Evaluation of the toxicology, pharmacokinetics, and local tolerance of recombinant factor IX Fc fusion protein in animals

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**ARTICLE INFO**

**Abstract**

**Introduction:** Recombinant factor IX Fc fusion protein (rFIXFc) is a recombinant coagulation factor composed of a single molecule of recombinant factor IX (rFIX) covalently fused to the Fc domain of human immunoglobulin G1 (IgG1) with no intervening sequence. An extensive nonclinical program was performed to support the clinical development of rFIXFc for treatment of people with hemophilia B.

**Materials and Methods:** Repeat-dose toxicology studies of rFIXFc were performed in 2 relevant species: Sprague Dawley rats (4-week study) and cynomolgus monkeys (5- and 27-week studies). Assessments included in-life observations, electrocardiograms (monkeys only), laboratory evaluations (including hematology and blood chemistry), postmortem analyses, local tolerance, and pharmacokinetics (PK). Allometric scaling was performed with PK data from multiple species, including humans. Local tolerance (single-dose study) and thrombogenic potential (Wessler stasis model) of rFIXFc were tested in New Zealand White rabbits.

**Results:** There were no significant local or systemic toxicity findings in the repeat-dose studies. Allometric scaling data suggested that animal rFIXFc PK results are predictive of human PK parameters. There were no findings from the local tolerance study in rabbits; thrombogenic activity was less than that elicited by rFIX and a prothrombin complex concentrate, and similar to vehicle control.

**Conclusions:** rFIXFc was well tolerated in toxicology studies and demonstrated a low thrombogenic potential. These results are consistent with phase 1/2a and phase 3 clinical studies of rFIXFc in people with hemophilia B.

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**Introduction**

Hemophilia B is an X-linked hereditary bleeding disorder resulting from factor IX (FIX) clotting factor deficiency. FIX prophylaxis is considered the optimal therapy for people with hemophilia B because it is associated with improved clinical outcomes compared with episodic treatment [1–3]. Conventional FIX products necessitate infusions 2 to 3 times weekly to achieve protective factor levels. The infusion frequency required for prophylaxis is likely related to the half-life of the FIX product; thus, products with longer half-lives would require less frequent infusions.

The first longer-acting recombinant factor IX (rFIX) product that is commercially available, recombinant factor IX Fc fusion protein (rFIXFc), is composed of a single molecule of rFIX covalently fused to the Fc domain of human immunoglobulin G1 (IgG1) with no intervening sequence [4]. The Fc portion of rFIXFc binds to the neonatal Fc receptor (FcRn), which is part of an endogenous pathway that delays lysosomal degradation of Fc-containing proteins by cycling them back into circulation and thus extending their plasma half-life [5,6]. An extensive non-clinical program was performed in multiple animal species to support clinical development of rFIXFc [7]. Additionally, a phase 1/2a study and the phase 3 B-LONG study have demonstrated the efficacy and safety of rFIXFc for the prevention and treatment of bleeding episodes in previously treated subjects with severe hemophilia B [8,9].

Pharmacokinetic (PK) studies in mice, rats, dogs, and monkeys have shown the prolonged half-life of rFIXFc relative to rFIX, while pharmacodynamic studies in mice and dogs demonstrated both immediate onset of action, as well as the prolonged clotting activity of rFIXFc.
relative to rFIX [7]. In this report, we describe the evaluation of the systemic and local toxicological effects of rFIXFc in 2 pharmaceutically relevant species, rats and monkeys; the local tolerance and thrombogenic safety in rabbits; and the degree to which rFIXFc PK parameters in animals predict those in humans.

Materials and Methods

Treatment Regimens

Study Drug

rFIXFc was produced using a stably transfected human embryonic kidney (HEK) 293H host cell line [10]. Dosing frequencies in the repeat-dose toxicity studies (every 4 days in rats and weekly in monkeys) were chosen based on elimination half-lives of approximately 1.5 days in rats and 2 to 3 days in monkeys to ensure that animals were exposed to rFIXFc on a continuous basis.

