Title

Recombinant factor IX-Fc fusion protein (rFIXFc) demonstrates safety and prolonged activity in a phase 1/2a study in hemophilia B patients

Left running head: SHAPIRO et al.

Short Title: PROLONGED rFIXFc ACTIVITY IN HEMOPHILIA B

Authors

Amy D Shapiro1*, Margaret V Ragni2, Leonard A Valentino3, Nigel S Key4, Neil C Josephson5, Jerry S Powell6, Gregory Cheng7, Arthur R Thompson5, Jaya Goyal8, Karen L Tubridy9, Robert T Peters9, Jennifer A Dumont9, Donald Euwart8, Lian Li9, Bengt Hallén10, Peter Gozzi10, Alan J Bitonti9, Haiyan Jiang9, Alvin Luk9, Glenn F Pierce9*

Affiliations

1Indiana Hemophilia and Thrombosis Center, Indianapolis, IN; ²University of Pittsburgh, Pittsburgh, PA; ³Rush University Medical Center, Chicago, IL; ⁴University of North Carolina, Chapel Hill, NC; ⁵Puget Sound Blood Center, Seattle, WA; ⁶University of California, Davis, CA; ⁷Chinese University of Hong Kong, Hong Kong, PRC; ⁸Biogen Idec, Cambridge, MA; ⁹Biogen Idec Hemophilia, Waltham, MA; ¹⁰Swedish Orphan Biovitrum AB (publ), Stockholm, Sweden

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*Correspondence:

Glenn F. Pierce MD, PhD, Biogen Idec Hemophilia, 133 Boston Post Road, Weston, MA 02493
Email: glenn.pierce@biogenidec.com, Phone: 781-464-4242, Fax: 888-689-6970

Amy Shapiro MD, Indiana Hemophilia and Thrombosis Center, 8402 Harcourt Road, Indianapolis, IN 46260

Email: ashapiro@IHTC.org, Phone: 317-256-8837, Fax: 317-871-0010

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Abstract

Current Factor IX (FIX) products display a half-life ($t_{1/2}$) of approximately 18 hours, requiring frequent intravenous infusions for prophylaxis and treatment in hemophilia B patients. This open-label, dose-escalation trial in previously treated adult subjects with hemophilia B examined the safety and pharmacokinetics (PK) of rFIXFc. rFIXFc is a recombinant fusion protein composed of FIX and the Fc domain of human IgG1, to extend circulating time. Fourteen subjects received a single dose of rFIXFc: one subject each received 1, 5, 12.5, or 25 IU/kg, and 5 subjects each received 50 or 100 IU/kg. rFIXFc was well tolerated and most AEs were mild or moderate in intensity. No inhibitors were detected in any subject. Dose-proportional increases in rFIXFc activity and antigen exposure were observed. Utilizing baseline subtraction, mean activity terminal $t_{1/2}$ and mean residence time for rFIXFc were 56.7 and 71.8 hours, respectively. This is approximately 3-fold longer than that reported for current rFIX products. The incremental recovery of rFIXFc was 0.93 IU/dL per IU/kg, similar to plasma-derived FIX. These results show that rFIXFc may offer a viable therapeutic approach to achieve prolonged hemostatic protection and less frequent dosing in patients with hemophilia B. The trial was registered at www.clinicaltrials.gov as NCT00716716.
Introduction

Hereditary deficiency of clotting factor IX (FIX), hemophilia B, results in spontaneous or traumatic bleeding into the joints, soft tissues, and body cavities.\(^1\) When not treated adequately with FIX replacement therapy, bleeding results in disability and increased risk of death.\(^2\) Early individualized prophylactic treatment can improve outcomes by reducing the incidence of hemarthroses and subsequent arthropathy.\(^3,4,5\)

