

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

21 November 2019 (21.11.2019)



(10) International Publication Number

WO 2019/222682 A1

(51) International Patent Classification:

A61K 38/36 (2006.01)

A61P 7/04 (2006.01)

A61K 38/37 (2006.01)

(21) International Application Number:

PCT/US2019/032956

(22) International Filing Date:

17 May 2019 (17.05.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/673,670 18 May 2018 (18.05.2018) US

62/712,880 31 July 2018 (31.07.2018) US

62/773,785 30 November 2018 (30.11.2018) US

62/801,576 05 February 2019 (05.02.2019) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,

KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,

MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,

TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: METHODS OF TREATING HEMOPHILIA A

(57) Abstract: The present disclosure provides a method of treating hemophilia A in a human subject in need thereof comprising administering to the subject a chimeric polypeptide comprising (i) a factor VIII (FVIII) protein and (ii) a von Willebrand factor (VWF) fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval.



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METHODS OF TREATING HEMOPHILIA A

REFERENCE TO PRIORITY APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 62/673,670, filed May 18, 2018, U.S. Provisional Application No. 62/712,880, filed July 31, 2018, U.S. Provisional Application No. 62/773,785 filed Nov. 30, 2018, and U.S. Provisional Application No. 62/801,576, filed Feb. 5, 2019, each of which are incorporated herein by reference in their entireties.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing in ASCII text file (Name: SA9-461PC_SeqListing.txt; Size: 922 kilobytes; Date of Creation: May 14, 2019) is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0003] Hemophilia A is a bleeding disorder caused by defects in the gene encoding coagulation factor VIII (FVIII) and affects 1-2 in 10,000 male births. Graw *et al.*, *Nat. Rev. Genet.* 6(6): 488-501 (2005). Patients affected with hemophilia A can be treated with infusions of purified or recombinantly produced FVIII. Many commercially available FVIII products are known to have a half-life of about 8-12 hours, requiring frequent intravenous administration to the patients. See Weiner M.A. and Cairo, M.S., *Pediatric Hematology Secrets*, Lee, M.T., 12. Disorders of Coagulation, Elsevier Health Sciences, 2001; Lillicrap, D. *Thromb. Res.* 122 Suppl 4:S2-8 (2008). In addition, a number of approaches have been tried in order to extend the FVIII half-life. For example, the approaches in development to extend the half-life of clotting factors include pegylation, glycopegylation, and conjugation with albumin. See Dumont *et al.*, *Blood.* 119(13): 3024-3030 (2012). Consistent results have been demonstrated in humans, for example, rFVIII^h was reported to improve half-life up to ~1.7-fold compared with ADVATE[®] in hemophilia A patients. See Powell *et al.*, *Blood.* 119(13): 3031-3037 (2012). Therefore, the half-life increases, despite minor improvements, indicate the presence of other half-life limiting factors. See Liu, T. *et al.*, 2007 ISTH meeting, abstract #P-M-035; Henrik, A. *et al.*, 2011 ISTH meeting, abstract #P=MO-181; Liu, T. *et al.*, 2011 ISTH meeting abstract #P-WE-131.

[0004] The current recommended standard of care involves the regular administration (routine prophylaxis) of FVIII to minimize the number of bleeding episodes. Routine prophylaxis has been associated with improvements in long-term outcomes, but is a demanding regimen limited by the need for frequent intravenous (IV) administration. See Manco-Johnson et al., *N Engl J Med.* 357(6):535-44 (2007). Extended half-life FVIII products have reduced the frequency of FVIII administration for prophylaxis; however, all interact with von Willebrand factor (VWF) and have comparable circulating half-lives, consistent with an upper limit on the half-life of rFVIII variants due to the half-life of endogenous VWF. See, e.g., Pipe et al., *Blood.* 128(16):2007-16 (2016). Prophylactic dosing for these FVIII products is every 3 to 5 days.

[0005] Next-generation extended half-life FVIII products that prevent and control bleeding episodes for longer periods of time, resulting in less frequent administration, would potentially address the challenges of adherence to demanding prophylactic regimens, which in turn could improve the quality of life for hemophilia patients.

BRIEF SUMMARY OF THE DISCLOSURE

[0006] Certain aspects of the present disclosure are directed to a method of treating hemophilia A in a human subject in need thereof comprising administering to the subject multiple doses of a chimeric polypeptide comprising (i) a factor VIII (FVIII) protein and (ii) a von Willebrand factor (VWF) fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval, wherein at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg and the dosing interval is at least about every 7 days.

[0007] In some embodiments, the multiple doses comprise at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, at least twelve doses, at least thirteen doses, at least fourteen doses, at least fifteen doses, at least sixteen doses, at least seventeen doses, at least eighteen doses, at least nineteen doses, at least twenty doses, or more.

[0008] In some embodiments, the treatment of hemophilia A comprises controlling or decreasing the incidence or frequency of a bleeding episode in a human subject in need thereof. In some embodiments, the treatment of hemophilia A comprises preventing or treating a bleeding episode in a human subject in need thereof.

[0009] In some embodiments, at least one of the multiple doses is from about 20 IU/kg to about 95 IU/kg, from about 20 IU/kg to about 90 IU/kg, from about 20 IU/kg to about 85 IU/kg, from about 20 IU/kg to about 80 IU/kg, from about 20 IU/kg to about 75 IU/kg, from about 20 IU/kg to about 70 IU/kg, from about 20 IU/kg to about 65 IU/kg, from about 20 IU/kg to about 60 IU/kg, from about 20 IU/kg to about 55 IU/kg, from about 20 IU/kg to about 50 IU/kg, from about 20 IU/kg to about 45 IU/kg, from about 20 IU/kg to about 40 IU/kg, from about 20 IU/kg to about 35 IU/kg, from about 20 IU/kg to about 30 IU/kg, or from about 20 IU/kg to about 25 IU/kg. In some embodiments, at least one of the multiple doses is from about 20 IU/kg to about 100 IU/kg, from about 25 IU/kg to about 100 IU/kg, from about 30 IU/kg to about 100 IU/kg, from about 35 IU/kg to about 100 IU/kg, from about 40 IU/kg to about 100 IU/kg, from about 45 IU/kg to about 100 IU/kg, from about 50 IU/kg to about 100 IU/kg, from about 55 IU/kg to about 100 IU/kg, from about 60 IU/kg to about 100 IU/kg, from about 65 IU/kg to about 100 IU/kg, from about 70 IU/kg to about 100 IU/kg, from about 75 IU/kg to about 100 IU/kg, from about 80 IU/kg to about 100 IU/kg, from about 85 IU/kg to about 100 IU/kg, or from about 90 IU/kg to about 100 IU/kg.

[0010] In some embodiments, at least one of the multiple doses is from about 20 IU/kg to about 80 IU/kg, from about 25 IU/kg to about 75 IU/kg, from about 30 IU/kg to about 70 IU/kg, from about 35 IU/kg to about 65 IU/kg, from about 40 IU/kg to about 60 IU/kg, or from about 45 IU/kg to about 55 IU/kg. In some embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg.

[0011] In some embodiments, at least one of the multiple doses is about 20 IU/kg, about 25 IU/kg, about 30 IU/kg, about 35 IU/kg, about 40 IU/kg, about 45 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, about 80 IU/kg, about 85 IU/kg, about 90 IU/kg, about 95 IU/kg, or about 100 IU/kg. In some embodiments, at least one of the multiple doses is about 25 IU/kg. In some embodiments, at least one of the multiple doses is about 50 IU/kg. In some embodiments, at least one of the multiple doses is about 65 IU/kg. In some embodiments, at least one of the multiple doses is about 80 IU/kg.

[0012] In some embodiments, the dosing interval is at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least

about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, at least about 30 days, or at least about 31 days.

[0013] In some embodiments, the dosing frequency is at least once every week, at least once every 2 weeks, at least once every 3 weeks, or at least once every 4 weeks. In some embodiments, the dosing interval is at least once every week. In some embodiments, the dosing interval is at least once every two weeks.

[0014] In some embodiments, the chimeric polypeptide is administered for prophylactic treatment.

[0015] In some embodiments, the FVIII protein is associated with the VWF fragment by a covalent bond. In some embodiments, the covalent bond is a peptide bond or a disulfide bond.

[0016] In some embodiments, the FVIII protein comprises a FVIII polypeptide and a first half-life extending moiety. In some embodiments, the first half-life extending moiety is fused to the C-terminus or the N-terminus of the FVIII polypeptide. In some embodiments, the first half-life extending moiety is inserted within the FVIII polypeptide. In some embodiments, the first half-life extending moiety is inserted within the B domain of the FVIII polypeptide. In some embodiments, the first half-life extending moiety is inserted within the FVIII polypeptide immediately downstream of an amino acid corresponding to amino acid residue 745 of SEQ ID NO: 65. In some embodiments, the first half-life extending moiety is fused to the FVIII polypeptide by a linker.

[0017] In some embodiments, the VWF fragment comprises a second half-life extending moiety. In some embodiments, the second half-life extending moiety is fused to the C-terminus or the N-terminus of the VWF fragment. In some embodiments, the second half-life extending moiety is inserted within the VWF fragment. In some embodiments, the second half-life extending moiety is fused to the C-terminus of the VWF fragment. In some embodiments, the second half-life extending moiety is fused to the VWF fragment by a linker.

[0018] In some embodiments, the first half-life extending moiety, the second half-life extending moiety, or both is selected from the group consisting of an albumin, an immunoglobulin Fc region, an XTEN sequence, the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, a PAS sequence, a HAP sequence, a transferrin, albumin-binding moieties, or any fragments, derivatives, variants, and any combination thereof.

[0019] In some embodiments, the first half-life extending moiety comprises a first XTEN.

[0020] In some embodiments, the first XTEN is inserted within the FVIII polypeptide immediately downstream of an amino acid corresponding to amino acid residue 745 of SEQ ID NO: 65.

[0021] In some embodiments, the second half-life extending moiety comprises a second XTEN. In some embodiments, the second XTEN is fused to the C-terminus of the VWF fragment.

5 [0022] In some embodiments, the FVIII protein comprises a first immunoglobulin (Ig) constant region or a portion thereof. In some embodiments, the first Ig constant region or the portion thereof is fused to the C-terminus or the N-terminus of the FVIII polypeptide. In some embodiments, the first Ig constant region or the portion thereof is inserted within the FVIII polypeptide. In some embodiments, the first Ig constant region or the portion thereof is fused to the C-terminus of the FVIII polypeptide.
10 In some embodiments, the first Ig constant region or the portion thereof is fused to the FVIII polypeptide by a linker. In some embodiments, the first Ig constant region or the portion thereof comprises a first Fc domain or a portion thereof.

[0023] In some embodiments, the VWF fragment comprises a second Ig constant region or a portion thereof. In some embodiments, the second Ig constant region or the portion thereof is fused to the C-terminus or the N-terminus of the VWF fragment. In some embodiments, the second Ig constant region or the portion thereof is inserted within the VWF fragment. In some embodiments, the second Ig constant region or the portion thereof is fused to the C-terminus of the VWF fragment. In some embodiments, the second Ig constant region or the portion thereof is fused to the VWF fragment by a linker. In some embodiments, the linker is a cleavable linker. In some embodiments,
15 the second Ig constant region or the portion thereof comprises a second Fc domain or a portion thereof.
20

[0024] In some embodiments, the FVIII protein and the VWF fragment are associated with each other through a covalent bond between the first Fc domain and the second Fc domain. In some embodiments, the FVIII protein and the VWF fragment are further associated with each other through
25 a non-covalent interaction between the FVIII protein and the VWF fragment.

[0025] In one aspect, disclosed herein is a method of treating hemophilia A in a human subject comprising administering to the subject in need thereof multiple doses of a chimeric polypeptide at a dosing interval, wherein the chimeric polypeptide comprises: (i) a FVIII protein comprising a first FVIII polypeptide fragment comprising the amino acid sequence of SEQ ID NO: 215; a first XTEN sequence
30 comprising the amino acid sequence of SEQ ID NO: 8 (AE288); a second FVIII polypeptide fragment comprising the amino acid sequence of SEQ ID NO: 216; and a first Fc region comprising the amino

acid sequence of SEQ ID NO: 217; and (ii) a VWF protein comprising: a D' domain of VWF comprising the amino acid sequence of SEQ ID NO: 210; a D3 domain of VWF comprising the amino acid sequence of SEQ ID NO: 214; a second XTEN sequence comprising the amino acid sequence of SEQ ID NO: 58 (AE144_5A); an a2 linker comprising the amino acid sequence of SEQ ID NO: 88; and a second Fc region comprising the amino acid sequence of SEQ ID NO: 217, and wherein the first Fc region is covalently linked to the second Fc region by a disulfide bond.

[0026] In some embodiments, the chimeric polypeptide comprises a FVIII protein comprising a FVIII polypeptide, a first XTEN sequence, a first Fc region, and a VWF protein comprising a D' domain of VWF, a D3 domain of VWF, a second XTEN sequence, an a2 linker of FVIII and a second Fc region, wherein the FVIII polypeptide comprises the amino acid sequence of SEQ ID NO: 215, the first XTEN sequence comprises the amino acid sequence of AE288 (SEQ ID NO: 8) and is fused to the C-terminus of SEQ ID NO: 215, the FVIII polypeptide further comprises the amino acid sequence of SEQ ID NO: 216, the first Fc region comprises the amino acid sequence of SEQ ID NO: 217 and is fused to the C-terminus of SEQ ID NO: 216; the D' domain of VWF comprises the amino acid sequence of SEQ ID NO: 210; the D3 domain of VWF comprises the amino acid sequence of SEQ ID NO: 214, the second XTEN sequence comprises the amino acid sequence of AE144_5A (SEQ ID NO: 58) and is fused to the C-terminus of the D3 domain of VWF; the a2 linker comprises the amino acid sequence of SEQ ID NO: 88 and is fused to the C-terminus of the second XTEN sequence; the second Fc region comprises the amino acid sequence of SEQ ID NO: 217 and is fused to the C-terminus of the a2 linker; and wherein the first Fc region is covalently linked to the second Fc region by a disulfide bond.

[0027] In some embodiments, the chimeric polypeptide comprises a FVIII protein comprising a FVIII signal peptide comprising the amino acid sequence of SEQ ID NO: 64. In some embodiments, the chimeric polypeptide comprises a VWF protein comprising a VWF signal peptide comprising the amino acid sequence of SEQ ID NO: 208. In some embodiments, the chimeric polypeptide comprises a VWF protein comprising a D1D2 domain of VWF comprising the amino acid sequence of SEQ ID NO: 209.

[0028] In some embodiments, the chimeric polypeptide comprises a FVIII protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to SEQ ID NO: 201, SEQ ID NO: 203, or SEQ ID NO: 207; and a VWF protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to SEQ ID NO: 202 or SEQ ID NO: 205.

[0029] In one embodiment, the chimeric polypeptide comprises a FVIII protein comprising the amino acid sequence of SEQ ID NO: 203 and a VWF protein comprising the amino acid sequence of SEQ ID NO: 205. In another embodiment, the chimeric polypeptide comprises a FVIII protein comprising the amino acid sequence of SEQ ID NO: 201 and a VWF protein comprising the amino acid sequence of SEQ ID NO: 202. In another embodiment, the chimeric polypeptide comprises a FVIII protein comprising the amino acid sequence of SEQ ID NO: 207 and a VWF protein comprising the amino acid sequence of SEQ ID NO: 202.

[0030] In some embodiments, the chimeric polypeptide comprises a FVIII protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from FVIII-161 (SEQ ID NO: 69), FVIII-169 (SEQ ID NO: 70), FVIII-170 (SEQ ID NO: 71), FVIII-173 (SEQ ID NO: 72); FVIII-195 (SEQ ID NO: 73); FVIII-196 (SEQ ID NO: 74), FVIII-199 (SEQ ID NO: 75), FVIII-201 (SEQ ID NO: 76); FVIII-203 (SEQ ID NO: 77), FVIII-204 (SEQ ID NO: 78), FVIII-205 (SEQ ID NO: 79), FVIII-266 (SEQ ID NO: 80), FVIII-267 (SEQ ID NO: 81), FVIII-268 (SEQ ID NO: 82), FVIII-269 (SEQ ID NO: 83), FVIII-271 (SEQ ID NO: 84), FVIII-272 (SEQ ID NO: 85), FVIII-312 (SEQ ID NO: 173), or FVIII-312A (SEQ ID NO: 203); and a VWF protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from VWF031 (SEQ ID NO: 86), VWF034 (SEQ ID NO: 87), VWF059 (SEQ ID NO: 197), VWF059A (SEQ ID NO: 202), or VWF036.

[0031] In some embodiments, the chimeric polypeptide is administered by a route selected from the group consisting of intravenous injection, intravenous infusion, subcutaneous administration, intramuscular administration, oral administration, nasal administration, and pulmonary administration.

[0032] In some embodiments, the chimeric polypeptide after the administration results in a FVIII plasma activity level of at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10%. In some embodiments, the FVIII plasma activity level is at least about 3%. In some embodiments, the FVIII plasma activity level is at least about 5%.

[0033] In some embodiments, the chimeric polypeptide after the administration results in a FVIII plasma activity level of at least about 1 IU/dL, at least about 2 IU/dL, at least about 3 IU/dL, at least about 4 IU/dL, at least about 5 IU/dL, at least about 6 IU/dL, at least about 7 IU/dL, at least about 8 IU/dL, at least about 9 IU/dL, or at least about 10 IU/dL. In some embodiments, the FVIII plasma

activity level is at least about 3 IU/dL. In some embodiments, the FVIII plasma activity level is at least about 5 IU/dL.

[0034] In some embodiments, the FVIII plasma activity level is at least about 10 IU/dL at least about 5 days after the administration of the chimeric polypeptide. In some embodiments, the FVIII plasma activity level is at least about 5 IU/dL at least about 7 days after the administration of the chimeric polypeptide. In some embodiments, the FVIII plasma activity level is at least about 3 IU/dL at least about 8 days after the administration of the chimeric polypeptide. In some embodiments, the FVIII plasma activity level is at least about 1 IU/dL at least about 10 days after the administration of the chimeric polypeptide.

[0035] In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg, and the dosing interval is at least about 7 days. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg, and the dosing interval is at least about 5 days. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg, and the dosing interval is at least about 5 days.

[0036] In some embodiments, at least one of the multiple doses is about 50 IU/kg. In some embodiments, the multiple doses are about 50 IU/kg, and the dosing interval is about 5 days. In some embodiments, the multiple doses are about 50 IU/kg, and the dosing interval is about 7 days. In some embodiments, the multiple doses are about 50 IU/kg, and the dosing interval is about 14 days.

[0037] In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg, and the dosing interval is at least about 7 days. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg, and the dosing interval is at least about 7 days. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg, and the dosing interval is at least about 7 days.

[0038] In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg, and the dosing interval is at least about 10 days. In some embodiments, at least one of the

multiple doses is from about 50 IU/kg to about 65 IU/kg, and the dosing interval is at least about 10 days. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg, and the dosing interval is at least about 10 days.

[0039] In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg, and the dosing interval is at least about 14 days. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg, and the dosing interval is at least about 14 days. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg, and the dosing interval is at least about 14 days.

[0040] In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg, and the dosing interval is at least about 1 week. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg, and the dosing interval is at least about 1 week. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg, and the dosing interval is at least about 1 week.

[0041] In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg, and the dosing interval is at least about 2 weeks. In some embodiments, at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg, and the dosing interval is at least about 2 weeks. In some embodiments, at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg, and the dosing interval is at least about 2 weeks.

[0042] In some embodiments, the dosing interval is at least about 5 days. In some embodiments, the dosing interval is at least about 7 days. In some embodiments, the dosing interval is about 5 days to about 14 days. In some embodiments, the dosing interval is about 7 days to about 14 days. In some embodiments, the dosing interval is at least about 10 days. In some embodiments, the dosing interval is about 10 days to about 21 days. In some embodiments, the dosing interval is about 14 days to about 21 days. In some embodiments, the dosing interval is about 14 days.

[0043] In some embodiments, the human subject is a female. In some embodiments, the human subject is a child. In some embodiments, the human subject is a child less than or equal to about 12 years old, less than about 11 years old, less than about 10 years old, less than about 9 years old, less than about 8 years old, less than about 7 years old, less than about 6 years old, less than about 5 years old, less than about 4 years old, less than about 3 years old, less than about 2 years old, or less than about 1 year old.

[0044] In some embodiments, the administration induces immune tolerance to FVIII in the human subject. In some embodiments, the administration reduces an inhibitory immune response to FVIII in the human subject. In some embodiments, the inhibitory immune response to FVIII comprises a high titer of anti-FVIII antibodies in the human subject.

5 **[0045]** In some embodiments, administration of the chimeric polypeptide induces no FVIII inhibitor after about 7 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 20 days, about 24 days, about 25 days, about 28 days, about 30 days, or about 35 days of the administration. In some embodiments, administration of the chimeric polypeptide induces no FVIII inhibitor after about 28 days of the administration.

10 **BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES**

[0046] FIG. 1 is a schematic representation of rFVIII_{FC}-VWF-XTEN. FVIII: factor VIII; VWF: von Willebrand Factor; A1, A2, A3, C1, C2: domains of FVIII; D'D3: domains of VWF; Fc: Fc region of immunoglobulin constant region.

15 **[0047]** FIGS. 2A and 2B show the protocol for testing the safety and efficacy of rFVIII_{FC}-VWF-XTEN in human patients in a low-dose cohort administered 25 IU/kg rFVIII_{FC}-VWF-XTEN (FIG. 2A) and a high-dose cohort administered 65 IU/kg rFVIII_{FC}-VWF-XTEN (FIG. 2B).

20 **[0048]** FIGS. 3A-3B are graphical representations of the baseline-corrected FVIII activity levels based on an activated partial thromboplastin time (aPTT) test in human subjects with severe hemophilia A administered 25 IU/kg rFVIII followed by a washout period and then 25 IU/kg rFVIII_{FC}-VWF-XTEN (FIG. 3A; low dose cohort) or 65 IU/kg rFVIII followed by a washout period and then 65 IU/kg rFVIII_{FC}-VWF-XTEN (FIG. 3B; high dose cohort). Horizontal dashed lines indicate 3%, 5%, 10%, and 20% FVIII activity.

25 **[0049]** FIG. 4 is a schematic representation of the design of a clinical study to assess the safety and tolerability of a total of four once-weekly doses of rFVIII_{FC}-VWF-XTEN at a dose of 50 IU/kg or 65 IU/kg in adult male, previously treated patients (PTPs), 18 to 65 years of age (inclusive), with severe hemophilia A. EOS = End of Study; ET = Early Termination; PK = Pharmacokinetics.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0050] The present disclosure is directed to a method of treating a bleeding disease or condition, e.g., hemophilia A, in a human subject in need thereof comprising administering to the subject multiple doses of a chimeric polypeptide comprising (i) a factor VIII (FVIII) polypeptide and (ii) a von Willebrand factor (VWF) fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval. In some embodiments, at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg. In some embodiments, the dosing interval is at least about every 5 days. In some embodiments, the dosing interval is at least about every 7 days.

I. Definitions

[0051] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0052] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0053] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0054] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0055] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings

provided herein are not limitations of the various aspects of the disclosure. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0056] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, e.g., 10 percent, up or down (higher or lower).

[0057] The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, e.g., DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a Factor VIII polypeptide contained in a vector is considered isolated for the purposes of the present disclosure. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present disclosure. Isolated polynucleotides or nucleic acids according to the present disclosure further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

[0058] Certain proteins secreted by mammalian cells are associated with a secretory signal peptide which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that signal peptides are generally fused to the N-terminus of the polypeptide, and are cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, a native signal peptide or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, e.g., a human tissue plasminogen activator (TPA) or mouse β -glucuronidase signal peptide, or a functional derivative thereof, can be used.

[0059] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a natural biological source or produced recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0060] An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the disclosure, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0061] Also included in the present disclosure are fragments or variants of polypeptides, and any combination thereof. The term "fragment" or "variant" when referring to polypeptide binding domains or binding molecules of the present disclosure include any polypeptides which retain at least some of the properties (e.g., FcRn binding affinity for an FcRn binding domain or Fc variant, coagulation activity for an FVIII variant, or FVIII binding activity for the VWF fragment) of the reference polypeptide. Fragments of polypeptides include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein, but do not include the naturally occurring full-length polypeptide (or mature polypeptide). Variants of polypeptide binding domains or binding molecules of the present disclosure include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can

be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions.

[0062] The term "VWF protein" or "VWF proteins" used herein means any VWF fragments that interact with FVIII and retain at least one or more properties that are normally provided to FVIII by full-length VWF, e.g., preventing premature activation to FVIIIa, preventing premature proteolysis, preventing association with phospholipid membranes that could lead to premature clearance, preventing binding to FVIII clearance receptors that can bind naked FVIII but not VWF-bound FVIII, and/or stabilizing the FVIII heavy chain and light chain interactions. A VWF fragment referred to herein is a VWF polypeptide that is less than the full-length VWF protein, wherein the VWF fragment retains the ability to interact with and/or bind to FVIII.

[0063] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In other embodiments, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0064] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present disclosure, the parameters are set, of

course, such that the percentage of identity is calculated over the full-length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0065] As used herein, an "amino acid corresponding to" or an "equivalent amino acid" in a VWF sequence or a FVIII protein sequence is identified by alignment to maximize the identity or similarity between a first VWF or FVIII sequence and a second VWF or FVIII sequence. The number used to identify an equivalent amino acid in a second VWF or FVIII sequence is based on the number used to identify the corresponding amino acid in the first VWF or FVIII sequence.

[0066] As used herein, the term "insertion site" refers to a position in a FVIII polypeptide, or fragment, variant, or derivative thereof, which is immediately downstream of the position at which a half-life extending moiety or heterologous moiety can be inserted. An "insertion site" is specified as a number, the number being the number of the amino acid in mature native FVIII (SEQ ID NO: 65) to which the insertion site corresponds, which is immediately C-terminal to the position of the insertion. For example, the phrase "a3 comprises an XTEN at an insertion site which corresponds to amino acid 1656 of SEQ ID NO: 65" indicates that the heterologous moiety is located between two amino acids corresponding to amino acid 1656 and amino acid 1657 of SEQ ID NO: 65.

[0067] The phrase "immediately downstream of an amino acid" as used herein refers to position right next to the terminal carboxyl group of the amino acid. For example, an insertion site immediately downstream of amino acid 745 corresponding to the mature wild type FVIII protein means that the insertion site is between amino acid 745 and amino acid 746 corresponding to the mature wild type FVIII protein. Similarly, the phrase "immediately upstream of an amino acid" refers to the position right next to the terminal amine group of the amino acid.

[0068] The phrase "between two amino acids of an insertion site" as used herein refers to a position in which an XTEN or any other polypeptide is inserted between two adjacent amino acids. Thus, the phrases "inserted immediately downstream of an amino acid" and "inserted between two amino acids of an insertion site" are used synonymously with "inserted at an insertion site."

[0069] The terms "inserted," "is inserted," "inserted into" or grammatically related terms, as used herein refers to the position of an XTEN in a chimeric polypeptide relative to the analogous position in native mature human FVIII. As used herein the terms refer to the characteristics of the recombinant FVIII polypeptide relative to native mature human FVIII, and do not indicate, imply or infer any methods or process by which the chimeric polypeptide was made. For example, in reference to a

chimeric polypeptide provided herein, the phrase "an XTEN is inserted into immediately downstream of residue 745 of the FVIII polypeptide" means that the chimeric polypeptide comprises an XTEN immediately downstream of an amino acid which corresponds to amino acid 745 in native mature human FVIII, *e.g.*, bounded by amino acids corresponding to amino acids 745 and 746 of native mature human FVIII.

[0070] A "fusion" or "chimeric" protein comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences which normally exist in separate proteins can be brought together in the fusion polypeptide, or the amino acid sequences which normally exist in the same protein can be placed in a new arrangement in the fusion polypeptide, *e.g.*, fusion of a Factor VIII domain of the disclosure with an Ig Fc domain. A fusion protein is created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. A chimeric polypeptide can further comprise a second amino acid sequence associated with the first amino acid sequence by a covalent, non-peptide bond or a non-covalent bond.

[0071] The term "linked" as used herein refers to a first amino acid sequence or nucleotide sequence covalently or non-covalently joined to a second amino acid sequence or nucleotide sequence, respectively. The first amino acid or nucleotide sequence can be directly joined or juxtaposed to the second amino acid or nucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first amino acid sequence to a second amino acid sequence at the C-terminus or the N-terminus, but also includes insertion of the whole first amino acid sequence (or the second amino acid sequence) into any two amino acids in the second amino acid sequence (or the first amino acid sequence, respectively). In some embodiments, the first amino acid sequence can be linked to a second amino acid sequence by a peptide bond or a linker. The first nucleotide sequence can be linked to a second nucleotide sequence by a phosphodiester bond or a linker. The linker can be a peptide or a polypeptide (for polypeptide chains) or a nucleotide or a nucleotide chain (for nucleotide chains) or any chemical moiety (for both polypeptide and polynucleotide chains). The term "linked" is also indicated by a hyphen (-).

[0072] As used herein the term "associated with" refers to a covalent or non-covalent bond formed between a first amino acid chain and a second amino acid chain. In some embodiments, the term "associated with" means a covalent, non-peptide bond or a non-covalent bond. This association can be indicated by a colon, *i.e.*, (:). In other embodiments, it means a covalent bond except a peptide

bond. For example, the amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a thiol group on a second cysteine residue. In most naturally occurring IgG molecules, the CH1 and CL regions are associated by a disulfide bond and the two heavy chains are associated by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system). Examples of covalent bonds include, but are not limited to, a peptide bond, a metal bond, a hydrogen bond, a disulfide bond, a sigma bond, a pi bond, a delta bond, a glycosidic bond, an agnostic bond, a bent bond, a dipolar bond, a Pi backbond, a double bond, a triple bond, a quadruple bond, a quintuple bond, a sextuple bond, conjugation, hyperconjugation, aromaticity, hapticity, or antibonding. Non-limiting examples of non-covalent bond include an ionic bond (e.g., cation-pi bond or salt bond), a metal bond, a hydrogen bond (e.g., dihydrogen bond, dihydrogen complex, low-barrier hydrogen bond, or symmetric hydrogen bond), van der Waals force, London dispersion force, a mechanical bond, a halogen bond, aurophilicity, intercalation, stacking, entropic force, or chemical polarity.

[0073] As used herein, the term "cleavage site" or "enzymatic cleavage site" refers to a site recognized by an enzyme. Certain enzymatic cleavage sites comprise an intracellular processing site. In some embodiments, a polypeptide has an enzymatic cleavage site cleaved by an enzyme that is activated during the clotting cascade, such that cleavage of such sites occurs at the site of clot formation. Exemplary such sites include, e.g., those recognized by thrombin, Factor XIa or Factor Xa. Exemplary FXIa cleavage sites include, e.g., TQSFNDFTR (SEQ ID NO: 1) and SVSQTSLKTR (SEQ ID NO: 3). Exemplary thrombin cleavage sites include, e.g., DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), ALRPR (SEQ ID NO: 7), ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), and IEPRSFS (SEQ ID NO: 194). Other enzymatic cleavage sites are known in the art and described in elsewhere herein.

[0074] As used herein, the term "processing site" or "intracellular processing site" refers to a type of enzymatic cleavage site in a polypeptide which is a target for enzymes that function after translation of the polypeptide. In some embodiments, such enzymes function during transport from the Golgi lumen to the trans-Golgi compartment. Intracellular processing enzymes cleave polypeptides prior to secretion of the protein from the cell. Examples of such processing sites include, e.g., those targeted by the PACE/furin (where PACE is an acronym for Paired basic Amino acid Cleaving Enzyme) family of endopeptidases. These enzymes are localized to the Golgi membrane and cleave proteins on the carboxyterminal side of the sequence motif Arg-[any residue]-(Lys or Arg)-Arg. As used herein the

"furin" family of enzymes includes, e.g., PCSK1 (also known as PC1/PC3), PCSK2 (also known as PC2), PCSK3 (also known as furin or PACE), PCSK4 (also known as PC4), PCSK5 (also known as PC5 or PC6), PCSK6 (also known as PACE4), or PCSK7 (also known as PC7/LPC, PC8, or SPC7). Other processing sites are known in the art.

5 **[0075]** In constructs that include more than one processing or cleavage site, it will be understood that such sites can be the same or different.

[0076] A "processable linker" as used herein refers to a linker comprising at least one intracellular processing site, which are described elsewhere herein.

[0077] As used herein, the term "half-life" refers to a biological half-life of a particular polypeptide
10 in vivo. Half-life can be represented by the time required for half the quantity administered to a subject to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given polypeptide is constructed as a function of time, the curve is usually biphasic with a rapid α -phase and longer β -phase. The α -phase typically represents an equilibration of the administered Fc polypeptide between the intra- and extra-vascular space and is, in part, determined by the size of the
15 polypeptide. The β -phase typically represents the catabolism of the polypeptide in the intravascular space. In some embodiments, FVIII and chimeric polypeptides comprising FVIII are monophasic, and thus do not have an alpha phase, but just the single beta phase. Therefore, in certain embodiments, the term half-life as used herein refers to the half-life of the polypeptide in the β -phase. The typical beta phase half-life of a human antibody in humans is 21 days. In certain embodiments, the half-life
20 is expressed as the half-life of the terminal phase.

[0078] Hemostatic disorder, as used herein, means a genetically inherited or acquired condition characterized by a tendency to hemorrhage, either spontaneously or as a result of trauma, due to an impaired ability or inability to form a fibrin clot. Examples of such disorders include the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency or
25 "Christmas disease") and hemophilia C (factor XI deficiency, mild bleeding tendency). Other hemostatic disorders include, e.g., Von Willebrand disease, Factor XI deficiency (PTA deficiency), Factor XII deficiency, deficiencies or structural abnormalities in fibrinogen, prothrombin, Factor V, Factor VII, Factor X or factor XIII, Bernard-Soulier syndrome, which is a defect or deficiency in GPIb. GPIb, the receptor for VWF, can be defective and lead to lack of primary clot formation (primary
30 hemostasis) and increased bleeding tendency), and thrombasthenia of Glanzman and Naegeli

(Glanzmann thrombasthenia). In liver failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this can increase bleeding risk.

[0079] "Administer" or "administering," as used herein refers to delivering to a subject a composition described herein, e.g., a chimeric polypeptide. The composition, e.g., the chimeric polypeptide, can be administered to a subject using methods known in the art. In particular, the composition can be administered intravenously, subcutaneously, intramuscularly, intradermally, or via any mucosal surface, e.g., orally, sublingually, buccally, nasally, rectally, vaginally or via pulmonary route. chimeric polypeptide. In some embodiments, the administration is self-administration. In some embodiments, a parent administers the chimeric polypeptide to a child. In some embodiments, the chimeric polypeptide is administered to a subject by a healthcare practitioner such as a medical doctor, a medic, or a nurse.

[0080] As used herein, the term "dose" refers to a single administration of a composition to a subject. A single dose can be administered all at once, e.g., as a bolus, or over a period of time, e.g., via an intravenous infusion. The term "multiple doses" means more than one dose, e.g., more than one administration.

[0081] When referring to co-administration of more than one composition, a dose of composition A can be administered concurrently with a dose of composition B. Alternatively, a dose of composition A can be administered before or after a dose of composition B. In some embodiments, composition A and composition B are combined into a single formulation.

[0082] As used herein, the term "interval" or "dosing interval" refers to the amount of time that elapses between a first dose of composition A and a subsequent dose of the same composition administered to a subject. A dosing interval can refer to the time that elapses between a first dose and a second dose, or a dosing interval can refer to the amount of time that elapses between multiple doses.

[0083] The term "dosing frequency" as used herein refers to the number of doses administered per a specific dosing interval. For example, a dosing frequency can be written as once a week, once every two weeks, etc. Therefore, a dosing interval of 7 days can be also written as a dosing interval of once in 7 days or once every week, or once a week.

[0084] As used herein the term "prophylactic treatment" refers to the administration of a therapy for the treatment of hemophilia, where such treatment is intended to prevent or reduce the severity of one or more symptoms of hemophilia, e.g., bleeding episodes, e.g., one or more spontaneous

bleeding episodes, and/or joint damage. See Jimenez-Yuste et al., Blood Transfus. 12(3):314-19 (2014). To prevent or reduce the severity of such symptoms, e.g., bleeding episodes and the progression of joint disease, hemophilia A patients may receive regular infusions of clotting factor as part of a prophylactic treatment regimen. The basis of such prophylactic treatment is the observation
5 that hemophilia patients with a clotting factor, e.g., FVIII, level of 1% or more rarely experience spontaneous bleeding episodes and have fewer hemophilia-related comorbidities as compared to patients with severe hemophilia. See, e.g., Coppola A. et al, Semin. Thromb. Hemost. 38(1): 79-94 (2012). . Health care practitioners treating these hemophilia patients surmised that maintaining factor levels at around 1% with regular infusions could potentially reduce the risk of hemophilia symptoms,
10 including bleeding episodes and joint damage. See id. Subsequent research has confirmed these benefits in pediatric hemophilia patients receiving prophylactic treatment with clotting factor, rendering prophylactic treatment the goal for people with severe hemophilia. See id.

[0085] A "prophylactic" treatment can also refer to the preemptive administration of the composition described herein, e.g., a chimeric polypeptide, to a subject in order to control, manage, prevent, or
15 reduce the occurrence or severity of one or more symptoms of hemophilia A, e.g., bleeding episodes. Prophylactic treatment with a clotting factor, e.g., FVIII, is the standard of care for subjects with severe hemophilia A. See, e.g., Oldenburg, Blood 125:2038-44 (2015). In some embodiments, prophylactic treatment refers to administering a composition disclosed herein to a subject in need thereof to reduce the occurrence of one or more symptom of hemophilia A. A prophylactic treatment can include
20 administration of multiple doses. The multiple doses used in prophylactic treatment are typically administered at particular dosing intervals. In certain embodiments, the annualized bleeding rate can be reduced to less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, less than 2, or less than 1.

[0086] The term "on-demand treatment" or "episodic treatment" refers to the "as needed"
25 administration of a chimeric molecule in response to symptoms of hemophilia A, e.g., a bleeding episode, or before an activity that can cause bleeding. In one aspect, the on-demand treatment can be given to a subject when bleeding starts, such as after an injury, or when bleeding is expected, such as before surgery. In another aspect, the on-demand treatment can be given prior to activities that increase the risk of bleeding, such as contact sports. In some embodiments, the on-demand
30 treatment is given as a single dose. In other embodiments, the on-demand treatment is given as a first dose, followed by one or more additional doses.. When the chimeric polypeptide is administered on-demand, the one or more additional doses can be administered at least about 12 hours, at least

about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, at least about 108 hours, or at least about 120 hours after the first dose. It should be noted, however, that the dosing interval associated with on-demand treatment is not the same as the dosing interval used for prophylactic treatment.

5 **[0087]** In some embodiments, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric polypeptide of the disclosure can be administered prior to or after surgery. The chimeric polypeptide of the disclosure can also be administered during or after surgery to control an acute bleeding episode. When the chimeric polypeptide is administered prior to surgery, the administration can be at least about 1 hour, at least about 2 hours, at least about 4 hours,
10 at least about 8 hours, at least about 12 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, or at least about 72 hours prior to surgery. When the chimeric polypeptide is administered to after surgery, the administration can be at least about 1 hour, at least about 2 hours, at least about 4 hours, at least about 8 hours, at least about 12 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, or at least about 72 hours after surgery. The surgery
15 can include, but is not limited to, liver transplantation, liver resection, dental procedures, or stem cell transplantation.

[0088] As used herein the term "acute bleeding" refers to a bleeding episode regardless of the underlying cause. For example, a subject can have trauma, uremia, a hereditary bleeding disorder (e.g., factor VII deficiency) a platelet disorder, or resistance owing to the development of antibodies
20 to clotting factors.