Repeat-dose Study in Rats

The systemic and local toxicological effects of rFIXFc were evaluated in Sprague Dawley rats in a 4-week, repeat-dose study in compliance with Good Laboratory Practice (GLP) standards. A total of 100 rats were included in the core toxicology group, and an additional 120 rats were included in a satellite group for PK and immunogenicity studies (Table 1). Rats received intravenous (IV) administration of rFIXFc every 4 days for 4 weeks and were euthanized 1 day after the last dose (terminal necropsy on Day 30). A cohort of rats in the high-dose and control groups were euthanized after a 4-week recovery period, followed by recovery necropsy (Day 57) to assess potential reversal of toxicological effects.

Repeat-dose Studies in Monkeys

rFIXFc was evaluated for local tolerance and systemic toxicological effects in cynomolgus monkeys in a 5-week, repeat-dose study in compliance with GLP standards. A total of 32 monkeys were included in the study (Table 1). Monkeys received IV administration of rFIXFc once weekly for 5 weeks, followed by a 4-week recovery period for a cohort of monkeys in the high-dose and control groups. Animals were euthanized for terminal necropsy 1 day after the last dose (Day 31) or recovery necropsy 4 weeks later (Day 58).

Local and systemic toxicological effects of rFIXFc in cynomolgus monkeys were also evaluated in a 27-week, repeat-dose study, also in compliance with GLP standards. A total of 40 monkeys were included in this study (Table 1). Monkeys received weekly IV administration of rFIXFc for 27 weeks, followed by a 4-week recovery period for a cohort of monkeys in the high-dose and control groups. Monkeys were euthanized for terminal necropsy on Day 184, and recovery necropsy on Day 212.

Single-dose Local Tolerance Study in Rabbits

A local tolerance study evaluated injection site reactions for both the liquid and lyophilized formulations of rFIXFc using 20 male New Zealand White rabbits. Each rabbit was given 4 infusions: single IV (intended clinical route of administration) and paravenous infusions on both the right ear (control) and the left ear (rFIXFc). For 10 rabbits, liquid rFIXFc was administered using a dose of 110 IU/kg in 1 ear and the saline control in the other ear. For the remaining 10 rabbits, lyophilized rFIXFc was administered using a dose of 198 IU/kg in 1 ear and the vehicle control in the other ear. For each treatment group, 5 rabbits were euthanized on Day 4, and 5 rabbits were euthanized on Day 14. Results were similar for both formulations of rFIXFc, and only results using the lyophilized formulation are reported here.

Toxicology

Toxicology parameters evaluated in the repeat-dose studies included in-life observations (eg, clinical observations, body weight, food consumption, ophthalmic examination), laboratory evaluations (eg, hematology, serum chemistry, coagulation parameters [prothrombin time (PT), activated partial thromboplastin time (aPTT), and FIX activity by modified 1-stage clotting assay (27-week monkey study only)]), postmortem analyses (eg, gross necropsy, organ weights, histopathology), and local tolerance evaluations (eg, gross and microscopic examination of IV injection sites). In addition, cardiovascular assessments were performed for all monkeys. In the 5-week monkey study, electrocardiograms (ECGs) were recorded pretest, 1 hour postdose on Days 22 and 27, and during the week prior to the scheduled Day 58 necropsy for animals in the recovery cohort. In the 27-week monkey study, ECGs were recorded pretest, 1 hour postdose on Days 64 and 120, during the week prior to the scheduled core cohort necropsy, and during the week prior to the recovery necropsy for monkeys in the recovery cohort. Heart rate was determined manually from the ECG recordings.

Immunogenicity (formation of anti-rFIXFc antibodies) was analyzed in a satellite group of rats (n = 30 per dose group) and in all monkeys in both studies via blood samples collected predose on Days 1, 17, 29, and 57 (rat study); Days 1, 15, 30, and 58 (5-week monkey study); or Days 1, 29, 92, 183, and 212 (27-week monkey study). After processing, each plasma sample was assessed for the presence of anti-rFIXFc antibodies by Prevalere Life Sciences, Inc. (Whitesboro, NY, USA) using a validated enzyme-linked immunosorbent assay (ELISA) [7]. For samples that tested positive, an immunocompetition assay was performed to confirm antibody response. Specificity of the antibodies against the Fc and FIX domains of the rFIXFc molecule was also determined during the immunocompetition assay. Specificity was assigned based on a change in signal upon the addition of Fc, FIX, or rFIXFc to the sample compared with the addition of saline; a change in signal of ≥25% indicated specificity. When antibody specificity to the Fc and/or FIX moieties could not be ascertained, specificity was assigned to the whole rFIXFc molecule.