FIX replacement therapy has been utilized for approximately 40 years, initially purified from plasma (pdFIX), and more recently manufactured as a recombinant human FIX (rFIX). Both pdFIX and rFIX achieve effective and safe hemostasis in the prophylactic and surgical settings.\(^1\) The current prophylaxis regimen is given two to three times weekly by intravenous infusion. The frequency is determined by the half-life (t\(_{1/2}\)) (approximately 18 hours) and recovery of rFIX. The requirement for frequent dosing can be a deterrent for adhering to a prophylactic regimen due to concerns about venous access and associated complications, such as thrombosis and infection.\(^6,7\) Thus, extending rFIX t\(_{1/2}\) and prolonging its protective hemostatic effect\(^8-10\) may reduce the number of injections needed to achieve effective hemostasis using a prophylaxis regimen or to control breakthrough bleeds in on-demand therapy.

rFIXFc is a recombinant monomeric fusion protein composed of a single molecule of FIX covalently fused to the human IgG1 Fc domain, demonstrating increased circulating t\(_{1/2}\) and bleeding control in several species.\(^10\) Activity was achieved with no cleavable linker between FIX and Fc, in contrast to other fusion proteins.\(^9\) The Fc domain permits binding to the neonatal Fc receptor (FcRn), expressed widely within endothelial cells and other cell types, and also represents a natural molecule with no known inherent toxicity.\(^10\) FcRn is constitutively expressed
throughout life and is responsible for protecting IgG1 and Fc-fusion proteins from lysosomal degradation.\textsuperscript{11,12} Fc-fusion proteins taken up by pinocytosis and/or endocytosis interact with FcRn, resident within endosomes, which, in turn, direct the Fc-fusion proteins back to the plasma membrane, reintroducing them into circulation in a pH-dependent manner.\textsuperscript{13} This recycling approach has been used to extend the $t_{1/2}$ of Fc fusion-based drugs used clinically (e.g., etanercept, romiplostim) and in development.\textsuperscript{14,15}

In mice, rats, dogs, and non-human primates, the $t_{1/2}$ of rFIXFc is 3 to 5-fold longer than rFIX,\textsuperscript{10} indicating for the first time that FIX is cleared, at least in part via lysosomal degradation, and that FIX $t_{1/2}$ can be extended via redirection to a natural protective pathway mediated by FcRn. This is a first-in-human clinical study which investigates the safety and pharmacokinetics (PK) of a long-acting FIX Fc-fusion protein in hemophilia B patients.
Methods

Study design

This study was performed in accordance with the US CFR and ICH Guidelines on Good Clinical Practices. Prior to any testing, approval from participating Institutional Review Boards and written informed consents from all subjects were obtained in accordance with the Declaration of Helsinki.

This was a Phase 1/2a, open-label, multicenter, dose-escalation study of single-dose rFIXFc in previously treated subjects with severe hemophilia B (ClinicalTrials.gov identifier NCT00716716). The primary objective was to assess the safety of rFIXFc over 30 days and the secondary objective was to estimate the PK parameters of rFIXFc at doses ranging from 12.5 to 100 IU/kg.

Subject eligibility was determined and one dose of rFIXFc was infused intravenously over approximately 10 minutes at 6 sequential dose levels: 1, 5, 12.5, 25, 50, and 100 IU/kg. Plasma samples to measure FIX activity at doses of 12.5 IU/kg and above were taken at baseline pre-infusion, immediately post-infusion, and 0.25, 1, 3, 6, 9, 24, 48, 72, 96, 120, 168, and 240 hours (10 days) post-infusion. At the 100 IU/kg dose, samples were also taken at 12 and 14 days.

Subjects

Male subjects at least 18 years of age with severe (defined as ≤2 IU/dL FIX:C) hemophilia B and at least 150 prior documented exposure days to other FIX products were included. Individuals with a history of inhibitors, allergic or anaphylactoid reactions associated with FIX or intravenous immunoglobulin, concurrent autoimmune disease, coagulation disorder other than hemophilia B, or who were taking medications that could affect hemostasis were excluded.
subjects with unknown genotypes, genotyping was performed (Mayo Clinic, Rochester, MN and Puget Sound Blood Center, Seattle, WA).

