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 illiopsoas 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 rFVIIIFc monomer.

[0028] FIG. 2. WBCT of rFVIIIFc 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 rFVIIIFc, ReFacto® and Advate®.

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

[0031] FIG. 5. Pharmacokinetics of intravenous rFVIIIIFc 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 rFVIIIFc and Xyntha after a single intravenous dose (125 IU/kg) in cynomolgus monkeys (n = 6, mean ± SD). Plasma concentrations were measured by ELISA.

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

[0035] FIG. 9. Group mean plasma chromogenic activity after a single intravenous dose (125 IU/kg) of rFVIIIFc and Xyntha in cynomolgus monkeys (n = 6, mean ± 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 rFVIIIFc and Xyntha in cynomolgus monkeys (n = 6,

mean ± SD). FVIII activity was measured using a FVIII-specific chromogenic activity assay. (A) rFVIIIFc 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 (±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 (±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 if 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 an 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 (t1/2) over a polypeptide consisting of the same Factor VIII portion without the non Factor VIII portion. A chimeric Factor VIII polypeptide with an increased t1/2 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 FVIIIFc 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 Oganesyan et al., Mol. Immunol. 46:1750 (2009), which is incorporated herein by reference in its entirety; H433K, N434F, and combinations thereof, as disclosed in Vaccaro et al., Nat. Biotechnol. 23:1283 (2005), which is incorporated herein by reference in its entirety; the mutants disclosed at pages 1-2, paragraph [0012], and Examples 9 and 10 of 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 rFVIIIFc 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 FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc

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 FVIIIFc 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, EF1α, in the expression vector pBUDCE4.1.

[0078] The rFVIIIFc 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 FVIIIFc for characterization in vivo. Recombinant FVIIIFc 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 rFVIIIFc hybrids, dimeric rFVIIIFc hybrids and dimeric Fc. However, there was essentially no dimeric rFVIIIFc detected in the conditioned medium from these cells. Rather, the conditioned medium contained Fc and monomeric rFVIIIFc. It is possible that the size of dimeric rFVIIIFc 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 t1/2, t1/2 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 t1/2beta (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 Vss (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 that 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 patent population that is about 1.5 fold longer than the mean MRT of a polypeptide comprising said Factor VIII without modification;

a mean t1/2beta (activity) in said patient population of about 18.3 ± 5.79 hours;

a mean t1/2beta (activity)in said patient population that is about 18 - 18.4 hours;

a mean t1/2beta (activity) in said patient population that is about 1.5 fold longer than the mean t1/2beta 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 Vss (activity) in said patient population of about 55.1 ± 12.3 mL/kg;

a mean Vss (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 illiopsoas 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 t1/2 compared to short acting FVIII. Preferably, prophylaxis is demonstrated by better Cmax, better Tmax, 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 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 phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation (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. Vss = 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 (rFVIIIFc) fusion protein was created to extend the half-life of FVIII. rFVIIIFc 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 rFVIIIFc compared to ReFacto®. In hemophilia A dogs, an intravenous dose of rFVIIIFc (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 rFVIIIFc 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 rFVIIIFc 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 (rFVIIIFc) chimeric protein and hybrid as an approach to extend the half-life of FVIII.

[00117] rFVIIIFc 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 FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIFc hybrid, and FVIIIFc 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, rFVIIIFc 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 FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc 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 FVIIIFc

[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 FVIIIFc 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, EF1α, in the expression vector pBUDCE4.1.

[00121] The rFVIIIFc 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 FVIIIFc for characterization *in vivo*. Recombinant FVIIIFc 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 rFVIIIFc hybrid, dimeric rFVIIIFc hybrid and dimeric Fc. However, there was essentially no dimeric rFVIIIFc detected in the conditioned medium from these cells. Rather, the conditioned medium contained Fc and monomeric rFVIIIFc. It is possible that the size of dimeric rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc, 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 rFVIIIFc, 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), FVIIIFc 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 FVIIIFc 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 rFVIIIFc was administered intravenously to the same dogs and blood samples were collected for WBCT, aPTT, FVIIIFc 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 FVIIIFc. Blood collections for clotting activity and FVIIIFc 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 rFVIIIFc 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 Siemans Healthcare Diagnostics (Dallas, TX, kit #B4238-40). Activity of rFVIIIFc 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 rFVIIIFc or FVIII by ELISA

FVIIIFc 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, $CaCl_2$ 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 (BioFx 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 (BioFx 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 (Vss), and clearance (Cl).

Results

Recombinant FVIII-Fc

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

[00137] Purified rFVIIIFc 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 FVIIIFc and ReFacto® (216 kDa and 170 kDa respectively), indicates that the two proteins have approximately equivalent specific activities (1970 IU/nmol for rFVIIIFc and 1521 – 2287 IU/nmol for ReFacto®). Thus the FVIII activity of rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc is approximately two to three times longer than for ReFacto®.

[00139] The chromogenic activity of rFVIIIFc, 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 rFVIIIFc was approximately 1.6 to 2 fold longer (11.1 hr) compared to Advate® (7 hr) and ReFacto® (5 hr). The Cmax was 1.6 ± 0.36 IU/mL for rFVIIIFc compared to 0.47 ± 0.30 IU/mL for Advate® and 0.67 ± 0.44 IU/mL for ReFacto®. The systemic exposure of rFVIIIFc was markedly greater for rFVIIIFc (22.6 hr·IU/mL) compared to ReFacto® (6.94 hr·IU/mL) and Advate® (3.90 hr·IU/mL) and clearance for rFVIIIFc 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 rFVIIIFc were studied in the Chapel Hill colony of hemophilia A dogs. A single intravenous dose of 125 IU/kg rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc was measured using a FVIII-specific chromogenic activity assay ($t_{1/2}$ = 15.4 ± 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 rFVIIIFc, 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 rFVIIIFc 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 rFVIIIFc. WBCT and aPTT were corrected to normal immediately after dosing with ReFacto®. However, the WBCT normalization after the single dose of rFVIIIFc lasted approximately twice as long compared to ReFacto® (Figure 4). Moreover, the plasma half-life of rFVIIIFc (15.7 ± 1.7 hr) was approximately twice as long for rFVIIIFc 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 rFVIIIFc or ReFacto®, platelet numbers and plasma fibrinogen concentration did not change from pre-dose values (data not shown).

Discussion

[00143] Recombinant FVIIIFc 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 rFVIIIFc and recombinant B-domain deleted FVIII (ReFacto®) indicated that the specific activities are similar for both proteins (1970 IU/nmol for rFVIIIFc and 1521 – 2287 IU/nmol for ReFacto®). It is somewhat surprising that the specific activity for rFVIIIFc 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 rFVIIIFc compared to a rFVIII product in hemophilia A mice and hemophilia A dogs. In both species, the half-life of rFVIIIFc 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 rFVIIIFc on WBCT was approximately twice as long compared to ReFacto®. In dogs, the C_{max} and clearance were similar for rFVIIIFc and ReFacto®, but the AUC and volume of distribution at steady state were approximately 1.5 fold and 2 fold greater for rFVIIIFc 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)

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Example 2

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

Materials and Methods

[00160] rFVIIIFc (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] rFVIIIFc 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 rFVIIIFc on Day 3, while Group 2 animals (n = 3) received rFVIIIFc on Day 0 followed by Xyntha on Day 4. The additional day between doses for group 2 was to ensure that the rFVIIIFc 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 rFVIIIFc or Xyntha by ELISA and a FVIII-specific chromogenic activity assay.

