# Efficacy of a Recombinant Factor IX in Mouse Models of Hemophilia B

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## Objective

Baxter is developing a new recombinant factor IX (rFIX) product for treating patients with hemophilia B. The aim of the presented preclinical studies was to evaluate the efficacy of Baxter's new rFIX in hemophilia B (FIX ko) mice in three primary pharmacodynamic models: A tail-tip bleeding model, a carotid occlusion model and a model using thrombelastography (TEG). These studies were incorporated into a preclinical program including pharmacokinetic and safety studies.

## Methods

To obtain dose-effect curves, Baxter's rFIX was tested in the tail-tip bleeding model at different doses of 10-100IU/kg. The carotid occlusion and TEG studies were used to compare the efficacy of Baxter's rFIX with that of other FIX products. The animals received intravenous prophylactic treatment with 75IU/kg of either Baxter's rFIX or the active control item, a commercially available rFIX. A commercially available plasma-derived factor IX (pdFIX) was also tested in the carotid occlusion model. Buffer served as the negative control item in all studies.

16 FIX ko mice (B6.129P2-*F9*$^{tm1Dws}$; 8m/8f) per group were used in the tail-tip bleeding model. The tip of the tail was cut off and total blood loss [mg] was assessed over 60 minutes. 10 FIX ko mice (5m/5f) per group were used in the carotid occlusion model. The left carotid artery was exposed and the endothelium was denuded by topical application of $FeCl_3$. Time to occlusion [min] was assessed. The observation period was 30min. Citrated whole blood was drawn by venipuncture of the V. cava caudalis in the TEG study (n=10, 5m/5f). The blood was mixed with $CaCl_2$ and TEG analysis was started immediately. The main variable assessed was R-time [min], which is the period of time of latency from the time that the blood is placed in the TEG analyzer until the initial fibrin formation. This represents the clot initiation phase and resembles the enzymatic portion of coagulation (function of coagulation factors).

Animals were anesthetized using ketamine and xylazine. All animal experiments were performed according to Austrian laws governing animal experimentation and were additionally approved by the Institutional Animal Care and Use Comittee.

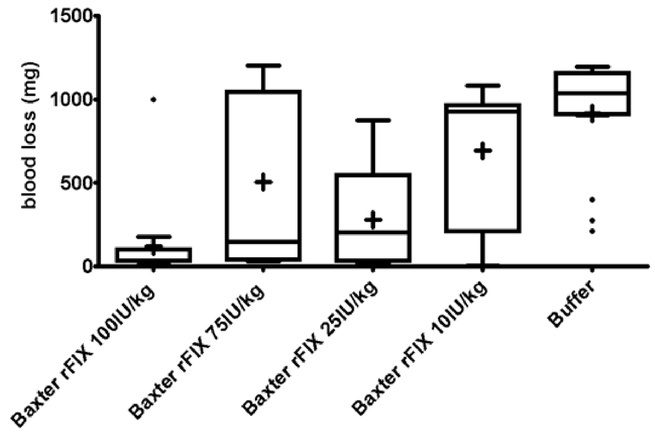


**Fig. 1: Blood loss in FIX ko mice after treatment with different doses of Baxter's rFIX or buffer**

Four different doses of Baxter's rFIX were tested to obtain a dose-effect curve. Buffer-treated animals served as negative controls and showed a median total blood loss of 1036mg.

Median blood loss could be reduced to 927mg by treatment with 10IU/kg of Baxter's rFIX and to 204mg by treatment with 25IU/kg rFIX. Treatment with 75IU/kg rFIX, the dose also used in the carotid occlusion and TEG studies, led to a median blood loss of 145mg, which was shown to be statistically significantly different from buffer (p=0.0085). Treatment with 100IU/kg further reduced median blood loss to 32mg. Statistical evaluation proved a monotone dose-response relation (p=0.0076).