Treatment product

rFIXFc was produced in HEK293 cells extensively tested for stability, sterility, and viral contamination to ensure safety. The purified drug product is composed of a monomeric rFIX covalently fused through its carboxy terminus to the N-terminus of a Fc monomer, which forms a disulfide bond with a second Fc monomer during synthesis and secretion from the cells.10,15 rFIXFc was supplied as a frozen (-70ºC) liquid containing 1000 IU of rFIXFc per 5 mL (200 IU/mL) of formulation buffer (10 mM sodium phosphate buffer [pH 7.0], 145 mM sodium chloride, and 0.1% polysorbate 20).

Outcome measures

Safety was evaluated by physical examination, vital signs, ECG, development of AEs, neutralizing and total binding antibody development, and laboratory changes over time. Secondary endpoints included estimated PK parameters. Laboratory assessments included viral status, levels of D-dimer and thrombin-antithrombin (TAT) complex, hematology, serum chemistry, and urinalysis. FIX antigen and rFIXFc concentrations in plasma were measured by ELISAs specific for FIX or rFIXFc (lower limit of quantification [LLOQ] of 10 and 16 ng/mL, respectively). FIX activity was determined by the one-stage (aPTT) clotting assay using Normal Reference Plasma as a calibrator (LLOQ 1 IU/dL), which has a potency assigned against the WHO 3rd International Standard for Factors II, VII, IX and X. FIX inhibitor was measured by Nijmegen-modified Bethesda assay (LLOQ 0.6 BU/mL), and the anti-FIXFc antibodies were
evaluated by a specific bridging electrochemiluminescent immunoassay (sensitivity of 250 ng/mL). ECGs were performed throughout the study and assessed for QT interval.

Pharmacokinetic analyses

FIX activity PK parameters were determined for the 25, 50, and 100 IU/kg dose groups (n = 11). A baseline subtraction method was used to account for endogenous FIX and incomplete elimination of the previous prophylactic dose when calculating the rFIXFc activity-versus-time profile. Baseline was defined based on the subjects’ pre-dose FIX antigen levels and the lowest FIX activity (at screening, pre-dose, or post-dose). Six of 11 subjects had baseline activity ≤ 1%, and the remaining 5 subjects had baseline activities of 2%. If residual drug was present pre-dose, it was decayed using ordinary first-order elimination with a t1/2 based on that reported for BeneFIX (18 hours).

The FIX activity-versus-time data were computer-fitted to a two-compartment model in WinNonlin, version 5.2.1 (Pharsight Inc., Mountain View, CA) for calculation of PK parameters including maximum activity (Cmax), t1/2, clearance (CL), volume of distribution at steady-state (Vss), area under the curve (time zero extrapolated to infinity) (AUCINF), mean residence time (MRT), and incremental recovery (K). In addition, plasma FIX activity above subject’s baseline at 168 hours (7 days) post dose, and time after dosing when FIX activity declined to 3 and 1 IU/dL above the subject’s baseline level were also estimated. Actual sampling times, doses, and infusion durations were used in all calculations.

Similar PK analyses were also applied to the rFIXFc antigen concentration-versus-time data. Since the sensitivity of the rFIXFc antigen ELISA was approximately 10-fold greater than that of the FIX one-stage (aPTT) clotting assay, antigen data from all subjects in the dose groups of 12.5
to 100 IU/kg up to 336 hours post dosing were included in the PK analysis. For derivation of rFIXFc antigen PK parameters, doses were converted to “mg/kg” based on rFIXFc specific activity. The assay was specific for rFIXFc antigen, thus, the pre-treatment baseline was below the limit of detection, irrespective of whether the subjects had circulating dysfunctional endogenous FIX antigen or residual exogenous FIX from their last infusion.