ELISA to measure rFVIIIFc and FVIII in plasma

Method to Measure rFVIIIFc in Monkey Plasma

[00166] This Enzyme Linked ImmunoSorbent Assay (ELISA) is designed to quantify rFVIIIFc 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 rFVIIIFc in the sample. Plates are read on an absorbance plate reader using a single detection wavelength, 650 nm. rFVIIIFc 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 αBDDA1 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 un-adsorbed 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 FVIIIFc 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, rFVIIIFc in the plasma samples is activated by thrombin. Activated Factor VIII (FVIIIa) then accelerates the conversion of Factor X (FX) to Factor Xa (FXa) in the presence of activated Factor IX (FIXa), phospholipids (PL) and calcium ions. The FXa activity is assessed by hydrolysis of a p-nitroanilide substrate specific to FXa. The initial rate of release of p-nitroaniline (pNA) measured at 405 nm is proportional to the FXa activity, and thus to the FVIII activity in the sample. The limit of quantitation of

FVIII activity due to rFVIIIFc 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 ± 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 1U/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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc and Xyntha are shown in Tables 9 and 10, respectively. The mean t1/2 for rFVIIIFc was 11.9 ± 1.7 hr (range 9.3 to 14.1 hr) and for Xyntha, the mean elimination t1/2 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 rFVIIIFc and Xyntha in Tables 9 and 10, respectively. The mean elimination t1/2 was 16.1 ± 6.9 hr (range 11.6 to 29.4 hr) for rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc, each subject will undergo an inhibitor assessment at 14–days (336 hours) post-injection of rFVIIIFc. 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 rFVIIIFc, 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 rFVIIIFc for a 10-day (240 hours) profiling. If a bleeding episode occurs before the first injection of rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc) 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 rFVIIIFc 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 Ca2+, 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 rFVIIIFc 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 Km and Vmax 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) ± the corresponding standard deviation. Based on these data, these proteins (rBDD FVIII and rFVIIIFc) were found to have comparable Km and Vmax values, within the variation of the assay. Therefore, the Xase complex formed with rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 Kd 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) ± the corresponding standard deviation (Table 12). Both proteins were found to have similar Kd and Vmax values, indicating that rFVIIIFc 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 FVIIIFc. FVIIIFc 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, FVIIIFc 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 FVIIIFc activity above baseline at approximately 168 h after
Cl	Clearance

MRT Mean residence time

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

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

TBLP5 Model-predicted time after dose when FVIIIFc activity has declined to approximately 5 IU/dL above baseline

Example 6

[00183] A recombinant B-domain-deleted factor VIII-Fc (rFVIIIFc) fusion protein has been created as an approach to extend the half-life of FVIII. The pharmacokinetics (PK) of rFVIIIFc were compared to rFVIII in hemophilia A mice. We found that the terminal half-life was twice as long for rFVIIIFc compared to rFVIII. In order to confirm that the underlying mechanism for the extension of half-life was due to the protection of rFVIIIFc 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 Cmax was similar between rFVIIIFc and rFVIII (XYNTHA®) in both mouse strains. However, while the half-life for rFVIIIFc was comparable to that of rFVIII in the FcRn knockout mice, the half-life for rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc in the hemophilia A mice using ROTEM. Hemophilia A mice were administered a single intravenous dose of 50 IU/kg rFVIIIFc, 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, rFVIIIFc 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 rFVIIIFc. 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, rFVIIIFc 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 rFVIIIFc heterodimeric hybrid protein, FVIIIFc monomeric Fc fusion protein, FVIIIFc monomer hybrid, monomeric FVIIIIFc hybrid, and FVIIIFc monomer-dimer. See Example 1, Fig. 1, and Table 2A.

[00186] Preclinical studies with rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc (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 rFVIIIFc 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 rFVIIIFc injection just prior to the administration of rFVIIIFc, at 10 and 30 minutes and 1, 3, 6, and 9 hours post-injection of rFVIIIFc; on Day 1 at 24 hours post-injection of rFVIIIFc; on Days 2 through 5 at 48, 72, 96, and 120 hours post-injection of rFVIIIFc; on Day 7 at 168 hours post-injection of rFVIIIFc; on Days 8, 9, and 10 at 192, 216, and 240 hours post-injection of high dose of rFVIIIFc (Cohort B only). FVIII activity was also measured at the final study visit (28 days post-injection of rFVIIIFc) at 672 hours post-injection of rFVIIIFc.

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 = Cmax_M/Actual Dose (IU/kg)

KV_OB = Cmax_OB/Actual Dose (IU/kg)

IVR_M = 100 × Cmax_M × Plasma Volume (dL) / Total Dose in IU; where plasma volume in mL = (23.7 × Ht in cm) + (9.0 × Wt in kg) – 1709.

IVR_OB = 100 × Cmax_OB × Plasma Volume (dL) / Total Dose in IU; where plasma volume in mL = (23.7 × Ht in cm) + (9.0 × 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 rFVIIIFc, with mean (±SD) model-predicted Cmax 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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. All Advate- and rFVIIIFc-treated patients had dose-related increases in FVIII activity. The observed increase in both Cmax 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 rFVIIIFc than for Advate with mean (±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 rFVIIIFc 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 ~ 48% and 61% greater following rFVIIIFc administration than Advate at 25 and 65 IU/kg dose levels, respectively. Mean (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

[00197] Similar to elimination half-life, the MRT was prolonged for rFVIIIFc relative to Advate. Mean (±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 rFVIIIFc 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 rFVIIIFc only accounted for ~ 66% of those observed for Advate at equivalent doses. Mean (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. V values were comparable between Advate and rFVIIIFc with mean (±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 rFVIIIFc 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 rFVIIIFc; 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 rFVIIIFc 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 rFVIIIFc demonstrating increased TBLP1and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIFc appeared to be comparable. A slight increase in TBLP1 and TBLP3 values were observed with increasing dose of Advate and rFVIIIFc. In contrast, slight decreases in mean IVR and K-values were noted with increasing dose of Advate and rFVIIIFc. As previously indicated, an assessment of the dose dependency of these parameters is confounded by limited dose levels.

