Hemophilia B Gene Therapy in Mice Using a Novel Chimeric AAV Capsid Combined With the Potency Enhanced CB 2679d-GT FIX Variant

P030

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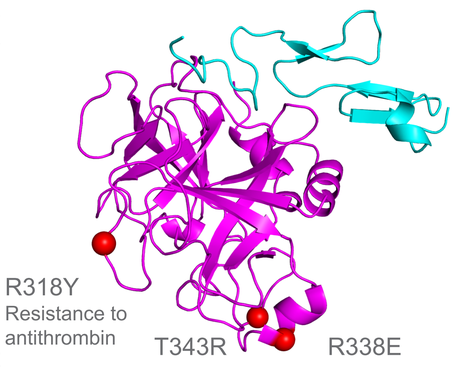
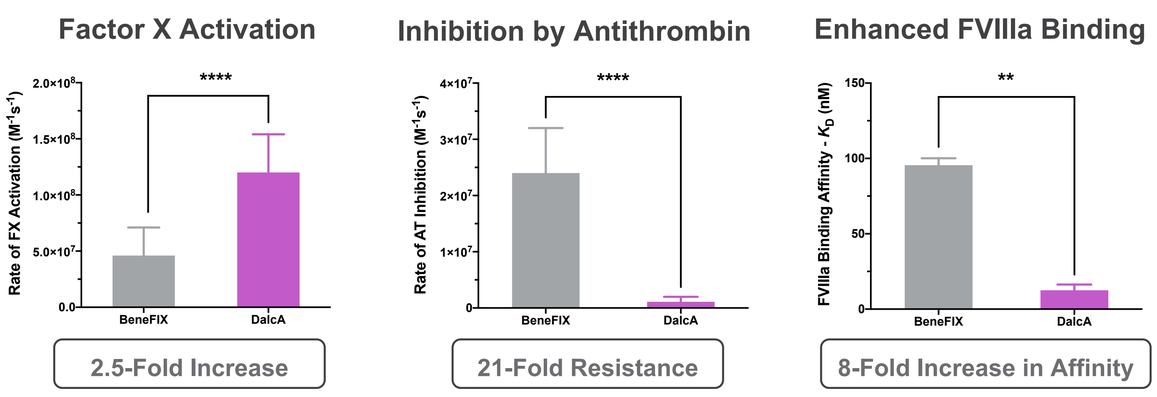
Conclusion

Combination of a next generation AAV vector with CB 2679d-GT, a rationally designed and potency enhanced FIX variant, has the potential to significantly improve transgene expression and FIX activity to effectively lower the viral dose needed for achieving relevant and protective FIX activity levels by 10-fold or greater

Background

+ We engineered a 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
+ A Phase 1/2 study demonstrated these enhancements resulted in a 22-fold improved activity over BeneFIX®
+ We have demonstrated that an AAV construct expressing CB 2679d-GT in hemophilia B mice significantly reduced tail clip bleeding time (4-5-fold) over that of Padua FIX, thus achieving a more rapid and robust hemostatic correction of bleeding and reduction in blood loss[1]



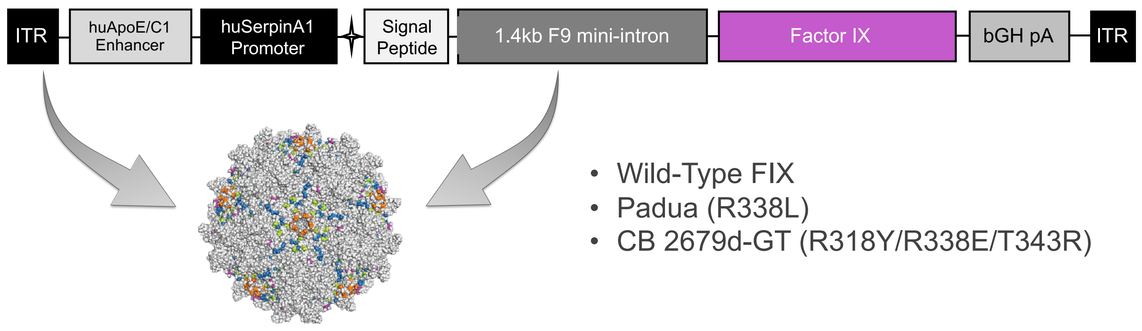


(**** P<0.0001, *** P<0.001, ** P<0.01 and * P<0.05)