To construct the activity-time profiles at steady state following different dosing regimens, Monte Carlo simulation was conducted using the population PK model of rFIXFc. The mean estimates of model parameters (CL, volume of distribution, inter-compartmental clearance, and volume of the second compartment) in the tested population, the inter-individual variance, and the residual variability were adopted from this Phase1/2a study. One thousand subjects were simulated per dosing regimen for a total of 5 dosing cycles for each subject. The body weight (BW) was generated according to the published method,17 ie, based on a power equation of $Z=\text{BW}^{0.5}$. The median BW in 1000 subjects was assumed to be 75 kg. Based on the simulated activity-time profiles, the mean and 95% CI of the model-simulated drug activity-time profiles of the 1000 subjects was constructed graphically for different dosing regimens.

**Statistical analyses**

Summary descriptive statistics were presented for all safety and PK parameters by dose level. The correlations between the K value, CL, and $V_{ss}$ of rFIXFc activity with body weight, age, and endogenous FIX antigen levels were determined by linear regression with associated $R^2$ and p value, using GraphPad Prism version 5 (La Jolla, CA).


**Results**

Subject disposition

The study enrolled 15 subjects; 14 received an infusion of rFIXFc (Table 1). One subject failed to return for study drug infusion and was withdrawn from the study. One subject each received 1, 5, 12.5, or 25 IU/kg, and 5 subjects each received either 50 or 100 IU/kg. All 14 subjects were included in the safety analysis; 12 subjects, who received 12.5 to 100 IU/kg doses of rFIXFc, were included in the PK analyses. The activity PK results for one subject (12.5 IU/kg) were excluded due to insufficient evaluable data to estimate the elimination phase. There were no clinically relevant differences among treatment groups. Subjects with a variety of hemophilia B genotypes, such as stop codon/nonsense and missense mutations, were included (Table 2). Several subjects had near absence of FIX antigen which correlated with markedly reduced FIX activity, while others with missense genotypes had more antigen than activity, indicating a dysfunctional circulating protein. The pre-treatment FIX activity in 2 subjects exceeded 2 IU/dL, likely due to an incomplete washout from their last infusion of FIX concentrate based on historical testing and disease phenotype.

Safety

A total of 16 AEs were reported by 7 subjects; AEs were distributed evenly across treatment groups (Table/Supplement). Most AEs were mild or moderate; the 2 treatment-related AEs included dysgeusia and headache, which occurred in one subject in the 50 IU/kg group. Two subjects experienced serious AEs that required hospitalization (abdominal adhesions and depression); neither was considered related to the study drug. No clinically relevant changes
occurred in laboratory values, QT interval, or vital signs. No allergic reactions were detected. All plasma samples tested negative for FIX inhibitors and anti-rFIXFc antibodies.

Six subjects experienced bleeding episodes between 9 and 28 days post-dose during the study, when infused rFIXFc had been washed out. There were no reports of thrombosis during the study. In laboratory testing of thrombogenic markers, two subjects experienced sporadic, variable increases in TAT complex (5.8-43.1 ng/mL) during frequent blood draws over the first 24 to 48 hours; increases were not correlated with FIX activity levels. The maximal TAT elevations were 4 and 8-fold above the upper limit of normal, and were not correlated with D dimer levels which remained normal, nor with consistent patterns post-dosing in both patients. The short t_{1/2} of TAT (15 minutes) suggested slight coagulation activation possibly due to documented difficult phlebotomies, as D-dimer formation (24 hour t_{1/2}) remained negative.