[00200] Mean (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (±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 rFVIIIFc 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 (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (±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 rFVIIIFc 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 rFVIIIFc, with mean (±SD) model-predicted Cmax 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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

[00203] All Advate- and rFVIIIFc-treated patients had dose-related increases in FVIII activity. The observed increase in both Cmax and AUCINF 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 rFVIIIFc than for Advate with mean (±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 rFVIIIFc 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 AUCINF) was ~ 53% and 84% greater following rFVIIIFc administration than Advate at 25 and 65 IU/kg dose levels, respectively. Mean (±SD) model-predicted AUCINF values were 1080 ± 236 and 2320 ± 784 hr*IU/dL for Advate and 1650 ± 408 and 4280 ± 1860 hr*IU/dL for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

[00206] Similar to elimination half-life, the MRT was prolonged for rFVIIIFc relative to Advate. Mean (±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 rFVIIIFc 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 rFVIIIFc only accounted for ~ 58-66% of those observed for Advate at equivalent doses. Mean (±SD) model-predicted CL values were 2.39 ± 0.527 and 3.21 ±

1.40 mL/hr/kg for Advate and 1.57 ± 0.349 and 1.86 ± 0.970 mL/hr/kg for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. V values were comparable between Advate and rFVIIIFc with mean (±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 rFVIIIFc 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 rFVIIIFc; 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 rFVIIIFc demonstrating increased TBLP1and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIFc appeared to be comparable.

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

[00210] Mean (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

[00211] 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 (±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 rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively. Mean (±SD) observed IVR values were 112 ± 14.5 and 116 ± 26.9 % for Advate and 113 ± 16.3 and 117 ± 33.6 % for rFVIIIFc for the 25 and 65 IU/kg dose groups, respectively.

CONCLUSIONS

[00212] All Advate- and rFVIIIFc-treated patients had comparable dose-related increases in Cmax and AUCINF over the dose range evaluated. Peak plasma levels of Advate and rFVIIIFc 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 rFVIIIFc; 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 rFVIIIFc 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 rFVIIIFc relative to Advate at comparable doses. Evidence of PK difference was also observed with rFVIIIFc demonstrating increased TBLP1 and TBLP3 values compared to Advate at equivalent doses. IVR and K-values for Advate and rFVIIIFc 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 Chomogenic Assay yielded a higher estimation of exposure parameters (e.g. Cmax, AUCINF, etc.).

[00215] With the observed improvements in PK, rFVIIIFc 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-inhuman 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 rFVIIIFc on this weekly prophylaxis regimen until PK results for rFVIIIFc are available. Base don 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 rFVIIIFc PK profiling as follows: Washout of at least 96 hours; a single dose of rFVIIIFc 65 IU/kg; Abbreviated sampling beginning on rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc every 12 to 24 hours. Prior to surgery, the physician will review the subject's rFVIIIFc 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 rFVIIIFc 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 rFVIIIFc administered as prophylaxis, on-demand, and surgical treatment regimens; and (b) to evaluate the efficacy of rFVIIIFc administered as prophylaxis, on-demand, and surgical treatment regimens. The secondary objectives of this study are (a) to characterize the PK profile of rFVIIIFc and compare the PK of FVIIIFc with the currently marketed product, ADVATE; (b) to evaluate individual responses with FVIIIFc; and (c) to evaluate FVIIIFc consumption.

Primary Objectives

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

Secondary Objectives

- To characterize the PK profile of rFVIIIFc and compare the PK of rFVIIIFc with the currently marketed product, Advate®
- To evaluate individual responses with rFVIIIFc
- 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 rFVIIIFc consumption (e.g., total annualized rFVIIIFc 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 rFVIIIFc and Advate in 2 subjects, specifically, 1 in the low dose cohort and 1 in the high dose cohort.

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

[00226] Following dosing with Advate and subsequently rFVIIIFc, 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 rFVIIIFc 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 rFVIIIFc relative to Advate in the subject dosed at this low dose. However, the differential improvement of rFVIIIFc versus Advate was much less appreciable at the 65 IU/kg dose.

Tables

Table 1: Polynucleotide Sequences

A. B-Domain Deleted FVIIIFc

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

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 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 AGTCAAACGG AGAAAGAAGA TGATAAAGTC TTCCCTGGTG
1141 GAAGCCATAC ATATGTCTGG 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 TCGGGCCTGG CCTAAAATGC
1441 ACACAGTCAA TGGTTATGTA AACAGGTCTC TGCCAGGTCT GATTGGATGC CACAGGAAAT
1501 CAGTCTATTG GCATGTGATT GGAATGGGCA CCACTCCTGA AGTGCACTCA ATATTCCTCG
1561 AAGGTCACAC ATTTCTTGTG AGGAACCATC GCCAGGCGTC CTTGGAAATC TCGCCAATAA
1621 CTTTCCTTAC TGCTCAAACA CTCTTGATGG ACCTTGGACA GTTTCTACTG TTTTGTCATA
1681 TCTCTTCCCA CCAACATGAT GGCATGGAAG CTTATGTCAA AGTAGACAGC TGTCCAGAGG
1741 AACCCCAACT ACGAATGAAA AATAATGAAG AAGCGGAAGA CTATGATGAT GATCTTACTG
1801 ATTCTGAAAT GGATGTGGTC AGGTTTGATG ATGACAACTC TCCTTCCTTT ATCCAAATTC
1861 GCTCAGTTGC CAAGAAGCAT CCTAAAACTT GGGTACATTA CATTGCTGCT GAAGAGGAGG
1921 ACTGGGACTA TGCTCCCTTA GTCCTCGCCC CCGATGACAG AAGTTATAAA AGTCAATATT
1981 TGAACAATGG CCCTCAGCGG ATTGGTAGGA AGTACAAAAA AGTCCGATTT ATGGCATACA
2041 CAGATGAAAC CTTTAAGACT CGTGAAGCTA TTCAGCATGA ATCAGGAATC TTGGGACCTT
2101 TACTTTATGG GGAAGTTGGA GACACACTGT TGATTATATT TAAGAATCAA GCAAGCAGAC
2161 CATATAACAT CTACCCTCAC GGAATCACTG ATGTCCGTCC TTTGTATTCA AGGAGATTAC
2221 CAAAAGGTGT AAAACACTTG AAGGATTTTC CAATTCTGCC AGGAGAAATA TTCAAATATA
2281 AATGGACAGT GACTGTAGAA GATGGGCCAA CTAAATCAGA TCCTCGGTGC CTGACCCGCT
2341 ATTACTCTAG TTTCGTTAAT ATGGAGAGAG ATCTAGCTTC AGGACTCATT GGCCCTCTCC
2401 TCATCTGCTA CAAAGAATCT GTAGATCAAA GAGGAAACCA GATAATGTCA GACAAGAGGA
2461 ATGTCATCCT GTTTTCTGTA TTTGATGAGA ACCGAAGCTG GTACCTCACA GAGAATATAC
2521 AACGCTTTCT CCCCAATCCA GCTGGAGTGC AGCTTGAGGA TCCAGAGTTC CAAGCCTCCA
2581 ACATCATGCA CAGCATCAAT GGCTATGTTT TTGATAGTTT GCAGTTGTCA GTTTGTTTGC
2641 ATGAGGTGGC ATACTGGTAC ATTCTAAGCA TTGGAGCACA GACTGACTTC CTTTCTGTCT
2701 TCTTCTCTGG ATATACCTTC AAACACAAAA TGGTCTATGA AGACACACTC ACCCTATTCC
2761 CATTCTCAGG AGAAACTGTC TTCATGTCGA TGGAAAACCC AGGTCTATGG ATTCTGGGGT
2821 GCCACAACTC AGACTTTCGG AACAGAGGCA TGACCGCCTT ACTGAAGGTT TCTAGTTGTG
2881 ACAAGAACAC TGGTGATTAT TACGAGGACA GTTATGAAGA TATTTCAGCA TACTTGCTGA
2941 GTAAAAACAA TGCCATTGAA CCAAGAAGCT TCTCTCAAAA CCCACCAGTC TTGAAACGCC
3001 ATCAACGGGA AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT GACTATGATC
3061 ATACCATATC AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC
3121 AGAGCCCCCG CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC
3181 TCTGGGATTA TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA
3241 GTGTCCCTCA GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC
3301 CCTTATACCG TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG
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3361  AAGTTGAAGA TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT
3421  ATTCTAGCCT TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT
3481  TTGTCAAGCC TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA
3541  CTAAAGATGA GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG
3601  ATGTGCACTC AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCCTG
3661  CTCATGGGAG ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA
3721  CCAAAAGCTG GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC
3781  AGATGGAAGA TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA
3841  TGGATACACT ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA
3901  GCATGGGCAG CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC
3961  GAAAAAAAGA GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG
4021  TGGAAATGTT ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT GGCGAGCATC
4081  TACATGCTGG GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG ACTCCCCTGG
4141  GAATGGCTTC TGGACACATT AGAGATTTTC ACATTACAGC TTCAGGACAA TATGGACAGT
4201  GGGCCCCAAA GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG
4261  AGCCCTTTTC TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC GGCATCAAGA
4321  CCCAGGGTGC CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA
4381  GTCTTGATGG GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT
4441  TCTTTGGCAA TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT 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 CAAGGGAGCA GTAATGCCTG GAGACCTCAG GTGAATAATC
4741  CAAAAGAGTG GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGCA GTAACTACTC
4801  AGGGAGTAAA ATCTCTGCTT ACCAGCATGT ACGTGAAGGA GTCCTCATC TCCAGCAGTC
4861  AAGATGGCCA TCAATGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA
4921  ATCAAGACTC CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC
4981  TTCGAATTCA CCCCCAGAGT TGCGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT
5041  GCGAGGCACA GGACCTCTAC GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCAGAAC
5101  TCCTGGGCGG ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT
5161  CCCGGACCCC 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 CCTGGTCAAA GGCTTCTATC
5521  CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC 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 Igκ 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  TGCCCACCGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA
8161  AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC
8221  GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT
8281  AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC
8341  CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC
8401  AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
8461  CCACAGGTGT ACACCCTGCC CCCATCCCGC GATGAGCTGA CCAAGAACCA GGTCAGCCTG
8521  ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
8581  CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGTTGG ACTCCGACGG CTCCTTCTTC
8641  CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC
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8701 TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
8761 GGTAAA