[0089] "Treat", "treatment", "treating", as used herein refers to, e.g., the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition, or the prophylaxis of
25 one or more symptoms associated with a disease or condition. In some embodiments, the term "treating" or "treatment" means maintaining a FVIII trough level at least about 1 IU/dL, 2 IU/dL, 3 IU/dL, 4 IU/dL, 5 IU/dL, 6 IU/dL, 7 IU/dL, 8 IU/dL, 9 IU/dL, 10 IU/dL, 11 IU/dL, 12 IU/dL, 13 IU/dL, 14 IU/dL, 15 IU/dL, 16 IU/dL, 17 IU/dL, 18 IU/dL, 19 IU/dL, or 20 IU/dL in a subject by administering a chimeric polypeptide or a VWF fragment of the disclosure. As used herein, a "trough level" in a
30 hemophilia patient is the measurement of the lowest concentration reached by a factor therapy, e.g., a FVIII therapy, before the next dose is administered. In other embodiments, treating or treatment means maintaining a FVIII trough level of at least about 1 IU/dL between the dosing interval. In other

embodiments, treating or treatment means maintaining a FVIII trough level of at least about 3 IU/dL between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII trough level of at least about 5 IU/dL between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII trough level between about 1 and about 20 IU/dL, about 2 and about 20 IU/dL, about 3 and about 20 IU/dL, about 4 and about 20 IU/dL, about 5 and about 20 IU/dL, about 6 and about 20 IU/dL, about 7 and about 20 IU/dL, about 8 and about 20 IU/dL, about 9 and about 20 IU/dL, or about 10 and about 20 IU/dL during the dosing interval.

[0090] “Treatment” or “treating” of a disease or condition can also include maintaining FVIII activity in a subject at a level comparable to at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% of the FVIII activity in a non-hemophiliac subject between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 1% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 2% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 3% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 4% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 5% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 6% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 7% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 8% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 9% between the dosing interval. In other embodiments, treating or treatment means maintaining a FVIII activity level of at least about 10% between the dosing interval. The minimum trough level required for treatment can be measured by one or more known methods (for example, the aPTT assays or chromogenic assays described herein) and can be adjusted (increased or decreased) for each person.

II. Methods of the Disclosure

[0091] Certain aspects of the present disclosure are directed to methods of treating hemophilia A in a subject in need thereof, comprising administering to the subject a chimeric polypeptide comprising a FVIII protein and a VWF fragment at a dosing interval. In some embodiments, the

method comprises administering to the subject multiple doses of a chimeric polypeptide, e.g., rFVIII-Fc-VWF-XTEN, comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval. In other aspects, the present disclosure is directed to methods of treating a bleeding disease or condition, e.g., hemophilia A, in a subject in need thereof, comprising administering to the subject multiple doses of a FVIII protein and multiple doses of a VWF fragment at a dosing interval.

[0092] In some embodiments, the multiple doses comprise at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, at least twelve doses, at least thirteen doses, at least fourteen doses, at least fifteen doses, at least sixteen doses, at least seventeen doses, at least eighteen doses, at least nineteen doses, at least twenty doses, or more. In some embodiments, the multiple doses are administered for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 12 months, at least about 18 months, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, at least about 10 years, at least about 15 years, at least about 20 years, or for at least about 25 years.

[0093] In certain embodiments, the methods of the present disclosure are directed to treating hemophilia A. In some embodiments, the treatment of hemophilia A comprises preventing a bleeding episode in a human subject in need thereof. In some embodiments, the treatment of hemophilia A comprises treating a bleeding episode in a human subject in need thereof. In some embodiments, the treatment of hemophilia A comprises controlling the incidence or frequency of a bleeding episode in a human subject in need thereof. In some embodiments, the treatment of hemophilia A comprises decreasing the incidence or frequency of a bleeding episode in a human subject in need thereof.

A. Doses

[0094] In some embodiments, the chimeric polypeptide, e.g., rFVIII-Fc-VWF-XTEN, is administered as a single dose or as multiple doses. In some embodiments, the amounts of each of the multiple doses are the same. In other embodiments, one or more of the multiple doses is different from one or more of the other multiple doses. In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 5 IU/kg to about 200 IU/kg or from about 10 IU/kg to about 150 IU/kg. In certain embodiments, at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg. In some embodiments, at least one of the multiple doses is from about 20 IU/kg to about 95

IU/kg, from about 20 IU/kg to about 90 IU/kg, from about 20 IU/kg to about 85 IU/kg, from about 20 IU/kg to about 80 IU/kg, from about 20 IU/kg to about 75 IU/kg, from about 20 IU/kg to about 70 IU/kg, from about 20 IU/kg to about 65 IU/kg, from about 20 IU/kg to about 60 IU/kg, from about 20 IU/kg to about 55 IU/kg, from about 20 IU/kg to about 50 IU/kg, from about 20 IU/kg to about 45 IU/kg, from about 20 IU/kg to about 40 IU/kg, from about 20 IU/kg to about 35 IU/kg, from about 20 IU/kg to about 30 IU/kg, or from about 20 IU/kg to about 25 IU/kg.

[0095] In some embodiments, at least one of the multiple doses is from about 20 IU/kg to about 100 IU/kg, from about 25 IU/kg to about 100 IU/kg, from about 30 IU/kg to about 100 IU/kg, from about 35 IU/kg to about 100 IU/kg, from about 40 IU/kg to about 100 IU/kg, from about 45 IU/kg to about 100 IU/kg, from about 50 IU/kg to about 100 IU/kg, from about 55 IU/kg to about 100 IU/kg, from about 60 IU/kg to about 100 IU/kg, from about 65 IU/kg to about 100 IU/kg, from about 70 IU/kg to about 100 IU/kg, from about 75 IU/kg to about 100 IU/kg, from about 80 IU/kg to about 100 IU/kg, from about 85 IU/kg to about 100 IU/kg, or from about 90 IU/kg to about 100 IU/kg. In some embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg.

[0096] In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 50 IU/kg to about 150 IU/kg, from about 50 IU/kg to about 140 IU/kg, from about 50 IU/kg to about 130 IU/kg, from about 50 IU/kg to about 120 IU/kg, from about 50 IU/kg to about 110 IU/kg, or from about 50 IU/kg to about 100 IU/kg. In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 50 IU/kg to about 95 IU/kg, from about 50 IU/kg to about 90 IU/kg, from about 50 IU/kg to about 85 IU/kg, from about 50 IU/kg to about 80 IU/kg, from about 50 IU/kg to about 75 IU/kg, from about 50 IU/kg to about 70 IU/kg, from about 50 IU/kg to about 65 IU/kg, from about 50 IU/kg to about 60 IU/kg, from about 50 IU/kg to about 55 IU/kg. In certain embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 50 IU/kg to about 80 IU/kg. In certain embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 50 IU/kg to about 65 IU/kg.

[0097] In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 60 IU/kg to about 150 IU/kg, from about 60 IU/kg to about 140 IU/kg, from about 60 IU/kg to about 130 IU/kg, from about 60 IU/kg to about 120 IU/kg, from about 60 IU/kg to about 110 IU/kg, or from about 60 IU/kg to about 100 IU/kg. In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 60 IU/kg to about 95 IU/kg, from about 60 IU/kg to about 90 IU/kg, from about 60 IU/kg to about 85 IU/kg, from about 60 IU/kg to about 80 IU/kg, from about 60

IU/kg to about 75 IU/kg, from about 60 IU/kg to about 70 IU/kg, or from about 60 IU/kg to about 65 IU/kg.

[0098] In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 65 IU/kg to about 150 IU/kg, from about 65 IU/kg to about 140 IU/kg, from about 65 IU/kg to about 130 IU/kg, from about 65 IU/kg to about 120 IU/kg, from about 65 IU/kg to about 110 IU/kg, or from about 65 IU/kg to about 100 IU/kg. In some embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 65 IU/kg to about 95 IU/kg, from about 65 IU/kg to about 90 IU/kg, from about 65 IU/kg to about 85 IU/kg, from about 65 IU/kg to about 80 IU/kg, from about 65 IU/kg to about 75 IU/kg, or from about 65 IU/kg to about 70 IU/kg. In certain embodiments, at least one of the multiple doses of the chimeric polypeptide is from about 65 IU/kg to about 80 IU/kg.

[0099] In some embodiments, at least one of the multiple doses is about 5 IU/kg, about 10 IU/kg, about 15 IU/kg, about 20 IU/kg, about 25 IU/kg, about 30 IU/kg, about 35 IU/kg, about 40 IU/kg, about 45 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, about 80 IU/kg, about 85 IU/kg, about 90 IU/kg, about 95 IU/kg, about 100 IU/kg, about 125 IU/kg, about 150 IU/kg, about 175 IU/kg, or about 200 IU/kg. In certain embodiments, at least one of the multiple doses is about 25 IU/kg. In certain embodiments, at least one of the multiple doses is about 30 IU/kg. In certain embodiments, at least one of the multiple doses is about 35 IU/kg. In certain embodiments, at least one of the multiple doses is about 40 IU/kg. In certain embodiments, at least one of the multiple doses is about 45 IU/kg. In certain embodiments, at least one of the multiple doses is about 50 IU/kg. In certain embodiments, at least one of the multiple doses is about 55 IU/kg. In certain embodiments, at least one of the multiple doses is about 60 IU/kg. In certain embodiments, at least one of the multiple doses is about 65 IU/kg. In certain embodiments, at least one of the multiple doses is about 70 IU/kg. In certain embodiments, at least one of the multiple doses is about 75 IU/kg. In certain embodiments, at least one of the multiple doses is about 80 IU/kg. In certain embodiments, at least one of the multiple doses is about 85 IU/kg. In certain embodiments, at least one of the multiple doses is about 90 IU/kg. In certain embodiments, at least one of the multiple doses is about 95 IU/kg. In certain embodiments, at least one of the multiple doses is about 100 IU/kg.

[0100] In some embodiments, each dose of the multiple doses is about 25 IU/kg. In some embodiments, each dose of the multiple doses is about 30 IU/kg. In some embodiments, each dose of the multiple doses is about 35 IU/kg. In some embodiments, each dose of the multiple doses is about 40 IU/kg. In some embodiments, each dose of the multiple doses is about 45 IU/kg. In some embodiments, each dose of the multiple doses is about 50 IU/kg. In some embodiments, each dose

of the multiple doses is about 55 IU/kg. In some embodiments, each dose of the multiple doses is about 60 IU/kg. In some embodiments, each dose of the multiple doses is about 65 IU/kg. In some embodiments, each dose of the multiple doses is about 70 IU/kg. In some embodiments, each dose of the multiple doses is about 75 IU/kg. In some embodiments, each dose of the multiple doses is about 80 IU/kg. In some embodiments, each dose of the multiple doses is about 85 IU/kg. In some embodiments, each dose of the multiple doses is about 90 IU/kg. In some embodiments, each dose of the multiple doses is about 95 IU/kg. In some embodiments, each dose of the multiple doses is about 100 IU/kg.

[0101] In some embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered prophylactically. When administered prophylactically, at least one of the multiple doses can be from about 15 IU/kg to about 100 IU/kg. In certain embodiments, at least one of the multiple doses administered prophylactically is about 25 IU/kg, about 30 IU/kg, about 35 IU/kg, about 40 IU/kg, about 45 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, about 80 IU/kg, about 85 IU/kg, about 90 IU/kg, about 95 IU/kg, or about 100 IU/kg. In some embodiments, at least one of the multiple doses administered prophylactically is about 25 IU/kg. In other embodiments, at least one of the multiple doses administered prophylactically is about 50 IU/kg. In other embodiments, at least one of the multiple doses administered prophylactically is about 65 IU/kg. In other embodiments, at least one of the multiple doses administered prophylactically is about 80 IU/kg.

[0102] In some embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered on-demand. When administered on-demand, the chimeric polypeptide can be administered as a single dose or as multiple doses. In some embodiments, the chimeric polypeptide is administered as one or more doses of from about 15 IU/kg to about 100 IU/kg. In certain embodiments, the chimeric polypeptide is administered on-demand as one or more doses of about 25 IU/kg, about 30 IU/kg, about 35 IU/kg, about 40 IU/kg, about 45 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, about 80 IU/kg, about 85 IU/kg, about 90 IU/kg, about 95 IU/kg, or about 100 IU/kg. In some embodiments, the chimeric polypeptide is administered on-demand as one or more doses of about 25 IU/kg. In other embodiments, the chimeric polypeptide is administered on-demand as one or more doses of about 50 IU/kg. In other embodiments, the chimeric polypeptide is administered on-demand as one or more doses of about 65 IU/kg. In other embodiments, the chimeric polypeptide is administered on-demand as one or more doses of about 80 IU/kg.

[0103] In some embodiments, the administration of the chimeric polypeptide according to the present methods induces no FVIII inhibitor after about 7 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 20 days, about 24 days, about 25 days, about 28 days, about 30 days, or about 35 days of the administration. In some embodiments, the administration of the chimeric polypeptide induces no FVIII inhibitor after about 28 days of the administration.

B. Dosing Interval

[0104] In certain embodiments, especially for prophylactic treatment, the chimeric polypeptide, e.g., rFVIII-Fc-VWF-XTEN, is administered as multiple doses at a dosing interval. In some embodiments, the dosing interval is at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, at least about 30 days, or at least about 31 days.

[0105] In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 5 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 6 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 8 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 9 days. In certain embodiments, the dosing interval is at least about 10 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 11 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 12 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 13 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 14 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 21 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 27 days. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 30 days.

[0106] In some embodiments, the dosing frequency, e.g., for prophylactic treatment of hemophilia A, is at least twice every week, at least once every week, at least once every 2 weeks, at least once every 3 weeks, at least once every 4 weeks, at least once every 5 weeks, or at least once every 6 weeks. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least once every week. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least 2 weeks. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least 3 weeks. In certain embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least 4 weeks.

[0107] In some embodiments, the dosing frequency, e.g., for prophylactic treatment of hemophilia A, is about 2 times every three months, about 1 time every month, about 2 times every month, about 3 times every month, about 4 times every month, about 5 times every month, about 6 times every month, about 7 times every month, or about 8 times every month. In certain embodiments, the dosing frequency is about 1 time every month. In certain embodiments, the dosing frequency is about 2 times every month. In certain embodiments, the dosing frequency is about 3 times every month. In certain embodiments, the dosing frequency is about 4 times every month. In certain embodiments, the dosing frequency is about 5 times every month. In certain embodiments, the dosing frequency is about 6 times every month.

[0108] In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 3 to 5 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 4 to 6 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 4 to 7 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 4 to 8 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 4 to 9 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 4 to 10 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 5 to 7 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 5 to 8 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 5 to 9 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 5 to 10 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 6 to 8 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 6 to 9 days. In some embodiments, the

dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 6 to 10 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 to 9 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 to 10 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 to 11 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 to 12 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 to 13 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 7 to 14 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 8 to 14 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 9 to 14 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 10 to 14 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 11 to 14 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 10 to 21 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 12 to 14 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 12 to 15 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 13 to 15 days. In some embodiments, the dosing interval, e.g., for prophylactic treatment of hemophilia A, is at least about 14 to 21 days.

[0109] In some embodiments, at least one of the multiple doses is administered according to a dose and dosing frequency described in Table 1.

Table 1A: Dosing amounts and corresponding dosing frequency, e.g., for prophylactic treatment of hemophilia.

	5 IU/kg	10 IU/kg	15 IU/kg	20 IU/kg	25 IU/kg	30 IU/kg	35 IU/kg	40 IU/kg	45 IU/kg	50 IU/kg
5 days	5 IU/kg Every 5 days	10 IU/kg Every 5 days	15 IU/kg Every 5 days	20 IU/kg Every 5 days	25 IU/kg Every 5 days	30 IU/kg Every 5 days	35 IU/kg Every 5 days	40 IU/kg Every 5 days	45 IU/kg Every 5 days	50 IU/kg Every 5 days
6 days	5 IU/kg Every	10 IU/kg Every	15 IU/kg Every	20 IU/kg Every	25 IU/kg Every	30 IU/kg Every	35 IU/kg Every	40 IU/kg Every	45 IU/kg Every	50 IU/kg Every

	6 days	6 days	6 days	6 days	6 days	6 days	6 days	6 days	6 days	6 days
7 days	5 IU/kg Every 7 days	10 IU/kg Every 7 days	15 IU/kg Every 7 days	20 IU/kg Every 7 days	25 IU/kg Every 7 days	30 IU/kg Every 7 days	35 IU/kg Every 7 days	40 IU/kg Every 7 days	45 IU/kg Every 7 days	50 IU/kg Every 7 days
8 days	5 IU/kg Every 8 days	10 IU/kg Every 8 days	15 IU/kg Every 8 days	20 IU/kg Every 8 days	25 IU/kg Every 8 days	30 IU/kg Every 8 days	35 IU/kg Every 8 days	40 IU/kg Every 8 days	45 IU/kg Every 8 days	50 IU/kg Every 8 days
9 days	5 IU/kg Every 9 days	10 IU/kg Every 9 days	15 IU/kg Every 9 days	20 IU/kg Every 9 days	25 IU/kg Every 9 days	30 IU/kg Every 9 days	35 IU/kg Every 9 days	40 IU/kg Every 9 days	45 IU/kg Every 9 days	50 IU/kg Every 9 days
10 days	5 IU/kg Every 10 days	10 IU/kg Every 10 days	15 IU/kg Every 10 days	20 IU/kg Every 10 days	25 IU/kg Every 10 days	30 IU/kg Every 10 days	35 IU/kg Every 10 days	40 IU/kg Every 10 days	45 IU/kg Every 10 days	50 IU/kg Every 10 days
11 days	5 IU/kg Every 11 days	10 IU/kg Every 11 days	15 IU/kg Every 11 days	20 IU/kg Every 11 days	25 IU/kg Every 11 days	30 IU/kg Every 11 days	35 IU/kg Every 11 days	40 IU/kg Every 11 days	45 IU/kg Every 11 days	50 IU/kg Every 11 days
12 days	5 IU/kg Every 12 days	10 IU/kg Every 12 days	15 IU/kg Every 12 days	20 IU/kg Every 12 days	25 IU/kg Every 12 days	30 IU/kg Every 12 days	35 IU/kg Every 12 days	40 IU/kg Every 12 days	45 IU/kg Every 12 days	50 IU/kg Every 12 days
13 days	5 IU/kg Every 13 days	10 IU/kg Every 13 days	15 IU/kg Every 13 days	20 IU/kg Every 13 days	25 IU/kg Every 13 days	30 IU/kg Every 13 days	35 IU/kg Every 13 days	40 IU/kg Every 13 days	45 IU/kg Every 13 days	50 IU/kg Every 13 days
14 days	5 IU/kg Every 14 days	10 IU/kg Every 14 days	15 IU/kg Every 14 days	20 IU/kg Every 14 days	25 IU/kg Every 14 days	30 IU/kg Every 14 days	35 IU/kg Every 14 days	40 IU/kg Every 14 days	45 IU/kg Every 14 days	50 IU/kg Every 14 days
15 days	5 IU/kg Every 15 days	10 IU/kg Every 15 days	15 IU/kg Every 15 days	20 IU/kg Every 15 days	25 IU/kg Every 15 days	30 IU/kg Every 15 days	35 IU/kg Every 15 days	40 IU/kg Every 15 days	45 IU/kg Every 15 days	50 IU/kg Every 15 days
16 days	5 IU/kg Every 16 days	10 IU/kg Every 16 days	15 IU/kg Every 16 days	20 IU/kg Every 16 days	25 IU/kg Every 16 days	30 IU/kg Every 16 days	35 IU/kg Every 16 days	40 IU/kg Every 16 days	45 IU/kg Every 16 days	50 IU/kg Every 16 days

17 days	5 IU/kg Every 17 days	10 IU/kg Every 17 days	15 IU/kg Every 17 days	20 IU/kg Every 17 days	25 IU/kg Every 17 days	30 IU/kg Every 17 days	35 IU/kg Every 17 days	40 IU/kg Every 17 days	45 IU/kg Every 17 days	50 IU/kg Every 17 days
18 days	5 IU/kg Every 18 days	10 IU/kg Every 18 days	15 IU/kg Every 18 days	20 IU/kg Every 18 days	25 IU/kg Every 18 days	30 IU/kg Every 18 days	35 IU/kg Every 18 days	40 IU/kg Every 18 days	45 IU/kg Every 18 days	50 IU/kg Every 18 days
19 days	5 IU/kg Every 19 days	10 IU/kg Every 19 days	15 IU/kg Every 19 days	20 IU/kg Every 19 days	25 IU/kg Every 19 days	30 IU/kg Every 19 days	35 IU/kg Every 19 days	40 IU/kg Every 19 days	45 IU/kg Every 19 days	50 IU/kg Every 19 days
20 days	5 IU/kg Every 20 days	10 IU/kg Every 20 days	15 IU/kg Every 20 days	20 IU/kg Every 20 days	25 IU/kg Every 20 days	30 IU/kg Every 20 days	35 IU/kg Every 20 days	40 IU/kg Every 20 days	45 IU/kg Every 20 days	50 IU/kg Every 20 days
21 days	5 IU/kg Every 21 days	10 IU/kg Every 21 days	15 IU/kg Every 21 days	20 IU/kg Every 21 days	25 IU/kg Every 21 days	30 IU/kg Every 21 days	35 IU/kg Every 21 days	40 IU/kg Every 21 days	45 IU/kg Every 21 days	50 IU/kg Every 21 days
	55 IU/kg	60 IU/kg	65 IU/kg	70 IU/kg	75 IU/kg	80 IU/kg	85 IU/kg	90 IU/kg	95 IU/kg	100 IU/kg
5 days	55 IU/kg Every 5 days	60 IU/kg Every 5 days	65 IU/kg Every 5 days	70 IU/kg Every 5 days	75 IU/kg Every 5 days	80 IU/kg Every 5 days	85 IU/kg Every 5 days	90 IU/kg Every 5 days	95 IU/kg Every 5 days	100 IU/kg Every 5 days
6 days	55 IU/kg Every 6 days	60 IU/kg Every 6 days	65 IU/kg Every 6 days	70 IU/kg Every 6 days	75 IU/kg Every 6 days	80 IU/kg Every 6 days	85 IU/kg Every 6 days	90 IU/kg Every 6 days	95 IU/kg Every 6 days	100 IU/kg Every 6 days
7 days	55 IU/kg Every 7 days	60 IU/kg Every 7 days	65 IU/kg Every 7 days	70 IU/kg Every 7 days	75 IU/kg Every 7 days	80 IU/kg Every 7 days	85 IU/kg Every 7 days	90 IU/kg Every 7 days	95 IU/kg Every 7 days	100 IU/kg Every 7 days
8 days	55 IU/kg Every 8 days	60 IU/kg Every 8 days	65 IU/kg Every 8 days	70 IU/kg Every 8 days	75 IU/kg Every 8 days	80 IU/kg Every 8 days	85 IU/kg Every 8 days	90 IU/kg Every 8 days	95 IU/kg Every 8 days	100 IU/kg Every 8 days
9 days	55 IU/kg Every 9 days	60 IU/kg Every 9 days	65 IU/kg Every 9 days	70 IU/kg Every 9 days	75 IU/kg Every 9 days	80 IU/kg Every 9 days	85 IU/kg Every 9 days	90 IU/kg Every 9 days	95 IU/kg Every 9 days	100 IU/kg Every 9 days

10 days	55 IU/kg Every 10 days	60 IU/kg Every 10 days	65 IU/kg Every 10 days	70 IU/kg Every 10 days	75 IU/kg Every 10 days	80 IU/kg Every 10 days	85 IU/kg Every 10 days	90 IU/kg Every 10 days	95 IU/kg Every 10 days	100 IU/kg Every 10 days
11 days	55 IU/kg Every 11 days	60 IU/kg Every 11 days	65 IU/kg Every 11 days	70 IU/kg Every 11 days	75 IU/kg Every 11 days	80 IU/kg Every 11 days	85 IU/kg Every 11 days	90 IU/kg Every 11 days	95 IU/kg Every 11 days	100 IU/kg Every 11 days
12 days	55 IU/kg Every 12 days	60 IU/kg Every 12 days	65 IU/kg Every 12 days	70 IU/kg Every 12 days	75 IU/kg Every 12 days	80 IU/kg Every 12 days	85 IU/kg Every 12 days	90 IU/kg Every 12 days	95 IU/kg Every 12 days	100 IU/kg Every 12 days
13 days	55 IU/kg Every 13 days	60 IU/kg Every 13 days	65 IU/kg Every 13 days	70 IU/kg Every 13 days	75 IU/kg Every 13 days	80 IU/kg Every 13 days	85 IU/kg Every 13 days	90 IU/kg Every 13 days	95 IU/kg Every 13 days	100 IU/kg Every 13 days
14 days	55 IU/kg Every 14 days	60 IU/kg Every 14 days	65 IU/kg Every 14 days	70 IU/kg Every 14 days	75 IU/kg Every 14 days	80 IU/kg Every 14 days	85 IU/kg Every 14 days	90 IU/kg Every 14 days	95 IU/kg Every 14 days	100 IU/kg Every 14 days
15 days	55 IU/kg Every 15 days	60 IU/kg Every 15 days	65 IU/kg Every 15 days	70 IU/kg Every 15 days	75 IU/kg Every 15 days	80 IU/kg Every 15 days	85 IU/kg Every 15 days	90 IU/kg Every 15 days	95 IU/kg Every 15 days	100 IU/kg Every 15 days
16 days	55 IU/kg Every 16 days	60 IU/kg Every 16 days	65 IU/kg Every 16 days	70 IU/kg Every 16 days	75 IU/kg Every 16 days	80 IU/kg Every 16 days	85 IU/kg Every 16 days	90 IU/kg Every 16 days	95 IU/kg Every 16 days	100 IU/kg Every 16 days
17 days	55 IU/kg Every 17 days	60 IU/kg Every 17 days	65 IU/kg Every 17 days	70 IU/kg Every 17 days	75 IU/kg Every 17 days	80 IU/kg Every 17 days	85 IU/kg Every 17 days	90 IU/kg Every 17 days	95 IU/kg Every 17 days	100 IU/kg Every 17 days
18 days	55 IU/kg Every 18 days	60 IU/kg Every 18 days	65 IU/kg Every 18 days	70 IU/kg Every 18 days	75 IU/kg Every 18 days	80 IU/kg Every 18 days	85 IU/kg Every 18 days	90 IU/kg Every 18 days	95 IU/kg Every 18 days	100 IU/kg Every 18 days
19 days	55 IU/kg Every	60 IU/kg Every	65 IU/kg Every	70 IU/kg Every	75 IU/kg Every	80 IU/kg Every	85 IU/kg Every	90 IU/kg Every	95 IU/kg Every	100 IU/kg Every

	19 days	19 days	19 days	19 days	19 days	19 days	19 days	19 days	19 days	19 days
20 days	55 IU/kg Every 20 days	60 IU/kg Every 20 days	65 IU/kg Every 20 days	70 IU/kg Every 20 days	75 IU/kg Every 20 days	80 IU/kg Every 20 days	85 IU/kg Every 20 days	90 IU/kg Every 20 days	95 IU/kg Every 20 days	100 IU/kg Every 20 days
21 days	55 IU/kg Every 21 days	60 IU/kg Every 21 days	65 IU/kg Every 21 days	70 IU/kg Every 21 days	75 IU/kg Every 21 days	80 IU/kg Every 21 days	85 IU/kg Every 21 days	90 IU/kg Every 21 days	95 IU/kg Every 21 days	100 IU/kg Every 21 days

Table 1B: Dosing amounts and corresponding dosing intervals, e.g., for prophylactic treatment of hemophilia.

	55 IU/kg	60 IU/kg	65 IU/kg	70 IU/kg	75 IU/kg	80 IU/kg	85 IU/kg	90 IU/kg	95 IU/kg	100 IU/kg
5 days	55 IU/kg Every 5 days	60 IU/kg Every 5 days	65 IU/kg Every 5 days	70 IU/kg Every 5 days	75 IU/kg Every 5 days	80 IU/kg Every 5 days	85 IU/kg Every 5 days	90 IU/kg Every 5 days	95 IU/kg Every 5 days	100 IU/kg Every 5 days
6 days	55 IU/kg Every 6 days	60 IU/kg Every 6 days	65 IU/kg Every 6 days	70 IU/kg Every 6 days	75 IU/kg Every 6 days	80 IU/kg Every 6 days	85 IU/kg Every 6 days	90 IU/kg Every 6 days	95 IU/kg Every 6 days	100 IU/kg Every 6 days
7 days	55 IU/kg Every 7 days	60 IU/kg Every 7 days	65 IU/kg Every 7 days	70 IU/kg Every 7 days	75 IU/kg Every 7 days	80 IU/kg Every 7 days	85 IU/kg Every 7 days	90 IU/kg Every 7 days	95 IU/kg Every 7 days	100 IU/kg Every 7 days
8 days	55 IU/kg Every 8 days	60 IU/kg Every 8 days	65 IU/kg Every 8 days	70 IU/kg Every 8 days	75 IU/kg Every 8 days	80 IU/kg Every 8 days	85 IU/kg Every 8 days	90 IU/kg Every 8 days	95 IU/kg Every 8 days	100 IU/kg Every 8 days
9 days	55 IU/kg Every 9 days	60 IU/kg Every 9 days	65 IU/kg Every 9 days	70 IU/kg Every 9 days	75 IU/kg Every 9 days	80 IU/kg Every 9 days	85 IU/kg Every 9 days	90 IU/kg Every 9 days	95 IU/kg Every 9 days	100 IU/kg Every 9 days
10 days	55 IU/kg Every 10 days	60 IU/kg Every 10 days	65 IU/kg Every 10 days	70 IU/kg Every 10 days	75 IU/kg Every 10 days	80 IU/kg Every 10 days	85 IU/kg Every 10 days	90 IU/kg Every 10 days	95 IU/kg Every 10 days	100 IU/kg Every 10 days
11 days	55 IU/kg Every	60 IU/kg Every	65 IU/kg Every	70 IU/kg Every	75 IU/kg Every	80 IU/kg Every	85 IU/kg Every	90 IU/kg Every	95 IU/kg Every	100 IU/kg Every

	11 days	11 days	11 days	11 days	11 days	11 days	11 days	11 days	11 days	11 days
12 days	55 IU/kg Every 12 days	60 IU/kg Every 12 days	65 IU/kg Every 12 days	70 IU/kg Every 12 days	75 IU/kg Every 12 days	80 IU/kg Every 12 days	85 IU/kg Every 12 days	90 IU/kg Every 12 days	95 IU/kg Every 12 days	100 IU/kg Every 12 days
13 days	55 IU/kg Every 13 days	60 IU/kg Every 13 days	65 IU/kg Every 13 days	70 IU/kg Every 13 days	75 IU/kg Every 13 days	80 IU/kg Every 13 days	85 IU/kg Every 13 days	90 IU/kg Every 13 days	95 IU/kg Every 13 days	100 IU/kg Every 13 days
14 days	55 IU/kg Every 14 days	60 IU/kg Every 14 days	65 IU/kg Every 14 days	70 IU/kg Every 14 days	75 IU/kg Every 14 days	80 IU/kg Every 14 days	85 IU/kg Every 14 days	90 IU/kg Every 14 days	95 IU/kg Every 14 days	100 IU/kg Every 14 days
15 days	55 IU/kg Every 15 days	60 IU/kg Every 15 days	65 IU/kg Every 15 days	70 IU/kg Every 15 days	75 IU/kg Every 15 days	80 IU/kg Every 15 days	85 IU/kg Every 15 days	90 IU/kg Every 15 days	95 IU/kg Every 15 days	100 IU/kg Every 15 days
16 days	55 IU/kg Every 16 days	60 IU/kg Every 16 days	65 IU/kg Every 16 days	70 IU/kg Every 16 days	75 IU/kg Every 16 days	80 IU/kg Every 16 days	85 IU/kg Every 16 days	90 IU/kg Every 16 days	95 IU/kg Every 16 days	100 IU/kg Every 16 days
17 days	55 IU/kg Every 17 days	60 IU/kg Every 17 days	65 IU/kg Every 17 days	70 IU/kg Every 17 days	75 IU/kg Every 17 days	80 IU/kg Every 17 days	85 IU/kg Every 17 days	90 IU/kg Every 17 days	95 IU/kg Every 17 days	100 IU/kg Every 17 days
18 days	55 IU/kg Every 18 days	60 IU/kg Every 18 days	65 IU/kg Every 18 days	70 IU/kg Every 18 days	75 IU/kg Every 18 days	80 IU/kg Every 18 days	85 IU/kg Every 18 days	90 IU/kg Every 18 days	95 IU/kg Every 18 days	100 IU/kg Every 18 days
19 days	55 IU/kg Every 19 days	60 IU/kg Every 19 days	65 IU/kg Every 19 days	70 IU/kg Every 19 days	75 IU/kg Every 19 days	80 IU/kg Every 19 days	85 IU/kg Every 19 days	90 IU/kg Every 19 days	95 IU/kg Every 19 days	100 IU/kg Every 19 days
20 days	55 IU/kg Every 20 days	60 IU/kg Every 20 days	65 IU/kg Every 20 days	70 IU/kg Every 20 days	75 IU/kg Every 20 days	80 IU/kg Every 20 days	85 IU/kg Every 20 days	90 IU/kg Every 20 days	95 IU/kg Every 20 days	100 IU/kg Every 20 days
21 days	55 IU/kg	60 IU/kg	65 IU/kg	70 IU/kg	75 IU/kg	80 IU/kg	85 IU/kg	90 IU/kg	95 IU/kg	100 IU/kg

	Every 21 days	Every 21 days	Every 21 days	Every 21 days	Every 21 days	Every 21 days	Every 21 days	Every 21 days	Every 21 days	Every 21 days
	55 IU/kg	60 IU/kg	65 IU/kg	70 IU/kg	75 IU/kg	80 IU/kg	85 IU/kg	90 IU/kg	95 IU/kg	100 IU/kg
5 days	55 IU/kg Every 5 days	60 IU/kg Every 5 days	65 IU/kg Every 5 days	70 IU/kg Every 5 days	75 IU/kg Every 5 days	80 IU/kg Every 5 days	85 IU/kg Every 5 days	90 IU/kg Every 5 days	95 IU/kg Every 5 days	100 IU/kg Every 5 days
6 days	55 IU/kg Every 6 days	60 IU/kg Every 6 days	65 IU/kg Every 6 days	70 IU/kg Every 6 days	75 IU/kg Every 6 days	80 IU/kg Every 6 days	85 IU/kg Every 6 days	90 IU/kg Every 6 days	95 IU/kg Every 6 days	100 IU/kg Every 6 days
7 days	55 IU/kg Every 7 days	60 IU/kg Every 7 days	65 IU/kg Every 7 days	70 IU/kg Every 7 days	75 IU/kg Every 7 days	80 IU/kg Every 7 days	85 IU/kg Every 7 days	90 IU/kg Every 7 days	95 IU/kg Every 7 days	100 IU/kg Every 7 days
8 days	55 IU/kg Every 8 days	60 IU/kg Every 8 days	65 IU/kg Every 8 days	70 IU/kg Every 8 days	75 IU/kg Every 8 days	80 IU/kg Every 8 days	85 IU/kg Every 8 days	90 IU/kg Every 8 days	95 IU/kg Every 8 days	100 IU/kg Every 8 days
9 days	55 IU/kg Every 9 days	60 IU/kg Every 9 days	65 IU/kg Every 9 days	70 IU/kg Every 9 days	75 IU/kg Every 9 days	80 IU/kg Every 9 days	85 IU/kg Every 9 days	90 IU/kg Every 9 days	95 IU/kg Every 9 days	100 IU/kg Every 9 days
10 days	55 IU/kg Every 10 days	60 IU/kg Every 10 days	65 IU/kg Every 10 days	70 IU/kg Every 10 days	75 IU/kg Every 10 days	80 IU/kg Every 10 days	85 IU/kg Every 10 days	90 IU/kg Every 10 days	95 IU/kg Every 10 days	100 IU/kg Every 10 days
11 days	55 IU/kg Every 11 days	60 IU/kg Every 11 days	65 IU/kg Every 11 days	70 IU/kg Every 11 days	75 IU/kg Every 11 days	80 IU/kg Every 11 days	85 IU/kg Every 11 days	90 IU/kg Every 11 days	95 IU/kg Every 11 days	100 IU/kg Every 11 days
12 days	55 IU/kg Every 12 days	60 IU/kg Every 12 days	65 IU/kg Every 12 days	70 IU/kg Every 12 days	75 IU/kg Every 12 days	80 IU/kg Every 12 days	85 IU/kg Every 12 days	90 IU/kg Every 12 days	95 IU/kg Every 12 days	100 IU/kg Every 12 days
13 days	55 IU/kg Every 13 days	60 IU/kg Every 13 days	65 IU/kg Every 13 days	70 IU/kg Every 13 days	75 IU/kg Every 13 days	80 IU/kg Every 13 days	85 IU/kg Every 13 days	90 IU/kg Every 13 days	95 IU/kg Every 13 days	100 IU/kg Every 13 days
14 days	55 IU/kg	60 IU/kg	65 IU/kg	70 IU/kg	75 IU/kg	80 IU/kg	85 IU/kg	90 IU/kg	95 IU/kg	100 IU/kg

	Every 14 days	Every 14 days	Every 14 days	Every 14 days	Every 14 days	Every 14 days	Every 14 days	Every 14 days	Every 14 days	Every 14 days
15 days	55 IU/kg Every 15 days	60 IU/kg Every 15 days	65 IU/kg Every 15 days	70 IU/kg Every 15 days	75 IU/kg Every 15 days	80 IU/kg Every 15 days	85 IU/kg Every 15 days	90 IU/kg Every 15 days	95 IU/kg Every 15 days	100 IU/kg Every 15 days
16 days	55 IU/kg Every 16 days	60 IU/kg Every 16 days	65 IU/kg Every 16 days	70 IU/kg Every 16 days	75 IU/kg Every 16 days	80 IU/kg Every 16 days	85 IU/kg Every 16 days	90 IU/kg Every 16 days	95 IU/kg Every 16 days	100 IU/kg Every 16 days
17 days	55 IU/kg Every 17 days	60 IU/kg Every 17 days	65 IU/kg Every 17 days	70 IU/kg Every 17 days	75 IU/kg Every 17 days	80 IU/kg Every 17 days	85 IU/kg Every 17 days	90 IU/kg Every 17 days	95 IU/kg Every 17 days	100 IU/kg Every 17 days
18 days	55 IU/kg Every 18 days	60 IU/kg Every 18 days	65 IU/kg Every 18 days	70 IU/kg Every 18 days	75 IU/kg Every 18 days	80 IU/kg Every 18 days	85 IU/kg Every 18 days	90 IU/kg Every 18 days	95 IU/kg Every 18 days	100 IU/kg Every 18 days
19 days	55 IU/kg Every 19 days	60 IU/kg Every 19 days	65 IU/kg Every 19 days	70 IU/kg Every 19 days	75 IU/kg Every 19 days	80 IU/kg Every 19 days	85 IU/kg Every 19 days	90 IU/kg Every 19 days	95 IU/kg Every 19 days	100 IU/kg Every 19 days
20 days	55 IU/kg Every 20 days	60 IU/kg Every 20 days	65 IU/kg Every 20 days	70 IU/kg Every 20 days	75 IU/kg Every 20 days	80 IU/kg Every 20 days	85 IU/kg Every 20 days	90 IU/kg Every 20 days	95 IU/kg Every 20 days	100 IU/kg Every 20 days
21 days	55 IU/kg Every 21 days	60 IU/kg Every 21 days	65 IU/kg Every 21 days	70 IU/kg Every 21 days	75 IU/kg Every 21 days	80 IU/kg Every 21 days	85 IU/kg Every 21 days	90 IU/kg Every 21 days	95 IU/kg Every 21 days	100 IU/kg Every 21 days

[0110] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 25 IU/kg to about 65 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg to about 65 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg to about 65 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one

[illegible]

doses is administered at a dose of about 25 IU/kg to about 65 IU/kg at a dosing interval of at least about 14 to 21 days.

[0111] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 6 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 7 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 7 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 7 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 7 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 7 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 8 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 9 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 10 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 11 to 14 days. In some embodiments, at least one of the multiple doses is administered at a

dose of about 25 IU/kg at a dosing interval of at least about 12 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 12 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 13 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 25 IU/kg at a dosing interval of at least about 14 to 21 days.

[0112] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 6 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 7 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 7 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 7 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 7 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 7 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 8 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 9 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 10 to

14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 11 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 12 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 12 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 13 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg at a dosing interval of at least about 14 to 21 days.

[0113] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 6 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 7 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 7 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 7 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 7 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 7 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one

of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 8 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 9 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 10 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 11 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 12 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 12 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 13 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg at a dosing interval of at least about 14 to 21 days.