Pharmacokinetics

A dose-proportional increase in FIX activity was observed based on C_{max} occurring immediately after infusion and AUC_{INF} (Table 3). FIX activity exhibited biexponential decay following infusion of rFIXFc, and was characterized by a short distribution (alpha) phase followed by a log-linear elimination (beta) phase (Figure 1A). The mean distribution t_{1/2} (t_{1/2\alpha}) was variable for individual subjects (mean of 3.31 and 10.3 hours for the two higher dose groups) (Table 3). The mean elimination t_{1/2} (t_{1/2\beta}) was dose-independent over the therapeutic dose range tested, ie, 53.5 hours, 57.6 ± 8.27 hours, and 56.5 ± 14.1 hours at 25 IU/kg, 50 IU/kg, and 100 IU/kg, respectively. The mean t_{1/2\beta} reported for rFIX (BeneFIX) is 19.3 ± 4.97 hours (range 11.1 – 36.4 hours). The average time to 1% (1 IU/dL) above baseline, a surrogate assessment of rFIXFc efficacy, showed a dose-proportional increase. It was 7.34, 10.1 ± 1.58, and 12.3 ± 2.49 days for
doses of 25, 50, and 100 IU/kg, respectively. The average time to 3% above baseline was 3.81, 6.28 ± 1.11, and 8.53 ± 1.58 days following doses of 25, 50, and 100 IU/kg, respectively. At 168 hours (1 week) post dose, the plasma FIX activity was sustained at an average of 1.11 IU/dL, 2.47 ± 0. 911 IU/dL, and 4.65 ± 1.73 IU/dL above baseline for the 25, 50, and 100 IU/kg dose groups, respectively. MRT, CL, and Vss were all dose independent. The mean MRT for all dose groups was 71.9 ± 9.66 hours (range 53.2 - 85.9 hours), while the corresponding value reported for rFIX was 26.0 ± 6.07 hours (range 15.8 – 46.1 hours). The mean CL of rFIXFc was 3.18 mL/hours/kg, while the reported value for rFIX was 8.40 ± 2.01 mL/hr/kg and the mean Vss of rFIXFc was 227 ± 57.1 mL/kg (range 162 – 296 mL/kg).16 Furthermore, each 1 IU/kg of infused rFIXFc raised plasma FIX activity by 0.930 ± 0.179 IU/dL on average (Table 3), and this incremental recovery showed weak positive correlation with body weight (R²=0.336, p=0.048) (Figure/Supplement). The corresponding values reported for rFIX were 0.75 ± 0.23 (range 0.34 to 1.38) IU/dL per IU/kg.16

Plasma rFIXFc antigen levels were measured by a rFIXFc-specific ELISA, and the concentration-versus-time curves are shown in Figure 1B. Findings from rFIXFc activity PK were corroborated by rFIXFc antigen PK (Table 4). Plasma rFIXFc concentrations also declined biexponentially following infusion, with the Cmax detected immediately after dosing. The plasma rFIXFc activity, as measured by the one-stage (aPTT) clotting assay, correlated well with rFIXFc antigen by ELISA in individual subjects (data not shown) and all subjects as a group (R²=0.946, p<0.0001) (Figure 1C). However, the mean dose-independent t1/2α (13.2 ± 3.95 hours), t1/2β (101 ± 20.9 hours), and MRT (110 ± 18.5 hours) of rFIXFc antigen were longer than those determined by plasma FIX activity measurements (Table 4).
Discussion

Studies have demonstrated clear medical benefits with early individualized prophylactic therapy in hemophilia. To achieve wider acceptance, prophylaxis therapy needs to be effective, convenient, simple, and safe. Introduction of rFIXFc replacement therapy with a prolonged $t_{1/2}$ may represent an important step towards achieving these goals. Moreover, a FIX product with an extended half-life compared to currently available products may have a greater impact in the treatment of episodic bleeds through a potential reduction in the number of follow-up treatments needed to control a bleed. A long-lasting FIX product may also influence defective wound healing, as has been described for hemophilia B.

rFIXFc was not associated with inhibitor formation in any subject, all of whom had prior exposure to FIX products. Moreover, there was no evidence of allergic reactions or thrombogenicity as reported previously for rFIX. Levels of activated FIX (FIXa) are extremely low in rFIXFc, less than 10% of the levels reported for currently marketed rFIX and 5% of the levels reported for pdFIX products.