B. Full Length FVIIIFc

(i) Full Length FVIIIFc 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 AGTGGAACTG TCATGGGACT ATATGCAAAG TGATCTCGGT GAGCTGCCTG TGGACGCAAG
841 ATTTCCTCCT AGAGTGCCAA AATCTTTTCC ATTCAACACC TCAGTCGTGT ACAAAAAGAC
901 TCTGTTTGTA GAATTCACGG ATCACCTTTT CAACATCGCT AAGCCAAGGC CACCCTGGAT
961 GGGTCTGCTA GGTCCTACCA TCCAGGCTGA GGTTTATGAT ACAGTGGTCA TTACACTTAA
1021 GAACATGGCT TCCCATCCTG TCAGTCTTCA TGCTGTTGGT 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 TGCTGTATTT GATGAAGGGA AAAGTTGGCA
1381 CTCAGAAACA AAGAACTCCT TGATGCAGGA TAGGGATGCT GCATCTGCTC GGGCCTGGCC
1441 TAAAATGCAC ACAGTCAATG GTTATGTAAA CAGGTCTCTG CCAGGTCTGA TTGGATGCCA
1501 CAGGAAATCA GTCTATTGGC ATGTGATTGG AATGGGCACC ACTCCTGAAG TGCACTCAAT
1561 ATTCCTCGAA GGTCACACAT TTCTTGTGAG GAACCATCGC CAGGCGTCCT 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 CTTCCTTTAT
1861 CCAAATTCGC TCAGTTGCCA AGAAGCATCC TAAAACTTGG GTACATTACA TTGCTGCTGA
1921 AGAGGAGGAC TGGGACTATG CTCCCTTAGT CCTCGCCCCC GATGACAGAA GTTATAAAAG
1981 TCAATATTTG AACAATGGCC CTCAGCGGAT TGGTAGGAAG TACAAAAAAG TCCGATTTAT
2041 GGCATACACA GATGAAACCT TTAAGACTCG TGAAGCTATT CAGCATGAAT CAGGAATCTT
2101 GGGACCTTTA CTTTATGGGG AAGTTGGAGA CACACTGTTG ATTATATTTA AGAATCAAGC
2161 AAGCAGACCA TATAACATCT ACCCTCACGG AATCACTGAT GTCCGTCCTT TGTATTCAAG
2221 GAGATTACCA AAAGGTGTAA AACATTTGAA GGATTTTCCA ATTCTGCCAG GAGAAATATT
2281 CAAATATAAA TGGACAGTGA CTGTAGAAGA TGGGCCAACT AAATCAGATC CTCGGTGCCT
2341 GACCCGCTAT TACTCTAGTT TCGTTAATAT GGAGAGAGAT CTAGCTTCAG GACTCATTGG
2401 CCCTCTCCTC ATCTGCTACA AAGAATCTGT AGATCAAAGA GGAAACCAGA TAATGTCAGA
2461 CAAGAGGAAT GTCATCCTGT TTTCTGTATT TGATGAGAAC CGAAGCTGGT ACCTCACAGA
2521 GAATATACAA CGCTTTCTCC CCAATCCAGC TGGAGTGCAG CTTGAGGATC CAGAGTTCCA
2581 AGCCTCCAAC ATCATGCACA GCATCAATGG CTATGTTTTT GATAGTTTGC AGTTGTCAGT
2641 TTGTTTGCAT GAGGTGGCAT ACTGGTACAT TCTAAGCATT GGAGCACAGA CTGACTTCCT
2701 TTCTGTCTTC TTCTCTGGAT ATACCTTCAA ACACAAAATG GTCTATGAAG ACACACTCAC
2761 CCTATTCCCA TTCTCAGGAG AAACTGTCTT CATGTCGATG GAAAACCCAG GTCTATGGAT
2821 TCTGGGGTGC CACAACTCAG ACTTTCGGAA CAGAGGCATG ACCGCCTTAC TGAAGGTTTC
2881 TAGTTGTGAC AAGAACACTG GTGATTATTA CGAGGACAGT TATGAAGATA TTTCAGCATA
2941 CTTGCTGAGT AAAAACAATG CCATTGAACC AAGAAGCTTC TCCCAGAATT CAAGACACCC
3001 TAGCACTAGG CAAAAGCAAT TTAATGCCAC CACAATTCCA GAAAATGACA TAGAGAAGAC
3061 TGACCCTTGG TTTGCACACA GAACACCTAT GCCTAAAATA CAAAATGTCT CCTCTAGTGA
3121 TTTGTTGATG CTCTTGCGAC 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 AAACTTGATT TCAAAGTTTC TAGTACATCA AATAATCTGA TTTCAACAAT
3421 TCCATCAGAC AATTTGGCAG CAGGTACTGA TAATACAAGT TCCTTAGGAC CCCCAAGTAT
3481 GCCAGTTCAT TATGATAGTC AATTAGATAC CACTCTATTT GGCAAAAAGT CATCTCCCCT
3541 TACTGAGTCT GGTGGACCTC TGAGCTTGAG TGAAGAAAAT AATGATTCAA AGTTGTTAGA

