# FACTOR IX POLYPEPTIDES AND METHODS OF USE THEREOF

## BACKGROUND OF THE INVENTION

### Field of the Invention

[0001] The present invention relates generally to the field of therapeutics for hemostatic disorders.

### Background Art

[0002] Hemophilia B (also known as Christmas disease) is one of the most common inherited bleeding disorders in the world. It results in decreased in vivo and in vitro blood clotting activity and requires extensive medical monitoring throughout the life of the affected individual. In the absence of intervention, the afflicted individual will suffer from spontaneous bleeding in the joints, which produces severe pain and debilitating immobility; bleeding into muscles results in the accumulation of blood in those tissues; spontaneous bleeding in the throat and neck may cause asphyxiation if not immediately treated; renal bleeding; and severe bleeding following surgery, minor accidental injuries, or dental extractions also are prevalent.

[0003] Normal in vivo blood coagulation at minimum requires the serine proteases Factors II (prothrombin), VII, IX, X and XI (soluble plasma proteins); cofactors including the transmembrane protein tissue factor and the plasma proteins Factors V and VIII; fibrinogen, the transglutaminase Factor XIII, phospholipid (including activated platelets), and calcium. Additional proteins including kallikrein, high molecular weight kininogen, and Factor XII are required for some in vitro clotting tests, and may play a role in vivo under pathologic conditions.

[0004] In hemophilia, blood clotting is disturbed by a lack of certain plasma blood clotting factors. Hemophilia B is caused by a deficiency in Factor IX that may result from either the decreased synthesis of the Factor IX protein or a defective molecule with reduced activity. The treatment of hemophilia occurs by replacement of the missing clotting factor by exogenous factor concentrates highly enriched in Factor IX. However, generating such a concentrate from blood is fraught with technical difficulties, as is described below.

[0005] Purification of Factor IX from plasma (plasma derived Factor IX; pdFIX) almost exclusively yields active Factor IX. However, such purification of factor IX from plasma is very difficult because Factor IX is only present in low concentration in plasma (5 ug/mL.

Andersson, Thrombosis Research 7: 451 459 (1975). Further, purification from blood requires the removal or inactivation of infectious agents such as HIV and HCV. In addition, pdFIX has a short half-life and therefore requires frequent dosing. Recombinant factor IX (rFIX) is also available, but suffers from the same short half-life and need for frequent dosing (e.g., 2-3 times per week for prophylaxis) as pdFIX. rFIX also has a lower incremental recovery (K value) compared to pdFIX, which necessitates the use of higher doses of rFIX than those for pdFIX.

**[0006]** Reduced mortality, prevention of joint damage and improved quality of life have been important achievements due to the development of plasma-derived and recombinant Factor IX. Prolonged protection from bleeding would represent another key advancement in the treatment of hemophilia B patients. However, to date, no products that allow for prolonged protection have been developed. Therefore, there remains a need for improved methods of treating hemophilia due to Factor IX deficiency that are more tolerable and more effective than current therapies.

## BRIEF SUMMARY OF THE INVENTION

**[0007]** The present invention provides methods of administering Factor IX using chimeric polypeptides comprising Factor IX and hybrids of such chimeric polypeptides; chimeric polypeptides comprising Factor IX and hybrids of such chimeric polypeptides; polynucleotides encoding such chimeric and hybrid polypeptides; cells comprising such polynucleotides; and methods of producing such chimeric and hybrid polypeptides using such cells. In some embodiments, the Factor IX chimeric polypeptide is a Factor IX FcRn binding partner (BP) chimeric polypeptide such as a Factor IX Fc chimeric polypeptide. In other embodiments, the Factor IX chimeric polypeptide is a Factor IX-XTEN polypeptide.

**[0008]** The present invention provides a method of administering Factor IX to a subject in need thereof, comprising administering to the subject a dose of at least about 10, at least about 20, or at least about 25 IU/kg of a Factor IX FcRn BP chimeric polypeptide, e.g., a Factor IX-Fc chimeric polypeptide or a Factor IX-XTEN chimeric polypeptide, at about a once weekly or longer dosing interval.

**[0009]** In some embodiments, the plasma level of the chimeric polypeptide reaches an average trough of at least about 1 IU/dl after at least about 6 days in at least about 70%, at least about 80%, at least about 90%, or about 100% of a patient population or reaches a trough of at least about 1, 2, 3, 4, or 5 IU/dl after at least about 6 days in a subject. In some

embodiments, the plasma level of said chimeric polypeptide reaches an average trough of about 1-5 or 1-3 IU/dl. Such trough or average trough may be reached after about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 days.

**[0010]** In some embodiments, the chimeric polypeptide has greatly reduced phosphorylation and sulfation in comparison to plasma derived Factor IX. In some embodiments the chimeric polypeptide is less than 25% phosphorylated and less than 25% sulfated, e.g., less than 25% fully phosphorylated and sulfated. In some embodiments, the chimeric polypeptide is less than about 10% phosphorylated and less than about 9% sulfated. In some embodiments, the chimeric polypeptide has a gamma carboxylation pattern/distribution, a gamma carboxylation content, a sialylation pattern/distribution, and/or a sialylation content similar to (i.e., within 10% of) or the same as those of the Factor IX Fc chimeric polypeptide in Examples 5-6.

**[0011]** In some embodiments, the chimeric polypeptide has an incremental recovery greater that 0.7 or greater than 0.75 ug/ml (antigen). In some embodiments, the chimeric polypeptide has a mean incremental recovery (K-Value) (activity; observed) of at least about 0.8, at least about 0.9, or at least about 1 IU/dL per IU/kg.

**[0012]** In some embodiments, the chimeric polypeptide exhibits one or more pharmacokinetic parameters, in said patient population or in said subject, selected from the group consisting of:

**[0013]** (a) a mean clearance (CL) (activity) in said patient population of about 3.36 ± 0.93 mL/hour/kg; a mean clearance (CL) (activity) in said patient population of about 3.0-3.72, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, or 3.72 mL/hour/kg; a mean clearance (CL) (activity) in said patient population that is about 2.5 fold lower than the clearance of a polypeptide comprising said Factor IX without said FcRn BP; a clearance (CL) (activity) in said subject of about 1.84-4.58 mL/hour/kg

**[0014]** (b) a mean mean residence time (MRT) (activity) in said patient population of at least about 68.05 ± 11.16 hours; a mean MRT (activity) in said patient population of about 60-78, 60, 62, 64, 66, 68, 70, 72, 74, 76, or 78 hours; a mean MRT (activity) in said patent population that is about 3 fold longer than the mean MRT of a polypeptide comprising said Factor IX without said FcRn BP; a mean residence time (MRT) (activity) in said subject of about 53.1-85.8 hours; a mean residence time (MRT) (activity) in said subject of at least

about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, or about 90 hours;

**[0015]** (c) a mean $t_{1/2beta}$ (activity) in said patient population of about 52.5 ± 9.2 hours; a mean $t_{1/2beta}$ (activity) in said patient population that is about 47-60 hours, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60 hours; a mean $t_{1/2beta}$ (activity) in said patient population that is about 3 fold longer than the mean $t_{1/2beta}$ of a polypeptide comprising said Factor IX without said FcRn BP; a $t_{1/2beta}$ (activity) in said subject of about 40-67.4, about 40, about 45, about 50, about 55, about 60, about 65, about 70, or about 75 hours;

**[0016]** (d) a mean incremental recovery (K value) (activity; observed) in said patient population of about 0.93 ± 0.18 IU/dL per IU/kg; a mean incremental recovery (K value) (activity; observed) in said patient population of about 0.85-1.0, about 0.85, about 0.86, about 0.87, about 0.88, about 0.89, about 0.90, about 0.91, about 0.92, about 0.93, about 0.94, about 0.95, about 0.96, about 0.97, about 0.98, about 0.99, about 1.0, about 1.05, about 1.10, or about 1.15 IU/dL per IU/kg; a mean incremental recovery (K value) (activity; observed) in said patient population that is about 24% better than the mean incremental recovery of a polypeptide comprising said Factor IX without said FcRn BP; an incremental recovery (K value) (activity; observed) in said subject of about 0.62-1.17 IU/dL per IU/kg;

**[0017]** (e) a mean Vss (activity) in said patient population of about 226 ± 67.76 (corrected to 69.8) mL/kg; a mean Vss (activity) in said patient population of about 200-300, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, or about 300 mL/kg; a Vss (activity) in said subject of about 145-365 mL/kg;

**[0018]** (f) a mean AUC/dose (activity) in said patient population of about 32.44 ± 10.75 IU*h/dL per IU/kg; a mean AUC/dose (activity) in said patient population of about 26-40, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 IU*h/dL per IU/kg; an AUC/dose in said subject of about 21.80-54.30 IU*h/dL per IU/kg.

**[0019]** In some embodiments, the dose of chimeric polypeptide contains a significantly lower (10-100 fold) level (0.01-0.001%) of activated FIX (FIXa), than currently marketed Factor IX products such as MONONINE™ (pdFIX; CSL Behring)) or BENEFIX™ (Wyeth; rFIX) (0.1%). Such level may be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 fold lower than currently marketed products, or 0.01, 0.05, 0.0033, 0.0025, 0.002, 0.00167, 0.00142, 0.00125, 0.00111, or 0.001%.

**[0020]** In some embodiments, the dosing interval is 6-18, 6-10, 9-18, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, or at least 18 days, weekly, two times monthly, or one time monthly. The dosing interval may be a prophylactic dosing interval, a fixed prophylactic dosing interval, or an individualized prophylactic dosing interval.

**[0021]** The methods of the invention are practiced on a subject in need of control or prevention of bleeding or bleeding episodes, in need of intermittent treatment, in need of prophylactic treatment, or in need of on-demand treatment.

**[0022]** The therapeutic doses that may be used in the methods of the invention are about 25-180, about 20-180, about 20-50, about 20-100, about 10-180, about 10-50, about 10-30, or about 50-100 IU/kg. The dose may be a fixed dose or an individualized dose.

**[0023]** In some embodiments, the chimeric polypeptide is administered intravenously or subcutaneously.

**[0024]** The subject in the methods of the invention may be a human subject or may be a non-human mammal. Non-human mammals include mice, dogs, primates, monkeys, cats, horses, cows, pigs, and other domestic animals and small animals.

**[0025]** The chimeric polypeptide may be in the form of a hybrid comprising a second polypeptide in association with said chimeric polypeptide, wherein said second polypeptide comprises or consists essentially of an FcRn BP, e.g., an Fc. The chimeric polypeptide may be at least 90%, at least 95%, or 100% identical to the Factor IX sequence, the Fc sequence, or both the Factor IX and Fc sequence in Tables 2A (SEQ ID NO:2) and/or 2B (SEQ ID NO:4), with or without the signal sequence(s) and propeptide.

**[0026]** The chimeric polypeptide or hybrid may be administered as part of a pharmaceutical composition comprising at least one excipient.

**[0027]** The invention also provides the above-described chimeric and hybrid polypeptides themselves, polynucleotides encoding them, a cultured human embryonic cells comprising the polynucleotides, and methods of producing such chimeric and hybrid polypeptides, and the polypeptides produced by such methods.

## BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

**[0028]** FIG. 1. Schematic of one type of Factor IX chimeric polypeptide, a Factor IX-Fc hybrid.

[0029] FIG. 2. Group mean FIXFc concentration versus time profiles; nominal dose comparison.

[0030] FIG. 3. Group mean FIXFc activity versus time profiles; nominal dose comparison.

[0031] FIG. 4. The baseline subtraction decision tree.

[0032] FIG. 5. Dose proportional increase in Cmax and AUC for FIX activity.

[0033] FIG. 6. Estimated Therapeutic Duration of rFIXFc at 50 (A) and 100 (B) IU/kg.

[0034] FIG. 7. Dose proportional increase in Cmax and AUC for FIX antigen.

[0035] FIG. 8. Pharmacokinetic estimates for rFIXFc antigen at 50 (A) and 100 (B) IU/kg nominal doses.

[0036] FIG. 9. Excellent correlation between rFIXFc activity and antigen levels. Note that due to recalculation of activity PK, as discussed in Example 11, $R^2 = 0.946$.

**[0037]** FIG. 10. rFIX-Fc domain structure and posttranslational modifications. PRO: Propeptide cleaved by processing enzyme. GLA: contains 12 γ-carboxylated glutamic acid (Gla) residues. ACT PEP: activation peptide cleaved to yield active protease. Other modifications: N- and O- glycosylation, Asp(64) β-hydroxylation, Tyr sulfation, Ser phosphorylation.

**[0038]** FIG. 11. SDS-PAGE gel of purification intermediates and purified FIXFc monomer. Samples from different steps in the purification of FIXFc were analyzed by non-reducing SDS-PAGE. Lane 1: SeeBlue Plus Molecular Weight Markers (Invitrogen). Lane 2: empty lane. Lane 3: Protein A load. Lane 4: Protein A eluate. Lane 5: Fractogel DEAE eluate. Lane 6: Q Seph FF eluate. Lane 7: final bulk FIXFc. Lane 8: empty lane. Lane 9: final bulk reduced FIXFc.

**[0039]** FIG. 12. Functional activity of FIXFc in FIX-deficient mice. FIX-deficient mice were dosed intravenously with 219 IU/kg FIXFc (3 or 4 per group, 6 groups, n = 23) or 200 IU/kg rFIX (3 or 4 per group, 5 groups, n = 23) at time = 0. Blood samples were collected at various times after dosing (0.25 hr to 96 hr) and analyzed for clotting activity using FIX activity assay. * rFIX activity is undetectable in all of the mice at time points later than 48 hr after dosing.

**[0040]** FIG. 13. Whole blood clotting time of FIXFc versus recombinant FIX in FIX-deficient mice. FIX-deficient mice (6 per group) were dosed intravenously with 50 IU/kg FIXFc or 50 IU/kg rFIX. Blood samples were collected before dosing and at various times after dosing. Blood samples were incubated at 37°C and were visually inspected for the presence of a blood clot once per minute. The time needed for a clot to form was recorded

and, once the clotting activity returned to baseline (i.e. no clot formation), no additional samples were obtained (samples collected 15 min to 144 hr for FIXFc or 15 min to 72 hr for rFIX).

**[0041]** FIG. 14. Pharmacodynamics of FIXFc in FIX-deficient mice. FIX-deficient mice were dosed with 219 IU/kg FIXFc (5 per group, 6 groups, n = 30) or 200 IU/kg rFIX (4 or 5 per group, 6 groups, n = 28) on Day 0, 4 and 8. Plasma samples were collected by cardiac puncture at 15 min and 96 hr after each dose and clotting activity was measured using a FIX activity assay. Plasma was also collected by tail bleeds at 8, 24, 48, and 72 hr after each dose. FIXFc levels were measured in all of the samples using an ELISA specific for FIXFc. (A) Measured v. Calculated Activity. Clotting activity for FIXFc was measured using FIX activity assay 15 min and 96 h after three doses. The in vitro clotting activity for FIXFc was determined to be 43.8 ± 5.4 IU/mg. Based on this activity (IU/mg) and the measured protein levels, a calculated plasma clotting activity level was determined for time points at 15 min, 8, 24, 48, 72 and 96 h after each dose. (B) In FIX-deficient mice treated with up to three doses of 200 IU/kg rFIX, FIX levels were measured using FIX-specific ELISA. Using the measured specific activities of FIXFc and rFIX, it was possible to compare calculated clotting activity for all samples analyzed by ELISA.

**[0042]** FIG. 15. Pharmacokinetics and pharmacodynamics of FIXFc in FIX-deficient dogs. Two dogs with hemophilia B were intravenously infused with 140 IU/kg FIXFc. Blood samples were collected at 5, 15, and 30 min, and at 1, 2, 4, 6, 8, 12, 24, 27, 30, 48, 51, 54, 72, 80, 96, 126, 144, and168 hr. (A) A sandwich ELISA utilizing a FIX capture antibody and Fc-HRP detection antibody was used to measure the concentration of intact FIXFc in the Hemophilic B dog plasma samples. (B) FIX clotting activity was measured for all time points with respect to a standard curve generated with FIXFc. (C) Blood collected from animals was immediately analyzed for whole blood clotting time. Blood samples were incubated at 28°C and were visually inspected for the presence of a clot once per minute, and the time in which a clot formed was recorded.

**[0043]** FIG. 16. Pharmacokinetics of FIXFc in Cynomolgus monkeys. Monkeys were administered a single dose (0.5, 2, and 10 mg/kg, corresponding to approximately 25, 100 or 500 IU/kg) of FIXFc (n=2, 3, and 3, respectively). Blood samples were collected at 0.25, 0.5, 1, 8, 24, 48, 72, 96, 120, 144 and 168 hr post-dose and plasma prepared for analysis of protein concentration by FIXFc-specific ELISA.

**[0044]** FIG. 17. rFIXFc and BENEFIX™ show comparable activity and dose response in whole blood from HemB mice. (A) ROTEM® Parameters. rFIX or BENEFIX™ were spiked

into HemB mouse blood and clotting parameters were measured by ROTEM®. (B)-(D) Dose response, measuring (B) CT, (C) CFT, and (D) Alpha-angle.

**[0045]** FIG. 18. Evaluation of acute efficacy in tail clip bleeding model of Hemophiliac mice.

**[0046]** FIG. 19. (A) Blood loss following tail clip in individual HemB mice treated with rFIXFc or BENEFIX™. (B) Dose response of rFIXFc and BENEFIX™ in median blood loss following tail clip in HemB mice.

**[0047]** FIG. 20. Tail vein transection (TVT) bleeding model of HemB mice: a model for the venous bleeding characteristic of severe hemophilia patients.

**[0048]** FIG. 21. Prolonged activity of rFIXFc relative to BENEFIX™ in treated HemB mice by whole blood ROTEM®. (A) CT, (B) CFT, (C) Alpha-angle, and (D) Partial correlation between whole blood clotting activity (CT) by ROTEM® versus plasma activity by aPTT.

**[0049]** FIG. 22. Prolonged efficacy of FIXFc relative to BENEFIX™ in tail vein transection (TVT) bleeding model of HemB mice. (A) Survival: Survival rates were comparable in mice receiving BENEFIX™ 24 hours pre TVT as in mice receiving rFIXFc 72 hours pre TVT, and (B) Rebleed: Bleeding rates were comparable in mice receiving BENEFIX™ 24 hours pre TVT as in mice receiving rFIXFc 72 hours pre TVT.

**[0050]** FIG. 23. Correlation between incremental recovery of rFIXFc activity versus body weight in 12 subjects who received a single dose of 12.5 to 100 IU/kg of rFIXFc.

**[0051]** FIG. 24. Monte Carlo simulation using the structural PK model of rFIXFc activity to construct the activity-time profiles to achieve trough of 1 IU/dL above baseline following weekly (A), every 10 days (B), or every two week dosing regimens (C). The median population PK parameters and relevant inter- and intra-subject variabilities were adopted from the clinical Phase1/2a study. 1000 subjects were simulated per dosing regimen with 14 to 16 sampling points for each subject, and the mean ± SD of the activity-time profiles of the 1000 subjects was constructed graphically for different dosing regimens.

**[0052]** FIG. 25. Monte Carlo simulation for rFIXFc doses to achieve trough of 1 IU/dL (1%), based on recalculated pharmacokinetic data. (A) once weekly, (B) every 10 days, and (C) every two weeks.

# DETAILED DESCRIPTION OF THE INVENTION

**[0053]** The present invention provides a method of treating Factor IX deficiency, e.g., Hemophilia B, with Factor IX using a longer dosing interval and/or improved pharmacokinetic parameters than is possible with currently known Factor IX products. The present invention also provides improved Factor IX chimeric polypeptides, Factor IX chimeric polynucleotides, and methods of production.

**[0054]** "Administering," as used herein, means to give a pharmaceutically acceptable Factor IX polypeptide of the invention to a subject via a pharmaceutically acceptable route. Preferred routes of administration are intravenous, e.g., intravenous injection and intravenous infusion, e.g., via central venous access. Additional routes of administration include subcutaneous, intramuscular, oral, nasal, and pulmonary administration, preferably subcutaneous. .Factor IX chimeric polypeptides and hybrid proteins may be administered as part of a pharmaceutical composition comprising at least one excipient. Advantages of the present invention include: improved regimen compliance; reduced break through bleeds; increased protection of joints from bleeds; prevention of joint damage; reduced morbidity; reduced mortality; prolonged protection from bleeding; decreased thrombotic events; and improved quality of life.

**[0055]** "Chimeric polypeptide," as used herein, means a polypeptide that includes within it at least two polypeptides (or portions thereof such as subsequences or peptides) from different sources. Chimeric polypeptides may include two, three, four, five, six, seven, or more polypeptides or portions thereof from different sources, such as different genes, different cDNAs, or different animal or other species. Chimeric polypeptides may include one or more linkers joining the different polypeptides or portions thereof. Thus, the polypeptides or portions thereof may be joined directly or they may be joined indirectly, via linkers, or both, within a single chimeric polypeptide. Chimeric polypeptides may include additional peptides such as signal sequences and sequences such as 6His and FLAG that aid in protein purification or detection. In addition, chimeric polypeptides may have amino acid or peptide additions to the N- and/or C-termini. Exemplary chimeric polypeptides of the invention are Factor IX-FcRn BP chimeric polypeptides, e.g., Factor IX-Fc chimeric polypeptides such as the FIXFc in Figure 1, SEQ ID NO:2 (Table 2) and Examples 1-4, with or without its signal sequence and propeptide. Another exemplary chimeric polypeptides of the invention include, but are not limited to, Factor IX-XTEN chimeric polypeptides. Factor IX can be fused to either N-terminus or C-terminus of XTEN.

**[0056]** The chimeric polypeptide may comprise a sequence at least 90% or at least 95% or 100% identical to the Factor IX and FcRn BP, e.g., the Fc amino acid sequence shown in Table 2A without a signal sequence and propeptide sequence (amino acids 1 to 642 of SEQ ID NO:2), or alternatively, with a propeptide sequence, or alternatively with a signal sequence and a propeptide sequence. .

**[0057]** "Culture," "to culture" and "culturing," as used herein, means to incubate cells under in vitro conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated in vitro.

**[0058]** "Factor IX" and "FIX," as used herein, means functional Factor IX polypeptide in its normal role in coagulation, unless otherwise specified. Thus, the term Factor IX includes variant polypeptides that are functional and the polynucleotides that encode such functional variant polypeptides. Preferred Factor IX polypeptides are the human, bovine, porcine, canine, feline, and murine Factor IX polypeptides. The full length polypeptide and polynucleotide sequences of Factor IX are known, as are many functional variants, e.g., fragments, mutants and modified versions. Factor IX polypeptides include full-length Factor IX, full-length Factor IX minus Met at the N-terminus, full-length Factor IX minus the signal sequence, mature Factor IX (minus the signal sequence and propeptide), and mature Factor IX with an additional Met at the N-terminus. Factor IX is preferably made by recombinant means ("recombinant Factor IX" or "rFIX"), i.e., it is not naturally occurring or derived from plasma.

**[0059]** A great many functional Factor IX variants are known. International publication number WO 02/040544 A3, which is herein incorporated by reference in its entirety, discloses mutants that exhibit increased resistance to inhibition by heparin at page 4, lines 9-30 and page 15, lines 6-31. International publication number WO 03/020764 A2, which is herein incorporated by reference in its entirety, discloses Factor IX mutants with reduced T cell immunogenicity in Tables 2 and 3 (on pages 14-24), and at page 12, lines 1-27. International publication number WO 2007/149406 A2, which is herein incorporated by reference in its entirety, discloses functional mutant Factor IX molecules that exhibit increased protein stability, increased in vivo and in vitro half-life, and increased resistance to proteases at page 4, line 1 to page 19, line 11. WO 2007/149406 A2 also discloses chimeric and other variant Factor IX molecules at page 19, line 12 to page 20, line 9. International publication number WO 08/118507 A2, which is herein incorporated by reference in its entirety, discloses Factor IX mutants that exhibit increased clotting activity at page 5, line 14 to page 6, line 5. International publication number WO 09/051717 A2, which is herein

incorporated by reference in its entirety, discloses Factor IX mutants having an increased number of N-linked and/or O-linked glycosylation sites, which results in an increased half-life and/or recovery at page 9, line 11 to page 20, line 2. International publication number WO 09/137254 A2, which is herein incorporated by reference in its entirety, also discloses Factor IX mutants with increased numbers of glycosylation sites at page 2, paragraph [006] to page 5, paragraph [011] and page 16, paragraph [044] to page 24, paragraph [057]. International publication number WO 09/130198 A2, which is herein incorporated by reference in its entirety, discloses functional mutant Factor IX molecules that have an increased number of glycosylation sites, which result in an increased half-life, at page 4, line 26 to page 12, line 6. International publication number WO 09/140015 A2, which is herein incorporated by reference in its entirety, discloses functional Factor IX mutants that an increased number of Cys residues, which may be used for polymer (e.g., PEG) conjugation, at page 11, paragraph [0043] to page 13, paragraph [0053].

**[0060]** In addition, hundreds of non-functional mutations in Factor IX have been identified in hemophilia patients, many of which are disclosed in Table 1, at pages 11-14 of International publication number WO 09/137254 A2, which is herein incorporated by reference in its entirety. Such non-functional mutations are not included in the invention, but provide additional guidance for which mutations are more or less likely to result in a functional Factor IX polypeptide.

**[0061]** The Factor IX (or Factor IX portion of a chimeric polypeptide) may be at least 90% or at least 95% or 100% identical to a Factor IX amino acid sequence shown in Table 2A without a signal sequence and propeptide sequence (amino acids 1 to 415 of SEQ ID NO:2), or alternatively, with a propeptide sequence, or with a propeptide and signal sequence (full length Factor IX).

**[0062]** Factor IX coagulant activity is expresses as International Unit(s) (IU). One IU of Factor IX activity corresponds approximately to the quantity of Factor IX in one milliliter of normal human plasma. Several assays are available for measuring Factor IX activity, including the one stage clotting assay (activated partial thromboplastin time; aPTT), thrombin generation time (TGA) and rotational thromboelastometry (ROTEM®). See, e.g., Example 3.

