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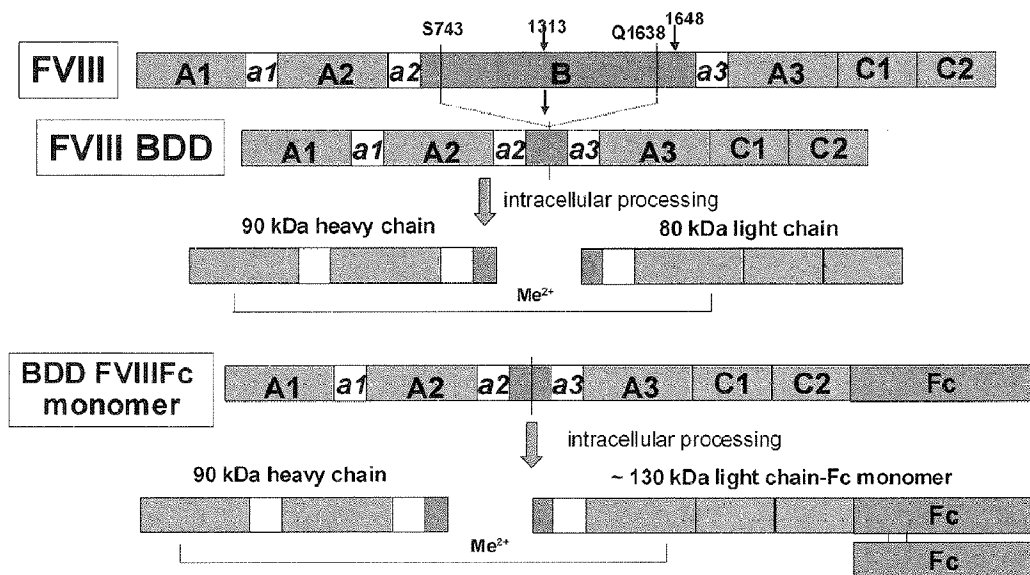
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(54) Titre : POLYPEPTIDES CHIMERIQUES ET HYBRIDES DE FACTEUR VIII-FC, ET PROCEDES D'UTILISATION DE CEUX-CI

(54) Title: FACTOR VIII-FC CHIMERIC AND HYBRID POLYPEPTIDES, AND METHODS OF USE THEREOF



(57) Abrégé/Abstract:

The present invention provides methods of administering Factor VIII; methods of administering chimeric and hybrid polypeptides comprising Factor VIII; chimeric and hybrid polypeptides comprising Factor VIII; polynucleotides encoding such chimeric and hybrid polypeptides; cells comprising such polynucleotides; and methods of producing such chimeric and hybrid polypeptides using such cells.

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ABSTRACT

The present invention provides methods of administering Factor VIII; methods of administering chimeric and hybrid polypeptides comprising Factor VIII; chimeric and hybrid polypeptides comprising Factor VIII; polynucleotides encoding such chimeric and hybrid polypeptides; cells comprising such polynucleotides; and methods of producing such chimeric and hybrid polypeptides using such cells.

FACTOR VIII-Fc CHIMERIC AND HYBRID 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 A is an X-linked bleeding disorder caused by mutations and/or deletions in the factor VIII (FVIII) gene resulting in a deficiency of FVIII activity (Peyvandi et al. 2006). The disease is characterized by spontaneous hemorrhage and excessive bleeding after trauma. Over time, the repeated bleeding into muscles and joints, which often begins in early childhood, results in hemophilic arthropathy and irreversible joint damage. This damage is progressive and can lead to severely limited mobility of joints, muscle atrophy and chronic pain (Rodriguez-Merchan, E.C., Semin. Thromb. Hemost. 29:87-96 (2003), which is herein incorporated by reference in its entirety).

[0003] The A2 domain is necessary for the procoagulant activity of the factor VIII molecule. Studies show that porcine factor VIII has six-fold greater procoagulant activity than human factor VIII (Lollar, P., and E. T. Parker, J. Biol. Chem. 266:12481-12486 (1991)), and that the difference in coagulant activity between human and porcine factor VIII appears to be based on a difference in amino acid sequence between one or more residues in the human and porcine A2 domains (Lollar, P., et al., J. Biol. Chem. 267:23652-23657 (1992)), incorporated herein by reference in its entirety.

[0004] Treatment of hemophilia A is by replacement therapy targeting restoration of FVIII activity to 1 to 5 % of normal levels to prevent spontaneous bleeding (Mannucci, P.M., et al., N. Engl. J. Med. 344:1773-1779 (2001), which is herein incorporated by reference in its entirety). There are plasma-derived and recombinant FVIII products available to treat bleeding episodes on-demand or to prevent bleeding episodes from occurring by treating prophylactically. Based on the half-life of these products treatment

regimens require frequent intravenous administration. Such frequent administration is painful and inconvenient.

[0005] Reduced mortality, prevention of joint damage and improved quality of life have been important achievements due to the development of plasma-derived and recombinant FVIII. Prolonged protection from bleeding would represent another key advancement in the treatment of hemophilia A 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 VIII deficiency that are more tolerable and more effective than current therapies.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides methods of administering Factor VIII; methods of administering chimeric polypeptides comprising Factor VIII and hybrids of such chimeric polypeptides; chimeric polypeptides comprising Factor VIII 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.

[0007] The present invention provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, at a dosing interval at least about one and one-half times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

[0008] The dosing interval may be at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., the Fc portion. The dosing interval may be at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., the Fc

portion. The dosing interval may be about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

[0009] The dosing interval may be at least about one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer.

[0010] The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, to obtain an area under the plasma concentration versus time curve (AUC) at least about one and one-quarter times greater than the AUC obtained by an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

[0011] The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a polypeptide comprising a Factor VIII and an Fc at a dosing interval of about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

[0012] The methods of the invention may be practiced on a subject in need of prophylactic treatment or on-demand treatment.

[0013] On-demand treatment includes treatment for a bleeding episode, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis (head trauma), 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 iliopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, e.g., 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.

[0014] For on-demand treatment, the dosing interval of said chimeric polypeptide is about once every 24-36, 24-48, 24-72, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 hours or longer.

- [0015] The therapeutic doses that may be used in the methods of the invention are about 10 to about 100 IU/kg, more specifically, about 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg, and more specifically, about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.
- [0016] The therapeutic doses that may be used in the methods of the invention are about 10 to about 150 IU/kg, more specifically, about 100-110, 110-120, 120-130, 130-140, 140-150 IU/kg, and more specifically, about 110, 115, 120, 125, 130, 135, 140, 145, or 150 IU/kg.
- [0017] The subject in the methods of the invention may be a human subject or may be a non-human mammal. Non-human mammals include, e.g., mice, dogs, primates, monkeys, cats, horses, cows, pigs, and other domestic animals and small animals. The determination of dosing interval and AUC may be carried out in a single subject or in a population of subjects.
- [0018] The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be a human Factor VIII, or a non-human Factor VIII, such as porcine, mouse or canine factor VIII. The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may have a full or partial deletion of the B domain.
- [0019] The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).
- [0020] The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence

shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

[0021] The Fc portion (or Fc portion of a chimeric polypeptide) may be at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12). The Fc portion (or Fc portion of a chimeric polypeptide) may be identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

[0022] The chimeric polypeptide may comprise a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2). The chimeric polypeptide may comprise a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

[0023] 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 Fc.

[0024] The second polypeptide may comprise or consist essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4). The second polypeptide may comprise or consist essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

[0025] The chimeric polypeptide or hybrid may be administered as part of a pharmaceutical composition comprising at least one excipient.

[0026] 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

[0027] FIG. 1. Schematic Representation of rFVIII Fc monomer.

[0028] FIG. 2. WBCT of rFVIII Fc compared to ReFacto[®] in hemophilia A mice after a 50 IU/kg intravenous dose (n = 6 mice per group).

[0029] FIG. 3. Chromogenic Activity in Plasma from hemophilia A mice after a single IV dose of 50 IU/kg rFVIII Fc, ReFacto[®] and Advate[®].

[0030] FIG. 4. WBCT of rFVIII Fc and ReFacto[®] in hemophilia A dogs (A) rFVIII Fc. (B) ReFacto[®] followed by rFVIII Fc in a Crossover Study.

[0031] FIG. 5. Pharmacokinetics of intravenous rFVIII Fc and ReFacto[®] in Hemophilia A Dogs (measured by ELISA).

[0032] FIG. 6. Activity of rFVIII and ReFacto[®] after a single intravenous dose in hemophilia A dogs (measured by FVIII-specific chromogenic activity assay).

[0033] FIG. 7. Group mean plasma concentration over time of rFVIII Fc and Xyntha after a single intravenous dose (125 IU/kg) in cynomolgus monkeys (n = 6, mean \pm SD). Plasma concentrations were measured by ELISA.

[0034] FIG. 8. Individual plasma concentration versus time curves of rFVIII Fc and Xyntha after a single intravenous dose (125 IU/kg) in cynomolgus monkeys (n = 6, mean \pm SD). Plasma concentrations were measured by ELISA. (A) rFVIII Fc by ELISA. (B) Xyntha by ELISA.

[0035] FIG. 9. Group mean plasma chromogenic activity after a single intravenous dose (125 IU/kg) of rFVIII Fc and Xyntha in cynomolgus monkeys (n = 6, mean \pm SD). FVIII activity was measured using a FVIII-specific chromogenic activity assay.

[0036] FIG. 10. Individual plasma chromogenic activity versus time curves after a single intravenous dose (125 IU/kg) of rFVIII Fc and Xyntha in cynomolgus monkeys (n = 6,

mean \pm SD). FVIII activity was measured using a FVIII-specific chromogenic activity assay. (A) rFVIII-Fc Chromogenic Activity. (B) Xyntha Chromogenic Activity.

[0037] FIG. 11. Biochemical characterization of rFVIII-Fc: Activation of Factor X as a function of Factor X concentration.

[0038] FIG. 12. Biochemical characterization of rFVIII-Fc: Activation of Factor X as a function of Factor IXa concentration.

[0039] FIG 13. Observed group mean FVIII activity (\pm SE) (one stage assay, 25 IU/kg (A) or 65 IU/kg (B); and chromogenic assay, 25 IU/kg (C) or 65 IU/kg (D)) versus time.

[0040] FIG. 14. Observed group mean FVIII activity (\pm SE) (one stage assay (A) or chromogenic assay (B)) versus time.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The present invention provides a method of treating Hemophilia A with Factor VIII using a longer dosing interval and/or greater AUC than is possible with currently known Factor VIII products. The present invention also provides improved Factor VIII chimeric polypeptides, Factor VIII chimeric polynucleotides, and methods of production.

[0042] Treatment of hemophilia A is by replacement therapy targeting restoration of FVIII activity to 1 to 5 % of normal levels to prevent spontaneous bleeding (Mannucci, P.M., et al., N. Engl. J. Med. 344:1773-9 (2001), herein incorporated by reference in its entirety). There are plasma-derived and recombinant FVIII products available to treat bleeding episodes on-demand or to prevent bleeding episodes from occurring by treating prophylactically. Based on the half-life of these products (10-12 hr) (White G.C., et al., Thromb. Haemost. 77:660-7 (1997); Morfini, M., Haemophilia 9 (suppl 1):94-99; discussion 100 (2003)), treatment regimens require frequent intravenous administration, commonly two to three times weekly for prophylaxis and one to three times daily for on-demand treatment (Manco-Johnson, M.J., et al., N. Engl. J. Med. 357:535-544 (2007)), each of which is incorporated herein by reference in its entirety. Such frequent administration is painful and inconvenient.

[0043] The present invention provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, or a hybrid of such a polypeptide at a dosing interval at least about one and one-half times longer than the

dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

[0044] The dosing interval may be at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion. The dosing interval may be at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion. The dosing interval may be about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

[0045] The dosing interval may be at least about one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer.

[0046] The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide, or a hybrid of such a polypeptide to obtain an area under the plasma concentration versus time curve (AUC) at least about one and one-quarter times greater than the AUC obtained by an equivalent amount of said Factor VIII without non-Factor VIII portion (a polypeptide consisting of said Factor VIII portion), e.g., without the Fc portion.

[0047] The present invention also provides a method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a polypeptide comprising a Factor VIII and an Fc or a hybrid of such a polypeptide at a dosing interval of about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

[0048] The methods of the invention may be practiced on a subject in need of prophylactic treatment or on-demand treatment.

[0049] "Administering," as used herein, means to give a pharmaceutically acceptable Factor VIII 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. Additional routes of administration include, e.g., subcutaneous, intramuscular, oral, nasal, and pulmonary administration. Chimeric polypeptides and hybrid proteins may be administered as part of a pharmaceutical composition comprising at least one excipient.

[0050] "Area under the plasma concentration versus time curve (AUC)," as used herein, is the same as the term of art in pharmacology, and is based upon the rate and extent of absorption of factor VIII 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. The determination of AUC may be carried out in a single subject, or in a population of subjects for which the average is calculated.

[0051] "B domain" of Factor VIII, as used herein, is the same as the B domain known in the art that is defined by internal amino acid sequence identity and sites of proteolytic cleavage by thrombin, e.g., residues Ser741-Arg1648 of full length human factor VIII. The other human factor VIII domains are defined by the following amino acid residues: A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Ile2032; C1, residues Arg2033-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-Tyr2332. The remaining sequence, residues Glu1649-Arg1689, is usually referred to as the factor VIII light chain activation peptide. The locations of the boundaries for all of the domains, including the B domains, for porcine, mouse and canine factor VIII are also known in the art. Preferably, the B domain of Factor VIII is deleted ("B domain deleted factor VIII" or "BDD FVIII"). An example of a BDD FVIII is REFACTO (recombinant BDD FVIII), which has the same sequence as the Factor VIII portion of the sequence in Table 2A(i) (amino acids -19 to 1438 or 1 to 1438 of SEQ ID NO:2).

[0052] A "B domain deleted factor VIII" may have the full or partial deletions disclosed in U.S. Patent Nos. 6,316,226, 6,346,513, 7,041,635, 5,789,203, 6,060,447, 5,595,886, 6,228,620, 5,972,885, 6,048,720, 5,543,502, 5,610,278, 5,171,844, 5,112,950, 4,868,112, and 6,458,563, each of which is incorporated herein by reference in its entirety. In some embodiments, a B domain deleted factor VIII sequence of the present invention comprises any one of the deletions disclosed at col. 4, line 4 to col. 5, line 28 and

examples 1-5 of U.S. Patent No. 6,316,226 (also in US 6,346,513). In some embodiments, a B domain deleted factor VIII of the present invention has a deletion disclosed at col. 2, lines 26-51 and examples 5-8 of U.S. Patent No. 5,789,203 (also US 6,060,447, US 5,595,886, and US 6,228,620). In some embodiments, a B domain deleted factor VIII has a deletion described in col. 1, lines 25 to col. 2, line 40 of US Patent No. 5,972,885; col. 6, lines 1-22 and example 1 of U.S. Patent no. 6,048,720; col. 2, lines 17-46 of U.S. Patent No. 5,543,502; col. 4, line 22 to col. 5, line 36 of U.S. Patent no. 5,171,844; col. 2, lines 55-68, figure 2, and example 1 of U.S. Patent No. 5,112,950; col. 2, line 2 to col. 19, line 21 and table 2 of U.S. Patent No. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 67, col. 7, line 43 to col. 8, line 26, and col. 11, line 5 to col. 13, line 39 of U.S. Patent no. 7,041,635; or col. 4, lines 25-53, of U.S. Patent No. 6,458,563. In some embodiments, a B domain deleted factor VIII has a deletion of most of the B domain, but still contains amino-terminal sequences of the B domain that are essential for *in vivo* proteolytic processing of the primary translation product into two polypeptide chain, as disclosed in WO 91/09122, which is incorporated herein by reference in its entirety. In some embodiments, a B domain deleted factor VIII is constructed with a deletion of amino acids 747-1638, i.e., virtually a complete deletion of the B domain. Hoeben R.C., *et al. J. Biol. Chem.* 265 (13): 7318-7323 (1990), incorporated herein by reference in its entirety. A B domain deleted factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of factor VIII. Meulien P., *et al. Protein Eng.* 2(4): 301-6 (1988), incorporated herein by reference in its entirety. Additional B domain deletions that are part of the invention include, e.g.,: deletion of amino acids 982 through 1562 or 760 through 1639 (Toole et al., *Proc. Natl. Acad. Sci. U.S.A.* (1986) 83, 5939-5942)), 797 through 1562 (Eaton, et al. *Biochemistry* (1986) 25:8343-8347)), 741 through 1646 (Kaufman (PCT published application No. WO 87/04187)), 747-1560 (Sarver, et al., *DNA* (1987) 6:553-564)), 741 though 1648 (Pasek (PCT application No.88/00831)), 816 through 1598 or 741 through 1689 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)), each of which is incorporated herein by reference in its entirety. Each of the foregoing deletions may be made in any Factor VIII sequence.

[0053] "Chimeric polypeptide," as used herein, means a polypeptide that includes within it at least two polypeptides (or subsequences or peptides) from different sources.

Chimeric polypeptides may include, e.g., two, three, four, five, six, seven, or more polypeptides from different sources, such as different genes, different cDNAs, or different animal or other species. Chimeric polypeptides may include, e.g., one or more linkers joining the different subsequences. Thus, the subsequences may be joined directly or they may be joined indirectly, via linkers, or both, within a single chimeric polypeptide. Chimeric polypeptides may include, e.g., 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.

[0054] In some embodiments, the chimeric polypeptide comprises a Factor VIII portion and a non-Factor VIII portion. Exemplary non-Factor VIII portions include, e.g., Fc, XTEN, and albumin. Exemplary chimeric polypeptides of the invention include, e.g., chimeric Factor VIII-Fc polypeptides, chimeric Factor VIII-XTEN polypeptides, and chimeric Factor VIII-albumin polypeptides.

[0055] Exemplary chimeric Factor VIII-Fc polypeptides include, e.g., SEQ ID NOs:2, 6, 8, 10, and 12 (Table 2), with or without their signal sequences and the chimeric Fc polypeptide of SEQ ID NO:4 (Table 2).

[0056] The chimeric polypeptide may comprise a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2). The chimeric polypeptide may comprise a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

[0057] As discussed above, exemplary chimeric polypeptides include Factor VIII 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 VIII can be fused to either the N-terminal end of the XTEN polypeptide or to the C-terminal end of the XTEN polypeptide, provided the Factor VIII component of the Factor VIII-XTEN fusion protein can be processed by a protease to yield a processed Factor VIII containing polypeptide.

A protease site may be included between the XTEN portion and the Factor VIII portion to allow such processing. XTEN polypeptides include, e.g., 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.

[0058] As discussed above, exemplary chimeric polypeptides also include Factor VIII fused to one or more albumin polypeptides. Preferably the albumin is human albumin. Factor VIII can be fused to either the N-terminal end of the albumin or to the C-terminal end of the albumin, provided the Factor VIII component of the Factor VIII-albumin fusion protein can be processed by an enzymatically-active proprotein convertase to yield a processed Factor VIII-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.

[0059] In some embodiments, a chimeric polypeptide comprising a Factor VIII portion has an increased half-life ($t_{1/2}$) over a polypeptide consisting of the same Factor VIII portion without the non Factor VIII portion. A chimeric Factor VIII polypeptide with an increased $t_{1/2}$ may be referred to herein as a long-acting Factor VIII. Long-acting chimeric Factor VIII polypeptides include, e.g., Factor VIII fused to Fc (including, e.g., chimeric Factor VIII polypeptides in the form of a hybrid such as a FVIII-Fc monomer dimer hybrid; see Example 1, Fig. 1, and Table 2A; and US Patent Nos. 7,404,956 and 7,348,004), Factor VIII fused to XTEN, and Factor VIII fused to albumin.

[0060] "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.

[0061] "Factor VIII," as used herein, means functional factor VIII polypeptide in its normal role in coagulation, unless otherwise specified. Thus, the term Factor VIII includes variant polypeptides that are functional. Preferred factor VIII proteins are the human, porcine, canine, and murine factor VIII proteins. As described in the Background Art section, the full length polypeptide and polynucleotide sequences are known, as are many functional fragments, mutants and modified versions. Examples of human factor VIII sequences are shown as subsequences in SEQ ID NOs:2, 6, 8, 10, and 12 (Table 2). Factor VIII polypeptides include, e.g., full-length factor VIII, full-length factor VIII

minus Met at the N-terminus, mature factor VIII (minus the signal sequence), mature factor VIII with an additional Met at the N-terminus, and/or factor VIII with a full or partial deletion of the B domain. Preferred Factor VIII variants include B domain deletions, whether partial or full deletions.

[0062] A great many functional factor VIII variants are known, as is discussed above and below. In addition, hundreds of nonfunctional mutations in factor VIII have been identified in hemophilia patients, and it has been determined that the effect of these mutations on factor VIII function is due more to where they lie within the 3-dimensional structure of factor VIII than on the nature of the substitution (Cutler et al., Hum. Mutat. 19:274-8 (2002)), incorporated herein by reference in its entirety. In addition, comparisons between factor VIII from humans and other species has identified conserved residues that are likely to be required for function (Cameron et al., Thromb. Haemost. 79:317-22 (1998); US 6,251,632), incorporated herein by reference in its entirety.

[0063] The human factor VIII gene was isolated and expressed in mammalian cells (Toole, J. J., et al., Nature 312:342-347 (1984); Gitschier, J., et al., Nature 312:326-330 (1984); Wood, W. I., et al., Nature 312:330-337 (1984); Vehar, G. A., et al., Nature 312:337-342 (1984); WO 87/04187; WO 88/08035; WO 88/03558; U.S. Pat. No. 4,757,006), each of which is incorporated herein by reference in its entirety, and the amino acid sequence was deduced from cDNA. Capon et al., U.S. Pat. No. 4,965,199, incorporated herein by reference in its entirety, disclose a recombinant DNA method for producing factor VIII in mammalian host cells and purification of human factor VIII. Human factor VIII expression in CHO (Chinese hamster ovary) cells and BHKC (baby hamster kidney cells) has been reported. Human factor VIII has been modified to delete part or all of the B domain (U.S. Pat. Nos. 4,994,371 and 4,868,112, each of which is incorporated herein by reference in its entirety), and replacement of the human factor VIII B domain with the human factor V B domain has been performed (U.S. Pat. No. 5,004,803, incorporated herein by reference in its entirety). The cDNA sequence encoding human factor VIII and predicted amino acid sequence are shown in SEQ ID NOs:1 and 2, respectively, of US Application Publ. No. 2005/0100990, incorporated herein by reference in its entirety.

[0064] U.S. Pat. No. 5,859,204, Lollar, J. S., incorporated herein by reference in its entirety, reports functional mutants of factor VIII having reduced antigenicity and

reduced immunoreactivity. U.S. Pat. No. 6,376,463, Lollar, J. S., incorporated herein by reference in its entirety, also reports mutants of factor VIII having reduced immunoreactivity. US Application Publ. No. 2005/0100990, Saenko et al., incorporated herein by reference in its entirety, reports functional mutations in the A2 domain of factor VIII.

[0065] A number of functional factor VIII molecules, including B-domain deletions, are disclosed in the following patents US 6,316,226 and US 6,346,513, both assigned to Baxter; US 7,041,635 assigned to In2Gen; US 5,789,203, US 6,060,447, US 5,595,886, and US 6,228,620 assigned to Chiron; US 5,972,885 and US 6,048,720 assigned to Biovitrum, US 5,543,502 and US 5,610,278 assigned to Novo Nordisk; US 5,171,844 assigned to Immuno Ag; US 5,112,950 assigned to Transgene S.A.; US 4,868,112 assigned to Genetics Institute, each of which is incorporated herein by reference in its entirety.

[0066] The porcine factor VIII sequence is published, (Toole, J. J., et al., Proc. Natl. Acad. Sci. USA 83:5939-5942 (1986)), incorporated herein by reference in its entirety, and the complete porcine cDNA sequence obtained from PCR amplification of factor VIII sequences from a pig spleen cDNA library has been reported (Healey, J. F., et al., Blood 88:4209-4214 (1996), incorporated herein by reference in its entirety). Hybrid human/porcine factor VIII having substitutions of all domains, all subunits, and specific amino acid sequences were disclosed in U.S. Pat. No. 5,364,771 by Lollar and Runge, and in WO 93/20093, incorporated herein by reference in its entirety. More recently, the nucleotide and corresponding amino acid sequences of the A1 and A2 domains of porcine factor VIII and a chimeric factor VIII with porcine A1 and/or A2 domains substituted for the corresponding human domains were reported in WO 94/11503, incorporated herein by reference in its entirety. U.S. Pat. No. 5,859,204, Lollar, J. S., also discloses the porcine cDNA and deduced amino acid sequences. 6,458,563, incorporated herein by reference in its entirety assigned to Emory discloses a B-domain deleted porcine Factor VIII.

[0067] The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a

chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

[0068] The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12). The Factor VIII (or Factor VIII portion of a chimeric polypeptide) may be identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

[0069] "Equivalent amount," as used herein, means the same amount of Factor VIII activity as expressed in International Units, which is independent of molecular weight of the polypeptide in question. One International Unit (IU) of factor VIII activity corresponds approximately to the quantity of factor VIII in one milliliter of normal human plasma. Several assays are available for measuring Factor VIII activity, including the European Pharmacopoeia chromogenic substrate assay and a one stage clotting assay.

[0070] "Fc," 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 Fc includes any variants of IgG Fc that are functional. 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. The FcRn binding partners include, e.g., 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 Fc may comprise the CH2 and CH3 domains of an immunoglobulin with or without the hinge region of the immunoglobulin. Exemplary Fc variants are provided in WO 2004/101740 and WO 2006/074199, incorporated herein by reference in its entirety.

[0071] Fc (or Fc portion of a chimeric polypeptide) may contain one or more mutations, and combinations of mutations.

[0072] Fc (or Fc portion of a chimeric polypeptide) may contain mutations conferring increased half-life such as M252Y, S254T, T256E, and combinations thereof, as disclosed in Oganessian 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 US 2009/0264627 A1, which is incorporated herein by reference in its entirety; and the mutants disclosed at page 2, paragraphs [0014] to [0021] of US 20090163699 A1, which is incorporated herein by reference in its entirety.

[0073] Fc (or Fc portion of a chimeric polypeptide) may also include, e.g., 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, e.g., 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 (Fcγ1) 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 wildtype proline substituted by alanine at position number 238. In addition to alanine other amino acids may be substituted for the wildtype 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 FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA, 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, e.g., T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591, which is incorporated herein by reference in its entirety).

[0074] The Fc (or Fc portion of a chimeric polypeptide) may be at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12). The Fc (or Fc portion of a chimeric polypeptide) may be identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

[0075] "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 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 disulfide or other covalent bond(s). Hybrids are described in WO 2004/101740 and WO 2006/074199, each of which is incorporated herein by reference in its entirety. See also US Patent Nos. 7,404,956 and 7,348,004, 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. See, e.g., Figure 1, Example 1, and Table 2. In preferred embodiments, the second polypeptide is a polypeptide comprising an Fc. In preferred embodiments, the chimeric polypeptide is a chimeric Factor VIII-Fc polypeptide and the second polypeptide consists essentially of Fc, e.g., the hybrid polypeptide of Example 1, which is a rFVIII-Fc recombinant fusion protein consisting of a single molecule of recombinant B-domain deleted human FVIII (BDD-rFVIII) fused to the dimeric Fc domain of the human IgG1, with no intervening linker sequence. This hybrid polypeptide is referred to herein as FVIII-Fc monomeric Fc fusion protein, FVIII-Fc monomer hybrid, monomeric FVIII-Fc hybrid, and FVIII-Fc

monomer-dimer. See Example 1, Fig. 1, and Table 2A. The Examples provide preclinical and clinical data for this hybrid polypeptide.

[0076] The second polypeptide in a hybrid may comprise or consist essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4). The second polypeptide may comprise or consist essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

[0077] Figure 1 is a schematic showing the structure of a B domain deleted factor VIII-Fc chimeric polypeptide, and its association with a second polypeptide that is an Fc polypeptide. To obtain this hybrid, the coding sequence of human recombinant B-domain deleted FVIII was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human liver poly A RNA (Clontech) using FVIII-specific primers. The FVIII sequence includes the native signal sequence for FVIII. The B-domain deletion was from serine 743 (S743; 2287 bp) to glutamine 1638 (Q1638; 4969 bp) for a total deletion of 2682 bp. Then, the coding sequence for human recombinant Fc was obtained by RT-PCR from a human leukocyte cDNA library (Clontech) using Fc specific primers. Primers were designed such that the B-domain deleted FVIII sequence was fused directly to the N-terminus of the Fc sequence with no intervening linker. The FVIII-Fc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter. A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, EF1 α , in the expression vector pBUDCE4.1.