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 ATAAAACTAC
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 TTCTTGTCTG AGAAAAACAA
4201 AGTGGTAGTA GGAAAGGGTG AATTTACAAA GGACGTAGGA CTCAAAGAGA TGGTTTTTCC
4261 AAGCAGCAGA AACCTATTTC TTACTAACTT GGATAATTTA CATGAAAATA ATACACACAA
4321 TCAAGAAAAA AAAATTCAGG AAGAAATAGA AAAGAAGGAA ACATTAATCC AAGAGAATGT
4381 AGTTTTGCCT CAGATACATA CAGTGACTGG CACTAAGAAT TTCATGAAGA ACCTTTTCTT
4441 ACTGAGCACT AGGCAAAATG TAGAAGGTTC ATATGACGGG GCATATGCTC CAGTACTTCA
4501 AGATTTTAGG TCATTAAATG ATTCAACAAA TAGAACAAAG AAACACACAG CTCATTTCTC
4561 AAAAAAAGGG GAGGAAGAAA ACTTGGAAGG CTTGGGAAAT CAAACCAAGC AAATTGTAGA
4621 GAAATATGCA TGCACCACAA GGATATCTCC TAATACAAGC CAGCAGAATT TTGTCACGCA
4681 ACGTAGTAAG AGAGCTTTGA AACAATTCAG ACTCCCACTA GAAGAAACAG AACTTGAAAA
4741 AAGGATAATT GTGGATGACA CCTCAACCCA GTGGTCCAAA AACATGAAAC ATTTGACCCC
4801 GAGCACCCTC ACACAGATAG ACTACAATGA GAAGGAGAAA GGGGCCATTA CTCAGTCTCC
4861 CTTATCAGAT TGCCTTACGA GGAGTCATAG CATCCCTCAA GCAAATAGAT CTCCATTACC
4921 CATTGCAAAG GTATCATCAT TTCCATCTAT TAGACCTATA TATCTGACCA GGGTCCTATT
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 TCTCCCGAAA CCAGACTTGC CCAAAACATC
5221 TGGCAAAGTT 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 CAAAAGAAGA 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 ATACCATATC
5761 AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC AGAGCCCCCG
5821 CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC TCTGGGATTA
5881 TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA GTGTCCCTCA
5941 GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC CCTTATACCG
6001 TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG AAGTTGAAGA
6061 TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT ATTCTAGCCT
6121 TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAACT TTGTCAAGCC
6181 TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT ATGGCACCCA CTAAAGATGA
6241 GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG ATGTGCACTC
6301 AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCCTG CTCATGGGAG
6361 ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA CCAAAAGCTG
6421 GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC AGATGGAAGA
6481 TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA TGGATACACT
6541 ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA GCATGGGCAG
6601 CAATGAAAAC ATCCATTCTA TTCATTTCAG TGGACATGTG TTCACTGTAC GAAAAAAAGA
6661 GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG TGGAAATGTT
6721 ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT GGCGAGCATC TACATGCTGG
6781 GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG ACTCCCCTGG GAATGGCTTC
6841 TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT GGGCCCCAAA
6901 GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG AGCCCTTTTC
6961 TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC 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 GTAACTACTC AGGGAGTAAA
7501    ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC AAGATGGCCA
7561    TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA ATCAAGACTC
7621    CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC TTCGAATTCA
7681    CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT GCGAGGCACA
7741    GGACCTCTAC GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCAGAAC TCCTGGGCGG
7801    ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC
7861    TGAGGTCACA TGCGTGGTGG TGGACGTGAG 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 GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT
8221    CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT
8281    GTTGGACTCC GACGGCTCCT TCTTCCTCTA 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     GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421     GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCTG
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 ACAAAGAACT CCTTGATGCA GGATAGGGAT
721     GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781     CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC
841     ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
901     CGCCAGGCGT CCTTGGAAAT CTCGCCAATA 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 CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
1201    TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT 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 CCAAAAGGTG TAAAACATTT GAAGGATTTT
1561 CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG TGACTGTAGA AGATGGGCCA
1621 ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA 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 AGTTTGTTTG CATGAGGTGG CATACTGGTA CATTCTAAGC
1981 ATTGGAGCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
2041 ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101 ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG GAACAGAGGC
2161 ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221 AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGA**GAC**
2281 AAAACTCACA CATGCCCACC GTGCCCAGCT CCAGAACTCC TGGGCGGACC GTCAGTCTTC
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 GAACAACTAC AAGACCACGC CTCCCGTGTT GGACTCCGAC
2821 GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC
2881 GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
2941 TCCCTGTCTC CGGGTAAA

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)