Pharmacokinetics

PK evaluations were performed for the repeat-dose studies. In the 4-week rat study, blood samples were collected on Days 1 and 29 (predose and 0.25, 1, 8, 24, 48, 72, 96, 120, and 144 hours postdose) for determination of plasma rFIXFc concentration. In the 27-week monkey study, blood samples were obtained for the first dose on Day 1 (predose and 0.25, 1, 8, 24, 48, 72, 96, 120, and 168 hours postdose); on Days 29 and 92 (predose and 0.25, 1, 8, and 24 hours postdose); and on Day 183 (predose and 0.25, 1, 8, and 24 hours postdose for all

![Table 1](image-url)
terminal and recovery necropsies; and 48, 72, 96, 120, and 168 hours postdose for recovery necropsies. PK results from the 5-week monkey study were similar to those from the 27-week study, and are reported in Table S1 and Figure S1.

Analysis of rFIXFc plasma concentration was performed using a validated ELISA [7] by Prevalere Life Sciences, Inc. PK analysis was based on individual animal concentration-time point data and dosage information and was performed by Battelle (Columbus, OH, USA). Plasma concentration-time data were analyzed by noncompartmental analysis using WinNonlin® software version 5.0.1 (Pharsight Corporation, Mountain View, CA, USA). The PK parameters determined and evaluated included maximum plasma concentration (Cmax), area under the time versus plasma concentration curve from the time of dose administration to the last measurable concentration (AUClast), elimination half-life (t1/2), volume of distribution at steady state (Vss), clearance, and mean residence time (MRT). Mean ± standard deviation (SD) plasma concentration-time curves for each group were constructed. PK parameters were summarized by group. No statistical analysis comparisons within a group or across groups were made, other than those that could be performed using simple descriptive statistical analysis.

**Allometric Scaling**

Plasma clearance and Vss were modeled using allometric scaling (log-log plot) to determine their relationship to body weight across 4 animal species (mice, rats, dogs, and monkeys) and subjects with hemophilia B. PK data were previously obtained (mouse data from 15 separate PK profiles [4–6 mice per time point]; rat data from 8 separate profiles [5–6 rats per time point]; dog data from 2 individual profiles; monkey data from 66 individual profiles; and human data from a phase 1/2a study [n = 12] and the phase 3 B-LONG clinical study [n = 117], which were plotted separately in this analysis [unpublished data and other studies [7–9,11]]).

**Local Tolerance Study in Rabbits**

Injection sites on rabbits were evaluated by limited gross examination and histopathology on Days 4 and 14 after dosing.

**Thrombogenicity Evaluation**

Early, crude replacement FIX preparations often contained small amounts of activated FIX (FIXa), which can cause thrombosis [12]. The potential thrombogenic activity of rFIXFc after IV administration to New Zealand White rabbits was assessed using an unmodified Wessler stasis model [13]. Forty-two male rabbits were assigned to 1 of 7 dose groups, with 6 rabbits per group. Each animal was infused with a bolus injection of saline, dilution vehicle, rFIXFc (50, 200, or 1000 IU/kg), plasma-derived prothrombin complex concentrate (Prothrombin® SD; 198 IU/kg), or rFIX (BeneFIX®; 991 IU/kg). The number and type of thrombi were assessed and scored (blinded reviewer) using a semiquantitative scale of 0 to 4 (0, no clots; 1, a few macroscopic strands of fibrin; 2, several small thrombi; 3, ≥2 large thrombi; and 4, single thrombus forming a cast of the isolated vein segment).