**[0063]** "FcRn binding partner," or "FcRn BP" as used herein, means functional neonatal Fc receptor (FcRn) binding partners, unless otherwise specified. An FcRn binding partner is any molecule that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the FcRn binding partner. Thus, the term FcRn BP includes

any variants of IgG Fc that are functional. For example, the region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379, incorporated herein by reference in its entirety). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. FcRn BPs include whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of immunoglobulins or immunoglobulin fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U. S. Department of Public Health, Bethesda; MD, incorporated herein by reference in its entirety. (The FcRn receptor has been isolated from several mammalian species including humans. The sequences of the human FcRn, rat FcRn, and mouse FcRn are known (Story et al. 1994, J. Exp. Med. 180: 2377), incorporated herein by reference in its entirety.) An FcRn BP may comprise the CH2 and CH3 domains of an immunoglobulin with or without the hinge region of the immunoglobulin. Exemplary FcRn BP variants are provided in WO 2004/101740 and WO 2006/074199, incorporated herein by reference in its entirety.

**[0064]** FcRn BP also include albumin and fragments thereof that bind to the FcRn. Preferably the albumin is human albumin. Factor IX can be fused to either the N-terminal end of the albumin or to the C-terminal end of the albumin, provided the Factor IX component of the Factor IX-albumin fusion protein can be processed by an enzymatically-active proprotein convertase to yield a processed Factor IX-containing polypeptide. Examples of albumin, e.g., fragments thereof, that may be used in the present invention are known. e.g., U.S. Patent No. 7,592,010; U.S. Patent No. 6,686,179; and Schulte, Thrombosis Res. 124 Suppl. 2:S6-S8 (2009), each of which is incorporated herein by reference in its entirety.

**[0065]** FcRn BP (or FcRn BP portion of a chimeric polypeptide) may contain one or more mutations, and combinations of mutations.

**[0066]** FcRn BP (or FcRn BP portion of a chimeric polypeptide) may contain mutations conferring increased half-life such as M252Y, S254T, T256E, and combinations thereof, as disclosed in Oganesyan et al., Mol. Immunol. 46:1750 (2009), which is incorporated herein by reference in its entirety; H433K, N434F, and combinations thereof, as disclosed in Vaccaro et al., Nat. Biotechnol. 23:1283 (2005), which is incorporated herein by reference in

its entirety; the mutants disclosed at pages 1-2, paragraph [0012], and Examples 9 and 10 of U.S. 2009/0264627 A1, which is incorporated herein by reference in its entirety; and the mutants disclosed at page 2, paragraphs [0014] to [0021] of U.S. 20090163699 A1, which is incorporated herein by reference in its entirety.

**[0067]** FcRn BP (or FcRn BP portion of a chimeric polypeptide) may also include the following mutations: The Fc region of IgG can be modified according to well recognized procedures such as site directed mutagenesis and the like to yield modified IgG or Fc fragments or portions thereof that will be bound by FcRn. Such modifications include modifications remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example the following single amino acid residues in human IgG1 Fc (Fcy1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, A330S, P331A, P331S, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wild type proline substituted by alanine at position number 238. In addition to alanine other amino acids may be substituted for the wild type amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred FcRn binding partners distinct from native Fc. Additionally, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to hundreds more FcRn binding partners. Certain of these mutations may confer new functionality upon the FcRn binding partner. For example, one embodiment incorporates N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the FcRn binding partner, and to render the FcRn binding partner incapable of binding to FcyRI, FcyRIIA, FcyRIIB, and FcyRIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847, which is incorporated herein by reference in its entirety; Friend et al. 1999, Transplantation 68:1632, which is incorporated herein by reference in its entirety;

Shields et al. 1995, J. Biol. Chem. 276:6591, which is incorporated herein by reference in its entirety). Additionally, at least three human Fc gamma receptors appear to recognize a binding site on IgG within the lower hinge region, generally amino acids 234-237. Therefore, another example of new functionality and potential decreased immunogenicity may arise from mutations of this region, as for example by replacing amino acids 233-236 of human IgG1 "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FcγRI, FcγRII, and FcγRIII which mediate various effector functions will not bind to IgG1 when such mutations have been introduced (Ward and Ghetie 1995, Therapeutic Immunology 2:77, which is incorporated herein by reference in its entirety; and Armour et al.1999, Eur. J. Immunol. 29:2613, which is incorporated herein by reference in its entirety). As a further example of new functionality arising from mutations described above, affinity for FcRn may be increased beyond that of wild type in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased "off" rate. Mutations believed to impart an increased affinity for FcRn include T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591, which is incorporated herein by reference in its entirety).

**[0068]** The FcRn BP (or FcRn BP portion of a chimeric polypeptide) may be at least 90% or at least 95% or 100% identical to the Fc amino acid sequence shown in Table 2A or B without a signal sequence (amino acids 1 to 227 of SEQ ID NO:2), or alternatively, with a signal sequence.

**[0069]** "Hybrid" polypeptides and proteins, as used herein, means a combination of a chimeric polypeptide with a second polypeptide. The chimeric polypeptide and the second polypeptide in a hybrid may be associated with each other via non-covalent protein-protein interactions, such as charge-charge or hydrophobic interactions. The chimeric polypeptide and the second polypeptide in a hybrid may be associated with each other via covalent bond(s) such as disulfide bonds. The chimeric peptide and the second peptide may be associated with each other via more than one type of bond, such as non-covalent and disulfide bonds. Hybrids are described in WO 2004/101740, WO2005/001025, US Pat. No. 7,404,956, US Pat. No. 7,348,004, and WO 2006/074199, each of which is incorporated herein by reference in its entirety. The second polypeptide may be a second copy of the same chimeric polypeptide or it may be a non-identical chimeric polypeptide. In preferred embodiments, the second polypeptide is a polypeptide comprising an FcRn BP, e.g., Fc. In preferred embodiments, the chimeric polypeptide is a Factor IX-FcRn BP, e.g., Factor IX-Fc chimeric polypeptide, and the second polypeptide consists essentially of Fc. See, e.g., Figure 1,

Examples 1-3, and Table 2 (SEQ ID NOs:2 and 4). See, e.g., US 7404956, which is incorporated herein by reference. in its entirety.

**[0070]** The second polypeptide in a hybrid may comprise or consist essentially of a sequence at least 90% or at least 95% or 100% identical to the amino acid sequence shown in Table 2B without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4), or alternatively, with a signal sequence.

**[0071]** The polypeptide of the present invention also includes Factor IX fused to one or more XTEN polypeptides. Schellenburger et al., Nat. Biotech. 27:1186-90 (2009), which is incorporated herein by reference in its entirety. Factor IX can be fused to either the N-terminal end of the XTEN polypeptide or to the C-terminal end of the XTEN polypeptide. XTEN polypeptides include, but not limited to, those disclosed in WO 2009/023270, WO 2010/091122, WO 2007/103515, US 2010/0189682, and US 2009/0092582, each of which is incorporated herein by reference in its entirety.

**[0072]** "Dosing interval," as used herein, means the amount of time that elapses between multiple doses being administered to a subject. The dosing interval in the methods of the invention using a chimeric FIX-FcRn BP, e.g., a chimeric FIX-Fc, may be at least about one and one-half to eight times longer than the dosing interval required for an equivalent amount (in IU/kg) of said Factor IX without the FcRn BP, e.g., Fc portion (i.e., a polypeptide consisting of said FIX). The dosing interval when administering, e.g., a Factor IX-Fc chimeric polypeptide (or a hybrid) of the invention may be at least about one and one-half times longer than the dosing interval required for an equivalent amount of said Factor IX without the FcRn BP, e.g., Fc, portion (i.e., a polypeptide consisting of said Factor IX). The dosing interval may be at least about one and one-half to eight times longer than the dosing interval required for an equivalent amount of said Factor IX without, e.g., the Fc portion (or a polypeptide consisting of said Factor IX).

**[0073]** In some embodiments, the dosing interval is 6-18, 6-10, 9-18, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, or at least 18 days. The dosing interval may be at least about once weekly, and may be 6-10 days, e.g., about 7-10, about 7-9, about 7-8, about 8-10, about 9-10, about 6-7, about 8-9, about 6, about 7, about 8, about 9, or about 10 days.

**[0074]** The dosing interval may be 9-18 days, e.g., about 9-17, about 9-16, about 9-15, about 9-14, about 9-13, about 9-12, about 9-11, about 9-10 days, about 10-18, about 11-18, about 12-18, about 13-18, about 14-18, about 15-18, about 16-18, about 17-18 days, about 10-11, about 11-12, about 12-13, about 13-14, about 14-15, about 15-16, and about 16-17

days, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, or about 18 days. The dosing interval may be about 10-14 days. The dosing interval may be about every two weeks or twice monthly. The dosing interval may be longer than 18 days, e.g., about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 days. The dosing interval may be a fixed interval, e.g., 7 days for 25-50 IU/kg, 10-13 days for 50-100 IU/kg, or 14 days for 100-150 IU/kg. The fixed interval and dose are determined such that the combination of interval and dose will result in a trough of at least about 1-5 or at least about 1-3, or at least about 1, at least about 2, or at least about 3 IU/dl FIX activity in a population of subjects or in an individual subject. The fixed dosing interval may also be 7 days for 20-50 IU/kg, 10-14 days for 50-100 IU/kg, 14-16 days for 100-150 IU/kg, 7 days for 10-50 IU/kg, 10-13 days for 15-100 IU/kg, or 14-15 days for 50-150 IU/kg. The fixed dosing interval may also be 7 days for 10-30 IU/kg, 10 days 15-50 IU/kg, 11 days 20-70 IU/kg, 12 days 25-85 IU/kg, 13 days 30 to 100 IU/kg, 14 days 40 to 125 IU/kg, and 15 days for 50–150 IU/kg.

**[0075]** In preferred embodiments, the dosing interval is 20 IU/kg once weekly, 40 IU/kg every 10 days, or 100 IU/kg every two weeks (twice monthly).

**[0076]** The dosing interval may, alternatively, be an individualized interval that is determined for each subject based on pharmacokinetic data or other information about that subject. The individualized dose/dosing interval combination may be the same as those for fixed interval regimens in the preceding paragraphs, or may differ, as illustrated in the Examples. The regimen may initially be at a fixed dosing interval, and then it may change to an individualized dosing interval.

**[0077]** "On-demand treatment," as used herein, means treatment that is intended to take place over a short course of time and is in response to an existing condition, such as a bleeding episode, or a perceived short term need such as planned surgery. Conditions that may require on-demand treatment include a bleeding episode, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the illiopsoas sheath. Bleeding episodes other than these are also included. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include minor surgery, major surgery, tooth extraction, tonsillectomy, other dental/thoraco-facial

surgeries, inguinal herniotomy, synovectomy, total knee replacement, other joint replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery. Surgeries other than these are also included. Additional conditions that may require on-demand treatment include those listed in Table 26.

[0078] Additional conditions that may require on-demand treatment include minor hemorrhage, hemarthroses, superficial muscle hemorrhage, soft tissue hemorrhage, moderate hemorrhage, intramuscle or soft tissue hemorrhage with dissection, mucous membrane hemorrhage, hematuria, major hemorrhage, hemorrhage of the pharynx, hemorrhage of the retropharynx, hemorrhage of the retroperitonium, hemorrhage of the central nervous system, bruises, cuts, scrapes, joint hemorrhage, nose bleed, mouth bleed, gum bleed, intracranial bleeding, intraperitoneal bleeding, minor spontaneous hemorrhage, bleeding after major trauma, moderate skin bruising, or spontaneous hemorrhage into joints, muscles, internal organs or the brain. Additional reasons for on-demand treatment include the need for peri-operative management for surgery or dental extraction, major surgery, extensive oral surgery, urologic surgery, hernia surgery, orthopedic surgery such as replacement of knee, hip, or other major joint.

[0079] Abbreviations:

| | |
|---|---|
| $AUC_{INF}$ | Area under the concentration-time curve from zero to infinity |
| $AUC_{\alpha}$ | Area under the concentration-time curve over the distribution phase |
| $AUC_{\beta}$ | Area under the concentration-time curve over the elimination phase |
| Alpha HL | Distribution phase half-life |
| Beta HL | Elimination phase half-life; also referred to as $t_{1/2}$ |
| C168 | Estimated FIXFc activity above baseline at approximately 168 h after dose |
| $C_{max}$ | Maximum concentration, occurring at $T_{max}$ |
| CV% | Percent coefficient of variation |
| Cl | Clearance |
| IVR | *in vivo* recovery (%) |
| K-Value | Incremental recovery |
| MRT | Mean residence time |
| N | Number |
| NC | Not Calculable |
| NR | Not Reported |
| SD | Standard Deviation |
| SE | Standard Error |
| TBLP1 | Model-predicted time after dose when FIXFc activity has declined to approximately 1 IU/dL above baseline |
| TBLP3 | Model-predicted time after dose when FIXFc activity has declined to approximately 3 IU/dL above baseline |
| TBLP5 | Model-predicted time after dose when FIXFc activity has declined to approximately 5 IU/dL above baseline |
| $V_{SS}$ | Volume of distribution at steady state |
| $V_1$ | Volume of distribution of the central compartment |

**[0080]** Pharmacokinetic (PK) parameters include the terms above and the following terms, which have their ordinary meaning in the art, unless otherwise indicated. Some of the terms are explained in more detail in the Examples. PK parameters may be based on FIX antigen level (often denoted parenthetically herein as "antigen") or FIX activity level (often denoted parenthetically herein as "activity"). In the literature, PK parameters are often based on FIX activity level due to the presence in the plasma of some patients of endogenous, inactive FIX, which interferes with the ability to measure administered (i.e., exogenous) FIX using antibody against FIX. However, when FIX is administered as part of a fusion protein containing a heterologous polypeptide such as a FcRn BP, administered (i.e., exogenous) FIX antigen may be accurately measured using antibody to the heterologous polypeptide. In addition, certain PK parameters may be based on model predicted data (often denoted parenthetically herein as "model predicted") or on observed data (often denoted parenthetically herein as "observed"), and preferably are based on observed data.

**[0081]** "Baseline," as used herein, is the lowest measured plasma Factor IX level in a subject prior to administering a dose. In the first-in-human study described in Example 1, the Factor IX plasma levels were measured at two time points prior to dosing: at a screening visit and immediately prior to dosing. Predose times were treated as zero (baseline) for the purpose of calculations, i.e., to generate "baseline subtracted" data. See, e.g., Figure 4. Alternatively, (a) the baseline in patients whose pretreatment FIX activity is <1%, who have no detectable FIX antigen, and have nonsense genotypes is defined as 0%, (b) the baseline for patients with pretreatment FIX activity <1% and who have detectable FIX antigen is set at 0.5%, (c) the baseline for patients whose pretreatment FIX activity is between 1 – 2% is Cmin (the lowest activity throughout the PK study), and (d) the baseline for patients whose pretreatment FIX activity is ≥2% is 2%. Activity above the baseline pre-dosing is considered residue drug from prior treatment, and was decayed to baseline and subtracted from the PK data following rFIXFc dosing. See Example 11.

**[0082]** "Area under the plasma concentration versus time curve" ("AUC"), which, as used herein, is based upon the rate and extent of elimination of Factor IX following administration. AUC is determined over a specified time period, such as 12, 18, 24, 36, 48, or 72 hours, or for infinity using extrapolation based on the slope of the curve. Unless otherwise specified herein, AUC is determined for infinity ($AUC_{INF}$). AUC may also be calculated on a per dose basis. As with many of the other PK parameters, the determination of AUC may be carried out in a single subject, or in a population of subjects for which the average is calculated. In

Example 1, the mean AUC/dose in the patient population was 32.44 IU*h/dL per IU/kg and the range for individual subjects was 21.80-54.30 IU*h/dL per IU/kg. (See Table 13 for mean AUC/dose based on activity.) Therefore, the mean AUC/dose in a patient population may be about 26-40, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 IU*h/dL per IU/kg. See Table 14 for AUC/dose and other AUC parameters based on antigen.

**[0083]** "In vivo recovery" ("IVR") is represented by the incremental recovery (K-value), which is the observed peak activity minus predose level and then divided by the dose. IVR may also be calculated on a percentage basis, as is described in the Examples. For clarity, the units (K value or IU/dl per IU/kg versus %) are used herein. The mean IVR can be determined in a patient population, or the individual IVR can be determined in a single subject. The FIXFc used in the first-in-human study described in Example 1 exhibited a mean IVR of about 0.93 IU/dl per IU/kg in the patient population; and an IVR in each subject that ranged from 0.62 to 1.17 IU/dl per IU/kg (Table 13). Therefore, the chimeric polypeptide of the invention exhibits an mean IVR in a patient population of 0.85-1.15 (e.g., about 0.85, about 0.86, about 0.87, about 0.88, about 0.89, about 0.90, about 0.91, about 0.92, about 0.93, about 0.94, about 0.95, about 0.96, about 0.97, about 0.98, about 0.99, about 1.0, about 1.05, about 1.10, about 1.15) and an IVR in a subject of at least about 0.6, about 0.7, 0.8, about 0.9, about 1.0, about 1.1, or about 1.2 IU/dl per IU/kg.

**[0084]** "Clearance rate" ("CL"), as used herein, is a measure of the body's ability to eliminate a drug, and is expressed as the volume of plasma cleared of drug over time. The FIXFc used in the study described in Example 1 exhibited a mean CL of about 3.36 ml/hour/kg (see Table 13), which is about 2.5 fold lower than the CL (8.2 ml/hour/kg) of a polypeptide consisting of Factor IX (BENEFIX™); the range of CL values in individual subjects was 1.84-4.58 ml/h/kg. Therefore, a chimeric polypeptide of the invention exhibits a mean CL in a population of 3.0-3.72, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, or 3.72 mL/hour/kg For CL based on antigen, see Table 14.

**[0085]** "Mean residence time" ("MRT"), as used herein, is a measure of the average lifetime of drug molecules in the body. The FIXFc used in the study described in Example 1 exhibited a mean MRT of about 68.05 hours (see Table 13); the range of MRT values was 53.1-85.8 hours in individual subjects. Therefore, a chimeric polypeptide of the invention exhibits a mean MRT in a population of 60-78, about 60, about 62, about 64, about 66, about 68, about 70, about 72, about 74, about 76, or about 78 hours and a MRT in a subject of at

least about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, or about 90 hours. For MRT based on antigen, see Table 14.

**[0086]** "$t_{1/2\beta}$," or $t_{1/2\ beta}$" or "Beta HL," as used herein, is half-life associated with elimination phase, $t_{1/2\beta}$=(ln2)/elimination rate constant associated with the terminal phase. In the study described in Example 1, the FIXFc used exhibited a mean $t_{1/2z}$ in a patient population that was about 52.5 hours (see Table 13) and the range of $t_{1/2\ \beta}$ values in individual subjects was 47-60 hours. Therefore, a chimeric polypeptide of the invention exhibits an average $t_{1/2\beta}$ greater than about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, or about 60 hours. For $t_{1/2\beta}$ based on antigen, see Table 14.

**[0087]** "Trough," as used herein, is the lowest plasma Factor IX activity level reached after administering a dose of chimeric polypeptide of the invention or another Factor IX molecule and before the next dose is administered, if any. Trough is used interchangeably herein with "threshhold." Baseline Factor IX levels are subtracted from measured Factor IX levels to calculate the trough level. In some embodiments, the trough is 1-5 or 1-3 IU/dl after about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13 or about 14 days. In some embodiments, the plasma level of the chimeric polypeptide reaches an average trough of at least about 1 IU/dl after at least about 6 days in at least about 70%, at least about 80%, at least about 90%, or about 100% of a patient population or reaches a trough of at least about 1, 2, 3, 4, or 5 IU/dl after at least about 6 days in a subject. In some embodiments, the plasma level of said chimeric polypeptide reaches an average trough of about 1-5 or 1-3 IU/dl. Such trough or average trough may be reached after about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 days.

**[0088]** "Volume of distribution at steady state (Vss)," as used herein, is the apparent space (volume) into which a drug distributes. Vss = the amount of drug in the body divided by the plasma concentration at steady state. In Example 1, the mean Vss found in the population was about 226 mL/kg and the range for subjects was about 145-365 mL/kg. (See Table 13.) Thus, the mean Vss in a patient population may be 200-300, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, or about 300 mL/kg. The Vss for individual subjects may be about 145, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240,

about 250, about 260, about 270, about 280, about 290, about 300, about 310, about 320, about 330, about 340, about 350, about 360, or about 370 ml/kg. For Vss based on antigen, see Table 14.

**[0089]** "Polypeptide," "peptide" and "protein" are used interchangeably and refer to a polymeric compound comprised of covalently linked amino acid residues.

**[0090]** "Polynucleotide" and "nucleic acid" are used interchangeably and refer to a polymeric compound comprised of covalently linked nucleotide residues. Polynucleotides may be DNA, cDNA, RNA, single stranded, or double stranded, vectors, plasmids, phage, or viruses. Polynucleotides include those in Table 1, which encode the polypeptides of Table 2 (see Table 1). Polynucleotides also include fragments of the polynucleotides of Table 1, e.g., those that encode fragments of the polypeptides of Table 2, such as the Factor IX, Fc, signal sequence, propeptide, 6His and other fragments of the polypeptides of Table 2.

**[0091]** "Prophylactic treatment," as used herein, means administering a Factor IX polypeptide in multiple doses to a subject over a course of time to increase the level of Factor IX activity in a subject's plasma. Preferably, the increased level is sufficient to decrease the incidence of spontaneous bleeding or to prevent bleeding in the event of an unforeseen injury. Prophylactic treatment decreases or prevents bleeding episodes, for example, those described under on-demand treatment. Prophylactic treatment may be fixed or may be individualized, as discussed under "dosing interval", e.g., to compensate for inter-patient variability.

**[0092]** "Subject," as used herein means a human or a non-human mammal. Non-human mammals include mice, dogs, primates, monkeys, cats, horses, cows, pigs, and other domestic animals and small animals. Subjects also include pediatric humans. Pediatric human subjects are birth to 20 years, preferably birth to 18 years, birth to 16 years, birth to 15 years, birth to 12 years, birth to 11 years, birth to 6 years, birth to 5 years, birth to 2 years, and 2 to 11 years of age.

**[0093]** The methods of the invention may be practiced on a subject in need of control or prevention of bleeding or bleeding episodes. Such subjects include those in need of control or prevention of bleeding in minor hemorrhage, hemarthroses, superficial muscle hemorrhage, soft tissue hemorrhage, moderate hemorrhage, intramuscle or soft tissue hemorrhage with dissection, mucous membrane hemorrhage, hematuria, major hemorrhage, hemorrhage of the pharynx, hemorrhage of the retropharynx, hemorrhage of the retroperitonium, hemorrhage of the central nervous system, bruises, cuts, scrapes, joint hemorrhage, nose bleed, mouth bleed, gum bleed, intracranial bleeding, intraperitoneal bleeding, minor spontaneous hemorrhage, bleeding after major trauma, moderate skin

bruising, or spontaneous hemorrhage into joints, muscles, internal organs or the brain. Such subjects also include those need of peri-operative management., such as management of bleeding associated with surgery or dental extraction.

**[0094]** "Therapeutic dose," as used herein, means a dose that achieves a therapeutic goal, as described herein. The calculation of the required dosage of plasma derived Factor IX (pdFIX) is based upon the empirical finding that, on average, 1 IU of pdFIX per kg body weight raises the plasma Factor IX activity by approximately 1 IU/dL (1%). On that basis, the required dosage is determined using the following formula:

Required units = body weight (kg) x desired Factor IX rise (IU/dL or % of normal) x 1 (IU/kg per IU/dL)

**[0095]** Because FIXFc, e.g., as described in the Examples and in Figure 1, has an incremental recovery similar to pdFIX (different from that of BENEFIX™), the required dose is determined using the formula above, or adjusting it slightly. See also Table 26 for specific recommended doses for various on-demand treatment needs. For pediatric subjects using pdFIX, dosage guidance is the same as for adults. However, pediatric patients may have a lower incremental recovery, and the dosage may therefore need to be adjusted upwards.

**[0096]** The therapeutic doses that may be used in the methods of the invention are 10-180, 20-180, or 25-180 IU/kg, more specifically, preferred doses for a 6-10 day dosing interval are as follows: about 25-110, about 30-110, about 40-110, about 50-110, about 60-110, about 70-110, about 80-110, about 90-110, and about 100-110; about 30-100, about 30-90, about 30-80, about 30-70, about 30-60, about 30-50, about 30-40 IU/kg; about 40-110, about 50-100, about 60-90, and about 70-80 IU/kg; about 40-50, about 50-60, about 60-70, about 70-80, about 80-90, about 90-100, and about 100-110 IU/kg; about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, and about 110 IU/kg. A 6-10 day dosing interval includes a weekly dosing interval. Additional therapeutic doses for a 6-10 day, e.g., weekly, dosing interval include 20-50, 20-100, and 20-180 IU/kg, more specifically, preferred doses for a 6-10 day, e.g., weekly, dosing interval are as follows: about 20-110, about 20-100, about 20-90, about 20-80, about 20-70, about 20-60, about 20-50, about 20-40, about 20-30, about 20-40, and about 20 IU/kg. See also Examples 10 and 11. Doses may be lower than 20 IU/kg if effective for a given patient, e.g., about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, or about 19 IU/kg.

[0097] Preferred therapeutic doses for a 9-18 day, e.g., two times monthly, dosing interval are as follows: about 50-180, about 60-180, about 70-180, about 80-180, about 90-180, about 100-180, about 110-180, about 120-180, about 130-180, about 140-180, about 150-180, about 160-180, and about 170-180 IU/kg; about 90-170, about 90-160, about 90-150, about 90-140, about 90-130, about 90-120, about 90-110, and about 90-100 IU/kg; about 100-170, about 110-160, about 120-150, and about 130-140 IU/kg; about 90-100, about 100-110, about 110-120, about 120-130, about 130-140, about 140-150, about 150-160, and about 160-170 IU/kg; about 60, about 70, about 80, about 90, about 95, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, about 160, about 165, about 170, about 175, and about 180 IU/kg. See also Examples 10 and 11.