[0078] The rFVIII-Fc expression vector was transfected into human embryonic kidney 293 cells (HEK293H; Invitrogen) using Lipofectamine 2000 transfection reagent (Invitrogen). Stable clonal cell lines were generated by selection with Zeocin (Invitrogen). One clonal cell line, 3C4-22 was used to generate FVIII-Fc for characterization in vivo. Recombinant FVIII-Fc was produced and purified (McCue et al. 2009) at Biogen Idec (Cambridge, MA). The transfection strategy described above was

expected to yield three products, i.e., monomeric rFVIII_h hybrids, dimeric rFVIII_h hybrids and dimeric Fc. However, there was essentially no dimeric rFVIII_h detected in the conditioned medium from these cells. Rather, the conditioned medium contained Fc and monomeric rFVIII_h. It is possible that the size of dimeric rFVIII_h was too great and prevented efficient secretion from the cell. This result was beneficial since it rendered the purification of the monomer less complicated than if all three proteins had been present. The material used in these studies had a specific activity of approximately 9000 IU/mg.

[0079] "Dosing interval," as used herein, means the amount of time that elapses between multiple doses being administered to a subject. The comparison of dosing interval may be carried out in a single subject or in a population of subjects and then the average obtained in the population may be calculated.

[0080] The dosing interval when administering a chimeric Factor VIII polypeptide, e.g., a chimeric Factor VIII-Fc polypeptide (a polypeptide comprising a Factor VIII 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 VIII without the non-Factor VIII portion, e.g., without the Fc portion (a polypeptide consisting of said Factor VIII). The dosing interval may be at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion, e.g., without the Fc portion (a polypeptide consisting of said Factor VIII). The dosing interval may be at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of said Factor VIII without the non-Factor VIII portion, e.g., without the Fc portion (a polypeptide consisting of said Factor VIII).. The dosing interval may be about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer. The dosing interval may be at least about one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer. For on-demand treatment, the dosing interval of said chimeric polypeptide or hybrid is about once every 24-36, 24-48, 24-72, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,

48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 hours or longer.

[0081] Preferably, the effective dose is 25-65 IU/kg (25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 62, 64, or 65 IU/kg) and the dosing interval is once every 3-5, 3-6, 3-7, 3, 4, 5, 6, 7, or 8 or more days, or three times per week, or no more than three times per week. Preferably, the effective dose is 65 IU/kg and the dosing interval is once weekly, or once every 6-7 days.

[0082] "Long-acting Factor VIII" is a Factor VIII having an increased half-life (also referred to herein as $t_{1/2}$, $t_{1/2}$ beta, elimination half-life and HL) over a reference Factor VIII. The increased half-life of a long-acting Factor VIII may be due to fusion to one or more non-Factor VIII polypeptides such as, e.g., Fc, XTEN or albumin. The increased half-life may be due to one or more modification, such as, e.g., pegylation. Exemplary long-acting Factor VIII polypeptides include, e.g., chimeric Factor VIII polypeptides comprising Fc, chimeric Factor VIII polypeptides comprising XTEN and chimeric Factor VIII polypeptides comprising albumin. Additional exemplary long-acting Factor VIII polypeptides include, e.g., pegylated Factor VIII.

[0083] The "reference" polypeptide, in the case of a long-acting chimeric Factor VIII polypeptide, is a polypeptide consisting essentially of the Factor VIII portion of the chimeric polypeptide, e.g., the same Factor VIII portion without the Fc portion, without the XTEN portion, or without the albumin portion. Likewise, the reference polypeptide in the case of a modified Factor VIII is the same Factor VIII without the modification, e.g., a Factor VIII without the pegylation.

[0084] In some embodiments, the long-acting Factor VIII has one or more of the following properties when administered to a subject:

- a mean residence time (MRT) (activity) in said subject of about 14–41.3 hours;
- a clearance (CL) (activity) in said subject of about 1.22-5.19 mL/hour/kg or less;
- a $t_{1/2}$ beta (activity) in said subject of about 11-26.4 hours;
- an incremental recovery (K value) (activity; observed) in said subject of about 1.38-2.88 IU/dL per IU/kg;
- a V_{ss} (activity) in said subject of about 37.7-79.4 mL/kg; and
- an AUC/dose in said subject of about 19.2-81.7 IU*h/dL per IU/kg.

[0085] In some embodiments, the long-acting Factor VIII has one or more of the following properties when administered to a patient population:

a mean incremental recovery (K-Value) (activity; observed) greater than 1.38 IU/dL per IU/kg;

a mean incremental recovery (K-Value) (activity; observed) of at least about 1.5, at least about 1.85, or at least about 2.46 IU/dL per IU/kg.

a mean clearance (CL) (activity) in said patient population of about 2.33 ± 1.08 mL/hour/kg or less;

a mean clearance (CL) (activity) in said patient population of about 1.8-2.69 mL/hour/kg;

a mean clearance (CL) (activity) in said patient population that is about 65% of the clearance of a polypeptide comprising said Factor VIII without modification;

a mean mean residence time (MRT) (activity) in said patient population of at least about 26.3 ± 8.33 hours;

a mean MRT (activity) in said patient population of about 25.9 - 26.5 hours;

a mean MRT (activity) in said patient population that is about 1.5 fold longer than the mean MRT of a polypeptide comprising said Factor VIII without modification;

a mean $t_{1/2\beta}$ (activity) in said patient population of about 18.3 ± 5.79 hours;

a mean $t_{1/2\beta}$ (activity) in said patient population that is about 18 - 18.4 hours;

a mean $t_{1/2\beta}$ (activity) in said patient population that is about 1.5 fold longer than the mean $t_{1/2\beta}$ of a polypeptide comprising said Factor VIII without modification;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 2.01 ± 0.44 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population of about 1.85 - 2.46 IU/dL per IU/kg;

a mean incremental recovery (K value) (activity; observed) in said patient population that is about 90 % of the mean incremental recovery of a polypeptide comprising said Factor VIII without modification;

a mean V_{ss} (activity) in said patient population of about 55.1 ± 12.3 mL/kg;

a mean V_{ss} (activity) in said patient population of about 45.3 - 56.1 mL/kg;

a mean AUC/dose (activity) in said patient population of about 49.9 ± 18.2 IU*h/dL per IU/kg;

a mean AUC/dose (activity) in said patient population of about 44.8 - 57.6 IU*h/dL per IU/kg.

[0086] "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 need such as planned surgery. Conditions that may require on-demand treatment include, e.g., 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 iliopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, e.g., 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.

[0087] Preferably, on-demand treatment resolves greater than 80% (greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or 100%) or 80-100%, 80-90%, 85-90%, 90-100%, 90-95%, or 95-100% of bleeds (e.g., spontaneous bleeds) in a single dose. Preferably, greater than 80% (greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or 100%) or 80-100%, 80-90%, 85-90%, 90-100%, 90-95%, or 95-100% of bleeding episodes are rated excellent or good by physicians after on-demand treatment. Preferably, greater than 5%, (greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 11%, greater than 12%, greater than 13%, greater than 14%, greater than 15%, greater than 16%, greater than 17%, greater than 18%, greater than 19%, greater than 20%), or 5-20%, 5-15%, 5-10%, 10-20%, or 10-15% of bleeding episodes are rated as fair by physicians after on-demand treatment.

[0088] "Polypeptide," "peptide" and "protein" are used interchangeably and refer to a polymeric compound comprised of covalently linked amino acid residues.

[0089] "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, e.g., those in Table 1, which encode the polypeptides of Table 2 (see Table 1). Polynucleotides also include, e.g., fragments of the polynucleotides of Table 1, e.g., those that encode fragments of the polypeptides of Table 2, such as the Factor VIII, Fc, signal sequence, 6His and other fragments of the polypeptides of Table 2.

[0090] "Prophylactic treatment," as used herein, means administering a Factor VIII polypeptide in multiple doses to a subject over a course of time to increase the level of Factor VIII activity in a subject's plasma. Preferably, the increased level is sufficient to decrease the incidence of spontaneous bleeding or to prevent bleeding, e.g., in the event of an unforeseen injury. Preferably, during prophylactic treatment, the plasma protein level in the subject does not fall below the baseline level for that subject, or below the level of Factor VIII that characterizes severe hemophilia (<1 IU/dl [1%]).

[0091] Preferably, the prophylaxis regimen is "tailored" to the individual patient, preferably by determining PK data for each patient and administering Factor VIII of the invention at a dosing interval that maintains a trough level of 1-3% FVIII activity. Adjustments may be made when a subject experiences unacceptable bleeding episodes defined as ≥ 2 spontaneous bleeding episodes over a rolling two-month period. In this case, adjustment will target trough levels of 3-5%. Preferably, prophylactic treatment results in prevention and control of bleeding, sustained control of bleeding, sustained protection from bleeding, and/or sustained benefit. Prophylaxis, e.g., sustained protection can be demonstrated by an increased AUC to last measured time point (AUC-LAST) and reduced clearance, resulting in increased terminal $t_{1/2}$ compared to short acting FVIII. Preferably, prophylaxis is demonstrated by better C_{max} , better T_{max} , and/or greater mean residence time versus short-acting FVIII. Preferably, prophylaxis results in no spontaneous bleeding episodes within about 24, 36, 48, 72, or 96 hours (e.g., 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 96, 87, 88, 89, 90, 91, 92, 93, 94, 95, or 96 hours, preferably within 72 hours), after injection (e.g., the last injection). Preferably, prophylaxis results in greater than 30% (e.g., greater than 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 96,

87, 88, 89, or 90%, preferably greater than 50%), mean reduction in annualized bleeding episodes with once weekly dosing (e.g., at 65 IU/kg).

[0092] "Subject," as used herein means a human or a non-human mammal. Non-human mammals include, e.g., mice, dogs, primates, monkeys, cats, horses, cows, pigs, and other domestic animals and small animals.

[0093] "Therapeutic dose," as used herein, means a dose that achieves a therapeutic goal, as described herein. The calculation of the required dosage of factor VIII is based upon the empirical finding that, on average, 1 IU of factor VIII per kg body weight raises the plasma factor VIII activity by approximately 2 IU/dL. The required dosage is determined using the following formula:

Required units = body weight (kg) x desired factor VIII rise (IU/dL or % of normal) x 0.5 (IU/kg per IU/dL)

[0094] The therapeutic doses that may be used in the methods of the invention are about 10-100 IU/kg, more specifically, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg, and more specifically, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.

[0095] Additional therapeutic doses that may be used in the methods of the invention are about 10 to about 150 IU/kg, more specifically, about 100-110, 110-120, 120-130, 130-140, 140-150 IU/kg, and more specifically, about 110, 115, 120, 125, 130, 135, 140, 145, or 150 IU/kg.

[0096] "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 VIII 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, e.g., polypeptide and polynucleotide fragments, deletions, insertions, and modified versions of original polypeptides.

[0097] 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, 3, 5, 7, 9, or 11 (the factor VIII portion, the Fc portion, individually or together) or the complementary strand thereto, the nucleotide coding sequence of known mutant and recombinant factor VIII 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, 4, 6, 8, 10, or 12 (the factor VIII 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.

[0098] 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, 4, 6, 8, 10, or 12 (the factor VIII portion, the Fc portion, individually or together), and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

[0099] 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.

[00100] 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.

[00101] 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.

[00102] 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.

[00103] 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.

[00104] 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 VIII portion, the Fc portion, individually or together) or 4, or a known factor VIII 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.

[00105] 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.

[00106] 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 be made for the purposes of the present invention.

[00107] 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*).

[00108] 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.

[00109] 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.)

[00110] 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.

[00111] As stated above, polypeptide variants include, e.g., modified polypeptides. Modifications include, e.g., 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 phosphatidylinositol, 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 (Mei et al., Blood 116:270-79 (2010), which is incorporated herein by reference in its entirety), proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. In some embodiments, Factor VIII is modified, e.g., pegylated, at any convenient location. In some embodiments, Factor VIII is pegylated at a surface exposed amino acid of Factor VIII, preferably a surface exposed cysteine, which may be an engineered cysteine. Mei et al. (2010). In some embodiments, modified Factor VIII, e.g., pegylated Factor VIII, is a long-acting Factor VIII.

[00112] "Volume of distribution at steady state (Vss)," as used herein, has the same meaning as the term used in pharmacology, which is the apparent space (volume) into which a drug distributes. $V_{ss} = \text{the amount of drug in the body divided by the plasma concentration at steady state}$.

[00113] "About," as used herein for a range, modifies both ends of the range. Thus, "about 10-20" means "about 10 to about 20."

[00114] 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

Abstract

[00115] A recombinant B-domain-deleted factor VIII-Fc (rFVIII-Fc) fusion protein was created to extend the half-life of FVIII. rFVIII-Fc was studied in mouse and dog models of severe hemophilia A and compared to rFVIII (ReFacto®). Whole blood clotting time (WBCT) in hemophilia A mice was corrected for approximately two to three times longer and the elimination half-life in plasma was nearly twice as long for rFVIII-Fc compared to ReFacto®. In hemophilia A dogs, an intravenous dose of rFVIII-Fc (125 IU/kg) corrected the WBCT to normal. The WBCT remained below 20 min, the time consistent with FVIII:C > 1%, through approximately 96 hr, compared to 48 hr for dogs treated with ReFacto®. The elimination half-life of rFVIII-Fc in dog plasma, when measured using ELISA or chromogenic activity assays, was 15.7 ± 1.7 hr and 15.4 ± 0.3 hr, respectively. ReFacto® corrected WBCT for approximately one half as long as rFVIII-Fc and the plasma half-life was 7.0 hr. Thus, fusion of FVIII to Fc produced a molecule with an increased plasma half-life and the ability to provide prolonged protection from bleeding.

Introduction

[00116] Reduced mortality, prevention of joint damage and improved quality of life have been important achievements due to the development of plasma-derived and recombinant FVIII. Prolonged protection from bleeding would represent another key advancement in the treatment of hemophilia A patients. The inventors have created a recombinant factor VIII-Fc (rFVIII-Fc) chimeric protein and hybrid as an approach to extend the half-life of FVIII.

[00117] rFVIII-Fc is a heterodimeric hybrid protein comprised of B-domain-deleted FVIII fused recombinantly to the Fc domain of human immunoglobulin G1 (IgG1) (Fig. 1, SEQ ID NO:2; Table 2A) (This protein is also referred to herein as FVIII-Fc monomeric Fc fusion protein, FVIII-Fc monomer hybrid, monomeric FVIII-Fc hybrid, and FVIII-Fc monomer-dimer.). The Fc enables binding to the neonatal Fc receptor (FcRn), which is responsible for protection of IgG from degradation and confers on IgG the three week half-life observed in humans (Ghetie V, and Ward ES., *Annu. Rev. Immunol.* 2000;18:739-766; Roopenian DC, and Akilesh S., *Nature Rev. Immunol.* 2007;7:715-725, each of which is incorporated herein by reference in its entirety).

[00118] The Fc domain of IgG1 has been fused to growth factors, cytokines, enzymes and ligand-binding regions of receptors (Ashkanazi A, et al., *Int. Rev. Immunol.* 1993:10:219-27; Chamow SM, and Ashkanazi A, *Trends Biotechnol.* 1996:14:52-60; Fisher et al., *N. Engl. J. Med.* 1996:334(26):1697-702, each of which is incorporated herein by reference in its entirety). Several of these have become important therapeutic molecules (e.g. etanercept, alefacept, abatacept). In these fusion proteins, two effector molecules are connected to two Fc molecules. In this example, rFVIII^h has been constructed as a monomeric Fc fusion protein (one copy of a polypeptide consisting of the sequence in Table 2A(i) (SEQ ID NO:2) with or without the signal sequence and one copy of a polypeptide consisting of the sequence in Table 2A(ii) (SEQ ID NO:4) with or without the signal sequence), i.e., with only one copy of the effector molecule (see Figure 1), and the studies presented herein compare the pharmacodynamics and pharmacokinetics of this novel protein to rFVIII in mouse and dog models of hemophilia A. The signal sequence is cleavage during secretion. This protein construct is referred to herein as FVIII^h monomeric Fc fusion protein, FVIII^h monomer hybrid, monomeric FVIII^h hybrid, and FVIII^h monomer-dimer. See Example 1, Fig. 1, Table 2A; and US Patent Nos. 7,404,956 and 7,348,004, each of which is incorporated herein by reference in its entirety, for the structure and production of this protein.

Methods and Materials

FVIII Preparations

Recombinant FVIII^h

[00119] The coding sequence of human recombinant B-domain deleted FVIII was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human liver poly A RNA (Clontech) using FVIII-specific primers. The FVIII sequence includes the native signal sequence for FVIII. The B-domain deletion was from serine 743 (S743; 2287 bp) to glutamine 1638 (Q1638; 4969 bp) for a total deletion of 2682 bp See Example 1, Fig. 1, Table 2A; and US Patent Nos. 7,404,956 and 7,348,004, each of which is incorporated herein by reference in its entirety, for the structure and production of this protein.

[00120] The coding sequence for human recombinant Fc was obtained by RT-PCR from a human leukocyte cDNA library (Clontech) using Fc specific primers. Primers were

designed such that the B-domain deleted FVIII sequence was fused directly to the N-terminus of the Fc sequence with no intervening linker. The FVIII_h DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter. A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, EF1 α , in the expression vector pBUDCE4.1.

[00121] The rFVIII_h expression vector was transfected into human embryonic kidney 293 cells (HEK293H; Invitrogen) using Lipofectamine 2000 transfection reagent (Invitrogen). Stable clonal cell lines were generated by selection with Zeocin (Invitrogen). One clonal cell line, 3C4-22 was used to generate FVIII_h for characterization *in vivo*. Recombinant FVIII_h was produced and purified (McCue JT, et al., J. Chromatogr. A 2009;7824-7830, incorporated by reference herein in its entirety) at Biogen Idec (Cambridge, MA). The transfection strategy described above was expected to yield three products, i.e., monomeric rFVIII_h hybrid, dimeric rFVIII_h hybrid and dimeric Fc. However, there was essentially no dimeric rFVIII_h detected in the conditioned medium from these cells. Rather, the conditioned medium contained Fc and monomeric rFVIII_h. It is possible that the size of dimeric rFVIII_h was too great and prevented efficient secretion from the cell. This result was beneficial since it rendered the purification of the monomer less complicated than if all three proteins had been present. The material used in these studies had a specific activity of approximately 9000 IU/mg. In addition, these human cells produced higher protein level than other cells that were attempted in this experiment.

Recombinant FVIII

[00122] Recombinant B-domain deleted FVIII (ReFacto[®]) was purchased from Novis Pharmaceuticals and was prepared according to manufacturer's instructions. ReFacto[®] (recombinant B-domain deleted FVIII) has the same amino acid sequence as amino acids 1 to 1438 of SEQ ID NO:2.

Hemophilia A animals

[00123] The hemophilia A mice are FVIII exon 16 knockouts on a 129 x B6 background that were obtained from Dr. Kazazian at the University of Pennsylvania (Bi L, et al., Nat. Genet. 1995;10(1):119-121, incorporated by reference herein in its entirety) and bred at

Syntonix. These mice exhibit prolonged whole blood clotting times (>60 min), and are thus a good model of severe hemophilia A.

[00124] Hemophilia A dogs were from the in-bred colony maintained at the Francis Owen Blood Research Laboratory at the University of North Carolina, Chapel Hill (Graham, JB, et al., J. Exp. Med. 1949;90:97-111, incorporated by reference herein in its entirety). These dogs have a severe hemophilic phenotype comparable to the severe form of the human disease (Graham, JB, et al., J. Exp. Med. 1949;90:97-111; Lozier, JN, et al., Proc. Natl. Acad. Sci. 2002;99:12991-12996, each of which is incorporated by reference herein in its entirety).

Study Designs

Hemophilia A Mouse Studies

[00125] The effect of rFVIII[®] and ReFacto[®] on whole blood clotting time (WBCT) was studied in FVIII-deficient mice. Each protein was administered intravenously at 50 IU/kg and blood was collected from the tail vein of each mouse pre-dose and various time points post-dosing. The blood samples were incubated in microtubes at 37°C and visually inspected once per minute for the presence of a clot. Time of clot formation was recorded. If no clot formed by 60 min, the clotting time was recorded as >60min. Blood from normal mice clots in approximately 4 min (range 2-7 min, n = 10 mice) in the WBCT assay.

[00126] In a second set of studies, hemophilia A mice were administered a single intravenous dose of 50 IU/kg rFVIII[®], ReFacto[®] or Advate[®] (4 mice per time point). Blood was collected by cardiac puncture in one tenth volume 3.2% sodium citrate at 0.25, 8, 24, 48 and 72 hr after dosing. Plasma was prepared and stored at -80°C until analysis for FVIII activity using a FVIII-specific chromogenic activity assay.

Hemophilia A Dog Studies

[00127] In a single dose PK/PD study of rFVIII[®], two hemophilia A dogs from the Chapel Hill colony were administered a single intravenous dose of 125 IU/kg and blood samples were collected pre-dose and after dosing at selected time points for WBCT, activated partial thromboplastin time (aPTT), FVIII[®] plasma concentration, hematology and serum chemistry. Time points for WBCT included pre-dose, 5 and 30 min and 1, 2,

4, 8, 24, 32, 48, 72, 96, 144, and 168 hr after dosing. Blood collections for clotting activity (aPTT) and FVIIIc plasma concentration included the time points listed above for WBCT as well as 15 min and 3, 6, 12 hours after dosing.

[00128] A second study was conducted in which ReFacto[®] (114 IU/kg for dog M12 and 120 IU/kg for dog M38) was administered intravenously. WBCT was measured until clotting times were ≥ 20 min (consistent with FVIII:C $> 1\%$), and then 125 IU/kg rFVIIIc was administered intravenously to the same dogs and blood samples were collected for WBCT, aPTT, FVIIIc plasma concentration, hematology and serum chemistry. Time points for WBCT included pre-dose, 5 and 30 min and 1, 2, 4, 8, 24, 32, 48, 72 hr after dosing. Blood was also collected at 96, 120, 144, and 168 hr after dosing with FVIIIc. Blood collections for clotting activity and FVIIIc plasma concentration included the time points listed above for WBCT as well as 15 min and 3, 6, 12 hours after dosing.

[00129] The WBCT procedure in hemophilia A dogs was slightly different than that in the hemophilia A mice. After dosing with rFVIIIc or ReFacto[®], one mL of blood was collected at various time points and 0.5 mL was distributed into two siliconized glass tubes which were subsequently placed into a 28°C water bath. Beginning at one minute, one tube was tilted every 30 sec, the second left undisturbed. When a clot formed in the tilted tube, the second tube was then tilted every 30 sec until a clot formed. The time for a fully gelled clot in the second tube was recorded as the WBCT.

FVIII activity in plasma

Measurement of FVIII activity in plasma by FVIII-specific chromogenic assay

[00130] Plasma samples were tested for FVIII activity by an automated chromogenic method using a Sysmex CA1500 instrument and reagents were from Siemens Healthcare Diagnostics (Dallas, TX, kit #B4238-40). Activity of rFVIIIc was determined using a standard curve created using the 7th International Standard Factor FVIII Concentrate (NIBSC code 99/678) spiked into human FVIII-depleted plasma (Stago USA) at concentrations ranging from 1.5 – 0.016 IU/mL.

Measurement of rFVIII^h or FVIII by ELISAFVIII^h in dog plasma by ELISA

[00131] A FVIII antibody specific to the A1 domain (Green Mountain Antibodies: GMA-8002) was coated on 96 well plates and incubated for 1 hr at 37°C. The coated plates were blocked with Tris-buffered saline containing Tween 20, CaCl₂ and bovine serum albumin for 1 hr at room temperature and then standards, controls and samples that were prepared in normal dog plasma, were diluted 1:10 and then added to the plates and incubated for 1 hour at 37°C. The plates were washed and then donkey (F(ab)₂) anti-human Fc-HRP (Jackson: 709-036-098) was added and incubated for 1 hr at 37°C. After washing, TMB (BioRx supersensitive substrate: TMBS-0100-01) was added to the plates, the substrate reaction was quenched with acid and absorbance was measured on a SpectraMax Plus plate reader (Molecular Devices) at 450 nm.

ReFacto® in dog plasma by ELISA

[00132] An anti-FVIII antibody specific to the A1 domain on the heavy chain (Green Mountain Antibodies: GMA-8002) was coated on 96 well plates and incubated for 2 hr at room temperature. The coated plates were blocked for 1 hr at 37 °C and after washing, the standards, controls and samples were prepared in normal dog plasma then diluted 1:10 were added to the plates and incubated for 2 hr at room temperature. The plates were washed then treated with the detection antibody, a pre-diluted anti-FVIII horse radish peroxidase conjugate (Affinity Biologicals: F8C-EIA-D), and incubated at room temperature for 1 hr. After washing TMB (BioRx supersensitive substrate: TMBS-0100-01) was added to the plates for 10 min. The substrate reaction was quenched with acid and the signal was measured on a SpectraMax Plus plate reader (Molecular Devices) at a wavelength of 450 nm.

Measurement of Fibrinogen

[00133] The concentration of fibrinogen in plasma was measured at Esoterix (Research Triangle Park, NC) using a kit that contains HemosIL™ PT-Fibrinogen-HS reagent (Instrumentation Laboratory, Lexington, MA, Catalog #0008468210) and an ACL 7000 Coagulation Analyzer (Beckman Coulter), according to the manufacturer's instructions.

Measurement of Platelets

[00134] Platelets were counted in EDTA anti-coagulated whole blood by automated methods using the Vet-ABC-Diff Hematology Analyzer programmed with a species specific smart card (SCIL Animal Care Co., Gurnee, IL).

Pharmacokinetic Analysis

[00135] The pharmacokinetic parameters were calculated by noncompartmental analysis using WinNonlin software from Pharsight, version 5.2 (Mountain View, Ca). PK parameters included the maximum concentration in plasma (C_{max}), area under the plasma concentration versus time curve (AUC), elimination half-life ($t_{1/2}$), volume of distribution (V_{ss}), and clearance (Cl).

Results

Recombinant FVIII-Fc

[00136] rFVIII-Fc is a recombinant fusion of human B-domain deleted FVIII with Fc from human IgG1, with no intervening linker sequence (rFVIII-Fc; Figure 1).

[00137] Purified rFVIII-Fc had a specific activity of approximately 9000 IU/mg as determined using a chromogenic activity assay. Recombinant B-domain deleted FVIII (ReFacto[®]) has a reported specific activity of 9110 – 13700 IU/mg. Conversion of specific activity into IU/nmol to take into account the size difference between FVIII-Fc and ReFacto[®] (216 kDa and 170 kDa respectively), indicates that the two proteins have approximately equivalent specific activities (1970 IU/nmol for rFVIII-Fc and 1521 – 2287 IU/nmol for ReFacto[®]). Thus the FVIII activity of rFVIII-Fc is not affected by fusion of the C-terminus of human FVIII to the N-terminus of human Fc.

Administration to Hemophilia A mice

[00138] A single 50 IU/kg dose of rFVIII-Fc or ReFacto[®] was administered intravenously to FVIII-deficient mice (n = 6/group). Blood samples were collected pre-dose and after dosing through 120 hr and WBCT determined as described in Materials and Methods. Baseline WBCT were greater than 60 min. Data from a representative experiment are shown in Figure 2 and Table 3. Immediately after dosing with either rFVIII-Fc or ReFacto[®], WBCT was corrected to 2-17 minutes. Blood from mice treated with

ReFacto[®] lost the ability to clot by 42 hr, whereas blood from all mice treated with rFVIII[®] still clotted at 96 hr, the blood from one of six was clotted at 113 hr, but all had lost the ability to clot by 120 hr. These data suggest that the duration of effect for rFVIII[®] is approximately two to three times longer than for ReFacto[®].