[0114] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 6 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 7 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 7 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 7 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of

about 80 IU/kg at a dosing interval of at least about 7 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 7 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 8 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 9 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 10 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 11 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 12 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 12 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 13 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 80 IU/kg at a dosing interval of at least about 14 to 21 days.

[0115] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 13 days. In

some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 8 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 9 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 10 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 11 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 12 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 12 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 13 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 80 IU/kg at a dosing interval of at least about 14 to 21 days.

[0116] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 65 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 65 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 50 IU/kg to about 65 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one

[illegible]

doses is administered at a dose of about 50 IU/kg to about 65 IU/kg at a dosing interval of at least about 14 to 21 days.

[0117] In some embodiments, such as for prophylactic treatment of hemophilia A, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 8 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 9 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 10 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to

about 80 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 11 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 12 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 12 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 13 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 14 to 21 days.

[0118] In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 8 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 4 to 9 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 4 to 10 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 4 to 11 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 4 to 12 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 4 to 13 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about

80 IU/kg at a dosing interval of at least about 4 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 5 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 5 to 14 days. In some
5 embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 to 14 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 8 to 14 days. In some
10 embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 10 to 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 10 to 21 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 13 to
15 15 days. In some embodiments, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 14 to 21 days.

[0119] In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 5 days. In some embodiments, such as for prophylactic
20 treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 7 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 6 days. In some embodiments, such as for prophylactic treatment of hemophilia A in
25 a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 5 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 4 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject,
30 at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 3 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of

about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 2 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 8 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 9 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 10 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 11 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 12 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 13 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 14 days. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 21 days.

[0120] In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing interval of at least about 1 week. In some embodiments, such as for prophylactic treatment of hemophilia A in a pediatric subject, at least one of the multiple doses is administered at a dose of about 65 IU/kg to about 80 IU/kg at a dosing frequency of at least twice about every 1 week.

[0121] In certain embodiments, such as for prophylactic treatment of hemophilia A, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF, e.g., rFVIII-Fc-VWF-XTEN, at a dosing interval, wherein at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg and the dosing interval is at least about 5 days. In certain embodiments, such as for prophylactic treatment of hemophilia A, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment

comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval, wherein at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg and the dosing interval is at least about 7 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 8 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 9 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 10 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 11 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 12 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 13 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 14 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 15 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 16 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 17 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 18 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 19 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 20 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg and the dosing interval is at least about 21 days.

[0122] In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF, e.g., rFVIII-Fc-VWF-XTEN, at a dosing interval, wherein at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 5 days. In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval, wherein at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 6 days. In some embodiments, the method is

for the prophylactic treatment of hemophilia A. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 7 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 8 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 9 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 10 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 11 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 12 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 13 days. In certain embodiments, at least one of the multiple doses is about 25 IU/kg and the dosing interval is at least about 14 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 15 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 16 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 17 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 18 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 19 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 20 days. In certain embodiments, at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 21 days.

[0123] In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF, e.g., rFVIII-Fc-VWF-XTEN, at a dosing interval, wherein at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 5 days. In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval, wherein at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 6 days. In some embodiments, the method is for the prophylactic treatment of hemophilia A. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 7 days. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 8 days. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least

about 9 days. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 10 days. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 11 days. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 12 days. In certain
5 embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 13 days. In certain embodiments, at least one of the multiple doses is about 50 IU/kg and the dosing interval is at least about 14 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at least about 15 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at least about 16
10 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at least about 17 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at least about 18 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at least about 19 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at
15 least about 20 days. In certain embodiments, at least one of the multiple doses is from about 50 IU/kg and the dosing interval is at least about 21 days.

[0124] In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF, e.g., rFVIII-Fc-VWF-XTEN, at a dosing interval, wherein
20 at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 5 days. In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval, wherein at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 6 days. In some embodiments, the method is
25 for the prophylactic treatment of hemophilia A. In certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 7 days. In certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 8 days. In certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 9 days. In certain embodiments, at least one of the multiple doses is about 65 IU/kg
30 and the dosing interval is at least about 10 days. In certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 11 days. In certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 12 days. In

certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 13 days. In certain embodiments, at least one of the multiple doses is about 65 IU/kg and the dosing interval is at least about 14 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 15 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 16 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 17 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 18 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 19 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 20 days. In certain embodiments, at least one of the multiple doses is from about 65 IU/kg and the dosing interval is at least about 21 days.

[0125] In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF, e.g., rFVIII-Fc-VWF-XTEN, at a dosing interval, wherein at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 5 days. In certain embodiments, the method comprises administering to the subject multiple doses of a chimeric polypeptide comprising (i) a FVIII protein and (ii) a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval, wherein at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 6 days. In some embodiments, the method is for the prophylactic treatment of hemophilia A. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 7 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 8 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 9 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 10 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 11 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 12 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 13 days. In certain embodiments, at least one of the multiple doses is about 80 IU/kg and the dosing interval is at least about 14 days. In certain embodiments, at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 15 days. In certain embodiments,

at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 16 days. In certain embodiments, at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 17 days. In certain embodiments, at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 18 days. In certain embodiments, at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 19 days. In certain embodiments, at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 20 days. In certain embodiments, at least one of the multiple doses is from about 80 IU/kg and the dosing interval is at least about 21 days.

[0126] In some embodiments, the chimeric polypeptide is administered for prophylactic treatment of hemophilia A. Prophylactic treatment of hemophilia A includes alleviating or reducing the severity of the symptoms of hemophilia A on a continuous or nearly continuous basis. In some embodiments, the prophylactic treatment is administered before the occurrence of a symptom of hemophilia A, e.g., before a bleeding incident. In other embodiments, the prophylactic treatment is administered on a regular basis, e.g., at a dosing interval described herein, to prevent the onset of or to lessen the severity of a symptom prior to the onset of the symptom. Any dosing interval disclosed herein can be used in a prophylactic treatment of hemophilia A.

[0127] In other embodiments, the chimeric polypeptide is administered before an activity that can bring on one or more symptom of hemophilia A, e.g., as an on-demand treatment. For example, a chimeric polypeptide of the present disclosure can be administered to a subject suffering from hemophilia A before the subject undergoes a surgery or engages in an activity that otherwise increases the risk of physical trauma and/or a bleeding incident. When administered on-demand, the chimeric polypeptide can be administered as a single dose or as multiple doses. In some embodiments, the on-demand treatment comprises administering multiple doses of the chimeric polypeptide at a dosing interval of at least about 12 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, at least about 108 hours or at least about 120 hours. In certain embodiments, the on-demand treatment comprises administering at least 2 doses, at least 3 doses, at least 4 doses or at least 5 doses of the chimeric polypeptide.

[0128] In some embodiments, the subject has previously been treated with one or more FVIII replacement therapy. In certain embodiments, the subject failed to respond to a previous FVIII replacement therapy. In certain embodiments, the FVIII replacement therapy is ELOCTATE® or ADVATE®. In some embodiments, the subject is an adult. In some embodiments, the subject is an

adult male. In other embodiments, the subject is an adult female. In other embodiments, the subject is a child, e.g., less than or equal to about 12 years old, less than or equal to about 11 years old, less than or equal to about 10 years old, less than or equal to about 9 years old, less than or equal to about 8 years old, less than or equal to about 7 years old, less than or equal to about 6 years old, less than or equal to about 5 years old, less than or equal to about 4 years old, less than or equal to about 3 years old, less than or equal to about 2 years old, or less than or equal to about 1 year old). In some embodiments, the subject is a female. In some embodiments, the subject is a male. In some embodiments, the subject is a female less than or equal to about 12 years old. In some embodiments, the subject is a female less than or equal to about 11 years old. In some embodiments, the subject is a female less than or equal to about 10 years old.

[0129] A chimeric polypeptide described herein can be administered by any means known in the art. In some embodiments, the chimeric polypeptide is administered by a route selected from the group consisting of intravenous injection, intravenous infusion, subcutaneous administration, intramuscular administration, oral administration, nasal administration, and pulmonary administration. In some embodiments, the chimeric polypeptide is administered intravenously. In other embodiments, the chimeric polypeptide is administered subcutaneously.

[0130] In some embodiments, the chimeric polypeptide, e.g., rFVIII_h-VWF-XTEN, after the administration results in a FVIII plasma activity level of at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10%. In certain embodiments, the FVIII plasma activity level is at least about 3%. In some embodiments, the FVIII plasma activity level is at least about 4%. In some embodiments, the FVIII plasma activity level is at least about 5%. In some embodiments, the FVIII plasma activity level is at least about 4%. In some embodiments, the FVIII plasma activity level is at least about 5%. In some embodiments, the FVIII plasma activity level is at least about 5.6%. In some embodiments, the FVIII plasma activity level is at least about 7%. In some embodiments, the FVIII plasma activity level is at least about 10%. In some embodiments, the FVIII plasma activity level is at least about 12%. In some embodiments, the FVIII plasma activity level is at least about 12.95%. As used herein, plasma activity level is expressed as a percent (%). Alternatively, plasma activity can be expressed in IU/dL units, wherein 1% is equal to 1 IU/dL.

[0131] In some embodiments, the FVIII plasma activity level is at least about 10% at least about 5 days after the administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg. In some embodiments, the FVIII plasma activity level is at least about 12% at least about 5 days after the

administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg. In some embodiments, the FVIII plasma activity level is at least about 12.95% at least about 5 days after the administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg. In some embodiments, the FVIII plasma activity level is at least about 5% at least about 7 days after the administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg. In some embodiments, the FVIII plasma activity level is at least about 5.6% at least about 7 days after the administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg. In some embodiments, the FVIII plasma activity level is at least about 3% at least about 8 days after the administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg. In some embodiments, the FVIII plasma activity level is at least about 1% at least about 10 days after the administration of the chimeric polypeptide, e.g., at a dose of 25 IU/kg.

[0132] The present disclosure also includes a method of treating hemophilia A comprising administering to a subject a composition comprising (i) a FVIII protein and (ii) a VWF fragment. In some embodiments, the FVIII protein is present in a first formulation and the VWF fragment is present in a second formulation. In other embodiments, the FVIII protein and the VWF fragment are present in the same formulation. In particular embodiments, the FVIII protein and the VWF fragment are separate components of a single formulation. In certain embodiments, the FVIII protein, e.g., single chain FVIII, FVIII-Fc, pegylated FVIII, full length mature FVIII, or B domain deleted FVIII, is administered at a dose disclosed herein, e.g., 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, and at a doing interval of at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, or at least 14 days, and the VWF fragment is administered at a dose of at least about 0.25:1, 0.5:1, 1:1, 2:1, 3:1, 4:1, 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 100:1, 200:1, 300:1, 400:1, 500:1, 1000:1, 2000:1, 3000:1, 4000:1, 5000:1, 10,000:1, 50,000:1, 100,000:1, or 500,000:1 VWF fragment to FVIII molecule administered. In other embodiments, the VWF fragment is administered to the subject concurrently with, right after, or right before the FVIII protein. In certain embodiments, the VWF fragment is administered about 0.5 hours, about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours, or about 6 hours before the FVIII protein. In other embodiments, the FVIII protein is administered about 0.5 hours, about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours, or about 6 hours before the VWF fragment.

C. Immune Tolerance Induction

[0133] Certain aspects of the present disclosure are directed to methods of treating hemophilia A in a subject in need thereof, comprising administering to the subject a chimeric polypeptide comprising a FVIII protein and a VWF fragment at a dosing interval, wherein the chimeric polypeptide induces immune tolerance to FVIII in the subject. Some aspects of the present disclosure are directed to methods of treating hemophilia A in a subject in need thereof, comprising administering to the subject a chimeric polypeptide comprising a FVIII protein and a VWF fragment at a dosing interval, wherein the chimeric polypeptide reduces an inhibitory response to FVIII in the subject. Some aspects of the present disclosure are directed to methods of treating hemophilia A in a subject in need thereof, comprising administering to the subject a chimeric polypeptide comprising a FVIII protein and a VWF fragment at a dosing interval, wherein the chimeric polypeptide does not induce an immune response to the chimeric polypeptide following the administration. Another aspect of the present disclosure is directed to a method of inducing immune tolerance in a human with hemophilia, comprising (1) administering to the human an effective amount of a chimeric polypeptide described herein, e.g., rFVIII-Fc-VWF-XTEN, wherein the effective amount of the chimeric polypeptide induces immune tolerance in the human. Various methods of immune tolerance induction using chimeric polypeptides comprising a FVIII and an Fc are disclosed in International Publication No. WO 2018/102760 A1, which is incorporated by reference herein in its entirety.

[0134] In certain embodiments, the method further comprises (2) following induction of immune tolerance, administering to the human a tapering regimen of the composition or the chimeric polypeptide. In certain embodiments, induction of immune tolerance occurs when the titer of the inhibitory antibodies in the human is less than about 0.6 BU. In certain embodiments, induction of immune tolerance occurs when the titer of the inhibitory antibodies in the human is less than about 0.6 BU, and 60% recovery of clotting factor activity as monitored in plasma. In some embodiments of the present disclosure, the method further comprises (3) following the tapering regimen, administering to the human a prophylactic dose of the clotting factor, e.g., a chimeric polypeptide described herein, e.g., rFVIII-Fc-VWF-XTEN.

[0135] In certain aspects, the human has not been treated with a previous immune tolerance therapy against the clotting factor, e.g., against a FVIII. The chimeric polypeptide, e.g., rFVIII-Fc-VWF-XTEN, can be administered to the human at any time it has been determined that the human has developed an inhibitor immune response, e.g., after measuring the level of an inhibitory immune response in the human. In other embodiments, the chimeric polypeptide, e.g., rFVIII-Fc-VWF-XTEN,

can be administered to the human who has not yet developed one or more inhibitor immune response to prevent development of an inhibitor immune response. In some embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered to the human who has a high likelihood (e.g., family history, genetic predisposition, or showing of a biomarker) of developing an inhibitor immune response. In some embodiments, the method further comprises measuring the level of an inhibitory immune response or the likelihood of developing an inhibitor immune response before the administration.

[0136] In some embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered to the human less than about 1 day, less than about 2 days, less than about 3 days, less than about 4 days, less than about 5 days, less than about 6 days, less than about 7 days, less than about 2 weeks, less than about 3 weeks, less than about 4 weeks, less than about 2 months, less than about 3 months, less than about 4 months, less than about 5 months, less than about 6 months, less than about 1 year, less than about 2 years, less than about 3 years, less than about 4 years, or less than about 5 years after it has been determined that the human has developed an inhibitor immune response or that the human has a likelihood of developing an inhibitor immune response, e.g., after measuring the level of an inhibitory immune response or the likelihood of developing an inhibitor immune response in the human. In certain embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered to the human immediately after it has been determined that the human has developed an inhibitor immune response or that the human has a likelihood of developing an inhibitor immune response, e.g., after measuring the level of an inhibitory immune response or the likelihood of developing an inhibitor immune response in the human. In particular embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered to the human less than about 5 minutes, less than about 10 minutes, less than about 15 minutes, less than about 20 minutes, less than about 30 minutes, less than about 45 minutes, less than about 1 hour, less than about 2 hours, less than about 3 hours, less than about 4 hours, less than about 5 hours, less than about 6 hours, less than about 7 hours, less than about 8 hours, less than about 9 hours, less than about 10 hours, less than about 11 hours, less than about 12 hours, about 18 hours, or less than about 24 hours after it has been determined that the human has developed an inhibitor immune response or that the human has a likelihood of developing an inhibitor immune response, e.g., after measuring the level of an inhibitory immune response or the likelihood of developing an inhibitor immune response in the human. In particular embodiments, the chimeric polypeptide, e.g., rFVIII^hFc-VWF-XTEN, is administered to the human about 5 minutes, about 10 minutes, about 15 minutes, about 20 minutes,

about 30 minutes, about 45 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 18 hours, or about 24 hours after it has been determined that the human has developed an inhibitor immune response or that the human has a likelihood of developing an inhibitor immune response, e.g., after measuring the level of an inhibitory immune response or the likelihood of developing an inhibitor immune response in the human. In certain embodiments, the chimeric polypeptide, e.g., rFVIII-Fc-VWF-XTEN, is administered to the human less than about 1 day after it has been determined that the human has developed an inhibitor immune response or that the human has a likelihood of developing an inhibitor immune response, e.g., after measuring the level of an inhibitory immune response or the likelihood of developing an inhibitor immune response in the human.

[0137] Induction of an immune response can be continued until the level of inhibitor is lower than a certain level or until inhibitors are not detectable. In certain embodiments, the induction period can continue for at least about 24 weeks, at least about 26 weeks, at least about 28 weeks, at least about 30 weeks, at least about 32 weeks, at least about 34 weeks, at least about 36 weeks, at least about 38 weeks, at least about 40 weeks, at least about 42 weeks, at least about 44 weeks, at least about 46 weeks, at least about 48 weeks, at least about 50 weeks, at least about 52 weeks, at least about 54 weeks, at least about 56 weeks, at least about 58 weeks, at least about 60 weeks, at least about 62 weeks, at least about 64 weeks, at least about 66 weeks, at least about 68 weeks, at least about 70 weeks. In a particular embodiment, the induction period is less than 60 weeks.

[0138] The inhibitory immune response treated by the methods of the present invention can include any response within the human that negatively impacts one or more effects of a clotting factor treatment. In some embodiments, the inhibitory immune response comprises production of inhibitory antibodies against the clotting factor, e.g., inhibitory anti-FVIII antibodies. In certain embodiments, the method of the present disclosure further comprises measuring the titer of one or more inhibitory antibodies in the human before (e.g., at baseline) and after administering an effective amount of a chimeric polypeptide described herein, e.g., rFVIII-Fc-VWF-XTEN, or a polynucleotide encoding the same. In some embodiments, titer of the inhibitory antibodies prior to the administration (e.g., at baseline) is at least about 0.6 Bethesda Units (BU). In certain embodiments, the titer of the inhibitory antibodies prior to the administration (e.g., at baseline) is at least about 1 BU, at least about 2 BU, at least about 3 BU, at least about 4 BU, at least about 5 BU, at least about 6 BU, at least about 7 BU, at least about 10 BU, at least about 20 BU, at least about 30 BU, at least about 40 BU, at least about

50 BU, at least about 100 BU, at least about 150 BU, or at least about 200 BU. In one particular embodiment, the titer of the inhibitory antibodies prior to the administration (e.g., at baseline) is at least about 5 BU.

[0139] In some embodiments, the methods of the present invention reduce the titer of inhibitory antibodies in a human subject relative to the titer of the inhibitory antibodies prior to the administration. In certain embodiments, the titer of the inhibitory antibodies after the administration is less than about 0.6 BU. In some embodiments, the titer of the inhibitory antibodies after the administration is less than about 0.5 BU, less than about 0.4 BU, less than about 0.3 BU, less than about 0.2 BU, or less than about 0.1 BU. In one particular embodiment, the titer of the inhibitory antibodies after the administration is 0 BU. In other embodiments, the titer of the inhibitory antibodies after the administration is less than 5 BU, less than 4 BU, less than 3 BU, less than 2 BU, less than 1 BU, less than 0.9 BU, less than 0.8 BU, less than 0.7 BU, or less than 0.6 BU.

[0140] In some embodiments, the administration of a chimeric polypeptide described herein, e.g., rFVIII-Fc-VWF-XTEN, increases the differentiation of macrophages in the human towards an M2-like phenotype, as compared to macrophage differentiation in untreated controls and humans treated with the clotting factor alone. In some embodiments, the M2-like phenotype comprises upregulation of the NRF2 pathway, the PPAR gamma pathway, or both the NRF2 pathway and the PPAR gamma pathway. In some embodiments, the M2-like phenotype comprises upregulation of CD206 (MRC1). In some embodiments, the M2-like phenotype comprises upregulation of ARG1. In some embodiments, the M2-like phenotype comprises upregulation of CD206 (MRC1) and ARG1.

[0141] In some embodiments, the administration of a chimeric polypeptide described herein, e.g., rFVIII-Fc-VWF-XTEN, results in greater expression of one or more genes in the human, relative to the expression of the one or more genes in an untreated subject or in a subject treated with the clotting factor alone. In some embodiments, the administration results in greater expression of one or more genes selected from the group consisting of Hmox1, PPAR gamma, LPL, EGR2, SLCO4A1, heme oxygenase 1 (HO-1), oxidative stress induced growth inhibitor 1 (OSGIN1), superoxide dismutase 1 (SOD1), glutathione-disulfide reductase (GSR), glutamate-cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase modifier subunit (GCLM), NAD(P)H quinone dehydrogenase 1 (NQO1), fatty acid binding protein 5 (FABP5), B7-H3 (CD276), SLAM family member 3 (SLAMF3; lymphocyte antigen 9; LY9), SLAM family member 7 (SLAMF7), mannose receptor C-type 1 (MRC1), solute carrier family 12 member 4 (SLC12A), neuropilin 1 (NRP1), and any combination thereof. In some embodiments, the administration results in greater expression of one or more genes of the NRF2

pathway. In certain embodiments, the one or more genes of the NRF2 pathway are selected from the group consisting of HO-1, OSGIN1, SOD1, GSR, GCLC, GCLM, NQO1, and any combination thereof. In some embodiments, the administration results in greater expression of one or more genes of the PPAR gamma pathway. In some embodiments, the one or more genes of the PPAR gamma pathway are selected from the group consisting of PPAR gamma, LPL, FABP5, EGR2, and any combination thereof. In some embodiments, the administration results in greater expression of one or more genes selected from the group consisting of B7-H3 (CD276), SLAMF3, SLAMF7, MRC1, SLC12A, NRP1, and any combination thereof. In particular embodiments, the administration results in greater expression of the one or more genes relative to the expression of the one or more genes in an untreated human or a human administered the clotting factor alone, wherein the expression is at least about 1.5 fold greater, at least about 2 fold greater, at least about 2.5 fold greater, at least about 3 fold greater, at least about 3.5 fold greater, at least about 4 fold greater, at least about 4.5 fold greater, or at least about 5 fold greater.

[0142] In some embodiments, the differential expression of the one or more genes is observed less than 6 hours after the administration of a chimeric polypeptide described herein, e.g., rFVIII^hFc-VWF-XTEN. In some embodiments, the differential expression is observed less than 12 hours after administration. In some embodiments, the differential expression is observed less than 18 hours after administration. In some embodiments, the differential expression is observed less than 24 hours after administration.

[0143] In some embodiments, the inhibitory immune response comprises a cell-mediated immune response. In certain embodiments, the cell-mediated immune response comprises the release of a cytokine. In some embodiments, the cytokine is any cytokine associated with an increased immune response. In some embodiments, the cytokine selected from the group consisting of IL-1, IL-6, IL-16, IL-12, IL-4, IL-17, tumor necrosis factor α (TNF- α), interferon α , interferon γ and any combination thereof. In one embodiment, the cell-mediated immune response comprises increased serum levels of IL-12. In another embodiment, the cell-mediated immune response comprises increased serum levels of IL-4. In another embodiment, the cell-mediated immune response comprises increased serum levels of IL-17. In another embodiment, the cell-mediated immune response comprises increased serum levels of TNF- α .

[0144] Various gene mutations have been linked with an increased risk of developing an inhibitory immune response. For example, the TNF- α -308G>A polymorphism within Hap2, which is associated with increased constitutive and inducible transcription levels of TNF has been linked with an increased

risk of developing an inhibitory immune response. See Astermark et al., Blood 108: 3739-3745 (2006), which is herein incorporated by reference in its entirety. Thus, in some embodiments, the human has a genetic polymorphism associated with increased TNF- α . In some embodiments, the polymorphism is the TNF- α -308G>A polymorphism. In some embodiments, the human has a polymorphism in an IL10 gene, e.g. a polymorphism associated with increased secretion of IL10. In some embodiments, FVIII-Fc is administered to a subject with the allele 134 of the IL10G microsatellite in the promote region of the IL10 gene. See Astermark et al. Hemostasis, Thrombosis, and Vascular Biology 108: 3739-3745 (2006), which is herein incorporated by reference in its entirety.

[0145] In some embodiments, the human has a genetic polymorphism associated with decreased CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4) expression. In some embodiments, the human has a mutation in DR15 (HLA-DR15) or DQB0602 MHC (Major histocompatibility complex) Class II molecules. Other MHC Class II molecules associated with the development of an inhibitory immune response in subjects with hemophilia are A3, B7, C7, DQA0102, C2, DQA0103, DQB0603, and DR13 (see Inhibitors in Patients with Hemophilia, E.C. Rodriguez-Merchan & C.A. Lee, Eds., Blackwell Science, Ltd., 2002).

[0146] In some embodiments, the methods of the present disclosure reduce the level of one or more cytokine in the subject compared to the level of the one or more cytokines in the subject after a previous treatment with a polypeptide consisting of a FVIII protein. In another embodiment, the methods of the present disclosure reduce the level of one or more cytokine in the subject compared to the level of the one or more cytokines in the subject prior to the administration. In other embodiments, the expression of one or more tolerogenic molecules is increased after the administration of the methods of the present disclosure relative to the expression level of the one or more tolerogenic molecules prior to the administration. In certain embodiments, the one or more tolerogenic molecules is selected from IL-10, TGF- β , IL-35, IDO-1, and any combination thereof.

[0147] In other embodiments, the immune response comprises a clinical symptom selected from the group consisting of: increased bleeding tendency, high clotting factor consumption, lack of response to clotting factor therapy, decreased efficacy of clotting factor therapy, shortened half-life of the clotting factor, and any combination thereof. In certain embodiments, the immune response comprises a clinical symptom selected from the group consisting of: increased bleeding tendency, high clotting factor consumption, lack of response to clotting factor therapy, decreased efficacy of clotting factor therapy, decreased recovery of clotting factor activity as monitored in the plasma, shortened half-life of the clotting factor, and any combination thereof.

[0148] In certain embodiments, the human was previously diagnosed as having an inhibitory immune response. Such a diagnosis can be made using any methods known in the art. For example, a human can be characterized as having an immune response to a clotting factor, e.g., a FVIII, if the human has one or more the following: (a) a titer of inhibitory antibodies to the clotting factor greater than or equal to 0.6 BU; (b) increased serum levels of one or more cytokine selected from the group consisting of IL-12, IL-4, IL-17, and TNF- α ; (c) increased bleeding tendency; (d) high clotting factor consumption; (e) a lack of response to clotting factor therapy; (f) decreased efficacy of clotting factor therapy; (g) shortened half-life of the clotting factor, and any combination thereof. In one particular embodiment, the human is characterized as having an immune response to a clotting factor if the human has a titer of inhibitory antibodies to the clotting factor greater than or equal to 0.6 BU.

[0149] In some embodiments, the human was previously diagnosed as having developed an inhibitory immune response to the clotting factor at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 13 months, at least about 14 months, at least about 15 months, at least about 16 months, at least about 17 months, at least about 18 months, at least about 19 months, at least about 20 months, at least about 21 months, at least about 22 months, at least about 23 months, at least about 24 months, at least about 27 months, at least about 30 months, at least about 33 months, at least about 36 months, at least about 39 months, at least about 42 months, at least about 45 months, at least about 48 years, at least about 51 months, at least about 54 months, at least about 57 months, at least about 60 months, at least about 6 years, at least about 7 years, at least about 8 years, at least about 10 years, at least about 15 years, or at least about 20 years prior to the administration. In one embodiment, the human was previously diagnosed as having developed an inhibitory immune response to the clotting factor at least about 5 years prior to the administration.

[0150] In some embodiments, the methods of the present disclosure provide an improved time to tolerance as compared to standard of care methods of inducing immune tolerance. The term "time to tolerance," as used herein refers to the amount of time between the administration of the first dose of the composition or the chimeric protein comprising a clotting factor and an Fc region and the development of immune tolerance in the human. Decreasing the time to tolerance can have significant benefits for the human, including but not limited to reducing the total financial burden required to achieve tolerance. In some embodiments, the time to tolerance is about 1 to about 24 weeks, about

1 to about 23 weeks, about 1 to about 22 weeks, about 1 to about 21 weeks, about 2 to about 20 weeks, about 2 to about 19 weeks, about 2 to about 18 weeks, about 2 to about 17 weeks, about 3 to about 16 weeks, about 3 to about 15 weeks, about 3 to about 14 weeks, about 3 to about 13 weeks, about 4 to about 12 weeks, about 4 to about 11 weeks, about 4 to about 10 weeks, about 4 to about 9 weeks, about 5 to about 8 weeks, about 5 to about 7 weeks, about 5 to about 6 weeks, about 1 to about 12 weeks, about 1 to about 11 weeks, about 1 to about 10 weeks, about 1 to about 9 weeks, about 1 to about 8 weeks, about 1 to about 7 weeks, about 1 to about 6 weeks, about 1 to about 5 weeks, or about 1 to about 4 weeks. In some embodiments, the time to tolerance is less than about 70 weeks, less than about 65 weeks, less than about 60 weeks, less than about 58 weeks, less than about 56 weeks, less than about 54 weeks, less than about 52 weeks, less than about 50 weeks, less than about 48 weeks, less than about 46 weeks, less than about 44 weeks, less than about 42 weeks, less than about 40 weeks, less than about 38 weeks, less than about 36 weeks, less than about 34 weeks, less than about 32 weeks, less than about 30 weeks, less than about 28 weeks, less than about 26 weeks, less than about 24 weeks, less than about 23 weeks, less than about 22 weeks, less than about 21 weeks, less than about 20 weeks, less than about 19 weeks, less than about 18 weeks, less than about 17 weeks, less than about 16 weeks, less than about 15 weeks, less than about 14 weeks, less than about 13 weeks, less than about 12 weeks, less than about 11 weeks, less than about 10 weeks, less than about 9 weeks, less than about 8 weeks, less than about 7 weeks, less than about 6 weeks, less than about 5 weeks, less than about 4 weeks, less than about 3 weeks, less than about 2 weeks, or less than about 1 week. In certain embodiments, the time to tolerance is about 4 to about 12 weeks. In one embodiment, the time to tolerance is about 4 weeks. In another embodiment, the time to tolerance is about 12 weeks. In some embodiments, the time to tolerance is less than about 10 months. In some embodiments, the time to tolerance is less than about 9 months. In some embodiments, the time to tolerance is less than about 8 months. In some embodiments, the time to tolerance is less than about 7 months. In some embodiments, the time to tolerance is less than about 6 months. In some embodiments, the time to tolerance is less than about 5 months. In some embodiments, the time to tolerance is less than about 4 months. In some embodiments, the methods of the present disclosure result in a shorter time to tolerance in the human following treatment with a composition or the chimeric protein comprising a clotting factor and an Fc region as compared to the time to tolerance following treatment with the clotting factor alone.

[0151] In some embodiments, development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of less than about 0.6 BU. In some embodiments,

development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of than about 0.5 BU. In some embodiments, development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of less than about 0.4 BU. In some embodiments, development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of less than about 0.3 BU. In some embodiments, development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of less than about 0.2 BU. In some embodiments, development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of less than about 0.1 BU. In some embodiments, development of immune tolerance is characterized by a titer of an inhibitory antibody to the clotting factor of 0.0 BU. In certain embodiments, the titer of inhibitory immune antibodies is observed at two consecutive measurements, e.g., in two consecutive weeks within a four-week period.

[0152] In some embodiments, the development of immune tolerance is characterized by incremental recovery >66% (e.g., incremental recovery of about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%). As used herein, "incremental recovery" refers to peak FVIII levels 15 -30 minutes after infusion.

[0153] After the induction period and tapering period are completed, the subject can then be on prophylactic treatment of the chimeric protein. The prophylactic dosing regimen can be any dosing regimen disclosed herein.

[0154] In some embodiments, the human treated using the methods of the present disclosure is receiving or has recently received an immunostimulatory therapy. For example, inhibitors have also been reported in HCV positive hemophilia A patients undergoing treatment with interferon as well as in HIV positive hemophilia A patients having an immune reconstitution inflammatory syndrome associated with anti-retroviral therapy. See Report of Expert Meeting on FVIII Products and Inhibitor Development, European Medicines Agency (February 28, 2006-March 2, 2006). Thus, in some embodiments, the human is receiving interferon therapy. In some embodiments, the human is receiving anti-viral therapy. In some embodiments, the human is receiving an anti-retroviral therapy and having an immune reconstitution inflammatory syndrome.

[0155] In certain embodiments, the human has had less than 150 exposure days (ED) to the clotting factor, e.g. FVIII. In one embodiment, the human has had less than 50 ED. In another embodiment, the human has had less than 20 ED.

[0156] Some aspects of the present disclosure are directed to methods of reducing the severity or occurrence of an allergic or anaphylactic reaction to a clotting factor in a subject in need thereof, comprising administering to the subject a composition or a chimeric protein comprising the clotting factor and an Fc region. In some embodiments, the administration of the composition or the chimeric protein reduces the severity of an anaphylactoid reaction to the clotting factor. In some embodiments, the administration of the composition or the chimeric protein reduces the severity of an allergic reaction to the clotting factor.

III. Chimeric Polypeptides

[0157] Chimeric polypeptides useful for the present disclosure comprise a FVIII protein comprising a FVIII polypeptide and a VWF fragment comprising a D' domain and a D3 domain of VWF. The VWF fragment is used to prevent or block the FVIII protein from interacting with endogenous VWF, thereby preventing the FVIII protein from being cleared through a VWF clearance pathway. In order to prevent dissociation of the FVIII protein from the VWF fragment, in some embodiments, the FVIII protein and the VWF fragment are associated directly or indirectly with each other through an association stronger than the natural interaction between FVIII and VWF.

[0158] In certain embodiments, the FVIII protein is associated with the VWF fragment by a covalent bond. The covalent bond can be any covalent bond known in the art. In some embodiments, the covalent bond is a disulfide bond. In some embodiments, the covalent bond is a peptide bond. In other embodiments, the FVIII protein is modified to increase the strength of the interaction between the FVIII protein and the VWF fragment. In other embodiments, the VWF fragment is modified to increase the strength of the interaction between the FVIII protein and the VWF fragment. In still other embodiments, the FVIII protein and the VWF protein both are modified to increase the strength of the interaction between the FVIII protein and the VWF fragment.

[0159] In particular embodiments, the FVIII protein comprising a FVIII polypeptide and the VWF fragment comprising a D' domain and a D3 domain of VWF associate directly through at least one covalent bond between at least one amino acid in the FVIII polypeptide sequence and at least one amino acid in the D' and D3 domains of VWF. In other embodiments, the FVIII protein and the VWF fragment associate indirectly through at least one covalent bond between at least one amino acid in

the FVIII polypeptide sequence and at least one amino acid in a heterologous sequence fused directly or indirectly with the D' and D3 domains of VWF. In other embodiments, the FVIII protein and the VWF fragment associate indirectly through at least one covalent bond between at least one amino acid in the VWF fragment sequence and at least one amino acid in a heterologous sequence fused directly or indirectly with the FVIII polypeptide. In still other embodiments, the FVIII protein and the VWF fragment associate indirectly through at least one covalent bond between at least one amino acid in a heterologous sequence fused directly or indirectly with the D' and D3 domains of VWF and at least one amino acid in a heterologous sequence fused directly or indirectly with the FVIII polypeptide.

[0160] The FVIII protein of the present disclosure can comprise a FVIII polypeptide and one or more heterologous moieties, e.g., half-life extending moieties, fused directly or indirectly with the FVIII polypeptide. The VWF fragment of the present disclosure can also comprise a D' domain and a D3 domain of VWF and one or more heterologous moieties, e.g., half-life extending moieties, fused directly or indirectly with the D' and D3 domains of VWF. In some embodiments, the FVIII protein useful for the present disclosure consists essentially of or consists of a FVIII polypeptide and the VWF fragment useful for the disclosure comprises a D' domain and a D3 domain of VWF and one or more heterologous moieties, e.g., half-life extending moieties, fused directly or indirectly with the D' and D3 domains of VWF. In some embodiments, the FVIII protein comprises a FVIII polypeptide and one or more heterologous moieties, e.g., half-life extending moieties, fused directly or indirectly with the FVIII polypeptide and the VWF fragment useful for the present disclosure consists essentially of or consists of a D' domain and a D3 domain of VWF. In some embodiments, the chimeric polypeptide comprises a FVIII protein consists essentially of or consists of a FVIII polypeptide and a VWF fragment consists essentially of or consists of a D' domain and a D3 domain of VWF.

[0161] In some embodiments, the chimeric polypeptide or protein disclosed herein is a FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer. In one embodiment, the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer chimeric polypeptide comprises: (i) a FVIII protein comprising a FVIII polypeptide, an XTEN inserted within the B domain of the FVIII polypeptide, and a first Fc region; and (ii) a VWF protein comprising a VWF fragment, a second XTEN sequence, an $\alpha 2$ linker, and a second Fc region. A schematic representation of an exemplary FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer, rFVIII-Fc-VWF-XTEN, is presented in FIG. 1.

[0162] In a specific embodiment, the chimeric polypeptide is rFVIII-Fc-VWF-XTEN. In another specific embodiment, rFVIII-Fc-VWF-XTEN comprises (i) a FVIII protein comprising the amino acid

sequence of SEQ ID NO: 203 and (ii) a VWF protein comprising the amino acid sequence of SEQ ID NO: 205. In yet another specific embodiment, rFVIII-Fc-VWF-XTEN comprises (i) a FVIII protein and (ii) a VWF protein that are covalently linked via a disulfide bond.

III.A. Factor VIII Polypeptides

[0163] "Factor VIII," abbreviated throughout the instant application as "FVIII," as used herein, means functional FVIII polypeptide in its normal role in coagulation, unless otherwise specified. Thus, the term FVIII includes variant polypeptides that are functional. "A FVIII protein" is used to refer to a FVIII polypeptide (or protein) alone, a FVIII polypeptide fused to additional polypeptides, and a FVIII polypeptide associated with one or more additional polypeptides as long as the FVIII protein exhibits a FVIII function/activity. The terms "FVIII polypeptide," "FVIII portion," and "FVIII" refer to the FVIII polypeptide sequence alone. Examples of the FVIII functions/activities include, but are not limited to, an ability to activate coagulation, an ability to act as a cofactor for factor IX, or an ability to form a tenase complex with factor IX in the presence of Ca^{2+} and phospholipids, which then converts Factor X to the activated form Xa. The FVIII polypeptide can be the human, porcine, canine, rat, or murine FVIII polypeptide. In addition, comparisons between FVIII from humans and other species have identified conserved residues that are likely to be required for function (Cameron et al., *Thromb. Haemost.* 79:317-22 (1998); US 6,251,632). The full-length polypeptide and polynucleotide sequences are known, as are many functional fragments, mutants and modified versions. Various FVIII amino acid and nucleotide sequences are disclosed in, e.g., US Publication Nos. 2015/0158929 A1, 2014/0308280 A1, and 2014/0370035 A1 and International Publication No. WO 2015/106052 A1. FVIII polypeptides include, e.g., full-length FVIII, full-length FVIII minus Met at the N-terminus, mature FVIII polypeptide (minus the signal sequence), mature FVIII polypeptide with an additional Met at the N-terminus, and/or FVIII polypeptide with a full or partial deletion of the B domain. FVIII variants include B domain deletions, whether partial or full deletions.

[0164] The FVIII polypeptide of the chimeric polypeptide used herein has FVIII activity in the plasma. FVIII activity can be measured by any known methods in the art. A number of tests are available to assess the function of the coagulation system: activated partial thromboplastin time (aPTT) test, chromogenic assay, ROTEM assay, prothrombin time (PT) test (also used to determine INR), fibrinogen testing (often by the Clauss method), platelet count, platelet function testing (often by PFA-100), TCT, bleeding time, mixing test (whether an abnormality corrects if the patient's plasma

is mixed with normal plasma), coagulation factor assays, antiphospholipid antibodies, D-dimer, genetic tests (e.g., factor V Leiden, prothrombin mutation G20210A), dilute Russell's viper venom time (dRVVT), miscellaneous platelet function tests, thromboelastography (TEG or Sonoclot), thromboelastometry (TEM®, e.g., ROTEM®), or euglobulin lysis time (ELT).

5 **[0165]** The aPTT test is a performance indicator measuring the efficacy of both the "intrinsic" (also referred to the contact activation pathway) and the common coagulation pathways. This test is commonly used to measure clotting activity of commercially available recombinant clotting factors, e.g., FVIII. It is typically used in conjunction with prothrombin time (PT), which measures the extrinsic pathway. (See, e.g., Kamal et al., Mayo Clin Proc., 82(7):864-873 (2007)). In one embodiment, aPTT
10 is tested using an assay where FVIII activity is measured using the Dade® Actin® FSL Activated PTT Reagent (Siemens Health Care Diagnostics) on a BCS® XP analyzer (Siemens Healthcare Diagnostics). In certain embodiments, the chimeric polypeptide has a plasma FVIII activity of at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%,
15 at least about 12 %, at least about 13%, at least about 14%, at least about 15%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, or at least about 20% as measured by aPTT test.