**[0098]** Preferred therapeutic doses are 10-50, 15-100, 20-100, 20-50, 50-100, 10, 20, 40, 50, and 100 IU/kg.

**[0099]** The therapeutic dose may be about 20-50, about 20-100, about 20-180, 25-110, about 30-110, about 40-110, about 50-110, about 60-110, about 70-110, about 80-110, about 90-110, about 100-110, about 30-100, about 30-90, about 30-80, about 30-70, about 30-60, about 30-50, about 30-40 IU/kg, about 40-110, about 50-100, about 60-90, about 70-80 IU/kg, about 40-50, about 50-60, about 60-70, about 70-80, about 80-90, about 90-100, about 100-110 IU/kg, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, and about 110 IU/kg. Such doses are preferred for dosing intervals of about 6-10, about 7-10, about 7-9, about 7-8, about 8-10, about 9-10, about 6-7, about 8-9, about 6, about 7, about 8, about 9, and about 10 days, and once weekly.

**[0100]** The therapeutic dose may about 90-180, about 100-180, about 110-180, about 120-180, about 130-180, about 140-180, about 150-180, about 160-180, and about 170-180 IU/kg. The dose may be about 90-170, about 90-160, about 90-150, about 90-140, about 90-130, about 90-120, about 90-110, and about 90-100 IU/kg. The dose may be about 100-170, about 110-160, about 120-150, and about 130-140 IU/kg. The dose may be about 90-100, about 100-110, about 110-120, about 120-130, about 130-140, about 140-150, about 150-160, and about 160-170 IU/kg. The dose may be about 90, about 95, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, about 160, about 165, about 170, about 175, and about 180 IU/kg. Such doses are preferred for dosing interval of about 9-18, about 9-17, about 9-16, about 9-15, about 9-14, about 9-13, about 9-12, about 9-11, about 9-10, about 10-18, about 11-18, about 12-18, about

13-18, about 14-18, about 15-18, about 16-18, about 17-18, about 10-11, about 11-12, about 12-13, about 13-14, about 14-15, about 15-16, and about 16-17 days, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, and about 18 days, one time monthly and two times monthly (every two weeks).

**[0101]** Preferred therapeutic dose and dosing intervals are as follows: 20 IU/kg once weekly, 40 IU/kg every 10 days, and 100 IU/kg every two weeks (twice monthly). Additional combinations of dose and dose interval include: a dose at least about 50 IU/kg and a dosing interval at least about 7 days, a dose at least about 100 IU/kg and a dosing interval at least about 9 days, a dose at least about 100 IU/kg and a dosing interval at least about 12 days, a dose at least about 150 IU/kg and a dosing interval at least about 14 days, 20-50 or 20-100 IU/kg and said dosing interval is one time weekly, a dose of 20-50 IU/kg and a dosing interval of 7 days, a dose of 50-100 IU/kg and a dosing interval of 10-14 days, or a dose of 100-150 IU/kg and a dosing interval of 14-16 days. Preferred combinations of dosing interval and dose also include 10-50 IU/kg for 7 days, 15-100 IU/kg for 10-13 days, 50-150 IU/kg for 14-15 days, 10-30 IU/kg for 7 days, 15-50 IU/kg for 10 days, 20-70 IU/kg for 11 days, 25-85 IU/kg for 12 days, 30 to 100 IU/kg for 13 days, 40 to 125 IU/kg for 14 days, and 50-150 IU/kg for 15 days.

**[0102]** "Variant," as used herein, refers to a polynucleotide or polypeptide differing from the original polynucleotide or polypeptide, but retaining essential properties thereof, e.g., Factor IX coagulant activity or Fc (FcRn binding) activity. Generally, variants are overall closely similar, and, in many regions, identical to the original polynucleotide or polypeptide. Variants include polypeptide and polynucleotide fragments, deletions, insertions, and modified versions of original polypeptides.

**[0103]** Variant polynucleotides may comprise, or alternatively consist of, a nucleotide sequence which is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:1 or 3 (the Factor IX portion, the Fc portion, individually or together) or the complementary strand thereto, the nucleotide coding sequence of known mutant and recombinant Factor IX or Fc such as those disclosed in the publications and patents cited herein or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or 4 (the Factor IX portion, the Fc portion, individually or together), and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also

included as variants, as are polypeptides encoded by these polynucleotides as long as they are functional.

**[0104]** Variant polypeptides may comprise, or alternatively consist of, an amino acid sequence which is at least 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:2 or 4 (the Factor IX portion, the Fc portion, individually or together), and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

**[0105]** By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be, for example, the entire sequence shown in SEQ ID NO:1 or 3, the ORF (open reading frame), or any fragment specified as described herein.

**[0106]** As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence or polypeptide of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (reference or original sequence) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245), which is herein incorporated by reference in its entirety In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

**[0107]** If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the

subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

**[0108]** For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

**[0109]** By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere

between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

**[0110]** As a practical matter, whether any particular polypeptide is at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences of SEQ ID NO:2 (the Factor IX portion, the Fc portion, individually or together) or 4, or a known Factor IX or Fc polypeptide sequence, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (reference or original sequence) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., Comp. App. Biosci. 6:237-245(1990), incorporated herein by reference in its entirety. In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

**[0111]** If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

**[0112]** For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

**[0113]** The polynucleotide variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

**[0114]** Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

**[0115]** Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J.

Biol. Chem. 268: 2984-2988 (1993), incorporated herein by reference in its entirety, reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988), incorporated herein by reference in its entirety.)

**[0116]** Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem. 268:22105-22111 (1993), incorporated herein by reference in its entirety) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild type.

**[0117]** As stated above, polypeptide variants include modified polypeptides. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

**[0118]** The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower).

**[0119]** Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for

purposes of illustration only and are not intended to be limiting of the invention. All patents and publications referred to herein are expressly incorporated by reference.

Example 1. First-In-Human (FiH) Trial

**[0120]** The first-in-human study was an open label, dose-escalation, Phase 1/2 study to determine the safety, tolerability and pharmacokinetic (PK) parameters of FIXFc (recombinant human coagulation factor IX fusion protein). FIXFc is a recombinant fusion protein comprising human clotting factor IX coupled to the Fc domain from human IgG1. The fusion protein is expressed in human embryonic kidney cells (HEK 293). See Example 3.

**[0121]** FIXFc is being developed for the control and prevention of hemorrhagic episodes in patients with hemophilia B (congenital factor IX deficiency or Christmas disease), including the control and prevention of bleeding in surgical settings.

**[0122]** FIXFc is a recombinant fusion protein comprised of coagulation Factor IX (FIX) and an Fc domain of a human antibody (IgG1 isotype). The FIXFc molecule is heterodimeric with a FIXFc single chain (FIXFc-sc) and an Fc single chain (Fc-sc) bound together through two disulfide bonds in the hinge region of Fc. See Figure 1 and Table 2.

**[0123]** rFIXFc drug product is a clear colorless solution intended for intravenous (IV) administration. rFIXFc is supplied as 1000 IU per a 5 mL volume in a 10 mL single use only vial. The Drug Product is packaged in USP Type I glass vials with bromobutyl stoppers and tear-off plain aluminum overseals. rFIXFc drug product contains 200 IU/mL in 10mM sodium phosphate buffer pH 7.0 with addition of 145 mM NaCl and 0.1% polysorbate 20. The rFIXFc solution should not be diluted.

**[0124]** Study Design. A total of 14 previously treated patients with severe hemophilia B were enrolled and treated with FIXFc as an intravenous (IV) infusion over approximately 10 minutes. Six dose levels, 1, 5, 12.5, 25, 50, and 100 IU/kg were evaluated in the study. One patient per dose level was enrolled at dose levels 1, 5, 12.5, and 25 IU/kg, and at least three evaluable patients per dose level were enrolled at 50 and 100 IU/kg.

**[0125]** After the screening (scheduled within 14 days of the FIXFc dose), the treatment period for the patients began. The treatment period for each dose level included a single dose of FIXFc (Day 1) up until the completion of the 72-hour safety observation period (3 days) for dose levels 1 and 5 IU/kg or until the last PK sample was taken for patients in dose levels 12.5 to 100 IU/kg (approximately 10 days). Patients treated with 1, 5, 12.5, or 25 IU/kg were

enrolled and treated in a sequential manner starting at 1 IU/kg. Patients receiving 50 IU/kg were not treated on the same day and at least one day separated dosing. After treatment of the 50 IU/kg patients, treatment of the 100 IU/kg patients began.

**[0126]** The post-treatment period was a 30-day safety observation period starting from the day the patient received the dose of FIXFc and overlapped with the treatment period since patients were undergoing the required study evaluations, such as PK sampling, during this time.

**[0127]** Patients assigned to dose levels 12.5 to 100 IU/kg had blood samples drawn to assess FIX activity and FIXFc concentration. Blood samples were to be drawn just prior to administration of FIXFc; 15 minutes following the end of the infusion; and at 1, 3, 6, 9, 24, 48, 72, 96, 120, 168, and 240 hours following the end of the infusion or until baseline FIX levels were reached. If a patient continued to have FIX levels above baseline at the 240-hour time point (Study Day 11), samples were taken at 288 hours (Study Day 13) and again at 336 hours (Study Day 15) if the FIX level was above baseline at Study Day 13.

**[0128]** Patient 10 received BENEFIX™ treatment for a bleed prior to scheduled FIXFc sampling at 216 hours post dosing. Consequently, FIXFc activity and antigen data for the 216 h and following time points were excluded from analysis. No additional deviations occurred that are felt to have affected the interim analysis results of this study.

**[0129]** For Factor IX antigen, pharmacokinetic analyses were performed on the individual patient observed FIXFc concentration versus time data following IV infusion of FIXFc. Primary analysis was performed using model-dependent methodology. FIXFc concentration data were computer-fitted to a two-compartment open model with elimination from the central compartment using user-defined initial parameter estimates for the calculation of initial parameter values. WinNonlin estimated microscopic rate constants were generated and FIXFc concentration data were weighted with the function of $1/(Y^{-hat} * Y^{-hat})$. Observed data for two subjects (e.g., Patients 5 and 6) were inadequately described by the two-compartment model. Consequently, model-independent analysis was performed on these two patients using WinNonlin noncompartmental analysis IV-Infusion input model (linear trapezoidal rule for AUC calculation). For noncompartmental analysis, the half-life was calculated from the beta phase using the data points that describe the terminal log-linear decline in the regression. A minimum of three points were used to describe elimination phase. This occurred approximately between 4 and 14 days. For PK analysis of antigen, the "mg/kg" dose equivalents were utilized. These values were determined based on a specific activity for FIXFc of 60.2 IU/mg. Actual sampling times, doses, and infusion durations were used for

calculations. Nominal sampling times and doses were used for the creation of tables and concentration-time figures. Individual and mean PK parameters and descriptive statistics are presented. Formal statistical analysis was not performed because the dose range and the number of subjects in each cohort were too small for meaningful analysis.

**[0130]** For Factor IX activity, a baseline subtraction method was applied to the activity versus time profile according the baseline subtraction decision tree (Figure 4). Activity values of < 1% were defined at 1 IU/dL for baseline decay. Predose times were treated as zero for the purpose of calculations. In addition, baseline corrected activity data were truncated at time points that represented a return to baseline levels. Pharmacokinetic analyses were performed on the baseline subtracted FIX activity versus time data obtained following IV infusion administration of FIXFc. A model-dependent assessment was utilized for analysis of the IV-infusion dose groups. The baseline subtracted data were computer-fitted to a two-compartment open model with elimination from the central compartment using WinNonlin-defined parameter boundaries for the calculation of the initial parameter values. WinNonlin estimated microscopic rate constants were generated and FIXFc activity data were weighted with the function of $1/(Y^{-hat} * Y^{-hat})$. Actual sampling times, doses, and infusion durations were used for calculations. Nominal sampling times and doses were used for the creation of tables and concentration-time figures.

**[0131]** When unavailable from the actual data, the activity at 168 h post dosing (C168) and time to 1 IU/dL above baseline (TBLP1) of rFIXFc were obtained using the WinNonlin generated microscopic rate constants to simulate the FIXFc activity level versus time data. Individual and mean PK parameters and descriptive statistics are presented in this Example. Formal statistical analysis was not performed, because the dose range and the number of subjects in each cohort were too small for meaningful analysis.

**[0132]** Results for FIXFc antigen pharmacokinetics showed that FIXFc plasma concentrations increased sharply after the short IV infusion of FIXFc, with mean (±SD) $C_{max}$ values of 1670 (n=1), 2730 (n=1), 7510 ± 2480 and 15400 ± 3960 ng/mL for the 12.5, 25, 50, and 100 IU/kg nominal dose levels, respectively, and was reached within the first half-hour for all patients All FIXFc-treated patients had dose-related increases in systemic FIXFc plasma exposure (as assessed by $C_{max}$ and $AUC_{INF}$). Although limited to a single evaluable patient at the 12.5 and 25 IU/kg nominal dose, the observed increase in both $C_{max}$ and $AUC_{INF}$ was reasonably proportional to dose over the dose range evaluated. (Table 3 shows individual patient and group mean FIXFc antigen concentration versus time data; sorted by nominal dose, actual dose, infusion duration, and patient number. Table 4 shows individual

patient and group mean FIXFc antigen PK summary data; sorted by nominal dose, actual dose, "mg/kg" equivalent dose, and patient number, shows individual patient and group mean FIXFc antigen PK summary data; sorted by nominal dose, actual dose, "mg/kg" equivalent dose, and patient number, and see Table 11.)

**[0133]** FIXFc plasma concentrations declined in a biexponential fashion following the short IV infusion. Both distribution (alpha) and elimination (beta) half-life values appeared to be dose-independent over the dose range evaluated with individual patient alpha and beta half-life values ranging from 9.79 to 21.2 hours and 71.0 to 140 hours, respectively. Mean alpha half-life values (±SD) for the 50 and 100 IU/kg nominal dose levels were 13.1 ± 4.77 and 12.1 ± 2.33 hours, respectively. Mean beta half-life values (±SD) for the 50 and 100 IU/kg nominal dose levels were 110 ± 26.5 and 95.8 ± 11.1 hours, respectively. In addition, primary PK parameter values for Cl, $V_{SS}$, and MRT were determined and, in general, all appeared to be dose-independent over the dose range evaluated. As indicated, this assessment is limited by single patient data at the 12.5 and 25 IU/kg nominal dose levels. (Table 12 and Figures 2, 7, and 8.)

**[0134]** Further, mean Cl values were 2.28 ± 0.374 and 2.11 ± 0.464 mL/h/kg for the 50 and 100 IU/kg nominal dose levels, respectively. Mean $V_{SS}$ values were 259 ± 78.5 and 238 ± 52.2 mL/kg for the 50 and 100 IU/kg nominal dose levels, respectively. In addition, mean MRT values were 112 ± 21.5 and 114 ± 17.1 h for the 50 and 100 IU/kg nominal dose levels,.

**[0135]** Results for baseline corrected FIXFc activity pharmacokinetics showed that FIXFc activity increased sharply after the short IV infusion of FIXFc, with mean (±SD) model-predicted $C_{max}$ values of 11.9 (n=1), 19.9 (n=1), 41.6 ± 8.97 and 98.2 ± 8.21 IU/dL for the 12.5, 25, 50, and 100 IU/kg nominal dose levels, respectively, and was reached within the first half-hour for all patients. (Table 5 shows individual patient and group mean baseline corrected FIXFc activity versus time data; sorted by nominal dose, actual dose, infusion duration, and patient number and. Table 6 shows individual patient and group mean FIXFc activity PK summary data; sorted by nominal dose, actual dose, "mg/kg" equivalent dose, and patient number.)

**[0136]** All FIXFc-treated patients had dose-related increases in FIX activity (relative to predose baseline response). Although limited to a single evaluable patient at both the 12.5 and 25 IU/kg nominal dose levels, the observed increase in both $C_{max}$ and $AUC_{INF}$ was reasonably proportional to dose over the dose range evaluated. (Tables 6, 9, and 13 and Figures 3 and 5.)

**[0137]** After the end of the infusion, the decline in baseline corrected FIX activity exhibited biexponential decay; characterized by a rapid distribution (alpha) phase followed by a log-linear elimination (beta) phase. During the alpha phase, the rate of decline in FIXFc activity was variable with individual patient alpha half-life values ranging from 0.140 to 16.6 hours. The seemingly dose-dependent increase in mean alpha half-life values was confounded by a single patient at the 12.5 and 25 IU/kg nominal dose levels. In contrast, elimination (beta) half-life values appeared to be dose-independent over the dose range with individual patient beta half-life values ranging from 42.1 to 67.4 hours over the 25 to 100 IU/kg dose range. Although estimated and reported, the elimination half-life for patient 1 treated with 12.5 IU/kg of rFIXFc are not included in summary evaluation due to this patient's FIX levels being detectable for only up to 96 hours resulting in a truncated terminal phase and contributing to an underestimation of the terminal elimination half-life. Mean beta half-life values (±SD) for the 50 and 100 IU/kg nominal dose levels were 52.1 ± 10.4 and 52.5 ± 10.1 hours, respectively, and 52.5 ± 9.2 (range 40-67.4) hours for combined 25, 50 and 100 IU/kg nominal doses. (Tables 6, 8 and 13).

**[0138]** In addition, primary PK parameter values for Cl, $V_1$, $V_{SS}$, and MRT were determined and, in general, all appeared to be dose-independent over the dose range evaluated.

**[0139]** Further, mean Cl values were 3.77 ± 1.12 and 2.89 ± 0.615 mL/h/kg for the 50 and 100 IU/kg nominal dose levels, respectively, and 3.36 ±0.928 mL/h/kg for the combined 25, 50, and 100 IU/kg nominal doses. (Tables 6, 8 and 13.)

**[0140]** Mean $V_{SS}$ values were 264 ± 77.6 and 179 ± 31.1 mL/kg for the 50 and 100 IU/kg nominal dose levels, respectively, and 226 ± 69.8 mL/kg for the combined 25, 50, and 100 IU/kg nominal doses. (Tables 6, 8 and 13.)In addition, mean MRT values were 71.7 ± 13.0 and 62.8 ± 8.82 h for the 50 and 100 IU/kg nominal dose levels, respectively, and 68.05 ± 11.16 h for the combined 25, 50, and 100 IU/kg nominal doses. (Tables 6, 8 and 13.)

**[0141]** In addition to the primary PK parameters, secondary PK parameters (e.g., C168, K-values, IVR, etc.) were determined to evaluate FIXFc duration of effect. As anticipated, dose-dependent increases in C168, TBLP1, TBLP3, and TBLP5 values were observed. In contrast, K-values and IVR values appeared to be dose-independent over the dose range evaluated. Over the full dose range, individual patient model-predicted and observed K-values ranged from 0.61 to 1.02 and 0.62 to 1.17 IU/dL per IU/kg, respectively. Mean model-predicted K-values for the 50 and 100 IU/kg nominal dose levels were 0.76 and 0.90 IU/dL per IU/kg, respectively, and 0.821 ± 0.1387 (range 0.61-1.02) IU/dL per 1 IU/kg for

combined 25, 50, and 100 IU/kg nominal doses. Mean model-predicted IVR values for the 50 and 100 IU/kg nominal dose levels were 34.5 and 35.1%, respectively. Mean observed K-values for the 50 and 100 IU/kg nominal dose levels were 0.86 and 1.02 IU/dL per IU/kg, respectively, and 0.926 ± 0.1787 (range 0.97-1.17) IU/dL per 1 IU/kg for combined 25, 50, and 100 IU/kg nominal doses. Mean observed IVR values for the 50 and 100 IU/kg nominal dose levels were 39.2 and 39.8%, respectively. (Tables 6, 7, 8 and 13.) Table 7A-7B show7 shows individual patient and group mean FIXFc activity secondary PK summary data; sorted by nominal dose, actual dose, and patient number.

**[0142]** Each 1 IU/kg of infused rFIXFc raised plasma FIX activity by 0.93 ± 0.18 IU/dl on average, and this incremental recovery (K value) showed weak positive correlation with body weight ($R^2$=0.336, p=0.048) (Figure 23).

**[0143]** Pharmacokinetic estimates for FIXFc activity were consistent with those for rFIXFc antigen (e.g., compare Tables 13 and 14). Further, there was excellent correlation between rFIXFc activity and antigen levels, indicating the preservation of rFIXFc in vivo activity. (Figure 9.) In addition, relative to historical data for BENEFIX™ (Wyeth), rFIXFc demonstrated (Table 8) the following:

Dose linearity from 25-100 IU/kg
3 fold increase in $t_{1/2beta}$
3 fold increase in mean residence time
24% improved incremental recovery
2.5 fold reduced clearance

**[0144]** FIXFc is a recombinant fusion protein comprised of FIX attached to the Fc domain of human IgG1. FIXFc has been designed to be a long-acting version of FIX. Preclinical studies with FIXFc have shown a prolongation of the half-life of FIX activity compared to BENEFIX™, the commercially available recombinant FIX product. The rationale for this study was to evaluate the safety and PK of FIXFc in severe hemophilia B patients. For this study, 12 evaluable subjects aged 18 to 76 years were available for PK evaluation. Each subject received a single administration of FIXFc at a nominal dose of 12.5, 25, 50, or 100 IU/kg of body weight infused intravenously over approximately 10 minutes. Plasma samples for PK assessments of both FIXFc activity and antigen concentrations were obtained before infusion as well as up to 14 days after dosing. The PK of both FIXFc antigen and activity were independently characterized in this study using model-dependent and model-independent methods.

**[0145]** FIXFc was well tolerated following administration of single IV doses of 12.5, 25, 50, and 100 IU/kg of body weight. There was no evidence of drug-related serious adverse

events in this study. No neutralizing or binding antibodies to rFIXFc were detected in any subject.

**[0146]** Approximate dose-proportional increases in $C_{max}$ and $AUC_{INF}$ were observed for both FIXFc antigen and activity following the administration of doses of 12.5 through 100 IU/kg, but the V and Cl were similar across all doses. These results indicate that FIXFc antigen and activity exhibited linear PK over the dose range evaluated. The relatively small V parameter values may indicate that FIXFc enters the interstitial fluid but does not cross the cell membrane into the intracellular fluids.

**[0147]** Peak plasma levels of FIXFc antigen and activity were observed within 0.5 h after the end of the infusion and remained detectable for several days after dosing. Evidence of reduced clearance and prolonged half-life was observed for both FIXFc antigen and activity.

**[0148]** Mean clearance and terminal elimination half-life values associated with FIXFc antigen concentrations for the 50 and 100 IU/kg dose levels were 2.28 and 2.11 mL/h/kg and 110 and 95.8 hours, respectively. Similarly, mean clearance and terminal elimination half-life values associated with FIXFc activity levels over the same dose range were 3.77 and 2.89 mL/h/kg and 52.1 and 52.5 hours, respectively. Comparison of FIXFc activity PK results observed in the current study to reported PK for BENEFIX™ activity (Summary of Product Characteristics of BENEFIX™; Nov 18, 2009) revealed an approximate 3-fold reduction in FIXFc clearance and an approximate 3-fold increase in both FIXFc terminal elimination half-life and mean residence time relative to BENEFIX™.

**[0149]** With the observed improvements in PK, FIXFc will provide a prolonged protection from bleeding, allowing less frequent injections for individuals with Hemophilia B. Based on the results of this trial, rFIXFc may be dosed every two weeks or twice monthly using doses of 100 IU/kg and at least weekly using lower doses. Such a regimen requires fewer injections. In addition, the use of rFIXFc will have other potential clinical impacts such as: central venous access; improved regimen compliance; reduced break through bleeds; and increased protection of joints from bleeds.

## Example 2. B-LONG Phase 1/2/3 Trial

**[0150]** This will be an open-label, multicenter evaluation of the safety, pharmacokinetics, and efficacy of recombinant, long-acting coagulant Factor IX Fc fusion (rFIXFc) in the prevention and treatment of bleeding in previously treated subjects with severe hemophilia B. Treatment with FIX products currently on the market necessitates dosing 2-3 times per week.

A product with a prolonged half-life that extends the required dosing interval to once weekly or longer would be considered by the medical community as a significant improvement for the treatment of severe hemophilia patients.

**[0151]** Dose levels vary widely for rFIX products in clinical prophylaxis studies: the reported doses range from 10 to 171 IU/kg (Roth et al., Blood 98:3600 (2001)) or 40 to 100 IU/kg (MASAC Recommendation 177, National Hemophilia Foundation (Oct. 2006)). Moreover, trough levels of FIX activity during prophylaxis treatment in subjects with no clinical signs of bleeding are predicted to range between 0.2 and 3.8 IU/dL (Carlsson et al., Hemophilia 4:83 (1998)). Considering the inter-individual patient variability, individualized dosage regimens based on the clinical status of a patient are common practice.

**[0152]** The results of a Phase 1/2a study (Example 1) evaluating the safety and pharmacokinetics of a single dose of a frozen liquid formulation of rFIXFc have demonstrated the drug is well tolerated at doses ranging from 1 to 100 IU/kg and the PK characterization suggests several advantages over currently available treatments, namely a half-life and MRT that are 3-fold longer than that previously reported for BENEFIX™ (61 hours vs. 19 hours). The purpose of this study is to determine the PK parameter estimates of the lyophilized rFIXFc in humans prospectively, to compare these with BENEFIX™ PK parameter estimates in humans, and to demonstrate the efficacy of lyophilized rFIXFc in the prevention and treatment of bleeding and the safety of its repeat dosing for previously treated subjects with severe hemophilia B.

**[0153]** The study will entail four arms: a low dose prophylaxis regimen (n=25), a high dose prophylaxis regimen (n=25), an on-demand regimen (n=20) and a major surgery regimen (n=5). The low dose regimen arm will include a PK subgroup (n=16) dosed with BENEFIX™, followed by crossover to rFIXFc.

**[0154]** The primary objectives of the study are: to evaluate the safety and tolerability of rFIXFc in all treatment arms; to evaluate the efficacy of rFIXFc in all treatment arms; and to evaluate the effectiveness of prophylaxis over on-demand therapy (comparison of the annualized number of bleeding episodes between Arms 1 and 2 versus on-demand regimen Arm 3).