[00139] The chromogenic activity of rFVIII[®], ReFacto[®] or Advate[®] (full-length recombinant FVIII) was studied in the FVIII-deficient mice after a single intravenous dose of 50 IU/kg. Blood was collected pre-dose and after dosing at 8, 24, 48, and 72 hr. The activity was measured using a FVIII-specific chromogenic activity assay and is shown in Figure 3. The pharmacokinetic parameters are reported in Table 4. The circulating half-life for rFVIII[®] was approximately 1.6 to 2 fold longer (11.1 hr) compared to Advate[®] (7 hr) and ReFacto[®] (5 hr). The C_{max} was 1.6 ± 0.36 IU/mL for rFVIII[®] compared to 0.47 ± 0.30 IU/mL for Advate[®] and 0.67 ± 0.44 IU/mL for ReFacto[®]. The systemic exposure of rFVIII[®] was markedly greater for rFVIII[®] (22.6 hr·IU/mL) compared to ReFacto[®] (6.94 hr·IU/mL) and Advate[®] (3.90 hr·IU/mL) and clearance for rFVIII[®] was notably lower (2.09 mL/hr/kg) compared to both ReFacto[®] (7.2 mL/hr/kg) and Advate[®] (12.8 hr/mL/kg) in the hemophilia A mice.

Administration to Hemophilia A dogs

[00140] The pharmacodynamics (PD) and pharmacokinetics (PK) of rFVIII[®] were studied in the Chapel Hill colony of hemophilia A dogs. A single intravenous dose of 125 IU/kg rFVIII[®] was administered to each of four hemophilia A dogs and the WBCT was immediately corrected to normal (Figure 4). The range of WBCT in normal dogs is 8-12 min. The WBCT remained below 20 min, the time consistent with FVIII:C >1%, through approximately 96 hr with the exception of one dog that had WBCT <20 min through 72 hr. In addition, aPTT was also immediately corrected to normal (Table 6). The concentration of rFVIII[®] in plasma was measured using a specific ELISA which was designed to detect both the FVIII and Fc portions of the molecule. The plasma concentration versus time curves are shown in Figure 5. PK analysis of the data showed that the $t_{1/2}$ was 15.7 ± 1.7 hr (Table 5). Similar results were obtained when rFVIII[®] was measured using a FVIII-specific chromogenic activity assay ($t_{1/2} = 15.4 \pm 0.3$ hr, Table 5) and the plasma concentration versus time curves were similar using both methods (Figures 5 and 6). When the activity data were converted from IU/mL to ng/mL using the

specific activity for rFVIII[®]Fc, there was a good correlation with the ELISA data, thereby demonstrating that the protein that was measured by ELISA was fully active.

[00141] Two of the dogs treated with rFVIII[®]Fc also received a single dose of ReFacto[®], 114 IU/kg for dog M12 and 120 IU/kg for dog M38, 72 hr prior to dosing with rFVIII[®]Fc. WBCT and aPTT were corrected to normal immediately after dosing with ReFacto[®]. However, the WBCT normalization after the single dose of rFVIII[®]Fc lasted approximately twice as long compared to ReFacto[®] (Figure 4). Moreover, the plasma half-life of rFVIII[®]Fc (15.7 ± 1.7 hr) was approximately twice as long for rFVIII[®]Fc compared to ReFacto[®] (7.0 and 6.7 hr) when the concentration of the proteins in plasma were measured by ELISA (Table 5). Similar results were obtained when the two molecules were measured by FVIII-specific chromogenic activity.

[00142] To assess the potential risk of thrombogenicity, platelets and fibrinogen were measured. After dosing with either rFVIII[®]Fc or ReFacto[®], platelet numbers and plasma fibrinogen concentration did not change from pre-dose values (data not shown).

Discussion

[00143] Recombinant FVIII[®]Fc was produced in human embryonic kidney 293 (HEK 293) cells from a stably transfected cell line and was purified from cell culture medium. Production in a human cell line represents a significant change in manufacturing compared to currently marketed rFVIII products which are produced in either Chinese Hamster Ovary cells or Baby Hamster Kidney cells. The rationale for this change was that it was expected that the human cells were best equipped to perform the necessary post-translational modifications for the FVIII portion of this molecule.

[00144] Conversion of the specific activity to IU/nmol to take into account the difference in molecular weights for rFVIII[®]Fc and recombinant B-domain deleted FVIII (ReFacto[®]) indicated that the specific activities are similar for both proteins (1970 IU/nmol for rFVIII[®]Fc and 1521 – 2287 IU/nmol for ReFacto[®]). It is somewhat surprising that the specific activity for rFVIII[®]Fc is not affected by fusion of the C terminus of FVIII with the N-terminus of Fc since the C1 and C2 domain of FVIII are involved in phospholipid binding which is essential for full FVIII activity (Fay, PJ, J. Hematology 83:103-8 (2006) and Raut, S, et al., Br. J. Haematol. 107:323 (1999), each of which is incorporated by reference herein in its entirety).

[00145] Treatment of hemophilia A is on-demand at the time of a bleeding episode or by prophylaxis for the prevention of bleeding. Although on-demand treatment is still frequently used, there is a trend toward prophylaxis and the prevention of joint damage (Blanchette P, et al., Haemophilia 2004; 10:679-683, Manco-Johnson, MJ, et al., N. Engl. J. Med. 2007;357:535-544, each of which is incorporated by reference herein in its entirety). Current FVIII products are administered every two to three days for prophylaxis due to the relatively short half-life of 10-12 hr in order to maintain a FVIII:C above 1 % in patients (Morfini, M, Haemophilia 2003;9 (suppl 1):94-99;discussion 100, White GC, et al., Thromb. Haemost. 1997;77:660-7, Blanchette, P, et al., J. Thromb. Haemost. 2008 Aug;6(8):1319-26, each of which is incorporated by reference herein in its entirety). Longer-acting FVIII therapies that provide prolonged protection from bleeding would represent a marked improvement in the quality of life for patients with hemophilia A. Strategies to extend the half-life of clotting factors include those that have been successful for other molecules, including pegylation (Rostin J, et al., Bioconj. Chem. 2000;11:387-96, incorporated by reference herein in its entirety), glycopegylation (Stennicke HR, et al., Thromb. Haemost. 2008;100:920-8, incorporated by reference herein in its entirety), formulation with pegylated liposomes (Spira J, et al., Blood 2006;108:3668-3673, Pan J, et al., Blood 2009;114:2802-2811, each of which is incorporated by reference herein in its entirety) and conjugation with albumin (Schulte S., Thromb. Res. 2008;122 Suppl 4:S14-9, incorporated by reference herein in its entirety). Pegylation represents an approach to reduce clearance, however, the effect of the modification *in vivo* is currently unknown. The outcome of direct pegylation of FVIII on *in vivo* is currently unknown, whereas FVIII formulated with pegylated liposomes has been studied clinically and showed a modest to no effect on bleeding periods (Spira J, et al., Blood 2006;108:3668-3673, Spira J, et al., Thromb. Haemost. 2008 Sep;100(3):429-34, each of which is incorporated by reference herein in its entirety).

[00146] The present approach to extend the half-life of FVIII was to recombinantly fuse FVIII to the Fc domain of IgG1. Fc binds to the naturally occurring receptor, FcRn, of which the normal function is protection of IgG from degradation. The results described herein represent the initial pharmacokinetic and efficacy characterization of rFVIII-Fc compared to a rFVIII product in hemophilia A mice and hemophilia A dogs. In both species, the half-life of rFVIII-Fc was approximately twice that of rFVIII when measured

by FVIII activity or ELISA (dogs only). These data also correlated well with the WBCT results from both animal models, i.e. the duration of the effect of rFVIIIIFc on WBCT was approximately twice as long compared to ReFacto[®]. In dogs, the C_{max} and clearance were similar for rFVIIIIFc and ReFacto[®], but the AUC and volume of distribution at steady state were approximately 1.5 fold and 2 fold greater for rFVIIIIFc compared to ReFacto[®], respectively. The PK parameters for ReFacto[®] in this animal model are consistent with the values reported in the literature (Brinkhous K, et al., Sem. Thromb. Haemost. 2002;28:269-272, incorporated by reference herein in its entirety).

[00147] If these findings translate to the same extension of half-life in humans, this could represent a significant advancement in the treatment of patients with hemophilia A.

Additional References (each of which is incorporated herein by reference in its entirety)

- [00148] Berkner K., Methods Enzymol. 1993;222:450-477.
- [00149] Bitonti AJ, and Dumont JA., Adv. Drug Del. Rev. 2006;58:1106-1118.
- [00150] Dumont JA, et al., J. Aerosol Med. 2005;18:294-303.
- [00151] Dumont JA, et al., BioDrugs 2006;20:151-160.
- [00152] Ellis CN, and Krueger GG., N. Engl. J. Med. 2001;345:248-55.
- [00153] Low SC, et al., Hum Reprod. 2005;7:1805-1813.
- [00154] Manco-Johnson, M., Haemophilia 2007;13 Suppl;2: 4-9.
- [00155] Mannucci, PM, and Tuddenham, EGD., N. Engl. J. Med. 2001;344:1773-1779.
- [00156] Peyvandi F, et al., Haemophilia 2006;12(Suppl 3):82-89.
- [00157] Rodriguez-Merchan, EC., Semin. Thromb. Hemost. 2003;29:87-96.
- [00158] Srour MA, et al., Ann. Hematol. 2008; 87:107-12.

Example 2

[00159] The objective of the study was to determine the pharmacokinetics and pharmacodynamics of rFVIIIIFc and BDD-rFVIII (Xyntha[®]) in cynomolgus monkeys after a single intravenous dose.

Materials and Methods

[00160] rFVIII-Fc (Biogen Idec), supplied as a frozen liquid at a concentration of 1.2 mg/mL, and 9882 IU/mL. The specific activity is 8235 IU/mg. Storage was at - 70°C. It was diluted prior to injection.

[00161] Name: Xyntha (Novis Pharmaceuticals), Supplied as a lyophilized powder which was reconstituted according to the manufacturer's instructions to produce a solution with a nominal concentration of 525 IU/mL. Storage was according to the manufacturer's recommendations.

Animals

[00162] Cynomolgus monkeys from the New Iberia Research Center (NIRC) colony were used, and the study (NIRC Study # 8733-0903) was conducted under an approved NIRC IACUC protocol (APS 2008-8733-058) at NIRC in New Iberia, LA.

[00163] Six naïve cynomolgus monkeys (three males, three females) that were determined to be in good health were used in the study.

[00164] The study was performed in compliance with the protocol and UL Lafayette-NIRC Standard Operating Procedures.

Study Design

[00165] rFVIII-Fc was administered intravenously at 125 IU/kg to each of six monkeys (three males, three females). Xyntha (BDD-rFVIII) was administered intravenously to the same animals at 125 IU/kg in a crossover design. Group 1 animals (n = 3) received Xyntha on Day 0 and rFVIII-Fc on Day 3, while Group 2 animals (n = 3) received rFVIII-Fc on Day 0 followed by Xyntha on Day 4. The additional day between doses for group 2 was to ensure that the rFVIII-Fc had sufficient time to decrease below projected baseline levels. Blood was collected for plasma in one-tenth volume 3.2 % sodium citrate from each animal predose and after dosing at 0.25, 4, 12, 24, 36, 48 and 72 hr for measurement of rFVIII-Fc or Xyntha by ELISA and a FVIII-specific chromogenic activity assay.

ELISA to measure rFVIII_{IFc} and FVIII in plasmaMethod to Measure rFVIII_{IFc} in Monkey Plasma

[00166] This Enzyme Linked ImmunoSorbent Assay (ELISA) is designed to quantify rFVIII_{IFc} in monkey plasma. In this ELISA method, goat anti-human IgG-(H+L) antibody (monkey absorbed) from Bethyl Laboratories (Cat#A80-319A) is diluted in Coating Buffer and immobilized onto a 96-well microtiter sample plate. The plate is aspirated, and all un-adsorbed sites are blocked with the addition of Blocking Buffer (3% BSA/1xTris) for approximately 2 hours at 37°C. Plasma samples are diluted 1:20 with High Calcium Sample Dilution Buffer (3% Non-Fat Dry Milk/TBST with 30 mM CaCl₂) and dispensed onto the sample plate. Plates are incubated for approximately 2 hours at 37°C. The plate is subsequently washed and mouse anti-B domain-deleted (α .BDDA1) Factor VIII (A1 domain) antibody from Green Mountain Antibodies (Cat#GMA-8002) is added to the plate and incubated for approximately 1 hour at 37°C. After washing the plate, HRP-conjugated goat anti-mouse IgG2a antibody from Southern Biotech (Cat#1080-05) is added to the plate and incubated for approximately 30 minutes at room temperature. The plate is washed again and a tetramethylbenzidine (TMB) peroxidase substrate solution is added and incubated for approximately 30 minutes at room temperature. The reaction is stopped by addition of a non-acidic Stop Solution. Color develops in proportion to the amount of rFVIII_{IFc} in the sample. Plates are read on an absorbance plate reader using a single detection wavelength, 650 nm. rFVIII_{IFc} concentrations are determined on a standard curve obtained by plotting optical density (OD) versus concentration using a four-parameter logistic curve-fitting program. The calibration curve range of this method is 0.400 ng/mL – 51.2 ng/mL in 5% monkey plasma (8.00 ng/mL – 1024 ng/mL in 100% monkey plasma). One calibrator outside the qualified range of the assay at 0.200 ng/mL in 5% monkey plasma may be included to serve as an anchor point to facilitate curve-fitting. The anchor point is removed or retained based on the best fit of the curve (i.e., the highest number of standards read within defined accuracy, %RE).

Method to Measure FVIII in Monkey Plasma

[00167] This Enzyme Linked ImmunoSorbent Assay (ELISA) is designed to quantify FVIII in monkey plasma. In this ELISA method, mouse α BDDA1 FVIII antibody from

Green Mountain Antibodies (Cat# GMA-8002) is diluted in Coating Buffer and immobilized onto a 96-well microtiter sample plate. The plate is aspirated, and all unadsorbed sites are blocked with the addition of Blocking Buffer (3% BST/1xTris) for approximately 1 hour at 37°C. Plasma samples are diluted 1:20 with High Calcium Sample Dilution Buffer (Blocking Buffer with 100 mM CaCl₂) and dispensed onto the sample plate. Plates are incubated for approximately 2 hours at 37°C. After washing the plate, a Detecting Antibody from the Affinity Biologicals Kit, an HRP labeled polyclonal antibody (Cat#F8C-EIA-D), is further diluted in TBS/0.05% Tween 20, and added to the plate and incubated for approximately 1 hour at room temperature. The plate is washed again and a tetramethylbenzidine (TMB) peroxidase substrate solution is added and incubated for approximately 30 minutes at room temperature. The reaction is stopped by addition acidic Stop Solution. Color develops in proportion to the amount of FVIII_{FC} in the sample. Plates are read on an absorbance plate reader using a single detection wavelength, 450 nm. FVIII concentrations are determined on a standard curve obtained by plotting optical density (OD) versus concentration using a four-parameter logistic curve-fitting program. The calibration curve range of this method is 0.625 ng/mL – 20 ng/mL in 5% monkey plasma (12.5 ng/mL – 400 ng/mL in 100% monkey plasma). Two calibrators outside the qualified range of the assay at 0.313 and 0.156 ng/mL in 5% monkey plasma may be included to serve as anchor points to facilitate curve-fitting. The anchor points can be removed or retained based on the best fit of the curve (i.e., the highest number of standards read within defined accuracy, %RE).

FVIII-Specific Chromogenic Assay

[00168] FVIII activity in cynomolgus monkey plasma samples was estimated based on administered dose, and then diluted to approximately 0.25 – 1 IU/ml in human FVIII-depleted plasma (Diagnostica Stago). Samples were analyzed in a Sysmex CA1500 (Siemens Diagnostic Healthcare) using a FVIII chromogenic kit (Siemens). In this chromogenic assay, rFVIII_{FC} in the plasma samples is activated by thrombin. Activated Factor VIII (FVIII_a) then accelerates the conversion of Factor X (FX) to Factor X_a (FX_a) in the presence of activated Factor IX (FIX_a), phospholipids (PL) and calcium ions. The FX_a activity is assessed by hydrolysis of a p-nitroanilide substrate specific to FX_a. The initial rate of release of p-nitroaniline (pNA) measured at 405 nm is proportional to the FX_a activity, and thus to the FVIII activity in the sample. The limit of quantitation of

FVIII activity due to rFVIII-Fc in this assay is ~ 0.3 IU/ml. The assay can measure total FVIII activity down to a lower limit of approximately 0.06 IU/ml with an accuracy of $\pm 20\%$. The calculated activity of the pre-dose sample for individual animals was subtracted from the value at each time point to generate the PD curves (FVIII activity vs. time).

[00169] A standard curve was generated from the NIBSC 7th International Standard FVIII concentrate diluted to 1 IU/ml in human FVIII-deficient plasma. Standard curves were diluted serially in the Sysmex instrument to yield concentrations of 0.15, 0.1, 0.05, 0.025, 0.0053 and 0.0026 IU/ml. Since the instrument dilutes all samples 1:10 internally, the FVIII standard concentrations correspond to plasma concentrations of 1.5 – 0.026 IU/ml, which is the range of FVIII activities that can be measured.

PK analysis

[00170] The concentration time profiles were evaluated using the non-compartmental analysis module in the WinNonlin software program (Version 5.2, Pharsight Corporation, Mountain View, CA).

RESULTS

[00171] The concentration of rFVIII-Fc in monkey plasma was measured using a sandwich ELISA format that measured both the FVIII and Fc portions of the molecule and the data are reported in Table 7. All predose samples were below the limit of quantitation. Figure 7 illustrates the group mean rFVIII-Fc and Xyntha plasma concentrations over time and individual plasma concentration versus time curves are shown in Figure 8. A summary of the PK parameters for rFVIII-Fc and Xyntha are shown in Tables 9 and 10, respectively. The mean $t_{1/2}$ for rFVIII-Fc was 11.9 ± 1.7 hr (range 9.3 to 14.1 hr) and for Xyntha, the mean elimination $t_{1/2}$ was 12.7 ± 4.4 hr (range 9.2 to 19.9 hr).

[00172] FVIII activity was measured using a FVIII-specific chromogenic activity assay and the data are reported in Table 8. Pre-dose activity due to endogenous FVIII was subtracted from all samples. A graph of the mean group data is shown in Figure 9 and the individual plasma concentration vs. time curves are shown in Figure 10. A summary of the PK parameters are reported for rFVIII-Fc and Xyntha in Tables 9 and 10, respectively. The mean elimination $t_{1/2}$ was 16.1 ± 6.9 hr (range 11.6 to 29.4 hr) for rFVIII-Fc and 12.5 ± 1.7 hr (range 10.4 to 14.3 hr) for Xyntha.

Discussion and Conclusions

[00173] The elimination half-lives were similar for rFVIII-Fc and Xyntha after a single intravenous dose of 125 IU/kg. whether the test article was measured by ELISA or a chromogenic activity assay.

Example 3

[00174] This will be a Phase I/IIa, open-label, crossover, dose-escalation, multi-center, and first-in-human study designed to evaluate the safety, tolerability, and pharmacokinetics of a single dose of rFVIII-Fc in subjects with severe (defined as <1 IU/dL [1%] endogenous factor VIII [FVIII]) hemophilia A. A total of approximately 12 previously treated patients will be enrolled and dosed with rFVIII-Fc at 25 or 65 IU/kg. After the screening (scheduled within 28 days prior to the first dose of the Advate® [rFVIII], the reference comparator agent) and a minimum of 4-days (96 hours) elapsing with no FVIII treatment prior to the first injection, approximately 6 subjects will receive a single 25 IU/kg dose of Advate® followed by a 3-day (72 hours) pharmacokinetic (PK) profile then crossover and receive a 25 IU/kg single, open-label dose of rFVIII-Fc for a 7-day (168 hours) PK profiling. The first 3 subjects will be dosed sequentially. For the first three (3) subjects dosed with 25 IU/kg of rFVIII-Fc, each subject will undergo an inhibitor assessment at 14-days (336 hours) post-injection of rFVIII-Fc. Dosing of the next subject (for the first three subjects only) will occur once the inhibitor testing is completed. After the 3rd subject completed the 14 day inhibitor assessment, the remaining three subjects at 25 IU/kg and the six subjects at 65 IU/kg will begin enrollment sequentially at least 1 day apart within each dose group.

[00175] One week after the last subject receives the 25 IU/kg dose of the rFVIII-Fc, approximately 6 unique subjects will be recruited for the 65 IU/kg cohort. Each subject in the 65 IU/kg cohort will receive a single 65 IU/kg dose of Advate® followed by a 4-day (96 hours) PK profiling then crossover and receive a 65 IU/kg single, open-label dose of rFVIII-Fc for a 10-day (240 hours) profiling. If a bleeding episode occurs before the first injection of rFVIII-Fc in any cohort, subject's pre-study FVIII product should be used for treatment and an interval of at least 4 days must then pass before receiving the first injection of rFVIII-Fc for the PK profile.

[00176] All subjects will be followed for a 14-day (336 hours) and 28 day safety evaluation period after administration of rFVIII-Fc 25 IU/kg or 65 IU/kg for safety. All subjects will undergo pharmacokinetic sampling pre- and post-dosing along with blood samples for analysis of FVIII activity at designated time points.

Example 4

Activity within the Xase Complex

[00177] To investigate the binding of the FVIII proteins (rBDD FVIII and rFVIII-Fc) with FIXa, and measure the ability of these proteins to activate FX, kinetic studies were performed examining these interactions in the context of the Xase complex. This assay involved the formation of the Xase complex with activated FIX and activated rBDD FVIII or rFVIII-Fc protein on a phospholipid surface in the presence of calcium, and monitoring the conversion of FX to FXa as measured by cleavage of a chromogenic or fluorogenic substrate.

[00178] Briefly, FVIII is first activated with α -thrombin for 5 min, then mixed with FIXa in the presence of Ca^{2+} , and synthetic phospholipid vesicles (25% phosphatidylserine (PS)/75% phosphatidylcholine (PC)) or platelets. Under conditions described below, FVIIIa and FIXa interact in the presence of a phospholipid surface and calcium ions to form an active Xase complex that mediates the conversion of FX into FXa through proteolytic processing. In turn, FXa cleaves a FXa-specific chromogenic or fluorogenic substrate. The cleaved substrate is chromogenic and therefore the amount of cleaved substrate in a solution is indicative of the amount of FXa generated. This is quantitated by measuring the absorbance of the solution at 405 nm.

A. Activation of Factor X

[00179] The ability of rBDD FVIII and rFVIII-Fc to activate FX were studied in the context of the Xase complex as described above. Thrombin-activated FVIII proteins were incubated with FIXa and phospholipids in the presence of calcium, then added to different concentrations of FX in the presence of a FX-specific substrate and the rates of FXa generation determined (Figure 11).

[00180] Based on these data, the K_m and V_{max} for the different FVIII proteins in the context of the Xase complex were calculated (Chang 1997) (Table 11). Data are

expressed as the mean of six analyses (3 experiments containing duplicate runs) \pm the corresponding standard deviation. Based on these data, these proteins (rBDD FVIII and rFVIII_{Fc}) were found to have comparable K_m and V_{max} values, within the variation of the assay. Therefore, the Xase complex formed with rFVIII_{Fc} behaves similarly to the Xase complex formed with the licensed product rBDD FVIII (ReFacto) with respect to interactions with phospholipids and ability to activate FX. Note that these comparable data also demonstrate that rFVIII_{Fc} is activated to a comparable degree as rBDD FVIII after a short incubation with thrombin.

B. Interaction with FIXa

[00181] The interaction between rBDD FVIII and rFVIII_{Fc} with FIXa were also examined in the context of the Xase complex. The Xase complex was assembled as above, using a fixed amount of FX and varying FIXa levels, and FXa generation rates determined (Figure 12). From these data, the K_d value for the Xase complex formed with both of the FVIII proteins to FIXa were determined (Chang 1997). Data are expressed as the mean of six analyses (3 experiments containing duplicate runs) \pm the corresponding standard deviation (Table 12). Both proteins were found to have similar K_d and V_{max} values, indicating that rFVIII_{Fc} has comparable interactions with FIXa as the licensed rBDD FVIII product.

Example 5

[00182] Interim pharmacokinetic data for the Phase I/IIa clinical trial discussed in Example 3 demonstrated the following results for FVIII_{Fc}. FVIII_{Fc} had about a 50% increase in systemic exposure (AUC_{INF}), about 50% reduction in clearance (Cl), and about 50-70% increase in elimination half-life and MRT compared to ADVATE (full length rFVIII). In addition, FVIII_{Fc} showed increased C168, TBLP1, TBLP3, and TBLP5 values compared to ADVATE.

AUC_{INF}	Area under the concentration-time curve from zero to infinity
Beta HL	Elimination phase half-life; also referred to as $t_{1/2\beta}$
C168 dose	Estimated FVIII _{Fc} activity above baseline at approximately 168 h after dose
Cl	Clearance

MRT	Mean residence time
TBLP1	Model-predicted time after dose when FVIII-Fc activity has declined to approximately 1 IU/dL above baseline
TBLP3	Model-predicted time after dose when FVIII-Fc activity has declined to approximately 3 IU/dL above baseline
TBLP5	Model-predicted time after dose when FVIII-Fc activity has declined to approximately 5 IU/dL above baseline

Example 6

[00183] A recombinant B-domain-deleted factor VIII-Fc (rFVIII-Fc) fusion protein has been created as an approach to extend the half-life of FVIII. The pharmacokinetics (PK) of rFVIII-Fc were compared to rFVIII in hemophilia A mice. We found that the terminal half-life was twice as long for rFVIII-Fc compared to rFVIII. In order to confirm that the underlying mechanism for the extension of half-life was due to the protection of rFVIII-Fc by FcRn, the PK were evaluated in FcRn knockout and human FcRn transgenic mice. A single intravenous dose (125 IU/kg) was administered and the plasma concentration measured using a chromogenic activity assay. The C_{max} was similar between rFVIII-Fc and rFVIII (XYNTHA®) in both mouse strains. However, while the half-life for rFVIII-Fc was comparable to that of rFVIII in the FcRn knockout mice, the half-life for rFVIII-Fc was extended to approximately twice longer than that for rFVIII in the hFcRn transgenic mice. These results confirm that FcRn mediates or is responsible for the prolonged half-life of rFVIII-Fc compared to rFVIII. Since hemostasis in whole blood measured by rotation thromboelastometry (ROTEM) has been shown to correlate with the efficacy of coagulation factors in bleeding models of hemophilia mice as well as in clinical applications, we sought to evaluate the ex vivo efficacy of rFVIII-Fc in the hemophilia A mice using ROTEM. Hemophilia A mice were administered a single intravenous dose of 50 IU/kg rFVIII-Fc, XYNTHA® (FVIII) or ADVATE® (FVIII). At 5 minutes post dose, clot formation was similar with respect to clotting time (CT), clot formation time (CFT) and α -angle. However, rFVIII-Fc showed significantly improved CT at 72 and 96 hr post dose, and CFT and α -angle were also improved at 96 hrs compared to both XYNTHA® (FVIII) and ADVATE® (FVIII), consistent with prolonged PK of rFVIII-Fc. Therefore construction of an Fc fusion of FVIII produces a molecule with a defined mechanism of

action that has an increased half-life and the potential to provide prolonged protection from bleeding.

Example 7

[00184] This Example presents final analysis results for FVIII activity from 16 patients treated with 25 and 65 IU/kg FVIII products. See Examples 3 and 5.

[00185] In this Example, rFVIII-Fc is a recombinant fusion protein comprised of a single molecule of recombinant B-domain deleted human FVIII (BDD-rFVIII) fused to the dimeric Fc domain of the human IgG1, with no intervening linker sequence. This protein construct is also referred to herein as rFVIII-Fc heterodimeric hybrid protein, FVIII-Fc monomeric Fc fusion protein, FVIII-Fc monomer hybrid, monomeric FVIII-Fc hybrid, and FVIII-Fc monomer-dimer. See Example 1, Fig. 1, and Table 2A.