Results provide insight into the PK of rFIXFc in patients with hemophilia B. $C_{max}$ was reached immediately after infusion, suggesting a rapid onset of action similar to rFIX. The incremental recovery observed for rFIXFc may represent an improvement compared with that reported for rFIX in 56 previously treated subjects. The improved recovery may be attributable to differences in posttranslational modification(s) from the HEK cell line, or the Fc moiety. In this study, the incremental recovery of rFIXFc is weakly correlated with body weight ($R^2=0.336$, p=0.048), consistent with earlier reports, but no correlation was observed with age ($R^2=0.2783$, p=0.078) or endogenous FIX antigen level ($R^2=0.0023$, p=0.881) (data not shown).
rFIXFc had a mean $t_{1/2\beta}$ and MRT approximately 3-fold longer than that reported for rFIX.\textsuperscript{16,23} Furthermore, the ranges for rFIXFc and rFIX do not overlap, and are consistent with the extended PK for rFIXFc observed in 4 different animal species.\textsuperscript{10} The $t_{1/2\alpha}$ distribution phase averages approximately 7 hours in rFIXFc patients, considerably longer than historical values for rFIX (2 to 3 hours). Similarly, the mean CL of rFIXFc activity is approximately 2.6-fold less than that reported for rFIX.\textsuperscript{16}

Although the same trend in improvement was observed in the rFIXFc antigen PK, both the $t_{1/2\alpha}$ and $t_{1/2\beta}$ of rFIXFc antigen were longer than those derived from FIX activity. This discrepancy could in part be attributed to the markedly more sensitive antigen assay that enabled inclusion of antigen values detectable at much later time points (up to 336 hours) in the calculation of the $t_{1/2\beta}$. If the terminal antigen half-life is terminated at the same time points as the lowest reliably detected activity, the half-lives are similar at 73.0 ± 19.7 hours. Further studies in larger number of subjects will be important to assess rFIXFc activity versus antigen levels over time.

Data from PK analyses provide a means of optimizing individualized prophylactic treatment to achieve target trough levels above 1% (1 IU/dL) of baseline, and reduce peak/trough variation.\textsuperscript{27,28} In comparison with the recommended dose regimen of 25 to 40 IU/kg of FIX twice weekly, the rFIXFc activity PK modeling results from this study suggest that once weekly dosing of rFIXFc at 20 IU/kg, or every 10 days at 40 IU/kg, or every 2 weeks at 100 IU/kg is sufficient to maintain a mean trough of 1% above baseline in the adult hemophilia B patient population (Figure 2). These model-simulated estimates, however, must consider the heterogeneity of reported clinical breakthrough bleeding events relative to trough level of plasma FIX activity,\textsuperscript{29,30} in addition to the heterogeneity in individual subjects’ PK parameters. Thus, prophylaxis dosing may require individual adjustment, as is often required now.
This study demonstrates the safety and prolonged t$\textsubscript{1/2}$ of a novel therapeutic, rFIXFc, resulting in rapid and prolonged circulating FIX activity. A larger clinical study to extend these observations is currently underway.
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Authorship

Contribution: A.D.S. and M.V.R. conducted the research, analyzed the data, and wrote the manuscript; L.A.V., N.S.K., N.C.J., J.S.P., G.C., K.L.T., R.T.P., A.J.B., and D.E. performed research; A.R.T. performed genotype/phenotype analyses; J.G., J.A.D., L.L., B.H., P.G., H.J. A.L., and G.F.P. designed the research, generated and analyzed data, and wrote the manuscript.


Correspondence: Glenn F. Pierce, Biogen Idec Hemophilia, 133 Boston Post Road, Weston, MA 02493; email: glenn.pierce@biogenidec.com; and Amy Shapiro, Indiana Hemophilia and Thrombosis Center, 8402 Harcourt Road, Indianapolis, IN 46260; email: ashapiro@IHTC.org
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Tables and Figures

Table 1. Subject demographics and disposition

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<th>Characteristic</th>
<th>1, 5, 12.5, and 25 IU/kg n=1/dose</th>
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### Table 2. Baseline FIX antigen and activity concentrations and genotype data for study subjects

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<th>Baseline Activity† (IU/dL)</th>
<th>Mutation‡</th>
<th>Previous Reports§</th>
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</table>
BLQ indicates below the limit of quantification; NA, not applicable

* FIX antigen was measured by ELISA and the lowest value from screening or pre-infusion was taken as the baseline value. The table is arranged by antigen level.