**[0155]** The secondary objectives of the study are: to compare the PK parameter estimates of rFIXFc and BENEFIX™; to evaluate the efficacy of rFIXFc in the on-demand and surgical arms; to evaluate and compare the PK parameter estimates of rFIXFc at baseline and Week 26 (± 1 week) in the PK subgroup; to evaluate subjects' response to treatment in all arms; and to evaluate rFIXFc consumption in all arms.

Main Inclusion Criteria:

Male and 12 years of age and older and weigh at least 40 kg
Diagnosed with hemophilia B (baseline Factor IX level less than or equal to 2%)
History of at least 100 exposure days to any Factor IX product
Platelet count ≥100,000 cells/μL
INR (international normalized ratio) ≤ 1.40 as defined by the testing laboratory's normal range
CD4 count ≥ 200 cells/μL

Main Exclusion Criteria:

History of Factor IX inhibitors
Kidney or liver dysfunction
Diagnosed with another coagulation defect other than hemophilia B
Prior history of anaphylaxis associated with any FIX or IV immunoglobulin administration
Taking systemic immunosuppressive drugs (e.g., systemic corticosteriods; however, HAART (highly active antiretroviral therapy) is permitted)

Example 3. FIXFc Production in HEK293 Cells

**[0156]** FIXFc was produced in stably transfected HEK293 cells containing an expression cassette for FIXFc (native FIX fused directly to the Fc region) and an expression cassette for Fc alone. The cells also were transfected with an expression cassette for PC5, which is a processing enzyme that allows for full processing of the FIX propeptide. The transfected cells were grown in serum-free suspension media containing vitamin K, and they secreted three proteins: FIXFc dimer, FIXFc monomer (one FIXFc chain and one Fc chain), and Fc dimer. FIXFc monomer ("FIXFc") was purified by column chromatography (Protein A, Fractogel DEAE, and Q Sepharose pseudo-affinity elution with low ionic strength $CaCl_2$), and viral inactivated and filtered for administration to human subjects. Also see Peters et al., Blood. 2010 Mar 11;115(10):2057-64 (Epub 2010 Jan 7); and U.S. Patent No. 7,566,565; each of which is incorporated by reference herein in its entirety.

**[0157]** Coagulant activity of FIXFc was measured by quantitating its ability to restore the clotting activity of FIX-deficient plasma using an MLA Electra 1600C (Medical Laboratory Automation/Instrument Labs, Pleasantville, NY). Results were compared to a calibration curve generated using serial dilutions of a World Health Organization FIX standard.

**[0158]** Serine phosphorylation and tyrosine sulfation of Factor IX are thought to be important for in vivo recovery. It has been reported that MONONINE™ (plasma purified Factor IX (pdFIX) marketed by CSL Berhing) has better in vivo recovery than BENEFIX™ (recombinant FIX (rFIX) marketed by Wyeth) because of the higher phosphorylation/sulfation level of MONONINE™ (>90%/>90% versus <10%/5%).

However, FIXFc produced in HEK293 cells has almost no phosphorylation/sulfation (<10%/4%, which is very similar to BENEFIX™), and shows better IVR (1.0 IU/dl per IU/kg) than BENEFIX™ (0.7).

**[0159]** In addition, FIXFc produced as described above had a significantly lower (10-100 fold) level (0.01-0.001%) of activated FIX (FIXa), a product related impurity, than either MONONINE™ (pdFIX) or BENEFIX™ (rFIX) (0.1%). The resulting FIXFc will have fewer unwanted thrombotic events upon administration than MONONINE™ or BENEFIX™.

Example 4. Pediatric Studies: Extrapolation and Interrelation Between the Development in Adult and Pediatric Populations

**[0160]** Patient characteristics that show relationships with FIX pharmacokinetics include age-dependent physiological changes (Björkman and Berntorp, Clin. Pharmacokinetics 40:815-32 (2001); and Bjorkman, Hemophilia 9(suppl 1):101-10 (2003)) and body size and composition (Shapiro, Hemophilia 11:571-82 (2005)). Thus, weight-adjusted clearance (CL) of FIX has generally been found to decrease with age and/or body weight during growth from infancy to adulthood, with a corresponding increase in terminal half-life ($t_{1/2}$). For rFIX product (BENEFIX™), CL and volume distribution at steady state (Vss) are increased in children and then remain constant during adulthood; thus, these parameters will be closely monitored in the pediatric studies.

**[0161]** Peak levels of FIX procoagulant activity (FIX:C) depend on the initial volume of distribution of FIX:C after single and/or repeated doses of FIX. The initial distribution of FIX is rapid. However, it has been shown that in vivo recovery (mean incremental recovery) for BENEFIX™ was typically 30% lower than that of a monoclonal antibody purified plasma derived coagulation factor (pdFIX) (Roth et al., Blood 98:3600-3606 (2001)). Furthermore, studies with pdFIX have shown that subjects 15 years of age and younger have a significantly lower recovery than those who are older (White et al., Thromb. Haemost. 73:779-84 (1995)). Therefore, monitoring of trough and peak levels will also be performed in the pediatric studies.

**[0162]** Since studies have shown that children may respond differently compared to adults, pharmacokinetic assessments at baseline with 50 IU/kg of rFIXFc will be performed in children with abbreviated pharmacokinetic sampling.

**[0163]** The Phase 1/2a study (SYN-FIXFc-07-001) evaluating the safety and pharmacokinetics profile of a single intravenous administration of rFIXFc in PTPs aged 18

years and above with severe hemophilia B was recently completed. Preliminary results from this initial exploration in humans demonstrates an approximately 3-fold increase in pharmacokinetic parameters (mean terminal half-life, MRT, and AUC) of rFIXFc compared with what has been reported in the literature for BENEFIX™ (see above). Additionally, rFIXFc was well tolerated and there were no sign of injection site reactions as well as no development of inhibitors. Together, these safety and pharmacokinetic results support the initiation of a Phase 1/2/3 registrational study (998HB102 Study (B-LONG), see above) evaluating the safety, pharmacokinetics, and efficacy of rFIXFc in prevention and treatment of bleeding in 104 PTPs (with at least 100 treatment EDs to previous products) 12 years and older with severe hemophilia B (<2%). Once sufficient safety data are available from the registrational study, a pediatrics program will be initiated to further investigate the safety and efficacy of rFIXFc in children. The demonstration of prolonged half-life of rFIX in humans will mean that less frequent injections will be needed for the prevention and treatment of bleeding to individuals with hemophilia B.

Phase 2/3 Pediatric PTPs Study In Previously Treated Children (<12 Years Old)

**[0164]** Once the data are available on 10 PTPs (≥12 years) for 26 EDs from the registrational study (998HB 102 Study), a Pediatric Study, phase 3 will be initiated. This Phase 2/3 pediatric study, in PTPs who had at least 50 EDs to FIX products prior to enrollment, will be conducted globally at approximately 25 clinical sites. Approximately 25 PTPs (to ensure 20 evaluable subjects), age 2-11 years with severe hemophilia B (<2 IU/dL [<2%] endogenous FIX), will be screened and selected according to the pre-defined criteria. All evaluable subjects will complete the pharmacokinetic portion of the study (PK with pre-study FIX product and then PK with rFIXFc) and will receive weekly dosing of rFIXFc for 52 weeks. This study will record incremental recovery, in vivo half-life, AUC, and clearance of rFIXFc. All subjects will undergo pharmacokinetic assessment at baseline with pre-study FIX and rFIXFc and the duration of the study for each subject will be approximately 69 weeks, including screening and follow-up.

**[0165]** Each subject will receive 50 IU/kg of rFIXFc at baseline for pharmacokinetic assessment followed by repeated weekly dosing with 50-60 IU/kg of rFIXFc. With regard to patient compliance, abbreviated pharmacokinetic sampling will be employed for pre-study product and for rFIXFc as follows: pre dose, end of injection, 30 + 10 minutes, 3 ± 1 hours, 24 ± 3 (Day 1), 72 ± 3 (Day 3), 120 ± 3 (Day 5), and 168 ± 3 hours (Day 7) after the end of injection. In order to address immunogenicity, all subjects will be treated with rFIXFc weekly

for a minimum of 50 EDs. Safety parameters will be included for immediate safety and tolerability assessment, such as: (a) vital signs (pulse, blood pressure, respiratory rate, temperature) at pre rFIXFc injection and 30 minutes post injection; (b) hematology and coagulation parameters; (c) clinical chemistry; (d) frequent FIX inhibitor determinations using the Nijmegen-modified Bethesda assay (immediately before first exposure, ED4 [Week 4], ED12, ED24, ED36, and ED50); and (e) adverse events.

**[0166]** Efficacy will be assessed by evaluation of number of bleeding episodes, bleeding intervals and number of treatments and consumption of FIX per annualized year and per event.

Phase 2/3 Pediatric PUPs Study In Previously Untreated Children (0 – 11 Years Old)

**[0167]** Once the data from 10 previously-treated children (2-11 years) with complete pharmacokinetics and 50 EDs are available in the preceding study, a Phase 2/3 pediatric PUPs study will be initiated. This study will be conducted globally at approximately 60 clinical sites. Up to 30 PUPs (to ensure 20 evaluable subjects) for 0 and above years with severe hemophilia B (<2 IU/dL [<2%] endogenous FIX) will be screened and selected according to the pre-defined criteria.

**[0168]** Participation in the study will vary since the initiation treatment may begin using rFIXFc as modified prophylaxis regimen. Per patient study participation is expected to be approximately four years including screening and follow-up. During this time most patients are expected to achieve 50 EDs to rFIXFc. In order to address immunogenicity, all subjects will be treated with approximately 50 EDs of rFIXFc or for up to 4 years. Safety parameters will be included for immediate safety and tolerability assessment: (a) frequent FIX inhibitor determinations using the Nijmegen-modified Bethesda assay; and (b) adverse events.

**[0169]** Efficacy will be assessed by evaluation of number of bleeding episodes, bleeding intervals and number of treatments and consumption of FIX per annualized year and per event.

## Example 5. Biochemical Characterization, Activity, and PK Analysis in Non-Human Animals

**[0170]** The rFIXFc produced in Example 3 was characterized for its posttranslational modification, and the following results were obtained (see Table 15 and Figure 11). The propeptide of rFIXFc was properly processed during production. rFIXFc's gamma-carboxylation pattern was similar to that of rFIX. Further, total Gla/molecule (11.2 ± 0.7) of

rFIXFc was comparable to rFIX. Because gamma-carboxylation at certain residues is essential for FIX activity, these are important results. In addition, Ser 158 phosphorylation and Tyr 155 sulfation of rFIXFc were comparable to rFIX. N-linked glycans in FIX are not fully sialylated, similar to rFIX. rFIXFc O-linked glycosylation in the first EGF domain was the same as FIX, albeit in different relative ratios. Asp 64 of rFIXFc had a higher degree of beta-hydroxylation than rFIX or plasma derived FIX (pdFIX). Activated FIX was present at a much lower level in the rFIXFc preparation than in the rFIX or pdFIX preparations, as is discussed in detail in Example 3.

**[0171]** In addition, rFIXFc was administered to various animal species to determine its activity and PK parameters. The results are shown in Table 16 and Figures 12-16.

## Example 6. Gamma-Carboxylation

**[0172]** The goals of this study were to analyze and characterize γ-carboxylation of the glutamic acids (Gla) in a preclinical lot of FIXFc material and commercially available FIX products, to characterize the Gla content of an enriched "peak" fraction and a high salt elution "strip" fraction originating from a pseudo-affinity chromatography ion-exchange step, and to further separate an enriched "peak" and a high salt elution "strip" fraction by anion-exchange HPLC and further characterize the separated species.

**[0173]** To achieve these goals, a number of complementary analytical methods were developed. These include amino acid analysis (AAA) using basic hydrolysis to determine (total) Gla content, peptide map (LC/MS) using Lys-C peptides to determine Gla distribution, analytical anion-exchange HPLC of intact molecules to separate isoforms, and activated partial thromboplastin time (aPTT) to determine biological activity.

**[0174]** The two Gla (E) containing peptides are:

–K1K2: YNSGKL$^{7}$***E***$^{8}$***E***FVQGNL$^{15}$***E***R$^{17}$***E***CM$^{20}$***E***$^{21}$***E***K

- [M+H]+6 Gla = 2953.9
- [M+H]+5 Gla = 2909.9

–K3: CSF$^{26}$***E***$^{27}$***E***AR$^{30}$***E***VF$^{33}$***E***NT$^{36}$***E***RTT$^{40}$***E***FWK

- [M+H]+6 Gla = 2959.9
- [M+H]+5 Gla = 2915.9
- [M+H]+4 Gla = 2871.9

**[0175]** Thirty micrograms of sample (originating from the enriched peak fraction, high salt strip fraction and each species from the analytical anion-exchange HPLC) was denatured,

reduced, alkylated and digested with Lys-C (1:20, E:S). The digest was quenched with 2% TFA and injected onto a Jupiter C18 (2.0 x 250 mm) Phenomenex column. Separation was performed on an Agilent 1100 system. The column was maintained at 25° C and peptides were eluted with a multi-step acetonitrile gradient. Mass spectrometry (Thermo-Fisher LCQ) was performed in "Triple Play" mode.

**[0176]** Complementary methods were developed to analyze and characterize the Gla content and distribution of preclinical rFIXFc material. The γ-carboxylation of glutamic acids (Gla) content and distribution in a preclinical lot of rFIXFc (enriched peak fraction) was performed and compared to commercially available products. Analysis demonstrated similar Gla content and distribution with respect to commercially available products. A high salt elution "strip" fraction was analyzed and compared to the enriched peak fraction. Analysis indicated a reduced level of γ-carboxylation.

**[0177]** The FIXFc (Enriched Peak Fraction) was isolated from pseudo-affinity chromatography ion-exchange step and further separated into 3 iso-forms by analytical anion exchange HPLC. AEX column load and separated species were highly γ-carboxylated. (The AEX column load is the strip fraction collected during a high salt elution step from the pseudo-affinity chromatography ion-exchange step.) AEX column load and separated species were biologically active. The Gla content and distribution was similar to rFIX. The peptide map indicates distribution of 4/5/6 Gla's on the K3 peptide. The peptide map indicates a high population of 6 Gla's on the K1K2 peptide and a trace level of 5 Gla's.

**[0178]** The FIXFc (Strip Peak Fraction) was isolated from pseudo-affinity chromatography ion-exchange step and further separated into 2 iso-forms by analytical anion exchange HPLC. AEX column load and separated species were reduced in γ-carboxylation level. There was reduced Gla content relative to FIXFc enriched peak fraction. A decreased level of biological activity was observed. The peptide map indicates an increased population of 5 Gla's in K1K2 relative to the enriched peak fraction and may suggest an impact on biological activity.

**[0179]** References (each of which is incorporated by reference herein in its entirety): Dumont JA, et al., Monomeric Fc Fusion Molecules in Therapeutic Abs-From Bench to Clinic, Ch. 33 p779-795; Gillis S, et al., Protein Science (1997) 6:185; White GC, et al., J. Thrombosis and Haemostasis (1997) 78:261; Hansson K, and Stenflo J, Journal Thrombosis and Haemostasis (2005) 3:2633; and Peters RT, et al., Blood (2010) 115:2057.

Example 7. Evaluation of rFIXFc Pro-coagulant Activity in HemB Mice Bleeding Models

**Comparable potency of rFIXFc and BENEFIX™ was demonstrated in HemB mouse whole blood ROTEM in vitro and in a HemB mouse Tail Clip bleeding model in vivo.**

**[0180]** The ability of rFIXFc to form firm and stable clots was evaluated by Rotation Thromoboelastometry (ROTEM®, Pentapharm GmbH, Munich, Germany) with Calcium Chloride as activator (NATEM). Pooled whole blood collected via the vena cava from HemB mice was divided into seven aliquots, which were spiked with rFIXFc to a final concentration of 7.4%, 0.74% and 0.074% of normal plasma FIX activity, or BENEFIX™ to 10%, 1%, 0.1% of normal. As a negative control, a blood sample was spiked with FIX formulation buffer. A total of 10 blood pools from 5 HemB mice were generated to complete the assessment. The NATEM reaction was initiated by the addition of $CaCl_2$. Coagulation parameters, including Clotting Time (CT), Clot Formation Time (CFT) and Alpha Angle were assessed. The mean and SD of CT, CFT and alpha angle are summarized in Table 17. The dose responses for the three parameters are plotted in Figure 17. All three parameters are comparable between rFIXFc and BENEFIX™ in the dose range tested ($p>0.05$ by one-way ANOVA (Kruskal-Wallis) analysis).

**[0181]** Acute efficacy of rFIXFc was also evaluated in HemB mouse Tail Clip bleeding model. (Figure 18.) Male HemB mice were stratified for equal presentation of body weight and age in different treatment groups. Prior to tail clip injury, mice were anesthetized with a cocktail of 50mg/kg Ketamine and 0.5 mg/kg Dexmedetomidine and placed on a heating pad to help maintain the body temperature. The tails of the mice were then immersed in 37°C water for 10 minutes to dilate the lateral vein. After the vein dilation, rFIXFc, BENEFIX™ or vehicle were injected via the tail vein and 5 min later, the distal 4mm of the tail were then cut off using a #11 scalpel with straight edge. The shed blood was collected into 13 ml of warm saline for 30 minutes and the blood loss was quantified gravimetrically. Six rFIXFc treatment groups (720, 360, 240, 120, 80, 40 IU/kg, n=15) and three BENEFIX™ treatment groups (360, 120, 40 IU/kg, n=15) were tested. The individual animal's blood loss value and dose response curve of median blood loss are shown in Figure 19(A), and the median blood loss volume of each treatment group is summarized in Table 18. The dose response in median blood loss volume for both rFIXFc and BENEFIX™ are comparable ($p = 0.9315$ by unpaired t test with Welch's correction).

**[0182]** To determine if the three-fold extended half-life of rFIXFc relative to BENEFIX™ resulted in prolonged efficacy of rFIXFc, the present inventors evaluated the efficacy of rFIXFc and BENEFIX™ in both ex-vivo ROTEM® assay and Tail Vein Transection bleeding model (TVT) in HemB mice. Figure 20.

**[0183]** For ex vivo ROTEM®, male HemB mice received 50 IU/kg of rFIXFc or 100 IU/kg of BENEFIX™ by intravenous injection. Whole blood was collected from the vena cava of treated animals at 5 min, 24, 72, 96, 120, 168, and 216 hour post rFIXFc dosing (n=8 mice at each time point) or at 5 min, 24, 48, 72, and 96 hour post BENEFIX™ dosing (n=4 mice/time point). Blood samples were analyzed immediately by NATEM. The mean and SD for CT, CFT, and alpha angle are shown in Table 19, and the CT, CFT and alpha-angle versus time curves are shown in Figure 21. In comparison to BENEFIX™, rFIXFc showed comparable CT, CFT, and alpha angle at 5 min, but significantly improved CT, CFT and alpha angle after 72 hrs despite a 2-fold lower dose relative to BENEFIX™.

**[0184]** To evaluate the prophylactic efficacy of rFIXFc and BENEFIX™, male HemB mice were stratified for equal representation of body weight and age in 9 different treatment groups. rFIXFc was administered by iv injection at a dose of 4 IU/kg, 13 IU/kg ,40 IU/kg and 120 IU/kg at 72 hours prior to tail vein transaction, whereas the same doses of BENEFIX™ was administered at 24 hour prior to the injury. Prior to tail vein transection, mice were anesthetized with a cocktail of 50 mg/kg Ketamine/0.125 mg/kg Dexmedetomidine/ 0.1 mg/kg Buprenex. In order to allow the mice to maintain normal activity following tail vein transection, 1 mg/kg Atipamezole solution was given to reverse the effect of Dexmedetomidine, which immediately followed by the lateral tail vein transection with a straight edged number 11 surgical blade at an area where the diameter of the tail is approximately 3 mm. The shedding blood was washed away with warm saline to ensure clear observation of the wound, and the mouse was then single-housed in a clean cage with white paper bedding for the next 24 hours. The re-bleed and the physical activity were observed and recorded hourly up to 12 hour post injury. Moribund mice were euthanized immediately after identification, and a 24 hour post injury checkup was performed to complete the study. The Kaplan-Meier curve for Time to Euthanasia and chart of survival rates 24 hour post TVT were shown in Figure 22. The Log-rank test determined that all treatment groups with higher than 4 IU/kg dose are significantly better than vehicle group ($p< 0.001$). Furthermore, survival is comparable between mice that received the same dose of rFIXFc at 72 hrs prior to injury as that of BENEFIX™ at 24 hrs prior to injury ($p= 0.4886$, 0.9268, 0.7279 and 0.5209 for 4, 13, 40 and 120 IU/kg dose groups respectively). The

survival rates at 24 hour post TVT were plotted and ED50 value for each molecule were extrapolated from the curve, the ED50 for the two treatments are similar at 17.8 IU/kg for rFIXFc and 15.4 IU/kg for rFIX. Therefore, rFIXFc provided 3-fold longer duration of protection in HemB mice relative to a comparable dose of BENEFIX™ as measured by survival and re-bleed following tail vein transection injury. Therefore, rFIXFc provided 3-fold longer duration of protection in HemB mice relative to a comparable dose of BENEFIX™ as measured by survival and rebleed following tail vein transection injury.

**[0185]** In conclusion, as the data show, whereas 15.4 IU/kg of BENEFIX™ resulted in 50% of HemB mice surviving the tail vein transection at 24 hrs post dosing, 17.8 IU/kg of rFIXFc achieved 50% survival in animals that were injured at 72 hrs post dosing. Therefore, rFIXFc demonstrates a 3-fold longer prophylactic efficacy in correlation with its half-life extension relative to BENEFIX™. The results from the bleeding models are further corroborated by ex vivo ROTEM® analysis of whole blood from HemB mice treated with either 100 IU/kg of BENEFIX™ or 50 IU/kg of rFIXFc. At 5 min post dosing, comparable improvement in clot formation were observed in both treatment groups. However, the major ROTEM® parameters such as the clotting time, clot formation time and alpha-angle were significantly improved in rFIXFc-treated mice at 72 to 216 hrs following dosing despite a 2-fold lower dose of rFIXFc relative to BENEFIX™.

**[0186]** In summary, the acute potency of rFIXFc is comparable to that of BENEFIX™ as shown in both whole blood ROTEM® in vitro and the tail clip bleeding model in HemB mice. The prolonged prophylactic efficacy of rFIXFc was shown in ex vivo whole blood ROTEM® from treated HemB mice and was determined to be approximately 3-fold longer in comparison to BENEFIX™ in the tail vein transection bleeding model in HemB mice. The prolonged efficacy of rFIXFc correlates well with the 3-fold longer $T_{1/2}$ of rFIXFc relative to BENEFIX™ previously demonstrated in pharmacokinetic study in HemB mice. Therefore, rFIXFc is fully active for on-demand treatment while achieving significantly prolonged prophylactic protection with the potential to reduce the dosing frequency, which are under investigation in the phase 3 study.

## Example 8. Pharmacokinetic and Pharmacodynamic Analysis of rFIXFc and BENEFIX™ Following a Single Subcutaneous Dose in FIX-Deficient Mice

**[0187]** The pharmacokinetic (PK) and pharmacodynamic (PD) profiles of recombinant Factor IX-Fc (rFIXFc) and BENEFIX™ (rFIX) were determined following a single

intravenous or subcutaneous injection of 200 or 400 IU/kg in FIX-deficient mice. Whole blood was collected via vena cava (n=4 mice/timepoint/treatment). The concentrations of rFIXFc and BENEFIX™ in plasma were determined using a human FIX-specific ELISA. The activities of rFIXFc and BENEFIX™ were determined using an activated partial thromboplastin time (aPTT) assay. PK analyses were performed using model-dependent methodology using WinNonLin. Results are shown in Tables 22 and 23.

**[0188]** For FIXFc, the bioavailability in FIX-deficient mice was 38% for the 200 IU/kg dose and 38-46% for the combined dose (antigen ELISA) and 29% for the 200 IU/kg dose and 29-39% for the combined dose (aPTT activity assay) compared to rFIX, 23% and 19%, respectively. The rFIXFc had 1.5-1.7 fold (200 IU/kg dose) and 1.5-2.5 fold (combined doses) improved bioavailability compared to BENEFIX™.

**[0189]** For rFIXFc, the terminal half-life (antigen ELISA) was 62 hr for the 200 IU/kg dose and 51-62 hr for the combined doses and the terminal half-life (aPTT activity assay) was 42 hr for the 200 IU/kg dose and 40-42 hr for the combined doses, whereas for BENEFIX™, the terminal half-life was 24 hr (antigen ELISA) for the 200 IU/kg dose and 17 hr (aPTT activity assay) for the 200 IU/kg dose. This indicates a 2.5-2.6 fold (200 IU/kg dose and combined dose) improvement in half-life with rFIXFc.

**[0190]** In addition, as Tables 22 and 23 show, rFIXFc had 4.5-5.6 fold increase in AUC/dose and a 1.9-3.7 fold increase in Cmax/dose versus BENEFIX™.

**[0191]** Recombinant factor IX Fc fusion (rFIXFc) protein is a long-acting form of recombinant FIX (rFIX) that will provide less frequent dosing of rFIX for treatment of hemophilia B. From mice to non-human primates and in hemophilia B patients, rFIXFc has an approximately 3-fold longer half-life versus rFIX (BENEFIX™). For prophylactic treatment, intravenous delivery of rFIX remains a burdensome delivery method, especially for children and in patients with poorly accessible veins. Subcutaneous administration of rFIX presents as a more attractive delivery route that is less invasive and with less frequent dosing. As such, subcutaneous delivery of rFIXFc will cause less pain and discomfort than intravenous delivery and result in improved compliance due to being easier to administer and administered in less time than an intravenous route. Prophylaxis regimens will also improve quality-of-life and clinical outcomes will include decreased bleeding incidences.