[00186] Preclinical studies with rFVIII-Fc have shown an approximately 2-fold prolongation of the half-life of rFVIII activity compared to commercially available rFVIII products. The rationale for this study was to evaluate the safety and tolerability of a single dose of rFVIII-Fc in frozen liquid formulation and provide data on the PK in severe hemophilia A subjects. For this study, 16 evaluable subjects were available for PK evaluation. Single administration of two doses of both rFVIII-Fc and Advate at a nominal dose of 25 (n=6) and 65 IU/kg of body weight (n=10) were infused intravenously over approximately 10 minutes. Blood samples for plasma PK assessments were obtained before infusion, as well as up to 10 days after dosing. The PK of FVIII activity for both Advate and rFVIII-Fc were characterized in this study using a model-dependent method.

OBJECTIVES

[00187] The primary objective of this study was to assess the safety and tolerability of single administration of two doses of rFVIII-Fc (25 and 65 IU/kg) in previously treated patients (PTPs) aged 12 and above with severe hemophilia A.

[00188] The secondary objectives were to determine the pharmacokinetics (PK) parameters determined by pharmacodynamic (PD) activity of FVIII over time after a single administration of 25 or 65 IU/kg of rFVIII-Fc compared to Advate in one-stage clotting and chromogenic assays.

Study Design (See Example 3)

[00189] Blood samples were collected for FVIII activity PK evaluations at the screening visit (within 28 days prior to dosing Advate); on Day 0 (injection of Advate) pre-injection and at 10 and 30 minutes and 1, 3, 6, and 9 hours post-injection; on Day 1 at 24 hours post-injection of Advate; on Day 2 at 48 hours post-injection of Advate; on Day 3 at 72 hours post-injection of Advate; and on Day 4 at 96 hours post-injection of high dose of Advate (Cohort B only).

[00190] Blood samples were collected for FVIII activity PK evaluations on the day of rFVIII-Fc injection just prior to the administration of rFVIII-Fc, at 10 and 30 minutes and 1, 3, 6, and 9 hours post-injection of rFVIII-Fc; on Day 1 at 24 hours post-injection of rFVIII-Fc; on Days 2 through 5 at 48, 72, 96, and 120 hours post-injection of rFVIII-Fc; on Day 7 at 168 hours post-injection of rFVIII-Fc; on Days 8, 9, and 10 at 192, 216, and 240 hours post-injection of high dose of rFVIII-Fc (Cohort B only). FVIII activity was also measured at the final study visit (28 days post-injection of rFVIII-Fc) at 672 hours post-injection of rFVIII-Fc.

Pharmacokinetic Modeling and Calculations

[00191] Abbreviations

TBLP1 = Model-predicted time after dose when FVIII activity has declined to approximately 1 IU/dL above baseline.

TBLP3 = Model-predicted time after dose when FVIII activity has declined to approximately 3 IU/dL above baseline

$KV_M = C_{max_M} / \text{Actual Dose (IU/kg)}$

$KV_OB = C_{max_OB} / \text{Actual Dose (IU/kg)}$

$IVR_M = 100 \times C_{max_M} \times \text{Plasma Volume (dL)} / \text{Total Dose in IU}$; where plasma volume in mL = $(23.7 \times \text{Ht in cm}) + (9.0 \times \text{Wt in kg}) - 1709$.

$IVR_OB = 100 \times C_{max_OB} \times \text{Plasma Volume (dL)} / \text{Total Dose in IU}$; where plasma volume in mL = $(23.7 \times \text{Ht in cm}) + (9.0 \times \text{Wt in kg}) - 1709$.

RESULTS

[00192] Figure 13. Observed group mean (+SE) FVIII activity versus time profiles, sorted by dose level, grouped by compound (one-stage assay, 25 IU/kg (A) and 65 IU/kg (B)) and (chromogenic assay, 25 IU/kg (C) and 65 IU/kg (D)).

[00193] Figure 14. Observed group mean (+SE) FVIII activity versus time profiles, grouped by dose level and compound (one-stage assay; A) (chromogenic assay; B).

Single-Dose Pharmacokinetics (One-Stage Assay)

[00194] Observed FVIII activity increased sharply after the short IV infusion of either Advate or rFVIII-Fc, with mean (\pm SD) model-predicted C_{max} values of 56.6 ± 4.74 and 121 ± 28.2 IU/dL for Advate and 55.6 ± 8.18 and 108 ± 16.9 IU/dL for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively. All Advate- and rFVIII-Fc-treated patients had dose-related increases in FVIII activity. The observed increase in both C_{max} and AUCINF was slightly less than proportional to dose over the dose range evaluated.

[00195] After the end of the infusion, the decline of the observed FVIII activity exhibited monoexponential decay characteristics until the baseline level was reached. The rate of decline in FVIII activity was slower for rFVIII-Fc than for Advate with mean (\pm SD) model-predicted elimination half-life values of 11.9 ± 2.98 and 10.4 ± 3.03 hr for Advate and 18.0 ± 3.88 and 18.4 ± 6.99 hr for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively. Elimination half-life values appeared to be dose-independent over the dose range evaluated for both FVIII products.

[00196] Total systemic FVIII exposure (assessed by AUCINF) was $\sim 48\%$ and 61% greater following rFVIII-Fc administration than Advate at 25 and 65 IU/kg dose levels, respectively. Mean (\pm SD) model-predicted AUCINF values were 974 ± 259 and 1810 ± 606 hr*IU/dL for Advate and 1440 ± 316 and 2910 ± 1320 hr*IU/dL for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively.

[00197] Similar to elimination half-life, the MRT was prolonged for rFVIII-Fc relative to Advate. Mean (\pm SD) model-predicted MRT values were 17.1 ± 4.29 and 14.9 ± 4.38 hr for Advate and 25.9 ± 5.60 and 26.5 ± 10.1 hr for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively. MRT values appeared to be dose-independent over the dose range evaluated for both FVIII products.

[00198] In addition, primary PK parameter values for CL and V were determined. CL values for rFVIIIIFc only accounted for ~ 66% of those observed for Advate at equivalent doses. Mean (\pm SD) model-predicted CL values were 2.70 ± 0.729 and 4.08 ± 1.69 mL/hr/kg for Advate and 1.80 ± 0.409 and 2.69 ± 1.25 mL/hr/kg for rFVIIIIFc for the 25 and 65 IU/kg dose groups, respectively. V values were comparable between Advate and rFVIIIIFc with mean (\pm SD) model-predicted V values of 43.9 ± 4.27 and 56.1 ± 13.4 mL/kg for Advate and 45.3 ± 7.23 and 61.6 ± 10.6 mL/kg for rFVIIIIFc for the 25 and 65 IU/kg dose groups, respectively. Slight increases in mean CL and V values were noted with increasing dose of Advate and rFVIIIIFc; however, the increase in standard deviations at the 65 IU/kg dose coupled with limited dose levels confounded an assessment of the dose-dependency of these parameters. For example, the CV% geometric mean CL value for the rFVIIIIFc treatment group increased from 23.0% (25 IU/kg) to 48.6% (65 IU/kg).

[00199] In addition to the primary PK parameters, secondary PK parameters (e.g. K-values, IVR, etc.) were determined to evaluate FVIII duration of effect. Evidence of PK difference was also observed with rFVIIIIFc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIIFc appeared to be comparable. A slight increase in TBLP1 and TBLP3 values were observed with increasing dose of Advate and rFVIIIIFc. In contrast, slight decreases in mean IVR and K-values were noted with increasing dose of Advate and rFVIIIIFc. As previously indicated, an assessment of the dose dependency of these parameters is confounded by limited dose levels.

[00200] Mean (\pm SD) observed TBLP1 were 2.88 ± 0.733 and 2.93 ± 0.848 IU/dL per IU/kg for Advate and 4.28 ± 0.873 and 5.16 ± 2.02 IU/dL per IU/kg for rFVIIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (\pm SD) observed TBLP3 were 2.06 ± 0.527 and 2.26 ± 0.666 IU/dL per IU/kg for Advate and 3.09 ± 0.623 and 3.93 ± 1.59 IU/dL per IU/kg for rFVIIIIFc for the 25 and 65 IU/kg dose groups, respectively.

[00201] Mean IVR and K-values calculated using observed Cmax values (subtracted with baseline and residual drug within the model) were generally greater than values determined using model-predicted Cmax values; consistent with slight underestimation of the observed peak activity using the one-compartment model. Mean (\pm SD) observed K-values were 2.57 ± 0.198 and 2.13 ± 0.598 IU/dL per IU/kg for Advate and 2.46 ± 0.330

and 1.85 ± 0.332 IU/dL per IU/kg for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively. Mean (\pm SD) observed IVR values were 94.1 ± 15.6 and 85.8 ± 16.5 % for Advate and 89.5 ± 11.9 and 74.8 ± 6.72 % for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively.

Single-Dose Pharmacokinetics (Chromogenic Assay)

[00202] Observed FVIII activity increased sharply after the short IV infusion of either Advate or rFVIII-Fc, with mean (\pm SD) model-predicted C_{max} values of 70.2 ± 9.60 and 157 ± 38.6 IU/dL for Advate and 70.3 ± 10.0 and 158 ± 34.7 IU/dL for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively.

[00203] All Advate- and rFVIII-Fc-treated patients had dose-related increases in FVIII activity. The observed increase in both C_{max} and AUC_{INF} was slightly less than proportional to dose over the dose range evaluated.

[00204] After the end of the infusion, the decline of the observed FVIII activity exhibited monoexponential decay characteristics until the baseline level was reached. The rate of decline in FVIII activity was slower for rFVIII-Fc than for Advate with mean (\pm SD) model-predicted elimination half-life values of 10.7 ± 1.98 and 10.3 ± 3.27 hr for Advate and 16.2 ± 2.92 and 19.0 ± 7.94 hr for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively. Elimination half-life values appeared to be dose-independent over the dose range evaluated for both FVIII products.

[00205] Total systemic FVIII exposure (assessed by AUC_{INF}) was $\sim 53\%$ and 84% greater following rFVIII-Fc administration than Advate at 25 and 65 IU/kg dose levels, respectively. Mean (\pm SD) model-predicted AUC_{INF} values were 1080 ± 236 and 2320 ± 784 hr*IU/dL for Advate and 1650 ± 408 and 4280 ± 1860 hr*IU/dL for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively.

[00206] Similar to elimination half-life, the MRT was prolonged for rFVIII-Fc relative to Advate. Mean (\pm SD) model-predicted MRT values were 15.3 ± 2.86 and 14.8 ± 4.72 hr for Advate and 23.4 ± 4.22 and 27.3 ± 11.4 hr for rFVIII-Fc for the 25 and 65 IU/kg dose groups, respectively. MRT values appeared to be dose-independent over the dose range evaluated for both FVIII products.

[00207] In addition, primary PK parameter values for CL and V were determined. CL values for rFVIII-Fc only accounted for $\sim 58\text{--}66\%$ of those observed for Advate at equivalent doses. Mean (\pm SD) model-predicted CL values were 2.39 ± 0.527 and $3.21 \pm$

1.40 mL/hr/kg for Advate and 1.57 ± 0.349 and 1.86 ± 0.970 mL/hr/kg for rFVIII Fc for the 25 and 65 IU/kg dose groups, respectively. V values were comparable between Advate and rFVIII Fc with mean (\pm SD) model-predicted V values of 35.8 ± 5.52 and 43.6 ± 11.2 mL/kg for Advate and 35.9 ± 6.65 and 42.7 ± 8.91 mL/kg for rFVIII Fc for the 25 and 65 IU/kg dose groups, respectively. Increases in mean CL and V values were noted with increasing dose of Advate and rFVIII Fc; however, the increase in standard deviations at 65 IU/kg coupled with limited dose levels confounded an assessment of the dose-dependency of these parameters.

[00208] In addition to the primary PK parameters, secondary PK parameters (e.g. K-values, IVR, etc.) were determined to evaluate FVIII duration of effect. Evidence of PK difference was also observed with rFVIII Fc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIII Fc appeared to be comparable.

[00209] A slight increase in TBLP1 and TBLP3 values were observed with increasing dose of Advate and rFVIII Fc. In contrast, slight decreases in mean IVR and K-values were noted with increasing dose of Advate and rFVIII Fc. As previously indicated, an assessment of the dose dependency of these parameters is confounded by limited dose levels.

[00210] Mean (\pm SD) observed TBLP1 were 2.70 ± 0.511 and 3.09 ± 0.978 IU/dL per IU/kg for Advate and 4.06 ± 0.798 and 5.66 ± 2.38 IU/dL per IU/kg for rFVIII Fc for the 25 and 65 IU/kg dose groups, respectively. Mean (\pm SD) observed TBLP3 were 1.98 ± 0.377 and 2.39 ± 0.718 IU/dL per IU/kg for Advate and 3.04 ± 0.598 and 4.44 ± 1.84 IU/dL per IU/kg for rFVIII Fc for the 25 and 65 IU/kg dose groups, respectively.

[00211] Mean IVR and K-values calculated using observed C_{max} values (subtracted with baseline and residual drug within the model) were generally greater than values determined using model-predicted C_{max} values; consistent with slight underestimation of the observed peak activity using the one-compartment model. Mean (\pm SD) observed K-values were 3.08 ± 0.429 and 2.85 ± 0.721 IU/dL per IU/kg for Advate and 3.12 ± 0.451 and 2.92 ± 0.985 IU/dL per IU/kg for rFVIII Fc for the 25 and 65 IU/kg dose groups, respectively. Mean (\pm SD) observed IVR values were 112 ± 14.5 and 116 ± 26.9 % for Advate and 113 ± 16.3 and 117 ± 33.6 % for rFVIII Fc for the 25 and 65 IU/kg dose groups, respectively.

CONCLUSIONS

- [00212] All Advate- and rFVIII-Fc-treated patients had comparable dose-related increases in C_{max} and AUC_{INF} over the dose range evaluated. Peak plasma levels of Advate and rFVIII-Fc activity were generally observed within the first hour after the end of the infusion and remained detectable for several days after dosing. After the end of infusion, the decline in baseline corrected FVIII activity exhibited monoexponential decay until the baseline was reached for both products. Parameter values for elimination half-life and MRT appeared to be dose-independent over the dose range evaluated for both FVIII products. Slight increases in mean CL and V values were noted with increasing dose of Advate and rFVIII-Fc; however, increased intersubject variability at the 65 IU/kg coupled with limited dose levels confounded an assessment of the dose-dependency of these parameters.
- [00213] Comparison of rFVIII-Fc and Advate activity PK revealed an approximate 48-61% (One-Stage Assay) or 53-84% (Chromogenic Assay) increase in systemic exposure, approximate 30-40% reduction in clearance, and an approximate 50-80% increase in both elimination half-life and MRT for rFVIII-Fc relative to Advate at comparable doses. Evidence of PK difference was also observed with rFVIII-Fc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIII-Fc appeared to be comparable.
- [00214] The PK parameters obtained from Chromogenic Assay results generally agreed with those from the One-Stage Assay, except that the Chromogenic Assay yielded a higher estimation of exposure parameters (e.g. C_{max}, AUC_{INF}, etc.).
- [00215] With the observed improvements in PK, rFVIII-Fc may provide a prolonged protection from bleeding, allowing less frequent injections for individuals with Hemophilia A.

Example 8

- [00216] On the basis of the interim PK analysis from the first-in-human study of rFVIII-Fc (Example 3), the A-LONG study was designed. A-LONG is an open label, multi-center evaluation of the safety, pharmacokinetics, and efficacy of recombinant Factor VIII Fc fusion (FVIII-Fc) in the prevention and treatment of bleeding in previously treated subjects with severe hemophilia A (defined as <1 IU/dL [$<1\%$] endogenous FVIII).

[00217] Approximately 106 subjects will be enrolled into one of three regimens: a tailored prophylaxis regimen (arm 1), a weekly dosing regimen (arm 2), and an on-demand regimen (arm 3).

Arm 1: Tailored Prophylaxis Regimen

[00218] Arm 1 will include an overall group and a PK subgroup. Approximately 66 subjects will be enrolled. The initial regimen will be twice weekly at 25 IU/kg on the first day, followed by 50 IU/kg on the fourth day of the week. Subjects will administer rFVIIIIFc on this weekly prophylaxis regimen until PK results for rFVIIIIFc are available. Based on these results, a tailored prophylaxis regimen will be established for each individual, in which the dose and interval will be determined to maintain a trough level of 1-3% FVIII activity. Each subject will then administer his individually tailored prophylaxis regimen throughout the study.

[00219] Subjects will be monitored throughout the study and ongoing dose and interval adjustments will be made. Adjustments will only be made when a subject experiences unacceptable bleeding episodes defined as ≥ 2 spontaneous bleeding episodes over a rolling two-month period. In this case, adjustment will target trough levels of 3-5%.

Arm 2: Weekly Dosing Regimen

[00220] Approximately 20 subjects will be enrolled/randomized and undergo abbreviated rFVIIIIFc PK profiling as follows: Washout of at least 96 hours; a single dose of rFVIIIIFc 65 IU/kg; Abbreviated sampling beginning on rFVIIIIFc Day 0, including pre-injection and 10 (± 2) minutes, 3 hours (± 15 minutes), 72 (± 2) hours [Day 3], and 96 (± 2) hours [Day 4] from the start of injection. Following the abbreviated PK profiling, subjects will then administer a fixed dose of 65 IU/kg rFVIIIIFc every 7 days.

Arm 3: On-demand Regimen

[00221] A minimum of 10 major surgeries in at least 5 subjects will be evaluated in the study. Major surgery is defined as any surgical procedure (elective or emergent) that involves general anesthesia and/or respiratory assistance in which a major body cavity is penetrated and exposed, or for which a substantial impairment of physical or physiological functions is produced (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation).

[00222] For prophylaxis during surgery, subjects will be treated with 35 to 50 IU/kg rFVIIIc every 12 to 24 hours. Prior to surgery, the physician will review the subject's rFVIIIc PK profile and assess the dose regimen of Factor VIII replacement generally required for the type of planned surgery and the clinical status of the subject. Recommendation for the appropriate dosing of rFVIIIc in the surgical treatment period, including any rehabilitation time, will take these factors into consideration.

[00223] The primary objectives of this study are (a) to evaluate the safety and tolerability of rFVIIIc administered as prophylaxis, on-demand, and surgical treatment regimens; and (b) to evaluate the efficacy of rFVIIIc administered as prophylaxis, on-demand, and surgical treatment regimens. The secondary objectives of this study are (a) to characterize the PK profile of rFVIIIc and compare the PK of FVIIIc with the currently marketed product, ADVATE; (b) to evaluate individual responses with FVIIIc; and (c) to evaluate FVIIIc consumption.

Primary Objectives

- To evaluate safety and tolerability of rFVIIIc administered as prophylaxis, weekly, on-demand, and surgical treatment regimens
- To evaluate the efficacy of rFVIIIc administered as tailored prophylaxis, on-demand, and surgical treatment regimens

Secondary Objectives

- To characterize the PK profile of rFVIIIc and compare the PK of rFVIIIc with the currently marketed product, Advate®
- To evaluate individual responses with rFVIIIc
- To characterize the range of dose and schedules required to adequately prevent bleeding in a prophylaxis regimen; maintain homeostasis in a surgical setting; or to treat bleeding episodes in an on-demand, weekly treatment, or prophylaxis setting
- To evaluate rFVIIIc consumption (e.g., total annualized rFVIIIc consumption per subject)

Example 9

Clinical ROTEM Assessment

[00224] In the study in Example 8, in addition to the measurement of plasma FVIII activity by one-stage activated partial thromboplastin time (aPTT) assay, whole blood rotational thromboelastometry (ROTEM) has also been explored to assess the improvement in global hemostasis by rFVIII-Fc and Advate in 2 subjects, specifically, 1 in the low dose cohort and 1 in the high dose cohort.

[00225] rFVIII-Fc and Advate appear to be comparably active in clot formation when spiked into subjects' blood prior to rFVIII-Fc treatment. The clotting time (CT) was linear with respect to the dose of rFVIII-Fc and Advate in the range of approximately 1% of 100% of normal, and the dose response was comparable between rFVIII-Fc and Advate in the same subject.

[00226] Following dosing with Advate and subsequently rFVIII-Fc, citrated whole blood was sampled at various time points and the clot formation following recalcification was monitored by ROTEM. Despite the variable baseline CT due to residue FVIII levels prior to Advate or rFVIII-Fc dosing, both products effectively corrected the CT to comparable levels 30 minutes post-injection. In addition, the improvement in CT was better sustained at and after 3 hours post-injection of 25 IU/kg of rFVIII-Fc relative to Advate in the subject dosed at this low dose. However, the differential improvement of rFVIII-Fc versus Advate was much less appreciable at the 65 IU/kg dose.

Tables

Table 1: Polynucleotide Sequences

A. B-Domain Deleted FVIII_hFc

(i) B-Domain Deleted FVIII_hFc Chain DNA Sequence (FVIII signal peptide underlined, Fc region in bold) (SEQ ID NO:1, which encodes SEQ ID NO:2)

661				A	TGCAAATAGA	GCTCTCCACC	TGCTTCTTTC
721	TGTGCCTTTT	GCGATTCTGC	TTTAGTGCCA	CCAGAAGATA	CTACCTGGGT	GCAGTGGAAC	
781	TGTCATGGGA	CTATATGCAA	AGTGATCTCG	GTGAGCTGCC	TGTGGACGCA	AGATTTCCTC	
841	CTAGAGTGCC	AAAATCTTTT	CCATTCAACA	CCTCAGTCGT	GTACAAAAAG	ACTCTGTTTG	
901	TAGAATTCAC	GGATCACCTT	TTCAACATCG	CTAAGCCAAG	GCCACCCTGG	ATGGGTCTGC	
961	TAGGTCCTAC	CATCCAGGCT	GAGGTTTATG	ATACAGTGGT	CATTACACTT	AAGAACATGG	
1021	CTTCCCATCC	TGTCAGTCTT	CATGCTGTTG	GTGTATCCTA	CTGGAAAGCT	TCTGAGGGAG	
1081	CTGAATATGA	TGATCAGACC	AGTCAAAGGG	AGAAAGAAGA	TGATAAAGTC	TTCCCTGGTG	
1141	GAAGCCATAC	ATATGTCCTG	CAGGTCCTGA	AAGAGAATGG	TCCAATGGCC	TCTGACCCAC	
1201	TGTGCCTTAC	CTACTCATAT	CTTTCTCATG	TGGACCTGGT	AAAAGACTTG	AATTCAGGCC	
1261	TCATTGGAGC	CCTACTAGTA	TGTAGAGAAG	GGAGTCTGGC	CAAGGAAAAG	ACACAGACCT	
1321	TGCACAAATT	TATACTACTT	TTTGCTGTAT	TTGATGAAGG	GAAAAGTTGG	CACTCAGAAA	
1381	CAAAGAACTC	CTTGATGCAG	GATAGGGATG	CTGCATCTGC	TCGGGCCCTGG	CCTAAAAATGC	
1441	ACACAGTCAA	TGGTTATGTA	AACAGGTCTC	TGCCAGGTCT	GATTGGATGC	CACAGGAAAT	
1501	CAGTCTATTG	GCATGTGATT	GGAATGGGCA	CCACTCCTGA	AGTGCACCTA	ATATTCTCTG	
1561	AAGGTCACAC	ATTTCTTGTG	AGGAACCATC	GCCAGGCGTC	CTTGGAAATC	TCGCCAATAA	
1621	CTTTCCTTAC	TGCTCAAACA	CTCTTGATGG	ACCTTGGAAC	GTTTCTACTG	TTTTGTCTATA	
1681	TCTCTTCCCA	CCAACATGAT	GGCATGGAAG	CTTATGTCAA	AGTAGACAGC	TGTCCAGAGG	
1741	AACCCCAACT	ACGAATGAAA	AATAATGAAG	AAGCGGAAGA	CTATGATGAT	GATCTTACTG	
1801	ATTCTGAAAT	GGATGTGGTC	AGGTTTGATG	ATGACAACCT	TCCTTCCTTT	ATCCAAATTC	
1861	GCTCAGTTGC	CAAGAAGCAT	CCTAAAACTT	GGGTACATTA	CATTGCTGCT	GAAGAGGAGG	
1921	ACTGGGACTA	TGCTCCCTTA	GTCTCTGCCC	CCGATGACAG	AAGTTATAAA	AGTCAATATT	
1981	TGAACAATGG	CCCTCAGCGG	ATTGGTAGGA	AGTACAAAAA	AGTCCGATTT	ATGGCATAAC	
2041	CAGATGAAAC	CTTTAAGACT	CGTGAAGCTA	TTGAGCATGA	ATCAGGAATC	TTGGGACCTT	
2101	TACTTTATGG	GGAAGTTGGA	GACACACTGT	TGATTATATT	TAAGAATCAA	GCAAGCAGAC	
2161	CATATAACAT	CTACCCTCAC	GGAATCACTG	ATGTCCGTCC	TTTGTATTCA	AGGAGATTAC	
2221	CAAAAGGTGT	AAAACATTTG	AAGGATTTTC	CAATTCTGCC	AGGAGAAATA	TTCAAATATA	
2281	AATGGACAGT	GACTGTAGAA	GATGGGCCAA	CTAAATCAGA	TCCTCGGTGC	CTGACCCGCT	
2341	ATTACTCTAG	TTTCGTTAAT	ATGGAGAGAG	ATCTAGCTTC	AGGACTCATT	GGCCCTCTCC	
2401	TCATCTGCTA	CAAAGAATCT	GATAGTCAAA	GAGGAAACCA	GATAATGTCA	GACAAGAGGA	
2461	ATGTCATCCT	GTTTTCTGTA	TTTGATGAGA	ACCGAAGCTG	GTACCTCACA	GAGAATATAC	
2521	AACGCTTTCT	CCCCAATCCA	GCTGGAGTGC	AGCTTGAGGA	TCCAGAGTTC	CAAGCCTCCA	
2581	ACAICATGCA	CAGCATCAAT	GGCTATGTTT	TTGATAGTTT	GCAGTTGTCA	GTTTGTGTTG	
2641	ATGAGGTGGC	ATACTGGTAC	ATTCTAAGCA	TTGGAGCACA	GACTGACTTC	CTTTCTGTCT	
2701	TCTTCTCTGG	ATATACCTTC	AAACACAAAA	TGGTCTATGA	AGACACACTC	ACCCTATTCC	
2761	CATTCTCAGG	AGAAACTGTC	TTTATGTCGA	TGGAAAACCC	AGGTCTATGG	ATTCTGGGGT	
2821	GCCACAACCT	AGACTTTTCG	AACAGAGGCA	TGACCGCCTT	ACTGAAGGTT	TCTAGTTGTG	
2881	ACAAGAACAC	TGGTGATTAT	TACGAGGACA	GTTATGAAGA	TATTTTCAGCA	TACTTGCTGA	
2941	GTAAAAACAA	TGCCATTGAA	CCAAGAAGCT	TCTCTCAAAA	CCCACCAGTC	TTGAAACGCC	
3001	ATCAACGGGA	AATAACTCGT	ACTACTCTTC	AGTCAGATCA	AGAGGAAATT	GACTATGATG	
3061	ATACCATATC	AGTTGAAATG	AAGAAGGAAG	ATTTTGACAT	TTATGATGAG	GATGAAAATC	
3121	AGAGCCCCCG	CAGCTTTCAA	AAGAAAACAC	GACACTATTT	TATTGCTGCA	GTGGAGAGGC	
3181	TCTGGGATTA	TGGGATGAGT	AGCTCCCCAC	ATGTTCTAAG	AAACAGGGCT	CAGAGTGGCA	
3241	GTGTCCCTCA	GTTCAAGAAA	GTTGTTTTTC	AGGAATTTAC	TGATGGCTCC	TTTACTCAGC	
3301	CCTTATACCG	TGGAGAACTA	AATGAACATT	TGGGACTCCT	GGGGCCATAT	ATAAGAGCAG	