[0166] The aPTT assay may also be used for assessing the potency of a chimeric polypeptide prior to administration to a patient or subject. (Hubbard AR, et al. J Thromb Haemost 11: 988–9 (2013)).
20 The aPTT assay may further be used in conjunction with any of the assays described herein, either prior to administration or following administration to a patient or subject.

[0167] ROTEM analysis provides information on the whole kinetics of haemostasis: clotting time, clot formation, clot stability and lysis. The different parameters in thromboelastometry are dependent on the activity of the plasmatic coagulation system, platelet function, fibrinolysis, or many factors
25 which influence these interactions. This assay can provide a complete view of secondary haemostasis.

[0168] The chromogenic assay mechanism is based on the principles of the blood coagulation cascade, where activated FVIII accelerates the conversion of Factor X into Factor Xa in the presence of activated Factor IX, phospholipids and calcium ions. The Factor Xa activity is assessed by
30 hydrolysis of a p-nitroanilide (pNA) substrate specific to Factor Xa. The initial rate of release of p-nitroaniline measured at 405 nM is directly proportional to the Factor Xa activity and thus to the FVIII

activity in the sample. In one embodiment, the chromogenic assay is the BIOPHEN FVIII:C assay (Hyphen Biomed, Neuville sur Oise, France)

[0169] The chromogenic assay is recommended by the FVIII and Factor IX Subcommittee of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH). Since 1994, the chromogenic assay has also been the reference method of the European Pharmacopoeia for the assignment of FVIII concentrate potency. Thus, in some embodiments, the chimeric polypeptide comprising a FVIII polypeptide has FVIII activity comparable to a chimeric polypeptide comprising mature FVIII polypeptide or a BDD FVIII polypeptide (e.g., RECOMBINATE®, KOGENATE FS®, HELIXATE FS®, XYNTHA/REFACTO AB®, HEMOFIL-M®, MONARC-M®, MONOCLATE-P®, HUMATE-P®, ALPHANATE®, KOATE-DVI®, AFSTYLA®, AND HYATE:C®).

[0170] The chromogenic assay may also be used for assessing the potency of a chimeric polypeptide prior to administration to a patient or subject. (Hubbard AR, et al. J Thromb Haemost 11: 988–9 (2013)). The chromogenic assay may further be used in conjunction with any of the assays described herein, either prior to administration or following administration to a patient or subject.

[0171] In other embodiments, the chimeric polypeptide comprising a FVIII polypeptide of this disclosure has a Factor Xa generation rate comparable to a chimeric polypeptide comprising a mature FVIII polypeptide or a BDD FVIII polypeptide (e.g., ADVATE®, REFACTO®, or ELOCTATE®).

[0172] In order to activate Factor X to Factor Xa, activated Factor IX (Factor IXa) hydrolyzes one arginine-isoleucine bond in Factor X to form Factor Xa in the presence of Ca²⁺, membrane phospholipids, and a FVIII cofactor. Therefore, the interaction of FVIII with Factor IX is critical in coagulation pathway. In certain embodiments, the chimeric polypeptide comprising a FVIII polypeptide can interact with Factor IXa at a rate comparable to a chimeric polypeptide comprising a mature FVIII polypeptide sequence or a BDD FVIII polypeptide (e.g., ADVATE®, REFACTO®, or ELOCTATE®).

[0173] In addition, FVIII is bound to von Willebrand Factor while inactive in circulation. FVIII degrades rapidly when not bound to VWF and is released from VWF by the action of thrombin. In some embodiments, the chimeric polypeptide comprising a FVIII polypeptide binds to von Willebrand Factor, e.g., a VWF fragment disclosed herein, at a level comparable to a chimeric polypeptide comprising a mature FVIII polypeptide sequence or a BDD FVIII polypeptide (e.g., ADVATE®, REFACTO®, or ELOCTATE®).

[0174] FVIII can be inactivated by activated protein C in the presence of calcium and phospholipids. Activated protein C cleaves FVIII heavy chain after Arginine 336 in the A1 domain, which disrupts a Factor X substrate interaction site, and cleaves after Arginine 562 in the A2 domain, which enhances the dissociation of the A2 domain as well as disrupts an interaction site with the Factor IXa. This cleavage also bisects the A2 domain (43 kDa) and generates A2-N (18 kDa) and A2-C (25 kDa) domains. Thus, activated protein C can catalyze multiple cleavage sites in the heavy chain. In some embodiments, the chimeric polypeptide comprising a FVIII polypeptide is inactivated by activated Protein C at a level comparable to a chimeric polypeptide comprising a mature FVIII polypeptide sequence or a BDD FVIII polypeptide (e.g., ADVATE®, REFACTO®, or ELOCTATE®).

[0175] In other embodiments, the chimeric polypeptide comprising a FVIII polypeptide has FVIII activity in vivo comparable to a chimeric polypeptide comprising a mature FVIII polypeptide sequence or a BDD FVIII polypeptide (e.g., ADVATE®, REFACTO®, or ELOCTATE®). In a particular embodiment, the chimeric polypeptide comprising a FVIII polypeptide is capable of protecting a HemA mouse at a level comparable to a chimeric polypeptide comprising a mature FVIII polypeptide sequence or a BDD FVIII polypeptide (e.g., ADVATE®, REFACTO®, or ELOCTATE®) in a HemA mouse tail vein transection model.

[0176] Examples of human FVIII sequences (full-length) are shown below.

Table 2: Amino Acid Sequence of Full-length Human Factor VIII

(Full-length FVIII: FVIII signal peptide underlined; FVIII heavy chain is double underlined; B domain is italicized; and FVIII light chain is in plain text)

Signal Peptide: (SEQ ID NO: 64)

MQIELSTCFFLCLLRFCFS

Mature Factor VIII (SEQ ID NO: 65)*

ATRRYYLGAVELSWDYMQSDLGELPVDARFPVRPKSFPFNTSVVYKKTLFVEFTDHLFNIAPRPPWMGLL
GPTIQAEVYDVTVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSOREKEDDKVFPGGSHTYVWQVLK
ENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGLAKEKTQTLHKFILLFAVFDEGKSWHSETKN
SLMODRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQA
SLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMD
VVRFDDDNPSFQIRSVAKKHPKTWVHYIAAEEDWDYAPLVLAPDDRSYKSOYLNNGPQRIGRKYKKVR
FMAYTDETFKTREAIQHESGILGPLLYGEVGDTLIIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDF
PILPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSFVNMERDLASGLIGPLLCYKESVDQRGNGQIMSDKRNVL
FSVFDENRSWYLTENIQRFPLNPAGVQLEDPEFOASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDF
LSVFFSGYTFKHKMVYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYY

EDSYEDISAYLLSKNNAIEPRSFQNSRHPSTRQKQFNATTIPENDIEKTDPWFAHRTMPKIQNVSSDLLMLLRQ
 SPTPHGLSLSDLQEAKYETFSDDPSPGAIDSNNLSSEMTHFRPQLHHSQDMVFTPESEGLQLRLNEKLGTTAATELKK
 LDFKVSSSTNNLISTIPSDNLAAGTDNTSSLGPPSMPVHYDSQLDITLFGKKSSPLTESGGPLSLSEENNDKLLSEGL
 MNSQESSWGKNVSTESGRLFKGKRAHGPALLTKDNALFKVISLLKTNKTSNNSATNRKTHIDGPSLLIENSPSVWQ
 NILESDETFKKVTPLIHDRMLMDKNATALRLNHMSNKTSSKNMEMVQOKKEGPIPPDAQNPDMSSFFKMLFLPES
 ARWIQRTHGKNSLNSGQGPSKQLVSLGPEKSVQGNFLSEKNKVVGKGEFTKDVGLKEMVFPSSRNLFNLND
 NLHENNTHNQEKKIQEEIEKKETLIQENVVLPQIHTVTGTFKNFMKNLFLSTRQNVESYDGAYAPVLQDFRSLNDS
 TNRTKKHTAHFSKKGEEENLEGLGNQTKQIVEKYACTTRISPNTSQQNFVTQRSKRALKQFRLPLEETELEKRIIVD
 DTSTQWSKNMKHLTPSTLTQIDYNEKEKGAITQSPLSDCLTRSHSIPQANRSPLPIAKVSSFPSIRPIYLTRVLFQDNSS
 HLPAAASYRKDSGVQESSHFLQGAKKNNLSLAILTLEMTGDQREVGSIGTSATNSVTYKKVENTVLPKPDLPKTSKG
 VELLPKVHIYQKDLFPTETSNNGSPGHLDLVEGSLLOQTEGAIKWNEANRPGKVPFLRVATESSAKTPSKLLDPLAWD
 NHYGTQIPKEEWKSQEKSPKTAFAKKKDTILSLNACESNHAIANAINEGQNKPEIEVTWAKQGRTERLCSQNPPVLKR
 HQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVL
 RNRAQSGSVQFKKVVFQEFDTGDSFTQPLYRGELNEHLGGLPYIRAEVEDNIMVTFRNQASRPYSFYSSLIS
 YEEDQRQGAEPKRNFKPNETKTYFWKVQHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLL VCHTN
 TLNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAPCNQIMEDPTFKENYRFHAINGYIMDTLPLV
 MAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPKAGIWRVECLIGE
 HLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYQWAPKLARLHYSGSINAWSTKEPFSWIKVDL
 LAPMIIHGKIQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPIIARYIR
 LHPHTYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQ
 VNNPKEWLQVDFQKTMKVTVTTQGVKSLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTP
 VVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

TABLE 3. Nucleotide Sequence Encoding Full-Length FVIII (SEQ ID NO: 66)*

*The underlined nucleic acids encode a signal peptide.

661	<u>ATG CAAATAGAGC TCTCCACCTG</u>
721	<u>CTTCTTTCTG TGCCTTTTGC GATTCTGCTT</u> TAGTGCCACC AGAAGATACT ACCTGGGTGC
781	AGTGGAAGCTG TCATGGGACT ATATGCAAAG TGATCTCGGT GAGCTGCCTG TGGACGCAAG
841	ATTTCTCCT AGAGTGCCAA AATCTTTTCC ATTCAACACC TCAGTCGTGT AAAAAAGAC
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	TAAATGCAC ACAGTCAATG GTTATGTAAA CAGGTCTCTG CCAGGTCTGA TTGGATGCCA
1501	CAGGAAATCA GTCTATTGGC ATGTGATTGG AATGGGCACC ACTCCTGAAG TGCACTCAAT
1561	ATTCTCGAA GGTCACACAT TTCTTGTTGAG GAACCATCGC CAGGCGTCTT TGGAAATCTC
1621	GCCAATAACT TTCCTTACTG CTCAAACACT CTTGATGGAC CTTGGACAGT TTCTACTGTT
1681	TTGTCATATC TCTTCCCACC AACATGATGG CATGGAAGCT TATGTCAAAG TAGACAGCTG
1741	TCCAGAGGAA CCCCAACTAC GAATGAAAAA TAATGAAGAA GCGGAAGACT ATGATGATGA
1801	TCTTACTGAT TCTGAAATGG ATGTGGTCAG GTTTGATGAT GACAACTCTC CTTCTTTTAT
1861	CCAAATTCGC TCAGTTGCCA AGAAGCATCC TAAAACTGG GTACATTACA TTGCTGCTGA
1921	AGAGGAGGAC TGGGACTATG CTCCCTTAGT CTCGCCCCC 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 CGCTTCTCC 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 AAAGTGTCTT CATGTCTGATG GAAAACCCAG GTCTATGGAT
2821	TCTGGGGTGC CACAACCTCAG ACTTTCGGAA CAGAGGCATG ACCGCCTTAC TGAAGGTTTC
2881	TAGTTGTGAC AAGAACACTG GTGATTATTA CGAGGACAGT TATGAAGATA TTTCAGCATA
2941	CTTGCTGAGT AAAAAAATG CCATTGAACC AAGAAGCTTC TCCCAGAATT CAAGACACCC
3001	TAGCACTAGG CAAAAGCAAT TTAATGCCAC CACAATTCCA GAAAATGACA TAGAGAAGAC
3061	TGACCCTTGG TTTGCACACA GAACACCTAT GCCTAAAATA CAAAATGTCT CCTCTAGTGA
3121	TTTGTGATG 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 AAAGTTGATT TCAAAGTTTC TAGTACATCA AATAATCTGA TTTCAACAAT
3421	TCCATCAGAC AATTTGGCAG CAGGTACTGA TAATACAAGT TCCTTAGGAC CCCCAGTAT
3481	GCCAGTTCAT TATGATAGTC AATTAGATAC CACTCTATTT GGCAAAAAGT CATCTCCCCT
3541	TACTGAGTCT GGTGGACCTC TGAGCTTGAG TGAAGAAAAT AATGATTCAA AGTTGTTAGA
3601	ATCAGGTTTA ATGAATAGCC AAGAAAGTTC ATGGGGAAAA AATGTATCGT CAACAGAGAG
3661	TGGTAGGTTA TTAAAGGGA AAAGAGCTCA TGGACCTGCT TTGTTGACTA AAGATAATGC
3721	CTTATTCAAA GTTAGCATCT CTTTGTTAAA GACAAACAAA ACTTCCAATA ATTCAGCAAC
3781	TAATAGAAAG ACTCACATTG ATGGCCCATC ATTATTAATT GAGAATAGTC CATCAGTCTG
3841	GCAAAATATA TTAGAAAGTG AACTGAGTT TAAAAAAGTG ACACCTTTGA TTCATGACAG
3901	AATGCTTATG GACAAAAATG CTACAGCTTT GAGGCTAAAT CATATGTCAA ATAAACTAC
3961	TTCATCAAAA AACATGGAAA TGGTCCAACA GAAAAAGAG GGCCCCATTC CACCATGTC
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 AACCTATTTT TACTAACTT 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 ACTCCCATA GAAGAAACAG AACTTGAAAA
4741	AAGGATAATT GTGGATGACA CCTCAACCCA GTGGTCCAAA AACATGAAAC ATTTGACCCC
4801	GAGCACCTC ACACAGATAG ACTACAATGA GAAGGAGAAA GGGGCCATTA CTCAGTCTCC
4861	CTTATCAGAT TGCCTTACGA GGAGTCATAG CATCCCTCAA GCAAATAGAT CTCCATTACC
4921	CATTGCAAAG GTATCATCAT TTCCATCTAT TAGACCTATA TATCTGACCA GGGTCTTATT
4981	CCAAGACAAC TCTTCTCATC TTCCAGCAGC ATCTTATAGA AAGAAAGATT CTGGGGTCCA
5041	AGAAAGCAGT CATTCTTAC AAGGAGCCAA AAAAAATAAC CTTTCTTTAG CCATTCTAAC
5101	CTTGAGATG ACTGGTGATC AAAGAGAGGT TGGCTCCCTG GGGACAAAGT CCACAAATTC

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5161 AGTCACATAC AAGAAAGTTG AGAACACTGT TCTCCCGAAA CCAGACTTGC CAAAAACATC
5221 TGGCAAAGTT GAATTGCTTC CAAAAGTTCA CATTATCAG AAGGACCTAT TCCCTACGGA
5281 AACTAGCAAT GGGTCTCCTG GCCATCTGGA TCTCGTGGA 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 GCCCCGAAATA 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 CAAAAGCTG
6421 GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC AGATGGAAGA
6481 TCCCACTTT AAAGAGAATT ATCGTTCCA TGCAATCAAT GGCTACATAA TGGATACACT
6541 ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTGATGG TATCTGCTCA GCATGGGCAG
6601 CAATGAAAAC ATCCATTCTA TTCATTTTCA TGGACATGTG TTTACTGTAC GAAAAAAGA
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 CTTTATTATT 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
7141 TGTGGATTCA TCTGGGATAA AACACAATAT TTTAACCCT CCAATTATTG CTCGATACAT
7201 CCGTTTGAC 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 GTAATACTC AGGGAGTAAA
7501 ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC AAGATGGCCA
7561 TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTAGGGAA ATCAAGACTC
7621 CTTACACCT GTGGTGAAC CTCTAGACCC ACCGTTACTG ACTCGCTACC TTCGAATTCA
7681 CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT GCGAGGCACA
7741 GGACCTCTAC

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[0177] FVIII polypeptides include full-length FVIII, full-length FVIII minus Met at the N-terminus, mature FVIII (minus the signal sequence), mature FVIII with an additional Met at the N-terminus,

and/or FVIII with a full or partial deletion of the B domain. In certain embodiments, FVIII variants include B domain deletions, whether partial or full deletions.

[0178] The sequence of native mature human FVIII polypeptide is presented as SEQ ID NO: 65. A native FVIII polypeptide has the following formula: A1-a1-A2-a2-B-a3-A3-C1-C2, where A1, A2, and A3 are the structurally-related "A domains," B is the "B domain," C1 and C2 are the structurally-related "C domains," and a1, a2 and a3 are acidic spacer regions. Referring to the primary amino acid sequence position in SEQ ID NO:65, the A1 domain of human FVIII extends from Ala1 to about Arg336, the a1 spacer region extends from about Met337 to about Val374, the A2 domain extends from about Ala375 to about Tyr719, the a2 spacer region extends from about Glu720 to about Arg740, the B domain extends from about Ser741 to about Arg 1648, the a3 spacer region extends from about Glu1649 to about Arg1689, the A3 domain extends from about Ser1690 to about Leu2025, the C1 domain extends from about Gly2026 to about Asn2072, and the C2 domain extends from about Ser2073 to Tyr2332. Other than specific proteolytic cleavage sites, designation of the locations of the boundaries between the domains and regions of FVIII can vary in different literature references. The boundaries noted herein are therefore designated as approximate by use of the term "about."

[0179] The human FVIII 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; and U.S. Pat. No. 4,757,006). The FVIII amino acid sequence was deduced from cDNA as shown in U.S. Pat. No. 4,965,199. In addition, partially or fully B-domain deleted FVIII is shown in U.S. Pat. Nos. 4,994,371 and 4,868,112. In some embodiments, the human FVIII B-domain is replaced with the human Factor V B-domain as shown in U.S. Pat. No. 5,004,803. The cDNA sequence encoding human Factor VIII and amino acid sequence are shown in SEQ ID NOs: 1 and 2, respectively, of US Application Publ. No. 2005/0100990.

[0180] The porcine FVIII sequence is published in Toole, J. J., et al., Proc. Natl. Acad. Sci. USA 83:5939-5942 (1986). Further, the complete porcine cDNA sequence obtained from PCR amplification of FVIII sequences from a pig spleen cDNA library has been reported in Healey, J. F., et al., Blood 88:4209-4214 (1996). Hybrid human/porcine FVIII 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. More recently, the nucleotide and corresponding amino acid sequences of the A1 and A2 domains of porcine FVIII and a chimeric FVIII with porcine A1 and/or A2 domains substituted for the corresponding human domains were reported in WO 94/11503. U.S. Pat.

No. 5,859,204, Lollar, J. S., also discloses the porcine cDNA and deduced amino acid sequences. U.S. Pat. No. 6,458,563 discloses a B-domain-deleted porcine FVIII.

[0181] U.S. Pat. No. 5,859,204 to Lollar, J. S. reports functional mutants of FVIII having reduced antigenicity and reduced immunoreactivity. U.S. Pat. No. 6,376,463 to Lollar, J. S. also reports mutants of FVIII having reduced immunoreactivity. US Appl. Publ. No. 2005/0100990 to Saenko et al. reports functional mutations in the A2 domain of FVIII.

[0182] In some embodiments, the FVIII polypeptide (or FVIII portion of a chimeric polypeptide) can be at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a FVIII amino acid sequence of amino acids 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 2332 of SEQ ID NO: 65 (without a signal sequence) or a FVIII amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 64 and 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 19 of SEQ ID NO: 64 and amino acids 1 to 2332 of SEQ ID NO: 65 (with a signal sequence), wherein the FVIII has a clotting activity, e.g., activates Factor IX as a cofactor to convert Factor X to activated Factor X. The FVIII polypeptide (or FVIII portion of a chimeric polypeptide) can be identical to a FVIII amino acid sequence of amino acids 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 2332 of SEQ ID NO: 65 (without a signal sequence). The FVIII polypeptide can further comprise a signal sequence.

[0183] The "B-domain" of FVIII, 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, e.g., residues Ser741-Arg1648 of full-length human FVIII. The other human FVIII domains are defined by the following amino acid residues: A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Asn2019; C1, residues Lys2020-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 a3 acidic region. The locations of the boundaries for all of the domains, including the B-domains, for porcine, mouse and canine FVIII are also known in the art. In some embodiments, the B domain of FVIII 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 4. (BDD FVIII heavy chain is double underlined; B domain is italicized; and BDD FVIII light chain is in plain text). In some embodiments, the B domain FVIII has a deletion of the full B domain except 5 amino acids, as shown in Table 5 (SEQ ID NO: 68) (B domain is italicized).

TABLE 4. Amino Acid Sequence of B-domain Deleted Factor VIII (B-domain deletion except 14 amino acid residues) (SEQ ID NO: 67)

ATRRYYLGAVELSWDYMQSDLGELPVDARFPFPRVPKSPFNTSVVYKKTLFVEFTDHLFNIAPRPPWMGLL
 GPTIQAEVYDVTVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLK
 ENGPMA SDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGLAKEKTQTLHKFILLFAVFDEGKSWHSETKN
 SLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQA
 SLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMD
 VVRFDDDNPSFQIRSVAKKHPKWTWVHYIAAEEEDWDYAPLVLAPDDRSYKSOYLNNGPQRIGRKYKKVR
 FMAYTDETFKTREAIQHESGILGPLYGEVGDTLIIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDF
 PILPGEIFKYKWTVTVEDGPTKSDPRCLTRYSSSFVNMERDLASGLIGPLLCYKESVDQRGNOIMSDKRNVL
 FSVFDENRSWYL TENIQRFLPNPAGVQLEDPEFOASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTD
 FLSVFFSGYTFKHKMVYEDTLTLFPFSGETVFM SMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDY
 EDSYEDISAYLLSKNNAIEPRSFQNEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSP
 RSFQKKTRHYFIAAVERLWDYGMSSSPHVLNRNAQSGSVPOFKKVVFQFTDGSFTQPLYRGELNEHLGLL
 PYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPKRFVKPNETKTYFWKVQHMAPTKDEFDC
 KAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAPC
 NIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMA
 LYNLYPGVFETVEMLPKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPGLMASGHIRDFQITASGQYQGW
 APKLARLH YSGSINAWSTKEPFSWIKVDLLAPMIIHGKIQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNST
 GTLMVFFGNVDSSGIKHNI FNPPIIARYIRLHPHTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSY
 FTNMFATWSPSKARLHLQGRSNAWRPVNNPKEWLQVDFQKTMKVTGVTQGVKSLTSMYVKEFLISS
 QDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

TABLE 5. Amino Acid Sequence of B-domain Deleted Factor VIII (B-domain deletion except 5 amino acid residues) (SEQ ID NO: 68)

ATRRYYLGAVELSWDYMQSDLGELPVDARFPFPRVPKSPFNTSVVYKKTLFVEFTDHLFNIAPRPPWMGLL
 GPTIQAEVYDVTVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLK
 ENGPMA SDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGLAKEKTQTLHKFILLFAVFDEGKSWHSETKN
 SLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQA
 SLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMD
 VVRFDDDNPSFQIRSVAKKHPKWTWVHYIAAEEEDWDYAPLVLAPDDRSYKSOYLNNGPQRIGRKYKKVR
 FMAYTDETFKTREAIQHESGILGPLYGEVGDTLIIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDF
 PILPGEIFKYKWTVTVEDGPTKSDPRCLTRYSSSFVNMERDLASGLIGPLLCYKESVDQRGNOIMSDKRNVL
 FSVFDENRSWYL TENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTD
 FLSVFFSGYTFKHKMVYEDTLTLFPFSGETVFM SMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDY
 EDSYEDISAYLLSKNNAIEPRSFQNEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRH
 YFIAAVERLWDYGMSSSPHVLNRNAQSGSVPOFKKVVFQFTDGSFTQPLYRGELNEHLGLLGPYIRAEVED
 NIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPKRFVKPNETKTYFWKVQHMAPTKDEFDC KAWAYFSD
 VDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTF
 KENYRFHAINGYIMDTLPGLVMAQDQIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMA LYNLYPGVF
 ETVEMLPKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPGLMASGHIRDFQITASGQYQGWAPKLARLH
 YSGSINAWSTKEPFSWIKVDLLAPMIIHGKIQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFF

GNVDSSGIKHNIFNPPIIARYIRLHPHTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFAT
 WSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTQGVKSLLTSMYVKEFLISSSQDGHQW
 TLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

[0184] A "B-domain-deleted FVIII" can have the full or partial deletions disclosed in U.S. Pat. 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, U.S. Publ. Nos. US 2017/0073393 A1 and US 2012/308641 A1, and Int'l Publ. Nos. WO 2011/041770 A1, WO 2015/106052 A1 (PCT/US2015/010738), and WO 2016/025764. In some embodiments, a B-domain-deleted FVIII sequence used in the methods of the present disclosure comprises any one of the deletions disclosed at col. 4, line 4 to col. 5, line 28 and Examples 1-5 of U.S. Pat. No. 6,316,226 (also in US 6,346,513). In other embodiments, a B-domain deleted Factor VIII is the S743/Q1638 B-domain deleted Factor VIII (SQ BDD FVIII) (e.g., Factor VIII having a deletion from amino acid 744 to amino acid 1637, e.g., Factor VIII having amino acids 1-743 and amino acids 1638-2332 of mature FVIII). In some embodiments, a B-domain-deleted FVIII used in the methods of the present disclosure 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 FVIII polypeptide 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 chains, as disclosed in WO 91/09122. In some embodiments, a B-domain-deleted FVIII polypeptide is constructed with a deletion of amino acids 747-1638, i.e., virtually a complete deletion of the B domain. Hoebe R.C., et al. J. Biol. Chem. 265 (13): 7318-7323 (1990). A B-domain-deleted Factor VIII polypeptide can also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of FVIII. Meulien P., et al. Protein Eng. 2(4): 301-6 (1988). Additional B domain deletions that are part of the disclosure include: 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 through 1648 (Pasek (PCT application No.88/00831)), or 816 through 1598 or 741 through 1648 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)). In particular embodiments, the B-domain-deleted FVIII polypeptide comprises a deletion of amino acid residues 746 to 1648 of mature FVIII (corresponding to a deletion of 765 to 1665 of full length FVIII). In other embodiments, the B-domain-deleted FVIII polypeptide comprises a deletion of amino acid residues 745 to 1648 of mature FVIII (corresponding to a deletion of 764 to 1665 of full length FVIII).

[0185] In other embodiments, BDD FVIII includes a FVIII polypeptide containing fragments of the B-domain that retain one or more N-linked glycosylation sites, e.g., residues 757, 784, 828, 900, 963, or optionally 943, which correspond to the amino acid sequence of the full-length FVIII sequence. Examples of the B-domain fragments include 226 amino acids or 163 amino acids of the B-domain as disclosed in Miao, H.Z., et al., Blood 103(a): 3412-3419 (2004), Kasuda, A, et al., J. Thromb. Haemost. 6: 1352-1359 (2008), and Pipe, S.W., et al., J. Thromb. Haemost. 9: 2235-2242 (2011) (i.e., the first 226 amino acids or 163 amino acids of the B domain are retained). In still other embodiments, BDD FVIII further comprises a point mutation at residue 309 (from Phe to Ser) to improve expression of the BDD FVIII polypeptide. See Miao, H.Z., et al., Blood 103(a): 3412-3419 (2004). In still other embodiments, the BDD FVIII includes a FVIII polypeptide containing a portion of the B-domain, but not containing one or more furin cleavage sites (e.g., Arg1313 and Arg 1648). See Pipe, S.W., et al., J. Thromb. Haemost. 9: 2235-2242 (2011). In some embodiments, the BDD FVIII comprises single chain FVIII that contains a deletion in amino acids 765 to 1652 corresponding to the mature full length FVIII (also known as rFVIII-SingleChain and AFSTYLA®). See US Patent No. 7,041,635. Each of the foregoing deletions can be made in any FVIII sequence.

[0186] In some embodiments, the FVIII has a partial B-domain. In some embodiments, the FVIII polypeptide with a partial B-domain is FVIII198. FVIII198 is a partial B-domain containing single chain FVIII-Fc molecule-226N6. Number 226 represents the N-terminus 226 amino acid of the FVIII B-domain, and N6 represents six N-glycosylation sites in the B-domain.

[0187] In certain embodiments, the FVIII polypeptide is selected from a FVIII polypeptide disclosed in Publication Nos. WO 2017/117630 A1, WO 2018/087271 A1, US 9,878,017 B2, US 8,575,104 B2, US 8,754,194 B2, US 7,939,632 B2, US 2018/0161402 A1, US 9,956,269 B2, US 9,107,902 B2, US 2017/209546 A1,

[0188] In some embodiments, FVIII is cleaved right after Arginine at amino acid 1648 (in full-length Factor VIII or SEQ ID NO: 65), amino acid 754 (in the S743/Q1638 B-domain deleted Factor VIII or SEQ ID NO: 67), or the corresponding Arginine residue (in other variants), thereby resulting in a heavy chain and a light chain. In other embodiments, a FVIII polypeptide comprises a heavy chain and a light chain, which are linked or associated by a metal ion-mediated non-covalent bond.

[0189] In other embodiments, FVIII is a single chain FVIII that has not been cleaved right after Arginine at amino acid 1648 (in full-length FVIII or SEQ ID NO: 65), amino acid 754 (in the S743/Q1638 B-domain-deleted FVIII or SEQ ID NO: 67), or the corresponding Arginine residue (in other variants). A single chain FVIII can comprise one or more amino acid substitutions. In some embodiments, the amino acid substitution is at a residue corresponding to residue 1648, residue 1645, or both of full-length mature Factor VIII polypeptide (SEQ ID NO: 65) or residue 754, residue 751, or both of SQ BDD Factor VIII (SEQ ID NO: 67). The amino acid substitution can be any amino acids other than Arginine, e.g., isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, selenocysteine, serine, tyrosine, histidine, ornithine, pyrrolysine, or taurine.

[0190] FVIII can further be cleaved by thrombin and then activated as FVIIIa, serving as a cofactor for activated Factor IX (FIXa). And the activated FIX together with activated FVIII forms a Xase complex and converts Factor X to activated Factor X (FXa). For activation, FVIII is cleaved by thrombin after three Arginine residues, at amino acids 372, 740, and 1689 (corresponding to amino acids 372, 740, and 795 in the B-domain deleted FVIII sequence), the cleavage generating FVIIIa having the 50kDa A1, 43kDa A2, and 73kDa A3-C1-C2 chains. In some embodiments, the FVIII polypeptide useful for the present disclosure is non-active FVIII. In other embodiments, the FVIII polypeptide is an activated FVIII.

[0191] The protein having FVIII polypeptide linked to or associated with the VWF protein can comprise a sequence at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or 67, wherein the sequence has the FVIII clotting activity, e.g., activating Factor IX as a cofactor to convert Factor X to activated Factor X (FXa).

[0192] "Hybrid" or "chimeric" polypeptides and proteins, as used herein, includes a combination of a first polypeptide and a second polypeptide. In some embodiments, the hybrid or chimeric polypeptide comprises a single polypeptide chain, e.g., a chimeric polypeptide comprising a FVIII polypeptide and an XTEN. See, e.g., US 2015/0158929 A1, which is incorporated by reference herein

in its entirety. In some embodiments, the hybrid or chimeric polypeptide comprises a combination of a first polypeptide chain, e.g., a VWF fragment fused to an XTEN sequence and a first Ig constant region or a portion thereof, with a second polypeptide chain, e.g., a FVIII polypeptide fused to a second Ig constant region or a portion thereof, thereby forming a heterodimer. See, e.g., US 2015/0266943 A1, US 2016/0251408 A1, US 2017/0073393 A1, each of which is incorporated by reference herein in its entirety. In some embodiments, the first polypeptide and the second polypeptide in a hybrid are associated with each other via protein-protein interactions, such as charge-charge or hydrophobic interactions. In other embodiments, a first polypeptide comprises a VWF protein-XTEN-Fc fusion protein, and a second polypeptide comprises FVIII-Fc fusion protein, making the hybrid a heterodimer, wherein the XTEN contains less than 288 amino acids. In other embodiments, the first polypeptide comprises a VWF protein-XTEN-Fc fusion protein, and the second polypeptide comprises FVIII(X)-Fc fusion protein, making the hybrid a heterodimer, wherein the XTEN contains less than 288 amino acids. The first polypeptide and the second polypeptide can be associated through a covalent bond, e.g., a disulfide bond, between the first Fc region and the second Fc region. The first polypeptide and the second polypeptide can further be associated with each other by binding between the VWF fragment and the FVIII polypeptide.

[0193] In certain embodiments, the chimeric polypeptide disclosed herein comprises a FVIII protein comprising a first FVIII polypeptide fragment, an XTEN sequence, a second FVIII polypeptide fragment, and a Fc region. In some embodiments, the FVIII protein comprises, as ordered from N-terminal to C-terminal, a first FVIII polypeptide fragment, fused to an XTEN sequence, fused to a second FVIII polypeptide fragment, fused to a Fc region. In a specific embodiment, the FVIII protein comprises a first FVIII polypeptide fragment comprising the amino acid sequence of SEQ ID NO: 215, an XTEN sequence comprising the amino acid sequence of SEQ ID NO: 8, a second FVIII polypeptide comprising the amino acid sequence of SEQ ID NO: 216, and/or a first Fc region comprising the amino acid sequence of SEQ ID NO: 217.

[0194] In one specific embodiment, the chimeric polypeptide is rFVIII-Fc-VWF-XTEN and comprises a FVIII protein comprising the amino acid sequence of SEQ ID NO: 201. In another specific embodiment, rFVIII-Fc-VWF-XTEN comprises the amino acid sequence of SEQ ID NO: 207.

[0195] In some embodiments, the FVIII protein further comprises a FVIII signal peptide sequence. In one specific embodiment, the FVIII protein comprises a FVIII signal peptide comprising the amino acid sequence of SEQ ID NO: 64, a first FVIII polypeptide fragment comprising the amino acid sequence of SEQ ID NO: 215, an XTEN sequence comprising the amino acid sequence of SEQ ID

NO: 8, a second FVIII polypeptide comprising the amino acid sequence of SEQ ID NO: 216, and/or a first Fc region comprising the amino acid sequence of SEQ ID NO: 217.

[0196] In one specific embodiment, the chimeric polypeptide is rFVIII-Fc-VWF-XTEN and comprises a FVIII protein comprising the amino acid sequence of SEQ ID NO: 203. In another specific embodiment, rFVIII-Fc-VWF-XTEN comprises a FVIII protein encoded by the nucleic acid sequence of SEQ ID NO: 204 or a fragment thereof. Additional exemplary polypeptide sequences relating to the FVIII proteins of the chimeric polypeptides disclosed herein are provided in Tables 18-19.

[0197] A great many functional FVIII variants are known, as is discussed above and below. In addition, hundreds of nonfunctional mutations in FVIII have been identified in hemophilia patients, and it has been determined that the effect of these mutations on FVIII function is due more to where they lie within the 3-dimensional structure of FVIII 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 FVIII from humans and other species have 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.

III.B. Von Willebrand Factor (VWF) Fragments

[0198] VWF (also known as F8VWF) is a large multimeric glycoprotein present in blood plasma and produced constitutively in endothelium (in the Weibel-Palade bodies), megakaryocytes (α -granules of platelets), and subendothelial connective tissue. The basic VWF monomer is a 2813 amino acid protein. Every monomer contains a number of specific domains with a specific function, the D'/D3 domain (which binds to Factor VIII), the A1 domain (which binds to platelet GPIb-receptor, heparin, and/or possibly collagen), the A3 domain (which binds to collagen), the C1 domain (in which the RGD domain binds to platelet integrin α IIb β 3 when this is activated), and the "cysteine knot" domain at the C-terminal end of the protein (which VWF shares with platelet-derived growth factor (PDGF), transforming growth factor- β (TGF β) and β -human chorionic gonadotropin (β HCG)).

[0199] The term "a VWF fragment" as used herein includes, but is not limited to, functional VWF fragments comprising a D' domain and a D3 domain, which are capable of inhibiting binding of endogenous VWF to FVIII. In some embodiments, the VWF fragment binds to the FVIII protein. In other embodiments, the VWF fragment blocks the VWF binding site on the FVIII protein, thereby inhibiting interaction of the FVIII protein with endogenous VWF. In other embodiments, the VWF fragment blocks the binding of FVIII to endogenous VWF, thereby preventing clearance of the FVIII

protein through a VWF-clearance pathway. The VWF fragments include derivatives, variants, mutants, or analogues that retain these activities of VWF.

[0200] The 2813 monomer amino acid sequence for human VWF is reported as Accession Number NP000543.2 in Genbank. The nucleotide sequence encoding the human VWF is reported as
 5 Accession Number NM000552.3 in Genbank. A nucleotide sequence of human VWF is designated as SEQ ID NO: 20. SEQ ID NO: 21 is the amino acid sequence of full-length VWF. Each domain of VWF is listed in Table 6.