**[0192]** The concentration of rFIXFc in mouse plasma was measured using a human FIX-specific ELISA that measured the FIX portion of the molecule and the mg/kg nominal dose was used in the analysis. A summary of the PK parameters for rFIXFc and BENEFIX™ are shown in Table 20 (antigen ELISA) and Table 21 (aPTT activity assay) for n=4/group. Both

analysis by antigen and activity showed that the Cmax and AUC were significantly improved for rFIXFc versus BENEFIX™. Using the antigen ELISA, the bioavailability (F %) was 38% for rFIXFc versus 23% for BENEFIX™. Similarly, using the aPTT activity assay, the bioavailability was 29% for rFIXFc versus 19% for BENEFIX™. Thus, rFIXFc demonstrated an increase in bioavailability over BENEFIX™ by 1.5 to 1.6 fold. Measurements of elimination half-life showed that rFIXFc markedly increased the half-life whether measured by antigen (rFIXFc 62 hr versus BENEFIX™ 24 hr) or activity (rFIXFc 42 hr versus BENEFIX™ 17 hr) assays. These data show that rFIXFc had an extended half-life compared to BENEFIX™ by 2.6 to 2.5 fold.

**[0193]** The rFIXFc given subcutaneously to FIX-deficient mice demonstrated a PK and PD profile with increases in Cmax and AUC for rFIXFc compared to BENEFIX™. Overall, the bioavailability for rFIXFc ranged from 29% (activity) to 38% (antigen) with a half-life of 42 hr (activity) to 62 hr (antigen) compared to BENEFIX™, which had bioavailability from 19-23% and half-life from 17-24%, respectively. Thus, the half-life for rFIXFc delivered subcutaneously in FIX-deficient mice demonstrated about a 2.2 (antigen) to 3.3 (activity) fold increase over currently marketed rFIX products given intravenously. Overall, these data support the notion that rFIXFc delivered subcutaneously will be of clinical benefit for prophylactic treatment in hemophilia B patients.

## Example 9. Pharmacokinetic Analysis of rFIXFc Following a Single Subcutaneous Dose in Cynomolgus Monkeys

**[0194]** The pharmacokinetic (PK) profile of recombinant Factor IX-Fc (rFIXFc) was studied after a single subcutaneous dose of 50 IU/kg, 100 IU/kg or 200 IU/kg in cynomolgus monkeys. The concentration of rFIXFc in plasma was measured using a FIX-specific ELISA. Primary analysis was performed using model-dependent methodology using WinNonLin. See Tables 22-25.

**[0195]** Pharmacokinetic analysis of the plasma concentration versus time data (measured by FIX-specific ELISA) demonstrated that the bioavailability and terminal half-life were similar among doses. The bioavailabilities for rFIXFc were 40% (50 IU/kg), 34% (100 IU/kg), 36% (200 IU/kg), and 36-45% (combined doses) The terminal half-lives for rFIXFc were 61 hr (50 IU/kg), 45 hr (100 IU/kg), 49 hr (200 IU/kg), and 44-58 hr (combined doses).

**[0196]** The concentration of rFIXFc in monkey plasma was measured using a FIX-specific ELISA that measured the FIX portion of the molecule and the mg/kg nominal dose

was used in the analysis. Spike and recovery analysis demonstrated the accuracy of this FIX-specific ELISA assay for detecting rFIXFc over the range of plasma concentrations assessed. A summary of the PK parameters for rFIXFc are shown in Table 22 (50 IU/kg), Table 23 (100 IU/kg) and Table 24 (200 IU/kg) for n=3/group. For rFIXFc SC, the geometric means and CV% of the geometric mean for Cmax were 860 + 22 (50 IU/kg), 1630 + 97 (100 IU/kg) and 3,750 + 26 (200 IU/kg), respectively indicating a dose-dependent increase. Similar increases were seen for AUC. The geometric means for bioavailability (F %) were 40 + 16 (50 IU/kg), 30 + 75 (100 IU/kg) and 36 + 27 (200 IU/kg), demonstrating that bioavailability was similar among doses. Measurements of terminal half-life showed that the half-life was similar among doses at 58 + 39 hr (50 IU/kg), 45 + 13 hr (100 IU/kg) and 46 + 44 hr (200 IU/kg).

**[0197]** The rFIXFc given subcutaneously to cynomolgus monkeys demonstrated a PK profile with dose-dependent increases in Cmax and AUC. Overall, the bioavailability ranged from 30-40% with a half-life of 45-58 hr. Thus, the half-life for rFIXFc delivered subcutaneously in monkeys demonstrated about a 2.8-fold increase over currently marketed rFIX products given intravenously. Overall, these data support the notion that rFIXFc delivered subcutaneously will be of clinical benefit for prophylactic treatment in hemophilia B patients.

## Example 10. Predicted Prophylactic Dosing Regimens

**[0198]** In comparison with the standard recommended dose regimen of 25 to 40 IU/kg of FIX twice or three times weekly, the median rFIXFc activity PK results from the Phase 1/2a study described above suggest that about once weekly dosing of rFIXFc at about 22.5 IU/kg, or about every 10 days at about 45 IU/kg, or about every 2 weeks at about 120 IU/kg is sufficient to maintain a trough of 1% above baseline (Figure 24). These model simulated estimates are validated by the available data from the Phase 1/2a trial, which fall entirely within the 95% confidence interval of the simulated activity-over-time curve. These regimens will often serve at the beginning of therapy. Considering the heterogeneity of reported clinical breakthrough bleeding events relative to trough level of plasma FIX activity, maintenance doses will need to be adjusted individually.

**[0199]** After recalculation of the PK results from the Phase 1/2 study (see Example 11), the new predicted dosing regimen, e.g., for prophylaxis, is 20 IU/kg once weekly, 40 IU/kg

every 10 days, or 100 IU/kg every two weeks (twice monthly). See also Table 27 and Figure 25.

## Example 11. Recalculation of Pharmacokinetic Data from First in Human (FiH) Study (Example 1)

**[0200]** Subjects with a variety of hemophilia B genotypes, such as stop codon/nonsense and missense mutations, were included in the FiH study discussed in Example 1. Several subjects had markedly reduced endogenous FIX antigen levels which correlated with markedly reduced FIX activity, while a few subjects with missense genotypes had more antigen than measured 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. Based on this information, the PK data from Example 1 was recalculated without baseline subtraction, as is described below in detail. See Table 27.

**[0201]** In contrast to the PK calculations (based on activity) in Example 1, if the rFIXFc activity PK is modeled without baseline subtraction, as was recently reported for the PK analysis of a glycoPEGylated rFIX (Negrier et al., *Blood* DOI 10.1182/blood 2011 02 335596 (2011), which is herein incorporated by reference in its entirety), the resulting estimates of elimination half-life and MRT are much longer than the estimates in Example 1, at 82.2 ± 21.6 and 96.8 ± 22.0 hours (mean ± SD), respectively. However, with the knowledge that not all severe hemophilia B patients have 0% endogenous FIX activity, and taking into account patient's genotype and endogenous FIX antigen level, the present inventors adopted a baseline subtraction analysis method in their PK modeling. Specifically, (a) the baseline in two patients was defined as 0% because their pretreatment FIX activity was <1%, they had no detectable FIX antigen and had nonsense genotypes, (b) the baseline for three patients was set at 0.5% because their pretreatment FIX activity was <1% and they had detectable FIX antigen, (c) for patients whose pretreatment FIX activity was between 1 – 2%, Cmin (the lowest activity throughout the PK study) was defined as baseline, and (d) for patients whose pretreatment FIX activity was ≥2%, 2% (which was the upper limit for enrollment into the trial) was the baseline. Activity above the baseline pre-dosing was considered residue drug from prior treatment, and was decayed to baseline and subtracted from the PK data following rFIXFc dosing.

**[0202]** The resulting mean terminal half-life (56.7 ± 10.9 hours, range 42.4 – 74.5 hours) and MRT (71.8 ± 10 hours, range 53.2 - 85.9 hours) of rFIXFc are approximately 3-fold longer than that reported for rFIX. The reported terminal half-life of rFIX is 19.3 ± 4.97 hours (range 11.1 - 36.4 hours) and MRT 26.0 ± 6.07 hours (range 15.8 - 46.1 hours). Roth *et al., Blood 98:3600-3606* (2001); and Summary of Product Characteristics for BENEFIX™, Electronic Medicines Compendium (2010) (http://www.medicines.org.uk/emc/medicine/20376/SPC/BENEFIX™/#PHARMACODYNAMIC_PROPS), each of which his incorporated herein by reference in its entirety. Thus, the ranges for rFIXFc do not overlap the ranges for rFIX. Similarly, the mean CL of rFIXFc activity (3.18 ± 0.78 mL/hr/kg, range 2.05 - 4.18 mL/hr/kg) is approximately 2.6-fold less than that reported for rFIX (8.40 ± 2.01 mL/hr/kg, range 4.66 - 13.64 mL/hr/kg), while the Vss of both proteins are comparable at 4-5 times the plasma volume.

**[0203]** Although the same trend toward improvement was observed in the rFIXFc antigen PK, both the $T_{1/2}\alpha$ and $T_{1/2}\beta$ of rFIXFc antigen were significantly longer than that derived from FIX activity measurements. The $T_{1/2}\alpha$ estimated for rFIXFc antigen clearly deviates from that normally associated with FIX (2 – 3 hours). Furthermore, the probable incomplete washout from the pre-study replacement therapy before infusion of rFIXFc sometimes resulted in a higher baseline value, which in turn could lead to an underestimation of the rFIXFc $T_{1/2}\beta$, as measured by FIX activity. A number of subjects had an aPTT activity up to 3 IU/dL, well above the limit of quantification (1 IU/dL) for the aPTT assay, at later time points up to 336 hrs (14 days) post-dose. However, these time points were excluded from the estimation of the terminal half-life because the values were at or only slightly above pretreatment baselines, thus deemed to have returned to baseline. In contrast, the low but detectable terminal levels of rFIXFc may be unmasked by the specific and highly sensitive rFIXFc antigen ELISA, which detects as low as 0.1 IU/dL as compared to aPTT lower limit of 1.0 IU/dl.

**[0204]** The remaining PK parameters (activity) changed a small amount relative to elimination half-life and MRT. See Table 27(B). A dose-proportional, linear increase in FIX activity was observed based on $C_{max}$ occurring immediately after infusion and $AUC_{INF}$ (Table 4). FIX activity exhibited biexponential decay following infusion of rFIXFc, and was characterized by a rapid distribution (alpha) phase followed by a log-linear elimination (beta) phase. The mean distribution half-life ($T_{1/2}\alpha$) was highly variable for individual subjects (mean of 3.4 and 10.3 hours for the two higher dose groups) (Table 27(B)). The mean

elimination half-life ($T_{1/2}\beta$) was dose independent over the therapeutic dose range tested, i.e., 53.5 hours, 57.5 ± 8.2 hours, and 56.5± 14.1 hours at 25 IU/kg, 50 IU/kg, and 100 IU/kg, respectively. The time to 1% (1 IU/dL) above baseline, an assessment of rFIXFc activity, showed a dose-proportional increase. It was 7.3, 10.1 ± 1.5, and 12.3 ± 2.5 days for doses of 25, 50, and 100 IU/kg, respectively. At 168 hours (1 week) post dose, the plasma FIX activity was sustained at 1.1 IU/dL, 2.5 ± 0.9 IU/dL, and 4.6 ± 1.7 IU/dL above baseline for the 25, 50, and 100 IU/kg dose groups, respectively. Also dose-independent were MRT, CL, and Vss over the dose range of 25 to 100 IU/kg. Furthermore, each 1 IU/kg of infused rFIXFc raised plasma FIX activity by 0.93 ± 0.18 IU/dL on average (Table 27(B)), and this incremental recovery (K) showed weak positive correlation with body weight ($R^2$=0.336, p=0.048)

**[0205]** Long-term empirical clinical experience has suggested that a sustained plasma factor activity as low as 1 to 2 IU/dL will be adequate to prevent spontaneous bleeding events in severe hemophilia A and B patients, (Nilsson *et al., J. Intern. Med. 232:25-32* (1992), which is herein incorporated by reference in its entirety), and increased bleeding events are associated with the amount of time under 1% of normal FVIII activity. Collins *et al., Thromb Haemost 7:413-420* (2009), which is herein incorporated by reference in its entirety. Thus, PK analyses provide a means to optimize prophylactic treatment with individualized dose modeling to achieve sustained trough levels above 1% (1 IU/dL ) of baseline, reduce peak/trough variation, and improve the cost effectiveness of treatment. Carlsson *et al., Haemophilia 4:83-88* (1998); Kisker *et al., Haemophilia 9:279-284* (2003), each of which is herein incorporated by reference in its entirety.

**[0206]** To construct the concentration-time profiles 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 for this Phase1/2a study. Wang *et al., J. Clin. Pharmacol. 49:1012-1024* (2009), which is herein incorporated by reference in its entirely. One thousand subjects were simulated per dosing regimen with 14 to 16 sampling points for each subject. There were 14 sampling points for weekly dosing, 15 for every 10 day dosing, and 16 for every other week dosing. The body weight (BW) was generated according to the published method, Wang *et al.* (2009). i.e., based on a power equation of Z=BW-0.5. The median BW in 1000 subjects was assumed to be 75 kg. Based on the simulated concentration-time profiles, the mean ± standard deviation (SD) of the drug

concentration-time profiles of the 1000 subjects was constructed graphically for different dosing regimens. Figure 25.

**[0207]** In comparison with the standard recommended dose regimen of 25 to 40 IU/kg of FIX twice weekly, the median rFIXFc activity PK modeling results from this study show 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 trough of 1% above baseline. Figure 25. These model-simulated estimates are validated by the available data from this Phase 1/2a study, which fall entirely within the 95% confidence interval of the simulated activity-over-time curve. However, considering the heterogeneity of reported clinical breakthrough bleeding events relative to trough level of plasma FIX activity (Bjorkman, *Haemophilia 9:101-110* (2003); Ahnstrom *et al., Haemophilia 10:689-697* (2004), each of which is herein incorporated by reference in its entirety), the maintenance dose would likely require individual adjustment.

# Tables

## Table 1: Polynucleotide Sequences: FIX-Fc

### A. FIX-Fc Chain DNA Sequence (SEQ ID NO:1, which encodes SEQ ID NO:2)

pSYN-FIX-030 Nucleotide sequence (nt 1 to 7583):

FIX exon 1 (signal peptide, 1st amino acid propeptide): nt 690-777
FIX mini intron: nt 778-1076
FIX propeptide sequence : nt 1077-1126
Mature FIX sequence : nt 1127-2371
Fc : nt 2372-3052

gcgcgcgttgacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatata
tggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgt
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cttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttctt
gaagtggtggcctaactacggctacactagaagaacagtatttggtatctgcgctctgctgaagccagttacctt
cggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagca
gcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttctacggggtctgacgctcagtggaa
cgaaaactcacgttaagggattttggtcatgacattaacctataaaaataggcgtatcacgaggccctttcgtct
cgcgcgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagacggtcacagcttgtctgtaagc
ggatgccgggagcagacaagcccgtcagggcgcgtcagcgggtgttggcgggtgtcggggctggcttaactatgc
ggcatcagagcagattgtactgagagtgcaccatatatgcggtgtgaaataccgcacagatgcgtaaggagaaaa
taccgcatcaggcgccattcgccattcaggctgcgcaactgttgggaagggcgatcggtgcgggcctcttcgcta
ttacgcca

B. Fc DNA sequence (mouse Igκ signal peptide underlined) (SEQ ID NO:3, which encodes SEQ ID NO:4) This is the Fc cassette from pSYN-FIX-030. In addition, there is a separate Fc expression cassette that was transfected into the cell line in plasmid pSYN-Fc-015 that encodes the same amino acid sequence, but contains a few noncoding changes. The second copy of Fc encoding sequence enables a better monomer: dimer ratio.

atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgacaaaactcacacat
gcccaccgtgcccagcacctgaactcctgggaggaccgtcagtcttcctcttccccccaaaacccaaggacacc
ctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttc
aactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctcc
aacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
acaccctgcccccatcccgcgatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatc
ccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtt
ggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtctt
ctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

Table 2: Polypeptide Sequences

**FIX-Fc Monomer Hybrid: created by coexpressing FIX-Fc and Fc chains.**

A. FIX-Fc chain (SEQ ID NO:2):

(28 amino acid signal sequence underlined, 18 amino acid propeptide double underlined, Fc portion in italics.) The C-terminal lysine is not present in either subunit; this processing is often observed in recombinant proteins produced in mammalian cell culture, as well as with plasma derived proteins.

**FIXFC-SC SUBUNIT:**

**FIX Signal Peptide : -46 MQRVNMIMAE SPGLITICLL GYLLSAEC**

**FIX Propeptide : -18 TVFLDHENAN KILNRPKR**

**1 YNSGKLEEFV QGNLERECME EKCSFEEARE *VFENTERTTE FWKQYVDGDQ***

**51 CESNPCLNGG SCKDDINSYE CWCPFGFEGK NCELDVTCNI KNGRCEQFCK**

**101 NSADNKVVCS CTEGYRLAEN QKSCEPAVPF PCGRVSVSQT SKLTRAETVF**

**151 PDVDYVNSTE AETILDNITQ STQSFNDFTR VVGGEDAKPG QFPWQVVLNG**

**201 KVDAFCGGSI VNEKWIVTAA HCVETGVKIT VVAGEHNIEE TEHTEQKRNV**

**251 IRIIPHHNYN AAINKYNHDI ALLELDEPLV LNSYVTPICI ADKEYTNIFL**

**301 KFGSGYVSGW GRVFHKGRSA LVLQYLRVPL VDRATCLRST KFTIYNNMFC**

**351 AGFHEGGRDS CQGDSGGPHV TEVEGTSFLT GIISWGEECA MKGKYGIYTK**

**401 VSRYVNWIKE KTKLT*DKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR***

***451 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV***

***501 LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR***

***551 DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF***

***601 LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK***

B. Fc chain (SEQ ID NO:4)

20 amino acid heterologous mouse Igκ light chain signal peptide (underlined):

-20 METDTLLLWV LLLWVPGSTG

Mature Fc sequence (corresponding to human IgG1 amino acids 221 to 447, EU numbering)

```
  1 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
 51 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
101 CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK
151 GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
201 NVFSCSVMHE ALHNHYTQKS LSLSPGK
```

Table 3. Individual Patient FIXFc Antigen Concentration versus Time Data; Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number

| Actual Time (h) | Concentration (ng/mL) | Actual Time (h) | Concentration (ng/mL) | Actual Time (h) | Concentration (ng/mL) | Actual Time (h) | Concentration (ng/mL) |
|---|---|---|---|---|---|---|---|
| Patient 1 | | Patient 2 | | Patient 3 | | Patient 4 | |
| -0.50 | 0.0 | -1.23 | 0.0 | -0.18 | 0.0 | -0.18 | 0.0 |
| 0.17 | 2325.3 | 0.17 | 3352.1 | 0.28 | 5915.3 | 0.17 | 8166.5 |
| 0.42 | 1632.4 | 0.40 | 3017.3 | 0.42 | 6574.3 | 0.42 | 7362.3 |
| 1.17 | 1497.7 | 1.15 | 2280.7 | 1.17 | 5764.7 | 1.17 | 6723.4 |
| 3.18 | 1466.4 | 3.15 | 2077.5 | 3.17 | 4204.8 | 3.17 | 5291.4 |
| 6.13 | 1268.2 | 6.15 | 2054.7 | 6.17 | 3956.2 | 6.18 | 4673.1 |
| 9.12 | 1100.7 | 9.15 | 1700.4 | 9.17 | 3567.7 | 9.17 | 3954.6 |
| 24.12 | 805.0 | 24.23 | 1417.3 | 24.17 | 2805.6 | 24.17 | 3327.6 |
| 48.03 | 544.5 | 48.40 | 766.0 | 48.98 | 1727.7 | 48.20 | 2148.7 |
| 72.23 | 377.7 | 70.73 | 719.0 | 72.40 | 1165.8 | 72.17 | 1632.2 |
| 96.75 | 215.3 | 92.57 | 480.2 | 96.98 | 917.1 | 96.17 | 1234.4 |
| 120.13 | 192.6 | 119.98 | 326.3 | 121.23 | 673.9 | 120.13 | 894.0 |
| 141.95 | 128.6 | 141.10 | 241.1 | 168.65 | 568.2 | 144.18 | 645.2 |
| 169.45 | 112.4 | 167.98 | 194.6 | 240.15 | 265.4 | 168.22 | 564.1 |
| 192.37 | 93.6 | 192.85 | 160.1 | 290.97 | 286.4 | 192.20 | 509.2 |
| 216.28 | 76.1 | 216.98 | 149.0 | 337.98 | 238.5 | 216.23 | 474.5 |
| 237.30 | 76.4 | 238.65 | 125.7 | | | 240.23 | 446.1 |

Table 3. Individual Patient FIXFc Antigen Concentration versus Time Data;
Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number (continued)

| **Actual Time (h)** | **Concentration (ng/mL)** | **Actual Time (h)** | **Concentration (ng/mL)** | **Actual Time (h)** | **Concentration (ng/mL)** | **Actual Time (h)** | **Concentration (ng/mL)** |
|---|---|---|---|---|---|---|---|
| Patient 5 | | Patient 6 | | Patient7 | | Patient 8 | |
| -0.18 | 0.0 | -0.07 | 0.0 | -1.27 | 0.0 | -1.37 | 0.0 |
| 0.17 | 7520.2 | 0.17 | 11671.7 | 0.22 | 7055.9 | 0.25 | 27413.4 |
| 0.43 | 7233.9 | 0.42 | 8654.5 | 0.42 | 6215.7 | 0.47 | 23640.8 |
| 1.20 | 6752.1 | 1.17 | 8880.4 | 1.17 | 5498.6 | 1.35 | 18505.6 |
| 3.15 | 5873.1 | 3.17 | 8509.3 | 3.17 | 4477.7 | 3.22 | 15708.1 |
| 6.23 | 5919.2 | 6.17 | 7618.7 | 6.17 | 4084.8 | 6.17 | 14915.6 |
| 9.20 | 5332.9 | 9.17 | 6584.2 | 9.17 | 3888.9 | 9.17 | 16486.4 |
| 24.17 | 4215.9 | 48.17 | 3217.7 | 24.17 | 2849.4 | 24.72 | 9937.8 |
| 48.15 | 2986.6 | 72.17 | 1651.6 | 48.82 | 1630.6 | 48.90 | 6383.5 |
| 72.15 | 1933.3 | 96.17 | 1580.1 | 72.57 | 1295.7 | 72.38 | 4190.6 |
| 96.03 | 1249.0 | 120.17 | 722.7 | 96.57 | 1150.7 | 96.40 | 3774.7 |
| 120.13 | 401.4 | 240.17 | 329.5 | 121.15 | 954.9 | 120.30 | 2514.9 |
| 144.03 | 482.3 | 288.17 | 292.7 | 144.10 | 780.6 | 168.77 | 1626.0 |
| 168.17 | 478.0 | 336.17 | 252.7 | 168.82 | 447.6 | 240.27 | 924.7 |
| 192.12 | 433.7 | | | 192.77 | 446.5 | 288.83 | 682.4 |
| 216.15 | 368.9 | | | 240.57 | 427.8 | 337.03 | 586.4 |
| 240.07 | 264.0 | | | | | | |

Table 3. Individual Patient FIXFc Antigen Concentration versus Time Data; Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number (continued)

| Actual Time (h) | Concentration (ng/mL) | Actual Time (h) | Concentration (ng/mL) | Actual Time (h) | Concentration (ng/mL) | Actual Time (h) | Concentration (ng/mL) |
|---|---|---|---|---|---|---|---|
| Patient 9 | | Patient 10 | | Patient 11 | | Patient 12 | |
| -0.82 | 0.0 | -0.48 | 0.0 | -0.15 | 0.0 | -1.12 | 0.0 |
| 0.28 | 15027.1 | 0.25 | 16760.0 | 0.23 | 19641.7 | 0.17 | 15194.5 |
| 0.63 | 13374.1 | 0.50 | 11529.0 | 0.47 | 17267.2 | 0.42 | 12255.7 |
| 1.17 | 12395.6 | 1.22 | 10566.3 | 1.22 | 15902.2 | 1.17 | 11171.3 |
| 3.20 | 10808.4 | 3.22 | 9889.0 | 3.22 | 13708.9 | 3.17 | 9835.4 |
| 6.22 | 9640.2 | 6.22 | 8290.2 | 6.25 | 12469.4 | 6.17 | 8513.2 |
| 9.15 | 10505.5 | 9.22 | 7114.7 | 9.22 | 12029.8 | 9.17 | 8413.0 |
| 23.15 | 6487.3 | 24.22 | 5877.0 | 24.22 | 8083.3 | 24.17 | 5538.2 |
| 46.62 | 5324.8 | 48.22 | 3980.4 | 47.72 | 4431.0 | 48.20 | 3885.5 |
| 70.10 | 2895.5 | 72.22 | 2455.6 | 71.88 | 2162.6 | 72.13 | 2959.9 |
| 94.15 | 3208.3 | 96.12 | 2052.6 | 191.72 | 1468.7 | 95.17 | 2215.4 |
| 118.13 | 2610.6 | 120.22 | 1302.5 | 263.72 | 428.6 | 119.17 | 1799.7 |
| 166.10 | 2007.2 | 144.22 | 1349.3 | | | 167.38 | 1339.7 |
| 238.15 | 1086.2 | 168.22 | 1221.0 | | | 239.50 | 892.4 |
| 286.15 | 942.8 | 192.18 | 910.2 | | | 287.25 | 646.9 |
| 335.57 | 621.3 | **216.22** | **136.2** | | | | |

Table 4. Individual Patient and Group Mean FIXFc Antigen Pharmacokinetic Summary Data

| Nominal Dose (IU/kg) | Actual Dose (IU/kg) | Equivalent Dose (mg/kg) | Patient | $C_{max}$ (ng/mL) | $AUC_{INF}$ (h*ng/mL) | Cl* (mL/h/kg) | $V_{SS}$* (mL/kg) | MRT* (h) | Alpha HL* (h) | Beta HL* (h) |
|---|---|---|---|---|---|---|---|---|---|---|
| 12.5 | 13.714 | 0.228 | 1 | 1670 | 91300 | 2.50 | 245 | 98.2 | 21.2 | 107 |
| | N | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 25 | 27.250 | 0.453 | 2 | 2730 | 144000 | 3.14 | 273 | 87.1 | 11.3 | 71.0 |
| | N | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 50 | 54.5 | 0.905 | 3 | 5470 | 356000 | 2.54 | 366 | 144 | 18.6 | 138 |
| | 54.5 | 0.905 | 4 | 6910 | 389000 | 2.32 | 244 | 105 | 10.6 | 85.3 |
| | *54.5* | *0.905* | *5* | *7520* | *416000* | *2.17* | *184* | *84.5* | *NC* | *94.3* |
| | *54.513* | *0.906* | *6* | *11700* | *531000* | *1.71* | *190* | *112* | *NC* | *140* |
| | 55.878 | 0.928 | 7 | 5950 | 348000 | 2.67 | 310 | 116 | 10.1 | 93.9 |
| | N | | | 5 | 5 | 5 | 5 | 5 | 3 | 5 |
| | Mean | | | 7510 | 408000 | 2.28 | 259 | 112 | 13.1 | 110 |
| | SD | | | 2480 | 73900 | 0.374 | 78.5 | 21.5 | 4.77 | 26.5 |
| | SE | | | 1110 | 33100 | 0.167 | 35.1 | 9.60 | 2.75 | 11.8 |
| | Geometric Mean | | | 7230 | 403000 | 2.26 | 250 | 111 | 12.6 | 108 |
| | CV% Geometric Mean | | | 30.3 | 17.1 | 17.6 | 30.8 | 19.4 | 34.9 | 23.8 |
| 100 | 109 | 1.81 | 10 | 12500 | 667000 | 2.72 | 263 | 96.8 | 9.79 | 78.0 |
| | 109 | 1.81 | 8 | 21600 | 1200000 | 1.51 | 156 | 103 | 15.7 | 94.3 |
| | 109 | 1.81 | 9 | 13400 | 998000 | 1.81 | 248 | 137 | 11.5 | 107 |
| | 109.176 | 1.81 | 11 | 17200 | 844000 | 2.15 | 226 | 105 | 13.0 | 97.1 |
| | 109.441 | 1.82 | 12 | 12500 | 778000 | 2.34 | 295 | 126 | 10.6 | 102 |
| | N | | | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Mean | | | 15400 | 897000 | 2.11 | 238 | 114 | 12.1 | 95.8 |
| | SD | | | 3960 | 206000[a] | 0.464[b] | 52.2[c] | 17.1 | 2.33 | 11.1 |
| | SE | | | 1770 | 92000 | 0.208 | 23.3 | 7.64 | 1.04 | 4.96 |
| | Geometric Mean | | | 15100 | 878000 | 2.06 | 232 | 113 | 11.9 | 95.2 |
| | CV% Geometric Mean | | | 24.5 | 22.9 | 22.9 | 24.7 | 14.8 | 118.7 | 12.2 |

* CL, Vss, MRT, T1/2 α and T1/2β for combined 12.5-100 IU/kg doses are 2.30±0.46 (1.51-2.72); 250±58.2 (156-366); 110±18.5 (84.5-144); 12.0±4.0 (10.1-18.6, not including two patients whose PK parameters were determined by non-compartmental analysis); and 101±20.9 (78-140), respectively. Due to correction of rounding or other errors, (a) should be 207,000, and (b) should be 0.468, (c) should be 52.1.