† FIX clotting activity was measured by the one-stage (aPTT) clotting assay where average normal is 100 IU/dL. The lowest level from screening or pre-infusion samples was taken as the closest to the actual baseline.

‡ Nucleotide and codon numbering according to Yoshitake et al (1985)\(^3\) as used in the international database\(^3\) where the first codon is of the circulating protein after signal- and pro-peptide cleavage.

§ From the 2004 updated international database, http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html; the three novel mutations have also not been noted in subsequently published series. Where novel, other changes reported for the same codon are indicated.

║ Data are below the limit of quantification of 10 ng/mL

¶ Although other exon 1 deletions have been reported, it is not known if this one has identical breakpoints.

<table>
<thead>
<tr>
<th>Transition</th>
<th>Nucleotide Position</th>
<th>Codon</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>233</td>
<td>C31008T</td>
<td>T296M</td>
<td>137 Missense, Recurrent CpG transition</td>
</tr>
<tr>
<td>2185</td>
<td>T31152C</td>
<td>I344T</td>
<td>2 Missense</td>
</tr>
<tr>
<td>3280</td>
<td>G20414A</td>
<td>R145H</td>
<td>63 Missense, Recurrent CpG transition</td>
</tr>
<tr>
<td>4247</td>
<td>A31301G</td>
<td>K394E</td>
<td>3 Missense</td>
</tr>
</tbody>
</table>

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Table 3. rFIXFc activity pharmacokinetic parameters in 11 subjects with hemophilia B

<table>
<thead>
<tr>
<th>Dose (IU/kg)</th>
<th>Parameter (mean±SD) (Range)</th>
<th>Incremental Recovery (IU/dL per IU/kg)*</th>
<th>Time to 1% above baseline (Day)‡</th>
<th>Time to 3% above baseline (Day)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n C&lt;sub&gt;max&lt;/sub&gt; (IU/dL)</td>
<td>AUC&lt;sub&gt;INF&lt;/sub&gt; (h·IU/dL)</td>
<td>CL (mL/h/kg)</td>
<td>Vss (mL/kg)</td>
</tr>
<tr>
<td>25</td>
<td>1 20.4 766 3.56 271 76.2 0.612 53.5 0.771 1.11 7.34 3.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>5 47.5±12.9 (33.0-61.1) 1700±548 (1300-2650) 3.44±0.833 (2.05-4.18) 262±54.2 (166-296) 77.0±6.80 (67.9-85.9) 3.31±3.13 (0.130-8.15) 57.6±8.27 (47.9-67.2) 0.870±0.214 (0.633-1.12) 2.47±0.911 (1.63-3.97) 10.1±1.58 (8.41-12.4) 6.28±1.11 (5.25-8.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5 98.5±7.84 (90.8-110) 4020±986 (3090-5130) 2.84±0.657 (2.13-3.55) 183±27.9 (162-221) 65.9±10.3 (53.2-76.5) 10.3±5.64 (3.97-16.6) 56.5±14.1 (42.4-74.5) 1.02±0.113 (0.890-1.18) 4.65±1.73 (3.08-6.85) 12.3±2.49 (9.87-15.0) 8.53±1.58 (7.07-10.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD for all dose groups</td>
<td>11 NA†</td>
<td>NA‡</td>
<td>3.18 ± 0.745 (2.05-4.18) 227 ± 57.1 (162-296) 71.9 ± 9.66 (53.2-85.9) NA‡</td>
<td>56.7 ± 10.4 (42.4-74.5) 0.930 ± 0.179 (0.633-1.18)</td>
</tr>
</tbody>
</table>

C<sub>max</sub> indicates maximum activity; AUC<sub>INF</sub>, area under the curve (time zero extrapolated to infinite time); CL, clearance; Vss, volume of distribution at steady state; MRT, mean residence time; t<sub>1/2α</sub>, distribution t<sub>1/2</sub>; t<sub>1/2β</sub>, elimination t<sub>1/2</sub>.