TABLE 6. VWF Sequences (human)

VWF domains	Amino acid Sequence		
VWF Signal Peptide	1	<u>MIPARFAGVL LALALILPGT LC</u>	22
SEQ ID NO: 208			
(Amino acids 1 to 22 of SEQ ID NO: 21)			
VWF D1D2 Domain	23	AEGTRGRS STARCSLFGS DFVNTFDGSM	
	51	YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG	
	101	TVTQGDQRV MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL	
	151	SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC	
SEQ ID NO: 209	201	ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC	
	251	EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME	
	301	YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTTEPC	
	351	VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD	
	401	NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG	
(Amino acids 23 to 763 of SEQ ID NO: 21)	451	LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM	
	501	DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG	
	551	NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS	
	601	PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL	
	651	NCPKGQVYLQ CGTPCNLT CR SLSYPDEECN EACLEGCFCP PGLYMDERGD	
	701	CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD	
	751	AVLSSPLSHR SKR	763
VWF D' Domain	764	<u>SLSCRPP MVKL VCPADN LRAEGLECTK TCONYDLECM</u>	
	801	<u>SMGCVSGCLC PPGMVRHENR CVALERCPCF HOGKEYAPGE TVKIGCNTCV</u>	
	851	<u>CRDRKWNCTD HVC DAT</u>	866

SEQ ID NO: 210 (Amino acids 764 to 866 of SEQ ID NO: 21)		
VWF D3 Domain SEQ ID NO: 211 (Amino acids 867 to 1240 of SEQ ID NO: 21)	867 901 951 1001 1051 1101 1151 1201	<u>CSTI</u> <u>GMAHYLTFDG</u> <u>LKYLFPGE</u> <u>CQ YVLVQDYCGS</u> <u>NPGTFRILVG</u> <u>NKGCSHPSVK</u> <u>CKKRVTILVE</u> <u>GGEIELFDGE</u> <u>VNVKRPMKDE</u> <u>THFEVVESGR</u> <u>YIILLGKAL</u> <u>SVVWDRHLSI</u> <u>SVVLKQTYQE</u> <u>KVCGLCGNFD</u> <u>GIONNDLTSS</u> <u>NLQVEEDPVD</u> <u>FGNSWKVSSQ</u> <u>CADTRKVPLD</u> <u>SSPATCHNNI</u> <u>MKQTMVDSSC</u> <u>RILTSDVFQD</u> <u>CNKLVDPPEY</u> <u>LDVCIYDTCS</u> <u>CESIGDCACF</u> <u>CDTIAAYAHV</u> <u>CAOHGKVVW</u> <u>RTATLCQOSC</u> <u>EERNLRENGY</u> <u>ECEWRYNSCA</u> <u>PACQVTCQHP</u> <u>EPLACPVOCV</u> <u>EGCHAHCPPG</u> <u>KILDELLQTC</u> <u>VPEDCPVCE</u> <u>VAGRRFASGK</u> <u>KVTLNPSDPE</u> <u>HCQICHCDVV</u> <u>NLTCEACQEP</u> 1240
VWF A1 Domain SEQ ID NO: 212 (Amino acids 1241 to 1479 of SEQ ID NO: 21)	1241 1251 1301 1351 1401 1451	<u>GGLVVPPTDA</u> <u>PVSPTTLYVE</u> <u>DISEPPLHDF</u> <u>YCSRLLDLVF</u> <u>LLDGSSRLSE</u> <u>AEFEVLKAFV</u> <u>VDMMERLRIS</u> <u>QKWVRVAVVE</u> <u>YHDGSHAYIG</u> <u>LKDRKRPSSEL</u> <u>RRIASQVKYA</u> <u>GSQVASTSEV</u> <u>LKYTLFQIFS</u> <u>KIDRPEASRI</u> <u>ALLLMASQEP</u> <u>QRMSRNFVRY</u> <u>VQGLKKKKVI</u> <u>VIPVGIGPHA</u> <u>NLKQIRLIEK</u> <u>QAPENKAFVL</u> <u>SSVDELEQQR</u> <u>DEIVSYLCDL</u> <u>APEAPPPTLP</u> <u>PDMAQVTVG</u> 1479
VWF A2 Domain to C-terminus SEQ ID NO: 213 (Amino acids 1480 to 2813 of SEQ ID NO: 21)	1480 1501 1551 1601 1651 1701 1751 1801 1851 1901 1951 2001 2051 2101 2151 2201 2251	<u>PGLLGVSTLGP</u> <u>KRNSMVLDVA</u> <u>FVLEGSDKIG</u> <u>EADFNRSKEF</u> <u>MEEVIQRMDV</u> <u>GQDSIHVTVL</u> <u>QYSYMTVEY</u> <u>PFSEAQSKGD</u> <u>ILQRVREIRY</u> <u>QGGNRTNTGL</u> <u>ALRYLSDHSF</u> <u>LVSQGDREQA</u> <u>PNLVYMTGN</u> <u>PASDEIKRLP</u> <u>GDIQVVPIGV</u> <u>GPNANVQELE</u> <u>RIGWPNAFIL</u> <u>IQDFETLPRE</u> <u>APDLVLQRC</u> <u>SGEGLQIPTL</u> <u>SPAPDCSQPL</u> <u>DVILLLDGSS</u> <u>SFPASYFDEM</u> <u>KSFAKAFISK</u> <u>ANIGPRLTQV</u> <u>SVLQYGSITT</u> <u>IDVPWNVVPE</u> <u>KAHLLSLVDV</u> <u>MQREGGPSQI</u> <u>GDALGFAVRY</u> <u>LTSEMHGARP</u> <u>GASKAVVILV</u> <u>TDVSVDSVDA</u> <u>AADAARSNRV</u> <u>TVFPIGIGDR</u> <u>YDAAQLRILA</u> <u>GPAGDSNVVK</u> <u>LQRIEDLPTM</u> <u>VTLGNSFLHK</u> <u>LCSGFVRICM</u> <u>DEDGNEKRP</u> <u>GDVWTLPDQCH</u> <u>TVTCQPDGQT</u> <u>LLKSHRVNCD</u> <u>RGLRPSCPNS</u> <u>QSPVKVEETC</u> <u>GCRWTCPCVC</u> <u>TGSSTRHIVT</u> <u>FDGQNFKLTG</u> <u>SCSYVLFQNK</u> <u>EQDLEVILHN</u> <u>GACSPGARQG</u> <u>CMKSIEVKHS</u> <u>ALSVEXHSDM</u> <u>EVTVNGRLVS</u> <u>VPYVGGNMEV</u> <u>NVYGAIMHEV</u> <u>RFNHLGHIFT</u> <u>FTPQNEFQL</u> <u>QLSPKTFASK</u> <u>TYGLCGICDE</u> <u>NGANDFMLRD</u> <u>GTVTTDWKTL</u> <u>VQEWTVQRP</u> <u>QTCQPILEEQ</u> <u>CLVPDSSHQ</u> <u>VLLPLFAEC</u> <u>HKVLAPATFY</u> <u>AICQQDSCHQ</u> <u>EQVCEVIASY</u> <u>AHLCRTNGVC</u> <u>VDWRTPDFCA</u> <u>MSCPPSLVYN</u> <u>HCEHGCPRHC</u> <u>DGNVSSCGDH</u> <u>PSEGCFCPPD</u> <u>KVMLEGSCVP</u> <u>EEACTQCIGE</u> <u>DGVQHQFLEA</u> <u>WVPDHPQCQI</u> <u>CTCLSGRKVN</u> <u>CTTQPCPTAK</u>

2301	APTCGLCEVA RLRQNADQCC PEYECVCDPV SCDLPPVPHC ERGLQPTLTN
2351	PGECPNFTC ACRKEECKRV SPPSCPPHRL PTLRKTQCCD EYECACNCVN
2401	STVSCPLGYL ASTATNDCGC TTTTCLPDKV CVHRSTIYPV GQFWEEGCDV
2451	CTCTDMEDAV MGLRVAQCSQ KPCEDSCRSG FTYVLHEGEC CGRCLPSACE
2501	VVTGSPRGDS QSSWKSQSG WASPENPCLI NECVRVKEEV FIIQRNVSCP
2551	QLEVPVCPSP FQLSCKTSAC CPSCRCERME ACMLNGTVIG PGKTVMIDVC
2601	TTCRCMVQVG VISGFKLECR KTTCNPCPLG YKEENNTGEC CGRCLPTACT
2651	IQLRGGQIMT LKRDETLQDG CDTHFCKVNE RGEYFWEKRV TGCPPFDEHK
2701	CLAEKGKIMK IPGTCCDTCE EPECNDITAR LQYVKVGSCK SEVEVDIHYC
2751	QGKCASKAMY SIDINDVQDQ CSCCSPTRTE PMQVALHCTN GSVVYHEVLN
2801	AMECKCSPRK CSK 2813

[0201] The VWF protein as used herein can be a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the VWF fragment binds to Factor VIII (FVIII) and inhibits binding of endogenous VWF (full-length VWF) to FVIII. The VWF fragment comprising the D' domain and the D3 domain can further comprise a VWF domain selected from the group consisting of an A1 domain, an A2 domain, an A3 domain, a D1 domain, a D2 domain, a D4 domain, a B1 domain, a B2 domain, a B3 domain, a C1 domain, a C2 domain, a CK domain, one or more fragments thereof, and any combinations thereof. In some embodiments, a VWF fragment comprises, consists essentially of, or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof. The VWF fragment described herein does not contain a site binding to a VWF clearance receptor. In other embodiments, the VWF fragment described herein is not amino acids 764 to 1274 of SEQ ID NO: 21. The VWF fragment of the present disclosure can comprise any other sequences linked to or fused to the VWF fragment. For example, a VWF fragment described herein can further comprise a signal peptide.

[0202] In some embodiments, the VWF fragment comprising a D' domain and a D3 domain binds to or is associated with a FVIII protein. See, e.g., US 2015/0023959 A1, US 2015/0266943 A1, US 2016/0251408 A1, US 2017/0073393 A1, US 2018/185455 A1, US 2018/0051067 A1, US 2017/0152300 A1, US 9,878,017 B2, US 9,458,223 B2, US 8,575,104 B2, WO 2017/117630 A1, US 2018/0161402 A1, WO 2017/117631 A1, WO 2018/087271 A1, US 9,107,902 B2, WO 2017/222337 A1, WO 2015/185758 A1, each of which is incorporated by reference herein in its entirety. By binding to or associating with a FVIII protein, a VWF fragment of the disclosure protects FVIII from protease cleavage and FVIII activation, stabilizes the heavy chain and light chain of FVIII, and prevents

clearance of FVIII by scavenger receptors. In other embodiments, the VWF fragment binds to or associates with a FVIII protein and blocks or prevents binding of the FVIII protein to phospholipid and activated Protein C. By preventing or inhibiting binding of the FVIII protein with endogenous, full-length VWF, the VWF fragment of the disclosure reduces the clearance of FVIII by VWF clearance
5 receptors and thus extends half-life of the chimeric polypeptide. The half-life extension of a chimeric polypeptide is thus due to the binding of or associating with the VWF fragment lacking a VWF clearance receptor binding site to the FVIII protein and shielding or protecting of the FVIII protein by the VWF fragment from endogenous VWF which contains the VWF clearance receptor binding site. The FVIII protein bound to or protected by the VWF fragment can also allow recycling of a FVIII
10 protein. By eliminating the VWF clearance pathway receptor binding sites contained in the full length VWF molecule, the FVIII/VWF heterodimers of the disclosure are shielded from the VWF clearance pathway, further extending FVIII half-life.

[0203] In some embodiments, a VWF protein useful for the present disclosure comprises a D' domain and a D3 domain of VWF, wherein the D' domain is at least 60%, 70%, 80%, 85%, 90%,
15 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In other embodiments, a VWF protein comprises the D' domain and the D3 domain of VWF, wherein the D3 domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF
20 to FVIII. In some embodiments, a VWF protein described herein comprises, consists essentially of, or consists of the D' domain and D3 domain of VWF, which are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In other embodiments, a VWF protein comprises, consists essentially of, or consists of the D1, D2, D', and D3 domains at least
25 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In still other embodiments, the VWF protein further comprises a signal peptide operably linked thereto.

[0204] In some embodiments, a VWF protein useful for the disclosure consists essentially of or consists of (1) the D'D3 domain, the D1D'D3 domain, D2D'D3 domain, or D1D2D'D3 domain and (2)
30 an additional VWF sequence up to about 10 amino acids (e.g., any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1250 of SEQ ID NO: 21), up to about 15 amino acids (e.g., any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1255 of

SEQ ID NO: 21), up to about 20 amino acids (e.g., any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1260 of SEQ ID NO: 21), up to about 25 amino acids (e.g., any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1265 of SEQ ID NO: 21), or up to about 30 amino acids (e.g., any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1260 of SEQ ID NO: 21). In some embodiments, the VWF protein comprising or consisting essentially of the D' domain and the D3 domain is neither amino acids 764 to 1274 of SEQ ID NO: 21 nor the full-length mature VWF. In some embodiments, the D1D2 domain is expressed in trans with the D'D3 domain. In some embodiments, the D1D2 domain is expressed in cis with the D'D3 domain.

[0205] In other embodiments, the VWF protein comprising the D'D3 domains linked to the D1D2 domains further comprises an intracellular cleavage site, e.g., (a cleavage site by PACE (furin) or PC5), allowing cleavage of the D1D2 domains from the D'D3 domains upon expression. Non-limiting examples of the intracellular cleavage site are disclosed elsewhere herein.

[0206] In yet other embodiments, a VWF protein comprises a D' domain and a D3 domain, but does not comprise an amino acid sequence selected from the group consisting of (1) amino acids 1241 to 2813 corresponding to SEQ ID NO: 21, (2) amino acids 1270 to amino acids 2813 corresponding to SEQ ID NO: 21, (3) amino acids 1271 to amino acids 2813 corresponding to SEQ ID NO: 21, (4) amino acids 1272 to amino acids 2813 corresponding to SEQ ID NO: 21, (5) amino acids 1273 to amino acids 2813 corresponding to SEQ ID NO: 21, (6) amino acids 1274 to amino acids 2813 corresponding to SEQ ID NO: 21, and any combinations thereof.

[0207] In still other embodiments, a VWF protein of the present disclosure comprises, consists essentially of, or consists of an amino acid sequence corresponding to the D' domain, D3 domain, and A1 domain, wherein the amino acid sequence is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acid 764 to 1479 of SEQ ID NO: 21, wherein the VWF protein prevents binding of endogenous VWF to FVIII. In particular embodiments, the VWF protein is not amino acids 764 to 1274 of SEQ ID NO: 21.

[0208] In some embodiments, a VWF protein of the disclosure comprises a D' domain and a D3 domain, but does not comprise at least one VWF domain selected from the group consisting of (1) an A1 domain, (2) an A2 domain, (3) an A3 domain, (4) a D4 domain, (5) a B1 domain, (6) a B2 domain, (7) a B3 domain, (8) a C1 domain, (9) a C2 domain, (10) a CK domain, (11) a CK domain and C2 domain, (12) a CK domain, a C2 domain, and a C1 domain, (13) a CK domain, a C2 domain,

a C1 domain, a B3 domain, (14) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, (15) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, and a B1 domain, (16) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, and a D4 domain, (17) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, and an A3 domain, (18) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, and an A2 domain, (19) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, an A2 domain, and an A1 domain, and (20) any combinations thereof.

[0209] In yet other embodiments, the VWF protein comprises the D'D3 domains and one or more domains or modules. Examples of such domains or modules include, but are not limited to, the domains and modules disclosed in Zhou et al., Blood published online April 6, 2012: DOI 10.1182/blood-2012-01-405134, which is incorporated herein by reference in its entirety. For example, the VWF protein can comprise the D'D3 domain and one or more domains or modules selected from the group consisting of A1 domain, A2 domain, A3 domain, D4N module, VWD4 module, C8-4 module, TIL-4 module, C1 module, C2 module, C3 module, C4 module, C5 module, C5 module, C6 module, and any combinations thereof.

[0210] In still other embodiments, the VWF protein is linked to a heterologous moiety, wherein the heterologous moiety is linked to the N-terminus or the C-terminus of the VWF protein or inserted immediately downstream of one or more amino acids (e.g., one or more XTEN insertion sites) in the VWF protein. For example, the insertion sites for the heterologous moiety in the VWF protein can be in the D' domain, the D3 domain, or both. The heterologous moiety can be a half-life extender.

[0211] In certain embodiments, a VWF protein useful for the disclosure forms a multimer, e.g., dimer, trimer, tetramer, pentamer, hexamer, heptamer, or the higher order multimers. In other embodiments, the VWF protein is a monomer having only one VWF protein. In some embodiments, the VWF protein of the present disclosure can have one or more amino acid substitutions, deletions, additions, or modifications. In some embodiments, the VWF protein can include amino acid substitutions, deletions, additions, or modifications such that the VWF protein is not capable of forming a disulfide bond or forming a dimer or a multimer. In other embodiments, the amino acid substitution is within the D' domain and the D3 domain. In a particular embodiment, a VWF protein useful for the disclosure contains at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 corresponding to SEQ ID NO: 21. The at least one amino acid substitution can be any amino acids that are not occurring naturally in the

wild type VWF. For example, the amino acid substitution can be any amino acids other than cysteine, e.g., Isoleucine, Alanine, Leucine, Asparagine, Lysine, Aspartic acid, Methionine, Phenylalanine, Glutamic acid, Threonine, Glutamine, Tryptophan, Glycine, Valine, Proline, Serine, Tyrosine, Arginine, or Histidine. In another example, the amino acid substitution has one or more amino acids
5 that prevent or inhibit the VWF proteins from forming multimers.

[0212] In certain embodiments, the VWF protein useful herein can be further modified to improve its interaction with FVIII, e.g., to improve binding affinity to FVIII. As a non-limiting example, the VWF protein comprises a serine residue at the residue corresponding to amino acid 764 of SEQ ID NO: 21 and a lysine residue at the residue corresponding to amino acid 773 of SEQ ID NO: 21. Residues
10 764 and/or 773 can contribute to the binding affinity of the VWF proteins to FVIII. In other embodiments, The VWF proteins useful for the disclosure can have other modifications, e.g., the protein can be pegylated, glycosylated, hesylated, or polysialylated.

[0213] In certain embodiments, the chimeric polypeptide disclosed herein comprises a VWF protein comprising a VWF fragment, an XTEN sequence, an $\alpha 2$ linker of FVIII, and a Fc region. In some
15 embodiments, the VWF protein comprises, as ordered from N-terminal to C-terminal, a VWF fragment, fused to an XTEN sequence, fused to an $\alpha 2$ linker, fused to a Fc region. In certain embodiments, the VWF protein comprises a D' domain of VWF comprising the amino acid sequence of SEQ ID NO: 210, a D3 domain of VWF comprising the amino acid sequence of SEQ ID NO: 214, an XTEN sequence comprising the amino acid sequence of SEQ ID NO: 58 (AE144_5A), an $\alpha 2$ linker
20 comprising the amino acid sequence of SEQ ID NO: 88, and/or a Fc region comprising the amino acid sequence of SEQ ID NO: 217.

[0214] In one specific embodiment, the chimeric polypeptide is rFVIII-Fc-VWF-XTEN and comprises a VWF protein comprising the amino acid sequence of SEQ ID NO: 202.

[0215] In some embodiments, the VWF protein comprises a VWF fragment comprising a D1, D2, D', and/or D3 domain of VWF. In one embodiment, the VWF fragment comprises a D1D2 domain of
25 VWF comprising the amino acid sequence of SEQ ID NO: 209. In some embodiments, the VWF protein further comprises a VWF signal peptide sequence. In one embodiment, the VWF signal peptide comprises the amino acid sequence of SEQ ID NO: 208. In one specific embodiment, the VWF protein comprises a VWF signal peptide comprising the amino acid sequence of SEQ ID NO:
30 208, a D1D2 region of VWF comprising the amino acid sequence of SEQ ID NO: 209, a D' domain of VWF comprising the amino acid sequence of SEQ ID NO: 210, a D3 domain of VWF comprising

the amino acid sequence of SEQ ID NO: 214, an XTEN sequence comprising the amino acid sequence of SEQ ID NO: 58 (AE144_5A), an a2 linker comprising the amino acid sequence of SEQ ID NO: 88, and/or a Fc region comprising the amino acid sequence of SEQ ID NO: 217.

[0216] In one specific embodiment, the chimeric polypeptide is rFVIII^hFc-VWF-XTEN and comprises a VWF protein comprising the amino acid sequence of SEQ ID NO: 205. In another specific embodiment, rFVIII^hFc-VWF-XTEN comprises a VWF protein encoded by the nucleic acid sequence of SEQ ID NO: 206 or a fragment thereof. Additional exemplary polypeptide sequences relating to the VWF proteins of the chimeric polypeptides disclosed herein are provided in Tables 18-19.

III.C. Half-Life Extending Moieties

[0217] In some embodiments, the chimeric polypeptide of the disclosure comprises one or more half-life extending moieties. In some embodiments, the FVIII polypeptide of the chimeric polypeptide is fused to or associated with one or more half-life extending moieties (i.e., FVIII protein). In other embodiments, the chimeric polypeptide comprises at least two half-life extending moieties, a first half-life extending moiety fused to a FVIII polypeptide and a second half-life extending moiety fused to D'D3 domains of VWF. In some embodiments, the first half-life extending moiety is fused to the C-terminus or the N-terminus of the FVIII polypeptide. In some embodiments, the first half-life extending moiety is inserted within the FVIII polypeptide. In some embodiments, the first half-life extending moiety is inserted within the B domain of the FVIII polypeptide. In some embodiments, the first half-life extending moiety is inserted within the FVIII polypeptide immediately downstream of an amino acid corresponding to amino acid residue 745 of SEQ ID NO: 65. In some embodiments, the first heterologous moiety is fused to the FVIII polypeptide by a linker.

[0218] In certain embodiments, the D'D3 domains of VWF in the VWF fragment can be fused to or associated with a second half-life extending moiety. In some embodiments, the second half-life extending moiety is fused to the C-terminus or the N-terminus of the VWF fragment. In some embodiments, the second half-life extending moiety is inserted within the VWF fragment. In some embodiments, the second half-life extending moiety is fused to the C-terminus of the VWF fragment. In certain embodiments, wherein the second half-life extending moiety is fused to the VWF fragment by a linker.

[0219] In other embodiments, the chimeric polypeptide comprises a FVIII polypeptide and a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the D'D3 domain of VWF is fused to one or more half-life extending moieties and wherein the D'D3 domain of VWF and the FVIII polypeptide are associated with a bond stronger than naturally occurring bond between FVIII and VWF.

[0220] In some embodiments, the chimeric polypeptide comprises a FVIII polypeptide and a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the D'D3 domain of VWF is fused to the FVIII polypeptide by a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the D' domain and D3 domain of VWF are further associated with the FVIII polypeptide by at least one non-covalent bond. In certain embodiments, the D' domain and the D3 domain of VWF are further fused to an Fc. In some embodiments, the D' domain and D3 domain of VWF are fused to an Fc by a linker. In some embodiments, the Fc is fused to a second Fc by an additional linker. In certain embodiments, the Fc and the second Fc are associated with each other by a covalent bond, e.g., a disulphide bond. In particular embodiments, the chimeric polypeptide comprises a FVIII polypeptide and a VWF fragment comprising a D' domain and a D3 domain of VWF as disclosed in International Publication No. WO 2017/222337 A1, which is incorporated by reference herein in its entirety.

[0221] The first half-life extending moiety, the second half-life extending moiety, or both can be selected from the group consisting of an FcRn binding partner, e.g., an albumin or an immunoglobulin Fc region, an XTEN sequence, the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, a PAS sequence, a HAP sequence, a transferrin, albumin-binding moieties, or any fragments, derivatives, variants, and any combination thereof.

III.C.1. Ig Constant Region or a portion thereof

[0222] In some embodiments, the chimeric polypeptide of the disclosure also includes a first Ig constant region or a portion thereof fused to a FVIII polypeptide by an optional linker. The first Ig constant region or the portion thereof can be inserted within the FVIII polypeptide or fused to the C-terminus or the N-terminus of the FVIII polypeptide. In some embodiments, the chimeric polypeptide further includes a second Ig constant region or a portion thereof fused to a VWF protein. The first Ig constant region or the portion thereof can be inserted within the VWF fragment or fused to the C-terminus or the N-terminus of the VWF fragment. In particular embodiments, the first Ig constant

region is linked to or associated with the second Ig constant region by a covalent bond, e.g., a disulfide bond.

[0223] The Ig constant region or a portion thereof can improve pharmacokinetic or pharmacodynamic properties of the chimeric polypeptide in combination with an additional heterologous moiety, e.g., an XTEN sequence, and the VWF protein. In certain embodiments, the Ig constant region or a portion thereof extends a half-life of a molecule fused to the Ig constant region or a portion thereof.

[0224] An Ig constant region is comprised of domains denoted CH (constant heavy) domains (CH1, CH2, etc.). Depending on the isotype, (i.e. IgG, IgM, IgA, IgD, or IgE), the constant region can be comprised of three or four CH domains. Some isotypes (e.g. IgG) constant regions also contain a hinge region. See Janeway et al. 2001, Immunobiology, Garland Publishing, N.Y., N.Y.

[0225] An Ig constant region or a portion thereof for producing the chimeric polypeptide of the present disclosure can be obtained from a number of different sources. In some embodiments, an Ig constant region or a portion thereof is derived from a human Ig. It is understood, however, that the Ig constant region or a portion thereof can be derived from an Ig of another mammalian species, including for example, a rodent (e.g. a mouse, rat, rabbit, guinea pig) or non-human primate (e.g. chimpanzee, macaque) species. Moreover, the Ig constant region or a portion thereof can be derived from any Ig class, including IgM, IgG, IgD, IgA, and IgE, and any Ig isotype, including IgG1, IgG2, IgG3, and IgG4. In some embodiments, the human isotype IgG1 is used.

[0226] A variety of the Ig constant region gene sequences (e.g., human constant region gene sequences) are available in the form of publicly accessible deposits. Constant region domains sequence can be selected having a particular effector function (or lacking a particular effector function) or with a particular modification to reduce immunogenicity. Many sequences of antibodies and antibody-encoding genes have been published and suitable Ig constant region sequences (e.g., hinge, CH2, and/or CH3 sequences, or portions thereof) can be derived from these sequences using art recognized techniques. The genetic material obtained using any of the foregoing methods can then be altered or synthesized to obtain polypeptides of the present disclosure. It will further be appreciated that the scope of this disclosure encompasses alleles, variants and mutations of constant region DNA sequences.

[0227] The sequences of the Ig constant region or a portion thereof can be cloned, e.g., using the polymerase chain reaction and primers which are selected to amplify the domain of interest. To clone

a sequence of the Ig constant region or a portion thereof from an antibody, mRNA can be isolated from hybridoma, spleen, or lymph cells, reverse transcribed into DNA, and antibody genes amplified by PCR. PCR amplification methods are described in detail in U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; and in, e.g., "PCR Protocols: A Guide to Methods and Applications" Innis et al. eds., Academic Press, San Diego, CA (1990); Ho et al. 1989. Gene 77:51; Horton et al. 1993. Methods Enzymol. 217:270). PCR can be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also can be used to isolate DNA clones encoding the antibody light and heavy chains. In this case the libraries can be screened by consensus primers or larger homologous probes, such as mouse constant region probes. Numerous primer sets suitable for amplification of antibody genes are known in the art (e.g., 5' primers based on the N-terminal sequence of purified antibodies (Benhar and Pastan. 1994. Protein Engineering 7:1509); rapid amplification of cDNA ends (Ruberti, F. et al. 1994. J. Immunol. Methods 173:33); antibody leader sequences (Larrick et al. 1989 Biochem. Biophys. Res. Commun. 160:1250)). The cloning of antibody sequences is further described in Newman et al., U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein.

[0228] An Ig constant region used herein can include all domains and the hinge region or portions thereof. In some embodiments, the Ig constant region or a portion thereof comprises CH2 domain, CH3 domain, and a hinge region, i.e., an Fc region or an FcRn binding partner.

[0229] As used herein, the term "Fc region" is defined as the portion of a polypeptide which corresponds to the Fc region of native Ig, i.e., as formed by the dimeric association of the respective Fc domains of its two heavy chains. A native Fc region forms a homodimer with another Fc region. In contrast, the term "genetically-fused Fc region" or "single-chain Fc region" (scFc region), as used herein, refers to a synthetic dimeric Fc region comprised of Fc domains genetically linked within a single polypeptide chain (i.e., encoded in a single contiguous genetic sequence).

[0230] In some embodiments, the "Fc region" refers to the portion of a single Ig heavy chain beginning in the hinge region just upstream of the papain cleavage site (i.e. residue 216 in IgG, taking the first residue of heavy chain constant region to be 114) and ending at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain.

[0231] The Fc region of an Ig constant region, depending on the Ig isotype can include the CH2, CH3, and CH4 domains, as well as the hinge region. Chimeric polypeptides comprising an Fc region of an Ig bestow several desirable properties on a chimeric polypeptide including increased stability, increased serum half-life (see Capon et al., 1989, Nature 337:525) as well as binding to Fc receptors
5 such as the neonatal Fc receptor (FcRn) (U.S. Pat. Nos. 6,086,875, 6,485,726, 6,030,613; WO 03/077834; US2003-0235536A1), which are incorporated herein by reference in their entireties.

[0232] An Ig constant region or a portion thereof can be an FcRn binding partner. FcRn is active in adult epithelial tissues and expressed in the lumen of the intestines, pulmonary airways, nasal surfaces, vaginal surfaces, colon and rectal surfaces (U.S. Pat. No. 6,485,726). An FcRn binding
10 partner is a portion of an Ig that binds to FcRn. Another example of an FcRn binding partner is albumin, further described below.

[0233] The FcRn receptor has been isolated from several mammalian species including humans. The sequences of the human FcRn, monkey FcRn, rat FcRn, and mouse FcRn are known (Story et al. 1994, J. Exp. Med. 180:2377). The FcRn receptor binds IgG (but not other Ig classes such as IgA,
15 IgM, IgD, and IgE) at relatively low pH, actively transports the IgG transcellularly in a luminal to serosal direction, and then releases the IgG at relatively higher pH found in the interstitial fluids. It is expressed in adult epithelial tissue (U.S. Pat. Nos. 6,485,726, 6,030,613, 6,086,875; WO 03/077834; US2003-0235536A1) including lung and intestinal epithelium (Israel et al. 1997, Immunology 92:69) renal proximal tubular epithelium (Kobayashi et al. 2002, Am. J. Physiol. Renal Physiol. 282:F358)
20 as well as nasal epithelium, vaginal surfaces, and biliary tree surfaces.

[0234] FcRn binding partners useful in the present disclosure encompass molecules that can be specifically bound by the FcRn receptor including whole IgG, the Fc fragment of IgG, and other fragments that include the complete binding region of the FcRn receptor. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography
25 (Burmeister et al. 1994, Nature 372:379). 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 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
30 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of Igs or Ig 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.

[0235] Fc regions or FcRn binding partners bound to FcRn can be effectively shuttled across epithelial barriers by FcRn, thus providing a non-invasive means to systemically administer a desired therapeutic molecule. Additionally, fusion proteins comprising an Fc region or an FcRn binding partner are endocytosed by cells expressing the FcRn. But instead of being marked for degradation, these fusion proteins are recycled out into circulation again, thus increasing the in vivo half-life of these proteins. In certain embodiments, the portions of Ig constant regions are an Fc region or an FcRn binding partner that typically associates, via disulfide bonds and other non-specific interactions, with another Fc region or another FcRn binding partner to form dimers and higher order multimers.

[0236] Two FcRn receptors can bind a single Fc molecule. Crystallographic data suggest that each FcRn molecule binds a single polypeptide of the Fc homodimer. In some embodiments, linking the FcRn binding partner, e.g., an Fc fragment of an IgG, to a biologically active molecule provides a means of delivering the biologically active molecule orally, buccally, sublingually, rectally, vaginally, as an aerosol administered nasally or via a pulmonary route, or via an ocular route. In other embodiments, the chimeric polypeptide can be administered invasively, e.g., subcutaneously, intravenously.

[0237] An FcRn binding partner region is a molecule or a portion thereof that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the Fc region. Specifically bound refers to two molecules forming a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished from nonspecific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the affinity constant K_A is higher than 10^6 M^{-1} , or higher than 10^8 M^{-1} . If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions such as concentration of the molecules, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (e.g. serum albumin, milk casein), etc., can be optimized by a skilled artisan using routine techniques.

[0238] In certain embodiments, a chimeric polypeptide of the disclosure comprises one or more truncated Fc regions that are nonetheless sufficient to confer Fc receptor (FcR) binding properties to the Fc region. For example, the portion of an Fc region that binds to FcRn (i.e., the FcRn binding portion) comprises from about amino acids 282-438 of IgG1, EU numbering (with the primary contact sites being amino acids 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. Thus, an Fc region of the

disclosure can comprise or consist of an FcRn binding portion. FcRn binding portions can be derived from heavy chains of any isotype, including IgG1, IgG2, IgG3 and IgG4. In some embodiments, an FcRn binding portion from an antibody of the human isotype IgG1 is used. In other embodiments, an FcRn binding portion from an antibody of the human isotype IgG4 is used.

5 **[0239]** In other embodiments, the "Fc region" includes an amino acid sequence of an Fc domain or derived from an Fc domain. In certain embodiments, an Fc region comprises at least one of: a hinge (e.g., upper, middle, and/or lower hinge region) domain (about amino acids 216-230 of an antibody Fc region according to EU numbering), a CH2 domain (about amino acids 231-340 of an antibody Fc region according to EU numbering), a CH3 domain (about amino acids 341-438 of an antibody Fc region according to EU numbering), a CH4 domain, or a variant, portion, or fragment thereof. In other
10 embodiments, an Fc region comprises a complete Fc domain (i.e., a hinge domain, a CH2 domain, and a CH3 domain). In some embodiments, an Fc region comprises, consists essentially of, or consists of a hinge domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a hinge domain (or a portion thereof) fused to a CH2 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to
15 both a hinge domain (or a portion thereof) and a CH3 domain (or a portion thereof). In still other embodiments, an Fc region lacks at least a portion of a CH2 domain (e.g., all or part of a CH2 domain). In a particular embodiment, an Fc region comprises or consists of amino acids corresponding to EU numbers 221 to 447.

20 **[0240]** The Fc regions denoted as F, F1, or F2 herein can be obtained from a number of different sources. In some embodiments, an Fc region of the polypeptide is derived from a human Ig. It is understood, however, that an Fc region can be derived from an Ig of another mammalian species, including for example, a rodent (e.g. a mouse, rat, rabbit, or guinea pig) or non-human primate (e.g. chimpanzee, macaque) species. Moreover, the polypeptide of the Fc domains or portions thereof can
25 be derived from any Ig class, including IgM, IgG, IgD, IgA and IgE, and any Ig isotype, including IgG1, IgG2, IgG3 and IgG4. In other embodiments, the human isotype IgG1 is used.

[0241] In certain embodiments, the Fc variant confers a change in at least one effector function imparted by an Fc region comprising said wild-type Fc domain (e.g., an improvement or reduction in the ability of the Fc region to bind to Fc receptors (e.g. FcγRI, FcγRII, or FcγRIII) or complement
30 proteins (e.g. C1q), or to trigger antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)). In other embodiments, the Fc variant provides an engineered cysteine residue.

[0242] The Fc regions of the disclosure can employ art-recognized Fc variants which are known to impart a change (e.g., an enhancement or reduction) in effector function and/or FcR or FcRn binding. Specifically, a binding molecule of the disclosure can include, for example, a change (e.g., a substitution) at one or more of the amino acid positions disclosed in International PCT Publications

5 WO88/07089A1, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2, WO04/029207A2, WO04/035752A2, WO04/063351A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1,

10 WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/0231329, US2007/0231329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243188, US20070248603, US20070286859, US20080057056 ; or US Patent Nos. 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; 7,083,784; 7,404,956, and 7,317,091, each of which is

15 incorporated by reference herein. In some embodiments, the specific change (e.g., the specific substitution of one or more amino acids disclosed in the art) can be made at one or more of the disclosed amino acid positions. In other embodiments, a different change at one or more of the disclosed amino acid positions (e.g., the different substitution of one or more amino acid position disclosed in the art) can be made.

20 **[0243]** The Fc region or FcRn binding partner of IgG can be modified according to well recognized procedures such as site directed mutagenesis and the like to yield modified IgG or Fc fragments or portions thereof that will be bound by FcRn. Such modifications include modifications remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example, the following single amino acid residues in human IgG1 Fc (Fc □1)

25 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, P331A,

30 E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A,

R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wild type proline substituted by alanine at position number 238. As an example, some embodiments incorporate the N297A mutation, removing a highly conserved N-glycosylation site. In addition to alanine other amino acids can be substituted for the wild type amino acids at the positions specified above. Mutations can be introduced singly into Fc giving rise to more than one hundred Fc regions distinct from the native Fc. Additionally, combinations of two, three, or more of these individual mutations can be introduced together, giving rise to hundreds more Fc regions. Moreover, one of the Fc region of a construct of the disclosure can be mutated and the other Fc region of the construct not mutated at all, or they both can be mutated but with different mutations.

[0244] Certain of the above mutations can confer new functionality upon the Fc region or FcRn binding partner. For example, some embodiments incorporate N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the Fc region, and to render the Fc region incapable of binding to FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et al. 1995, J. Biol. Chem. 276:6591). As a further example of new functionality arising from mutations described above affinity for FcRn can be increased beyond that of wild type in some instances. This increased affinity can reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased "off" rate. Examples of mutations believed to impart an increased affinity for FcRn include, but not limited to, T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).

[0245] 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 can 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 and Armour et al. 1999, Eur. J. Immunol. 29:2613.

[0246] In some embodiments, the Ig constant region or a portion thereof, e.g., an Fc region, is a polypeptide including the sequence PKNSSMISNTP (SEQ ID NO: 89 or SEQ ID NO: 3 of U.S. Pat.

No. 5,739,277) and optionally further including a sequence selected from HQSLGTQ (SEQ ID NO: 90), HQNLSDGK (SEQ ID NO: 91), HQNISDGK (SEQ ID NO: 92), or VISSHLGQ (SEQ ID NO: 93) (or SEQ ID NOs: 11, 1, 2, and 31, respectively of U.S. Pat. No. 5,739,277).

[0247] In other embodiments, the immunoglobulin constant region or a portion thereof comprises an amino acid sequence in the hinge region or a portion thereof that forms one or more disulfide bonds with another immunoglobulin constant region or a portion thereof. The disulfide bond by the immunoglobulin constant region or a portion thereof places the first polypeptide comprising a FVIII polypeptide and the second polypeptide comprising the VWF fragment together so that endogenous VWF does not replace the VWF fragment and does not bind to the FVIII polypeptide. Therefore, the disulfide bond between the first immunoglobulin constant region or a portion thereof and a second immunoglobulin constant region or a portion thereof prevents interaction between endogenous VWF and the FVIII polypeptide. This inhibition of interaction between the VWF and the FVIII polypeptide allows the half-life of the chimeric polypeptide to go beyond the two fold limit. The hinge region or a portion thereof can further be linked to one or more domains of CH1, CH2, CH3, a fragment thereof, and any combinations thereof. In a particular embodiment, the immunoglobulin constant region or a portion thereof is a hinge region and CH2.

[0248] In certain embodiments, the Ig constant region or a portion thereof is hemi-glycosylated. For example, the chimeric polypeptide comprising two Fc regions or FcRn binding partners can contain a first, glycosylated, Fc region (e.g., a glycosylated CH2 region) or FcRn binding partner and a second, aglycosylated, Fc region (e.g., an aglycosylated CH2 region) or FcRn binding partner. In some embodiments, a linker can be interposed between the glycosylated and aglycosylated Fc regions. In other embodiments, the Fc region or FcRn binding partner is fully glycosylated, i.e., all of the Fc regions are glycosylated. In other embodiments, the Fc region can be aglycosylated, i.e., none of the Fc moieties are glycosylated.

[0249] In certain embodiments, a chimeric polypeptide of the disclosure comprises an amino acid substitution to an Ig constant region or a portion thereof (e.g., Fc variants), which alters the antigen-independent effector functions of the Ig constant region, in particular the circulating half-life of the protein.

[0250] Such proteins exhibit either increased or decreased binding to FcRn when compared to proteins lacking these substitutions and, therefore, have an increased or decreased half-life in serum, respectively. Fc variants with improved affinity for FcRn are anticipated to have longer serum half-

lives, and such molecules have useful applications in methods of treating mammals where long half-life of the administered polypeptide is desired, e.g., to treat a chronic disease or disorder (see, e.g., US Patents 7,348,004, 7,404,956, and 7,862,820). In contrast, Fc variants with decreased FcRn binding affinity are expected to have shorter half-lives, and such molecules are also useful, for example, for administration to a mammal where a shortened circulation time can be advantageous, e.g. for in vivo diagnostic imaging or in situations where the starting polypeptide has toxic side effects when present in the circulation for prolonged periods. Fc variants with decreased FcRn binding affinity are also less likely to cross the placenta and, thus, are also useful in the treatment of diseases or disorders in pregnant women. In addition, other applications in which reduced FcRn binding affinity can be desired include those applications in which localization the brain, kidney, and/or liver is desired. In one exemplary embodiment, the chimeric polypeptides of the disclosure exhibit reduced transport across the epithelium of kidney glomeruli from the vasculature. In other embodiments, the chimeric polypeptides of the disclosure exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In some embodiments, a protein with altered FcRn binding comprises at least one Fc region or FcRn binding partner (e.g, one or two Fc regions or FcRn binding partners) having one or more amino acid substitutions within the "FcRn binding loop" of an Ig constant region. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering) of a wild-type, full-length, Fc region. In other embodiments, an Ig constant region or a portion thereof in a chimeric polypeptide of the disclosure having altered FcRn binding affinity comprises at least one Fc region or FcRn binding partner having one or more amino acid substitutions within the 15 Å FcRn "contact zone." As used herein, the term 15 Å FcRn "contact zone" includes residues at the following positions of a wild-type, full-length Fc moiety: 243-261, 275-280, 282-293, 302-319, 336- 348, 367, 369, 372-389, 391, 393, 408, 424, 425-440 (EU numbering). In other embodiments, a Ig constant region or a portion thereof of the disclosure having altered FcRn binding affinity comprises at least one Fc region or FcRn binding partner having one or more amino acid substitutions at an amino acid position corresponding to any one of the following EU positions: 256, 277-281, 283-288, 303-309, 313, 338, 342, 376, 381, 384, 385, 387, 434 (e.g., N434A or N434K), and 438. Exemplary amino acid substitutions which altered FcRn binding activity are disclosed in International PCT Publication No. WO05/047327 which is incorporated by reference herein.

[0251] An Fc region or FcRn binding partner used in the disclosure can also comprise an art recognized amino acid substitution which alters the glycosylation of the chimeric polypeptide. For example, the Fc region or FcRn binding partner of the chimeric polypeptide linked to the D'D3 domain

of VWF or a FVIII polypeptide can comprise an Fc region having a mutation leading to reduced glycosylation (e.g., N- or O-linked glycosylation) or can comprise an altered glycoform of the wild-type Fc moiety (e.g., a low fucose or fucose-free glycan).