Table 5. Individual Patient and Group Mean FIXFc Activity and Baseline Corrected FIXFc Activity versus Time Data; Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number

| Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) | Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) | Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) |
|---|---|---|---|---|---|---|---|---|
| Patient 1 | | | Patient 2 | | | Patient 3 | | |
| -309.80 | 2 | NC | -310.60 | 3 | NC | -524.08 | < 1.0 | NC |
| -0.50 | 3 | 0.0 | -1.23 | 2 | 0.0 | -0.18 | 2 | 0.0 |
| 0.17 | 16 | 13.0 | 0.17 | 23 | 21.0 | 0.28 | 44 | 42.0 |
| 0.42 | 11 | 8.1 | 0.40 | 19 | 17.0 | 0.42 | 31 | 29.0 |
| 1.17 | 10 | 7.1 | 1.15 | 15 | 13.0 | 1.17 | 27 | 25.1 |
| 3.18 | 12 | 9.4 | 3.15 | 13 | 11.0 | 3.17 | 22 | 20.2 |
| 6.13 | 9 | 6.6 | 6.15 | 11 | 9.0 | 6.17 | 18 | 16.4 |
| 9.12 | 10 | 7.9 | 9.15 | 13 | 11.0 | 9.17 | 17 | 15.6 |
| 24.12 | 7 | 5.0 | 24.23 | 8 | 6.0 | 24.17 | 12 | 11.0 |
| 48.03 | 6 | 4.0 | 48.40 | 6 | 4.0 | 48.98 | 7 | 6.0 |
| 72.23 | 4 | 2.0 | 70.73 | 6 | 4.0 | 72.40 | 6 | 5.0 |
| 96.75 | 3 | 1.0 | 92.57 | 4 | 2.0 | 96.98 | 6 | 5.0 |
| **120.13** | **3** | **1.0** | 119.98 | 4 | 2.0 | 121.23 | 5 | 4.0 |
| **141.95** | **3** | **1.0** | 141.10 | 4 | 2.0 | 168.65 | 3 | 2.0 |
| **169.45** | **2** | **0.0** | 167.98 | 3 | 1.0 | **240.15** | **1** | **0.0** |
| **192.37** | **3** | **1.0** | **192.85** | **2** | **0.0** | **290.97** | **1** | **0.0** |
| **216.28** | **3** | **1.0** | **216.98** | **3** | **1.0** | **337.98** | **1** | **0.0** |
| **237.30** | **3** | **1.0** | **238.65** | **3** | **1.0** | **675.22** | **2** | **1.0** |
| **746.22** | **3** | **1.0** | **891.90** | **2** | **0.0** | | | |

Note: Data in bold represent a return to baseline and were excluded from analysis.

Table 5. Individual Patient and Group Mean FIXFc Activity and Baseline Corrected FIXFc Activity versus Time Data; Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number (continued)

| Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) | Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) | Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) |
|---|---|---|---|---|---|---|---|---|
| Patient 4 | | | Patient 5 | | | Patient 6 | | |
| -285.52 | 1 | NC | -104.18 | < 1.0 | NC | -503.20 | 3 | NC |
| -0.18 | < 1.0 | 0.0 | -0.18 | < 1.0 | 0.0 | -0.07 | 3 | 0.0 |
| 0.17 | 59 | 58.0 | 0.17 | 35 | 34.0 | 0.17 | 3 | 0.0 |
| 0.42 | 45 | 44.0 | 0.43 | 30 | 29.0 | 0.42 | 64 | 61.0 |
| 1.17 | 40 | 39.0 | 1.20 | 25 | 24.0 | 1.17 | 57 | 54.1 |
| 3.17 | 30 | 29.0 | 3.15 | 21 | 20.0 | 3.17 | 54 | 51.3 |
| 6.18 | 26 | 25.0 | 6.23 | 19 | 18.0 | 6.17 | 42 | 39.6 |
| 9.17 | 22 | 21.0 | 9.20 | NR | NR | 9.17 | 43 | 40.9 |
| 24.17 | 14 | 13.0 | 24.17 | 13 | 12.0 | 24.17 | 26 | 24.0 |
| 48.20 | 9 | 8.0 | 48.15 | 9 | 8.0 | 48.17 | 17 | 15.0 |
| 72.17 | 8 | 7.0 | 72.15 | 7 | 6.0 | 72.17 | 13 | 11.0 |
| 96.17 | 5 | 4.0 | 96.03 | 5 | 4.0 | 96.17 | 10 | 8.0 |
| 120.13 | 4 | 3.0 | 120.13 | 4 | 3.0 | 120.17 | 9 | 7.0 |
| 144.18 | 4 | 3.0 | 144.03 | 3 | 2.0 | 168.17 | 6 | 4.0 |
| 168.22 | 3 | 2.0 | 168.17 | 2 | 1.0 | 240.17 | 4 | 2.0 |
| 192.20 | 3 | 2.0 | **192.12** | **2** | **1.0** | 288.17 | 3 | 1.0 |
| 216.23 | 2 | 1.0 | **216.15** | **2** | **1.0** | **336.17** | **4** | **2.0** |
| **240.23** | **2** | **1.0** | **240.07** | **2** | **1.0** | **504.17** | **3** | **1.0** |
| **720.73** | **< 1.0** | **0.0** | **547.07** | **< 1.0** | **0.0** | | | |

Note: Data in bold represent a return to baseline and were excluded from analysis.

Table 5. Individual Patient and Group Mean FIXFc Activity and Baseline Corrected FIXFc Activity versus Time Data; Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number (continued)

| Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) | Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) | Actual Time (h) | Result (IU/dL) | Baseline Corrected Result (IU/dL) |
|---|---|---|---|---|---|---|---|---|
| Patient 7 | | | Patient 8 | | | Patient 9 | | |
| -438.43 | < 1.0 | NC | -120.42 | < 1.0 | NC | -193.05 | 8 | NC |
| -1.27 | 4 | 0.0 | -1.37 | < 1.0 | 0.0 | -0.82 | 3 | 0.0 |
| 0.22 | 46 | 42.0 | 0.25 | 129 | 128.0 | 0.28 | 100 | 97.0 |
| 0.42 | 38 | 34.1 | 0.47 | 117 | 116.0 | 0.63 | 93 | 90.1 |
| 1.17 | 30 | 26.2 | 1.35 | 102 | 101.0 | 1.17 | 94 | 91.1 |
| 3.17 | 28 | 24.5 | 3.22 | 98 | 97.0 | 3.20 | 80 | 77.3 |
| 6.17 | 24 | 20.8 | 6.17 | 80 | 79.0 | 6.22 | 69 | 66.6 |
| 9.17 | 22 | 19.2 | 9.17 | 72 | 71.0 | 9.15 | 64 | 61.9 |
| 24.17 | 14 | 12.4 | 24.72 | 53 | 52.0 | 23.15 | 47 | 45.0 |
| 48.82 | 10 | 9.0 | 48.90 | 30 | 29.0 | 46.62 | 25 | 23.0 |
| 72.57 | 6 | 5.0 | 72.38 | 19 | 18.0 | 70.10 | 17 | 15.0 |
| 96.57 | 5 | 4.0 | 96.40 | 14 | 13.0 | 94.15 | 13 | 11.0 |
| 121.15 | 4 | 3.0 | 120.30 | 9 | 8.0 | 118.13 | 9 | 7.0 |
| 144.10 | 3 | 2.0 | 168.77 | 6 | 5.0 | 166.10 | 5 | 3.0 |
| 168.82 | 2 | 1.0 | 240.27 | 3 | 2.0 | 238.15 | 3 | 1.0 |
| **192.77** | **2** | **1.0** | 288.83 | 2 | 1.0 | **286.15** | **2** | **0.0** |
| **240.57** | **2** | **1.0** | **337.03** | **2** | **1.0** | **335.57** | **2** | **0.0** |
| **744.57** | **3** | **2.0** | **840.28** | **< 1.0** | **0.0** | **741.77** | **3** | **1.0** |

Note: Data in bold represent a return to baseline and were excluded from analysis.

Table 5. Individual Patient and Group Mean FIXFc Activity and Baseline Corrected FIXFc Activity versus Time Data; Sorted by Nominal Dose, Actual Dose, Infusion Duration, and Patient Number (continued)

| **Actual Time (h)** | **Result (IU/dL)** | **Baseline Corrected Result (IU/dL)** | **Actual Time (h)** | **Result (IU/dL)** | **Baseline Corrected Result (IU/dL)** | **Actual Time (h)** | **Result (IU/dL)** | **Baseline Corrected Result (IU/dL)** |
|---|---|---|---|---|---|---|---|---|
| Patient 10 | | | Patient 11 | | | Patient 12 | | |
| -334.63 | 1 | NC | -912.28 | 2 | NC | -342.58 | 2 | NC |
| -0.48 | 2 | 0.0 | -0.15 | 2 | 0.0 | -1.12 | 2 | 0.0 |
| 0.25 | 120 | 118.0 | 0.23 | 110 | 108.0 | 0.17 | 108 | 106.0 |
| 0.50 | 104 | 102.0 | 0.47 | 106 | 104.0 | 0.42 | 90 | 88.0 |
| 1.22 | 84 | 82.1 | 1.22 | 96 | 94.0 | 1.17 | 70 | 68.0 |
| 3.22 | 75 | 73.2 | 3.22 | 92 | 90.0 | 3.17 | 69 | 67.0 |
| 6.22 | 60 | 58.4 | 6.25 | 81 | 79.0 | 6.17 | 55 | 53.0 |
| 9.22 | 56 | 54.6 | 9.22 | 70 | 68.0 | 9.17 | 55 | 53.0 |
| 24.22 | 36 | 35.0 | 24.22 | 53 | 51.0 | 24.17 | 37 | 35.0 |
| 48.22 | 21 | 20.0 | 47.72 | 33 | 31.0 | 48.20 | 25 | 23.0 |
| 72.22 | 14 | 13.0 | 71.88 | 25 | 23.0 | 72.13 | 14 | 12.0 |
| 96.12 | 11 | 10.0 | 167.72 | 8 | 6.0 | 95.17 | 10 | 8.0 |
| 120.22 | 7 | 6.0 | 191.72 | 8 | 6.0 | 119.17 | 7 | 5.0 |
| 144.22 | 6 | 5.0 | 263.72 | 4 | 2.0 | 167.38 | 6 | 4.0 |
| 168.22 | 6 | 5.0 | 359.72 | 3 | 1.0 | 239.50 | 3 | 1.0 |
| 192.18 | 4 | 3.0 | **383.97** | **3** | **1.0** | **287.25** | **2** | **0.0** |
| **216.22** | **85** | **84.0** | **890.97** | **14** | **12.0** | **526.42** | **4** | **2.0** |
| **744.95** | **2** | **1.0** | | | | | | |

Note: Data in bold represent a return to baseline and were excluded from analysis.

Table 6. Individual Patient and Group Mean FIXFc Activity Pharmacokinetic Summary Data; Sorted by Nominal Dose, Actual Dose, and Patient Number

| Nominal Dose (IU/kg) | Actual Dose (IU/kg) | Patient | $C_{max}$ (IU/dL) | $AUC_{INF}$ (h*IU/dL) | $AUC_{\alpha}$ (%) | $AUC_{\beta}$ (%) | AUC/Dose (IU*h/dL per IU/kg) | Cl (mL/h/kg) | $V_1$ (mL/kg) | $V_{SS}$ (mL/kg) | MRT (h) | Alpha HL (h) | Beta HL (h) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12.5 | 13.714 | 1 | 11.9 | 418 | 0.231 | 99.8 | 30.5 | 3.28 | 102 | 157 | 48.0 | 0.140 | 33.3 |
| | N | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 25 | 27.25 | 2 | 19.9 | 753 | 2.50 | 97.8 | 27.6 | 3.62 | 134 | 275 | 76.0 | 1.20 | 54.0 |
| | N | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 50 | 54.5 | 3 | 34.5 | 1280 | 5.7 | 94.5 | 23.5 | 4.26 | 155 | 365 | 85.8 | 2.32 | 62.9 |
| | 54.5 | 4 | 48.5 | 1450 | 12.4 | 87.7 | 26.6 | 3.76 | 111 | 282 | 75.1 | 3.64 | 58.9 |
| | 54.5 | 5 | 33.0 | 1190 | 1.5 | 98.3 | 21.8 | 4.58 | 160 | 274 | 59.9 | 0.840 | 42.1 |
| | 54.513 | 6 | 53.5 | 2960 | 1.0 | 99.1 | 54.3 | 1.84 | 100 | 149 | 81.1 | 1.07 | 56.7 |
| | 55.878 | 7 | 38.6 | 1270 | 2.2 | 97.9 | 22.7 | 4.41 | 141 | 248 | 56.4 | 1.07 | 40.0 |
| | N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Mean | | 41.6 | 1630 | 4.56 | 95.5 | 29.8 | 3.77 | 133 | 264 | 71.7 | 1.79 | 52.1 |
| | SD | | 8.97[a] | 750 | 4.75 | 4.70 | 13.8 | 1.12 | 26.7 | 77.6 | 13.0 | 1.19 | 10.4 |
| | SE | | 4.01 | 335 | 2.13 | 2.10 | 6.18 | 0.501 | 11.9 | 34.7 | 5.79 | 0.531 | 4.65 |
| | Geometric Mean | | 40.9 | 1530 | 2.98 | 95.4 | 27.9 | 3.59 | 131 | 254 | 70.7 | 1.52 | 51.3 |
| | CV% Geometric Mean | | 21.4 | 39.1 | 136.5 | 5.0 | 39.4 | 39.4 | 21.1 | 33.8 | 18.8 | 68.6 | 21.0 |
| 100 | 109 | 10 | 98.9 | 3330 | 18.5 | 81.3 | 30.6 | 3.28 | 109 | 216 | 65.9 | 6.53 | 54.6 |
| | 109 | 8 | 111 | 4580 | 28.9 | 71.1 | 42.0 | 2.38 | 98.0 | 145 | 61.1 | 13.2 | 54.2 |
| | 109 | 9 | 92.1 | 3540 | 17.0 | 82.9 | 32.5 | 3.08 | 118 | 163 | 53.1 | 9.43 | 42.4[d] |
| | 109.176 | 11 | 99.1 | 5150 | 28.6 | 71.3 | 47.2 | 2.12 | 110 | 162 | 76.2 | 16.6 | 67.4 |
| | 109.441 | 12 | 89.9 | 3060 | 9.2 | 90.8 | 28.0 | 3.58 | 121 | 207 | 57.9 | 4.19 | 43.8 |
| | N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Mean | | 98.2 | 3930 | 20.4 | 79.5 | 36.1 | 2.89 | 111 | 179 | 62.8 | 9.99 | 52.5 |
| | SD | | 8.21[b] | 893[c] | 8.37 | 8.37 | 8.17 | 0.615 | 8.98 | 31.1 | 8.82 | 4.99 | 10.1 |
| | SE | | 3.67 | 399 | 3.74 | 3.74 | 3.65 | 0.275 | 4.02 | 13.9 | 3.95 | 2.23 | 4.51 |
| | Geometric Mean | | 97.9 | 3860 | 18.9 | 79.1 | 35.4 | 2.83 | 111 | 177 | 62.4 | 8.92 | 51.7 |
| | CV% Geometric Mean | | 8.2 | 22.4 | 49.7 | 10.5 | 22.4 | 22.5 | 8.2 | 17.4 | 13.8 | 59.4 | 19.0 |

Due to correction of rounding or other errors, (a) should be 8.98, (b) should be 8.23, (c) should be 892, and (d) should be 42.2.

Table 7A-7B. Individual Patient and Group Mean FIXFc Activity Secondary Pharmacokinetic Summary Data; Sorted by Nominal Dose, Actual Dose, and Patient Number

| Nominal Dose (IU/kg) | Actual Dose (IU/kg) | Patient | C168[a] (IU/dL) | TBLP1[b] (Day) | TBLP3[c] (Day) | TBLP5[d] (Day) | K Value[e] (IU/dL per IU/kg) | K Value[f] (IU/dL per IU/kg) | In Vivo Recovery[g] (%) | In Vivo Recovery[h] (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 12.5 | 13.714 | 1 | *0.264* | *4.34* | 2.13 | 1.11 | 0.87 | 0.95 | 30.8 | 33.6 |
| | N | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 25 | 27.25 | 2 | 1.09 | 7.28 | 3.72 | 2.06 | 0.73 | 0.77 | 31.8 | 33.5 |
| | N | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 50 | 54.5 | 3 | 2.09 | *9.79* | 5.64 | 3.70 | 0.63 | 0.77 | 33.0 | 40.2 |
| | 54.5 | 4 | 2.08 | 9.58 | 5.69 | 3.89 | 0.89 | 1.06 | 37.8 | 45.2 |
| | 54.5 | 5 | 1.22 | 7.50 | 4.72 | 3.42 | 0.61 | 0.62 | 33.6 | 34.6 |
| | 54.513 | 6 | 4.61 | 12.2 | 8.47 | 6.72 | 0.98 | 1.12 | 38.2 | 43.6 |
| | 55.878 | 7 | 1.17 | 7.37 | 4.74 | 3.51 | 0.69 | 0.75 | 29.9 | 32.6 |
| | N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Mean | | 2.23 | 9.29 | 5.85 | 4.25 | 0.76 | 0.86 | 34.5 | 39.2 |
| | SD | | 1.40 | 1.98 | 1.54 | 1.39 | 0.17 | 0.22 | 3.5 | 5.5 |
| | SE | | 0.627 | 0.886 | 0.687 | 0.623 | 0.074 | 0.0963 | 1.6 | 2.5 |
| | Geometric Mean | | 1.96 | 9.12 | 5.71 | 4.10 | 0.75 | 0.84 | 34.4 | 38.9 |
| | CV% Geometric Mean | | 60.0 | 21.2 | 24.1 | 28.6 | 21.5 | 25.4 | 10.2 | 14.4 |

[a] C168 = Estimated FIX activity above baseline at approximately 168 h after dose. Value in italics was estimated from simulations performed using a one-compartment model and patient microscopic rate constants.

[b] TBLP1 = Model-predicted time after dose when FIX activity has declined to approximately 1 IU/dL above baseline. Values in italics were estimated from simulations performed using a one-compartment model and patient microscopic rate constants.

[c] TBLP3 = Model-predicted time after dose when FIX activity has declined to approximately 3 IU/dL above baseline.

[d] TBLP5 = Model-predicted time after dose when FIX activity has declined to approximately 5 IU/dL above baseline.

[e] K-Value was calculated using model predicted $C_{max}$ value generated from background subtracted results divided by dose.

[f] K-Value was calculated using the observed maximum post dose sample result; K-value = (Baseline Subtracted $C_{max}$ observed)/Dose).

[g] In-vivo Recovery = 100 × (Model Predicted $C_{max}$ from baseline subtracted data/Dose) × Plasma Volume (dL)/ Dose in IU; where plasma volume in mL = (23.7 × Ht in cm) + (9.0 × Wt in kg) – 1709.

[h] In-vivo Recovery = 100 × (Baseline Subtracted Observed $C_{max}$) × Plasma Volume (dL)/Dose in IU; where plasma volume in mL = (23.7 × Ht in cm) + (9.0 × Wt in kg) – 1709.

Table 7B

| Nominal Dose (IU/kg) | Actual Dose (IU/kg) | Patient | C168[a] (IU/dL) | TBLP1[b] (Day) | TBLP3[c] (Day) | TBLP5[d] (Day) | K Value[e] (IU/dL per IU/kg) | K Value][f] (IU/dL per IU/kg) | In Vivo Recovery[g] (%) | In Vivo Recovery[h] (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 100 | 109 | 10 | 4.08 | *11.6* | 8.01 | 6.34 | 0.91 | 1.08 | 43.4 | 51.8 |
| | 109 | 8 | 4.88 | 12.1 | 8.57 | 6.92 | 1.02 | 1.17 | 28.7 | 33.1 |
| | 109 | 9 | 3.09 | 9.87 | 7.07 | 5.78 | 0.84 | 0.89 | 39.7 | 41.8 |
| | 109.176 | 11 | 6.77 | 14.7 | 10.3 | 8.21 | 0.91 | 0.99 | 27.8 | 30.3 |
| | 109.441 | 12 | 3.09 | 9.96 | 7.07 | 5.72 | 0.82 | 0.97 | 35.8 | 42.2 |
| | N | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Mean | | 4.38 | 11.6 | 8.20 | 6.59 | 0.90 | 1.02 | 35.1 | 39.8 |
| | SD | | 1.53 | 1.97 | 1.34 | 1.03 | 0.0784 | 0.11 | 6.8 | 8.5 |
| | SE | | 0.685 | 0.881 | 0.597 | 0.459 | 0.0351 | 0.0482 | 3.0 | 3.8 |
| | Geometric Mean | | 4.19 | 11.5 | 8.12 | 6.53 | 0.90 | 1.02 | 34.5 | 39.1 |
| | CV% Geometric Mean | | 34.1 | 16.5 | 15.7 | 15.0 | 8.6 | 10.5 | 19.8 | 21.6 |

Table 8. Phase 1/2a Study: Comparison of PK Parameters for rFIXFc and BENEFIX™

| Parameters | *rFIXFc [Mean±SD (min – max)] [N=11] | †BENEFIX™ [Mean±SD (min – max)] [N=11] |
|---|---|---|
| $t_{1/2}$ (hours) | 52.5 ±9.2 (40 – 67.4) | 19.3 ±4.97 (11.1 – 36.4) |
| MRT (hours) | 68.05 ±11.16 (53.1 – 85.8) | 26.0 ±6.07 (15.81 – 46.09) |
| CL (mL/hour/kg) | 3.36 ±0.93 (1.84 – 4.58) | 8.4 ±2.01 (4.66 – 13.64) |
| Incremental Recovery (IU/dL per IU/kg) | 0.93 ±0.18 (0.62 – 1.17)[a] | 0.75 ±0.23 (0.34 – 1.38) |
| $C_{max}$ (IU/dL per IU/kg) | 24 hrs post-injection | |
| AUC | 48 hrs post-injection | |

* Estimates from 2-compartmental analysis of FIX activity at the nominal doses 25, 50 and 100 IU/kg (n=11)
†Summary of Product Characteristics of BENEFIX™ (Nov 18, 2009); Median and range (n=56)

a. Range corrected due to rounding or other errors as 0.63 – 1.18.

Relative to Historical Data for BENEFIX™, rFIX-Fc demonstrated:

- 3x increase in half-life and mean residence time
- 24% improved incremental recovery relative
- 2.5x reduced clearance

Table 9. Phase 1/2a Study: Dose Proportional Increase in Cmax and AUC of rFIXFc (activity)

| Dose (IU/kg) | # of Patients | Cmax (IU/dL) [Mean±SD (min – max)] | AUC (h*IU/dL) [Mean±SD (min – max)] |
|---|---|---|---|
| 25 | 1 | 19.9 | 753 |
| 50 | 5 | 41.6 ±8.97 (33.0 – 53.5) | 1630 ±750 (1190 – 2960) |
| 100 | 5 | 98.2 ±8.21 (89.9 – 111.0) | 3930 ±893 (3060 – 5150) |

Also see Figure 5.