**Statistical Analyses**

For the 27-week, repeat-dose monkey study, all appropriate in-life and postmortem data collected were analyzed statistically (when n ≥ 3). Data were analyzed for effects of rFIXFc by analysis of variance. Homogeneity was assessed using Bartlett’s test at the 0.05 level. For data for which variances were considered homogenous across test groups, tests for differences between the control group and comparison groups were made using Dunnett’s test. For nonhomogenous data, tests for pair-wise differences between the control group and each of the comparison groups were made using Cochran and Cox’s modified
2-sample t test. Statistical significance for each comparison was reported at the 0.05 level.

Results

Repeat-dose Toxicology Studies in Rats and Monkeys

Toxicology

rFIXFc was well tolerated in the repeat-dose toxicology studies in rats and monkeys. There were no adverse toxicological findings directly related to effects of rFIXFc, as assessed by multiple in-life parameters (Table 2). These parameters included cardiovascular evaluation of monkeys in both the 5-week and 27-week studies, which showed ECGs and heart rates within normal limits for the control and rFIXFc dosing groups. There were also no local tolerance findings related to rFIXFc. The single abnormal clinical observation attributed to rFIXFc administration was a mild hypersensitivity reaction in 2 male monkeys in the high-dose group during the 27-week study. The reactions consisted primarily of splotchy reddening of the face, ears, nose, and/or scrotal areas. In 1 monkey, the reaction was observed intermittently beginning at Week 5 and ending at Week 9; the other monkey presented reactions sporadically beginning at Week 9 and ending at Week 18. Each instance of the reaction was typically noted within a few hours postdose and resolved within 4 days postdose.

Laboratory evaluations of rats and monkeys also showed no adverse effects attributed to rFIXFc treatment (Table 2). During the 27-week study in monkeys, globulin levels for vehicle control–treated animals varied between 2.7 g/dL and 2.9 g/dL in males, and between 2.7 g/dL and 3.2 g/dL in females. There was no impact of rFIXFc treatment on globulin levels; all values from treated animals were comparable to the vehicle control ranges for males and females. A lack of effect on globulin levels was also seen in the 4-week study in rats and in the 5-week study in monkeys. Furthermore, modified aPTT and aPTT analyses in the 27-week monkey study (performed prior to dosing on Days 1, 29, 92, and 183, and on Day 212 of the recovery period) confirmed that there were no effects on endogenous FIX activity. Postmortem evaluations also showed no direct toxicological effects of rFIXFc in the repeat-dose studies (Table 2).

Immunogenicity assessments showed that repeated administration of rFIXFc resulted in a number of rats and monkeys testing positive for the presence of anti-rFIXFc antibodies (Table 3). This observation was expected because rFIXFc is a fully human, and thus foreign, protein in both rats and monkeys. For all repeat-dose studies, antibody formation was dose- and time-dependent. In the 4-week rat study, 40 of 72 rFIXFc–treated animals tested positive for antibodies during the study. The majority of these antibody-positive rats (n = 37) had antibodies specific to the Fc portion of the drug, while antibodies from 1 rat were specific to both the Fc and FIX regions; the antibody specificity to Fc or FIX could not be determined for 2 rats based on the defined signal threshold in the immunocompetition assay, and thus specificity was assigned to the whole rFIXFc molecule. The percentage of dosed animals with detectable antibody response to rFIXFc increased slightly as dose increased. Of note, 6 animals in the vehicle control group and 1 animal in the mid-dose group (on Day 1 prior to the first dose, and at other time points) tested positive for anti-rFIXFc antibodies; however, the signals measured in these animals were just above the assay cut-point and are unlikely to indicate an antibody response to rFIXFc.

In the 5-week monkey study, 12 of 22 animals dosed with rFIXFc tested positive for antibody formation. For those monkeys that tested positive at the end of the study (Day 30; n = 9), 3 had antibodies specific to the Fc portion of the drug, 3 had antibodies specific to the FIX domain, and 3 had antibodies of undetermined specificity. Overall, the specificity of antibodies that developed in the 5-week monkey study was roughly divided between the FIX and Fc domains; in contrast, the majority of antibodies showed specificity for the Fc domain in the 4-week rat study.

In the 27-week monkey study, 18 of 28 monkeys dosed with rFIXFc tested positive for anti-rFIXFc antibodies. In addition, 1 monkey in the control group tested positive on Days 92 and 183.