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3361 AAGTTGAAGA TAATATCATG GTAAC TTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT
3421 ATTCTAGCCT TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT
3481 TTGTCAAGCC TAATGAAACC AAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA
3541 CTAAGATGA GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGA AAAAG
3601 ATGTGCACTC AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCCCTG
3661 CTCATGGGAG ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA
3721 CCAAAAGCTG GTACTTCACT GAAAATATGG AAAGAACTG CAGGGCTCCC TGCAATATCC
3781 AGATGGAAGA TCCCAC TTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA
3841 TGGATACACT ACCTGGCTTA GTAAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA
3901 GCATGGGCAG CAATGAAAAC ATCCATTCTA TTCATTTT CAG TGGACATGTG TTCACTGTAC
3961 GAAAAAAGA GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG
4021 TGGAAATGTT ACCATCCAAA GCTGGAATTT GCGGGTGGG ATGCCTTATT GCGGAGCATC
4081 TACATGCTGG GATGAGCACA CTTTTCTGG TGTACAGCAA TAAGTGT CAG ACTCCCCTGG
4141 GAATGGCTTC TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT
4201 GGGCCCCAAA GCTGGCCAGA CTTTCTTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG
4261 AGCCCTTTTC TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTAC GGCATCAAGA
4321 CCCAGGGTGC CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA
4381 GTCTTGATGG GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT
4441 TCTTTGGCAA TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCCT CCAATTATTG
4501 CTCGATACAT CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT
4561 TGATGGGCTG TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT
4621 CAGATGCACA GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT
4681 CAAAAGCTCG ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC
4741 CAAAAGAGTG GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACTACTC
4801 AGGGAGTAAA ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC
4861 AAGATGGCCA TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAAGGGA
4921 ATCAAGACTC CTTACACCTT GTGGTGAAC CTCTAGACCC ACCGTTACTG ACTCGTACC
4981 TTCGAATTCA CCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT
5041 GCGAGGCACA GGACCTCTAC GACAAAAC TC ACACATGCC ACCGTGCCCC GCTCCAGAAC
5101 TCCTGGGCGG ACCGTCAGTC TTCCTCTTCC CCCC AAAACC CAAGGACACC CTCATGATCT
5161 CCCGACCCC TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA
5221 AGTTCAACTG GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG
5281 AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC
5341 TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA
5401 AAACCATCTC CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT
5461 CCCGGGATGA GCTGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAA GGCCTTCTATC
5521 CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAA TACAAGACCA
5581 CGCCTCCCGT GTTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA
5641 AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA
5701 ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA A

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(ii) Fc DNA sequence (mouse Igk signal peptide underlined) (SEQ ID NO:3, which encodes SEQ ID NO:4)

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7981 ATGGA GACAGACACA
8041 CTCCTGCTAT GGGTACTGCT GCTCTGGGTT CCAGGTTCCA CTGGTGACAA AACTCACACA
8101 TGCCACCGT GCCAGCACC TGAACCTCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCA
8161 AAACCAAGG ACACCCTCAT GATCTCCCG ACCCTGAGG TCACATGCGT GGTGGTGGAC
8221 GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT
8281 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTGAGCGTC
8341 CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC
8401 AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCGAGAA
8461 CCACAGGTGT ACACCCTGCC CCCATCCCGC GATGAGCTGA CCAAGAACCA GGTGAGCCTG
8521 ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
8581 CAGCCGAGGA ACAACTACAA GACCACGCCT CCCGTGTTGG ACTCCGACGG CTCCTTCTTC
8641 CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC

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8701 TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
8761 GGTAAG

B. Full Length FVIII_h

(i) Full Length FVIII_h DNA Sequence (FVIII signal peptide underlined, Fc region in bold) (SEQ ID NO:5, which encodes SEQ ID NO:6)

661 ATG CAAATAGAGC TCTCCACCTG
721 CTTCTTTCTG TGCCTTTTGC GATTCTGCTT TAGTGCCACC AGAAGATACT ACCTGGGTGC
781 AGTGGAAGCTG TCATGGGACT ATATGCAAAG TGATCTCGGT GAGCTGCCTG TGGACGCAAG
841 ATTTCTCTCT AGAGTGCCAA AATCTTTTCC ATTCAACACC TCAGTCGTGT AAAAAAGAC
901 TCTGTTTGTA GAATTCACGG ATCACCTTTT CAACATCGCT AAGCCAAGGC CACCCTGGAT
961 GGGTCTGCTA GGTCTACCA TCCAGGCTGA GGTTTATGAT ACAGTGGTCA TTACTTTAA
1021 GAACATGGCT TCCCATCCTG TCAGTCTTCA TGCTGTGGT GTATCCTACT GGAAAGCTTC
1081 TGAGGGAGCT GAATATGATG ATCAGACCAG TCAAAGGGAG AAAGAAGATG ATAAAGTCTT
1141 CCCTGGTGGA AGCCATACAT ATGTCTGGCA GGTCCTGAAA GAGAATGGTC CAATGGCCTC
1201 TGACCCACTG TGCCTTACCT ACTCATATCT TTCTCATGTG GACCTGGTAA AAGACTTGAA
1261 TTCAGGCCTC ATTGGAGCCC TACTAGTATG TAGAGAAGGG AGTCTGGCCA AGGAAAAGAC
1321 ACAGACCTTG CACAAATTTA TACTACTTTT IGCTGTATTT GATGAAGGGA AAAGTTGGCA
1381 CTCAGAAACA AAGAACTCCT TGATGCAGGA TAGGGATGCT GCATCTGCTC GGGCCTGGCC
1441 TAAATGCAC ACAGTCAATG GTTATGTAAG CAGGTCCTG CCAGGTCTGA TTGGATGCCA
1501 CAGGAAATCA GTCTATTGGC ATGTGATTGG AATGGGCACC ACTCCTGAAG TGCATCAAT
1561 ATTCCTCGAA GGTACACAT TTCTTGTGAG GAACCATCGC CAGGCGTCTT TGGAAATCTC
1621 GCCAATAACT TTCCTTACTG CTCAAACACT CTTGATGGAC CTTGGACAGT TTCTACTGTT
1681 TTGTCATATC TCTTCCCACC AACATGATGG CATGGAAGCT TATGTCAAAG TAGACAGCTG
1741 TCCAGAGGAA CCCCAACTAC GAATGAAAAA TAATGAAGAA GCGGAAGACT ATGATGATGA
1801 TCTTACTGAT TCTGAAATGG ATGTGGTCAG GTTTGATGAT GACAACTCTC TTTCTTTTAT
1861 CCAATTCGC TCAGTTGCCA AGAAGCATCC TAAAACCTGG GTACATTACA TTGCTGTCTGA
1921 AGAGGAGGAC TGGGACTATG CTCCCTTAGT CCTCGCCCCC GATGACAGAA GTTATAAAG
1981 TCAATATTTG AACAAATGGC CTCAGCGGAT TGGTAGGAAG TACAAAAAAG TCCGATTTAT
2041 GGCATACACA GATGAAACCT TTAAGACTCG TGAAGCTATT CAGCATGAAT CAGGAATCTT
2101 GGGACCTTTA CTTTATGGGG AAGTTGGAGA CACACTGTTG ATTATATTTA AGAATCAAGC
2161 AAGCAGACCA TATAACATCT ACCCTCACGG AATCACTGAT GTCCGTCTCT TGTATTCAAG
2221 GAGATTACCA AAAGGTGTAA AACATTTGAA GGATTTTCCA ATTCTGCCAG GAGAAATATT
2281 CAAATATAAA TGGACAGTGA CTGTAGAAGA TGGGCCAACT AAATCAGATC CTCGGTGCCT
2341 GACCCGCTAT TACTCTAGTT TCGTAAATAT GGAGAGAGAT CTAGCTTCAG GACTCATTTG
2401 CCCTCTCCTC ATCTGCTACA AAGAATCTGT AGATCAAAGA GGAACACAGA TAATGTCTGA
2461 CAAGAGGAAT GTCATCCTGT TTTCTGTATT TGATGAGAAC CGAAGCTGGT ACCTCACAGA
2521 GAATATACAA CGCTTTCTCC CCAATCCAGC TGGAGTGCAG CTTGAGGATC CAGAGTTCCA
2581 AGCCTCCAAC ATCATGCACA GCATCAATGG CTATGTTTTT GATAGTTTGC AGTTGTCACT
2641 TTGTTTGCAT GAGGTGGCAT ACTGGTACAT TCTAAGCATT GGAGCACAGA CTGACTTCCT
2701 TTCTGTCTTC TTCTCTGGAT ATACCTTCAA ACACAAAATG GTCTATGAAG ACACACTCAC
2761 CCTATTCCCA TTCTCAGGAG AAACCTGTCTT CATGTCGATG GAAAACCCAG GTCTATGGAT
2821 TCTGGGGTGC CAAACTCAG ACTTTCGGAA CAGAGGCATG ACCGCCTTAC TGAAGGTTTC
2881 TAGTTGTGAC AAGAACAATG GTGATTATTA CGAGGACAGT TATGAAGATA TTTTACGATA
2941 CTTGCTGAGT AAAAAAATG CCATTGAACC AAGAAGCTTC TCCAGAAATT CAAGACACCC
3001 TAGCACTAGG CAAAAGCAAT TTAATGCCAC CACAATTCCA GAAAATGACA TAGAGAAGAC
3061 TGACCCTTGG TTTGCACACA GAACACCTAT GCCTAAAATA CAAAATGTCT CCTCTAGTGA
3121 TTTGTTGATG CTCTTGGCAG AGAGTCCTAC TCCACATGGG CTATCCTTAT CTGATCTCCA
3181 AGAAGCCAAA TATGAGACTT TTTCTGATGA TCCATCACCT GGAGCAATAG ACAGTAATAA
3241 CAGCCTGTCT GAAATGACAC ACTTCAGGCC ACAGCTCCAT CACAGTGGGG ACATGGTATT
3301 TACCCCTGAG TCAGGCCTCC AATTAAGATT AAATGAGAAA CTGGGGACAA CTGCAGCAAC
3361 AGAGTTGAAG AAACCTGATT TCAAAGTTTC TAGTACATCA AATAATCTGA TTTCAACAAT
3421 TCCATCAGAC AATTTGGCAG CAGGTACTGA TAATACAAGT TCCTTAGGAC CCCCAAGTAT
3481 GCCAGTTCAT TATGATAGTC AATTAGATAC CACTCTATTT GGCAAAAAGT CACTCCCTCT
3541 TACTGAGTCT GGTGGACCTC TGAGCTTGAG TGAAGAAAAT AATGATTCAA AGTTGTTAGA

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3601 ATCAGGTTTA ATGAATAGCC AAGAAAGTTC ATGGGGAAAA AATGTATCGT CAACAGAGAG
3661 TGGTAGGTTA TTTAAAGGGA AAAGAGCTCA TGGACCTGCT TTGTTGACTA AAGATAATGC
3721 CTTATTCAAA GTTAGCATCT CTTTGTTAAA GACAAACAAA ACTTCCAATA ATTCAGCAAC
3781 TAATAGAAAG ACTCACATTG ATGGCCCATC ATTATTAATT GAGAATAGTC CATCAGTCTG
3841 GCAAAATATA TTAGAAAGTG ACACTGAGTT TAAAAAAGTG ACACCTTTGA TTCATGACAG
3901 AATGCTTATG GACAAAAATG CTACAGCTTT GAGGCTAAAT CATATGTCAA ATAAACTAC
3961 TTCATCAAAA AACATGGAAA TGGTCCAACA GAAAAAAGAG GGCCCCATTC CACCAGATGC
4021 ACAAAATCCA GATATGTCGT TCTTTAAGAT GCTATTCTTG CCAGAATCAG CAAGGTGGAT
4081 ACAAAGGACT CATGGAAAGA ACTCTCTGAA CTCTGGGCAA GGCCCCAGTC CAAAGCAATT
4141 AGTATCCTTA GGACCAGAAA AATCTGTGGA AGGTCAGAAT TTCTTGCTCG AGAAAAACAA
4201 AGTGGTAGTA GGAAAGGGTG AATTTACAAA GGACGTAGGA CTCAAAGAGA TGGTTTTTCC
4261 AAGCAGCAGA AACCTATTTT TACTAACTT GGATAATTTA CATCAAAATA ATACACACAA
4321 TCAAGAAAAA AAAATTTCAGG AAGAAATAGA AAAGAAGGAA ACATTAAATCC AAGAGAATGT
4381 AGTTTTGCCT CAGATACATA CAGTGACTGG CACTAAGAAT TTCATGAAGA ACCTTTTCTT
4441 ACTGAGCACT AGGCAAAATG TAGAAGGTTC ATATGACGGG GCATATGCTC CAGTACTTCA
4501 AGATTTTAGG TCATTAAATG ATTCAACAAA TAGAACAAAG AAACACACAG CTCATTTTCTC
4561 AAAAAAAGGG GAGGAAGAAA ACTTGGAAAG CTTGGGAAAT CAAACCAAGC AAATTGTAGA
4621 GAAATATGCA TGCACCACAA GGATATCTCC TAATACAAGC CAGCAGAATT TTGTCACGCA
4681 ACGTAGTAAG AGAGCTTTGA AACAAATTCAG ACTCCCACTA GAAGAAACAG AACTTGAAAA
4741 AAGGATAATT GTGGATGACA CCTCAACCCA GTGGTCCAAA AACATGAAAC ATTTGACCCC
4801 GAGCACCTC ACACAGATAG ACTACAATGA GAAGGAGAAA GGGGCCATTA CTCAGTCTCC
4861 CTTATCAGAT TGCCTTACGA GGAGTCATAG CATCCCTCAA GCAAATAGAT CTCCATTACC
4921 CATTGCAAAAG GTATCATCAT TTCCATCTAT TAGACCTATA TATCTGACCA GGGTCTTATT
4981 CCAAGACAAC TCTTCTCATC TTCCAGCAGC ATCTTATAGA AAGAAAGATT CTGGGGTCCA
5041 AGAAAGCAGT CATTTCTTAC AAGGAGCCAA AAAAAATAAC CTTTCTTTAG CCATTCTAAC
5101 CTTGGAGATG ACTGGTGATC AAAGAGAGGT TGGCTCCCTG GGGACAAGTG CCACAAATTC
5161 AGTCACATAC AAGAAAGTTG AGAACACTGT TCTCCGAAA CCAGACTTGC CCAAAACATC
5221 TGGCAAAAGT GAATTGCTTC CAAAAGTTCA CATTTATCAG AAGGACCTAT TCCCTACGGA
5281 AACTAGCAAT GGGTCTCCTG GCCATCTGGA TCTCGTGGAA GGGAGCCTTC TTCAGGGAAC
5341 AGAGGGAGCG ATTAAGTGGA ATGAAGCAAA CAGACCTGGA AAAGTTCCCT TTCTGAGAGT
5401 AGCAACAGAA AGCTCTGCAA AGACTCCCTC CAAGCTATTG GATCCTCTTG CTTGGGATAA
5461 CCACTATGGT ACTCAGATAC CAAAAGAGA GTGGAAATCC CAAGAGAAGT CACCAGAAAA
5521 AACAGCTTTT AAGAAAAAGG ATACCATTTT GTCCCTGAAC GCTTGTGAAA GCAATCATGC
5581 AATAGCAGCA ATAAATGAGG GACAAAATAA GCCCGAAATA GAAGTCACCT GGGCAAAGCA
5641 AGGTAGGACT GAAAGGCTGT GCTCTCAAAA CCCACCAGTC TTGAAACGCC ATCAACGGGA
5701 AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT GACTATGATG ACTCATATC
5761 AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC AGAGCCCCCG
5821 CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC TCTGGGATTA
5881 TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA GTGTCCCTCA
5941 GTTCAAGAAA GTTGTTTTCC AGGAATTAC TGATGGCTCC TTTACTCAGC CCTTATACCG
6001 TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG AAGTTGAAGA
6061 TAATATCATG GTAACCTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT ATTCTAGCCT
6121 TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAACT TTGTCAAGCC
6181 TAATGAAACC AAAACTTACT TTTGAAAAGT GCAACATCAT ATGGCACCCA CTAAAGATGA
6241 GTTTGACTGC AAAGCCTGGG CTTATTCTC TGATGTTGAC CTGGAAAAAG ATGTGCACTC
6301 AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCTCG CTCATGGGAG
6361 ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCAACATC TTTGATGAGA CCAAAGCTG
6421 GTACTTCACT GAAAATATGG AAAGAACTG CAGGGCTCCC TGCAATATCC AGATGGAAGA
6481 TCCCCTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA TGGATACACT
6541 ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTGATGG TATCTGCTCA GCATGGGCAG
6601 CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC GAAAAAAGA
6661 GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG TGGAAATGTT
6721 ACCATCCAAA GCTGGAATTT GCGGGTGGA ATGCCTTATT GCGGAGCATC TACATGCTGG
6781 GATGAGCACA CTTTTTCTGG GTACAGCAA TAAGTGTGAG ACTCCCTGGG GAATGGCTTC
6841 TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT GGGCCCCAAA
6901 GCTGGCCAGA CTTCAATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG AGCCCTTTTC
6961 TTGGATCAAG GTGGATCTGT TGGCACC AAT GATTATTAC GGCATCAAGA CCCAGGGTGC
7021 CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA GTCTTGATGG
7081 GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT TCTTTGGCAA

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7141   TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG CTCGATACAT
7201   CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT TGATGGGCTG
7261   TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT CAGATGCACA
7321   GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT CAAAAGCTCG
7381   ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC CAAAAGAGTG
7441   GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACACTC AGGGAGTAAA
7501   ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC AAGATGGCCA
7561   TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA ATCAAGACTC
7621   CTTACACACT GTGGTGAAC CTCTAGACCC ACCGTTACTG ACTCGCTACC TTCGAATTCA
7681   CCCCCAGAGT TGGGTGCACC AGATTGCCTT GAGGATGGAG GTTCTGGGCT GCGAGGCACA
7741   GGACCTCTAC GACAAAAC ACACATGCCC ACCGTGCCCA GCTCCAGAAC TCCTGGGCGG
7801   ACCGTCAGTC TTCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC
7861   TGAGGTCACA TGCGTGGTGG TGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG
7921   GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA
7981   CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA
8041   GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC
8101   CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA
8161   GCTGACCAAG AACCAGGTCA GCGTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT
8221   CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT
8281   GTTGGACTCC GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG
8341   GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC
8401   GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA A

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(ii) Fc (same sequence as A (ii) (SEQ ID NO:3))

C.

(i) Heavy Chain (HC)-Fc DNA sequence (no linker between HC and Fc) (signal peptide underlined, Fc region in bold) (SEQ ID NO:7, which encodes SEQ ID NO:8)

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1      ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG CTTTAGTGCC
61     ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGATCTC
121    GGTGAGCTGC CTGTGGACGC AAGATTTCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
181    ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
241    GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
301    GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT TCATGCTGTT
361    GGTGATCCTT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421    GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCCTG
481    AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
541    GTGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
601    GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661    TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAAGT CCTTGATGCA GGATAGGGAT
721    GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781    CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC
841    ACCACTCCTG AAGTGCATC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
901    CGCCAGGCGT CCTTGGAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
961    GACCTTGGAC AGTTTCTACT GTTTGTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021   GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
1081   GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
1141   GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAAC
1201   TGGGTACATT ACATTGCTGC TGAAGAGGAG GAAGGTCACA ATGCTCCCTT AGTCCTCGCC
1261   CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTGGTAGG
1321   AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
1381   ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG

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1441 TTGATTATAT TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAAGGTG TAAAACATTT GAAGGATTTT
1561 CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG TGA CTGTAGA AGATGGGCCA
1621 ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA TATGGAGAGA
1681 GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAAAGAATC TG TAGATCAA
1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCTATC TGTTTCTGT ATTTGATGAG
1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
1861 CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
1921 TTTGATAGTT TGCAGTTGTC AGTTTGTTTG CATGAGGTGG CATACTGGTA CATTCTAAGC
1981 ATTGGAGCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
2041 ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAACTGT CTTCATGTCG
2101 ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG GAACAGAGGC
2161 ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221 AGTTATGAAG ATATTTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGAGAC
2281 AAACTCACA CATGCCCACC GTGCCCAGCT CCAGAACTCC TGGGCGGACC GTCACTCTTC
2341 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC
2401 GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
2461 GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT
2521 GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC
2581 AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
2641 CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGATGAGCT GACCAAGAAC
2701 CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
2761 GAGAGCAATG GGCAGCCGGA GAACAACCTAC AAGACCACGC CTCCCGTGTT GGACTCCGAC
2821 GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
2881 GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
2941 TCCCTGTCTC CGGGTAAA

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C.

(ii) Heavy Chain (HC)-Fc DNA sequence (5 amino acid linker between HC and Fc)
(signal peptide underlined, Fc region in bold, 5 amino acid linker is double-underlined)
(SEQ ID NO:9, which encodes SEQ ID NO:10)

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1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGIGCCTTT TGCGATTCTG CTTTAGTGCC
61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGATCTC
121 GGTGAGCTGC CTGTGGACGC AAGATTTCCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
181 ACCTCAGTCG TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
241 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCCCTA CCATCCAGGC TGAGGTTTAT
301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT TCATGCTGTT
361 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421 GAGAAAGAAG ATGATAAAGT CTTCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCCTG
481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAGAAGCT CCTTGATGCA GGATAGGGAT
721 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAAATGGGC
841 ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
901 CGCCAGGCGT CCTTGGAAT CTGCGCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
1141 GATGACAACT CTCCTTCTTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACCT
1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCCTCGCC
1261 CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTGGTAGG
1321 AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT

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1381  ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGAAGTTGG AGACACACTG
1441  TTGATTATAT TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501  GATGTCCGTC CTTTGTATTC AAGGAGATTA CAAAAGGTG TAAAACATTT GAAGGATTTT
1561  CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG TGAAGTAGA AGATGGGCCA
1621  ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTAA TATGGAGAGA
1681  GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA
1741  AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801  AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
1861  CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
1921  TTTGATAGTT TGCAGTTGTC AGTTTGTGTT CATGAGGTGG CATACTGGTA CATTCTAAGC
1981  ATTGGAGCAC AGACTGACTT CCTTCTGTCT TTCTTCTCTG GATATACCTT CAAACACAAA
2041  ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAACTGT CTCATGTCG
2101  ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACCT CAGACTTTCG GAACAGAGGC
2161  ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221  AGTTATGAAG ATATTTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGAAGC
2281  TTCTCCGAGA ATGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCAGA ACTCCTGGGC
2341  GGACCGTCAG TCTTCTCTT CCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC
2401  CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC
2461  TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
2521  AACAGCACGT ACCGTGTGGT CAGCGTCTCT ACCGTCTGCT ACCAGGACTG GCTGAATGGC
2581  AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCCATCGA GAAAACCATC
2641  TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT
2701  GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
2761  ATCGCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCTCCC
2821  GTGTTGACT CCGACGGCTC CTTCTTCTCT TACAGCAAGC TCACCGTGGA CAAGAGCAGG
2881  TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
2941  ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAA

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C.

(iii) Light Chain (LC)-Fc DNA sequence (signal peptide underlined, Fc region in bold)
 (SEQ ID NO:11, which encodes SEQ ID NO:12)

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1  ATGGAGACAG ACACACTCCT GCTATGGGTA CTGCTGCTCT GGGTTCCAGG TTCCACTGGT
61  GAAATAACTC GTACTACTCT TCAGTCAGAT CAAGAGGAAA TTGACTATGA TGATACCATA
121 TCAGTTGAAA TGAAGAAGGA AGATTTTGAC ATTTATGATG AGGATGAAAA TCAGAGCCCC
181 CGCAGCTTTC AAAAGAAAAC ACGACACTAT TTTATTGCTG CAGTGGAGAG GCTCTGGGAT
241 TATGGGATGA GTAGCTCCCC ACATGTTCTA AGAAACAGGG CTCAGAGTGG CAGTGTCCCT
301 CAGTTCAAGA AAGTTGTTTT CCAGGAATTT ACTGATGGCT CCTTTACTCA GCCCTTATAC
361 CGTGGAGAAC TAAATGAACA TTTGGGACTC CTGGGGCCAT ATATAAGAGC AGAAGTTGAA
421 GATAATATCA TGGTAACTTT CAGAAATCAG GCCTCTCGTC CCTATTCCTT CTATTCTAGC
481 CTTATTTCTT ATGAGGAAGA TCAGAGGCAA GGAGCAGAAC CTAGAAAAAA CTTTGTCAAG
541 CCTAATGAAA CCAAAACCTA CTTTTGAAAA GTGCAACATC ATATGGCACC CACTAAAGAT
601 GAGTTTGACT GCAAAGCCTG GGCTTATTTT TCTGATGTTG ACCTGGAAAA AGATGTGCAC
661 TCAGGCCTGA TTGGACCCCT TCTGGTCTGC CACACTAACA CACTGAACCC TGCTCATGGG
721 AGACAAGTGA CAGTACAGGA ATTTGCTCTG TTTTTCACCA TCTTTGATGA GACCAAAAGC
781 TGGTACTTCA CTGAAAATAT GGAAAGAAAC TGCAGGGCTC CCTGCAATAT CCAGATGGAA
841 GATCCCACTT TTAAAGAGAA TTATCGCTTC CATGCAATCA ATGGCTACAT AATGGATACA
901 GTACCTGGCT TAGTAATGGC TCAGGATCAA AGGATTCGAT GGTATCTGCT CAGCATGGGC
961 AGCAATGAAA ACATCCATTC TATTCATTTT AGTGGACATG TGTTCACTGT ACGAAAAAAA
1021 GAGGAGTATA AAATGGCACT GTACAATCTC TATCCAGGTG TTTTTGAGAC AGTGGAAATG
1081 TTACCATCCA AAGCTGGAAT TTGGCGGGTG GAATGCCTTA TTGGCGAGCA TCTACATGCT
1141 GGGATGAGCA CACTTTTTCT GGTGTACAGC AATAAGTGTC AGACTCCCCT GGGAATGGCT
1201 TCTGGACACA TTAGAGATTT TCAGATTACA GCTTCAGGAC AATATGGACA GTGGGCCCCA
1261 AAGCTGGCCA GACTTCATTA TTCCGGATCA ATCAATGCCT GGAGCACCAA GGAGCCCTTT
1321 TCTTGATCA AGGTGGATCT GTTGGCACCA ATGATTATTC ACGGCATCAA GACCCAGGGT

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1381 GCCCCTCAGA AGTTCTCCAG CCTCTACATC TCTCAGTTTA TCATCATGTA TAGTCTTGAT
1441 GGGGAAGAAGT GGCAGACTTA TCGAGGAAAT TCCACTGGAA CCTTAATGGT CTTCTTTGGC
1501 AATGTGGATT CATCTGGGAT AAAACACAAT ATTTTAAACC CTCCAATTAT TGCTCGATAC
1561 ATCCGTTTGC ACCCAACTCA TTATAGCATT CGCAGCACTC TTCGCATGGA GTTGATGGGC
1621 TGTGATTTAA ATAGTTGCAG CATGCCATTG GGAATGGAGA GTAAAGCAAT ATCAGATGCA
1681 CAGATTACTG CTTTCATCCTA CTTTACCAAT ATGTTTGCCA CCTGGTCTCC TTCAAAAGCT
1741 CGACTTCACC TCCAAGGGAG GAGTAATGCC TGGAGACCTC AGGTGAATAA TCCAAAAGAG
1801 TGGCTGCAAG TGGACTTCCA GAAGACAATG AAAGTCACAG GAGTAACTAC TCAGGGAGTA
1861 AAATCTCTGC TTACCAGCAT GTATGTGAAG GAGTTCCTCA TCTCCAGCAG TCAAGATGGC
1921 CATCAGTGGA CTCTCTTTTT TCAGAATGGC AAAGTAAAGG TTTTTCAGGG AAATCAAGAC
1981 TCCTTCACAC CTGTGGTGAA CTCTCTAGAC CCACCGTTAC TGACTCGCTA CCTTCGAATT
2041 CACCCCAGA GTTGGGTGCA CCAGATTGCC CTGAGGATGG AGGTTCTGGG CTGCGAGGCA
2101 CAGGACCTCT ACGACAAAAC **TCACACATGC CCACCGTGCC CAGCTCCAGA ACTCCTGGGC**
2161 GGACCGTCAG TCTTCCTCTT CCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC
2221 CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC
2281 TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
2341 AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC
2401 AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCATCGA GAAAACCATC
2461 TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT
2521 GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
2581 ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
2641 GTGTTGGACT CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG
2701 TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
2761 ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAA

Table 2: Polypeptide Sequences

**A. B-Domain Deleted FVIII-Fc Monomer Hybrid (BDD FVIII-Fc monomer dimer):
created by coexpressing BDD FVIII-Fc and Fc chains.**

Construct = HC-LC-Fc fusion. An Fc expression cassette is cotransfected with BDDFVIII-Fc to generate the BDD FVIII-Fc monomer-. For the BDD FVIII-Fc chain, the Fc sequence is shown in bold; HC sequence is shown in double underline; remaining B domain sequence is shown in italics. Signal peptides are underlined.

