANOVA was used to determine statistically significant differences.

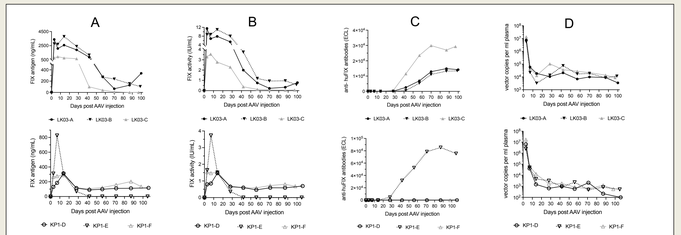


Figure 2. Juvenile male rhesus macaques were injected with $3.5x10^{12}$ vg/kg of the Cb 2679-GT rAAV construct (n=3) packaged with LK03 (upper panel) or KP1 (lower panel) Cb 2679-GT rAAV construct (n=3). Antigen levels (A), baseline-corrected activity levels (B), α-huFIX antibodies (C), and vector copies (D) were determined in plasma samples over a course of 14 weeks.

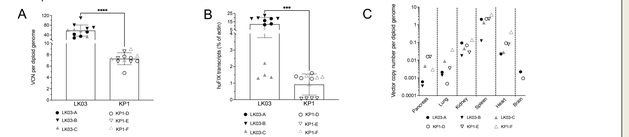


Figure 3. Rhesus macaques were euthanized 14 weeks post administration and each liver lobe was analyzed for vector copies (A) and huFIX transcript levels (B). A select number of other tissues were analyzed for vector copy numbers (C). Only one animal from each group was analyzed for the presence of vector in the brain.

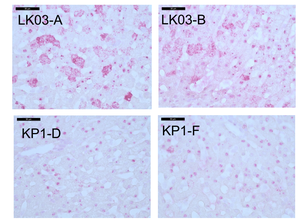


Figure 4. RNAScope *in situ* hybridization performed on rhesus liver sections using a huFIX specific probe. Representative images are shown at a 40x magnification.

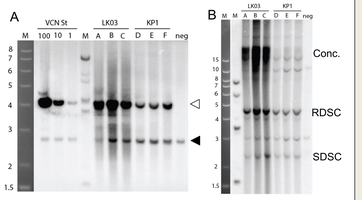


Figure 5. Analysis of rhesus macaque livers for vector copies (A) and episomal forms (B). 10 ug gDNA was digested with two enzymes that cut close to the ITRs (A), or with two enzymes that do not cut within the vector sequence (B). Open arrow: rAAV monomers, closed arrow: endogenous albumin, Conc.: concatemeric forms, RDSC: relaxed double-stranded circular monomers, SDSC: supercoiled double-stranded circular monomers.

REFERENCES AND ACKNOWLEDGEMENTS

1. Mahlangu, J., et al. (2021). Efficacy and safety of subcutaneous prophylaxis with dalcinocog alfa in adults with hemophilia B. *Haemophilia 27: 574-580*
2. Nair, N., et al. (2021). Gene therapy for hemophilia B using CB 2679d-GT: a novel factor IX variant with higher potency than factor IX Padua. *Blood 137: 2902-2906*
3. Lisowski L., et al. (2014). Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. *Nature 506: 382-386*
4. George, L.A., et al. (2021). Multiyear factor VIII expression after AAV gene transfer for hemophilia A. *N Engl J Med* 385: 1961-1973
5. Pekrun, K. et al. (2019). Using a barcoded AAV capsid library to select for clinically relevant gene therapy vectors. *JCI Insight* 4: e131610

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