Table 3

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<th>92</th>
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<th>212 (recovery group)</th>
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rFIXFc, recombinant factor IX Fc fusion protein; NA, not applicable.

a Data shown are the number of animals with anti-rFIXFc antibodies/total number of animals per group (equal number male and female).

b One antibody-positive monkey also tested positive for the presence of antibodies on Day 30, while the remaining 3 monkeys had not previously tested positive.

rFIXFc, recombinant factor IX Fc fusion protein; NA, not applicable.

a Data shown are the number of animals with anti-rFIXFc antibodies/total number of animals per group (equal number male and female).

b One antibody-positive monkey also tested positive for the presence of antibodies on Day 30, while the remaining 3 monkeys had not previously tested positive.
first detected on Day 29 and the incidence increased through Day 92, but no further increase was observed on Day 183. The majority of antibodies were specific to the FIX domain (n = 13, including 1 monkey from the control group), while antibodies from 2 monkeys were specific to the Fc domain, and 4 monkeys had antibodies specific to both the Fc and FIX domains. As demonstrated in the following PK analyses, the presence of antibodies in rats and monkeys led to more rapid elimination of rFIXFc in some animals later in the studies.

Table 4
Key PK Parameters for rFIXFc in the 4-week, Repeat-dose Study in Rats.*

<table>
<thead>
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<th>Dose group</th>
<th>Cmax (μg/mL)</th>
<th>AUClast (h·μg/mL)</th>
<th>t1/2 (h)</th>
<th>Vss (mL/kg)</th>
<th>CL (mL/h/kg)</th>
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PK, pharmacokinetic; rFIXFc, recombinant factor IX Fc fusion protein; Cmax, maximum plasma concentration; AUClast, area under the time versus plasma concentration curve from the time of dose administration to the last measurable concentration; t1/2, elimination half-life; Vss, volume of distribution at steady state; CL, clearance.

* For each dosing group, n = 24 (males and females).

Table 5
Key PK Parameters for rFIXFc in the 27-week, Repeat-dose Study in Monkeys.*

<table>
<thead>
<tr>
<th>Dose group</th>
<th>n</th>
<th>Cmax (μg/mL)</th>
<th>AUClast (h·μg/mL)</th>
<th>t1/2 (h)</th>
<th>Vss (mL/kg)</th>
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<td>50 IU/kg</td>
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<td>7.1 ± 1.1</td>
<td>8.5 ± 0.7</td>
<td>168 ± 22.8</td>
<td>92.0 ± 13.3</td>
<td>47.1 ± 6.9</td>
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<td>200 IU/kg</td>
<td>8</td>
<td>42.3 ± 7.8</td>
<td>40.3 ± 4.4</td>
<td>848 ± 145</td>
<td>410 ± 129</td>
<td>58.0 ± 13.1</td>
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<td>1000 IU/kg</td>
<td>12</td>
<td>240 ± 48.0</td>
<td>240 ± 36.2</td>
<td>3486 ± 451</td>
<td>1396 ± 434</td>
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PK, pharmacokinetic; rFIXFc, recombinant factor IX Fc fusion protein; Cmax, maximum plasma concentration; AUClast, area under the time versus plasma concentration curve from the time of dose administration to the last measurable concentration; t1/2, elimination half-life; Vss, volume of distribution at steady state; CL, clearance; NR, not reported.

* Data are reported as mean ± standard deviation.

Fig. 1. rFIXFc plasma concentration during the 27-week, repeat-dose monkey study. (A) Plasma concentration versus time curves (semi-log plot) for each dosing group on Day 1,* and (B) AUClast versus study day for high-dose group (rFIXFc 1000 IU/kg; n = 12).* rFIXFc, recombinant factor IX Fc fusion protein; AUClast, area under the time versus plasma concentration curve from the time of dose administration to the last measurable concentration. *Data represent mean ± standard deviation.
Results of the 3 repeat-dose studies each showed a no-observed-adverse-effect level (NOAEL) of 1000 IU/kg, the highest dose tested, in rats and monkeys.