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 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCTG
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 ACAAAGAACT CCTTGATGCA GGATAGGGAT
721 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC
841 ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
901 CGCCAGGCGT CCTTGGAAAT CTCGCCAATA 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 CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCC1AAAACT
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 GGGAAGTTGG AGACACACTG
1441  TTGATTATAT TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
1501  GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT GAAGGATTTT
1561  CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG TGACTGTAGA AGATGGGCCA
1621  ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA 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 AGTTTGTTTG CATGAGGTGG CATACTGGTA CATTCTAAGC
1981  ATTGCAGCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
2041  ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101  ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG GAACAGAGGC
2161  ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221  AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGAAGC
2281  TTCTCCCAGA ATGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCAGA ACTCCTGGGC
2341  GGACCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC
2401  CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC
2461  TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
2521  AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC
2581  AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC
2641  TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT
2701  GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
2761  ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
2821  GTGTTGGACT CCGACGGCTC CTTCTTCCTC 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 CCAAAACTTA CTTTTGGAAA GTGCAACATC ATATGGCACC CACTAAAGAT
601   GAGTTTGACT GCAAAGCCTG GGCTTATTTC 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   CTACCTGGCT TAGTAATGGC TCAGGATCAA AGGATTCGAT GGTATCTGCT CAGCATGGGC
961   AGCAATGAAA ACATCCATTC TATTCATTTC AGTGGACATG TGTTCACTGT ACGAAAAAAA
1021  GAGGAGTATA AAATGGCACT CTACAATCTC TATCCAGGTG TTTTTGAGAC AGTGGAAATG
1081  TTACCATCCA AAGCTGGAAT TTGGCGGGTG GAATGCCTTA TTGGCGAGCA TCTACATGCT
1141  GGGATCAGCA CACTTTTTCT GGTGTACAGC AATAAGTGTC AGACTCCCCT GGGAATGGCT
1201  TCTGGACACA TTAGAGATTT TCAGATTACA GCTTCAGGAC AATATGGACA GTGGGCCCCA
1261  AAGCTGGCCA GACTTCATTA TTCCGGATCA ATCAATGCCT GGAGCACCAA GGAGCCCTTT
1321  TCTTGGATCA AGGTGGATCT GTTGGCACCA ATGATTATTC ACGGCATCAA GACCCAGGGT
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1381  GCCCGTCAGA AGTTCTCCAG CCTCTACATC TCTCAGTTTA TCATCATGTA TAGTCTTGAT
1441  GGGAAGAAGT GGCAGACTTA TCGAGGAAAT TCCACTGGAA CCTTAATGGT CTTCTTTGGC
1501  AATGTGGATT CATCTGGGAT AAAACACAAT ATTTTTAACC CTCCAATTAT TGCTCGATAC
1561  ATCCGTTTGC ACCCAACTCA TTATAGCATT CGCAGCACTC TTCGCATGGA GTTGATGGCC
1621  TGTCATTTAA ATAGTTGCAC CATGCCATTG GGAATGCAGA GTAAAGCAAT ATCAGATGCA
1681  CAGATTACTG CTTCATCCTA CTTTACCAAT ATGTTTGCCA CCTGGTCTCC TTCAAAAGCT
1741  CGACTTCACC TCCAAGGGAG GAGTAATGCC TGGAGACCTC AGGTGAATAA TCCAAAACAG
1801  TGGCTGCAAG TGGACTTCCA GAAGACAATG AAAGTCACAG GAGTAACTAC TCAGGGAGTA
1861  AAATCTCTGC TTACCAGCAT GTATGTGAAG GAGTTCCTCA TCTCCAGCAG TCAAGATGGC
1921  CATCAGTGGA CTCTCTTTTT TCAGAATGGC AAAGTAAAGC TTTTTCAGGG AAATCAAGAC
1981  TCCTTCACAC CTGTGGTGAA GTCTCTAGAC CCACCGTTAC TGACTCGCTA CCTTCGAATT
2041  CACCCCCAGA GTTGGGTGCA CCAGATTGCC CTGAGGATGG AGGTTCTGGG CTGCGAGGCA
2101  CAGGACCTCT ACGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCAGA ACTCCTGGGC
2161  GGACCGTCAG TCTTCCTCTT CCCCCCAAAA 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 CCCCCATCGA 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
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Table 2: Polypeptide Sequences

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

Construct = HC-LC-Fc fusion. An Fc expression cassette is cotransfected with BDDFVIII-Fc to generate the BDD FVIIIFc monomer-. For the BDD FVIIIFc 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
ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR
PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEE
EDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
PTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENRSW
YLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
GDYYEDSYEDISAYLLSKNNAIEPR*SFSQNPPVLKRHQR*EITRTTLQSDQEEIDYDDTISVEMKK
EDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFT
DGSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPRK
NFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGR
QVTVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVM
AQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAGIWRVE
CLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYGQWAPKLARLHYSGSINAWST
KEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGN
VDSSGIKHNIFNPPIIARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASS
YFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLLTSMYVKE
FLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVL
GCEAQDLY**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**

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

METDTLLLWVLLLWVPGSTG
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT

LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

B. Full length FVIIIFc monomer hybrid (Full length FVIIIFc monomer dimer): created by coexpressing FVIIIFc 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 FVIIIFc monomer. For the FVIIIFc 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 FVIIIFc chain (FVIII signal peptide underlined (SEQ ID NO:6)

MQIELSTCFFLCLLRFCFS
ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR
PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEE
EDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
PTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENRSW
YLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
GDYYEDSYEDISAYLLSKNNAIEPR*SFSQNSRHPSTRQKQFNATTIPENDIEKTDPWFAHRTPMP*
KIQNVSSSDLLMLLRQSPTPHGLSLSDLQEAKYETFSDDPSPGAIDSNNSLSEMTHFRPQLHHSG
DMVFTPESGLQLRLNEKLGTTAATELKKLDFKVSSTSNNLISTIPSDNLAAGTDNTSSLGPPSMP
VHYDSQLDTTLFGKKSSPLTESGGPLSLSEENNDSKLLESGLMNSQESSWGKNVSSTESGRLFKG
KRAHGPALLTKDNALFKVSISLLKTNKTSNNSATNRKTHIDGPSLLIENSPSVWQNILESDTEFK
KVTPLIHDRMLMDKNATALRLNHMSNKTTSSKNMEMVQQKKEGPIPPDAQNPDMSFFKMLFLPES
ARWIQRTHGKNSLNSGQGPSPKQLVSLGPEKSVEGQNFLSEKNKVVVGKGEFTKDVGLKEMVFPS
SRNLFLTNLDNLHENNTHNQEKKIQEEIEKKETLIQENVVLPQIHTVTGTKNFMKNLFLLSTRQN
VEGSYDGAYAPVLQDFRSLNDSTNRTKKHTAHFSKKGEEENLEGLGNQTKQIVEKYACTTRISPN
TSQQNFVTQRSKRALKQFRLPLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEKGAI
TQSPLSDCLTRSHSIPQANRSPLPIAKVSSFPSIRPIYLTRVLFQDNSSHLPAASYRKKDSGVQE
SSHFLQGAKKNNLSLAILTLEMTGDQREVGSLGTSATNSVTYKKVENTVLPKPDLPKTSGKVELL
PKVHIYQKDLFPTETSNGSPGHLDLVEGSLLQGTEGAIKWNEANRPGKVPFLRVATESSAKTPSK
LLDPLAWDNHYGTQIPKEEWKSQEKSPEKTAFKKKDTILSLNACESNHAIAAINEGQNKPEIEVT
*WAKQGRTERLCSQNPPVLKRHQR*EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRS
FQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEH
LGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPRKNFVKPNETKTYFWKVQ
HHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDE
TKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYLLSMGSN
ENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFL
VYSNKCQTPLGMASGHIRDFQITASGQYGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMI
IHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPII
ARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARL

HLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFF
QNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY**DKTHTCPP**
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

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

METDTLLLWVLLLWVPGSTG
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
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 Igκ 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
ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR
PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEE
EDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
PTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENRSW
YLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
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

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR
PPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFP
GGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKF
ILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVK
VDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEE
EDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYG
EVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
PTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENRSW
YLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT
GDYYEDSYEDISAYLLSKNNAIEPRSFSQN**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR**
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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

METDTLLLWVLLLWVPGSTG
EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGNSS
SPHVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQ
ASRPYSFYSSLISYEEDQRQGAEPRKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDL
EKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQME
DPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKM
ALYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQIT
ASGQYGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQFII
MYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPTHYSIRSTLRMELMG
CDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVD
FQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLD
PPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL**
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

Table 3. Whole blood clotting time (WBCT) determination in hemophilia A mice after a single intravenous dose of 50 IU/kg rFVIIIFc 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 rFVIIIFc	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

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 rFVIIIFc	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)	Vss (mL/kg)
rFVIIIFc	1.56	22.6	11.1	2.09	28.4
ReFacto®[E]	0.67	6.94	5.0	7.2	43.8
Advate[E]	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 rFVIIIFc, 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)	Vz (mL/kg)
rFVIIIFc	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)	Vz (mL/kg)
rFVIIIFc	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 rFVIIIFc, 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 rFVIIIFc or ReFacto®.