Study Objective

To demonstrate that combination of a novel high transducing AAV chimeric capsid with the CB 2679d-GT transgene would provide significantly enhanced *in vivo* expression and FIX activity over previously observed levels

AAV Vector Design



- Wild-Type FIX
- Padua (R338L)
- CB 2679d-GT (R318Y/R338E/T343R)

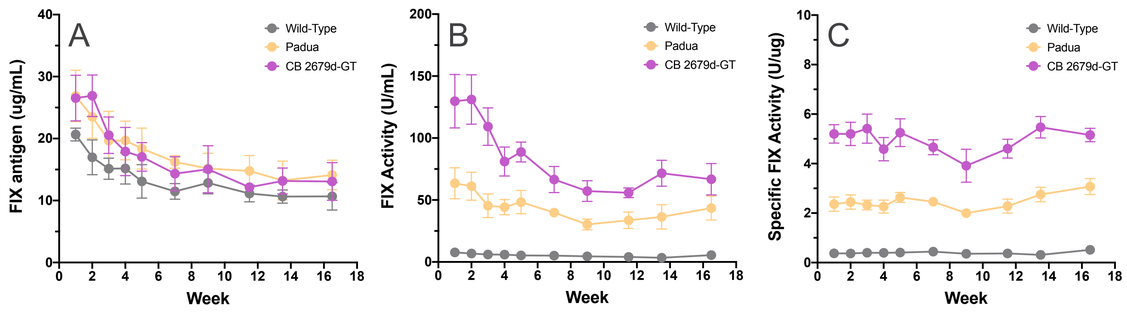
FIX minigene constructs were packaged into a novel AAV capsid designed through DNA shuffling

Methods

+ Codon optimized Wild-Type, CB 2679d-GT and R338L Padua FIX AAV constructs were prepared on the T148 background downstream of a robust hepatocyte-specific promoter in the ApoE/HCR-hAAT-hFIX minigene-bGH pA vector and packaged into a novel chimeric AAV capsid identified through DNA shuffling
+ C57BL/6 FIX-deficient mice (3-5 mice/group) were injected via the tail vein with either $2.0x10^8$, $2.0x10^9$ or $2.0x10^{10}$ vector genomes per mouse (vg/mouse) corresponding to $8x10^{11}$, $8x10^{10}$ or $8x10^9$ vg/kg assuming a nominal mouse weight of 25 grams with of AAV FIX vectors expressing either CB 2679d-GT, Padua, or wild-type FIX. Blood was collected for examination of the antigen levels and FIX activity levels
+ FIX activity was assessed by an activated partial thromboplastin time (aPTT) Factor IX single-stage clotting assay on an ACL-TOP instrument (Instrumentation Laboratories) using the recommended HemosIL® or SynthasIL® reagents and calibrators

