AAV BASED HEMOPHILIA B GENE THERAPY IN MICE USING FIX-CB2679d-GT

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STUDY OBJECTIVES

To provide preclinical proof of concept in hemophilia B mice for an AAV-based gene therapy and demonstrate the superiority of an AAV vector encoding FIX-CB2679d-GT over an AAV vector encoding FIX-R338L Padua

CONCLUSIONS

+ FIX antigen and activity levels remained steady and durable for up to 20 weeks with both FIX-CB2679d-GT and FIX-Padua
+ FIX-CB2679d-GT demonstrated a ~3-fold superior and statistically significant improvement in clotting activity and sustained potency when compared to FIX-Padua
+ FIX-CB2679d-GT showed a significant 4-5 fold reduction in tail clip bleeding time over FIX-Padua, thus achieving a more rapid and robust hemostatic correction of bleeding and reduction in blood loss

INTRODUCTION

+ Catalyst Biosciences has developed a next-generation engineered coagulation Factor IX, dalcinonacog alfa 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
+ A Phase 1/2 study demonstrated these enhancements result in a 22-fold improved potency over BeneFIX® in humans enabling administration by subcutaneous injection for routine prophylaxis
+ The FIX-CB2679d-GT variant may be an attractive candidate for development of a gene therapy approach for hemophilia B

METHODS

+ Codon optimized versions of FIX-CB2679d-GT and FIX-R338L Padua were prepared on the T148A background and cloned into an AAV vector downstream of a constitutive liver and hepatocyte-specific promoter (alpha1-antitrypsin). The AAV vectors were packaged with an AAV/DJ8 capsid
+ The *in vivo* performance of FIX-CB2679d-GT and FIX-Padua were assessed in FIX-deficient hemophilia B mice injected with $1x10^9$ vg/mouse, $5x10^9$ vg/mouse, $1x10^{10}$ vg/mouse or vector alone for 20 weeks
+ Plasma levels of FIX protein were evaluated a human specific ELISA (ASSERACHROM IX:Ag, Diagnostica Stago)
+ The potency of each vector was assessed throughout the study by measuring the FIX activity levels with an aPTT-based single-stage clotting assay on an ACL-TOP with quantification using the manufacturer reference was traceable to the WHO standard (09/172) (Haematologic Technologies, Inc.)
+ The *in vivo* efficacy at week 20 was evaluated for the $5X10^9$ and $1X10^{10}$ vg/mouse doses in a murine bleeding model (2.5-3 mm tail clip)

Statistical Analyses

+ A comprehensive global two-way repeated measures analysis of variance for the the $5x10^9$ vg/mouse and $1x10^{10}$ vg/mouse data sets was used to evaluate the FIX activity levels (U/mL) and normalized FIX activity levels (mU/ng)
+ Phenotypic analyses were evaluated by ordinary one-way ANOVA and Bonferroni's multiple comparisons test

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FIGURE 1: FIX ANTIGEN LEVELS INCREASE WITH VECTOR DOSE AND REMAIN STABLE FOR UP TO 20 WEEKS

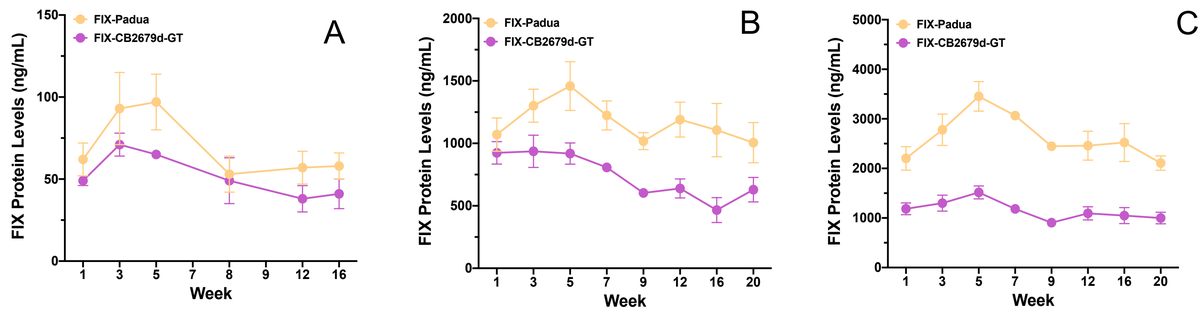


Figure 1: FIX levels for both FIX-Padua and FIX-CB2679d-GT increased with vector dose and remained durable for 20 weeks. The FIX-CB2679d-GT AAV construct produced consistently lower levels of protein than FIX-Padua at all dose levels. (A) $1x10^9$ vg/mouse, (B) $5x10^9$ vg/mouse and (C) $1x10^{10}$ vg/mouse