**Pharmacokinetics**

After the first dose in rats, \( C_{\text{max}} \) values were generally dose-proportional across dose groups (Table 4). Systemic rFIXFc exposure, as indicated by AUC values, was approximately proportional with dose for the 50 and 200 IU/kg doses, with a somewhat less than proportional increase at the 1000 IU/kg dose level. Clearance values ranged from 7.66 to 13.88 mL/h/kg, and group mean \( t_{1/2} \) values ranged from 26.7 to 34.5 hours for male and female rats at all dose levels. Repeated administration of rFIXFc was associated with decreases in AUC on Day 29, particularly with the mid and high doses, and concomitant increases in clearance (with clearance values ranging from 9.35-24.8 mL/h/kg; Table 4); these changes correlated with the appearance of anti-rFIXFc antibodies upon repeated dosing. Consistent with the higher incidence of antibody-positive rats at the higher dose levels, mean \( t_{1/2} \) values were slightly reduced for the 1000 and 200 IU/kg dose levels compared with the 50 IU/kg dose (16.8, 23.5, and 27.1 hours, respectively).

After the first dose on Day 1 in the 27-week monkey study, rFIXFc exposure increased in a dose-proportional manner, as measured by \( C_{\text{max}} \) and AUC (Table 5 and Fig. 1A); exposure was similar for males and females within each dose group, and combined data are shown. PK parameters, including \( t_{1/2} \), \( V_{\text{ss}} \), and clearance, were generally similar across groups. Clearance ranged from 4.0 to 5.0 mL/h/kg, and the group mean \( t_{1/2} \) ranged from 47 to 58 hours. PK parameters were assessed again on Day 29 (Table 5); compared with Day 1, \( C_{\text{max}} \) was unchanged across all dose groups. However, Day 29 group mean values for \( t_{1/2} \), \( AUC_{\text{ss}} \), MRT, and \( V_{\text{ss}} \) had decreased for the high-dose group compared with Day 1, and mean clearance increased. PK parameters on Day 29 correlated with the presence of anti-rFIXFc antibodies. On Days 92 and 183, mean PK parameters were similar to values on Day 29 across all dose groups, indicating no further effect on clearance; \( AUC_{\text{ss}} \) for the high-dose group also remained relatively constant after Day 29 (Fig. 1B). PK parameters were similar in the 5- and 27-week, repeat-dose monkey studies; results for Day 1 of the 5-week study are reported in Table S1 and Figure S1.

**Allometric Scaling**

The allometric scaling log-log plot and equation for plasma clearance and \( V_{\text{ss}} \) are shown in Figs. 2A and 2B, respectively. Based on the exponent from the allometric scaling plot for clearance (0.84), rFIXFc was eliminated more rapidly in small animals (mice, rats, dogs, and monkeys) compared with humans. In contrast, \( V_{\text{ss}} \) was proportional to body weight across species (with an exponent of 0.98). The exponents for clearance and \( V_{\text{ss}} \) are consistent with the range of exponents for other small molecules or other biotherapeutic proteins, which typically range from 0.6 to 0.8 for clearance (including an exponent of 0.73 for rFIX [14]), and from 0.8 to 1.1 for \( V_{\text{ss}} \) [14,15].

**Single-dose Study in Rabbits (Local Tolerance)**

There were no local tolerance findings attributed to administration of rFIXFc in rabbits. Microscopic findings of dermal inflammation and edema were similar in incidence and severity among all treated sites, including vehicle and control sites.

**Thrombogenic Safety**

Using the Wessler stasis model [13], the mean thrombogenic activity of rFIXFc administered at doses of 50, 200, and 1000 IU/kg was similar to
that of the dilution vehicle, and was less than that elicited by plasma-derived prothrombin complex concentrate and rFIX (Fig. 3).