i) B domain deleted FVIII-Fc chain (19 amino acid signal sequence underlined) (SEQ ID NO:2)

MQIELSTCFFLCLLRFCFS
 ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFNFNTSVVYKKTLFVEFTDHLFNIAPR
 PPWMGLLGPTIQAEVYDVTIVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
 GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGLAKEKTQTLHKF
 ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRSVYWHVIGM
 GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
 VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSVAKKHPKTWVHYIAEE
 EDWDYAPLVLPDDRYSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
 EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGKHLKDFPILPGEIFKYKWTVTVEDG
 PTKSDPRCLTRYSSFVNMERDLASGLIGPLLCYKESVDQGNQIMSDKRNVLFSVFDENRSW
 YLTENIQRFPLNPAGVQLEDPEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTDFLS
 VFFSGYTFKKHVMYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
 GDYEDSYEDISAYLLSKNNAIEPRSFSONPPVLKRHQREITRTTLQSDQEEIDYDDTISVEMKK
 EDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLNRNRAQSGSVPPQFKKVVFOEFT
 DGSFTQPLYRGELNEHLGLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPK
 NEVKPNETKTYFWKVQHMAPTKDEFDCKAWAYFSVDLEKDVHSGSLIGPLLCHTNTLNPAHGR
 QVTVQEFALFFTIFFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVM
 AQDQIRIRWYLLSMGSENENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPKAGIWRVE
 CLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGOYQWAPKLARLHYSGSINAWST
 KEPFSWIKVDLLAPMIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGN
 VDSSGIKHNI FNPIIARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASS
 YFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLTSMYVKE
 FLISSQDGHQWTLFFQNGKVVFQGNQDSFTPVVNSLDPPLLTRYLRHPQSWVHQIALRMEVL
 GCEAQDLYDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLY
 SKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK

ii) Fc chain (20 amino acid heterologous signal peptide from mouse Igk chain underlined) (SEQ ID NO:4)

METDTLLLWVLLLWVPGSTG
 DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT

LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK

**B. Full length FVIII_{FC} monomer hybrid (Full length FVIII_{FC} monomer dimer):
created by coexpressing FVIII_{FC} and Fc chains.**

Construct = HC-B-LC-Fc fusion. An Fc expression cassette is cotransfected with full length FVIII-Fc to generate the full length FVIII_{FC} monomer. For the FVIII_{FC} chain, the Fc sequence is shown in bold; HC sequence is shown in double underline; B domain sequence is shown in italics. Signal peptides are underlined.

i) Full length FVIII_{FC} chain (FVIII signal peptide underlined (SEQ ID NO:6))

MQIELSTCFFLCLLRFCFS
ATRRYYLGAVELSDYMQSDLGELPVDARFPPRPVPSFPFNTSVVYKKTLEFVEFTDHLFNIAPR
PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSVAKKHPKTWVHYIAAE
EDWDYAPLV LAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
PTKSDPRCLTRYISSFVNMERDLASGLIGPLLYCYKESVDQGNQIMSDKRNVLFSVFDENRSW
YLTENIQRF LNPAGVQLEDPEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
GDYYEDSYEDISAYLLSKNNAIEPRSFQNSRHPSTRQKQFNATTIPENDIEKTD PWFARHTPMP
KIQNVSSDLLMLLRQSPTPHGLSLSDLQEAKEYETFSDDPSPGAIDSNNSLSEMTHFRPQLHHS
DMVFTPESGQLRLNEKLGTTAATELKKLDFKVSSTSNLLISTIPSDNLAAGTDNTSSLGPPSMP
VHYDSQLD TTF LGKKSSPLTESGGPLSLSEENND SKLLESGLMNSQESSWGKNVSSTESGRLFKG
KRAHG PALLTKDNALFKVSI SLLKTNKTSNNSATNRKTHIDGPSLLIENSPSVWQNI LESTFEK
KVTPLIHDRMLMDKNATALRLNHMSNKTTSKNMEMVQQKKEGP IPPDAQNPDMSSFFKMLFLPES
ARWIQ RTHGKNSLNSGQGPSKQLVSLGPEKSVEGQNFLSEKNKVVVGKGFTKDVGLKEMVFP
SRNLFLTNDNLHENNTHNQEKIQEEIEKKETLIQENVVLPQIHTVTGTKNFMKNLFLSTRQN
VEGSYDGAYAPVLQDFRSLNDSTNRKHTAHFSKKGEEENLEGLGNQTKQIVEKYACTTRISP
TSQQNFVTQRSKRALKQFRLPLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEKGAI
TQSPLSDCLTRSHSIPQANRSP LPIAKVSSFPSIRPIYLTRVLFQDNSSHLPAASYRKD SGVQE
SSHFLQGA KKNLSLAILTLEMTGDQREVGS LGTSATNSVTYKKVENTVLPKPDLPKTS GKVELL
PKVHIYQKDLFPTETSNGSPGHLDLVEGSL LQGTEGAIKWNEANRPGKVPFLRVATESSAKTPSK
LLDPLAWDNHYGTQIPKEEWSQEKSP EKTAFKKKDTILSLNACESNHAIAAINEGQNKPEIEVT
WAKQGRTERLC SQNPVLKRHQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRS
FQKKTRHYFIAAVERLWDYGMSSSPHVLNRNAQSGSV PQFKKVVFQEFTDGSFTQPLYRGELNEH
LGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPKRFVKNPNETKTYFWKVQ
HMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDE
TKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQIRIRWYLLSMGSN
ENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLP SKAGIWRVECLIGEHLHAGMSTLFL
VYSNKCQTPLGMASGHIRDQITASGQYQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMI
IHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPII
ARYIRLHPHTYSISIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARL

HLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLTSMYVKEFLISSSQDGHQWTLFF
 QNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY**DKTHTCPP**
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

ii) Fc chain (20 amino acid heterologous signal peptide from mouse Igk chain underlined)
 (SEQ ID NO:4)

METDTLLLWVLLLWVPGSTG
 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
 RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

C. FVIII-Fc Heterodimer Hybrid

This is made by cotransfecting HC-Fc and LC-Fc constructs. Two HC-Fc constructs have been made. One has no linker between HC and Fc (HC-Fc) while the other has a 5 amino acid linker between HC and Fc (HC+5-Fc). The FVIII signal peptide was used for the HC-Fc constructs, while the mouse Igk signal sequence was used for the LC-Fc construct.

(i) HC-Fc (Fc sequence is shown in bold, signal peptide underlined) (SEQ ID NO:8)

MQIELSTCFFLCLLRFCFS
 ATRRYYLGAVELSWDYMQSDLGELPVDARFPFPRVPKSPFNTSVVYKKTTFVEFTDHLFNIAPR
 PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
 GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
 ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVMYWHVIGM
 GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLFCHISSHQHDGMEAYVK
 VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSVAKKHPKTWVHYIAAEE
 EDWDYAPLV LAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
 EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
 PTKSDPRCLTRYISSFVNMERDLASGLIGPLLYCYKESVDQRGNQIMSDKRNVLFSVFDENRSW
 YLTENIQRF LNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
 VFESGYTFKHKMVEYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
 GDYYEDSYEDISAYLLSKNNAIEPR**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT**
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

(ii) HC+5-Fc (Fc sequence is shown in bold, 5 amino acid linker sequence (from the B domain of FVIII) is shown in italics, signal peptide underlined.)(SEQ ID NO:10)

MQIELSTCFFLCLLRFCFS

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRPVPSFPPNTSVVYKKTLFVEFTDHLFNIAPR
 PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
 GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
 ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
 GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
 VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSVAKKHPKTWVHYIAAEE
 EDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
 EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
 PTKSDPRCLTRYSSFVNMERDLASGLIGPLLYCYKESVDQRGNQIMSDKRNVLFSVFDENRSW
 YLTENIQRFPLPNPAGVQLEDPEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTDFLS
 VFFSGYTFKHKMYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
 GDYYEDSYEDISAYLLSKNNAIEPRSFQMDKTHTCPPCPAPELLGGPSVFLFPPKPKD~~TL~~MI SR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

(iii) LC-Fc6His (Fc sequence is shown in bold, signal peptide underlined.) (SEQ ID NO:12)

METDTLLLWVLLLWVPGSTG

EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSS
 SPHVLRNRAQSGSVPQFKKVVFQEFDTGDSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQ
 ASRPYSFYSSLISYEEDQRQGAEPKRFVKNPNETKTYFWKVQHMAPTKDEFDCWAYFSDVDL
 EKDVHSGLLIGPLLCHTNTLNPAHGRQVTQEFALFFTI FDETKSWYFTENMERNCRAPCNIQME
 DPTFKENYRFHAINGYIMDTLPGLVMAQDQIRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKM
 ALYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQIT
 ASGQYQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMI IHGIKTQGARQKFSSLYISQFII
 MYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNI FNPPIIARYIRLHPHTHYSIRSTLRMELMG
 CDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVD
 FQKTMKVTGVTQGVKSLTSMYVKEFLISSQDGHQWTLFFQNGKVVFQGNQDSFTPVVNSLD
 PPLLTRYLRHPQSWVHQIALRMEVLGCEAQDLYDKTHTCPPCPAPELLGGPSVFLFPPKPKD~~TL~~
MI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
 K

Table 3. Whole blood clotting time (WBCT) determination in hemophilia A mice after a single intravenous dose of 50 IU/kg rFVIII^h or ReFacto®.

A.

		Time of Blood Collection, hr							
Treatment	Animal Number	Pre-dose	0.25	24	36	42	96	113	120
		WBCT, min							
50 IU/kg ReFacto®	1	>60	18	>60	ND	ND			
	2	>60	5	16	>60	ND			
	3	>60	4	7	>60	ND			
	4	>60	7	8	10	>60			
	5	>60	6	9	16	>60			
	6	>60	5	15	>60	ND			
50 IU/kg rFVIIIIFc	7	>60	7				8	>60	ND
	8	>60	5				8	>60	ND
	9	>60	4				16	>60	ND
	10	>60	3				11	4	>60
	11	>60	3				9	>60	ND
	12	>60	4				6	>60	ND

ND = not determined since previous time point was >60 min

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B.

Treatment	Animal Number	Time of Blood Collection, hr					
		Pre-dose	0.25	24	48	96	120
		WBCT, min					
50 IU/kg ReFacto®	1	>60	11	15	>60	>60	ND
	2	>60	3	3	>60	>60	>60
	3	>60	4	6	>60	>60	>60
50 IU/kg rFVIII Fc	4	>60	3	5	5	>60	>60
	5	>60	3	6	7	13	>60
	6	>60	5	8	9	9	>60

ND = Not determined since previous time point was >60 min

Table 4. PK Parameters after a single intravenous dose in hemophilia A mice (50 IU/kg)

Treatment	C _{max} (IU/mL)	AUC (hr·IU/mL)	T _{1/2} (hr)	CL (mL/hr/kg)	V _{ss} (mL/kg)
rFVIII-Fc	1.56	22.6	11.1	2.09	28.4
ReFacto®	0.67	6.94	5.0	7.2	43.8
Advate®	0.47	3.90	7.1	12.8	103

Table 5. PK Parameters after a single intravenous dose in hemophilia A dogs
(125 IU/kg rFVIII Fc, 114 and 120 IU/kg ReFacto®)

A. PK determined from chromogenic activity data

Treatment	C _{max} (IU/mL)	AUC (hr·IU/mL)	T _{1/2} (hr)	CL (mL/hr/kg)	V _z (mL/kg)
rFVIII Fc	2.0 ± 0.54	25.9 ± 6.47	15.4 ± 0.3	5.1 ± 1.4	113 ± 29
ReFacto®*	2.0	18.2	7.4	6.5	68.7

B. PK determined from ELISA data

Treatment	C _{max} (ng/mL)	AUC (hr·ng/mL)	T _{1/2} (hr)	CL (mL/hr/kg)	V _z (mL/kg)
rFVIII Fc	210 ± 33	2481 ± 970	15.7 ± 1.7	6.2 ± 3.0	144 ± 83
ReFacto®*	211	1545	6.9	8.7	85

Mean ± sd, n = 4 for rFVIII Fc, n = 2 for ReFacto®

*sd not reported for ReFacto® since there were just two dogs

Table 6. Clotting activity measured by aPTT in hemophilia A dogs after a single intravenous dose with rFVIII^{IFc} or ReFacto®.

Dog ID	Treatment	aPTT, sec	
		PreDose	5 min post dose
M10	rFVIII ^{IFc}	86.5	53.6
M11	rFVIII ^{IFc}	99.8	56.4
M12	rFVIII ^{IFc}	119	68.7
	ReFacto®	108	60.7
M38	rFVIII ^{IFc}	115	76.6
	ReFacto®	118	68.0

Table 7. Plasma Concentration of rFVIII^h or Xyntha in monkeys administered as a single intravenous dose of 125 IU/kg measured by **ELISA**.

A. rFVIII^h concentration in plasma (µg/mL)

Time, hr	Group 1			Group 2			Mean	SD
	604376	606595	C36195	C36066	C36174	604362		
Pre	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ		
0.25	0.400	0.334	0.374	0.348	0.383	0.323	0.360	0.030
4	0.266	0.259	0.236	0.233	0.259	0.217	0.245	0.019
12	0.165	0.152	0.12	0.15	0.161	0.149	0.150	0.016
24	0.079	0.074	0.047	0.08	0.088	0.076	0.074	0.014
36	0.035	0.04	0.022	0.04	0.041	0.046	0.037	0.008
48	0.019	0.021	BLQ	0.021	0.024	0.025	0.022	0.002

B. Xyntha concentration in plasma (µg/mL)

Time, hr	Group 1			Group 2			Mean	SD
	604376	606595	C36195	C36066	C36174	604362		
Pre	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ		
0.25	0.252	0.074	0.155	0.317	0.217	0.167	0.197	0.084
4	0.197	0.159	0.152	0.229	0.19	0.082	0.168	0.051
12	0.137	0.099	0.104	0.166	0.158	0.081	0.124	0.035
24	0.09	0.068	0.051	0.082	0.08	0.084	0.076	0.014
36	0.037	0.043	0.015	0.041	0.035	BLQ	0.034	0.011
48	0.022	BLQ	BLQ	0.017	0.013	BLQ	0.017	0.005

Table 8. Plasma Concentration of rFVIII_h or Xyntha in monkeys administered a single intravenous dose of 125 IU/kg measured by the **FVIII-specific chromogenic activity assay** (reported in IU/mL).

A. Xyntha

Time (hr)	Group 1			Group 2		
Predose	604376	606595	C36195	C36066	C36174	604362
0.25	5.62	4.55	5.01	4.5	5.15	3.77
4	3.9	4.05	3.2	3.19	3.46	2.36
12	2.51	2.82	1.69	2.17	2.5	2.01
24	1.67	1.66	1.18	0.95	1.57	1.5
36	0.7	0.85	0.48	0.44	0.85	0.82
48	BLQ	BLQ	BLQ	BLQ	0.38	0.48

B. rFVIII_h

Time (hr)	Group 1			Group 2		
Predose	604376	606595	C36195	C36066	C36174	604362
0.25	4.31	3.82	3.54	4.13	4.12	3.68
4	3	3.36	2.53	2.7	2.74	2.81
12	2	2.15	1.42	2.28	2.75	2.22
24	1.01	1.17	0.5	1.5	1.61	1.01
36	BLQ	0.52	0.48	0.88	0.72	0.64
48	0.31	BLQ	BLQ	BLQ	BLQ	BLQ
72	BLQ	BLQ	BLQ	BLQ	0.31	BLQ

BLQ = below the limit of quantitation

Table 9. PK Parameters of **rFVIIIIFc** after a single 125 IU/kg dose

PK Parameter	rFVIIIIFc ELISA Data								
	units	Group 1			Group 2			Average	SD
		604376	606595	C36195	C36066	C36174	604362		
T _{max}	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.00
C _{max}	µg/mL	0.4	0.334	0.374	0.348	0.383	0.323	0.368	0.030
T _{1/2}	hr	11.4	13.3	9.3	12.7	12.7	14.1	11.9	1.7
AUC	µg*hr/mL	5.86	5.65	4.37	5.56	4.37	5.58	5.16	0.68
CL	mL/hr/kg	2.15	2.23	2.88	2.27	2.07	2.26	2.32	0.29
V _z	mL/kg	35.3	42.5	38.8	37.9	37.9	46.1	38.5	3.9
MRT	hr	15.3	17	12.1	17.1	17.3	19.2	15.8	2.4

PK Parameter	rFVIIIIFc Chromogenic Activity Data								
	units	Group 1			Group 2			Average	SD
		604376	606595	C36195	C36066	C36174	604362		
T _{max}	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.00
C _{max}	IU/mL	4.31	3.82	3.54	4.13	4.12	3.68	3.93	0.30
T _{1/2}	hr	13.4	12.0	11.6	17.5	12.4	29.4	16.1	6.9
AUC	IU*hr/mL	74.7	75.5	53.5	92.9	88.9	92.7	79.7	15.2
CL	mL/hr/kg	1.67	1.65	2.34	1.35	1.41	1.35	1.63	0.38
V _z	mL/kg	32.3	28.7	39.2	33.9	25.2	57.2	36.1	11.4
MRT	hr	17.8	16.8	16.9	25	19.2	33.3	21.5	6.5

Table 10. PK Parameters of **Xyntha** after a single IV dose (125 IU/kg)

PK Parameter	Xyntha ELISA Data								
	units	Group 1			Group 2			Average	SD
		604376	606595	C36195	C36066	C36174	604362		
T _{max}	hr	0.25	4	0.25	0.25	0.25	0.25	0.88	1.53
C _{max}	IU/mL	0.252	0.159	0.155	0.317	0.217	0.167	0.21	0.06
T _{1/2}	hr	13.6	19.9	9.7	11	9.2	nd	12.7	4.4
AUC	IU*hr/mL	5.15	4.39	3.17	5.53	4.79	6.32	5.24	0.74
CL	mL/hr/kg	2.21	2.6	3.59	2.06	2.38	nd	2.57	0.61
V _z	mL/kg	43.4	74.7	50.1	32.9	31.5	nd	46.5	17.5
MRT	hr	19	28.4	14	16.1	15.9	nd	18.7	5.7

PK Parameter	Xyntha Chromogenic Activity Data								
	units	Group 1			Group 2			Average	SD
		604376	606595	C36195	C36066	C36174	604362		
T _{max}	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0
C _{max}	IU/mL	5.62	4.55	5.01	4.5	5.15	3.77	4.77	0.64
T _{1/2}	hr	12.8	14.3	11.4	10.4	11.7	14.6	12.5	1.7
AUC	IU*hr/mL	97.1	104.2	71.3	70.7	94.0	82.8	86.7	14.0
CL	mL/hr/kg	1.29	1.20	1.75	1.77	1.33	1.51	1.48	0.24
V _z	mL/kg	23.7	24.8	28.9	26.6	22.5	31.8	26.4	3.5
MRT	hr	17.8	20.1	16.0	14.8	18.4	23.2	18.4	3.0

Table 11. Activation of Factor X

	K _m (nM)	V _{max} (nM/min)
rFVIII _{IFc}	55.0 ± 5.9	65.6 ± 8.6
BDD FVIII	51.0 ± 8.7	73.5 ± 10.1

Table 12. Interaction with Factor IXa

	K _d (nM)	V _{max} (nM/min)
rFVIII _{IFc}	2.8 ± 0.4	4.5 ± 0.3
BDD FVIII	2.5 ± 0.3	4.0 ± 1.0

WHAT IS CLAIMED IS:

1. A method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric polypeptide comprising a Factor VIII portion and a second portion at a dosing interval at least about one and one-half times longer than the dosing interval required for an equivalent amount of a polypeptide consisting of said Factor VIII portion.
2. The method of claim 1, wherein said chimeric polypeptide comprises a Fc portion.
3. The method of claim 1 or 2, where said dosing interval is at least about one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer, than the dosing interval required for an equivalent amount of a polypeptide consisting of said Factor VIII portion.
4. The method of claim 3, where said dosing interval is at least about one and one-half, two, two and one-half, three, three and one-half, four, four and one-half, five, five and one-half or six times longer than the dosing interval required for an equivalent amount of a polypeptide consisting of said Factor VIII portion.
5. The method of any of claims 1-4, wherein said dosing interval of said chimeric polypeptide is about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.
6. The method of any of claims 1-5, wherein said subject is in need of prophylactic treatment.
7. The method of any of claims 1-4, wherein said subject is in need of on-demand treatment.
8. The method of claim 7, wherein said subject is in need of treatment for a bleeding episode.
9. The method of claim 8, 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 iliopsoas sheath.
10. The method of claim 7, wherein said subject is in need of surgical prophylaxis, peri-operative management, or treatment for surgery.

11. The method of claim 10, 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.

12. The method of any of claims 7-11, wherein said dosing interval of said chimeric polypeptide is about once every 24-36, 24-48, 24-72, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 hours or longer.

13. The method of any of claims 1-12, wherein said therapeutic dose is 10-100 IU/kg.

14. The method of claim 12, wherein said therapeutic dose is 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg.

15. The method of claim 13, wherein said therapeutic dose is 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.

16. The method of any of claims 1-15, wherein said subject is human.

17. The method of any of claims 1-16, wherein said Factor VIII is human Factor VIII.

18. The method of any of claims 1-17, wherein said Factor VIII has a full or partial deletion of the B domain.

19. The method of claim 17, wherein the Factor VIII portion of said chimeric polypeptide is at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

20. The method of claim 19, wherein the Factor VIII portion of said chimeric polypeptide is identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

21. The method of claim 17, wherein the Factor VIII portion of said chimeric polypeptide is at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

22. The method of claim 21, wherein the Factor VIII portion of said chimeric polypeptide is identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

23. The method of claim 17 or 18, wherein said second portion of said chimeric polypeptide is at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

24. The method of claim 23, wherein said second portion of said chimeric polypeptide is identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

25. The method of any of claims 1-24, 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 consists essentially of an Fc.

26. The method of claim 25, wherein said chimeric polypeptide comprises a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

27. The method of claim 26, wherein said chimeric polypeptide comprises a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

28. The method of any of claims 25-27, wherein said second polypeptide consists essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

29. The method of claim 28, wherein said second polypeptide consists essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) without a signal sequence (amino acids 1 to 227 of SEQ ID NO:4) or identical to the amino acid sequence shown in Table 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

30. The method of any of claims 1-29, wherein said chimeric polypeptide is administered as part of a pharmaceutical composition comprising at least one excipient.

31. A method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a chimeric polypeptide comprising a Factor VIII portion and a second portion to obtain an area under the plasma concentration versus time curve (AUC) at least about one and one-quarter times greater than the AUC obtained by an equivalent amount of a polypeptide consisting of said Factor VIII portion.

32. The method of claim 31, wherein said chimeric polypeptide is administered at a dosing interval of about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

33. The method of any of claims 31-32, wherein said subject is in need of prophylactic treatment.

34. The method of claim 31, wherein said subject is in need of on-demand treatment.

35. The method of claim 32, wherein said subject is in need of treatment for a bleeding episode.

36. The method of claim 33, 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 iliopsoas sheath.

37. The method of claim 34, wherein said subject is in need of surgical prophylaxis, peri-operative management, or treatment for surgery.

38. The method of claim 37, 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.

39. The method of any of claims 34-38, wherein said dosing interval of said chimeric polypeptide is about once every 24-36, 24-48, 24-72, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 hours or longer.

40. The method of any of claims 31-39, wherein said therapeutic dose is 10-100 IU/kg.

41. The method of claim 40, wherein said therapeutic dose is 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg.

42. The method of claim 41, wherein said therapeutic dose is 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.

43. The method of any of claims 31-42, wherein said subject is human.

44. The method of any of claims 31-43, wherein said Factor VIII is human Factor VIII.

45. The method of any of claims 31-44, wherein said Factor VIII has a full or partial deletion of the B domain.

46. The method of claim 44, wherein the Factor VIII portion of said chimeric polypeptide is at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

47. The method of claim 46, wherein the Factor VIII portion of said chimeric polypeptide is identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

48. The method of claim 44, wherein the Factor VIII portion of said chimeric polypeptide is at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

49. The method of claim 48, wherein the Factor VIII portion of said chimeric polypeptide is identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to

740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

50. The method of claim 43 or 44, wherein said second portion of said chimeric polypeptide is at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

51. The method of claim 50, wherein said second portion of said chimeric polypeptide is identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

52. The method of any of claims 31-51, 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 consists essentially of an Fc.

53. The method of claim 52, wherein said chimeric polypeptide comprises a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

54. The method of claim 53, wherein said chimeric polypeptide comprises a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

55. The method of any of claims 52-54, wherein said second polypeptide consists essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

56. The method of claim 55, wherein said second polypeptide consists essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

57. The method of any of claims 31-56, wherein said chimeric polypeptide is administered as part of a pharmaceutical composition comprising at least one excipient.

58. A method of administering Factor VIII to a subject in need thereof, comprising administering to the subject a therapeutic dose of a polypeptide comprising a Factor VIII and an Fc at a dosing interval of about every five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen days or longer.

59. The method of any of claim 58, wherein said subject is in need of prophylactic treatment.

60. The method of any of claims 58-59, wherein said therapeutic dose is 10-100 IU/kg.

61. The method of claim 60, wherein said therapeutic dose is 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 IU/kg.

62. The method of claim 61, wherein said therapeutic dose is 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 IU/kg.

63. The method of any of claims 58-62, wherein said subject is human.

64. The method of any of claims 58-63, wherein said Factor VIII is human Factor VIII.

65. The method of any of claims 58-64, wherein said Factor VIII has a full or partial deletion of the B domain.

66. The method of claim 64, wherein said Factor VIII is at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

67. The method of claim 66, wherein said Factor VIII is identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

68. The method of claim 64, wherein said Factor VIII is at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

69. The method of claim 68, wherein said Factor VIII is identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

70. The method of claim 64 or 65, wherein said second portion is at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

71. The method of claim 70, wherein said second portion is identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

72. The method of any of claims 58-71, wherein said chimeric polypeptide is a Factor VIII-Fc chimeric polypeptide in the form of a hybrid comprising a second polypeptide in association with said chimeric polypeptide, wherein said second polypeptide consists essentially of an Fc.

73. The method of claim 72, wherein said chimeric polypeptide comprises a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

74. The method of claim 73, wherein said chimeric polypeptide comprises a sequence identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

75. The method of any of claims 72-74, wherein said second polypeptide consists essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

76. The method of claim 75, wherein said second polypeptide consists essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

77. The method of any of claims 58-76, wherein said polypeptide is administered as part of a pharmaceutical composition comprising at least one excipient.

78. A polypeptide comprising a Factor VIII at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12), and an Fc.

79. The polypeptide of claim 78, wherein said Factor VIII is identical to a Factor VIII amino acid sequence shown in Table 2 without a signal sequence (amino acids 1 to 1438 of SEQ ID NO:2; amino acids 1 to 2332 of SEQ ID NO:6; amino acids 1 to 740 of SEQ ID NO:8; amino acids 1 to 745 of SEQ ID NO:10; or amino acids 1 to 684 of SEQ ID NO:12).

80. A polypeptide comprising a Factor VIII at least 90% or 95% identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12), and an Fc.

81. The polypeptide of claim 80, wherein said Factor VIII is identical to a Factor VIII amino acid sequence shown in Table 2 with a signal sequence (amino acids -19 to 1438 of SEQ ID NO:2; amino acids -19 to 2332 of SEQ ID NO:6; amino acids -19 to 740 of SEQ ID NO:8; amino acids -19 to 745 of SEQ ID NO:10; or amino acids -20 to 684 of SEQ ID NO:12).

82. The polypeptide of any of claims 78 to 81, wherein said Fc is at least 90% or 95% identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12)

83. The polypeptide of claim 82, wherein said Fc is identical to the Fc amino acid sequence shown in Table 2 (amino acids 1439 to 1665 of SEQ ID NO:2; amino acids 2333 to 2559 of SEQ ID NO:6; amino acids 741 to 967 of SEQ ID NO:8; amino acids 746 to 972 of SEQ ID NO:10; amino acids 685 to 924 of SEQ ID NO:12).