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

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

A. rFVIIIFc concentration in plasma (μg/mL)

	Group 1			Group 2			Mean	SD
Time, hr	**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)

	Group 1			Group 2				
Time, hr	**604376**	**606595**	**C36195**	**C36066**	**C36174**	**604362**	**Mean**	**SD**
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 rFVIIIFc 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. rFVIIIFc

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 **rFVIIIFc** after a single 125 IU/kg dose

PK Parameter	rFVIIIFc ELISA Data								
	units	Group 1			Group 2			Average	SD
		604376	606595	C36195	C36066	C36174	604362		
Tmax	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.00
Cmax	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
Vz	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	rFVIIIFc Chromogenic Activity Data								
	units	Group 1			Group 2			Average	SD
		604376	606595	C36195	C36066	C36174	604362		
Tmax	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.00
Cmax	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
Vz	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		
Tmax	hr	0.25	4	0.25	0.25	0.25	0.25	0.88	1.53
Cmax	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
Vz	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		
Tmax	hr	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0
Cmax	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
Vz	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

	Km (nM)	Vmax (nM/min)
rFVIIIFc	55.0 ± 5.9	65.6 ± 8.6
BDD FVIII	51.0 ± 8.7	73.5 ± 10.1

Table 12. Interaction with Factor IXa

	Kd (nM)	Vmax (nM/min)
rFVIIIFc	2.8 ± 0.4	4.5 ± 0.3
BDD FVIII	2.5 ± 0.3	4.0 ± 1.0

CLAIMS

1. A composition for use in decreasing the incidence of a bleeding episode in a human subject comprising a therapeutic dose of a chimeric polypeptide comprising a functional Factor VIII (FVIII) portion having coagulant activity and a Fc portion, wherein the composition is to be administered in multiple doses, each of the multiple doses being 25 IU/kg to 100 IU/kg of the chimeric polypeptide at a dosing interval of 72 hours to seven days between two doses.

2. Use of a composition comprising a therapeutic dose of a chimeric polypeptide in the manufacture of a medicament for the treatment of a bleeding episode in a subject suffering from hemophilia A, by decreasing the incidence of the bleeding episode, wherein the chimeric polypeptide comprises a functional Factor VIII (FVIII) portion having coagulant activity and a Fc portion, and wherein the medicament is to be administered in multiple doses, each of the multiple doses being 25 IU/kg to 100 IU/kg of the chimeric polypeptide at a dosing interval of 72 hours to seven days between two doses.

3. The use of claim 2, wherein the decreasing the incidence of a bleeding episode prevents or treats the bleeding episodes.

4. The use of any one of claims 2 to 3, wherein the administration is for routine prophylaxis.

5. The use of any one of claims 2 to 3, wherein the administration is for on-demand treatment.

6. The use of any one of claims 2 to 3, wherein the administration is for tailored prophylaxis.

7. The use of any one of claims 2 to 6, wherein a trough level of plasma Factor VIII:C in the subject is maintained above 1 IU/dl.

8. The use of any one of claims 2 to 7, wherein each of the multiple doses is in a therapeutic dose of 25-30 IU/kg, 30-40 IU/kg, 40-50 IU/kg, 50-60 IU/kg, 60-70 IU/kg, 70-80 IU/kg, 80-90 IU/kg, or 90 to 100 IU/kg of the chimeric polypeptide.

9. The use of any one of claims 2 to 8, wherein each of the multiple doses is in a therapeutic dose of 25 IU/kg, 30 IU/kg, 35 IU/kg, 40 IU/kg, 45 IU/kg, 50 IU/kg, 55 IU/kg, 60 IU/kg, 65 IU/kg, 70 IU/kg, 75 IU/kg, 80 IU/kg, 85 IU/kg, 90 IU/kg, or 100 IU/kg of the chimeric polypeptide.

10. The use of any one of claims 2 to 9, wherein the dosing interval is 72 hours to seven days.

11. The use of any one of claims 2 to 9, wherein the dosing interval is 72 hours, four days, five days, six days, or seven days.

12. The use of any one of claims 2 to 9, wherein the dosing interval is five days to seven days.

13. The use of any one of claims 2 to 10, wherein the dosing interval is twice a week.

14. The use of any one of claims 2 to 13, wherein each of the multiple doses is in a therapeutic dose of 65 IU/kg to 100 IU/kg of the chimeric polypeptide.

15. The use of claim 14, wherein the dosing interval is seven days.

16. The use of any one of claims 2 to 7, wherein the composition is to be administered to the subject twice weekly, in a first therapeutic dose of 25 IU/kg to 65 IU/kg of the chimeric polypeptide and a second therapeutic dose of 25 IU/kg to 65 IU/kg of the chimeric polypeptide.

17. The use of claim 16, wherein the dosing interval between the first dose and the second dose is 72 hours, four days, five days, six days, or seven days.

18. The use of any one of claims 2 to 17, wherein the administration resolves 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% of bleeding episodes.

19. The use of any one of claims 2 to 17, wherein the administration resolves 80-100%, 80-90%, 85-90%, 90-100%, 90-95%, or 95-100% of bleeding episodes.

20. The use of any one of claims 2 to 19, wherein each of the multiple doses is 25 IU/kg to 65 IU/kg.

21. The use of any one of claims 2 to 19, wherein each of the multiple dose is 25 IU/kg, 30 IU/kg, 35 IU/kg, 40 IU/kg, 45 IU/kg, 50 IU/kg, 55 IU/kg, 60 IU/kg, or 65 IU/kg.

22. The use of claim 20, wherein the dosing interval is three days to five days.

23. The use of any one of claims 2 to 19, wherein each of the multiple doses is 65 IU/kg and the dosing interval is five days to seven days.

24. The use of any one of claims of 2 to 23, wherein each of the multiple doses is 50 IU/kg or 65 IU/kg.

25. The use of claim 24, wherein the dosing interval is five days to seven days.

26. The use of any claims 2 to 23, wherein each of the multiple doses is 50 IU/kg and the dosing interval is five days.

27. A composition for use in maintaining homeostasis of a level of Factor VIII ("FVIII") in a human subject in need of a surgery comprising a therapeutic dose of a long-acting FVIII polypeptide comprising functional FVIII having coagulant activity and a Fc, wherein the composition is to be administered in multiple doses, each of the multiple doses being about 10 IU/kg to about 50 IU/kg of the long-acting FVIII polypeptide at a dosing interval of 12 hours to 7 days between two doses.