Discussion

rFIXFc has been evaluated for its toxicological effects in 2 species, Sprague Dawley rats and cynomolgus monkeys, and for local tolerance and thrombogenic potential in New Zealand White rabbits. The repeat-dose studies in rats and monkeys were of durations up to 4 and 27 weeks, respectively. Because rFIXFc binds to FcRn in both rats and monkeys, they were pharmacologically relevant species. Moreover, the activities of the clotting factors in the coagulation cascade are well conserved between these species and humans, and the IV route of administration for the toxicology studies matched the route of administration used in clinical studies of rFIXFc [8,9].

rFIXFc was well tolerated in the repeat-dose toxicology studies and did not cause any local tolerance reactions. In 2 high-dose males in the 27-week monkey study, a pattern consistent with a mild hypersensitivity reaction related to the development of anti-rFIXFc antibodies was observed; both of these monkeys had developed anti-rFIXFc antibodies by Day 29. These reactions were transient and resolved before the end of the study. In both rats and monkeys, the incidence of antibody development increased with increasing dose and time, and antibodies were “clearing” antibodies, resulting in more rapid elimination of rFIXFc later in the study compared with Day 1. Following the development of these antibodies, mean AUC remained dose-dependent in both rats and monkeys, although the absolute values were lower compared with Day 1 values. In the 27-week monkey study, there was no indication that these antibodies cross-reacted with endogenous FIX, based on a modified aPTT analysis (data not shown). Furthermore, the lack of histopathological changes in rats and monkeys that were treated with rFIXFc showed that there was no evidence of organ toxicity due to antibody/antigen complex formation. The development of antibodies to rFIXFc was an expected finding in rats and monkeys because this molecule is a foreign protein in both species; despite these antibodies, the toxico logic of rFIXFc was adequately assessed in the repeat-dose studies, as exposure was maintained throughout the dosing period in both rats and monkeys.

Importantly, administration of rFIXFc did not impact globulin levels in the repeat-dose rat and monkey studies. Globulin levels consist primarily of IgG and, as a fragment of IgG1, the Fc portion of rFIXFc binds to the same cellular receptor as IgG [6,16]. Despite rFIXFc doses of up to 1000 IU/kg, globulin levels were similar in the animals treated with rFIXFc and those treated with the vehicle control in the 3 repeat-dose studies. Thus, the results in rats and monkeys showed that administration of rFIXFc did not interfere with IgG homeostasis. This lack of effect is expected because the endogenous serum concentration of IgG is considerably higher than that of the pharmacological levels of rFIXFc.

rFIXFc has been evaluated for its pharmacokinetic properties in both rats and monkeys. The maximum serum concentration of rFIXFc is 1.3 mg/dL. Thus, the serum concentration of rFIXFc in a therapeutic dose range (eg, 50–100 IU/kg) is much lower than the serum concentration of endogenous IgG.

Based on empirically derived allometric scaling equations, the PK parameters of rFIXFc follow well-established guidelines for many biotherapeutic proteins: smaller animal species (based on body weight) eliminate proteins more rapidly than humans, and Vd is proportional to body weight across species. While the exponent for rFIXFc clearance (0.84) does not identify a mechanism of plasma elimination, it is nevertheless likely that the derived allometric scaling equation is due to a similarity in clearance mechanisms across species, which includes the role of FcRn in prolonging the elimination half-life of rFIXFc, as well as other clearance mechanisms specific to FIX itself.

The low thrombogenic potential of rFIXFc observed in rabbits and the normal profile of PT and hematology values following repeated rFIXFc doses of up to 1000 IU/kg in both rats and monkeys are in contrast to the high thrombogenic potential reported for some other, earlier FIX products in people with hemophilia B [12]. The small, variable amounts of FIXa contained in FIX products can lead to thrombosis, and most highly purified FIX concentrates have shown much lower thrombogenic potential than earlier, cruder preparations of FIX products. Consistent with the low thrombogenic potential observed in this study using a Wessler stasis model, the level of FIXa has been reported to be approximately 10 to 20 times lower in rFIXFc preparations (<0.013%) compared with conventional rFIX (0.11% ± 0.0019%) and plasma-derived FIX (0.21% ± 0.010%) products [7].

Conclusions

The highest dose used in the toxicology studies, 1000 IU/kg, provides a sufficient safety margin for use of rFIXFc as a replacement coagulation factor. The results of these toxicology studies support the clinical safety profile of rFIXFc for use in people with hemophilia B.

Conflict of Interest Statement

J. A. Dumont, K. S. Loveday, D. R. Light, and H. Jiang are shareholders and employees of Biogen Idec. G. F. Pierce is a shareholder and former employee of Biogen Idec.
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Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2015.01.020.

References