Table 10A-10B. Estimated Therapeutic Duration of rFIXFc at 50 and 100 IU/kg Doses.

| Parameter | Geo Median |
|---|---|
| FIX:C on Day 7 | 2.0 IU/dL (above baseline) |
| Time to 1 IU/dL above baseline | 9.1 days |
| Time to 3 IU/dL above baseline | 5.7 days |

| Parameter | Geo Median |
|---|---|
| FIX:C on Day 7 | 4.2 IU/dL (above baseline) |
| Time to 1 IU/dL above baseline | 11.5 days |
| Time to 3 IU/dL above baseline | 8.1 days |

Also see Figure 6A-6B.

Table 11. Dose Proportional Increase in Cmax and AUC for rFIXFc Antigen.

| Dose (IU/kg) | # of patients | Cmax (ng/mL) [Mean ±SD] | AUC (h*ng/mL) [Mean ±SD] |
|---|---|---|---|
| 25 | 1 | 2,730 | 144,000 |
| 50 | 5 | 7,510 ± 2,480 | 408,000 ± 73,900 |
| 100 | 5 | 15,400 ± 3,960 | 897,000 ± 206,000 |

Also see Figure 7.

Table 12. Pharmacokinetic Estimates for rFIXFc Antigen

| **Parameters** | **50 IU/kg [Mean ±SD] (N=5)** | **100 IU/kg [Mean ±SD] (N=5)** |
|---|---|---|
| CL (mL/hour/kg) | 2.28 ± 0.37 | 2.11 ± 0.46 |
| Vss (mL/kg) | 259 ± 78.5 | 238 ± 52.2 |
| MRT (hours) | 112 ± 21.5 | 114 ± 17.1 |
| $t_{1/2}$ (hours) | 110 ± 26.5 | 95.8 ± 11.1 |

Also see Figure 8A-8B.

Table 13. Mean PK Values Based on Activity

| | Cmax (IU/dL) | AUCINF (h*IU/dL) | AUCa (%) | AUCb (%) | AUC/Dose (IU*h/dL per IU/kg) | Cl (mL/kg) | Vl (mL/h/kg) | VSS (mL/kg) | MRT (h) | t1/2α (h) | t1/2β (h) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| n | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| Mean | 65.364 | 2596.636 | 11.591 | 88.427 | 32.436 | 3.555 | 123.364 | 226.000 | 68.045 | 5.463 | 52.455 |
| Std Dev | 32.9708 | 1497.1234 | 10.4490 | 10.5210 | 10.7506 | 0.9257 | 21.2804 | 69.7582 | 11.1637 | | 9.1674 |
| | | | | | | | | | | 5.4197 | |
| %CV | 50.4420 | 57.6563 | 90.1479 | 11.8980 | 33.1435 | 27.5890 | 17.2501 | 30.8664 | 16.4063 | | 17.4768 |
| | | | | | | | | | | 99.2128 | |
| Median | 53.500 | 2960.000 | 9.200 | 90.800 | 28.000 | 3.580 | 118.000 | 216.000 | 65.900 | 3.640 | 54.200 |
| Minimum | 19.90 | 753.00 | 1.00 | 71.10 | 21.80 | 1.84 | 98.00 | 145.00 | 53.10 | 0.84 | 40.00 |
| Maximum | 111.00 | 5150.00 | 28.90 | 99.10 | 54.30 | 4.58 | 160.00 | 365.00 | 85.80 | 16.60 | 67.40 |
| Geo. mean | 56.951 | 2181.294 | 6.781 | 87.826 | 31.020 | 3.226 | 121.767 | 216.533 | 67.218 | 3.326 | 51.715 |
| | | | | **C168 [1] (IU/dL)** | **TBLP1 [2] (Day)** | **TBLP3 [3] (Day)** | **TBLP5 [4] (Day)** | **Incremental Recovery [5] (IU/dL per IU/kg)** | **Incremental Recovery [6] (IU/dL per IU/kg)** | **In Vivo Recovery [7] (%)** | **In Vivo Recovery [8] (%)** |
| | | | n | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| | | | Mean | 3.106 | 10.177 | 6.727 | 5.115 | 0.821 | 0.926 | 34.518 | 38.991 |
| | | | Std Dev | 1.8231 | 2.3315 | 2.0089 | 1.8975 | 0.1387 | 0.1787 | 4.9250 | 6.6636 |
| | | | %CV | 58.6897 | 22.9088 | 29.8623 | 37.0944 | 16.8921 | 19.2940 | 14.2678 | 17.0900 |
| | | | Median | 3.090 | 9.870 | 7.070 | 5.720 | 0.840 | 0.970 | 33.600 | 40.200 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Minimum | 1.09 | 7.28 | 3.72 | 2.06 | 0.61 | 0.62 | 27.80 | 30.30 |
| | | | Maximum | 6.77 | 14.70 | 10.30 | 8.21 | 1.02 | 1.17 | 43.40 | 51.80 |
| | | | Geo. mean | 2.621 | 9.938 | 6.447 | 4.761 | 0.810 | 0.910 | 34.202 | 38.486 |

Footnotes:

Note: PK parameter values were determined by 2-compartmental method.

Geo. Mean = Geometric Mean

[1] C168 = FIX activity above baseline at 168 hr after dose.

[2] TBLP1 = Estimated time after dose when FIX activity has declined to 1 IU/dL above baseline.

[3] TBLP3 = Estimated time after dose when FIX activity has declined to 3 IU/dL above baseline.

[4] TBLP5 = Estimated time after dose when FIX activity has declined to 5 IU/dL above baseline.

[5] Incremental Recovery was calculated using model predicted Cmax value generated from background subtracted results divided by dose.

[6] Incremental Recovery was calculated using the observed maximum post-dose sample result;

Incremental Recovery = (Baseline Subtracted Cmax observed)/Dose.

[7] In-vivo Recovery = 100 x (Model Predicted Cmax from baseline subtracted data/Dose) x Plasma Volume (dL)/Dose in IU;

where plasma volume in mL = (23.7 x Ht in cm) + (9.0 x Wt in kg) - 1709.

[8] In-vivo Recovery = 100 x (Baseline Subtracted Observed Cmax) x Plasma Volume (dL)/Dose in IU; where plasma volume in mL = (23.7 x Ht in cm) + (9.0 x Wt in kg) - 1709.

Table 14. Mean PK Values Based on Antigen Level.

| Nominal Dose (IU/kg) | Actual Dose (IU/kg) | Equivalent Dose (mg/kg) | Patient | $C_{max}$ (ng/mL) | $AUC_{INF}$ (h*ng/mL) | Cl (mL/h/kg) | $V_{SS}$ (mL/kg) | MRT (h) | Alpha HL (h) | Beta HL (h) |
|---|---|---|---|---|---|---|---|---|---|---|
| 12.5 | 13.714 | 0.228 | 1 | 1670 | 91300 | 2.5 | 245 | 98.2 | 21.2 | 107 |
| 25 | 27.25 | 0.453 | 2 | 2730 | 144000 | 3.14 | 273 | 87.1 | 11.3 | 71 |
| | 54.5 | 0.905 | 3 | 5470 | 356000 | 2.54 | 366 | 144 | 18.6 | 138 |
| | 54.5 | 0.905 | 4 | 6910 | 389000 | 2.32 | 244 | 105 | 10.6 | 85.3 |
| | *54.5* | *0.905* | *5* | *7520* | *416000* | *2.17* | *184* | *84.5* | *NC* | *94.3* |
| | *54.513* | *0.906* | *6* | *11700* | *531000* | *1.71* | *190* | *112* | *NC* | *140* |
| 50 | 55.878 | 0.928 | 7 | 5950 | 348000 | 2.67 | 310 | 116 | 10.1 | 93.9 |
| | 109 | 1.81 | 10 | 12500 | 667000 | 2.72 | 263 | 96.8 | 9.79 | 78 |
| | 109 | 1.81 | 8 | 21600 | 1200000 | 1.51 | 156 | 103 | 15.7 | 94.3 |
| | 109 | 1.81 | 9 | 13400 | 998000 | 1.81 | 248 | 137 | 11.5 | 107 |
| | 109.176 | 1.81 | 11 | 17200 | 844000 | 2.15 | 226 | 105 | 13 | 97.1 |
| 100 | 109.441 | 1.82 | 12 | 12500 | 778000 | 2.34 | 295 | 126 | 10.6 | 102 |
| | N | | | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Mean | | | 9929.0 | 563525.0 | 2.3 | 250.0 | 109.6[b] | 13.2[c] | 100.7[e] |
| | SD | | | 5940.0 | 339925.0 | 0.5[a] | 58.2 | 18.5 | 4.0[d] | 20.9 |
| | SE | | | 1715.0 | 98128.0 | 0.1 | 16.8 | 5.3 | 1.3 | 6.0 |
| | Geometric Mean | | | 8014.0 | 452356.0 | 2.3 | 243.7 | 108.2 | 12.8 | 98.8 |

Due to correction of rounding or other errors, (a) should be 0.46, (b) should be 110, (c) should be 12.0, (d) should be 3.95, and (e) should be 101.

Table 15: Biochemical characterization of Factor IX

| | | FIXFc | rFIX | pdFIX |
|---|---|---|---|---|
| **Gamma-carboxylation** | | | | |
| aa 1-23 (K1K2 peptide) | % 6 Gla | 97.8 | 96.9 | 99.6 |
| | % 5 Gla | 2.2 | 3.1 | 0.4 |
| | % 4 Gla | 0 | 0 | 0 |
| aa 24-43 (K3 peptide) | % 6 Gla | 61.3 | 63.7 | 98.9 |
| | % 5 Gla | 26.3 | 30.9 | 1.1 |
| | % 4 Gla | 12.5 | 5.4 | 0 |
| **Total Gla/mol, peptide map** | | 11.5 | 11.6 | 12.0 |
| **Total Gla/mol, AAA** | | 11.3 ± 0.3 | 11.5 ± 0.3 | (12) |
| **Propeptide content** | | none detected | none detected | none detected |
| **β-hydroxylation Asp 64** | | 70% | 49% | 37% |
| **Sulfation of Tyr 155** | | 4% | 5% | (>90%) |
| **Phosphorylation of Ser 158** | | <10% | <10% | (>90%) |
| **Ala 148/Thr 148** | | 0/100% | 100/0% | 30/70% |
| **Activated FIX** | | <0.0125% | 0.109 +/- 0.00185% | 0.21 +/- 0.010% |
| **FXIa Activation** | | 94.8 +/- 2.4% | 96.6 +/- 1.8% | Not done |

Table 16: Summary of terminal half-lives of FIXFc and BENEFIX™ after a single intravenous dose.

| Species | BENEFIX™ | FIXFc |
|---|---|---|
| **Normal mice** | 12.3 hr | 47.2 ± 4.8 hr |
| **FIX-deficient mice** | 13.2 hr | 46.2 ± 10.1 hr |
| **FcRN KO mice** | 16.5 ± 3.0 hr | 16.9 ± 2.1 hr |
| **FcRN Tg32b mice** | 14.2 ± 2.9 hr | 53.0 ± 6.6. hr |
| **Rats** | 5.8 hr | 34.8 ± 5.3 hr |
| **FIX-deficient dogs** | 14-18 hr * | 47.5 hr |
| **Monkey** | 12.7 hr † | 47.3± 9.1 hr |

* Brinkhous et al, Blood, 1996; 88: 2603-2610

† McCarthy et al, 2002, Thromb Haemost, 2002; 87: 824-830

**Table 17. Summary of in vitro ROTEM® parameters for rFIXFc and BENEFIX™ spiked in pooled HemB mouse whole blood**

| | % of Normal Activity | CT (sec) (Mean ± SD) | CFT (sec) (Mean ± SD) | Alpha Angle (°) (Mean ± SD) |
|---|---|---|---|---|
| rFIXFc (n=10 pools) | 0.074 | 2263 ± 209 | 1152 ± 170 | 24 ± 5 |
| | 0.74 | 1371 ± 82 | 459 ± 45 | 34 ± 5 |
| | 7.4 | 790.8 ± 30 | 226 ± 20 | 52 ± 2 |
| BENEFIX™ (n=10 pools) | 0.1 | 2019 ± 178 | 732 ± 123 | 30 ± 3 |
| | 1 | 1090 ± 38 | 324 ± 33 | 43 ± 3 |
| | 10 | 551.1 ± 38 | 127 ± 10 | 67 ± 2 |

**Table 18. Median blood loss following tail clip in HemB mice treated with rFIXFc or BENEFIX™**

| Dose (IU/kg) | Median Blood Loss (mL) | | |
|---|---|---|---|
| | rFIXFc (n=15/dose) | BENEFIX™ (n=15/dose) | Vehicle (n=18) |
| 720 | 0.101 | | |
| 360 | 0.651 | 0.218 | |
| 240 | 0.298 | | |
| 120 | 0.4567 | 0.564 | |
| 80 | 0.8474 | | |
| 40 | 1.0097 | 0.918 | |
| 0 | | | 1.1586 |

**Table19 . Ex vivo ROTEM® parameter in HemB mice treated with rFIXFc and BENEFIX™**

| | Time (hour) | CT (sec) (Mean ± SD) | CFT (sec) (Mean ± SD) | Alpha Angle (degree) (Mean ± SD) |
|---|---|---|---|---|
| 100 IU/kg BENEFIX™ (n=4 mice/time point) | 0.083 | 599 ± 23 | 174 ± 16 | 58 ± 2 |
| | 24 | 682 ± 49 | 184 ± 34 | 57 ± 5 |
| | 48 | 897 ± 114 | 310 ± 89 | 45 ± 7 |
| | 72 | 1141 ± 155 | 508 ± 123 | 32 ± 7 |
| | 96 | 1613 ± 181 | 605 ± 92 | 27 ± 3 |
| 50 IU/kg rFIXFc (n=8 mice/time point) | 0.083 | 700 ± 18 | 213 ± 9 | 53 ± 1 |
| | 24 | 836 ± 31 | 261 ± 15 | 47 ± 2 |
| | 72 | 845 ± 38 | 285 ± 17 | 45 ± 2 |
| | 96 | 957 ± 30 | 296 ± 26 | 43 ± 2 |
| | 120 | 1014 ± 83 | 342 ± 50 | 42 ± 4 |
| | 168 | 1139 ± 65 | 408 ± 41 | 36 ± 3 |
| | 216 | 1366 ± 96 | 453 ± 48 | 34 ± 3 |

Table 20A. PK parameters of rFIXFc and BENEFIX™ (200 IU/kg) following subcutaneous injection of a single dose in FIX-deficient mice (Antigen ELISA)

| Compound | Dose ng/kg | V/F mL/kg | Tlag Hr | $AUC_{INF}$ Hr*ng/mL | Absorption HL Hr | Elimination HL Hr | CL/F mL/Hr/kg | Tmax Hr | Cmax ng/mL | $AUC_{INF}$ /Dose Hr.kg/mL | Cmax/Dose g/mL | F % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BeneFIX | 727273 | 3920 | 2.86 | 6397 | 1.96 | 23.9 | 114 | 10.6 | 148 | 0.00880 | 0.204 | 23.3 |
| rFIXFc | 3278689 | 2071 | 0.896 | 141370 | 7.67 | 61.9 | 23.2 | 27.3 | 1178 | 0.0431 | 0.359 | 38.1 |

Table 20B. PK parameters of rFIXFc and BENEFIX™ (200 IU/kg) following subcutaneous injection of a single dose in FIX-deficient mice (aPTT activity assay)

| Compound | Dose IU/kg | V/F dL/kg | Tlag Hr | $AUC_{INF}$ Hr*IU/dL | Absorption HL Hr | Elimination HL Hr | CL/F dL/Hr/kg | Tmax Hr | Cmax IU/dL | $AUC_{INF}$ /Dose Hr*kg/dL | Cmax/Dose g/dL | F % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BeneFIX | 207 | 54.8 | 0.631 | 93.9 | 7.01 | 17.2 | 2.20 | 16.0 | 2.04 | 0.454 | 9.86 | 18.9 |
| rFIXFc | 172 | 25.1 | 2.32 | 418 | 6.84 | 42.4 | 0.411 | 23.8 | 4.82 | 2.43 | 28.0 | 29.1 |

Table 21. PK and PD Analysis of rFIXFc and BENEFIX™ After a Single Subcutaneous Dose in FIX-Deficient Mice.

| | Assay | AUC/Dose (Hr*kg/mL) | Elim. Half-Life (Hr) | CL/F (mL/Hr/kg)/% | Tmax (Hr) | Cmax/Dose (kg/mL) | F (%) |
|---|---|---|---|---|---|---|---|
| rFIXFc 200 IU/kg | Antigen | 0.041 | 61.9 | 23.2 | 27.3 | 0.00035 | 38.1 |
| BENEFIX™ 200 IU/kg | Antigen | 0.0073 | 23.9 | 114 | 10.6 | 0.00017 | 23.3 |
| **Ratio (rFIXFc/BENEFIX™)** | **Antigen** | **5.62** | **2.59** | **0.20** | **2.58** | **2.05** | **1.63** |
| | | | | | | | |
| rFIXFc 400 IU/kg | Antigen | 0.042 | 50.9 | 23.7 | 18.3 | 0.00045 | 45.6 |
| BENEFIX™ 400 IU/kg | Antigen | 0.0089 | 20.2 | 113 | 8.13 | 0.00024 | 20.2 |
| **Ratio (rFIXFc/BENEFIX™)** | **Antigen** | **4.72** | **2.52** | **0.21** | **2.25** | **1.91** | **2.26** |
| | | **AUC/Dose (Hr*kg/mL)** | **Elim. Half-Life (Hr)** | **CL/F (mL/Hr/kg)/%** | **Tmax (Hr)** | **Cmax/Dose (kg/mL)** | **F (%)** |
| rFIXFc 200 IU/kg | Activity | 0.021 | 42.4 | 41.1 | 23.8 | 0.00024 | 29.1 |
| BENEFIX™ 200 IU/kg | Activity | 0.0047 | 17.2 | 220 | 16.0 | 0.00010 | 18.9 |
| **Ratio (rFIXFc/BENEFIX™)** | **Activity** | **4.47** | **2.46** | **0.19** | **1.49** | **2.40** | **1.54** |
| | | | | | | | |
| rFIXFc 400 IU/kg | Activity | 0.028 | 40.3 | 35.6 | 15.9 | 0.00037 | 39.2 |
| BENEFIX™ 400 IU/kg | Activity | 0.0052 | 15.6 | 193 | 18.1 | 0.00010 | 15.5 |
| **Ratio (rFIXFc/BENEFIX™)** | **Activity** | **5.38** | **2.58** | **0.18** | **0.88** | **3.70** | **2.53** |

Table 22. PK parameters of rFIXFc (50 IU/kg) following subcutaneous injection of a single dose in cynomolgus monkeys.

| Group | Animal_ID | V/F (mL/kg) | AUC (Hr*ng/mL) | Absorption HL (Hr) | Terminal HL (Hr) | CL/F (mL/Hr/kg) | Tmax (Hr) | Cmax (ng/mL) | AUC/D (Hr*kg/mL) | F(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 50 IU/kg rFIXFc | 5C4 | 545 | 109000 | 6.42 | 50.2 | 7.53 | 26.1 | 1050 | 0.133 | 43.7 |
| | C37716 | 975 | 108000 | 6.4 | 89 | 7.6 | 26.2 | 685 | 0.132 | 43.3 |
| | C41440 | 622 | 82500 | 8.54 | 43.4 | 9.93 | 24.9 | 885 | 0.101 | 33.1 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | Mean | 714 | 99800 | 7.79 | 60.9 | 8.35 | 25.7 | 873 | 0.122 | 40.1 |
| | SD | 229 | 14900 | 1.2 | 24.6 | 1.37 | 0.685 | 182 | 0.0183 | 6.03 |
| | SE | 132 | 8630 | 0.695 | 14.2 | 0.79 | 0.396 | 105 | 0.0106 | 3.48 |
| | Geometric Mean | 691 | 99000 | 7.72 | 57.9 | 8.28 | 25.7 | 860 | 0.121 | 39.7 |
| | CV% Geometric Mean | 31.2 | 15.8 | 16.4 | 39.4 | 15.8 | 2.68 | 21.7 | 15.9 | 15.9 |

Table 23. PK parameters of rFIXFc (100 IU/kg) following subcutaneous injection of a single dose in cynomolgus monkeys.

| Group | Animal_ID | V/F (mL/kg) | AUC (Hr*ng/mL) | Absorption HL (Hr) | Terminal HL (Hr) | CL/F (mL/Hr/kg) | Tmax (Hr) | Cmax (ng/mL) | AUC/D (Hr*kg/mL) | F(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 100 IU/kg rFIXFc | 29109 | 1630 | 69800 | 11.4 | 48.1 | 23.5 | 31 | 644 | 0.0426 | 14.0 |
| | 605097 | 561 | 207000 | 5.12 | 49.2 | 7.9 | 18.6 | 2250 | 0.126 | 41.5 |
| | C35785 | 387 | 238000 | 6.37 | 39 | 6.89 | 19.9 | 2970 | 0.145 | 47.8 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | Mean | 858 | 172000 | 7.62 | 45.4 | 12.8 | 23.2 | 1950 | 0.105 | 34.4 |
| | SD | 671 | 89600 | 3.31 | 5.58 | 9.3 | 6.79 | 1190 | 0.0546 | 18.0 |
| | SE | 388 | 51700 | 1.91 | 3.22 | 5.37 | 3.92 | 687 | 0.0315 | 10.4 |
| | Geometric Mean | 707 | 151000 | 7.18 | 45.2 | 10.9 | 22.6 | 1630 | 0.0921 | 30.3 |
| | CV% Geometric Mean | 86.2 | 75.5 | 43.1 | 12.8 | 75.5 | 28.2 | 96.9 | 75.5 | 75.5 |

Table 24. PK parameters of rFIXFc (200 IU/kg) following subcutaneous injection of a single dose in cynomolgus monkeys.

| Group | Animal_ID | V/F (mL/kg) | AUC (Hr*ng/mL) | Absorption HL (Hr) | Terminal HL (Hr) | CL/F (mL/Hr/kg) | Tmax (Hr) | Cmax (ng/mL) | AUC/D (Hr*kg/mL) | F(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 200 IU/kg rFIXFc | 50883 | 855 | 408000 | 3.36 | 73.7 | 8.03 | 15.7 | 3310 | 0.124 | 40.9 |
| | C31129 | 461 | 415000 | 6.42 | 40.4 | 7.91 | 20.2 | 5030 | 0.127 | 41.6 |
| | C41410 | 147 | 262000 | 11.5 | 32.6 | 3.12 | 26.7 | 3160 | 0.0799 | 26.3 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | Mean | 487 | 362000 | 7.08 | 48.9 | 6.36 | 20.9 | 3830 | 0.110 | 36.3 |
| | SD | 354 | 86100 | 4.08 | 21.8 | 2.8 | 5.51 | 1040 | 0.0263 | 8.67 |
| | SE | 205 | 49700 | 2.36 | 12.6 | 1.62 | 3.18 | 598 | 0.0152 | 5.00 |
| | Geometric Mean | 387 | 354000 | 6.27 | 46 | 5.83 | 20.4 | 3750 | 0.108 | 35.5 |
| | CV% Geometric Mean | 110 | 26.4 | 67.6 | 44.2 | 58.3 | 27 | 25.9 | 26.5 | 26.5 |

Table 25. PK Analysis of rFIXFc Following a Single Subcutaneous Dose in Cynomolgus Monkeys

| rFIXFc (IU/kg) | | AUC (Hr*ng/mL) | Abs Half-Life (Hr) | Elim. Half-Life (Hr) | CL/F (mL/Hr/kg)/% | Tmax (Hr) | Cmax (ng/mL) | F(%) |
|---|---|---|---|---|---|---|---|---|
| 50 | **Geo. Mean** | **99,000** | **7.72** | **57.9** | **8.28** | **25.7** | **860** | **39.7** |
| | CV% Geo. Mn | 15.8 | 16.4 | 39.4 | 15.8 | 2.68 | 21.7 | 15.9 |
| 100 | **Geo. Mean** | **221,959** | **5.71** | **43.8** | **7.38** | **19.2** | **2,585** | **44.5** |
| | CV% Geo. Mn | 9.89 | 15.5 | 16.5 | 9.70 | 4.78 | 19.8 | 10.0 |
| 200 | **Geo. Mean** | **354,000** | **6.27** | **46** | **5.83** | **20.4** | **3,750** | **35.5** |
| | CV% Geo. Mn | 26.4 | 67.6 | 44.2 | 58.3 | 27 | 25.9 | 26.5 |

Bioavailability ranged from 35.5 to 44.5% for rFIXFc
Elimination half-life ranged 43.8 to 57.9 hrs for rFIXFc

Table 26. Dosing Guidelines For rFIXFc Therapy In Hemophilia B

| Type of Hemorrhage | Factor IX Level Required (%) | Frequency of Doses (hrs) |
|---|---|---|
| ***Minor*** | | |
| Epistaxis | | |
| Hemarthroses, uncomplicated | 20-30 | 48 |
| Superficial muscular | 20-30 | 48 |
| Superficial soft tissue | 20-30 | 48 |
| ***Moderate*** | | |
| Epistaxis | | |
| Intramuscular with dissection | 25-50 | 48 |
| Soft tissue with dissection | 25-50 | 48 |
| Mucous membranes | 25-50 | 48 |
| Dental extractions | 25-50 | 48 |
| Hematuria | 25-50 | 48 |
| Hemarthroses, with limited motion | 40-80 | 48 |
| ***Major*** | | |
| Epistaxis | 50-100 | 24-48 |
| Pharynx | 50-100 | 24-48 |
| Retropharynx | 50-100 | 24-48 |
| Retroperitoneum | 50-100 | 24-48 |
| Surgery | 50-100 | 24-48 |
| CNS | 50-100 | 24-48 |

Patient should consult with the their physicians, but should take only 1 follow-up dose not less than 24-48 hours after the initial dose.

Tables 27A and 27B. Comparison of data using calculations in (A) Example 1 and (B) Example 11.