28. Use of a composition comprising a therapeutic dose of a long-acting FVIII polypeptide comprising functional FVIII having coagulant activity and a Fc, in the manufacture of a medicament for maintaining homeostasis of a level of Factor VIII ("FVIII") in a human

subject in need of a surgery, wherein the composition is to be administered in multiple doses, each of the multiple doses being about 10 IU/kg to about 50 IU/kg of the long-acting FVIII polypeptide at a dosing interval of 12 hours to 7 days between two doses.

29. The use of claim 28, wherein a trough level of plasma Factor VIII:C in the subject after the administration is maintained above 3 IU/dl.

30. The use of claim 28 or claim 29, wherein the administration prevents a bleeding episode in the subject.

31. The use of any one of claims 28 to 30, wherein the subject is in need of surgical prophylaxis, peri-operative management, or treatment for surgery.

32. The use of any one of claims 28 to 31, wherein the 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.

33. The use of any one of claims 28 to 32, wherein the surgery is an emergency surgery.

34. The use of any one of claims 28 to 33, wherein the therapeutic dose is about 10 IU/kg, about 20 IU/kg, about 30 IU/kg, about 40 IU/kg, or about 50 IU/kg.

35. The use of any one of claims 28 to 34, wherein the dosing interval is 12 hours to 24 hours, 24 hours to 48 hours, or 48 hours to 72 hours.

36. The use of any one of claims 28 to 30, wherein the dosing interval is 12 hours to 24 hours.

37. A composition for use in maintaining homeostasis of a level of Factor VIII ("FVIII") in a human subject in need of surgical prophylaxis, peri-operative management, or treatment of surgery wherein the composition comprises a FVIII polypeptide which is to be administered

in multiple doses of about 35 IU/kg to about 50 IU/kg of a long-acting chimeric FVIII polypeptide at a dosing interval of 12 hours to 24 hours, wherein the long-acting FVIII polypeptide is a FVIIIFc monomer dimer hybrid comprising a functional FVIII having coagulant activity and two Fcs, wherein one of the Fcs is fused to the C-terminus of the light chain of the functional FVIII, and wherein a trough level of plasma Factor VIII:C in the subject is maintained above 3 IU/dl between the administration of the doses.

38. Use of a composition for use in maintaining homeostasis of a level of Factor VIII ("FVIII") in a human subject in need of surgical prophylaxis, peri-operative management, or treatment of surgery wherein the composition comprises a FVIII polypeptide which is to be administered in multiple doses of about 35 IU/kg to about 50 IU/kg of a long-acting chimeric FVIII polypeptide at a dosing interval of 12 hours to 24 hours, wherein the long-acting FVIII polypeptide is a FVIIIFc monomer dimer hybrid comprising a functional FVIII having coagulant activity and two Fcs, wherein one of the Fcs is fused to the C-terminus of the light chain of the functional FVIII, and wherein a trough level of plasma Factor VIII:C in the subject is maintained above 3 IU/dl between the administration of the doses.

39. A composition for use in prophylactically treating hemophilia A in a human subject in need thereof comprising a therapeutic dose of a chimeric Factor VIII (FVIII) polypeptide, comprising a functional FVIII having coagulant activity and a Fc, wherein the composition is to be administered in multiple doses, each of the multiple doses being about 25 IU/kg to about 80 IU/kg of the chimeric FVIII polypeptide at a dosing interval of 72 hours to seven days between two doses.

40. Use of a composition comprising a therapeutic dose of a chimeric Factor VIII (FVIII) polypeptide, in the manufacture of a medicament for prophylactically treating hemophilia A in a human subject in need thereof, wherein the composition comprises a functional FVIII having coagulant activity and a Fc, wherein the composition is to be administered in multiple doses, each of the multiple doses being about 25 IU/kg to about 80 IU/kg of the chimeric FVIII polypeptide at a dosing interval of 72 hours to seven days between two doses.

41. The use of claim 40, wherein a trough level of plasma Factor VIII:C in the subject is maintained above 1 IU/dL.

42. The use of claim 40 or claim 41, wherein each of the multiple doses is about 25 IU/kg, about 30 IU/kg, about 40 IU/kg, about 50 IU/kg, about 55 IU/kg, about 60 IU/kg, about 65 IU/kg, about 70 IU/kg, about 75 IU/kg, or about 80 IU/kg.

43. The use of any one of claims 40 to 42, wherein the dosing interval is 72 hours, four days, five days, six days, or seven days.

44. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 43, wherein a mean clearance (CL) (activity) in the subject is 2.33 ± 1.08 mL/hour/kg or less.

45. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 44, wherein a mean residence time (MRT) (activity) in the subject is 1.5 fold longer than the mean MRT of a polypeptide consisting of said functional FVIII portion.

46. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 45, wherein the MRT is 14 to 41.3 hours.

47. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 46, wherein a T1/2 (activity) in the subject is 1.5 fold longer than the mean T1/2 (activity) of a polypeptide consisting of said functional FVIII portion.

48. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 47, wherein a T1/2 (activity) is 11 to 26.4 hours.

49. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 48, wherein a mean incremental recovery (K value) in the subject is 90% of the mean incremental recovery of a polypeptide consisting of said functional FVIII portion.

50. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 49, wherein a mean incremental recovery (K value) in the subject is 1.38 to 2.88 IU/dL per IU/kg.

51. The use of claim 50, wherein the mean incremental recovery (K value) in the subject is 70% to 125% of the mean incremental recovery of a polypeptide consisting of said functional FVIII portion.

52. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 51, wherein a mean Vss (activity) in the subject is 37.7 to 79.9 mL/kg.

53. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 52, wherein a mean AUC/dose (activity) in the subject is 19.2IU*h/dL per IU/kg to 81.7IU*h/dL per IU/kg.

54. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 53, wherein the chimeric polypeptide is pegylated.

55. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 54, wherein the chimeric polypeptide is a FVIIIFc monomer dimer hybrid.

56. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 55, wherein the functional Factor VIII portion comprises full-length factor VIII, mature factor VIII, or factor VIII with a full or partial deletion of the B domain.

57. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 56, wherein the functional FVIII portion comprises an amino acid sequence at least 90% or 95% identical to amino acids 1 to 1438 of SEQ ID NO: 2 or amino acids 1 to 2332 of SEQ ID NO: 6.

58. The use of claim 57, wherein the functional FVIII portion comprises amino acids 1 to 1438 of SEQ ID NO: 2 or amino acids 1 to 2332 of SEQ ID NO: 6.

59. The use of any of claims 2 to 26, 28 to 36, 38, or 40 to 56, wherein the Fc portion is at least 90% or 95% identical to amino acids 1439 to 1665 of SEQ ID NO: 2 or amino acids 2333 to 2559 of SEQ ID NO: 6.

60. The use of any of claims 2 to 26, 28 to 36, 38, or 40 to 59, wherein the Fc portion comprises amino acids 1439 to 1665 of SEQ ID NO: 2 or amino acids 2333 to 2559 of SEQ ID NO: 6.

61. The use of any one of claims 2 to 26, 28 to 36, 38, or 40 to 60, further comprising at least one excipient.