* Incremental recovery was calculated using observed C<sub>max</sub> subtracted with pretreatment baseline value and divided by dose.

† Plasma FIX activity above baseline at 168 hours (7 days) post dose.

‡ Model-predicted time after dose when FIX activity declined to 1% or 3% (1 or 3 IU/dL) above subject’s baseline.

§ Data are not applicable because parameters are not dose independent, thus the mean and SD values were not calculated across the different dose groups.
Table 4. rFIXFc antigen pharmacokinetic parameters in 12 subjects with hemophilia B

<table>
<thead>
<tr>
<th>Dose (IU/kg)</th>
<th>Parameter (mean±SD) (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>12.5</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>50§</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Mean ±SD for all dose groups</td>
<td>12</td>
</tr>
</tbody>
</table>

C_max indicates maximum concentration; AUC_INF, area under the curve (time zero extrapolated to infinite time); CL, clearance; Vss, volume of distribution at steady state; MRT, mean residence time; t_1/2α, distribution t_1/2; t_1/2β, elimination t_1/2; NA, not applicable

§ For 2 subjects in the 50 IU/kg dose group, data were inadequately described by the two-compartment model, therefore, a non-compartmental analysis was used

* Not including two subjects whose PK parameters were determined by non-compartmental analysis

I Data are not applicable because parameters are not dose independent, thus the mean and SD values were not calculated across the different dose groups
Figure 1. Dose dependent pharmacokinetic profiles and correlation of rFIXFc activity and antigen in plasma. (A) Plasma FIX activity and (B) plasma rFIXFc antigen levels over time following a single intravenous infusion of 12.5 (n=1), 25 (n =1), 50 (n = 5), or 100 (n = 5) IU/kg of rFIXFc. Results presented are group mean ± standard error of mean (SEM). (C) Correlation between plasma rFIXFc activity and antigen levels in 12 subjects who received a single dose of 12.5 to 100 IU/kg of rFIXFc. Samples were collected up to 336 hours post dosing.

Figure 2. Monte Carlo simulation for rFIXFc doses to achieve a trough of 1 IU/dL (1%) or 3IU/dL (3%) above baseline. The rFIXFc dosing intervals considered were (A) weekly, (B) every 10 days, or (C) every two weeks. The mean population PK parameters and relevant inter- and intra-subject variability were adopted from this Phase1/2a study. Based on the simulated activity-time profiles, the mean and 95% CI of the activity-time profiles of the 1000 subjects was constructed graphically for different dosing regimens for a total of five dosing cycles when the steady state was achieved.
Figure 1

(A) Plasma FIX Activity (IU/dL) (Mean+/−SEM) over time post dosing (Hr)

(B) Plasma rFIXFc Antigen (ng/mL) (Mean+/−SEM) over time (Hr)
Plasma rFIXFc Antigen (ng/mL) vs. Plasma rFIXFc Activity (IU/dL)

$R^2 = 0.946$

$p < 0.0001$
Figure 2

(A) 20 IU/kg of rFIXFc once weekly

FIX activity (IU/dL)

Time (Day)

0 4 8 12 16 20 24 28 32 36 40

0.1 1.0 10.0 100.0
(B) 40 IU/kg of rFIXFc every 10 days

(C) 100 IU/kg of rFIXFc every 14 days
Recombinant factor IX-Fc fusion protein (rFIXFc) demonstrates safety and prolonged activity in a phase 1/2a study in hemophilia B patients

Amy D Shapiro, Margaret V Ragni, Leonard A Valentino, Nigel S Key, Neil C Josephson, Jerry S Powell, Gregory Cheng, Arthur R Thompson, Jaya Goyal, Karen L Tubridy, Robert T Peters, Jennifer A Dumont, Donald Euwart, Lian Li, Bengt Hallén, Peter Gozzi, Alan J Bitonti, Haiyan Jiang, Alvin Luk and Glenn F Pierce