[0252] In some embodiments, an unprocessed chimeric polypeptide of the disclosure can comprise a genetically fused Fc region (i.e., scFc region) having two or more of its constituent Ig constant region or a portion thereof independently selected from the Ig constant region or a portion thereof described herein. In some embodiments, the Fc regions of a dimeric Fc region are the same. In other embodiments, at least two of the Fc regions are different. For example, the Fc regions or FcRn binding partners of the proteins of the disclosure comprise the same number of amino acid residues or they can differ in length by one or more amino acid residues (e.g., by about 5 amino acid residues (e.g., 1, 2, 3, 4, or 5 amino acid residues), about 10 residues, about 15 residues, about 20 residues, about 30 residues, about 40 residues, or about 50 residues). In yet other embodiments, the Fc regions or FcRn binding partners of the protein of the disclosure can differ in sequence at one or more amino acid positions. For example, at least two of the Fc regions or FcRn binding partners can differ at about 5 amino acid positions (e.g., 1, 2, 3, 4, or 5 amino acid positions), about 10 positions, about 15 positions, about 20 positions, about 30 positions, about 40 positions, or about 50 positions).

III.C.2. XTEN Sequences

[0253] As used herein "XTEN sequence" refers to extended length polypeptides with non-naturally occurring, substantially non-repetitive sequences that are composed mainly of small hydrophilic amino acids, with the sequence having a low degree or no secondary or tertiary structure under physiologic conditions. As a chimeric polypeptide partner, XTENs can serve as a carrier, conferring certain desirable pharmacokinetic, physicochemical and pharmaceutical properties when linked to a VWF protein or a FVIII sequence of the disclosure to create a chimeric polypeptide. Such desirable properties include but are not limited to enhanced pharmacokinetic parameters and solubility characteristics. As used herein, "XTEN" specifically excludes antibodies or antibody fragments such as single-chain antibodies or Fc fragments of a light chain or a heavy chain.

[0254] In some embodiments, a shorter XTEN sequence provides an improved half-life extending property compared to a longer XTEN sequence when the XTEN sequence is fused to a VWF protein and/or a second Ig constant region or a portion thereof. Therefore, in some embodiments, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof contains less than 288 amino acids in length, i.e., is shorter than 288 amino acids. In some embodiments, the

XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof consists of an amino acid sequence having a length of between 12 amino acids and 287 amino acids. In other embodiments, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof comprise at least about 36 amino acids, at least about 42 amino acids, at least about 72 amino acids, or at least about 144 amino acids, but less than 288 amino acids. In other embodiments, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof is selected from AE36, AG36, AE42, AG42, AE72, AG72, AE144, or AG144. In some embodiments, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof is an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 14, wherein the chimeric polypeptide exhibits an improved half-life compared to a chimeric polypeptide without the XTEN sequence.

[0255] The chimeric polypeptide of the disclosure can further comprise an additional (second, third, or more) XTEN sequences. The additional XTEN sequence can further be fused to the FVIII polypeptide or the first Ig constant region or a portion thereof. The additional XTEN sequences can be any length. For example, the additional XTEN sequence fused to the FVIII polypeptide or the first Ig constant region or a portion thereof is a peptide or a polypeptide having greater than about 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues. In certain embodiments, the additional XTEN sequence is a peptide or a polypeptide having greater than about 20 to about 3000 amino acid residues, greater than about 30 to about 2500 residues, greater than about 40 to about 2000 residues, greater than about 50 to about 1500 residues, greater than about 60 to about 1000 residues, greater than about 70 to about 900 residues, greater than about 80 to about 800 residues, greater than about 90 to about 700 residues, greater than about 100 to about 600 residues, greater than about 110 to about 500 residues, or greater than about 120 to about 400 residues. In certain embodiments, the additional XTEN that is fused to the FVIII polypeptide comprises at least about 288 amino acids. In particular embodiments, the additional XTEN that is fused to the FVIII polypeptide comprises about 288 amino acids. In particular embodiments, the additional XTEN that is fused to the FVIII polypeptide comprises an amino acid sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence of AE288 (SEQ ID NO: 8).

[0256] The XTEN sequences (i.e., the XTEN sequence fused to the VWF protein and/or the second Ig constant region or a portion thereof or the XTEN sequence fused to the FVIII polypeptide and/or the first Ig constant region or a portion thereof or inserted at one or more insertion sites within the FVIII polypeptide) can comprise one or more sequence motif of 9 to 14 amino acid residues or an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence motif, wherein the motif comprises, consists essentially of, or consists of 4 to 6 types of amino acids selected from the group consisting of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P). See US 2010-0239554 A1.

[0257] In some embodiments, the XTEN sequence comprises non-overlapping sequence motifs in which at least about 80%, or at least about 85%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% or about 100% of the sequence consists of multiple units of non-overlapping sequences selected from a single motif family selected from Table 7, resulting in a family sequence. As used herein, "family" means that the XTEN has motifs selected only from a single motif category from Table 7; i.e., AD, AE, AF, AG, AM, AQ, BC, or BD XTEN, and that any other amino acids in the XTEN not from a family motif are selected to achieve a needed property, such as to permit incorporation of a restriction site by the encoding nucleotides, incorporation of a cleavage sequence, or to achieve a better linkage to FVIII or VWF. In some embodiments of XTEN families, an XTEN sequence comprises multiple units of non-overlapping sequence motifs of the AD motif family, or of the AE motif family, or of the AF motif family, or of the AG motif family, or of the AM motif family, or of the AQ motif family, or of the BC family, or of the BD family, with the resulting XTEN exhibiting the range of homology described above. In other embodiments, the XTEN comprises multiple units of motif sequences from two or more of the motif families of Table 7. These sequences can be selected to achieve desired physical/chemical characteristics, including such properties as net charge, hydrophilicity, lack of secondary structure, or lack of repetitiveness that are conferred by the amino acid composition of the motifs, described more fully below. In the embodiments hereinabove described in this paragraph, the motifs incorporated into the XTEN can be selected and assembled using the methods described herein to achieve an XTEN of about 36 to about 3000 amino acid residues.

Table 7. XTEN Sequence Motifs of 12 Amino Acids and Motif Families

Motif Family*	MOTIF SEQUENCE
AD	GESPGGSSGSES (SEQ ID NO: 24)
AD	GSEGSSGPGESS (SEQ ID NO: 25)
AD	GSSESGSSEGGP (SEQ ID NO: 26)
AD	GSGGEPSESGSS (SEQ ID NO: 27)
AE, AM	GSPAGSPTSTEE (SEQ ID NO: 28)
AE, AM, AQ	GSEPATSGSETP (SEQ ID NO: 29)
AE, AM, AQ	GTSESATPESGP (SEQ ID NO: 30)
AE, AM, AQ	GTSTEPSEGSAP (SEQ ID NO: 31)
AF, AM	GSTSESPSGTAP (SEQ ID NO: 32)
AF, AM	GTSTPESGSASP (SEQ ID NO: 33)
AF, AM	GTSPSGESSTAP (SEQ ID NO: 34)
AF, AM	GSTSSTAESPGP (SEQ ID NO: 35)
AG, AM	GTPGSGTASSSP (SEQ ID NO: 36)
AG, AM	GSSTPSGATGSP (SEQ ID NO: 37)
AG, AM	GSSPSASTGTGP (SEQ ID NO: 38)
AG, AM	GASPGTSSTGSP (SEQ ID NO: 39)
AQ	GEPAGSPTSTSE (SEQ ID NO: 40)
AQ	GTGEPSSTPASE (SEQ ID NO: 41)
AQ	GSGPSTESAPTE (SEQ ID NO: 42)
AQ	GSETPSGPSETA (SEQ ID NO: 43)
AQ	GPSETSTSEPGA (SEQ ID NO: 44)
AQ	GSPSEPTEGTSA (SEQ ID NO: 45)
BC	GSGASEPTSTEP (SEQ ID NO: 46)
BC	GSEPATSGTEPS (SEQ ID NO: 47)
BC	GTSEPSTSEPGA (SEQ ID NO: 48)
BC	GTSTEPSEPGSA (SEQ ID NO: 49)
BD	GSTAGSETSTEA (SEQ ID NO: 50)
BD	GSETATSGSETA (SEQ ID NO: 51)
BD	GTSESATSESGA (SEQ ID NO: 52)
BD	GTSTEASEGSAS (SEQ ID NO: 53)

*Denotes individual motif sequences that, when used together in various permutations, results in a "family sequence"

[0258] In some embodiments, the XTEN sequence used in the disclosure is at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of AE42, AG42, AE48, AM48, AE72, AG72, AE108, AG108, AE144, AF144, AG144, AE180, AG180, AE216, AG216, AE252, AG252, AE288, AG288, AE324, AG324, AE360, AG360, AE396, AG396, AE432, AG432, AE468, AG468, AE504, AG504, AF504, AE540, AG540, AF540, AD576, AE576, AF576, AG576, AE612, AG612, AE624, AE648, AG648,

AG684, AE720, AG720, AE756, AG756, AE792, AG792, AE828, AG828, AD836, AE864, AF864, AG864, AM875, AE912, AM923, AM1318, BC864, BD864, AE948, AE1044, AE1140, AE1236, AE1332, AE1428, AE1524, AE1620, AE1716, AE1812, AE1908, AE2004A, AG948, AG1044, AG1140, AG1236, AG1332, AG1428, AG1524, AG1620, AG1716, AG1812, AG1908, and AG2004.

5 See US 2010-0239554 A1.

[0259] In some embodiments, the XTEN sequence is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of AE42 (SEQ ID NO: 9), AE72 (SEQ ID NO: 10), AE144_2A (SEQ IDNO: 55), AE144_3B (SEQ ID NO: 56), AE144_4A (SEQ ID NO: 57), AE144_5A (SEQ ID NO: 58), AE144_6B (SEQ ID NO: 59),
 10 AG144_A (SEQ ID NO: 60), AG144_B (SEQ ID NO: 61), AG144_C (SEQ ID NO: 62), AG144_F (SEQ IDNO: 63), AE864 (SEQ ID NO: 15), AE576 (SEQ ID NO: 16), AE288 (SEQ ID NO: 8), AE288_2 (SEQ ID NO: 54), AE144 (SEQ ID NO: 11), AG864 (SEQ ID NO: 17), AG576 (SEQ ID NO: 18), AG288 (SEQ ID NO: 19), AG144 (SEQ ID NO: 14), and any combinations thereof. In other embodiments, the XTEN sequence is selected from the group consisting of AE42 (SEQ ID NO: 9),
 15 AE72 (SEQ ID NO: 10), AE144_2A (SEQ IDNO: 55), AE144_3B (SEQ ID NO: 56), AE144_4A (SEQ ID NO: 57), AE144_5A (SEQ IDNO: 58), AE144_6B (SEQ ID NO: 59), AG144_A (SEQ ID NO: 60), AG144_B (SEQ ID NO: 61), AG144_C (SEQ ID NO: 62), AG144_F (SEQ IDNO: 63), AE864 (SEQ ID NO: 15), AE576 (SEQ ID NO: 16), AE288 (SEQ ID NO: 8), AE288_2 (SEQ ID NO: 54), AE144 (SEQ ID NO: 11), AG864 (SEQ ID NO: 17), AG576 (SEQ ID NO: 18), AG288 (SEQ ID NO: 19),
 20 AG144 (SEQ ID NO: 14), and any combinations thereof. In some embodiments, the XTEN sequence is AE288. The amino acid sequences for certain XTEN sequences of the disclosure are shown in Table 8.

TABLE 8. XTEN Sequences

XTEN	Amino Acid Sequence
AE42 SEQ ID NO: 9	GAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPASS
AE72 (SEQ ID NO: 10)	GAP TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGSAPGASS
AE144 (SEQ ID NO:11)	GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEG SAPGSEPATSGSETPGSEPATSGSETPGSEPATSGSETPGTSTEPSEGSAPGTSESA PESGPGSEPATSGSETPGTSTEPSEGSAP

AE144_2A (SEQ ID NO: 55)	TSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSTEPS EGSAPGTSESATPESGPGSEPATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGP GTSESATPESGPG
AE144_3B (SEQ ID NO: 56)	SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEPS EGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEE GTSTEPSEGSAPG
AE144_4A (SEQ ID NO: 57)	TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPS EGSAPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTSTEEGTSESATPESGP GTSTEPSEGSAPG
AE144_5A (SEQ ID NO: 58)	TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPS EGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEE GSPAGSPTSTEEG
AE144_6B (SEQ ID NO: 59)	TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPAT GSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPG TSTEPSEGSAPG
AG144 SEQ ID NO:14	GTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSST GSPGASPGTSSTGSPGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSA STGTGPGTTPGSGTASSSPGSSTPSGATGSP
AG144_A (SEQ ID NO: 60)	GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTTPGSGTASSSPGSSTPSGATGSPGSSPS ASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGASPGTSSTG SPGASPGTSSTGSP
AG144_B (SEQ ID NO: 61)	GTPGSGTASSSPGSSTPSGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSPS ASTGTGPGSSPSASTGTGPGSSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTG SPGASPGTSSTGSP
AG144_C (SEQ ID NO: 62)	GTPGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGS GTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTPSGATG SPGASPGTSSTGSP
AG144_F (SEQ ID NO: 63)	GSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSPS ASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATG SPGASPGTSSTGSP
AE288 SEQ ID NO: 8	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESG PGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPES GPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPE SGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSE GSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AE288_2	GSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEE GTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESA

(SEQ ID NO: 54)	TPESGPGTSTEPSEGSAPGTSESATPESGPSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTSTEE GTSESATPESGPGTSTEPSEGSAP
AG288 SEQ ID NO:19	PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS SPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSSPSASTG TGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSPSAST GTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSPSAS TGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS
AE576 SEQ ID NO:16	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGPSAGSPTSTEEGTSTEPSEGSA PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGPSAGSPTSTEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGPSAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGPSAG ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP
AG576 SEQ ID NO:18	PGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSSTPSGATG SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTAS SSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTA SSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTPSG ATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSSTPS GATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGASPG TSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSST PSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSS TPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGS STPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGS
AE864 SEQ ID NO:15	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGPSAGSPTSTEEGTSTEPSEGSA PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGPSAGSPTSTEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGSPAGSP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGPSAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEP ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGS EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPG SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEE GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESG PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGS APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AG864 SEQ ID NO:17	GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGASPGTSST GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSSTPSGA TGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGT

ASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSG TASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTP SGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSST PSGATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGAS PGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGS STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPG SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSP GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGS PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASS SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP
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[0260] In those embodiments wherein the XTEN component(s) have less than 100% of its amino acids consisting of 4, 5, or 6 types of amino acid selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), or less than 100% of the sequence consisting of the sequence motifs from Table 7 or the XTEN sequences of Table 8, the other amino acid residues of the XTEN are selected from any of the other 14 natural L-amino acids, but are preferentially selected from hydrophilic amino acids such that the XTEN sequence contains at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% hydrophilic amino acids. The XTEN amino acids that are not glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) are either interspersed throughout the XTEN sequence, are located within or between the sequence motifs, or are concentrated in one or more short stretches of the XTEN sequence, e.g., to create a linker between the XTEN and the FVIII or VWF components. In such cases where the XTEN component comprises amino acids other than glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), it is preferred that less than about 2% or less than about 1% of the amino acids be hydrophobic residues such that the resulting sequences generally lack secondary structure, e.g., not having more than 2% alpha helices or 2% beta-sheets, as determined by the methods disclosed herein. Hydrophobic residues that are less favored in construction of XTEN include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. Additionally, one can design the XTEN sequences to contain less than 5% or less than 4% or less than 3% or less than 2% or less than 1% or none of the following amino acids: cysteine (to avoid disulfide formation and oxidation), methionine (to avoid oxidation), asparagine and glutamine (to avoid desamidation). Thus, in some embodiments, the XTEN component comprising other amino acids in addition to glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) have a sequence with less than 5% of the residues contributing to alpha-helices and beta-sheets as measured by the Chou-Fasman algorithm and have at least 90%, or at least about 95% or more random coil formation as measured by the GOR algorithm.

[0261] In further embodiments, the XTEN sequence used in the disclosure affects the physical or chemical property, e.g., pharmacokinetics, of the chimeric polypeptide of the present disclosure. The XTEN sequence used in the present disclosure can exhibit one or more of the following advantageous properties: conformational flexibility, enhanced aqueous solubility, high degree of protease resistance, low immunogenicity, low binding to mammalian receptors, or increased hydrodynamic (or Stokes) radii. In some embodiments, the XTEN sequence linked to a FVIII protein in this disclosure increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that the chimeric polypeptide described herein stays in vivo for an increased period of time compared to wild type FVIII. In further embodiments, the XTEN sequence used in this disclosure increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that FVIII protein stays in vivo for an increased period of time compared to wild type FVIII.

[0262] A variety of methods and assays can be employed to determine the physical/chemical properties of proteins comprising the XTEN sequence. Such methods include, but are not limited to analytical centrifugation, EPR, HPLC-ion exchange, HPLC-size exclusion, HPLC-reverse phase, light scattering, capillary electrophoresis, circular dichroism, differential scanning calorimetry, fluorescence, HPLC-ion exchange, HPLC-size exclusion, IR, NMR, Raman spectroscopy, refractometry, and UV/Visible spectroscopy. Additional methods are disclosed in Amau et al., *Prot Expr and Purif* 48, 1-13 (2006).

[0263] Additional examples of XTEN sequences that can be used according to the present disclosure and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, WO 2011028344 A2, or WO 20130122617 A1.

III.C.2. Albumins

[0264] An albumin or a portion thereof can be an FcRn binding partner. In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one albumin polypeptide or fragment, variant, or derivative thereof. Human serum albumin (HSA, or HA), a protein of 609 amino acids in its full-length form, is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. The term "albumin" as used herein includes full-length albumin or a functional fragment, variant, derivative, or

analog thereof. Examples of albumin or the fragments or variants thereof are disclosed in US Pat. Publ. Nos. 2008/0194481A1, 2008/0004206 A1, 2008/0161243 A1, 2008/0261877 A1, or 2008/0153751 A1 or PCT Appl. Publ. Nos. 2008/033413 A2, 2009/058322 A1, or 2007/021494 A2, which are incorporated herein by reference in their entireties.

5 **[0265]** The albumin-binding polypeptides (ABPs) can comprise, without limitation, bacterial albumin-binding domains, albumin-binding peptides, or albumin-binding antibody fragments that can bind to albumin. Domain 3 from streptococcal protein G, as disclosed by Kraulis et al., FEBS Lett. 378:190-194 (1996) and Linhult et al., Protein Sci. 11:206-213 (2002) is an example of a bacterial albumin-binding domain. Examples of albumin-binding peptides are disclosed in Dennis et al., J. Biol.
10 Chem. 2002, 277: 35035-35043 (2002). Examples of albumin-binding antibody fragments are disclosed in Muller and Kontermann, Curr. Opin. Mol. Ther. 9:319-326 (2007); Roovers et al., Cancer Immunol. Immunother. 56:303-317 (2007), and Holt et al., Prot. Eng. Design Sci., 21:283-288 (2008), which are incorporated herein by reference in their entireties.

[0266] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure
15 comprises at least one attachment site for a non-polypeptide small molecule, variant, or derivative that can bind to albumin thereof. For example, the chimeric polypeptide can include one or more organic albumin-binding moieties. An example of such albumin-binding moieties is 2-(3-maleimidopropanamido)-6-(4-(4-iodophenyl)butanamido)hexanoate ("Albu" tag) as disclosed by Trussel et al., Bioconjugate Chem. 20:2286-2292 (2009).

20 **[0267]** In certain embodiments, the a VWF fragment described herein is fused to an albumin. In some embodiments, the VWF fragment-albumin constructs form homodimers. In some embodiments, the D'D3 domain of VWF that is fused to the albumin associates with a FVIII polypeptide. In certain embodiments, the D'D3 domain of VWF fused to the albumin associated with the FVIII polypeptide by an interaction stronger than the natural non-covalent interactions between wild-type FVIII and
25 VWF. In particular embodiments, the D'D3 domain of VWF and/or the FVIII polypeptide comprises one or more mutation that increases the affinity between the D'D3 domain of VWF and the FVIII polypeptide. In certain embodiments, the D'D3 domain of VWF and/or the FVIII polypeptide comprises one or more mutation that allows for a disulfide bond to form between the D'D3 domain of VWF and/or the FVIII polypeptide.

III.C.3. CTP

[0268] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin or fragment, variant, or derivative thereof. CTP peptides are known to increase the half-life of that protein. See, e.g., U.S. Patent No. 5,712,122, incorporated by reference herein in its entirety. Non-limiting exemplary CTP peptides are disclosed in U.S. Patent Application Publication No. US 2009/0087411 A1, incorporated by reference.

III.C.4. PAS

[0269] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one PAS peptide or fragment, variant, or derivative thereof. A PAS peptide or PAS sequence, as used herein, means an amino acid sequence comprising mainly alanine and serine residues or comprising mainly alanine, serine, and proline residues, the amino acid sequence forming random coil conformation under physiological conditions. Accordingly, the PAS sequence is a building block, an amino acid polymer, or a sequence cassette comprising, consisting essentially of, or consisting of alanine, serine, and proline which can be used as a part of the heterologous moiety in the chimeric polypeptide. An amino acid polymer also can form random coil conformation when residues other than alanine, serine, and proline are added as a minor constituent in the PAS sequence. By "minor constituent" is meant that that amino acids other than alanine, serine, and proline can be added in the PAS sequence to a certain degree, e.g., up to about 12%, i.e., about 12 of 100 amino acids of the PAS sequence, up to about 10%, up to about 9%, up to about 8%, about 6%, about 5%, about 4%, about 3%, i.e. about 2%, or about 1%, of the amino acids. The amino acids different from alanine, serine and proline can be selected from the group consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, and Val. Under physiological conditions, a PAS peptide forms a random coil conformation and thereby can mediate an increased in vivo and/or in vitro stability to a recombinant protein of the disclosure, and has procoagulant activity.

[0270] Non-limiting examples of the PAS peptides are disclosed in, e.g., US Pat. Publ. No. 2010/0292130 A1; PCT Appl. Publ. No. WO 2008/155134 A1; and European issued patent EP2173890.

III.C.5. HAP

[0271] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one homo-amino acid polymer (HAP) peptide or fragment, variant, or derivative thereof. A HAP peptide can comprise a repetitive sequence of glycine, which has at least 50 amino acids, at least 100 amino acids, 120 amino acids, 140 amino acids, 160 amino acids, 180 amino acids, 200 amino acids, 250 amino acids, 300 amino acids, 350 amino acids, 400 amino acids, 450 amino acids, or 500 amino acids in length. A HAP sequence is capable of extending half-life of a moiety fused to or linked to the HAP sequence. Non-limiting examples of the HAP sequence includes, but are not limited to (Gly)_n, (Gly4Ser)_n or S(Gly4Ser)_n, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, n is 20, 21, 22, 23, 24, 25, 26, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40. In other embodiments, n is 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200. See, e.g., Schlapschy M et al., Protein Eng. Design Selection, 20: 273-284 (2007).

III.C.6. Transferrin

[0272] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one transferrin peptide or fragment, variant, or derivative thereof. Any transferrin can fuse with the chimeric polypeptide used in the methods of the present disclosure. As an example, wild-type human Tf (Tf) is a 679 amino acid protein, of approximately 75 kDa (not accounting for glycosylation), with two main domains, N (about 330 amino acids) and C (about 340 amino acids), which appear to originate from a gene duplication. See GenBank accession numbers NM001063, XM002793, M12530, XM039845, XM 039847 and S95936 (www.ncbi.nlm.nih.gov), all of which are herein incorporated by reference in their entirety.

[0273] Transferrin transports iron through transferrin receptor (TfR)-mediated endocytosis. After the iron is released into an endosomal compartment and Tf-TfR complex is recycled to cell surface, the Tf is released back extracellular space for next cycle of iron transporting. Tf possesses a long half-life that is in excess of 14-17 days (Li et al., Trends Pharmacol. Sci. 23:206-209 (2002)). Transferrin fusion proteins have been studied for half-life extension, targeted deliver for cancer therapies, oral delivery and sustained activation of proinsulin (Brandsma et al., Biotechnol. Adv., 29: 230-238 (2011); Bai et al., Proc. Natl. Acad. Sci. USA 102:7292-7296 (2005); Kim et al., J. Pharmacol. Exp. Ther., 334:682-692 (2010); Wang et al., J. Controlled Release 155:386-392 (2011)).

III.C.7. PEG

[0274] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one attachment site for a non-polypeptide heterologous moiety or fragment, variant, or derivative thereof. For example, a chimeric polypeptide used in the methods of the present disclosure can include one or more polyethylene glycol (PEG) moieties attached to one or more amino acid residues in the clotting factor and/or the Fc region.

[0275] PEGylation of a protein can refer to a conjugate formed between the protein and at least one polyethylene glycol (PEG) molecule. PEG is commercially available in a large variety of molecular weights and average molecular weight ranges. Typical examples of PEG average molecular weight ranges include, but are not limited to, about 200, about 300, about 400, about 600, about 1000, about 1300-1600, about 1450, about 2000, about 3000, about 3000-3750, about 3350, about 3000-7000, about 3500-4500, about 5000-7000, about 7000-9000, about 8000, about 10000, about 8500-11500, about 16000-24000, about 35000, about 40000, about 60000, and about 80000 Daltons. These average molecular weights are provided merely as examples and are not meant to be limiting in any way.

[0276] A chimeric polypeptide used in the methods of the present disclosure can be PEGylated to include mono- or poly-(e.g., 2-4) PEG moieties. PEGylation can be carried out by any of the PEGylation reactions known in the art. Methods for preparing a PEGylated protein product will generally include (i) reacting a polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the peptide of the disclosure becomes attached to one or more PEG groups; and (ii) obtaining the reaction product(s). In general, the optimal reaction conditions for the reactions will be determined case by case based on known parameters and the desired result.

[0277] There are a number of PEG attachment methods available to those skilled in the art, for example Malik F et al., Exp. Hematol. 20:1028-35 (1992); Francis, Focus on Growth Factors 3(2):4-10 (1992); European Pat. Pub. Nos. EP0401384, EP0154316, and EP0401384; and International Pat. Appl. Pub. Nos. WO92/16221 and WO95/34326. As a non-limiting example, FVIII variants can contain cysteine substitutions, and the cysteines can be further conjugated to PEG polymer. See Mei et al., Blood 116:270-279 (2010) and U.S. Patent No. 7,632,921, which are incorporated herein by reference in their entireties.

[0278] In certain embodiments, the chimeric polypeptide comprises a single chain FVIII polypeptide covalently linked to a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the FVIII polypeptide comprises a deletion of all or a portion of the B domain, wherein the FVIII polypeptide is pegylated, and wherein the VWF fragment is fused directly or indirectly to the N-terminus of the FVIII polypeptide (e.g., PEG-scFVIII-D'D3, "MG1121," or LAFATE). In other embodiments, the chimeric polypeptide comprises a single chain FVIII polypeptide covalently linked to a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the FVIII polypeptide comprises a deletion of all or a portion of the B domain, wherein the VWF fragment is pegylated, and wherein the VWF fragment is fused directly or indirectly to the N-terminus of the FVIII polypeptide (e.g., scFVIII-D'D3-PEG). In other embodiments, the chimeric polypeptide comprises a single chain FVIII polypeptide covalently linked to a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the FVIII polypeptide comprises a deletion of all or a portion of the B domain, wherein FVIII polypeptide and the VWF fragment are pegylated, and wherein the VWF fragment is fused directly or indirectly to the N-terminus of the FVIII polypeptide (e.g., scFVIII-D'D3-PEG).

III.C.8. HES

[0279] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one hydroxyethyl starch (HES) polymer. HES is a derivative of naturally occurring amylopectin and is degraded by alpha-amylase in the body. HES exhibits advantageous biological properties and is used as a blood volume replacement agent and in hemodilution therapy in the clinics. See, e.g., Sommermeyer et al., *Krankenhauspharmazie* 8:271-278 (1987); and Weidler et al., *Arzneim.-Forschung/Drug Res.* 41: 494-498 (1991).

[0280] HES is mainly characterized by the molecular weight distribution and the degree of substitution. HES has a mean molecular weight (weight mean) of from 1 to 300 kD, from 2 to 200kD, from 3 to 100 kD, or from 4 to 70kD. Hydroxyethyl starch can further exhibit a molar degree of substitution of from 0.1 to 3, from 0.1 to 2, from 0.1 to 0.9, or from 0.1 to 0.8, and a ratio between C2:C6 substitution in the range of from 2 to 20 with respect to the hydroxyethyl groups. HES with a mean molecular weight of about 130 kD is VOLUVEN® from Fresenius. VOLUVEN® is an artificial colloid, employed, e.g., for volume replacement used in the therapeutic indication for therapy and prophylaxis of hypovolaemia. There are a number of HES attachment methods available to those skilled in the art, e.g., the same PEG attachment methods described above.

III.C.9. PSA

[0281] In certain aspects, a chimeric polypeptide used in the methods of the present disclosure comprises at least one polysialic acid (PSA) polymer. PSAs are naturally occurring unbranched polymers of sialic acid produced by certain bacterial strains and in mammals in certain cells. See, e.g., Roth J. et al. (1993) in *Polysialic Acid: From Microbes to Man*, eds. Roth J., Rutishauser U., Troy F. A. (BirkhäuserVerlag, Basel, Switzerland), pp. 335–348. PSAs can be produced in various degrees of polymerization from n=about 80 or more sialic acid residues down to n=2 by limited acid hydrolysis or by digestion with neuraminidases, or by fractionation of the natural, bacterially derived forms of the polymer. There are a number of PSA attachment methods available to those skilled in the art, e.g., the same PEG attachment methods described above. In certain aspects, an activated PSA can also be attached to a cysteine amino acid residue within the clotting factor, e.g., on FVIII, or within the Fc region. See, e.g., U.S. Patent No. 5846951.

III.C.10. Clearance Receptors

[0282] In certain aspects, the half-life of a chimeric polypeptide used in the methods of the present disclosure can be extended where the clotting factor of the chimeric polypeptide comprises a FVIII polypeptide and at least one fragment of a FVIII clearance receptor or FVIII-binding fragment, variant, or derivative thereof. Insertion of soluble forms of clearance receptors, such as the low-density lipoprotein-related protein receptor LRP1, or fragments thereof, can block binding of FVIII to clearance receptors and thereby extend its half-life, e.g., in vivo half-life. LRP1 is a 600 kDa integral membrane protein that is implicated in the receptor-mediate clearance of a variety of proteins, including FVIII. See, e.g., Lenting et al., *Haemophilia* 16:6-16 (2010). Other suitable FVIII clearance receptors are, e.g., LDLR (low-density lipoprotein receptor), VLDLR (very low-density lipoprotein receptor), and megalin (LRP-2), or fragments thereof. See, e.g., Bovenschen et al., *Blood* 106:906-912 (2005); Bovenschen, *Blood* 116:5439-5440 (2010); Martinelli et al., *Blood* 116:5688-5697 (2010).

III.D. Insertion Sites

[0283] In some aspects, the insertion site in the FVIII polypeptide is located in one or more domains of the FVIII polypeptide, which is the N-terminus, the A1 domain, the A2 domain, the A3 domain, the B domain, the C1 domain, the C2 domain, the C-terminus, or two or more combinations thereof or between two domains of the FVIII polypeptide, which are the A1 domain and a1 acidic region, and the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B

domain, the B domain and A3 domain, and the A3 domain and C1 domain, the C1 domain and C2 domain, or any combinations thereof. For example, the insertion sites in which the XTEN sequence can be inserted are selected from the group consisting of the N-terminus and A1 domain, the N-terminus and A2 domain, the N-terminus and A3 domain, the N-terminus and B domain, the N-terminus and C1 domain, the N-terminus and C2 domain, the N-terminus and the C-terminus, the A1 and A2 domains, the A1 and A3 domains, the A1 and B domains, the A1 and C1 domains, the A1 and C2 domains, the A1 domain and the C-terminus, the A2 and A3 domains, the A2 and B domains, the A2 and C1 domains, the A2 and C2 domains, the A2 domain and the C-terminus, the A3 and B domains, the A3 and C1 domains, the A3 and C2 domains, the A3 domain and the C-terminus, the B and C1 domains, the B and C2 domains, the B domain and the C-terminus, the C1 and C2 domains, the C1 and the C-terminus, the C2 domain, and the C-terminus, and two or more combinations thereof.

[0284] The FVIII polypeptide, in which the XTEN sequence is inserted immediately downstream of one or more amino acids (e.g., one or more XTEN insertion sites) in the FVIII polypeptide or linked at the C-terminus or the N-terminus, retains the FVIII activity after linkage to or insertion by the XTEN sequence. The XTEN sequence can be inserted in the FVIII polypeptide once or more than once, twice, three times, four times, five times, or six times such that the insertions do not affect the FVIII activity (i.e., the FVIII protein still retains the coagulation property).

[0285] The FVIII polypeptide useful in the present disclosure can be linked to one or more XTEN polypeptides at the N-terminus or C-terminus of the FVIII polypeptide by an optional linker or inserted immediately downstream of one or more amino acids (e.g., one or more XTEN insertion sites) in the FVIII polypeptide by one or more optional linkers.

[0286] In yet other embodiments, one or more XTENs are inserted in the B domain of FVIII. In one example, an XTEN is inserted between amino acids 740 and 1640 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 740 and 1640 is optionally not present. In another example, an XTEN is inserted between amino acids 741 and 1690 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 740 and 1690 is optionally not present. In other examples, an XTEN is inserted between amino acids 741 and 1648 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 741 and 1648 is optionally not present. In yet other examples, an XTEN is inserted between amino acids 743 and 1638 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 743 and 1638 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1656 corresponding to SEQ

ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1656 is optionally not present. In some examples, an XTEN is inserted between amino acids 745 and 1657 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1657 is optionally not present. In certain examples, an XTEN is inserted between amino acids 745 and 1667 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1667 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1686 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1686 is optionally not present. In some other examples, an XTEN is inserted between amino acids 747 and 1642 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 747 and 1642 is optionally not present. In still other examples, an XTEN is inserted between amino acids 751 and 1667 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 751 and 1667 is optionally not present. In some embodiments, an XTEN sequence is inserted between amino acids 745 and 746 of a mature FVIII polypeptide or the corresponding insertion site of the B-domain deleted FVIII polypeptide. In a particular embodiment, an XTEN sequence comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence of AE288 (SEQ ID NO: 8) is inserted between amino acids 745 and 746 of a mature FVIII, wherein the mature FVIII has a deletion of amino acid residues 746-1648. In particular embodiments, the XTEN is inserted immediately downstream of amino acid 745 of the B domain deleted FVIII of Table 5 (SEQ ID NO: 68).

III.E. Linkers

[0287] In certain embodiments, the chimeric polypeptide of the present disclosure further comprises one or more linkers in the FVIII protein and/or in the VWF fragment. One type of the linkers is a cleavable linker, which can be cleaved by various proteases when administered to a subject in vivo, e.g., at a site of coagulation. In some embodiments, the cleavable linker allows cleavage of moiety, e.g., a VWF protein, from the XTEN sequence, thus from the chimeric polypeptide at the site of the coagulation cascade, thereby allowing activated FVIII (FVIIIa) to have its FVIIIa activity. Another type of the linkers is a processable linker, which contains an intracellular cleavage site and thus can be cleaved by an intracellular processing enzyme in a host cell, allowing convenient expression of a polypeptide and formation of a chimeric polypeptide.

[0288] One or more linkers can be present between any two proteins in the chimeric polypeptide. In some embodiments, a chimeric polypeptide comprises a first polypeptide which comprises (i) a FVIII polypeptide and (ii) a first Ig constant region or a portion thereof and a second polypeptide which comprises (iii) a VWF protein, (iv) a linker (e.g., a cleavable linker), (v) an XTEN sequence, and (vi) a second Ig constant region or a portion thereof. In other embodiments, a chimeric polypeptide comprises a first polypeptide which comprises (i) a FVIII polypeptide and (ii) a first Ig constant region or a portion thereof and a second polypeptide which comprises (iii) a VWF protein, (iv) an XTEN sequence, (v) a linker (e.g., a cleavable linker), and (vi) a second Ig constant region or a portion thereof. In other embodiments, a chimeric polypeptide comprises a first polypeptide which comprises (i) a FVIII polypeptide and (ii) a first Ig constant region or a portion thereof and a second polypeptide which comprises (iii) a VWF protein, (iv) a first linker (e.g., a cleavable linker), (v) an XTEN sequence, (vi) a second linker (e.g., a cleavable linker), and (vii) a second Ig constant region or a portion thereof. In some embodiments, the first polypeptide further comprises a linker, e.g., a cleavable linker between the FVIII polypeptide and the first Ig constant region.

[0289] In certain embodiments, a chimeric polypeptide comprises a single chain comprising (i) a FVIII polypeptide, (ii) a first Ig constant region or a portion thereof, (iii) a linker (e.g., a processable linker), (iv) a VWF protein, (v) an XTEN sequence, and (vi) a second Ig constant region or a portion thereof. In other embodiments, a chimeric polypeptide comprises a single chain comprising (i) a FVIII polypeptide, (ii) a first Ig constant region or a portion thereof, (iii) a first linker (e.g., a processable linker), (iv) a VWF protein, (v) a second linker (e.g., a cleavable linker), (vi) an XTEN sequence, and (vii) a second Ig constant region or a portion thereof. The processable linker can be processed after the chimeric polypeptide is expressed in the host cell; thus the chimeric polypeptide produced in the host cell can be in the final form comprising two or three polypeptide chains.

[0290] The linker useful in the present disclosure can comprise any organic molecule. Example of linkers that can be used in the chimeric polypeptides of the present disclosure are disclosed, for example, in US 2011/0183907 A1, US 2016/0229903 A1, and US 2016/0251408 A1, each of which is incorporated by reference herein in its entirety. In some embodiments, the linker comprises a polymer, e.g., polyethylene glycol (PEG) or hydroxyethyl starch (HES). In other embodiments, the linker comprises an amino acids sequence. The linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. The linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-

300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, or 900-1000 amino acids. In some embodiments, the linker comprises an XTEN sequence. Additional examples of XTEN can be used according to the present disclosure and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, or WO 2011028344 A2. In other embodiments, the linker is a PAS sequence.

[0291] In certain embodiments, the linker is a polymer, e.g., polyethylene glycol (PEG) or hydroxyethyl starch (HES). In other embodiments, the linker is an amino acid sequence. The linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. The linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, or 900-1000 amino acids.

[0292] Examples of linkers are well known in the art. In certain embodiments, the linker comprises the sequence G_n . The linker can comprise the sequence $(GA)_n$. The linker can comprise the sequence $(GGS)_n$. In other embodiments, the linker comprises $(GGGS)_n$ (SEQ ID NO: 101). In still other embodiments, the linker comprises the sequence $(GGS)_n(GGGGS)_n$ (SEQ ID NO: 95). In these instances, n can be an integer from 1-100. In other instances, n can be an integer from 1-20, i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. Examples of linkers include, but are not limited to, GGG, SGGSGGS (SEQ ID NO: 96), GGSGGSGGSGGSGGG (SEQ ID NO: 97), GGSGGSGGGSGGGGS (SEQ ID NO: 98), GGSGGSGGSGGSGGSGGGS (SEQ ID NO: 99), or GGGGSGGGGSGGGGS (SEQ ID NO: 100). The linker does not eliminate or diminish the VWF protein activity or the clotting activity of Factor VIII. Optionally, the linker enhances the VWF protein activity or the clotting activity of Factor VIII protein, e.g., by further diminishing the effects of steric hindrance and making the VWF protein or Factor VIII portion more accessible to its target binding site.

[0293] In certain embodiments, the linker useful for the chimeric polypeptide is 15-25 amino acids long. In other embodiments, the linker useful for the chimeric polypeptide is 15-20 amino acids long. In some embodiments, the linker for the chimeric polypeptide is 10-25 amino acids long. In other

embodiments, the linker for the chimeric polypeptide is 15 amino acids long. In still other embodiments, the linker for the chimeric polypeptide is (GGGGS)_n (SEQ ID NO: 94) where G represents glycine, S represents serine and n is an integer from 1-20.

III.F. Cleavage Sites

[0294] A cleavable linker can incorporate a moiety capable of being cleaved either chemically (e.g., hydrolysis of an ester bond), enzymatically (i.e., incorporation of a protease cleavage sequence), or photolytically (e.g., a chromophore such as 3-amino-3-(2-nitrophenyl) propionic acid (ANP)) in order to release one molecule from another. Examples of cleavable linkers suitable for the chimeric polypeptide of the present disclosure can be found, for example, in US 2016/0229903 A1 and US 2016/0251408 A1, each of which is incorporated by reference herein in its entirety.

