Improved pharmacokinetics and bleeding efficacy of recombinant Factor IX Fc-XTEN in hemophilia-B mice

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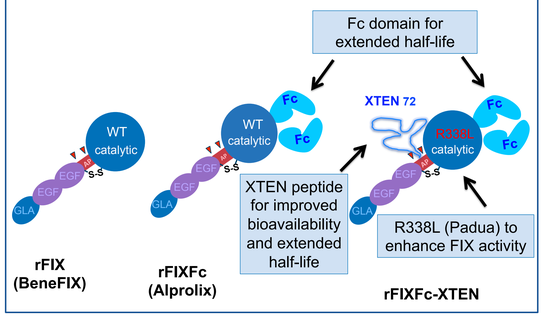
INTRODUCTION

- Prophylactic treatment for severe hemophilia B patients is considered the optimal therapy to reduce bleeding frequency and prevent joint damage.
- Alprolix (rFIXFc) is the first of a new generation of long acting (LA) rFIX replacement products generated to improve patient care by reducing the frequency of infusions[1]. All rFIX preparations are administered by intravenous dosing, which can be particularly challenging for young patients and patients with limited venous access.
- We are using XTEN recombinant protein technology[2], in combination with rFIXFc in order to develop LA rFIX-XTEN molecules that are suitable for prophylactic subcutaneous dosing in hemophilia B.
- XTEN are unstructured polypeptide sequences that consist of a limited set of natural amino acids (Pro, Ala, Gly, Glu, Ser, Thr).

Figure 1. Molecular design of rFIXFc-XTEN



- Activation of FIX cleaves and releases the AP domain and attached XTEN so that the resulting active FIXFc molecules are identical (except for R388L).

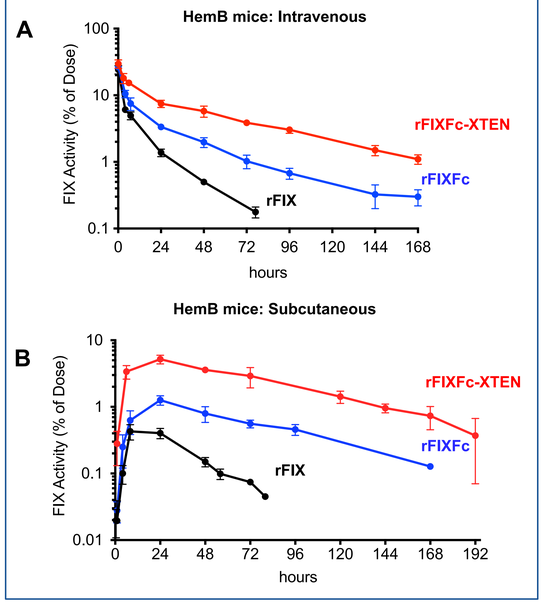
A 72 amino acid XTEN polypeptide was inserted into the activation peptide (AP) domain of rFIXFc, containing the natural Arg[338]Leu (R338L, Padua) variant with improved activity[3]. The fusion proteins were prepared by transient expression in human HEK293 cells followed by affinity purification.

Table 1. Relative specific activity of rFIXFc-XTEN

Molecule	IU/mg	IU/nmole (aPTT)	γ- carboxylated
FIX^{wt}	220	10	95 %
FIX^{R338L}	1227	57	88 %
FIXFc-XTEN	109-176	12-19	94 %

- Higher specific activity at molar basis of rFIXFc-XTEN compared to rFIX as measured by aPTT compared to WHO standard.