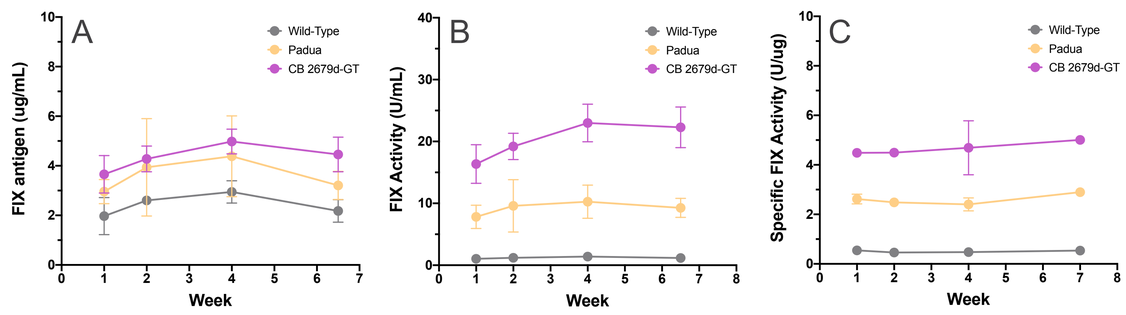
Results

The *in vivo* performance of the novel capsid / transgene at $8x10^{11}$ vg/kg



FIX antigen and activity levels were stable by week 5 and remained durable for the remaining 12 weeks of the study. (A) FIX antigen as determined by ELISA, (B) FIX activity levels as determined by aPTT (C) FIX specific activity calculated as the ratio of FIX activity/antigen. Data are presented as mean ± S.D

The *in vivo* performance of the novel capsid / transgene at $8x10^{10}$ vg/kg



FIX antigen and activity levels increased over the first three weeks and remained stable through the 6.5 weeks of the ongoing study (data cut-off). (A) FIX antigen as determined by ELISA, (B) FIX activity levels as determined by aPTT (C) FIX specific activity calculated as the ratio of FIX activity/antigen. Data are presented as mean ± S.D

The *in vivo* performance of the novel capsid / transgene at $8x10^9$ vg/kg

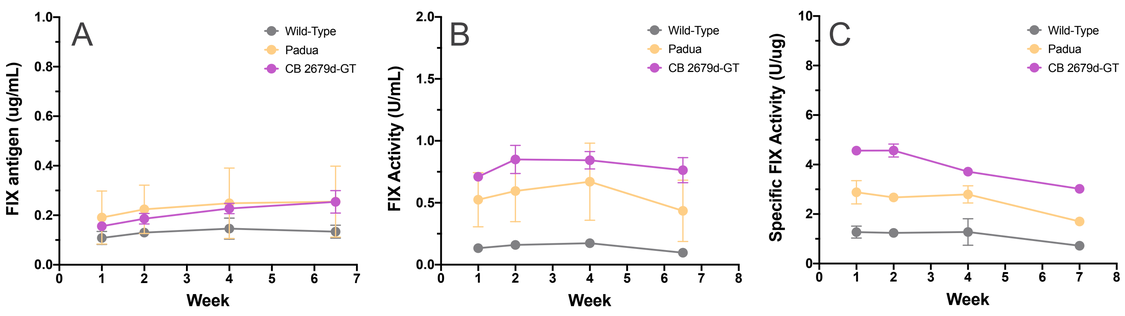


Figure 3: FIX antigen and activity levels remained stable through the 6.5 weeks of the ongoing study (data cut-off). (A) FIX antigen as determined by ELISA, (B) FIX activity levels as determined by aPTT (C) FIX specific activity calculated as the ratio of FIX activity/antigen. Data are presented as mean ± S.D

Combination of the CB 2679d-GT transgene with a novel capsid provides superior FIX levels at 1/10 the AAV dose compared to previous studies[1] and currently reported constructs using the Padua transgene[2]

FIX Transgene	AAV Capsid	Study Dose (vg/kg)	FIX Activity (U/mL)
CB 2679d-GT	**Novel Chimeric**	**$8.0x10^{10}$**	**20**
Padua	TAK-748[2]	$7.4x10^{11}$	20
Padua	TAK-748[2]	$7.4x10^{10}$	1
CB 2679d-GT	DJ/8[1]	$2.0x10^{11}$	4
CB 2679d-GT	DJ/8[1]	$4.0x10^{10}$	1

AAV dose levels and reported FIX activity were compared to the mid-dose data in the present study ($8.0x10^{10}$ vg/kg)

CB 2679d-GT in combination with a novel chimeric capsid provides a significant improvement in FIX activity levels (10-fold to 20-fold) depending on the vector construct and dose of the comparator

Bibliography

+ Blouse GE, Nair N, Vandendriessche T, Chuah MK, Landau, J. (2019) *Haemophilia*, Vol 25, Supplement S1 P124
+ Weiller M, Wang H, Coulibaly S, Schuster M, Rottensteiner H, Sun K, Chuah MK, Vandendriessche T. (2019) *Blood* Vol. 134, Supplement S1 P4633

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