[0295] In certain embodiments, a cleavable linker comprises one or more cleavage sites at the N-terminus or C-terminus or both. In other embodiments, the cleavable linker consists essentially of or consists of one or more cleavable sites. In other embodiments, the cleavable linker comprises heterologous amino acid linker sequences described herein or polymers and one or more cleavable sites.

[0296] In certain embodiments, a cleavable linker comprises one or more cleavage sites that can be cleaved in a host cell (i.e., intracellular processing sites). Non limiting examples of the cleavage site include RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), and RRRRS (SEQ ID NO: 104).

[0297] In some embodiments, a cleavable linker comprises an a1 region from FVIII, an a2 region from FVIII, an a3 region from FVIII, a thrombin cleavable site which comprises X-V-P-R (SEQ ID NO: 105) and a PAR1 exosite interaction motif, wherein X is an aliphatic amino acid, or any combinations thereof. comprises the a2 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Glu720 to Arg740 corresponding to full-length FVIII, wherein the a2 region is capable of being cleaved by thrombin. In particular embodiments, a cleavable linker useful for the disclosure comprises an a2 region which comprises ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106) or DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88). In other embodiments, a cleavable linker for the disclosure comprises the a1 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Met337 to Arg372 corresponding to full-length FVIII, wherein the a1 region is capable of being cleaved by thrombin. In particular embodiments, the a1 region comprises ISMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSV

(SEQ ID NO: 107). In some embodiments, a cleavable linker of the disclosure comprises the a3 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Glu1649 to Arg1689 corresponding to full-length FVIII, wherein the a3 region is capable of being cleaved by thrombin. In certain embodiments, a cleavable linker for the disclosure
 5 comprises an a3 region comprising ISEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDEN QSPRSFQ (SEQ ID NO: 108).

[0298] In other embodiments, a cleavable linker comprises the thrombin cleavage site which comprises X-V-P-R (SEQ ID NO: 105) and the PAR1 exosite interaction motif and wherein the PAR1 exosite interaction motif comprises S-F-L-L-R-N (SEQ ID NO: 109). The PAR1 exosite interaction
 10 motif can further comprise an amino acid sequence selected from P, P-N, P-N-D, P-N-D-K (SEQ ID NO: 110), P-N-D-K-Y (SEQ ID NO: 111), P-N-D-K-Y-E (SEQ ID NO: 112), P-N-D-K-Y-E-P (SEQ ID NO: 113), P-N-D-K-Y-E-P-F (SEQ ID NO: 114), P-N-D-K-Y-E-P-F-W (SEQ ID NO: 115), P-N-D-K-Y-E-P-F-W-E (SEQ ID NO: 116), P-N-D-K-Y-E-P-F-W-E-D (SEQ ID NO: 117), P-N-D-K-Y-E-P-F-W-E-D-E (SEQ ID NO: 118), P-N-D-K-Y-E-P-F-W-E-D-E-E (SEQ ID NO: 119), P-N-D-K-Y-E-P-F-W-E-D-E-E-S (SEQ ID NO: 120), or any combination thereof. In some embodiments, the aliphatic amino
 15 acid is selected from Glycine, Alanine, Valine, Leucine, or Isoleucine.

[0299] In other embodiments, a cleavable linker comprises one or more cleavage sites that are cleaved by a protease after a chimeric polypeptide comprising the cleavable linker is administered to a subject. In some embodiments, the cleavage site is cleaved by a protease selected from the group
 20 consisting of factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, MMP-12, MMP-13, MMP-17, and MMP-20. In other embodiments, the cleavage site is selected from the group consisting of a FXIa cleavage site (e.g., KLTR↓AET (SEQ ID NO: 121)), a FXIa cleavage site (e.g., DFTR↓VVG (SEQ ID NO: 122)), a FXIIa cleavage site (e.g., TMTR↓IVGG (SEQ ID NO: 123)), a Kallikrein cleavage site (e.g., SPFR↓STGG (SEQ ID NO: 124)), a FVIIa
 25 cleavage site (e.g., LQVR↓IVGG (SEQ ID NO: 125)), a FIXa cleavage site (e.g., PLGR↓IVGG (SEQ ID NO: 126)), a FXa cleavage site (e.g., IEGR↓TVGG (SEQ ID NO: 127)), a FIIa (thrombin) cleavage site (e.g., LTPR↓SLLV (SEQ ID NO: 128)), a Elastase-2 cleavage site (e.g., LGPV↓SGVP (SEQ ID NO: 129)), a Granzyme-B cleavage (e.g., VAGD↓SLEE (SEQ ID NO: 130)), a MMP-12 cleavage site (e.g., GPAG↓LGGA (SEQ ID NO: 131)), a MMP-13 cleavage site (e.g., GPAG↓LRGA (SEQ ID NO: 132)), a MMP-17 cleavage site (e.g., APLG↓LRLR (SEQ ID NO: 133)), a MMP-20 cleavage site (e.g., PALP↓LVAQ (SEQ ID NO: 134)), a TEV cleavage site (e.g., ENLYFQ↓G (SEQ ID NO: 135)), a
 30 Enterokinase cleavage site (e.g., DDDK↓IVGG (SEQ ID NO: 136)), a Protease 3C

(PRESCISSION™) cleavage site (e.g., LEVLFQ↓GP (SEQ ID NO: 137)), and a Sortase A cleavage site (e.g., LPKT↓GSES) (SEQ ID NO: 138). In certain embodiments, the FXIa cleavage sites include, but are not limited to, e.g., TQSFNDFTR (SEQ ID NO: 1) and SVSQTSKLTR (SEQ ID NO: 3). Non-limiting exemplary thrombin cleavage sites include, e.g., DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), or IEPRSFS (SEQ ID NO: 194), and a sequence comprising, consisting essentially of, or consisting of ALRPR (SEQ ID NO: 7) (e.g., ALRPRVVGGA (SEQ ID NO: 145)).

[0300] In some embodiments, the cleavage site is TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146). In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88) or a fragment thereof. In particular embodiments, the cleavage site comprises IEPRSFS (SEQ ID NO: 194). In other embodiments, the cleavage site comprises EPRSFS (SEQ ID NO: 195), wherein the cleavage site is not the full-length a2 region of FVIII. In still other embodiments, the cleavage site comprises IEPR (SEQ ID NO: 200). In yet other embodiments, the cleavage site comprises IEPR (SEQ ID NO: 200), wherein the cleavage site is not the full-length a2 region of FVIII or does not comprise the full-length a2 region of FVIII. In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), KNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 139), NTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 140), TGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 141), GDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 142), DYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 143), YYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 144), YEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 176), EDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 177), DSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 178), SYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 179), YEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 180), EDISAYLLSKNNAIEPRSFS (SEQ ID NO: 181), DISAYLLSKNNAIEPRSFS (SEQ ID NO: 182), ISAYLLSKNNAIEPRSFS (SEQ ID NO: 183), SAYLLSKNNAIEPRSFS (SEQ ID NO: 184), AYLLSKNNAIEPRSFS (SEQ ID NO: 185), YLLSKNNAIEPRSFS (SEQ ID NO: 186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNAIEPRSFS (SEQ ID NO: 190), NNAIEPRSFS (SEQ ID NO: 191), NAIIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or IEPRSFS (SEQ ID NO: 194). In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), KNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 139),

NTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 140),
 TGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 141),
 GDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 142), DYYEDSYEDISAYLLSKNNAIEPRSFS
 (SEQ ID NO: 143), YYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 144),
 5 YEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 176), EDSYEDISAYLLSKNNAIEPRSFS (SEQ ID
 NO: 177), DSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 178), SYEDISAYLLSKNNAIEPRSFS (SEQ
 ID NO: 179), YEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 180), EDISAYLLSKNNAIEPRSFS (SEQ ID
 NO: 181), DISAYLLSKNNAIEPRSFS (SEQ ID NO: 182), ISAYLLSKNNAIEPRSFS (SEQ ID NO:
 183), SAYLLSKNNAIEPRSFS (SEQ ID NO: 184), AYLLSKNNAIEPRSFS (SEQ ID NO: 185),
 10 YLLSKNNAIEPRSFS (SEQ ID NO: 186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS
 (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNNAIEPRSFS (SEQ ID NO: 190),
 NNAIEPRSFS (SEQ ID NO: 191), NAIIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or
 IEPRSFS (SEQ ID NO: 194), wherein the cleavage site is not the full-length FVIII a2 region. In certain
 embodiments the cleavable linker is cleavable in a thrombin cleavage assay as provided herein or as
 15 known in the art.

[0301] In particular embodiments, the chimeric polypeptide of the present disclosure comprises: (i)
 a FVIII protein comprising a FVIII polypeptide, a first XTEN sequence, and a first Fc region; and (ii) a
 VWF fragment comprising a D' domain of VWF and a D3 domain of VWF, a second XTEN sequence,
 an a2 linker of FVIII, and a second Fc region; wherein:

- 20 (a) the FVIII protein has a deletion of amino acids 746 to 1648 corresponding to mature FVIII;
- (b) the first XTEN sequence is inserted within the FVIII polypeptide immediately downstream of
 amino acid 745 corresponding to mature FVIII;
- (c) the first XTEN sequence comprises an amino acid sequence having at least about 70%, at
 least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at
 25 least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence
 identity to the amino acid sequence of AE288 (SEQ ID NO: 8);
- (d) the first Fc region is fused to the C-terminus of the FVIII polypeptide;
- (e) the second XTEN sequence is fused to the C-terminus of the VWF fragment;
- (f) the second XTEN sequence comprises an amino acid sequence having at least about 70%,
 30 at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%,

at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence of AE144_5A (SEQ ID NO: 58);

(g) the a2 linker is fused to the C-terminus of the XTEN;

(h) the a2 linker comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence of SEQ ID NO: 88;

(i) the second Fc region is fused to the C-terminus of the a2 linker; and

(j) the first Fc region is covalently linked to the second Fc region by a disulfide bond.

10 **[0302]** In particular embodiments, a chimeric polypeptide of the disclosure comprises two polypeptide sequences, a first polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from FVIII-161 (SEQ ID NO: 69), FVIII-169 (SEQ ID NO: 70), FVIII-170 (SEQ ID NO: 71), FVIII-173 (SEQ ID NO: 72); FVIII-195 (SEQ ID NO: 73); FVIII-196 (SEQ ID NO: 74), FVIII-199 (SEQ ID NO: 75), FVIII-201 (SEQ ID NO: 76); FVIII-203 (SEQ ID NO: 77), FVIII-204 (SEQ ID NO: 78), FVIII-205 (SEQ ID NO: 79), FVIII-266 (SEQ ID NO: 80), FVIII-267 (SEQ ID NO: 81), FVIII-268 (SEQ ID NO: 82), FVIII-269 (SEQ ID NO: 83), FVIII-271 (SEQ ID NO: 84) or FVIII-272 (SEQ ID NO: 85) and a second polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from VWF031 (SEQ ID NO: 86), VWF034 (SEQ ID NO: 87), or VWF-036.

20 **[0303]** In some embodiments, the chimeric polypeptide of the disclosure comprises two polypeptide sequences, a first polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 201; and a second polypeptide sequence comprising a VWF fragment comprising a D' domain of VWF and a D3 domain of VWF and an Fc region. In some embodiments, the chimeric polypeptide of the disclosure
25 comprises two polypeptide sequences, a first polypeptide sequence comprising FVIII polypeptide and an Fc region; and a second polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 202. In certain
30 embodiments, the chimeric polypeptide of the disclosure comprises two polypeptide sequences, a first polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 201 and a second polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to

the amino acid sequence set forth in SEQ ID NO: 202. In particular embodiments, the chimeric polypeptide of the disclosure comprises two polypeptide sequences, a first polypeptide sequence comprising the amino acid sequence set forth in SEQ ID NO: 201 and a second polypeptide sequence comprising the amino acid sequence set forth in SEQ ID NO: 202. In other particular embodiments, the chimeric polypeptide of the disclosure comprises two polypeptide sequences, a first polypeptide sequence comprising the amino acid sequence set forth in SEQ ID NO: 201 and a second polypeptide sequence comprising the amino acid sequence set forth in SEQ ID NO: 202, wherein the first polypeptide sequence and the second polypeptide sequence are linked to each other by a disulphide bond.

IV. Pharmaceutical Compositions

[0304] Compositions containing the chimeric polypeptide of the present disclosure can contain a suitable pharmaceutically acceptable carrier. For example, they can contain excipients and/or auxiliaries that facilitate processing of the active compounds into preparations designed for delivery to the site of action.

[0305] The pharmaceutical composition can be formulated for parenteral administration (i.e. intravenous, subcutaneous, or intramuscular) by bolus injection. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multidose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., pyrogen free water.

[0306] Suitable formulations for parenteral administration also include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions can be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions can contain substances, which increase the viscosity of the suspension, including, for example, sodium carboxymethyl cellulose, sorbitol and dextran. Optionally, the suspension can also contain stabilizers. Liposomes also can be used to encapsulate the molecules of the disclosure for delivery into cells or interstitial spaces. Exemplary pharmaceutically acceptable carriers are physiologically compatible solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying

agents, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like. In some embodiments, the composition comprises isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride. In other embodiments, the compositions comprise pharmaceutically acceptable substances such as wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the active ingredients.

[0307] Compositions of the disclosure can be in a variety of forms, including, for example, liquid (e.g., injectable and infusible solutions), dispersions, suspensions, semi-solid and solid dosage forms. The preferred form depends on the mode of administration and therapeutic application.

[0308] The composition can be formulated as a solution, micro emulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0309] The active ingredient can be formulated with a controlled-release formulation or device. Examples of such formulations and devices include implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations and devices are known in the art. See e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0310] Injectable depot formulations can be made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to

polymer, and the nature of the polymer employed, the rate of drug release can be controlled. Other exemplary biodegradable polymers are polyorthoesters and polyanhydrides. Depot injectable formulations also can be prepared by entrapping the drug in liposomes or microemulsions.

[0311] Supplementary active compounds can be incorporated into the compositions. In some embodiments, the chimeric polypeptide of the disclosure is formulated with another clotting factor, or a variant, fragment, analogue, or derivative thereof. For example, the clotting factor includes, but is not limited to, factor V, factor VII, factor VIII, factor IX, factor X, factor XI, factor XII, factor XIII, prothrombin, fibrinogen, von Willebrand factor or recombinant soluble tissue factor (rsTF) or activated forms of any of the preceding. The clotting factor of hemostatic agent can also include anti-fibrinolytic drugs, e.g., epsilon-amino-caproic acid, tranexamic acid.

[0312] Dosage regimens can be adjusted to provide the optimum desired response. For example, a single bolus can be administered, several divided doses can be administered over time, or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. See, e.g., Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, Pa. 1980).

[0313] In addition to the active compound, the liquid dosage form can contain inert ingredients such as water, ethyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan.

[0314] Non-limiting examples of suitable pharmaceutical carriers are also described in Remington's Pharmaceutical Sciences by E. W. Martin. Some examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition can also contain pH buffering reagents, and wetting or emulsifying agents.

[0315] In some embodiments, a pharmaceutical composition comprises a chimeric polypeptide and a pharmaceutically acceptable carrier. The FVIII protein in a chimeric polypeptide has extended half-life compared to wild type FVIII protein or the corresponding FVIII protein without the VWF fragment. In some embodiments, wherein the half-life of the chimeric polypeptide is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least

about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than wild type FVIII. In other embodiments, the half-life of Factor VIII is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In some embodiments, the half-life of the chimeric polypeptide is extended about 3 times compared to wild type FVIII.

[0316] In some embodiments, the composition is administered by parenteral administration, which can be intravenous or subcutaneous administration.

[0317] In other embodiments, the composition is used to treat a bleeding disease or condition in a subject in need thereof. The bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, 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, bleeding in the iliopsoas sheath and any combinations thereof. In still other embodiments, the subject is scheduled to undergo a surgery. In yet other embodiments, the treatment is prophylactic or on-demand.

[0318] Having now described the present disclosure 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 disclosure. All patents, publications, and articles referred to herein are expressly and specifically incorporated herein by reference.

EXAMPLES

Example 1: First in human study of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0319] Factor-based replacement therapy is the cornerstone of hemophilia care. Conventional recombinant FVIII (rFVIII) products have a short half-life, requiring frequent administration. Significant

advances to patient care have been brought by rFVIII Fc (Eloctate®), including the longest dosing interval for hemophilia A; adequate bleed control, including prophylactically, in acute, perioperative, and emergency situations; protection beyond bleed control, including joint protection and improved quality of life; and long-term consistent and well-characterized safety profile. However, weekly or longer factor VIII prophylactic dosing still remains an unmet need for the majority of people with hemophilia A.

[0320] The present study investigates the safety and pharmacokinetics (PK) of rFVIII Fc-VWF-XTEN, a chimeric polypeptide comprising a (i) a FVIII polypeptide, an XTEN inserted within the B domain of the FVIII polypeptide, and a first Fc region; and (ii) a VWF fragment, a second XTEN sequence, an a2 linker, and a second Fc region (FIG. 1).

[0321] Study Design

[0322] The object of the present study was to assess the safety, tolerability, and FVIII activity (PK) of a single dose of rFVIII Fc-VWF-XTEN compared with rFVIII. The patient population included previously treated adult males with severe hemophilia A (<1 IU/dL [$<1\%$] endogenous FVIII activity level) with at least 150 exposure days to FVIII products, and with no history of positive inhibitor test or clinical signs of decreased response to FVIII administration. The primary endpoints were adverse events and clinical abnormalities in laboratory tests, including development of inhibitors. Key secondary endpoints included pharmacokinetics parameters, including but not limited to elimination half-life ($t_{1/2}$), clearance (CL), area under the curve from 0 to infinity ($AUC_{0-\infty}$), mean residence time (MRT), and incremental recovery (IR).

[0323] Patients were enrolled into a low-dose cohort (7 patients; FIG. 2A) or a high-dose cohort (9 patients; FIG. 2B). Each patient was dosed with rFVIII (ADVATE®) followed by a PK sampling period. Following a brief washout period (≥ 3 days for the low dose cohort (FIG. 2A) and ≥ 4 days for the high-dose cohort (FIG. 2B)), each subject was then administered rFVIII Fc-VWF-XTEN followed by a PK sampling period. Subjects underwent safety observation for 28 days following the injection of rFVIII Fc-VWF-XTEN, including sample collection for inhibitor assessments at 14 and 28 days after the injection of rFVIII Fc-VWF-XTEN. Patients in the low-dose cohort received 25 IU/kg of rFVIII and 25 IU/kg of rFVIII Fc-VWF-XTEN. Patients in the high-dose cohort received 65 IU/kg of rFVIII and 65 IU/kg of rFVIII Fc-VWF-XTEN.

[0324] Methods

[0325] Pharmacokinetic parameters were measured by a modified activated partial thromboplastin time (aPTT) clotting assay ("one stage" assay). Briefly, FVIII activity is measured in a modified aPTT assay using the Dade® Actin® FSL Activated PTT Reagent (Siemens Health Care Diagnostics) on a BCS® XP analyzer (Siemens Healthcare Diagnostics). 7-point calibration curves (High: 1.500-0.050 IU/mL; Low: 0.150-0.0025 IU/mL) were prepared using a rFVIII-Fc-VWF-XTEN specific calibrator. For the high calibration curve, the rFVIII-Fc-VWF-XTEN calibrator [1.000 IU/mL] is diluted into Tris/BSA buffer (50 mM, 150 mM sodium chloride, pH 7.4 +/- 0.2, containing 1% BSA). This dilution is carried out by the BCS® XP analyzer to generate pre-selected calibration FVIII activity levels. These are then tested at the 1:2 working dilution prepared in Tris/BSA buffer. For the low calibration curve, the rFVIII-Fc-VWF-XTEN calibrator [1.000 IU/mL] is diluted in immuno-depleted FVIII deficient plasma (Siemens Healthcare Diagnostics) by the BCS® XP analyzer, and tested neat (in the absence of a working dilution). Calibration is performed in duplicate. Curves are plotted by FVIII activity in IU/mL (x-axis) and clotting time in seconds (y-axis). Both high and low calibration curves use a linear log-lin regression curve fit.

[0326] Samples are tested in duplicate using the high calibration curve metatest (1:2, 1:4, and 1:8 working dilutions in Tris/BSA) or the low calibration curve metatest (neat, 1:2, and 1:4 working dilutions in immuno-depleted FVIII deficient plasma). After the appropriate working dilution, all calibrators, patient samples and quality control samples are mixed 1:1 with immuno-depleted FVIII deficient plasma, containing less than or equal to 0.010 IU/mL FVIII activity and at least 0.400 IU/mL of the other clotting factors. After the samples are mixed with this plasma, the APTT reagent is added and the mixture is incubated. Following incubation, calcium chloride is added and the time to clot formation is measured optically. An algorithm then examines whether the individual working dilution results fall within +/- 15.0% of the mean to determine reported FVIII activity. Minimum of two metatest working dilution results are necessary to determine the reported FVIII activity, except near the Lower Limit of Quantification (LLOQ).

[0327] Results: Low Dose Cohort

[0328] Seven subjects enrolled in the low dose cohort; one subject withdrew for reasons unrelated to the study prior to receiving rFVIII-Fc-VWF-XTEN. Six subjects received a single, low dose of rFVIII-Fc-VWF-XTEN (25 IU/kg). Characteristics of the first four subjects treated in the low dose cohort included: previously treated adult male subjects with severe hemophilia A (<1 IU/dL [$<1\%$] endogenous FVIII activity level); all subjects were Caucasian; the age of the subjects ranged from

19-60 years, with an average age of 33; and the average weight was 85 kg (ranging from 67.2-100.6 kg).

[0329] In all patients, rFVIII^hFc-VWF-XTEN was generally well tolerated, and no inhibitors were observed at Day 14 or Day 28 following dosing.

- 5 **[0330]** The average FVIII activity post-infusion for the first six subjects receiving a single low dose of rFVIII^hFc-VWF-XTEN (25 IU/kg) at 5 days was about 12.0% and at 7 days was about 5.3% (FIG. 3A). Average FVIII activity remained above 1% through 10 days (FIG. 3A). Pharmacokinetic parameters, measured by activated partial thromboplastin time (aPTT) measurement, for rFVIII^hFc-VWF-XTEN (25 IU/kg) and rFVIII (25 IU/kg) are shown in **Table 9**. rFVIII^hFc-VWF-XTEN was observed
- 10 to have an average half-life of about 37.6 hours in the human subjects, which was over 4-fold higher than the average half-life for rFVIII (about 9.12 hours) administered at the same dose.

Table 9: Pharmacokinetic (PK) Parameters for the Low-Dose Cohort

PK Parameter	rFVIII-Fc-VWF-XTEN Geometric Mean [Range] (N=6)	rFVIII Geometric Mean [Range] (N=7)	Mean Ratio [95% CI]
Half-life, h	37.61 [33.28–42.50]	9.12 [6.24–13.33]	4.13 [2.94, 5.79] P < 0.001
C _{max} (IU/dL)	70.1 [49.7–98.9]	51.80 [43.3–61.9]	1.35 [1.04, 1.77] P = 0.032
AUC _∞ , h x IU/dL	4470 [3280–6080]	638 [495–822]	7.00 [5.78, 8.48] P < 0.001
MRT, h	56.93 [49.19–65.89]	12.54 [9.82–16.10]	4.54 [3.64, 5.66] P < 0.001
CL, mL/h/kg	0.56 [0.41–0.76]	3.91 [3.05–5.02]	0.14 [0.12, 0.17] P < 0.001
IR, IU/dL per IU/kg	2.72 [1.95–3.80]	2.00 [1.60–2.50]	1.36 [0.98, 1.89] P = 0.063

[0331] Results: High Dose Cohort

[0332] Nine subjects were enrolled in the high dose cohort. The subjects were administered a single, high dose of rFVIII-Fc-VWF-XTEN (65 IU/kg). One subject could not be tested for PK parameters due to reasons unrelated to the study. Characteristics of the subjects treated in the high dose cohort included: previously treated adult male subjects with severe hemophilia A (<1 IU/dL [$<1\%$] endogenous FVIII activity level); 7 of 9 subjects were Caucasian; the age of the subjects ranged from 32-63 years, with an average age of 44; and the average weight was 81.1 kg (ranging from 61.5-100.7 kg).

[0333] The average FVIII activity post-infusion for the eight evaluated subjects receiving a single high dose of rFVIII-Fc-VWF-XTEN (65 IU/kg) at 5 days was about 38.0% and at 7 days was about 17% (FIG. 3B). Average FVIII activity remained above 1% through 14 days (FIG. 3B). Pharmacokinetic parameters, measured activated partial thromboplastin time (aPTT) measurement, for rFVIII-Fc-VWF-XTEN (65 IU/kg) and rFVIII (65 IU/kg) are shown in **Table 10**. rFVIII-Fc-VWF-XTEN was observed to have an average half-life of about 42.5 hours in the human subjects, which was over 3-fold higher than the average half-life for rFVIII (about 13 hours) administered at the same dose.

Table 10: Pharmacokinetic (PK) Parameters for the High-Dose Cohort

PK parameter	rFVIII-Fc-VWF-XTEN Geometric Mean [Range] (N=8)	rFVIII Geometric Mean [Range] (N=9)	Mean Ratio [95% CI]
Half-life (h)	42.54 [39.72-45.56]	13.15 [10.89-15.87]	3.24 [2.76, 3.79] P < 0.001
C _{max} (IU/dL)	161 [142-183]	138 [117-162]	1.17 [1.09-1.25] P < 0.001
AUC _{0-inf} (h x IU/dL)	12,800 [11,100-14,900]	1960 [1670-2310]	6.54 [5.89-7.27] P < 0.001
MRT (h)	67.66 [62.59-73.14]	15.66 [14.06-17.45]	4.32 [3.96-4.72] P < 0.001
CL (mL/h/kg)	0.51 [0.44-0.59]	3.31 [2.81-3.88]	0.15 [0.14-0.17] P < 0.001
IR (IU/dL per IU/kg)	2.48 [2.18-2.82]	2.11 [1.79-2.49]	1.18 [1.10-1.26] P < 0.001

[0334] Conclusions:

[0335] rFVIII-Fc-VWF-XTEN was well tolerated in all patients with severe hemophilia A who were treated with either a single low dose (25 IU/kg) or a single high dose (65 IU/kg). No patient developed an inhibitor to FVIII. rFVIII-Fc-VWF-XTEN demonstrated a half-life that was 3-fold to 4-fold higher than rFVIII, suggesting a breakthrough in the VWF-imposed half-life ceiling. Sustained, high FVIII activity has the potential to provide extended protection against all bleed types.

Example 2: Repeat Dose Study of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0336] rFVIII-Fc-VWF-XTEN was well-tolerated and provided sustained FVIII levels in a cohort of subjects who received a single dose of rFVIII-Fc-VWF-XTEN. (Example 1). The present study will evaluate the safety and tolerability and characterize the pharmacokinetics of repeat doses of rFVIII-Fc-VWF-XTEN (FIG. 1).

[0337] Study Design

[0338] A total of 4 once-weekly doses of rFVIII-Fc-VWF-XTEN will be administered to adult male patients with severe hemophilia A who have been previously treated with FVIII treatment and received at least 150 exposure days of such FVIII treatment. Two cohorts, each consisting of approximately

ten adult males 18 to 65 years of age will be enrolled. Each subject in Cohort 1 will receive a total of four once-weekly doses of 50 IU/kg rFVIII Fc-VWF-XTEN on Days 1, 8, 15, and 22. Each subject in Cohort 2 will receive a total of four once-weekly doses of 65 IU/kg rFVIII Fc-VWF-XTEN on Days 1, 8, 15, and 22.

5 **[0339]** A schematic representation of the design of the clinical study is depicted as FIG. 4. A pre-dose pharmacokinetic sample will be taken on Day 1. In addition, there will be multiple PK samples taken after dosing on Days 1 and 22, and a trough (168h) sample occurring prior to dosing on Days 8, 15, and 22. Subjects will undergo a safety observation period for 28 days after the last dose of rFVIII Fc-VWF-XTEN, including inhibitor assessments at 14 and 28 days after the last dose of
10 rFVIII Fc-VWF-XTEN. For subjects who complete all four once-weekly doses of rFVIII Fc-VWF-XTEN, the safety observation period will end with the end-of-study (EOS) visit at Day 50 (+3 days). For subjects who discontinue treatment early, the safety observation period will end with the early termination (ET) visit 28 days after the last dose of rFVIII Fc-VWF-XTEN.

[0340] The primary objective of the study is to assess the safety and tolerability of a total of four
15 once-weekly doses of intravenous (IV) rFVIII Fc-VWF-XTEN. The primary endpoints are the occurrence of adverse events (AEs) and the occurrence of clinically significant abnormalities in laboratory tests, including development of inhibitors (neutralizing antibodies directed against FVIII) as determined via the Nijmegen-modified Bethesda assay. (Verbruggen et al, Thrombosis and Haemostasis, 73: 247–251 (1995)).

20 **[0341]** The secondary objective of the study is the characterization of the pharmacokinetics of rFVIII Fc-VWF-XTEN after a total of four once-weekly IV doses, with FVIII activity determined by the one-stage activated partial thromboplastin time (aPTT) clotting assay, as described in Example 1. The secondary endpoints are pharmacokinetics assessed via the estimation of parameters, including but not limited to maximum activity (C_{max}), half-life (t_{1/2}), total clearance at steady state (CL_{ss}),
25 accumulating index (AI), area under the activity-time curve from hour 0 over the dosing interval (AUC_{0-tau}), volume of distribution at steady state (V_{ss}), mean residence time (MRT), IR, lowest (trough) concentration that a drug reaches before the next dose is administered (C_{trough}), and time to 1% above baseline for FVIII activity.

[0342] The exploratory objective of the study is to characterize the pharmacokinetics of rFVIII Fc-VWF-XTEN after a total of four once-weekly IV doses, with FVIII activity determined by the two-stage
30 chromogenic coagulation assay. The exploratory endpoints are PK assessed via the estimation of

parameters, including but not limited to the C_{max}, t_{1/2}, CL_{ss}, A_I, AUC_{0-tau}, V_{ss}, MRT, IR, C_{trough}, and time to 1% above baseline for FVIII activity.

[0343] Methods

[0344] FVIII activity was determined by the modified activated partial thromboplastin time (aPTT) clotting assay ("one stage" assay), as detailed above in Example 1.

[0345] The two-stage chromogenic coagulation assay measures FVIII activity using the BIOPHEN FVIII:C assay (Hyphen Biomed 221402-RUO) on the BCS® XP analyzer (Siemens Healthcare Diagnostics). Briefly, phospholipids, calcium and thrombin activated FVIII:C form an enzymatic complex in the presence of a constant amount of Factor IXa. This enzymatic complex in turn activates Factor X (FX), which is supplied at a constant concentration and in excess to Factor Xa (FXa). The activation of FX to FXa is directly related to the amount of FVIII:C, which is the limiting factor in the assay in the presence of a constant and in an excess amount of Factor IXa. The activity of the generated FXa is then measured using a specific chromogenic FXa substrate, Sxa-11 (Hyphen Biomed). Factor Xa cleaves Sxa-11 and releases paranitroaniline (pNA). The amount of pNA released is directly proportional to the amount of FXa generated. There is a direct relationship between the amount of FVIII:C in the sample, the amount of FXa generated, and the amount of pNA released. The release of pNA is determined by color development at a wavelength of 405 nm.

[0346] 8-point calibration curves (High: 1.500 – 0.150 IU/mL; Low: 0.200 – 0.008 IU/mL) are prepared using a rFVIII₁Fc-VWF-XTEN specific calibrator. For the high calibration curve, rFVIII₁Fc-VWF-XTEN at 1IU/mL is diluted in Tris/BSA buffer to generate pre-selected calibration levels of FVIII activity. This dilution is carried out by the BCS® XP analyzer. The pre-selected calibration levels of FVIII activity are tested at a 1:4 working dilution in Tris/BSA buffer. For the low calibration curve, rFVIII₁Fc-VWF-XTEN at 1 IU/mL is pre-diluted 1:4 in congenital FVIII deficient plasma (Helena Laboratories) and then further diluted by the BCS® XP analyzer in Tris/BSA buffer to generate pre-selected calibration levels of FVIII activity which are tested in the absence of further dilution in the reaction setup.

[0347] Calibration is performed in duplicate. Curves are plotted for FVIII activity in IU/mL (x-axis) and change in absorbance/min (y-axis). For the high calibration curve, a linear log-log regression curve fit is used. For the low calibration curve, a linear lin-lin regression curve fit is used. Samples are tested in duplicate on either the high calibration curve at a 1:4 working dilution or the low

calibration curve using the 1:4 dilution in congenital FVIII deficient plasma or neat (in the absence of a working dilution).

[0348] Results

[0349] Demographics and Baseline Characteristics

5 **[0350]** 10 adult male subjects 18 to 65 years of age (inclusive) enrolled in Cohort 1 (50 IU/kg) and 5 adult male subjects 18 to 65 years of age (inclusive) enrolled in Cohort 2 (65 IU/kg). All 10 subjects in the 50 IU/kg dosing cohort received 4 doses of rFVIII-Fc-VWF-XTEN. Three of the 5 subjects enrolled in the 65 IU/kg dosing cohort received at least one dose of rFVIII-Fc-VWF-XTEN. All 15 subjects enrolled were from a single site in Sofia, Bulgaria. Of the 15 subjects enrolled, 13 subjects
10 received at least one dose of BIVV001 and were included in the summary of demographic and baseline characteristics. All subjects were male. The median age was 35 years (range: 25 to 55 years) and all subjects were white. The median weight was 86.7 kg (range: 51.0 to 130.9 kg) and the median body mass index was 25.9 kg/m² (range: 16.4 to 40.9 kg/m²).

[0351] Safety Analysis

15 **[0352]** For the interim analysis a total of 13 subjects were included in the Safety Analysis Set (n=10 from the 50 IU/kg cohort and n=3 from the 65 IU/kg cohort). All safety analyses were performed based on the Safety Analysis Set and included all subjects who received at least one dose of rFVIII-Fc-VWF-XTEN.

[0353] A treatment-emergent AE (TEAE) is defined as any adverse event that began on or after
20 the first rFVIII-Fc-VWF-XTEN administration and within 28 days after the final rFVIII-Fc-VWF-XTEN administration. AEs with missing start dates are assumed to be treatment-emergent, unless the stop date was before the dosing date. In general, AEs were summarized by subject incidence by cohort and overall as well as presented in a data listing by cohort and subject. Treatment-emergent serious adverse events (TESAEs) were also also evaluated for each subject, but none were observed in
25 treated subjects (**Table 11**).

[0354] Of the 15 subjects enrolled, 13 subjects received at least one dose of rFVIII-Fc-VWF-XTEN and were included in the safety analysis. 19 TEAEs were reported in 8 of the 13 subjects (61.5%) (**Table 11**). There were no serious or related TEAEs reported and no subject discontinued the study due to a TEAE. The most common TEAEs were arthralgia, upper respiratory tract infection,
30 headache, and rhinitis (2/13 subjects each; 15.4%). No inhibitor development to FVIII was detected

and there were no reports of serious hypersensitivity or anaphylaxis. At each level of subject summarization, a subject is counted once if the subject reported one or more events. Percentages reported in Table 11 are based on the number of subjects who received at least one dose of rFVIII-Fc-VWF-XTEN in each cohort and overall.

5

TABLE 11. Summary of Treatment Emergent Adverse Events (TEAEs) in Safety Analysis Set

	rFVIII-Fc-VWF-XTEN 50 IU/kg	rFVIII-Fc-VWF-XTEN 65 IU/kg	Major Surgical Period (N=0)	rFVIII-Fc-VWF-XTEN Total treated
Number of Subjects treated with rFVIII-Fc-VWF-XTEN	10	3	0	13
Total number of TEAEs	17	2	0	19
Subjects with at least one TEAE	7 (70.0%)	1 (33.3%)	0	8 (61.5%)
Subjects with at least one related TEAE	0	0	0	0
Subjects who discontinued study due to TEAE	0	0	0	0
Total Number of TESAEs	0	0	0	0
Subjects with at least one TESA	0	0	0	0
Subjects with at least one related TESA	0	0	0	0

[0355] Immunogenicity Analysis

[0356] All subjects were assessed for inhibitor development and anti-rFVIII-Fc-VWF-XTEN antibodies at Screening, Day 1, Day 8, Day 15, Day 22, Day 36, and Day 50 (EOS or ET visit). Testing for inhibitors was performed by the central laboratory using the Nijmegen-modified Bethesda assay.

5 If a Nijmegen-modified Bethesda assay result returned as ≥ 0.6 BU/mL, a separate sample was collected and tested for confirmation of inhibitor development within 2 to 4 weeks. Testing for potential antibody formation was performed at a central laboratory using a validated rFVIII-Fc-VWF-XTEN-specific anti-drug antibody (ADA) assay. Confirmed positive samples were further characterized for antibodies specific to FVIII, D'D3, or XTEN. The number and percentage of subjects who tested
10 positive for inhibitor development and the number and percentage of subjects who tested positive for anti-rFVIII-Fc-VWF-XTEN antibodies were summarized by cohort using the Safety Analysis Set.

[0357] No subject developed a positive anti-drug antibody assay result following rFVIII-Fc-VWF-XTEN administration.

[0358] Pharmacokinetic Analysis and Results

15 **[0359]** In general, safety and PK data were summarized using standard summary statistics for continuous and categorical data. Data was summarized by cohort. Continuous variables were summarized using descriptive statistics including the number of non-missing values (n), mean, standard deviation (SD), median, minimum, and maximum. Pharmacokinetic parameters were also summarized using geometric mean and percent coefficient of variation (%CV). Categorical variables
20 were summarized by counts and percentages.

[0360] All PK analyses were performed based on the PK analysis set (PKAS) and included 9 subjects from the 50 IU/kg dosing cohort who had adequate blood sample collections following rFVIII-Fc-VWF-XTEN administration.

[0361] **FVIII Activity**

25 **[0362]** To characterize the pharmacokinetics of rFVIII-Fc-VWF-XTEN after a total of four once-weekly IV doses, FVIII activity was determined in each subject at the prespecified time points. FVIII activity was summarized by cohort, scheduled PK visit, and timepoint for both the one-stage (activated partial thromboplastin time [aPTT]) clotting assay and the chromogenic coagulation assay. FVIII activity versus time profiles were plotted in both original and log scale for both FVIII activity

assays. Individual FVIII activity was listed for each subject by cohort, scheduled PK visit and timepoint for both FVIII activity assays. Results for FVIII activity according to the one-stage [aPTT] assay are depicted in Table 12. Results for FVIII activity according to the chromogenic assay are depicted in Table 13.

5 **[0363]** Briefly, following Day 1 dosing, mean FVIII activities based on one-stage and chromogenic assay at 72 h were 45.53% and 29.37%, at 120 h were 21.56% and 13.49%, and at 168 h were 7.91% and 5.97%, respectively. Following Day 22 dosing, mean FVIII activities based on one stage and chromogenic assay at 72 h were 46.28% and 30.46%, at 120 h were 22.30% and 14.48%, and at 168 h were 9.83% and 6.74%, respectively. Generally across all patients, time points, and with both
10 assays, the Ctrough for rFVIIIIFc-VWF-XTEN at 50 IU/kg QW in this interim analysis ranged between 7-10%.

[0364] PK Parameters

[0365] Individual PK parameter estimates were listed for each subject and summarized descriptively by cohort, scheduled PK visit, and time point for both FVIII activity assays.

15 **[0366]** The geometric mean elimination half-life ($t_{1/2}$) for all subjects receiving rFVIIIIFc-VWF-XTEN at 50 IU/kg QW in this interim analysis was 41.24 hours according to the one stage assay (**Table 16B**) and 43.87 hours according to the chromogenic assay (**Table 17B**).

[0367] Following Day 1 dosing, the geometric mean maximum FVIII activity (C_{max}) was 113 IU/dL with the one stage assay (**Table 14**) and 108 IU/dL with the chromogenic assay (**Table 15**). Following
20 Day 22 dosing, the geometric mean FVIII activity at steady state (C_{maxss}) was 127 IU/dL according to the one stage assay (**Table 16A**) and 115 IU/dL according to the chromogenic assay (**Table 17A**).