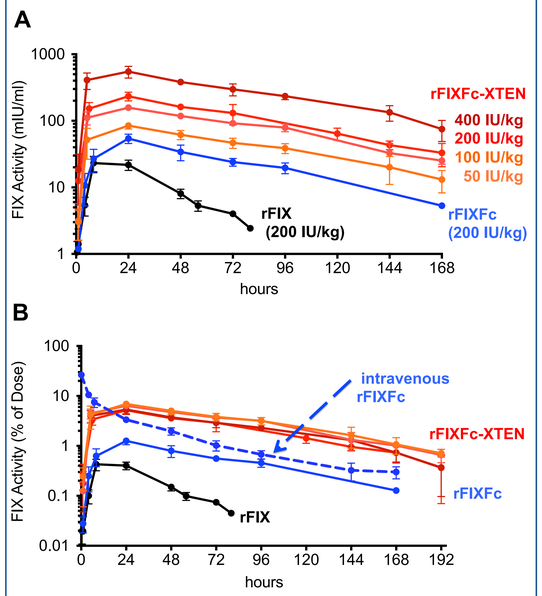
Figure 2. rFIXFc-XTEN shows greatly improved AUC/D and bioavailability for subcutaneous dosing



	Intravenous				Subcutaneous			
	I.R. (%)	$t_{1/2}$ (hr)	MRT (hr)	AUC/D (h*kg/mL)	F (%)	$t_{1/2}$ (hr)	MRT (hr)	AUC/D (h*kg/mL)
rFIX	21	17	17	0.043	14	21	48	0.006
rFIXFc	29	44	42	0.091	27	43	72	0.025
rFIXFc-XTEN	32	49	63	0.226	51	50	75	0.115

Hemophilia B mice were dosed by either intravenous or subcutaneous injection with a single bolus of the rFIX, rFIXFc or rFIXFc-XTEN fusion proteins (50-400 IU/kg, as indicated). Plasma activity levels were determined over time by one-stage activity assay. PK parameters were determined using non-compartmental modeling with Phoenix WinNonlin.

Figure 3. Subcutaneous rFIXFc-XTEN shows a linear dose response in HemB mice and improved PK compared to intravenously dosed FIXFc

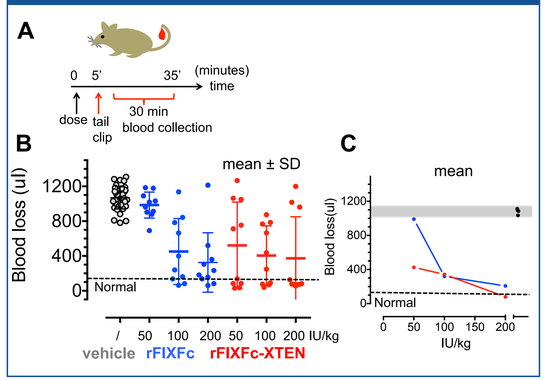


Intravenous (200 IU/kg)	C_{max}/dose (kg/mL)	$t_{1/2}$ (hr)	MRT (hr)	AUC/D (h*kg/mL)	Cl (mL/h/kg)
rFIXFc	0.0070	43	42	0.091	11
Subcutaneous (200 IU/kg)					
rFIX	0.0001	21	38	0.006	175
rFIXFc	0.0003	43	72	0.025	40
rFIXFc-XTEN	0.0013	50	75	0.115	9

- rFIXFc-XTEN shows a 19 -fold improved AUC/D and a 3.5 -fold improved bioavailability compared to rFIX for subcutaneous dosing.
- Subcutaneously dosed rFIXFc-XTEN shows better AUC/D than intravenously dosed rFIXFc.

In vivo efficacy of rFIXFc and rFIXFc-XTEN was compared by IV dosing of equal IU/kg in HemB mice. Five minutes post dosing an 4 µm tail tip amputation was performed under complete anesthesia and blood loss over 30 minutes was measured volumetrically and plotted as mean ± SD.

Figure 4. Acute efficacy in HemB mouse tail clip bleeding model of intravenously dosed rFIXFc-XTEN



- Intravenously dosed rFIXFc-XTEN shows improved efficacy in acute tail clip bleeding model, compared to rFIXFc.

CONCLUSIONS

- rFIXFc-XTEN has an higher specific activity than rFIX.
- When compared to IV administered rFIXFc, preclinical subcutaneous dosing data in hemophilia B mice suggest the potential of once weekly or less frequent prophylactic subcutaneous dosing of rFIXFc-XTEN in patients.
- rFIXFc-XTEN shows acute efficacy in the HemB mouse tail bleeding model.
- Studies are ongoing to address *in vivo* efficacy and allometric scaling in preclinical animal models.

References

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Disclosures

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