FIGURE 2: IMPROVED EFFICACY DEMONSTRATES SUPERIOR HEMOSTATIC POTENCY OF FIX-CB2679d-GT AT $5x10^9$ vg/mouse

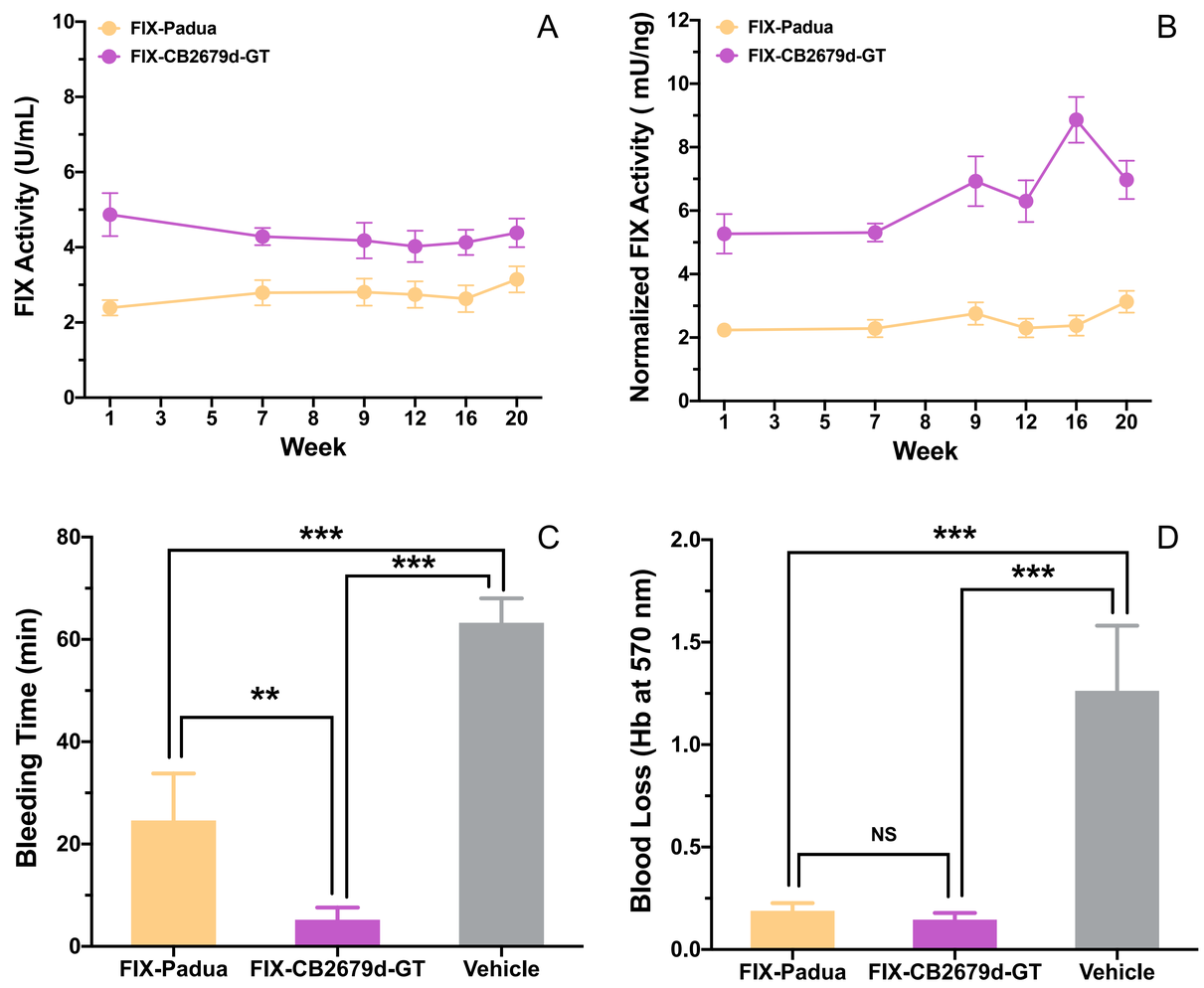
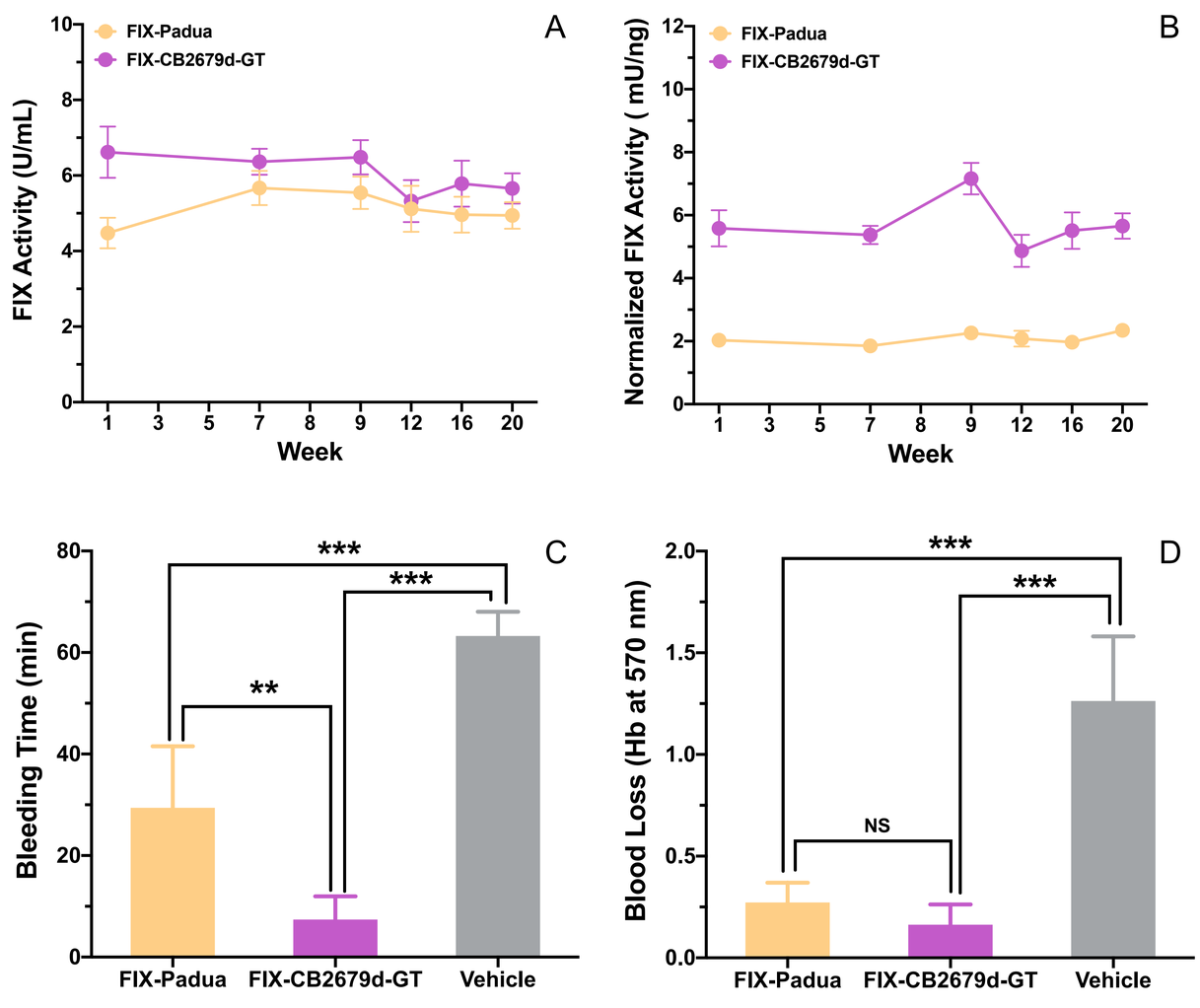


Figure 2: When normalized for the differential protein expression, enhancement of clotting activity was 2-3 fold greater (P=0.04). (A) FIX activity in U/mL ± SEM (B) FIX activity normalized to FIX protein and expressed as mU/ng ± SEM (C) *in vivo* efficacy expressed as bleeding time ± SD (D) *in vivo* efficacy expressed as blood loss ± SD (*** P<0.001, ** P<0.01, * P<0.05 and NS – Not Significant)

FIGURE 3: IMPROVED EFFICACY DEMONSTRATES SUPERIOR HEMOSTATIC POTENCY OF FIX-CB2679d-GT AT $1x10^{10}$ vg/mouse



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DISCLOSURES

G.E. Blouse & J. Landau: employees and shareholders of CATALYST BIOSCIENCES, T. VandenDriessche and M.K. Chuah: grant/research support from CATALYST BIOSCIENCES, T. VandenDriessche, M.K. Chuah and N. Nair: employees of VRIJE UNIVERSITEIT BRUSSEL