A

| **Dose** | **Parameter (mean±SD)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (IU/kg) | n | $C_{max}$ (IU/dL) | $AUC_{INF}$ (h·IU/dL) | CL (mL/h/kg) | Vss (mL/kg) | MRT (h) | T1/2α(h) | T1/2β (h) | Incremental Recovery (IU/dL per IU/kg)* | $C_{168h}$ (IU/dL)† | Time to 1% above baseline (Day)‡ |
| 25 | 1 | 19.9 | 753 | 3.62 | 275 | 76.0 | 1.20 | 54.0 | 0.77 | 1.09 | 7.28 |
| 50 | 5 | 41.6±8.97 /8.98 | 1630±750 | 3.77±1.12 | 264±77.6 | 71.7±13.0 | 1.79±1.19 | 52.1±10.4 | 0.86±0.22 | 2.23±1.40 | 9.29±1.98 |
| 100 | 5 | 98.2±8.21 /8.23 | 3930±893 | 2.89±0.615 | 179±31.1 | 62.8±8.82 | 9.99±4.99 | 52.5±10.1 | 1.02±0.11 | 4.38±1.53 | 11.6±1.97 |
| 25-100 | 11 | NA§ | NA§ | ND | ND | ND | ND | ND | ND | NA§ | NA§ |

B

| Dose (IU/kg) | Parameter (mean±SD) (Range) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | $C_{max}$ (IU/dL) | $AUC_{INF}$ (h·IU/dL) | CL (mL/h/kg) | Vss (mL/kg) | MRT (h) | T1/2α□(h) | T1/2β (h) | Incremental Recovery (IU/dL per IU/kg)* | $C_{168h}$ (IU/dL)† | Time to 1% above baseline (Day)‡ |
| 25 | 1 | 20.4 | 766 | 3.56 | 271 | 76.2 | 0.61 | 53.5 | 0.77 | 1.11 | 7.3 |
| 50 | 5 | 47.5±12.8 (33.0-61.1) | 1700±550 (1300-2660) | 3.44±0.84 (2.05-4.18) | 262±55.4 (163-296) | 76.8±6.7 (67.9-85.9) | 3.4±3.4 (0.13-8.72) | 57.5±8.2 (47.9-67.2) | 0.87±0.21 (0.63-1.12) | 2.46 ± 0.89 (1.63-3.92) | 10.1 ± 1.5 (8.4-12.3) |
| 100 | 5 | 98.5±7.9 (90.8-110) | 4020±986 (3090-5130) | 2.84±0.66 (2.13-3.55) | 183±27.9 (162-221) | 65.9±10.3 (53.2-76.5) | 10.3±5.6 (3.97-16.6) | 56.5±14.1 (42.4-74.5) | 1.02±0.11 (0.89-1.18) | 4.65 ± 1.73 (3.08-6.85) | 12.3 ±2.5 (9.9-15.0) |
| 25-100 | 11 | NA§ | NA§ | 3.18 ± 0.78 (2.05-4.18) | 227 ± 58.6 (162-296) | 71.8 ± 10.0 (53.2-85.9) | NA§ | 56.7 ± 10.9 (42.4-74.5) | 0.93 ± 0.18 (0.63-1.18) | NA§ | NA§ |

Results presented are mean ± SD with range listed in the parentheses

$C_{max}$ 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; T1/2□, distribution half-life; T1/2□, elimination half-life; NA, not applicable

* Incremental recovery was calculated using observed $C_{max}$ 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 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

WHAT IS CLAIMED IS:

1. A method of administering Factor IX to a human subject in need thereof, comprising administering to the subject a dose of at least about 25 IU/kg of a chimeric polypeptide comprising Factor IX and a FcRn binding partner (FcRn BP) at about a once weekly or longer dosing interval.

2. A method of administering Factor IX to a human subject in need thereof, comprising administering to the subject a dose of at least about 10 or at least about 20 IU/kg of a chimeric polypeptide comprising Factor IX and a FcRn binding partner (FcRn BP) at about a once weekly or longer dosing interval.

3. A method of administering Factor IX to a human subject in need thereof, comprising administering to the subject a dose of at least about 10 IU/kg of a chimeric polypeptide comprising Factor IX and XTEN at about a once weekly or longer dosing interval.

4. The method of any of claims 1-3, wherein the plasma level of said chimeric polypeptide reaches a trough of at least about 1 IU/dl after at least about 6 days in at least about 80% of a patient population or reaches a trough of at least about 1 IU/dl after at least about 6 days in said subject.

5. The method of any of claims 1-3, wherein the plasma level of said chimeric polypeptide reaches

an average trough of about 1-5 IU/dl in a patient population; or

a trough of about 1-5 IU/dl in said subject.

6. The method of any of claims 1-5, wherein less than 25% of the Factor IX chimeric polypeptide in said dose is fully phosphorylated and less than 25% of the Factor IX chimeric polypeptide in said dose is fully sulfated.

7. The method of claim 6, wherein less than about 10% of said chimeric polypeptide in said dose is phosphorylated and less than about 9% of said chimeric polypeptide in said dose is sulfated.

8. The method of any of claims 1-7, wherein said dose has a mean incremental recovery (K-Value) (activity; observed) greater than 0.75 IU/dL per IU/kg .

9. The method of claim 8, wherein said dose has a mean incremental recovery (K-Value) (activity; observed) of at least about 0.8, at least about 0.9, or at least about 1 IU/dL per IU/kg.

10. The method of any of claims 1-7, wherein said chimeric polypeptide exhibits one or more pharmacokinetic parameters, in said patient population or in said subject, selected from the group consisting of:

a mean clearance (CL) (activity) in said patient population of about 3.36 ± 0.93 mL/hour/kg;

a mean clearance (CL) (activity) in said patient population of about 3.0-3.72, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, or 3.72 mL/hour/kg;

a mean clearance (CL) (activity) in said patient population that is about 2.5 fold lower than the clearance of a polypeptide comprising said Factor IX without said FcRn BP;

a clearance (CL) (activity) in said subject of about 1.84-4.58 mL/hour/kg

a mean mean residence time (MRT) (activity) in said patient population of at least about 68.05 ± 11.16 hours;

a mean MRT (activity) in said patient population of about 60-78, 60, 62, 64, 66, 68, 70, 72, 74, 76, or 78 hours;

a mean MRT (activity) in said patent population that is about 3 fold longer than the mean MRT of a polypeptide comprising said Factor IX without said FcRn BP;

a mean residence time (MRT) (activity) in said subject of about 53.1-85.8 hours;

a mean residence time (MRT) (activity) in said subject of at least about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, or about 90 hours;

a mean $t_{1/2beta}$ (activity) in said patient population of about 52.5 ± 9.2 hours;

a mean $t_{1/2beta}$ (activity)in said patient population that is about 47-60 hours, , about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60 hours; a mean $t_{1/2beta}$ (activity) in said patient population that is about 3 fold longer than the mean $t_{1/2beta}$ of a polypeptide comprising said Factor IX without said FcRn BP;

a $t_{1/2beta}$ (activity) in said subject of about 40-67.4, about 40, about 45, about 50, about 55, about 60, about 65, about 70, or about 75, hours;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 0.93 ± 0.18 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 0.85-1.15, about 0.85, about 0.86, about 0.87, about 0.88, about 0.89, about 0.90, about 0.91, about 0.92, about 0.93, about 0.94, about 0.95, about 0.96, about 0.97, about 0.98, about 0.99, about 1.0, about 1.05, about 1.10, or about 1.15 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population that is about 24% better than the mean incremental recovery of a polypeptide comprising said Factor IX without said FcRN BP;

an incremental recovery (K value) (activity; observed) in said subject of about 0.62-1.17 IU/dL per IU/kg;

a mean Vss (activity) in said patient population of about 226 ± 67.76 mL/kg;

a mean Vss (activity) in said patient population of about 200-300, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, or about 300 mL/kg;

a Vss (activity) in said subject of about 145-365 mL/kg;

a mean AUC/dose (activity) in said patient population of about 32.44 ± 10.75 IU*h/dL per IU/kg;

a mean AUC/dose (activity) in said patient population of about 26-40, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 IU*h/dL per IU/kg;

an AUC/dose in said subject of about 21.80-54.30 IU*h/dL per IU/kg.

11. The method of any of claims 1-10, wherein said dosing interval is 6-10 days.

12. The method of claim 11, wherein said dose is selected from the group consisting of about 25-110, about 30-110, about 40-110, about 50-110, about 60-110, about 70-110, about 80-110, about 90-110, and about 100-110 IU/kg.

13. The method of claim 11, wherein said dose is selected from the group consisting of, about 30-100, about 30-90, about 30-80, about 30-70, about 30-60, about 30-50, and about 30-40 IU/kg.

14. The method of claim 11, wherein said dose is selected from the group consisting of about 40-110, about 50-100, about 60-90, and about 70-80 IU/kg.

15. The method of claim 11, wherein said dose is selected from the group consisting of about 40-50, about 50-60, about 60-70, about 70-80, about 80-90, about 90-100, and about 100-110 IU/kg.

16. The method of claim 11, wherein said dose is selected from the group consisting of about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, and about 110 IU/kg.

17. The method of any of claims 11-16, wherein said dosing interval is selected from the group consisting of about 7-10, about 7-9, and about 7-8 days.

18. The method of any of claims 11-16, wherein said dosing interval is selected from the group consisting of about 8-10 and about 9-10 days.

19. The method of any of claims 11-16, wherein said dosing interval is selected from the group consisting of about 6-7 and about 8-9 days.

20. The method of any of claims 11-16, wherein said dosing interval is selected from the group consisting of about 6, about 7, about 8, about 9, and about 10 days.

21. The method of claim 11, wherein said dose is about 30-50 IU/kg and said dosing interval is about 7 days.

22. The method of any of claim 4-21, wherein said trough is reached in at least about 90% of said patient population.

23. The method of claim 22, wherein said trough is reached in about 100% of said patient population.

24. The method of any of claims 1-11, 22, and 23, wherein said dose is about 50 IU/kg, and said dosing interval is about 7 days.

25. The method of any of claims 4-11, wherein said dose is about 50 IU/kg, said dosing interval is about 7 days, and said trough is reached in about 100% of said patient population.

26. The method of any of claims 1-10, wherein said dosing interval is 9-18 days.

27. The method of claim 26, wherein said dose is selected from the group consisting of about 90-180, about 100-180, about 110-180, about 120-180, about 130-180, about 140-180, about 150-180, about 160-180, and about 170-180 IU/kg.

28. The method of claim 26, wherein said dose is selected from the group consisting of about 90-170, about 90-160, about 90-150, about 90-140, about 90-130, about 90-120, about 90-110, and about 90-100 IU/kg.

29. The method of claim 26, wherein said dose is selected from the group consisting of about 100-170, about 110-160, about 120-150, and about 130-140 IU/kg.

30. The method of claim 26, wherein said dose is selected from the group consisting of about 90-100, about 100-110, about 110-120, about 120-130, about 130-140, about 140-150, about 150-160, and about 160-170 IU/kg.

31. The method of claim 26, wherein said dose is selected from the group consisting of about 90, about 95, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, about 160, about 165, about 170, about 175, and about 180 IU/kg.

32. The method of any of claims 26-31, wherein said dosing interval is selected from the group consisting of about 9-17, about 9-16, about 9-15, about 9-14, about 9-13, about 9-12, about 9-11, and about 9-10 days.

33. The method of any of claims 26-31, wherein said dosing interval is selected from the group consisting of about 10-18, about 11-18, about 12-18, about 13-18, about 14-18, about 15-18, about 16-18, and about 17-18 days.

34. The method of any of claims 26-31, wherein said dosing interval is selected from the group consisting of about 10-11, about 11-12, about 12-13, about 13-14, about 14-15, about 15-16, and about 16-17 days.

35. The method of any of claims 26-31, wherein said dosing interval is selected from the group consisting of about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, and about 18 days.

36. The method of claim 26, wherein said dose is about 100 IU/kg and said dosing interval is at least about 12 days.

37. The method of claim 36, wherein said trough is at least about 1 IU/dl in at least about 70%, about 80%, or about 90% of said patient population.

38. The method of claim 26, wherein said dosing interval is about 12-13 days.

39. The method of claim 26, wherein said dose is about 100 IU/kg and said dosing interval is at least about 9 days.

40. The method of claim 39, wherein the trough is at least about 1 IU/dl in about 100% of said patient population.

41. The method of claim 26 or 39, wherein said dosing interval is about 9-15 days.

42. The method of claim 26, wherein said dose is about 150 IU/kg and said dosing interval is at least about 14 days.

43. The method of claim 42, where said trough is at least about 1 IU/dl in about 100% of said patient population.

44. The method of any of claims 1-43, wherein said subject is in need of control or prevention of bleeding or bleeding episodes.

45. The method of claim 44, wherein said subject is in need of control or prevention of bleeding in minor hemorrhage, hemarthroses, superficial muscle hemorrhage, soft tissue hemorrhage, moderate hemorrhage, intramuscle or soft tissue hemorrhage with dissection, mucous membrane hemorrhage, hematuria, major hemorrhage, hemorrhage of the pharynx, hemorrhage of the retropharynx, hemorrhage of the retroperitonium, hemorrhage of the central nervous system, bruises, cuts, scrapes, joint hemorrhage, nose bleed, mouth bleed,

gum bleed, intracranial bleeding, intraperitoneal bleeding, minor spontaneous hemorrhage, bleeding after major trauma, moderate skin bruising, or spontaneous hemorrhage into joints, muscles, internal organs or the brain.

46. The method of any of claims 1-43, wherein said subject is in need of peri-operative management.

47. The method of claim 46, wherein said subject is in need of management of bleeding associated with surgery or dental extraction.

48. The method of claim 46, wherein said subject will undergo, is undergoing, or has undergone major surgery.

49. The method of claim 48, wherein said major surgery is orthopedic surgery, extensive oral surgery, urologic surgery, or hernia surgery.

50. The method of claim 49, wherein said orthopedic surgery is replacement of knee, hip, or other major joint.

51. The method of any of claims 1-43, wherein said subject is in need of prophylactic treatment.

52. The method of any of claims 1-43, wherein said subject is in need of on-demand treatment.

53. The method of claim 52, wherein said subject is in need of treatment for a bleeding episode.

54. The method of claim 53, wherein said subject is in need of treatment for hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the illiopsoas sheath.

55. The method of claim 51, wherein said subject is in need of surgical prophylaxis, peri-operative management, or treatment for surgery.

56. The method of claim 53, wherein said surgery is minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.

57. The method of any of claims 1-56, wherein said subject is human.

58. The method of any of claims 1-57, wherein said Factor IX in said chimeric polypeptide is a human Factor IX.

59. The method of any of claims 1-58, wherein said Factor IX in said chimeric polypeptide is a mutant Factor IX.

60. The method of any of claims 1-59, wherein said FcRn BP in said chimeric polypeptide is a human Fc.

61. The method of any of claims 1-60, wherein said FcRn BP in chimeric polypeptide is a mutant Fc.

62. The method of claim 58, wherein said Factor IX is at least 90% or 95% identical to a Factor IX amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 415 of SEQ ID NO:2).

63. The method of claim 62, wherein said Factor IX is identical to a Factor IX amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 415 of SEQ ID NO:2).

64. The method of claim 60, wherein said Fc is at least 90% or 95% identical to a Fc amino acid sequence shown in Table 2B without a signal sequence (amino acids 1 to 227 SEQ ID NO:4).

65. The method of claim 64, wherein said Fc is identical to a Fc amino acid sequence shown in Table 2B without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4).

66. The method of any of claims 1-65, wherein said chimeric polypeptide is in the form of a hybrid comprising a second polypeptide in association with said chimeric polypeptide, wherein said second polypeptide comprises a FcRn BP.

67. The method of claim 66, wherein said chimeric polypeptide comprises a sequence at least 90% or 95% identical to the Factor IX and Fc amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 642 of SEQ ID NO:2).

68. The method of claim 67, wherein said chimeric polypeptide comprises a sequence identical to the Factor IX and Fc amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 642 of SEQ ID NO:2).

69. The method of any of claims 66-68, wherein said second polypeptide comprises a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2B without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4).

70. The method of claim 69, wherein said second polypeptide comprises a sequence identical to the amino acid sequence shown in Table 2B without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4).

71. The method of any of claims 1-70, wherein said patient is in need of long-term treatment at weekly or longer dosing intervals.

72. The method of any of claims 1-71, wherein said chimeric polypeptide is administered as part of a pharmaceutical composition comprising at least one excipient.

73. A polypeptide comprising a Factor IX at least 90% or 95% identical to a Factor IX amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 415 of SEQ ID NO:2), and a FcRn BP.

74. The polypeptide of claim 73, wherein said Factor IX is identical to a Factor IX amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 415 of SEQ ID NO:2).

75. A polypeptide comprising a Factor IX at least 90% or 95% identical to a Factor IX amino acid sequence shown in Table 2A with a signal sequence and propeptide and propeptide (amino acids -46 to 415 of SEQ ID NO:2), and a FcRn BP.

76. The polypeptide of claim 75, wherein said Factor IX is identical to a Factor IX amino acid sequence shown in Table 2A with a signal sequence and propeptide (amino acids -46 to 415 of SEQ ID NO:2).

77. The polypeptide of any of claims 73-65, wherein said FcRn BP is at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2B (amino acids 1 to 227 of SEQ ID NO:4).

78. The polypeptide of claim 77, wherein said Fc is identical to the Fc amino acid sequence shown in Table 2B (amino acids 1 to 227of SEQ ID NO:4).

79. The polypeptide of claim 73 or 75, which comprises a sequence at least 90% or 95% identical to the Factor IX and Fc amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 642 of SEQ ID NO:2).

80. The polypeptide of claim 79, which comprises a sequence identical to the Factor IX and Fc amino acid sequence shown in Table 2A without a signal sequence and propeptide (amino acids 1 to 642 of SEQ ID NO:2).

81. The polypeptide of any of claims 73-80, which is in the form of a hybrid comprising a second polypeptide, wherein said second polypeptide comprises a FcRn BP.

82. The polypeptide of claim 81, wherein said second polypeptide comprises a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2B without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or at least 90% or 95% identical to the amino acid sequence shown in Table 2B with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

83. The polypeptide of claim 81, wherein said second polypeptide comprises a sequence identical to the amino acid sequence shown in Table 2B without a signal sequence

(amino acids 1 to 227 of SEQ ID NO:4) or at least 90% or 95% identical to the amino acid sequence shown in Table 2B with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

84. The polypeptide of any of claims 73-83, which has greatly reduced phosphorylation and sulfation in comparison to plasma derived Factor IX.

85. The polypeptide of claim 84, which is less than about 10% phosphorylated and less than about 9% sulfated.

86. The polypeptide of any of claims 73-85, which has a K value greater that 0.7 or 0.75.

87. The polypeptide of claim 86, which has a K value of at least about 0.8, at least about 0.9, or at least about 1.

88. The polypeptide of any of claims 73-87, which exhibits one or more pharmacokinetic parameters, in said patient population or in said subject, selected from the group consisting of:

a mean clearance (CL) (activity) in said patient population of about 3.36 ± 0.93 mL/hour/kg;

a mean clearance (CL) (activity) in said patient population of about 3.0-3.72, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, or 3.72 mL/hour/kg;

a mean clearance (CL) (activity) in said patient population that is about 2.5 fold lower than the clearance of a polypeptide comprising said Factor IX without said FcRn BP;

a clearance (CL) (activity) in said subject of about 1.84-4.58 mL/hour/kg

a mean mean residence time (MRT) (activity) in said patient population of at least about 68.05 ± 11.16 hours;

a mean MRT (activity) in said patient population of about 60-78, 60, 62, 64, 66, 68, 70, 72, 74, 76, or 78 hours;

a mean MRT (activity) in said patent population that is about 3 fold longer than the mean MRT of a polypeptide comprising said Factor IX without said FcRn BP;

a mean residence time (MRT) (activity) in said subject of about 53.1-85.8 hours;

a mean residence time (MRT) (activity) in said subject of at least about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, or about 90 hours;

a mean $t_{1/2beta}$ (activity) in said patient population of about 52.5 ± 9.2 hours;

a mean $t_{1/2beta}$ (activity)in said patient population that is about 47-60 hours, , about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60 hours; a mean $t_{1/2beta}$ (activity) in said patient

population that is about 3 fold longer than the mean $t_{1/2beta}$ of a polypeptide comprising said Factor IX without said FcRn BP;

a $t_{1/2beta}$ (activity) in said subject of about 40-67.4, about 40, about 45, about 50, about 55, about 60, about 65, about 70, or about 75 hours;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 0.93 ± 0.18 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 0.85-1.15, about 0.85, about 0.86, about 0.87, about 0.88, about 0.89, about 0.90, about 0.91, about 0.92, about 0.93, about 0.94, about 0.95, about 0.96, about 0.97, about 0.98, about 0.99, about 1.0, about 1.05, about 1.10, or about 1.15 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population that is about 24% better than the mean incremental recovery of a polypeptide comprising said Factor IX without said FcRN BP;

an incremental recovery (K value) (activity; observed) in said subject of about 0.62-1.17 IU/dL per IU/kg;

a mean Vss (activity) in said patient population of about 226 ± 67.76 mL/kg;

a mean Vss (activity) in said patient population of about 200-300, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, or about 300 mL/kg;

a Vss (activity) in said subject of about 145-365 mL/kg;

a mean AUC/dose (activity) in said patient population of about 32.44 ± 10.75 IU*h/dL per IU/kg;

a mean AUC/dose (activity) in said patient population of about 26-40, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 IU*h/dL per IU/kg;

an AUC/dose in said subject of about 21.80-54.30 IU*h/dL per IU/kg.

89. A polynucleotide encoding the polypeptide of any of claims 73-80.

90. A polynucleotide encoding the Factor IX-FcRn BP polypeptide and the second peptide of any one of claims 81-88.

91. The polynucleotide of claim 89 or 90, which is a vector, plasmid, phage, or virus.

92. The polynucleotide of any of claims 89-91, which is DNA or RNA.

93. A cultured human embryonic cell comprising the polynucleotide of any one of claims 89-92.

94. The cell of claim 93, which is a HEK293 cell.

95. A method of producing a Factor IX-FcRn BP hybrid protein comprising

culturing the cell of claim 93 or 94 under conditions that allow expression of the encoded Factor IX-FcRn BP chimeric polypeptide and the encoded polypeptide consisting essentially of FcRn BP; and

recovering the encoded Factor IX-FcRn BP hybrid protein.

96. A hybrid protein produced by the method of claim 95.

97. The hybrid protein of claim 96, which has greatly reduced phosphorylation and sulfation in comparison to plasma derived Factor IX.

98. The hybrid protein of claim 97, which is less than about 10% phosphorylated and less than about 9% sulfated.

99. The hybrid protein of any of claims 96-98, which has a K value greater that 0.7 or 0.75.

100. The hybrid protein of claim 99, which has a K value of at least about 8, about 9, or about 1.

101. The method of any of claims 66-72, wherein said chimeric polypeptide is in the form of a hybrid, wherein said hybrid consists essentially of a single chain of said chimeric polypeptide and a single chain of said second polypeptide, and wherein said chains are associated through (a) noncovalent interactions, (b) two disulfide bonds or (c) both (a) and (b).

102. The polypeptide of any of claims 81-88 and 96-100, wherein said chimeric polypeptide is in the form of a hybrid, wherein said hybrid consists essentially of a single chain of said chimeric polypeptide and a single chain of said second polypeptide, and wherein said chains are associated through (a) noncovalent interactions, (b) two disulfide bonds or (c) both (a) and (b).

103. A recombinant factor IX (rFIX) preparation, which has an incremental recovery (K-Value) in humans greater than 0.75 IU/dL per IU/kg and wherein less than 25% of the rFIX in the preparation is fully phosphorylated and sulfated.

104. The rFIX preparation of claim 103, which has an incremental recovery (K-Value) of at least about 0.8, at least about 0.9, or at least about 1 IU/dL per IU/kg.

105. The rFIX preparation of claim 103, wherein the rFIX comprises a FcRn BP.

106. The rFIX preparation of claim 105, which comprises the FcRn binding region of Fc.

107. The rFIX preparation of claim 105, which comprises the FcRn binding region of albumin.

108. The method of any of claims 1-10, wherein said dose is 10-50, 10-30, 20-50, 20-100, 10, or 20 IU/kg and said dosing interval is one time weekly.

109. The method of any of claims 1-10, wherein said dose is 15-50 or 40 IU/kg and said dosing interval is every 10 days.

110. The method of any of claims 1-10, wherein said dose is 100 IU/kg and said dosing interval is every two weeks or twice monthly.

111. The method of any of claims 1-10, wherein said dosing interval is one time weekly.

112. The method of any of claims 1-10, wherein said dose is 15-100 IU/kg and said dosing interval is 10-13 days.

113. The method of any of claims 1-10, wherein said dose 50-100 IU/kg and said dosing interval is 10-14 days, said dose is 50-150 IU/kg and said dosing interval is 14-15 days, or said dose is 100-150 IU/kg and said dosing interval is 14-16 days.

114. The method of any of claims 1-10, wherein said dose is 15-50 IU/kg and said dosing interval is 10 days, said dose is 20-70 IU/kg and said dosing interval is 11 days, said dose is 25-85 IU/kg and said dosing interval is 12 days, said dose is 30-100 IU/kg and said dosing interval is 13 days, said dose is 40-125 IU/kg and said dosing interval is 14 days, or said dose is 50–150 IU/kg and said dosing interval is 15 days.

115. The method of any of claims 1-10, which consists of a one time weekly prophylactic dosing interval.

116. The method of any of claims 1-10, which consists of a 10-14 day prophylactic dosing interval.

117. The method of any of claims 1-10, which consists of a 15-18 or 16-18 day prophylactic dosing interval.

118. The method of any of claims 1-10, which consists of a two times monthly prophylactic dosing interval.

119. The method of any of claims 1-10, which consists of a one time monthly prophylactic dosing interval.

120. The method of any of claims 1-10, which is a fixed or individualized prophylactic dose and/or dosing interval.

121. The method of any of claims 1-10, wherein said dose is administered intravenously or subcutaneously.