84. The polypeptide of claim 78, which comprises a sequence at least 90% or 95% identical to the Factor VIII and Fc amino acid sequences shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

85. The polypeptide of claim 84, which comprises a sequence identical to the Factor VIII and Fc amino acid sequences shown in Table 2A(i) without a signal sequence (amino acids 1 to 1665 of SEQ ID NO:2) or at least 90% or 95% identical to the Factor VIII and Fc amino acid sequence shown in Table 2A(i) with a signal sequence (amino acids -19 to 1665 of SEQ ID NO:2).

86. The polypeptide of any of claims 78-85, which is in the form of a hybrid comprising a second polypeptide, wherein said second polypeptide consists essentially of an Fc.

87. The polypeptide of claim 85, wherein said second polypeptide consists essentially of a sequence at least 90% or 95% identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

88. The polypeptide of claim 86, wherein said second polypeptide consists essentially of a sequence identical to the amino acid sequence shown in Table 2A(ii) 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 2A(ii) with a signal sequence (amino acids -20 to 227 of SEQ ID NO:4).

89. The polypeptide of any of claims 78-88, which has a half-life at least one and one-half to six times longer, one and one-half to five times longer, one and one-half to four times longer, one and one-half to three times longer, or one and one-half to two times longer to a polypeptide consisting of said Factor VIII.

90. A polynucleotide encoding the polypeptide of any of claims 78 -85.

91. The polynucleotide of claim 90, which comprises the Factor VIII-Fc nucleotide sequences of Table 1 (SEQ ID NOs:1, 5, 7, 9, or 11).

92. A polynucleotide encoding the Factor VIII-Fc polypeptide and the second peptide of any one of claims 86-89.

93. The polynucleotide of any of claims 90-92, which is a vector, plasmid, phage, or virus.

94. The polynucleotide of any of claims 90-93, which is DNA or RNA.

95. A cultured human embryonic cell comprising the polynucleotide of any one of claims 89-94.

96. The cell of claim 95, which is a HEK293 cell.

97. A method of producing a FactorVIII-Fc hybrid protein comprising culturing the cell of claim 95 or 96 under conditions that allow expression of the encoded Factor VIII-Fc chimeric polypeptide and the encoded polypeptide consisting essentially of Fc; and

recovering the encoded FactorVIII-Fc hybrid protein.

98. A protein produced by the method of claim 97.

99. The method of any one of claims 1-57, wherein said chimeric polypeptide has one or more characteristics selected from the group consisting of:

an ability to interact with phospholipid vesicles comparable to the ability of a polypeptide consisting of said Factor VIII portion;

an ability to form a Xase complex that activates Factor X comparable to the ability of a polypeptide consisting of said Factor VIII portion;

an ability to be activated by alpha-thrombin within five minutes comparable to the ability of a polypeptide consisting of said Factor VIII portion; and

an ability to interact with Factor IXa comparable to the ability of a polypeptide consisting of said Factor VIII portion.

100. The method of claim 99, wherein said chimeric polypeptide has one or more characteristics selected from the group consisting of:

forms a Xase complex that activates Factor X at a K_m within about one, about one and one half, or about two standard deviations of the K_m of a polypeptide consisting of said Factor VIII portion, wherein K_m is measured as a function of Factor X concentration;

forms a Xase complex that activates Factor X at a V_{max} within about one, about one and one half, or about two standard deviations of the V_{max} of a polypeptide consisting of said Factor VIII portion, wherein V_{max} is measured as a function of Factor X concentration;

forms a Xase complex that activates Factor X at a K_d within about one, about one and one half, or about two standard deviations of the K_d of a polypeptide consisting of said Factor VIII portion, wherein K_d is measured as a function of Factor IXa concentration; and

forms a Xase complex that activates Factor X at a V_{max} within about one, about one and one half, or about two standard deviations of the V_{max} of a polypeptide consisting of said Factor VIII portion, wherein V_{max} is measured as a function of Factor IXa concentration.

101. The method of any one of claims 58-77, wherein said polypeptide has one or more characteristics selected from the group consisting of:

an ability to interact with phospholipid vesicles comparable to the ability of a polypeptide consisting of said Factor VIII portion;

an ability to form a Xase complex that activates Factor X comparable to the ability of a polypeptide consisting of said Factor VIII portion;

an ability to be activated by alpha-thrombin within five minutes comparable to the ability of a polypeptide consisting of said Factor VIII portion; and

an ability to interact with Factor IXa comparable to the ability of a polypeptide consisting of said Factor VIII portion.

102. The method of claim 101, wherein said polypeptide has one or more characteristics selected from the group consisting of:

forms a Xase complex that activates Factor X at a K_m within about one, about one and one half, or about two standard deviations of the K_m of a polypeptide consisting of said Factor VIII portion, wherein K_m is measured as a function of Factor X concentration;

forms a Xase complex that activates Factor X at a V_{max} within about one, about one and one half, or about two standard deviations of the V_{max} of a polypeptide consisting of said Factor VIII portion, wherein V_{max} is measured as a function of Factor X concentration;

forms a Xase complex that activates Factor X at a K_d within about one, about one and one half, or about two standard deviations of the K_d of a polypeptide consisting of said Factor VIII portion, wherein K_d is measured as a function of Factor IXa concentration; and

forms a Xase complex that activates Factor X at a V_{max} within about one, about one and one half, or about two standard deviations of the V_{max} of a polypeptide consisting of said Factor VIII portion, wherein V_{max} is measured as a function of Factor IXa concentration.

103. The polypeptide of any one of claims 78-89 and 98, which has one, about one and one half, or about two or more characteristics selected from the group consisting of:

an ability to interact with phospholipid vesicles comparable to the ability of a polypeptide consisting of said Factor VIII portion;

an ability to form a Xase complex that activates Factor X comparable to the ability of a polypeptide consisting of said Factor VIII portion;

an ability to be activated by alpha-thrombin within five minutes comparable to the ability of a polypeptide consisting of said Factor VIII portion; and

an ability to interact with Factor IXa comparable to the ability of a polypeptide consisting of said Factor VIII portion.

104. The method of claim 101, wherein said polypeptide has one or more characteristics selected from the group consisting of:

forms a Xase complex that activates Factor X at a K_m within about one, about one and one half, or about two standard deviations of the K_m of a polypeptide consisting of said Factor VIII portion, wherein K_m is measured as a function of Factor X concentration;

forms a Xase complex that activates Factor X at a V_{max} within about one, about one and one half, or about two standard deviations of the V_{max} of a polypeptide consisting of said Factor VIII portion, wherein V_{max} is measured as a function of Factor X concentration;

forms a Xase complex that activates Factor X at a K_d within about one, about one and one half, or about two standard deviations of the K_d of a polypeptide consisting of said Factor VIII portion, wherein K_d is measured as a function of Factor IXa concentration; and

forms a Xase complex that activates Factor X at a V_{max} within about one, about one and one half, or about two standard deviations of the V_{max} of a polypeptide consisting of said Factor VIII portion, wherein V_{max} is measured as a function of Factor IXa concentration.

105. The method of any of claims 1-77, wherein said dose has a mean incremental recovery (K-Value) (activity; observed) greater than 1.38 IU/dL per IU/kg.

106. The method of any of claims 1-77 and 104, wherein said dose has a mean incremental recovery (K-Value) (activity; observed) of at least about 1.5, at least about 1.85, or at least about 2.46 IU/dL per IU/kg.

107. The method of any of claims 1-75 and 104-106, 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 2.33 ± 1.08 mL/hour/kg or less;

a mean clearance (CL) (activity) in said patient population of about 1.8-2.69 mL/hour/kg;
a mean clearance (CL) (activity) in said patient population that is about 65% of the clearance of a polypeptide consisting of said Factor VIII portion;
a clearance (CL) (activity) in said subject of about 1.22 - 5.19 mL/hour/kg
a mean mean residence time (MRT) (activity) in said patient population of at least about 26.3 ± 8.33 hours;
a mean MRT (activity) in said patient population of about 25.9 - 26.5 hours;
a mean MRT (activity) in said patent population that is about 1.5 fold longer than the mean MRT of a polypeptide consisting of said Factor VIII portion;
a mean residence time (MRT) (activity) in said subject of about 14 – 41.3 hours;
a mean $t_{1/2\beta}$ (activity) in said patient population of about 18.3 ± 5.79 hours;
a mean $t_{1/2\beta}$ (activity) in said patient population that is about 18 - 18.4 hours;
a mean $t_{1/2\beta}$ (activity) in said patient population that is about 1.5 fold longer than the mean $t_{1/2\beta}$ of a polypeptide consisting of said Factor VIII portion;
a $t_{1/2\beta}$ (activity) in said subject of about 11 - 26.4 hours;
a mean incremental recovery (K value) (activity; observed) in said patient population of about 2.01 ± 0.44 IU/dL per IU/kg;
a mean incremental recovery (K value) (activity; observed) in said patient population of about 1.85 - 2.46 IU/dL per IU/kg;
a mean incremental recovery (K value) (activity; observed) in said patient population that is about 90 % of the mean incremental recovery of a polypeptide consisting of said Factor VIII portion;
an incremental recovery (K value) (activity; observed) in said subject of about 1.38 - 2.88 IU/dL per IU/kg;
a mean V_{ss} (activity) in said patient population of about 55.1 ± 12.3 mL/kg;
a mean V_{ss} (activity) in said patient population of about 45.3 - 56.1 mL/kg;
a V_{ss} (activity) in said subject of about 37.7 - 79.4 mL/kg;
a mean AUC/dose (activity) in said patient population of about 49.9 ± 18.2 IU*h/dL per IU/kg;
a mean AUC/dose (activity) in said patient population of about 44.8 - 57.6 IU*h/dL per IU/kg; and
an AUC/dose in said subject of about 19.2 - 81.7 IU*h/dL per IU/kg.

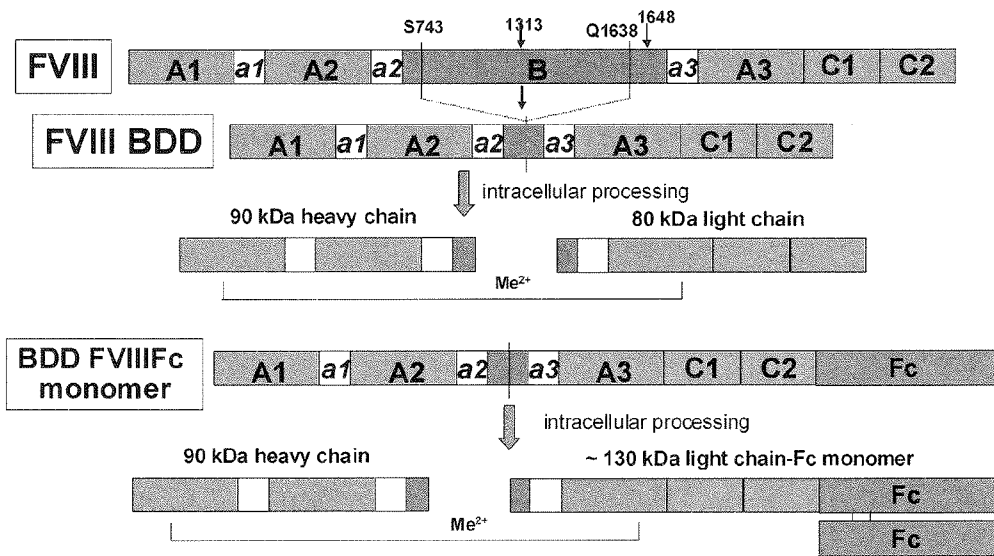
108. The method of any of claims 1-77 and 104-106, wherein said second portion is XTEN or albumin.

109. The method of any of claims 1-77 and 104-106, wherein the therapeutic dose is about 10 to about 150, 100-110, 110-120, 120-130, 130-140, 140-150, 110, 115, 120, 125, 130, 135, 140, 145, or 150 IU/kg.

110. The method of any of claims 1-77 and 104-106, wherein the dosing interval is one and one-half to 5, one and one-half, 2, 3, 4, or 5 days or longer.

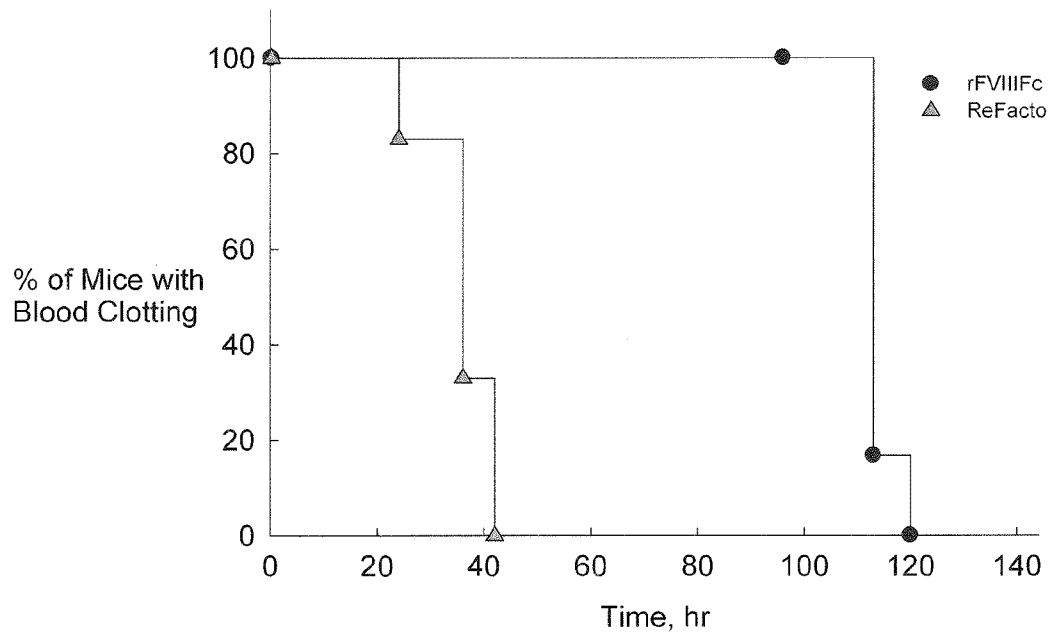
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Figure 1



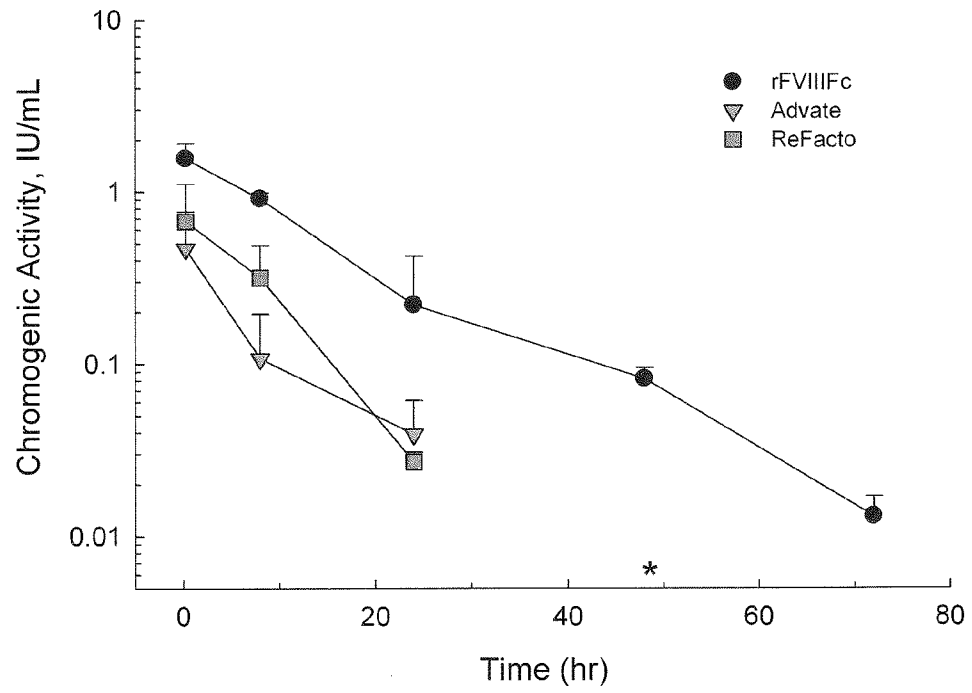
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Figure 2



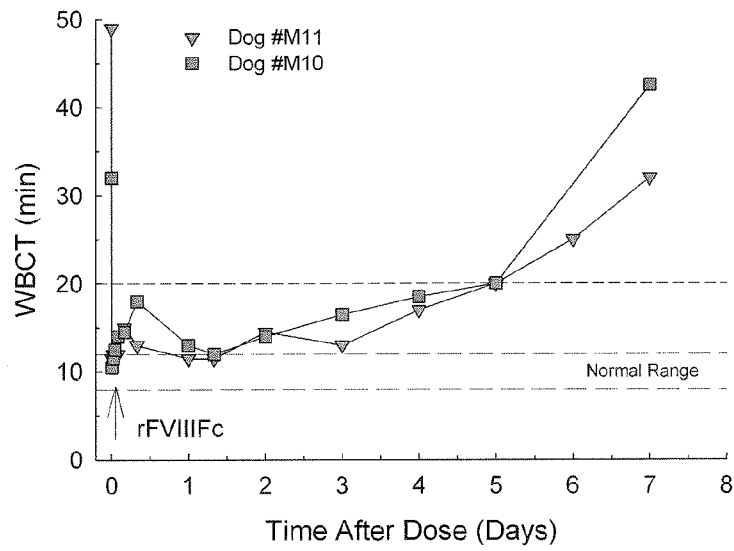
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Figure 3



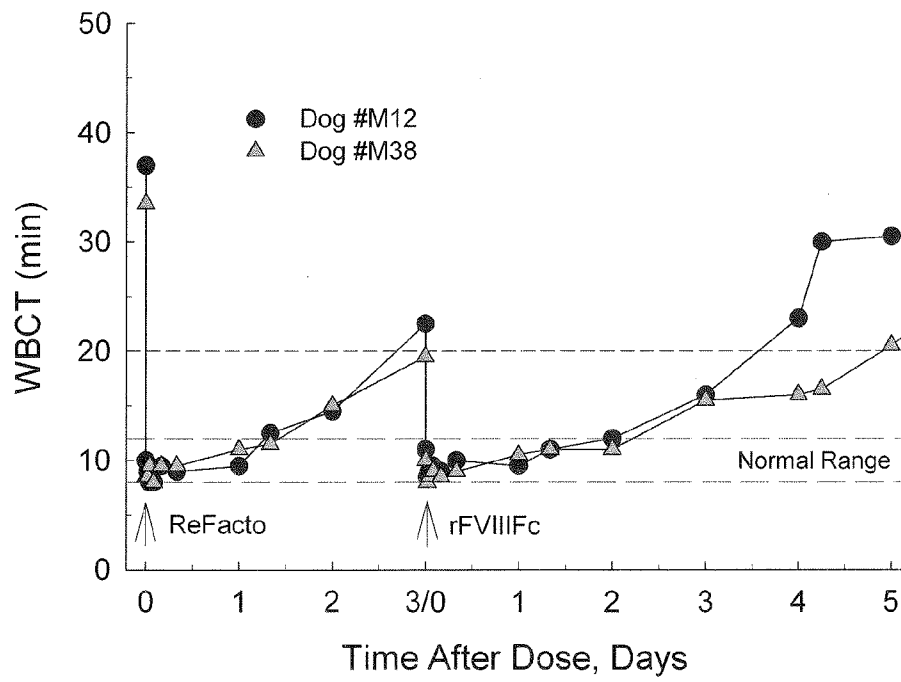
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Figure 4A



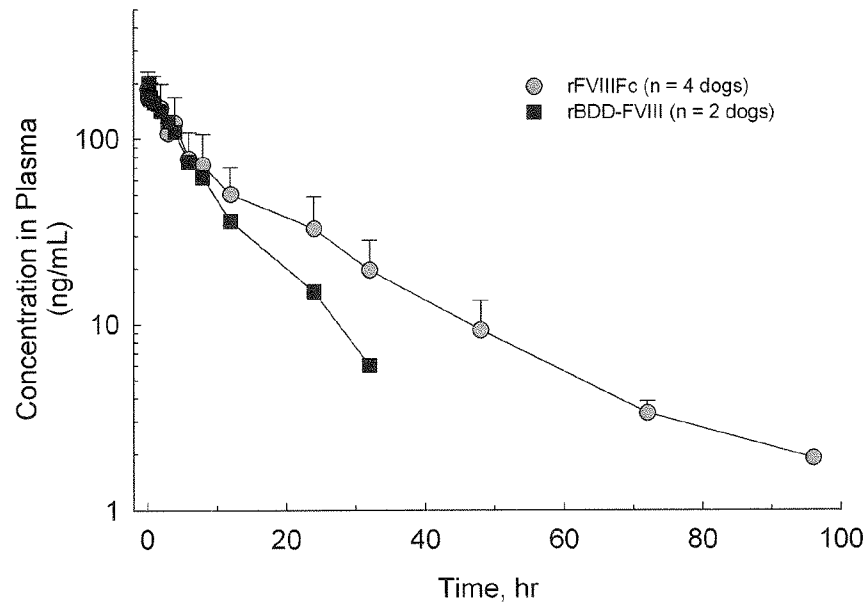
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Figure 4B



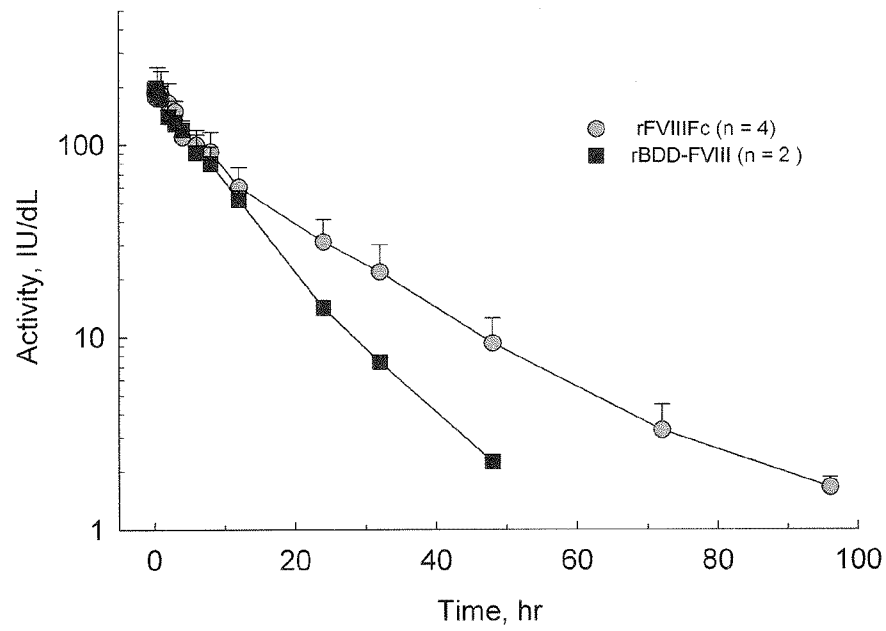
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Figure 5



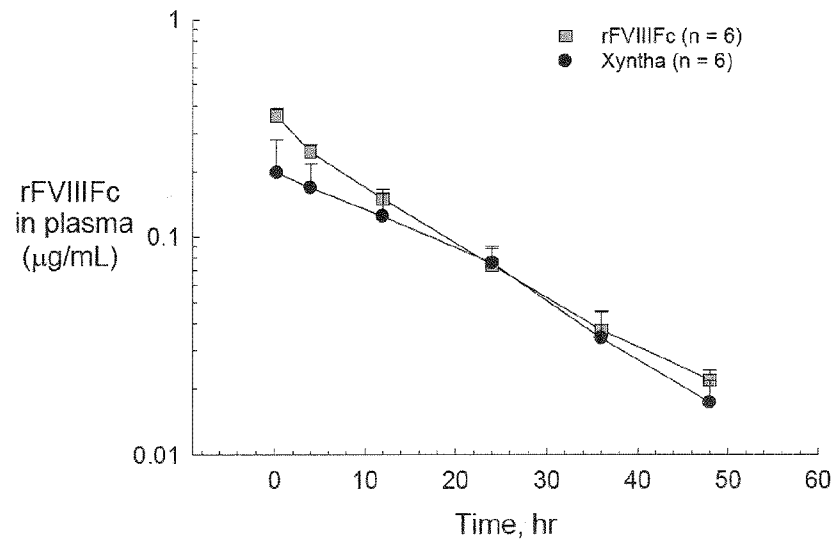
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Figure 6



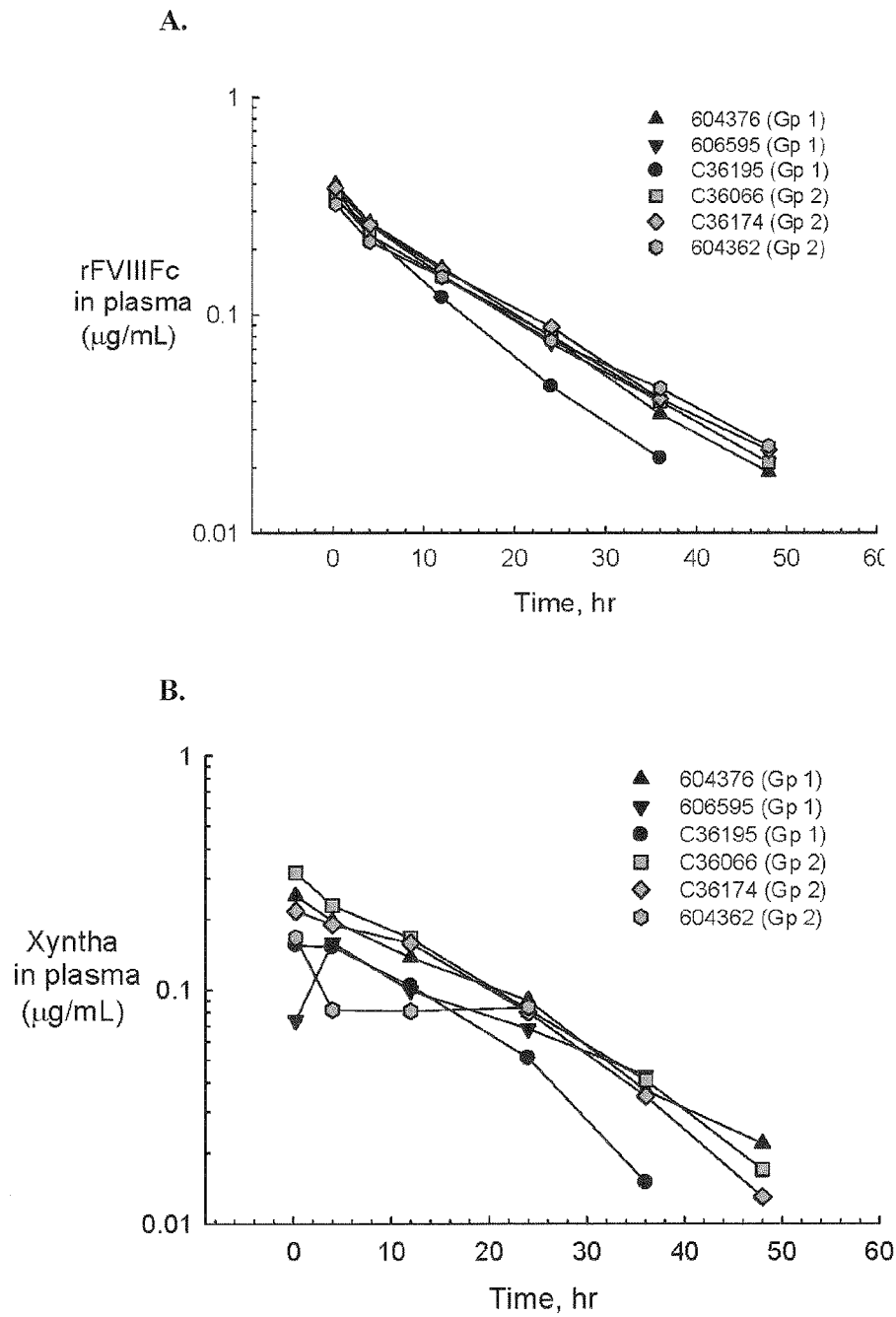
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Figure 7