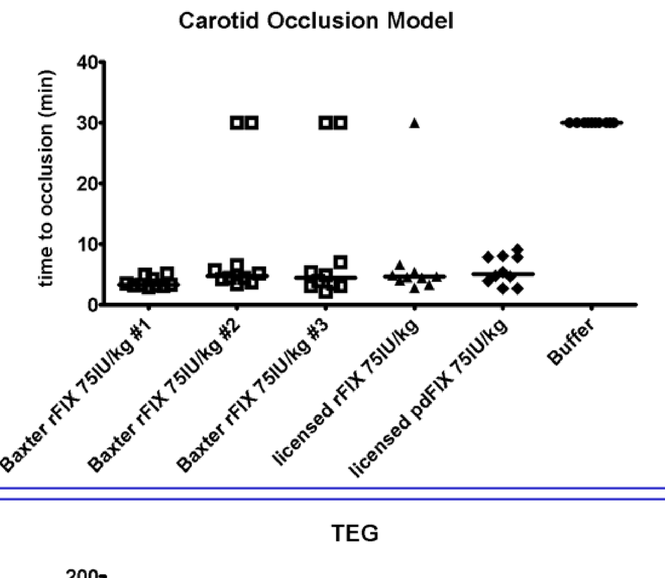


**Fig. 2: Time to occlusion after treatment with rFIX, pdFIX or buffer**

Three lots of Baxter's rFIX (□) were tested at 75IU/kg to show lot-to-lot consistency. All lots were considered efficacious as median time to occlusion (3.3-4.8min) was markedly reduced compared with buffer-treated animals (●; >30min = no occlusion; p=0.00035, assessed for Lot #2 only).

Median time to occlusion was 4.6min after treatment with 75IU/kg of the licensed rFIX (▲) and 5.1min after treatment with the licensed pdFIX (◆) product.

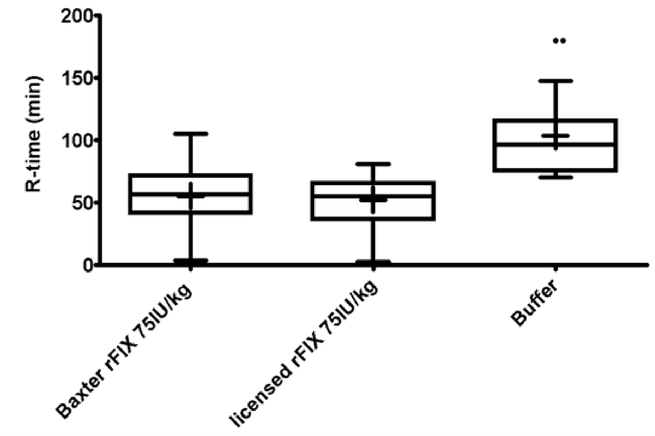


**Fig. 3: Time to clotting (R-time) of rFIX-treated and buffer-treated FIX ko mice**

Median R-time in buffer-treated animals was 98.7min. Treatment with 75IU/kg of Baxter's rFIX led to a statistically significant reduction of time to clotting (54.5min; p=0.00074).

Median R-time was similarly reduced to 56.2min by treatment with 75IU/kg of the active control item, a licensed rFIX product.

## Results

**Efficacy was defined as a statistically significant different result compared with buffer-treated animals. Baxter's rFIX was shown to be efficacious at 75IU/kg in all the primary pharmacodynamic studies performed (p≤0.0076; Figs. 1-3).**

**The dose-dependency of the hemostatic effect of Baxter's rFIX was demonstrated in a tail-tip bleeding model (Fig. 1).**

**The hemostatic effect of Baxter's rFIX at 75IU/kg was compared with the effect of a licensed rFIX product and a licensed pdFIX product. Treatment with Baxter's rFIX and the active control items led to similar results when administered at the same dose (Figs. 2, 3). Furthermore, treatment with different lots of Baxter's rFIX led to similar results, showing lot-to-lot consistency (Fig. 2).**

**The i.v. administration of Baxter's rFIX was well tolerated in all animals across all treatment groups without any signs of acute toxicity.**

## Conclusions

- **Dose-dependent hemostatic effect of Baxter's rFIX could be shown in a mouse model of hemophilia B**
- **No statistically significant differences from a licensed rFIX and a licensed pdFIX**
- **Baxter's new rFIX is effective in animal models closely reflecting the condition in hemophilia B patients**

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