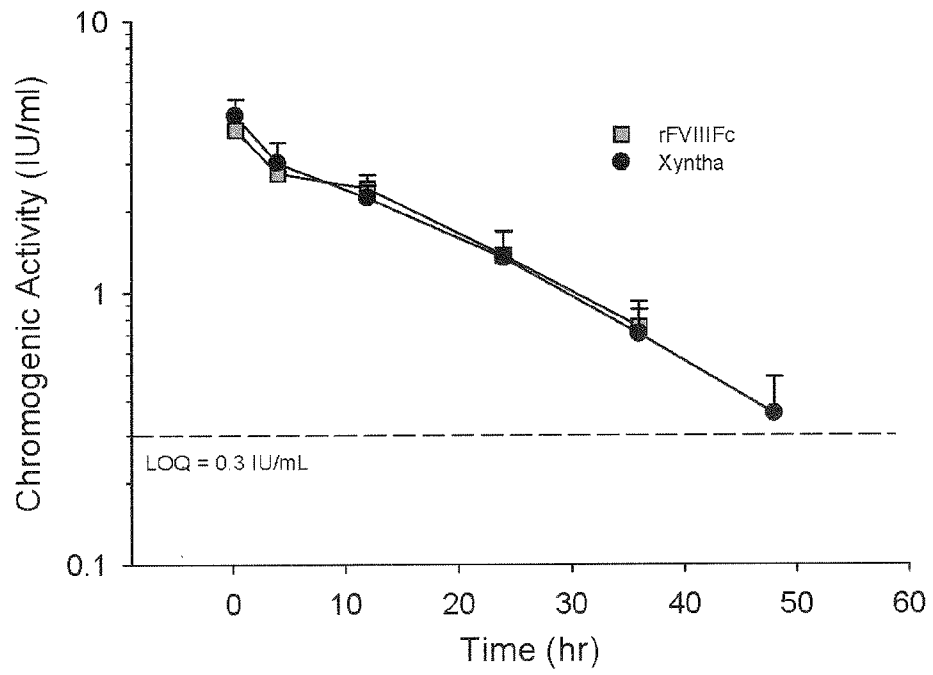
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Figure 8



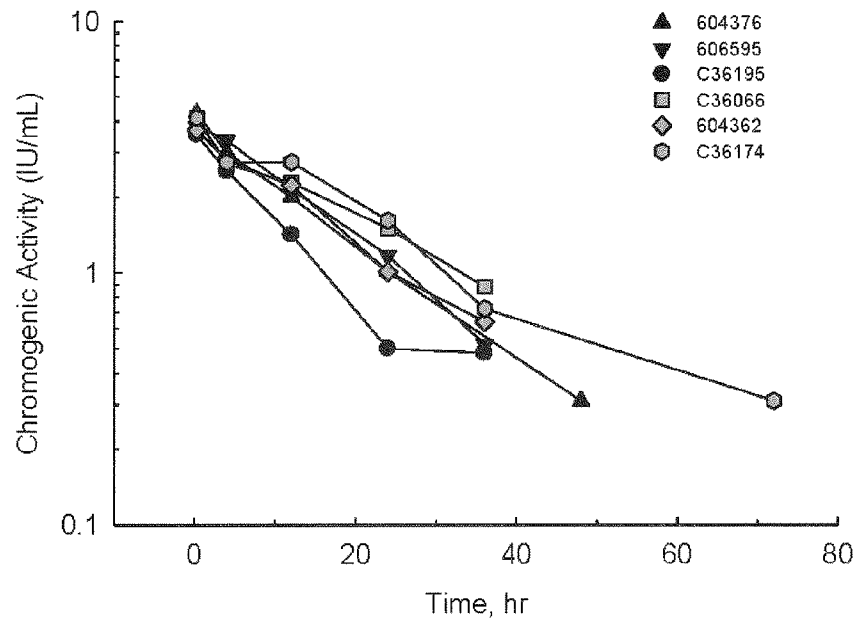
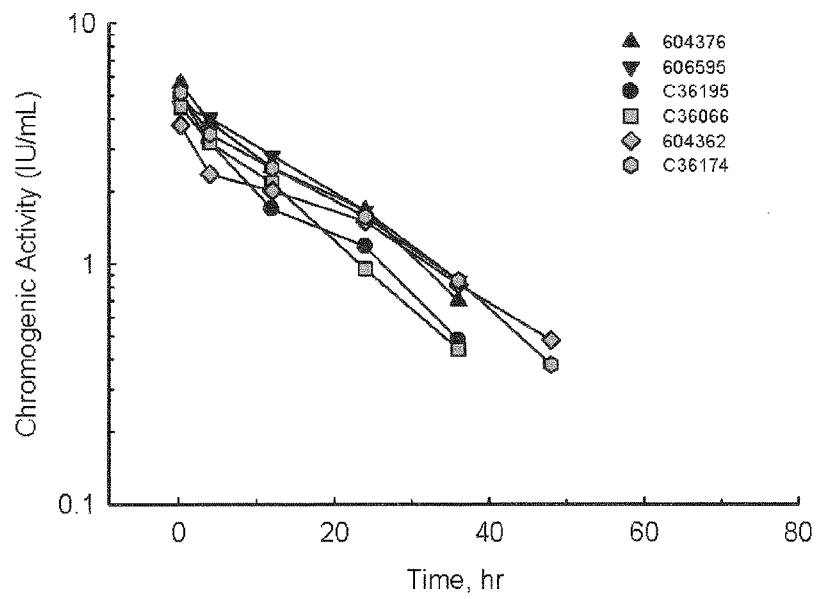
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Figure 9



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Figure 10

A.**B.**

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Figure 11

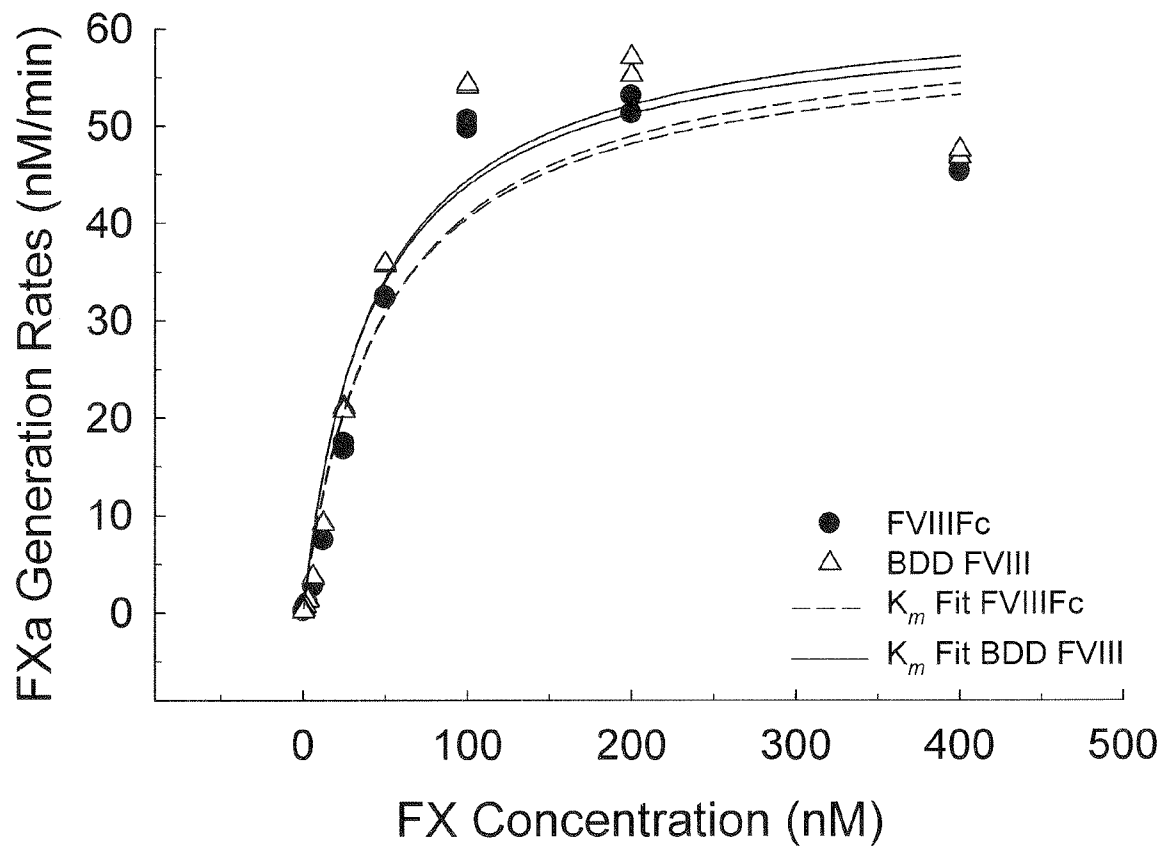
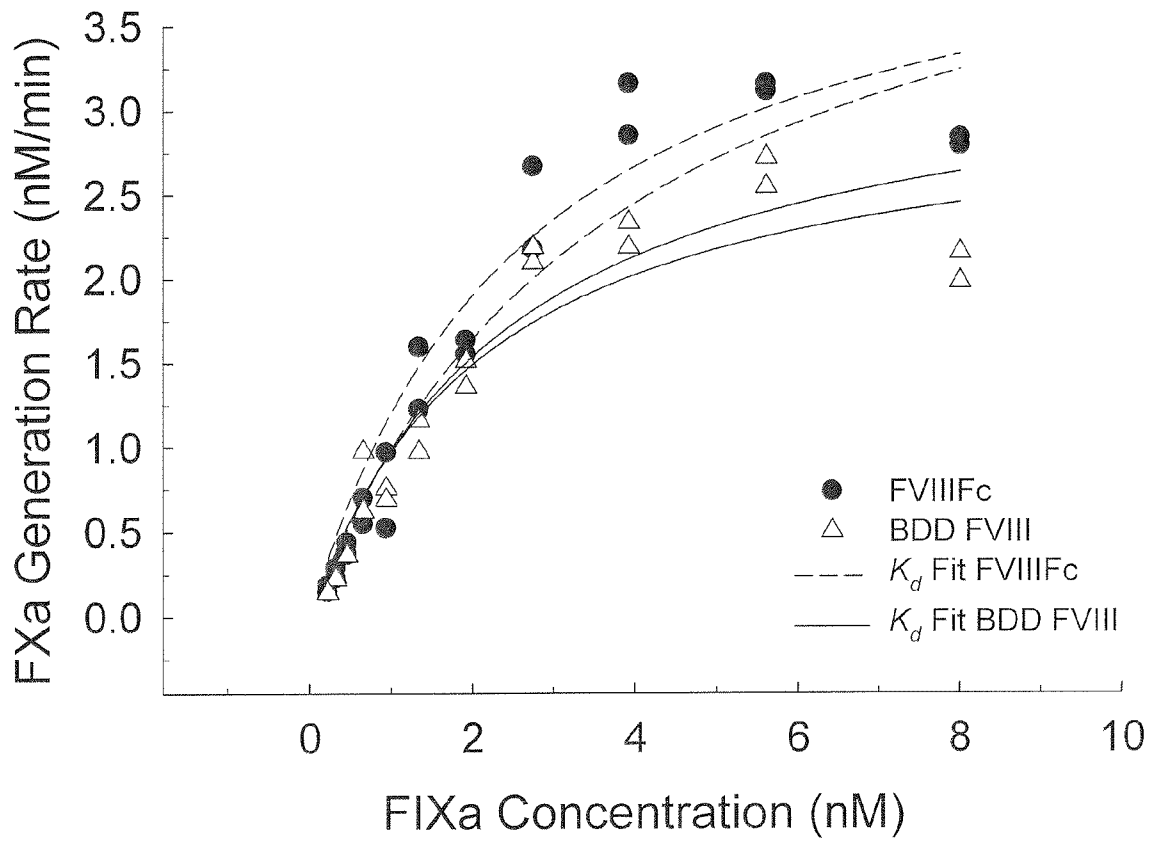


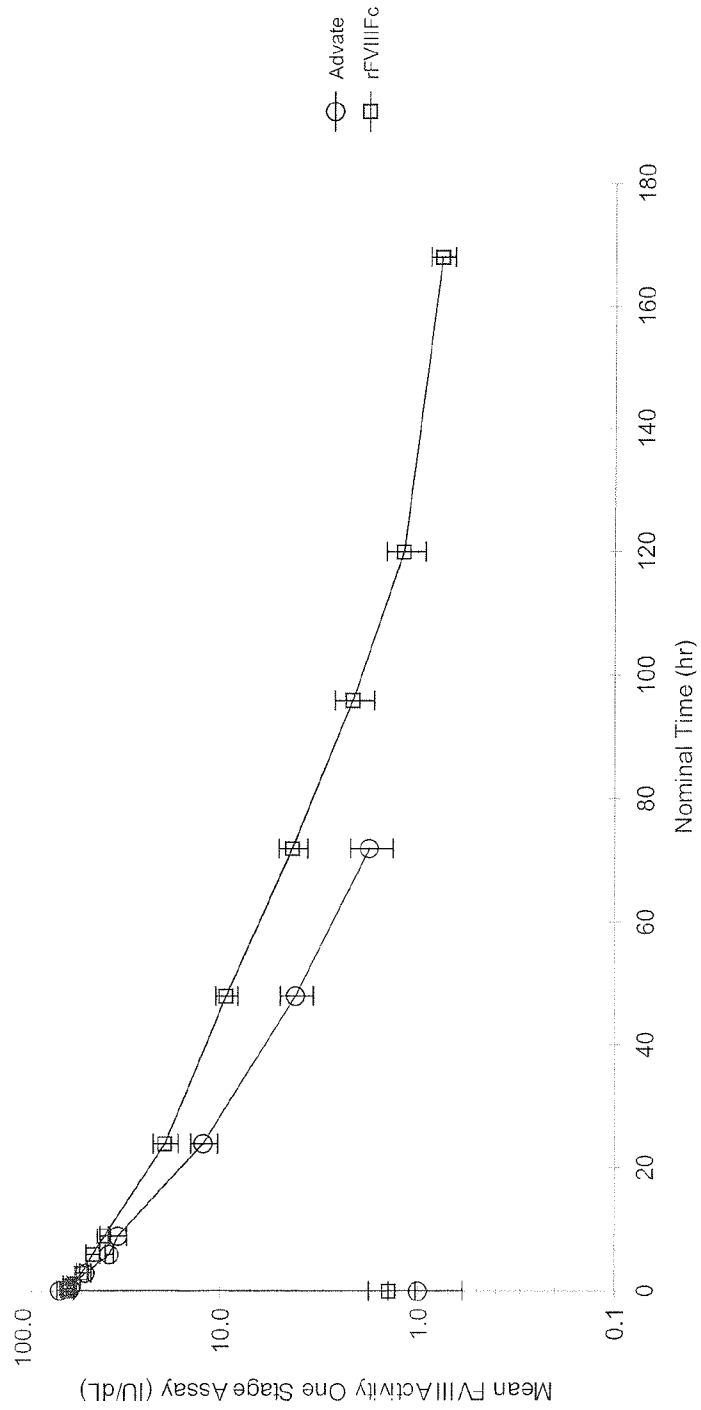
Figure 12



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Figure 13A

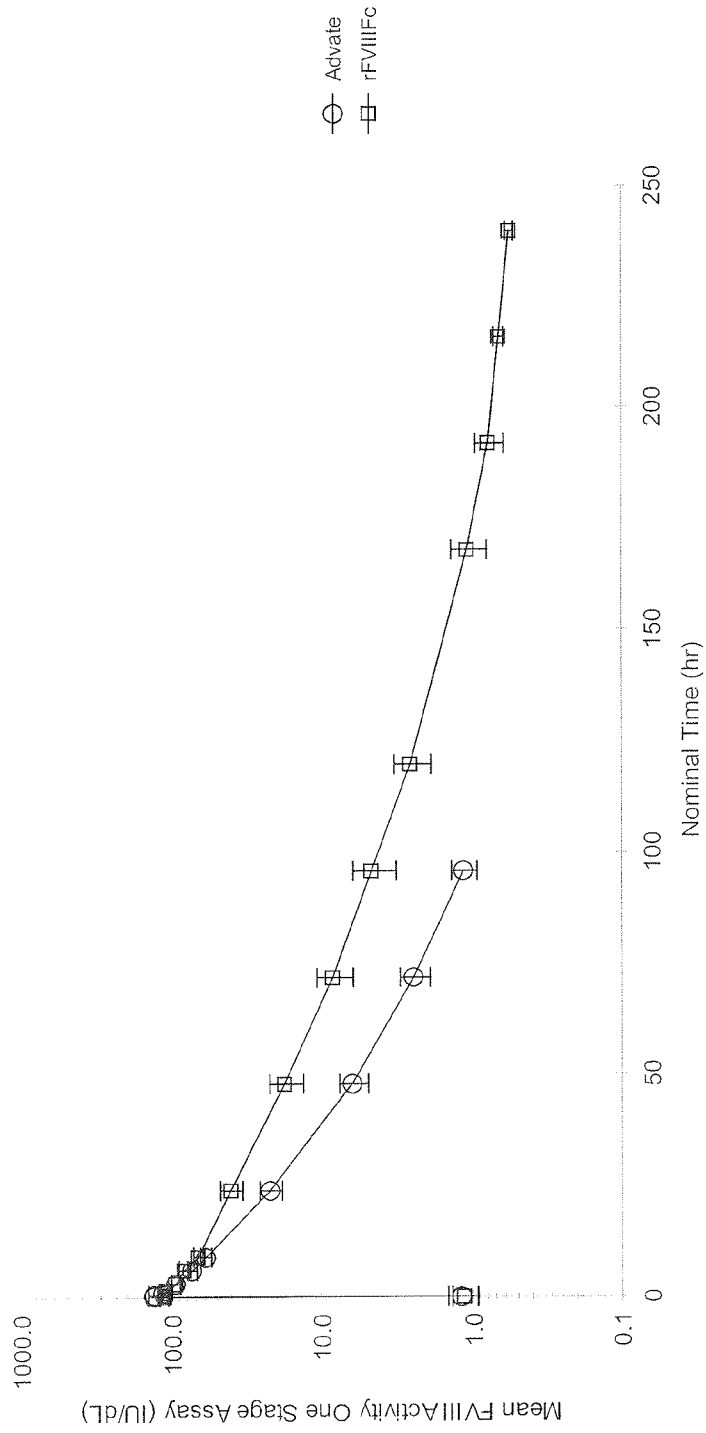
Dose_Level=25



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Figure 13B

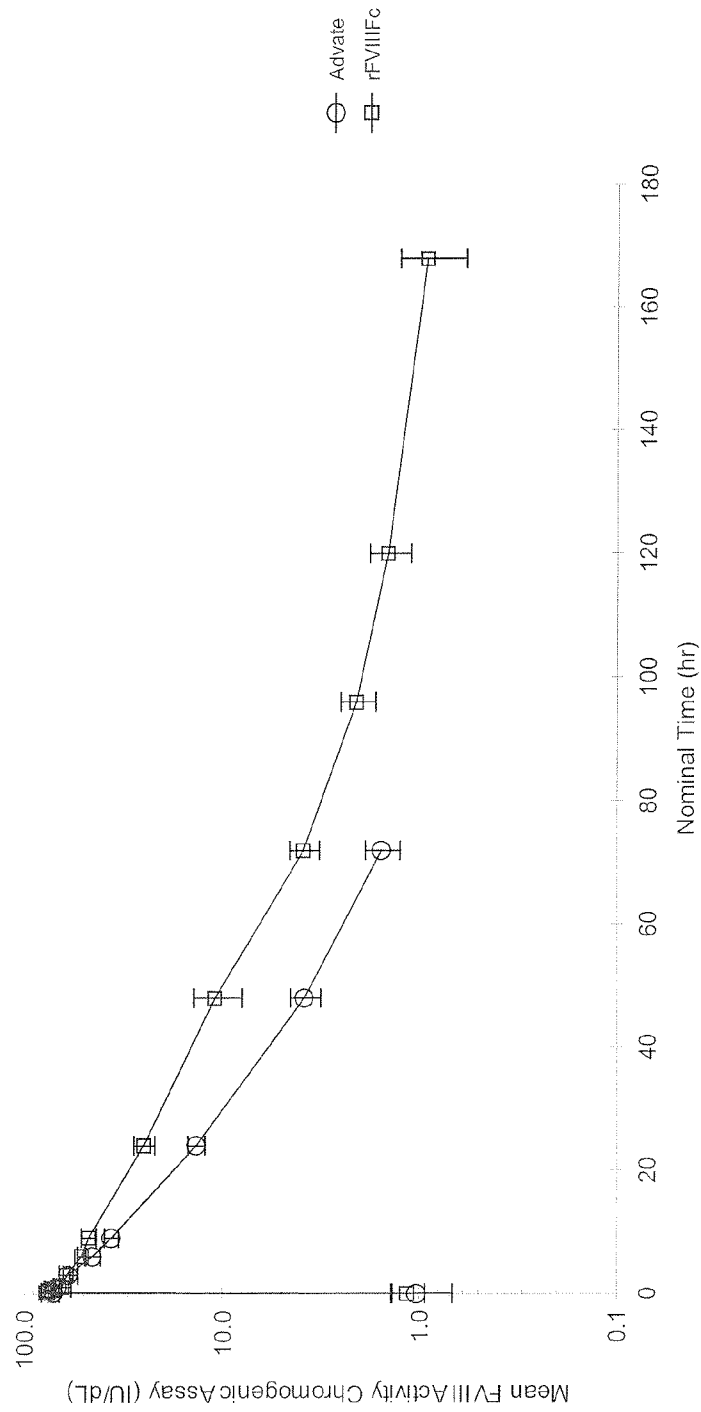
Dose_Level=65



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Figure 13C

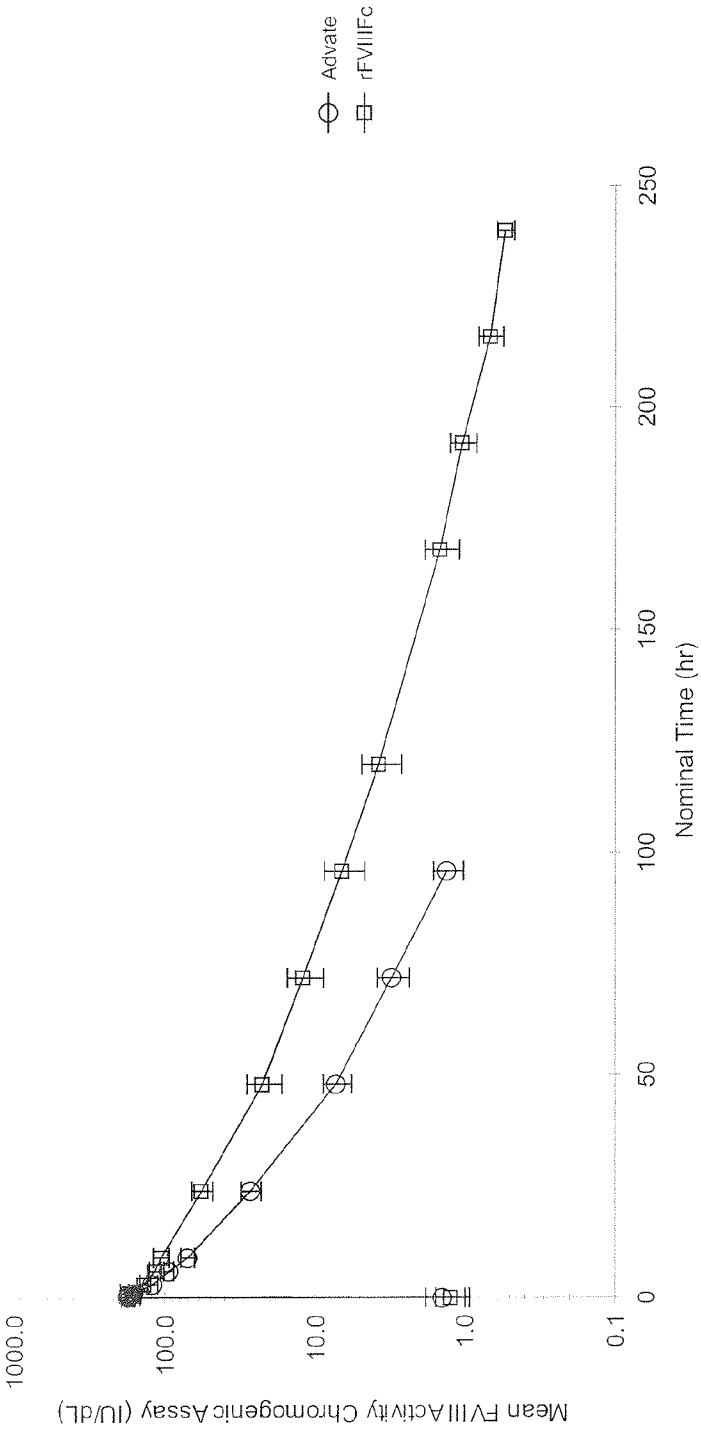
Dose_Level=25



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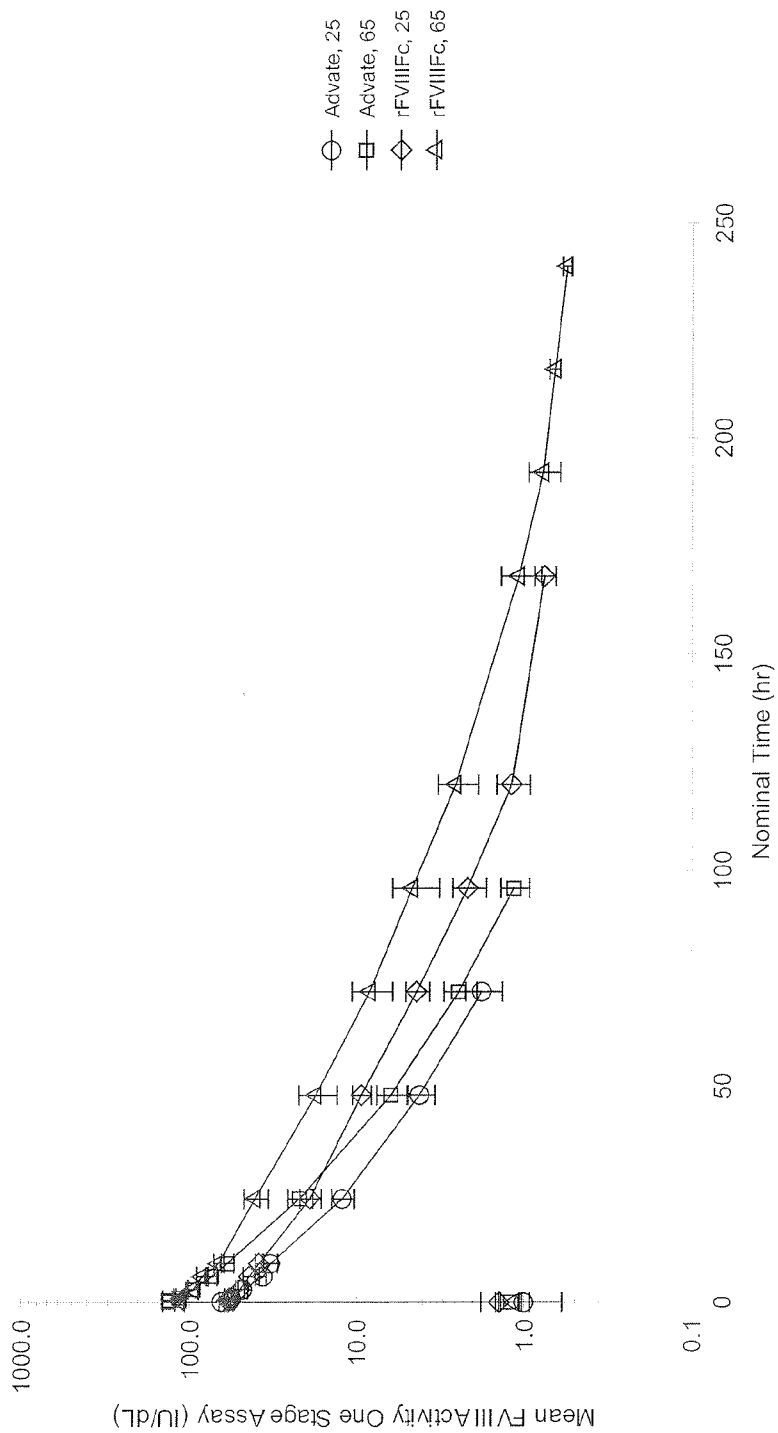
Figure 13D

Dose_Level=65



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Figure 14A



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Figure 14B