[0368] Following Day 1 dosing, the geometric mean cumulative FVIII activity (AUC_{0-tau}) was 7650 hr*IU/dL according to the one stage assay (**Table 14**) and 5630 hr*IU/dL according to the chromogenic assay (**Table 15**). Following Day 22 dosing, the geometric mean AUC_{0-tau} was 8270
25 hr*IU/dL according to the one stage assay (**Table 16A**) and 5880 hr*IU/dL according to the chromogenic assay (**Table 17A**).

[0369] C_{max} and AUC_{0-tau} data on Day 1 and Day 22 dosing indicated minimal accumulation supported by accumulation index (AI) of 1.07 (one-stage assay, **Table 16B**) and 1.08 (chromogenic assay, **Table 17B**). For all of the PK parameter data presented in Tables 12-17, values below the
30 limit of quantification (BLQ) were treated as 0 for the purpose of analysis. Where necessary for

calculation of PK parameters, as infusion end time was not captured, infusion duration was imputed as 8 minutes for each subject in line with administration guidance in the study's Directions for Handling and Administration document.

5 **TABLE 12. Summary of Baseline-Corrected FVIII Activity Levels (IU/dL) based on one-stage aPTT clotting assay: PK Analysis Set (Dose Level: 50 IU/kg)**

Dosing Day	Timepoint	n	Mean (SD)	Median	Geo. Mean	%CV	Min, Max
Day1	Pre-injection	9	0.00 (0.000)	0.00	0.00	-----	0.0, 0.0
	30 mins from SOI	9	113.17 (20.214)	118.10	111.51	17.862	82.0, 140.2
	3 hrs from SOI	9	109.07 (19.243)	109.90	107.49	17.643	77.4, 135.5
	24 hrs from SOI	9	91.04 (15.231)	92.80	89.88	16.730	68.9, 115.8
	48 hrs from SOI	9	66.38 (12.231)	65.90	65.30	18.426	46.7, 80.3
	72 hrs from SOI	9	45.53 (6.742)	45.60	45.08	14.807	36.7, 53.9
	120 hrs from SOI	9	21.56 (4.473)	22.20	21.11	20.750	14.2, 27.1
Day8	Pre injection	9	7.91 (2.869)	7.40	7.48	36.265	4.0, 13.1
Day 15	Pre injection	9	8.94 (3.322)	7.80	8.34	37.140	3.9, 13.5
Day 22	Pre injection	9	9.91 (2.838)	11.61	9.49	28.633	5.0, 12.9
	30 mins from SOI	9	132.77 (31.741)	131.80	129.56	23.907	96.0, 190.7
	3 hrs from SOI	9	120.94 (22.168)	126.60	119.03	18.329	85.2, 152.0
	24 hrs from SOI	9	103.79 (18.513)	110.20	102.21	17.838	74.0, 126.3
	48 hrs from SOI	9	69.89 (12.812)	68.10	68.84	18.332	49.8, 91.7
	72 hrs from SOI	9	46.28 (7.720)	47.90	45.68	16.682	33.4, 58.3
	120 hrs from SOI	9	22.30 (4.979)	21.70	21.77	22.329	13.7, 29.8
	168 hrs from SOI	9	9.83 (3.847)	9.60	9.16	39.120	4.9, 15.5
	240 hrs from SOI	9	2.84 (0.999)	2.80	2.68	35.117	1.4, 4.4
	336 hrs from SOI	9	1.28 (2.651)	0.00	0.00	207.494	0.0, 8.2

Abbreviations: SD = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, Min = minimum value, Max = maximum value, SOI = start of injection

TABLE 13. Summary of Baseline-Corrected FVIII Activity Levels (IU/dL) based on chromogenic assay: PK Analysis Set (Dose Level: 50 IU/kg)

Dosing Day	Timepoint	n	Mean (SD)	Median	Geo. Mean	%CV	Min, Max
Day1	Pre-injection	9	0.00 (0.000)	0.00	0.00	-----	0.0, 0.0
	30 mins from SOI	9	108.94 (15.126)	113.00	107.99	13.884	87.4, 129.9
	3 hrs from SOI	9	101.86 (13.782)	102.50	100.99	13.531	79.1, 118.3
	24 hrs from SOI	9	71.68 (14.654)	73.50	70.24	20.445	47.8, 93.5
	48 hrs from SOI	9	43.84 (7.086)	43.40	43.32	16.163	32.5, 54.0
	72 hrs from SOI	9	29.37 (5.319)	30.10	28.93	18.112	22.3, 37.2
	120 hrs from SOI	9	13.49 (2.680)	13.80	13.24	19.870	9.4, 17.2
Day8	Pre injection	9	5.97 (1.764)	6.20	5.74	29.568	3.6, 9.4
Day 15	Pre injection	9	6.93 (1.736)	6.70	6.74	25.044	4.8, 10.0
Day 22	Pre injection	9	7.13 (1.817)	7.50	6.93	25.466	5.0, 10.4
	30 mins from SOI	9	113.58 (14.920)	113.25	112.72	13.137	91.3, 137.6
	3 hrs from SOI	9	109.08 (16.394)	112.50	107.96	15.029	85.4, 132.6
	24 hrs from SOI	9	72.13 (14.985)	71.40	70.78	20.774	56.6, 95.8
	48 hrs from SOI	9	45.23 (4.907)	46.10	44.99	10.847	37.4, 52.3
	72 hrs from SOI	9	30.46 (5.247)	31.20	30.04	17.230	21.4, 39.6
	120 hrs from SOI	9	14.48 (2.862)	13.70	14.23	19.769	10.5, 18.7
	168 hrs from SOI	9	6.74 (1.911)	6.20	6.51	28.328	4.4, 9.7
	240 hrs from SOI	9	2.20 (1.025)	2.20	0.00	46.577	0.0, 3.7
	336 hrs from SOI	9	0.46 (1.021)	0.00	0.00	224.158	0.0, 3.0

Abbreviations: SD = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, Min = minimum value, Max = maximum value, SOI = start of injection

TABLE 14. Day 1 Dosing Summary of Pharmacokinetic Parameters for Baseline-Corrected FVIII Activity based on one-stage aPTT clotting assay: PK Analysis Set

Subject No.	Actual Dose (IU/kg)	C _{MAX} (IU/dL)	T _{MAX} (hr)	AUC _{0-tau} (hr*IU/dL)	Dose-normalized AUC _{0-tau} (hr*kg*IU/dL/IU)	IVR (%)	IR (IU/dL per IU/kg)
Cohort 1 (50 IU/kg): BIVV001 Day 1 Dosing							
170-001	50.0	91.7	3.13	7940	159	52.5	1.83
170-002	49.9	140	0.50	8310	166	97.4	2.81
170-003	50.2	82.0	0.50	6000	119	53.1	1.63
170-004	50.1	131	3.00	9300	186	95.9	2.61
170-006	50.0	139	0.50	7960	159	89.4	2.78
170-007	49.8	109	0.50	7480	150	71.1	2.19
170-009	50.0	121	2.85	8170	163	85	2.42
170-010	50.2	99.1	0.50	5750	115	61.3	1.97
170-011	49.8	118	0.50	8740	176	64.2	2.37
n	9	9	9	9	9	9	9
Mean	50.00	115	1.33	7740	155	74.4	2.29
Median	50.00	118	0.5	7960	159	71.1	2.37
Std. Dev	0.150	20.7	1.25	1180	23.8	17.9	0.42
%CV	0	18	94	15	15	24	18
Geo. Mean	50.00	113	0.91	7650	153	72.5	2.26
(95% CI)	(49.88, 50.12)	(97.6, 130)	(0.46, 1.81)	(6750, 8670)	(135, 174)	(60.1, 87.5)	(1.95, 2.61)
Minimum	49.8	82.0	0.50	5750	115	52.5	1.63
Maximum	50.2	140	3.13	9300	186	97.4	2.81

Abbreviations: Std Dev = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, C_{MAX} = maximum concentration at steady state, T_{MAX} = time to reach maximum concentration, C_{MAXSS} = maximum concentration at steady state, IVR = in vivo recovery, AUC_{0-inf} = area under the activity-time curve from time 0 to infinity, AUC_{0-tau} = area under the activity-time curve from hour 0 over the dosing interval, MRT_{INF} = mean residence time extrapolated to infinity, AI = accumulating index, IR = incremental recovery, C_{trough} = lowest concentration that a drug reaches before the next dose is administered, C_{avg} = average concentration after reaching steady state, t_{1/2} = elimination half-life, CL_{ss} = clearance at steady state, V_{ss} = volume of distribution at steady state.

TABLE 15: Day 1 Summary of Pharmacokinetic Parameters for Baseline-Corrected FVIII Activity based on chromogenic assay: PK Analysis Set

Subject No.	Actual Dose (IU/kg)	C _{MAX} (IU/dL)	T _{MAX} (hr)	AUC _{0-tau} (hr*IU/dL)	Dose-normalized AUC _{0-tau} (hr*kg*IU/dL/IU)	IVR (%)	IR (IU/dL per IU/kg)
Cohort 1 (50 IU/kg): BIVV001 Day 1 Dosing							
170-001	50	90.8	0.63	5850	117	52	1.82
170-002	49.9	130	0.5	6160	123	90.2	2.6
170-003	50.2	87.4	0.5	4290	85.4	56.6	1.74
170-004	50.1	124	0.5	7030	140	90.5	2.47
170-006	50	117	0.5	5530	111	74.9	2.33
170-007	49.8	105	0.5	5580	112	68.3	2.1
170-009	50	119	0.5	6500	130	84	2.39
170-010	50.2	95.4	0.5	4360	86.9	59	1.9
170-011	49.8	113	0.5	5970	120	61.4	2.27
n	9	9	9	9	9	9	9
Mean	50	109	0.51	5700	114	70.8	2.18
Median	50	113	0.5	5850	117	68.3	2.27
Std. Dev	0.15	15.1	0.04	905	18.2	14.8	0.3
%CV	0	14	9	16	16	21	14
Geo. Mean	50	108	0.51	5630	113	69.4	2.16
(95% CI)	(49.88, 50.12)	(96.8, 120)	(0.48, 0.55)	(4950, 6400)	(98.9, 128)	(59.1, 81.5)	(1.93, 2.41)
Minimum	49.8	87.4	0.5	4290	85.4	52	1.74
Maximum	50.2	130	0.63	7030	140	90.5	2.6

Abbreviations: Std Dev = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, C_{MAX} = maximum concentration at steady state, T_{MAX} = time to reach maximum concentration, C_{MAXSS} = maximum concentration at steady state, IVR = in vivo recovery, AUC_{0-inf} = area under the activity-time curve from time 0 to infinity, AUC_{0-tau} = area under the activity-time curve from hour 0 over the dosing interval, MRT_{INF} = mean residence time extrapolated to infinity, AI = accumulating index, IR = incremental recovery, C_{trough} = lowest concentration that a drug reaches before the next dose is administered, C_{avg} = average concentration after reaching steady state, t_{1/2} = elimination half-life, CL_{ss} = clearance at steady state, V_{ss} = volume of distribution at steady state.

TABLE 16A. Day 22 - Summary of Pharmacokinetic Parameters for Baseline-Corrected FVIII Activity based on one-stage aPTT clotting assay: PK Analysis Set (Part 1)

Subject No.	Actual Dose (IU/kg)	C _{MAX} SS (IU/dL)	AUC _{0-inf} (hr*IU/dL)	Dose-normalized AUC _{0-inf} (hr*kg*IU/dL/IU)	AUC _{0-tau} (hr*IU/dL)	Dose-normalized AUC _{0-tau} (hr*kg*IU/dL/IU)
Cohort 1 (50 IU/kg): BIVV001 Day 22 Dosing						
170-001	50.1	106	9230	184	8240	165
170-002	49.9	135	8690	174	8310	167
170-003	50	104	7520	150	6950	139
170-004	50	143	11000	219	10100	201
170-006	50	165	9480	190	9210	184
170-007	49.9	132	9300	186	8550	171
170-009	49.8	154	8980	180	8580	172
170-010	49.8	96	6130	123	5810	117
170-011	49.9	129	10200	205	9560	192
n	9	9	9	9	9	9
Mean	49.93	129	8940	179	8370	168
Median	49.9	132	9230	184	8550	171
Std. Dev	0.1	23.2	1420	28.4	1310	26.1
%CV	0	18	16	16	16	16
Geo. Mean	49.93	127	8830	177	8270	166
(95% CI)	(49.86, 50.01)	(110, 147)	(7740, 10100)	(155, 202)	(7260, 9410)	(145, 188)
Minimum	49.8	96	6130	123	5810	117
Maximum	50.1	165	11000	219	10100	201

Abbreviations: Std Dev = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, C_{MAX} = maximum concentration at steady state, T_{MAX} = time to reach maximum concentration, C_{MAXSS} = maximum concentration at steady state, IVR = in vivo recovery, AUC_{0-inf} = area under the activity-time curve from time 0 to infinity, AUC_{0-tau} = area under the activity-time curve from hour 0 over the dosing interval, MRT_{INF} = mean residence time extrapolated to infinity, AI = accumulating index, IR = incremental recovery, C_{trough} = lowest concentration that a drug reaches before the next dose is administered, C_{avg} = average concentration after reaching steady state, t_{1/2} = elimination half-life, CL_{ss} = clearance at steady state, V_{ss} = volume of distribution at steady state.

TABLE 16B: Day 22 - Summary of Pharmacokinetic Parameters for Baseline-Corrected FVIII Activity based on one-stage aPTT clotting assay: PK Analysis Set (Part 2)

Subject No.	t _{1/2} (hr)	MRTINF (hr)	CL _{ss} (mL/hr/kg)	V _{ss} (mL/kg)	AI	C _{trough} (IU/dL)	C _{avg} (IU/dL)
Cohort 1 (50 IU/kg): BIVV001 Day 22 Dosing							
170-001	50.05	78.56	0.61	47.8	1.11	15.2	49.1
170-002	36.86	57.13	0.6	34.3	1.04	7	49.5
170-003	43.86	66.35	0.72	47.8	1.08	9.6	41.4
170-004	45.45	69.54	0.5	34.5	1.08	15.5	60
170-006	34.21	49.79	0.54	27	1.03	4.9	54.8
170-007	48.87	67.08	0.58	39.1	1.1	11.5	50.9
170-009	34.63	56.19	0.58	32.6	1.04	7.8	51.1
170-010	38.29	58.71	0.86	50.3	1.05	5.8	34.6
170-011	42.34	63.41	0.52	33.1	1.07	11.2	56.9
n	9	9	9	9	9	9	9
Mean	41.62	62.97	0.61	38.5	1.07	9.83	49.8
Median	42.34	63.41	0.58	34.5	1.07	9.6	50.9
Std. Dev	5.93	8.57	0.11	8.21	0.03	3.85	7.79
%CV	14	14	18	21	3	39	16
Geo. Mean	41.24	62.46	0.6	37.7	1.07	9.16	49.2
(95% CI)	(36.95, 46.04)	(56.27, 69.33)	(0.531, 0.688)	(32.0, 44.4)	(1.05, 1.09)	(6.70, 12.5)	(43.2, 56.0)
Minimum	34.21	49.79	0.5	27	1.03	4.9	34.6
Maximum	50.05	78.56	0.86	50.3	1.11	15.5	60

Abbreviations: Std Dev = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, C_{MAX} = maximum concentration at steady state, T_{MAX} = time to reach maximum concentration, C_{MAXSS} = maximum concentration at steady state, I_{VR} = in vivo recovery, AUC_{0-inf} = area under the activity-time curve from time 0 to infinity, AUC_{0-tau} = area under the activity-time curve from hour 0 over the dosing interval, MRTINF = mean residence time extrapolated to infinity, AI = accumulating index, I_R = incremental recovery, C_{trough} = lowest concentration that a drug reaches before the next dose is administered, C_{avg} = average concentration after reaching steady state, t_{1/2} = elimination half-life, CL_{ss} = clearance at steady state, V_{ss} = volume of distribution at steady state.

TABLE 17A. Day 22 - Summary of Pharmacokinetic Parameters for Baseline-Corrected FVIII Activity based on chromogenic assay: PK Analysis Set (Part 1)

Subject No.	Actual Dose (IU/kg)	C _{MAX} SS (IU/dL)	AUC ₀ -inf (hr*IU/dL)	Dose-normalized AUC ₀ -inf (hr*kg*IU/dL/IU)	AUC ₀ -tau (hr*IU/dL)	Dose-normalized AUC ₀ -tau (hr*kg*IU/dL/IU)
Cohort 1 (50 IU/kg): BIVV001 Day 22 Dosing						
170-001	50.1	103	6560	131	5810	116
170-002	49.9	124	6830	137	6430	129
170-003	50.0	91.3	5210	104	4780	95.5
170-004	50.0	126	7050	141	6410	128
170-006	50.0	138	6520	130	6300	126
170-007	49.9	117	6460	129	5990	120
170-009	49.8	123	6620	133	6290	126
170-010	49.8	103	5080	102	4800	96.5
170-011	49.9	113	6910	138	6450	129
n	9	9	9	9	9	9
Mean	49.93	115	6360	127	5920	119
Median	49.9	117	6560	131	6290	126
Std. Dev	0.100	14.5	718	14.3	674	13.5
%CV	0	13	11	11	11	11
Geo. Mean	49.93	115	6320	127	5880	118
(95% CI)	(49.86, 50.01)	(104, 126)	(5760, 6940)	(115, 139)	(5360, 6460)	(107, 129)
Minimum	49.8	91.3	5080	102	4780	95.5

Abbreviations: Std Dev = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, C_{MAX} = maximum concentration at steady state, T_{MAX} = time to reach maximum concentration, C_{MAX}SS = maximum concentration at steady state, IVR = in vivo recovery, AUC₀-inf = area under the activity-time curve from time 0 to infinity, AUC₀-tau = area under the activity-time curve from hour 0 over the dosing interval, MRT_{INF} = mean residence time extrapolated to infinity, AI = accumulating index, IR = incremental recovery, C_{trough} = lowest concentration that a drug reaches before the next dose is administered, C_{avg} = average concentration after reaching steady state, t_{1/2} = elimination half-life, CL_{ss} = clearance at steady state, V_{ss} = volume of distribution at steady state.

TABLE 17B. Day 22 - Summary of Pharmacokinetic Parameters for Baseline-Corrected FVIII Activity based on chromogenic assay: PK Analysis Set (Part 2)

Subject No.	t _{1/2} (hr)	MRTINF (hr)	CL _{ss} (mL/hr/kg)	V _{ss} (mL/kg)	AI	C _{trough} (IU/dL)	C _{avg} (IU/dL)
Cohort 1 (50 IU/kg): BIVV001 Day 22 Dosing							
170-001	53.31	76.94	0.86	66.3	1.13	9.6	34.6
170-002	44.73	55.57	0.78	43.1	1.08	6	38.3
170-003	48.29	64.39	1.05	67.4	1.1	6.2	28.4
170-004	46.03	71.12	0.78	55.5	1.09	9.7	38.2
170-006	35.59	47.89	0.79	38	1.04	4.4	37.5
170-007	45.35	62.51	0.83	52	1.08	7	35.7
170-009	40.09	54.93	0.79	43.5	1.06	5.5	37.4
170-010	40.2	56.26	1.04	58.3	1.06	4.8	28.6
170-011	43.61	60.71	0.77	47	1.07	7.5	38.4
n	9	9	9	9	9	9	9
Mean	44.13	61.15	0.85	52.4	1.08	6.74	35.2
Median	44.73	60.71	0.79	52	1.08	6.2	37.4
Std. Dev	5.15	8.88	0.11	10.4	0.03	1.91	4.01
%CV	12	15	13	20	2	28	11
Geo. Mean	43.87	60.59	0.85	51.4	1.08	6.51	35
(95% CI)	(40.08, 48.01)	(54.25, 67.67)	(0.773, 0.932)	(44.1, 60.0)	(1.06, 1.10)	(5.26, 8.07)	(31.9, 38.4)
Minimum	35.59	47.89	0.77	38	1.04	4.4	28.4
Maximum	53.31	76.94	1.05	67.4	1.13	9.7	38.4

- 5 Abbreviations: Std Dev = standard deviation, Geo. Mean = geometric mean, %CV = percent coefficient of variation, C_{MAX} = maximum concentration at steady state, T_{MAX} = time to reach maximum concentration, C_{MAXSS} = maximum concentration at steady state, I_{VR} = in vivo recovery, AUC_{0-inf} = area under the activity-time curve from time 0 to infinity, AUC_{0-tau} = area under the activity-time curve from hour 0 over the dosing interval, MRTINF = mean residence time extrapolated to infinity, AI = accumulating index, I_R = incremental recovery, C_{trough} = lowest concentration that a drug reaches before the next dose is administered, C_{avg} = average concentration after reaching steady state, t_{1/2} = elimination half-life, CL_{ss} = clearance at steady state, V_{ss} = volume of distribution at steady state.
- 10

[0370] Conclusions

[0371] Consistent with the results observed in the single dose study (Example 1), the weekly dosing of rFVIII Fc-VWF-XTEN was generally well tolerated and no inhibitors were detected through 28 days following the final rFVIII Fc-VWF-XTEN administration. rFVIII Fc-VWF-XTEN at a dose of 50 IU/kg exhibited mean elimination half-life ($t_{1/2}$) of 41.24 and 43.87 hours with the one stage and chromogenic assays, respectively. These interim results demonstrate that the enhanced half-life observed in single dosing of rFVIII Fc-VWF-XTEN is maintained with repeat dosing QW, strongly signaling that the advantages of rFVIII Fc-VWF-XTEN can be realized by hemophilia A patients receiving treatment according to a prophylactic FVIII dosing schedule.

Example 3: Phase 3 Study of rFVIII Fc-VWF-XTEN in Previously Treated Patients With Severe Hemophilia A

[0372] The present study will evaluate the safety and tolerability and characterize the pharmacokinetics of once weekly administration of 50 IU/kg rFVIII Fc-VWF-XTEN (FIG. 1). The primary objective of the study is to evaluate the efficacy of rFVIII Fc-VWF-XTEN prophylactic treatment compared with rFVIII Fc-VWF-XTEN on-demand treatment. The secondary objection of the study is to evaluate rFVIII Fc-VWF-XTEN as a prophylactic treatment, its effect on bleeding episodes and consumption of rFVIII Fc-VWF-XTEN, joint health, and quality of life as measured by Patient-Reported Outcomes (PROs), and evaluate rFVIII Fc-VWF-XTEN for on-demand treatment.

[0373] In addition, the pharmacokinetic (PK) objective is to characterize the PK of rFVIII Fc-VWF-XTEN prophylaxis at baseline for all subjects and repeated at three months for a predefined subset of subjects, based on the aPTT FVIII activity assay. Additional objectives are to evaluate the efficacy of rFVIII Fc-VWF-XTEN in subjects who undergo surgery, and to evaluate the safety and tolerability of rFVIII Fc-VWF-XTEN. Further, joint health structural outcomes will be assessed via ultrasound, and the impact of treatment on other quality of life measurements and on healthcare resource utilization will be assessed.

[0374] Study Design

[0375] This is a pivotal, Phase 3, open-label, multicenter study of the safety, efficacy and PK of intravenous rFVIII Fc-VWF-XTEN in patients ≥ 12 years of age with severe hemophilia A (defined as <1 IU/dL [$<1\%$] endogenous FVIII). Approximately twenty-five subjects currently on an on-demand

regimen will receive rFVIII Fc-VWF-XTEN at a dose of 50 IU/kg IV on an on-demand basis for twenty-six weeks, then receive rFVIII Fc-VWF-XTEN at a dose of 50 IU/kg IV once-weekly on a prophylactic treatment regimen for twenty-six weeks. Subjects currently on a prophylactic treatment regimen will receive rFVIII Fc-VWF-XTEN at a dose of 50 IU/kg IV once-weekly on a prophylactic treatment regimen for fifty-two weeks.

[0376] Approximately 140 subjects ≥ 12 years of age will be enrolled; repeat PK assessments will be performed on a subset of approximately sixteen subjects at 12 weeks.

[0377] Subjects will be screened and, after meeting enrollment criteria, proceed to one of two treatment regimens (i.e., on demand or prophylaxis) with rFVIII Fc-VWF-XTEN at a dose of 50 IU/kg IV. All subjects will undergo baseline PK assessments after a washout period (at least 4 to 5 days, depending on current therapy). Subjects will undergo efficacy and safety assessments throughout the study. These safety assessments will include testing for potential inhibitor formation. A follow-up safety visit or telephone call will occur twenty-eight days after the last dose of rFVIII Fc-VWF-XTEN, unless the subject enrolls in the open-label extension study.

[0378] End of study (EOS) may occur when both criteria have been met: (i) at least 104 subjects have reached 50 exposure days (EDs) and have completed a valid inhibitor test after the 50th ED; and (ii) at least twenty-five subjects have completed the on-demand arm of the study, including the 26-week prophylactic treatment period, or withdrawn from the study early. In addition, subjects from any arm who undergo surgery during the study will be included in the surgery subset. A minimum of ten major surgeries in at least five subjects will be targeted to assess control and prevention of bleeding in the surgical setting.

[0379] Subjects will come to the clinic within an approximately 28-day Screening Period for determination of eligibility. In addition to the Screening Visit(s), subjects will return to the clinic for visits at Baseline, Week 4, Week 12, Week 26, Week 36, Week 52, and EOS/ET. The Dosing Period will be over 52 weeks. Subjects who enter on the prophylactic treatment arm will receive 52 once-weekly doses of rFVIII Fc-VWF-XTEN and additional doses as necessary to treat bleeding episodes. Subjects who enter on the on-demand treatment arm will receive rFVIII Fc-VWF-XTEN on-demand during the 26-week on-demand treatment period and 26 once-weekly doses of rFVIII Fc-VWF-XTEN and additional doses as necessary to treat bleeding episodes during the subsequent 26-week prophylactic treatment period. Safety will be assessed throughout the study. A Follow-Up Safety Visit

or Telephone Call will occur 28 days after the last dose of rFVIII-Fc-VWF-XTEN, unless the subject enrolls in the open-label extension study.

[0380] The primary endpoint of the study will be an intra-subject comparison of the Annualized Bleeding Rate (ABR) while subjects are on rFVIII-Fc-VWF-XTEN on-demand treatment (26 weeks) versus once-weekly prophylactic treatment (26 weeks).

[0381] The secondary endpoints of the study will include (i) an intra-subject comparison of ABR on rFVIII-Fc-VWF-XTEN prophylaxis versus the historical ABR (for subjects on prophylaxis who participated in a previous observational study); (ii) the occurrence of bleeding episodes and consumption (overall ABR, ABR by type and location, and total, annualized rFVIII-Fc-VWF-XTEN consumption per subject); (iii) the response to treatment; (iv) efficacy index (percentage of subjects that maintain trough level of L3% at day 7; (v) joint health; and (vi) changes in quality of life measures (haem-A-QoL and PROMIS-SF physical Function for subjects at least 18 years old; and HAEMO-QoL and PROMIS Pediatric-SF Physical Activity for subjects less than 18 years old).

[0382] The response to treatment will include the (i) number of infusions and dose of rFVIII-Fc-VWF-XTEN required to resolve a bleeding episode; (ii) percentage of bleeding episodes that require a single infusion for resolution; (iii) subject's assessment of response to rFVIII-Fc-VWF-XTEN treatment of individual bleeding episodes based on a 4-point response scale; (iv) and investigator's global assessment of subject's response to rFVIII-Fc-VWF-XTEN treatment based on a 4-point response scale.

[0383] Joint health evaluation will include (i) ABR of joint bleeding episodes; (ii) Target joint resolution at 52 weeks, based on the International Society on Thrombosis and Haemostasis (ISTH) criteria; (iii) Functional outcomes based on absolute change from baseline to 52 weeks assessed by the modified Hemophilia Joint Health Score (mHJHS) or HJHS; (iv) Percentage of subjects with no joint deterioration at 52 weeks assessed by the mHJHS or HJHS.

[0384] Additional endpoints include (i) Maximum (peak) plasma drug concentration (C_{max}), elimination half-life (t_{1/2}), accumulation index (AI), area under the plasma concentration-time curve (AUC), apparent volume of distribution at steady state (V_{ss}), mean residence time (MRT), incremental recovery (IR), trough plasma concentration (C_{trough}), and time above predefined FVIII activity levels; (ii) Investigators' or Surgeons' assessments of subjects' hemostatic response to rFVIII-Fc-VWF-XTEN treatment on a 4-point response scale, number and dose of infusions required to maintain hemostasis during the surgery, total rFVIII-Fc-VWF-XTEN consumption during the surgical period, estimated

blood loss during surgery, number and type of blood component transfusions required during surgery, and bleeding episodes post-surgery during the surgical period; (iii) the occurrence of adverse events (AEs) and serious adverse events (SAEs), clinically significant abnormalities in physical examination, vital signs, and laboratory tests, and the development of inhibitors (neutralizing antibodies directed against FVIII as determined via the Nijmegen-modified Bethesda assay; and (iv) anatomical structural joint health outcomes via ultrasound imaging in a subpopulation, changes in other QoL measures (EQ-5D-5L, PGIC (week 52 only), PROMIS Pain Intensity, PROMIS-SF Pain Interference (subjects ≥ 18 years old), PROMIS Pediatric-SF Pain Interference (subjects < 18 years old)), and HRU.

[0385] Inclusion/Exclusion Criteria

[0386] Eligible candidates must meet the following eligibility criteria: (1) ≥ 12 years of age at the time of informed consent; (2) severe hemophilia A, defined as < 1 IU/dL ($< 1\%$) endogenous FVIII as documented by a certified clinical laboratory; (3) previous treatment for hemophilia A (prophylactic or on demand) with any recombinant and/or plasma-derived FVIII, or cryoprecipitate for at least 150 EDs; (4) currently on a prophylactic treatment regimen with a marketed FVIII product and had at least four bleeding episodes in the twelve months prior to study enrollment, or currently on an on-demand treatment regimen with a marketed FVIII product and had at least twelve bleeding episodes in the twelve months prior to study enrollment; and (5) platelet count $\geq 100,000$ cells/ μ L at screening (test performed by the central laboratory and reviewed prior to the Day 1 dose). Subjects will be excluded for any of the following reasons: (1) other known coagulation disorder(s) in addition to hemophilia A; (2) history of hypersensitivity or anaphylaxis associated with any FVIII product; (3) history of a positive inhibitor test or clinical signs of decreased response to FVIII administrations (family history of inhibitors will not exclude the subject); (4) positive inhibitor result, taken at screening, defined as ≥ 0.6 BU/mL; (5) abnormal renal function, defined as serum creatinine > 2.0 mg/dL taken at Screening; (6) serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $> 5 \times$ upper limit of normal (ULN) taken at Screening; (7) serum total bilirubin $> 3 \times$ ULN, taken at Screening; (8) fitusiran or emicizumab use within the 12 weeks prior to Screening; (9) treatment within 12 weeks prior to screening with a monoclonal antibody therapeutic, an Fc fusion protein other than rFVIII-Fc, or IV immunoglobulin; (10) Treatment with acetylsalicylic acid (ASA) within 2 weeks prior to screening or treatment with non-steroidal anti-inflammatory drugs (NSAIDs) at or above the maximum dose specified in the regional prescribing information for each product; (11) systemic treatment within 12 weeks prior to screening with chemotherapy and/or other immunosuppressive drugs (except for the treatment of hepatitis C virus [HCV] or HIV; use of corticosteroids is allowed, except for systemic

corticosteroid treatment given daily or on alternate days at 20 mg/day of prednisone or its equivalent for >14 days; local, topical, and/or inhaled steroid use is permitted; and (12) major surgery within 8 weeks prior to screening (major surgery is defined as any surgical procedure (elective or emergent) that usually, but not always, involves general anesthesia and/or respiratory assistance, in which a
5 major body cavity is penetrated and exposed, or a substantial impairment of physical or physiological functions is produced (e.g., laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation)).

SEQUENCES

VWF031 nucleotide Sequence (SEQ ID NO: 147)

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VWF031 protein Sequence (SEQ ID NO: 86)

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 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 50 251 EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTPCPC

351 VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401 NRYFTFSGIC QYLLARDCQD HSFIVIVETV QCADDRDAVC TRSVTVRLPG
 451 LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 5 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL
 651 NCPKGQVYLQ CGTPCNLTCT SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751 AVLSSPLSHR SKRSLSCRPP MVKLVCADN LRAEGLECTK TCQNYDLECM
 10 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
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 901 NPGTFRILVG NKGCSPSVK CKKRVTILVE GGEIELFDGE VNVKRPMDKE
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 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 15 1051 MKQTMVDSSC RILTSDVFQD CNKLVDP EY LDVCIYDTCS CESIGDCAAF
 1101 CDTIAAYAHV CAQH GKVV TW RTATLCPQSC EERNLRENGY EAEWRYNSCA
 1151 PACQVTCQHP EPLACPVCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGGGGSGGG
 1251 GSGGGSGGG GSGGGSGGG GSVLPRGSGG GSGGGGSGDK THTCP PCPAP
 20 1301 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
 1351 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
 1401 EKTISKAKGQ PREPQVYTL PPSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
 1451 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRLWQQGNV FSCSV MHEAL
 1501 HNHYTQKSLS LSPGK*

VWF034 nucleotide Sequence (SEQ ID NO: 148)

1 ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
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 301 ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351 GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
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 40 651 GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
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 20 2201 GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
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 25 2451 TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
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 50 3701 GTGAA GCCTG CCAGG AGCCG ATATC GGGTA CCTCA GAGTC TGCTA CCCCC
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 3801 GACTT CCGAG AGTGC CACCC CTGAG TCCGG ACCCG GTTCC GAGCC CGCCA
 3851 CTTCC GGCTC CGAAA CTCCC GGCAC AAGCG AGAGC GCTAC CCCAG AGTCA

3901 GGACC AGGAA CATCT ACAGA GCCCT CTGAA GGCTC CGCTC CAGGG TCCCC
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 5 4101 GAGCC CCACC TCCAC TGAGG AGGGA TCTCC TGCTG GCTCT CCAAC ATCTA
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 15 4601 GTGGA GGTTC CGGTG GCGGG GGATC CCGTG GCGGG GGATC CCGTG GCGGG
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 20 4851 GGTCA AGTTC AACTG GTACG TGGAC GCGGT GGAGG TGCAT AATGC CAAGA
 4901 CAAAG CCGCG GGAGG AGCAG TACAA CAGCA CGTAC CGTGT GGTCA GCGTC
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 5051 CCAAA GGGCA GCCCC GAGAA CCACA GGTGT ACACC CTGCC CCCAT CCCGG
 25 5101 GATGA GCTGA CCAAG AACCA GGTC A CCTG ACCTG CCTGG TCAAA GGCTT
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 5301 CTTCT CATGC TCCGT GATGC ATGAG GCTCT GCACA ACCAC TACAC GCAGA
 30 5351 AGAGC CTCTC CCTGT CTCCG GTTAA ATGA

VWF034 Protein Sequence (SEQ ID NO: 87)

35 1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNVTDFGSM
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
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 40 251 ECTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
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 45 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
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 651 NCPKGQVYLQ CGTPCNLT CR SLSYPDEECN EACLEGCFCP PGLYMDERGD
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 50 751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
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901 NPGTFRILVG NKGC SHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
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 5 1101 CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
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 1501 SGSETPGTSE SATPESGPGT STEPSEGSAP DIGGGGGSGG GGS LVPRGSG
 1551 GDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 15 1601 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 1651 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
 1701 KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 1751 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

20

VWF050 nucleotide sequence (IHH triple mutant) (SEQ ID NO: 149)

1 ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
 51 GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GTTCA TCCAC GGCCC
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 25 151 TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
 201 ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
 251 TCTCC GTGTA TCTTG GGGAA TTTT TGACA TCCAT TTGTT TGTC AATGGT
 301 ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
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 451 TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
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 551 CTTAT GACTT TGCCA ACTCA TGGGC TCTGA GCAGT GGAGA ACAGT GGTGT
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 35 651 GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
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 15 2251 GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
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 25 2751 CTCAG TGAAA TGCAA GAAAC GGGTC ACCAT CCTGG TGGAG GGAGG AGAGA
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 4101 GTACC GTGTG GTCAG CGTCC TCACC GTCCT GGCCC AGGAC TGGCT GAATG
 4151 GCAAG GAGTA CAAGT GCAAG GTCTC CAACA AAGCC CTCCC AGCCC CCATC

4201 GAGAA AACCA TCTCC AAAGC CAAAG GGCAG CCCC AGAAC CACAG GTGTA
 4251 CACCC TGCCC CCATC CCGCG ATGAG CTGAC CAAGA ACCAG GTCAG CCTGA
 4301 CCTGC CTGGT CAAAG GCTTC TATCC CAGCG ACATC GCCGT GGAGT GGGAG
 4351 AGCAA TGGGC AGCCG GAGAA CAACT ACAAG ACCAC GCCTC CCGTG TTGGA
 5 4401 CTCCG ACGGC TCCTT CTTCC TCTAC AGCAA GCTCA CCGTG GACAA GAGCA
 4451 GGTGG CAGCA GGGGA ACGTC TTCTC ATGCT CCGTG ATGCA TGAGG CTCTG
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VWF050 protein sequence (IHH triple mutant) (SEQ ID NO: 150)

10 1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNNTFDGSM
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVLSVYLGE FFDIHLFVNG
 101 TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 15 251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTCEPC
 351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 451 LHNSLVKLKH GAGVAMDQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
 20 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL
 651 NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 25 751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
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 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901 NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
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 1151 PACQVTCQHP EPLACPVCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
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 35 1251 GSGGGSGGGG GSGGGSGGGG GSVLPRGSGG GSGGGGSDK THTPPCPAP
 1301 ELLGGPSVFL FPPKPKDTLM ASRTEVTCV VVDVSHEDPE VKFNWYVDGV
 1351 EVHNAKTKPR EEQYNSTYRV VSVLTVLAQD WLNGKEYKCK VSNKALPAPI
 1401 EKTISKAKGQ PREPQVYTL PPSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
 1451 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMEAL
 40 1501 HNAYTQKSLS LSPGK*

VWF057 nucleotide sequence (SEQ ID NO: 151)

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251 TCTCC GTGTA TCTTG GGGAA TTTT TGACA TCCAT TTGTT TGTCA ATGGT
 301 ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351 GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
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 5 451 TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
 501 CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
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 2351 AAGGG CTCGA GTGTA CCAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
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 45 2451 TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
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 30 4351 AAACC CAAGG ACACC CTCAT GATCT CCCGG ACCCC TGAGG TCACA TGCCT
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 35 4601 CAGCC CCCAT CGAGA AAACC ATCTC CAAAG CCAAA GGGCA GCCCC GAGAA
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 40 4851 GGACA AGAGC AGGTG GCAGC AGGGG AACGT CTTCT CATGC TCCGT GATGC
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 4951 GGTAAT ATGA

VWF057 protein sequence (SEQ ID NO: 152)

45 1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNFTFDGSM
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQ RVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 50 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
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 351 VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD

401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
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 5 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL
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 10 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGEQ YVLVQDYCGS
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 20 1351 TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA SSGGGGSGGG
 1401 GSGGGGSGGG GSGGGGSLVP RSGGGDKTHT CPPCPAPPELL GGPSVFLFPP
 1451 KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 1501 YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
 1551 PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYK TTP
 25 1601 PVLDS DGSFF LYSKLTVDKS RWQQGNVFC SVMHEALHNH YTQKSLSLSP
 1651 GK*

VWF058 nucleotide sequence (VWF034 with IHH mutation) (SEQ ID NO: 153)

30 1 ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
 51 GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GTTCA TCCAC GGCCC
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 201 ACGCT CCTTC TCGAT TATTG GGGGA TTCCA GAATG GCAAG AGAGT GAGCC
 35 251 TCTCC GTGTA TCTTG GGGAA TTTT TGACA TCCAT TTGTT TGTC AATGGT
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 351 GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
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 3201 CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
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 50 3651 TGACC CTGAG CACTG CCAGA TTTGC CACTG TGATG TTGTC AACCT CACCT
 3701 GTGAA GCCTG CCAGG AGCCG ATATC GGGTA CCTCA GAGTC TGCTA CCCCC
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 4251 ACCAG AGTCC GGTCC CGGGA CTTCA GAATC AGCAA CACCC GAGTC CGGCC
 10 4301 CTGGG TCTGA ACCCG CCACA AGTGG TAGTG AGACA CCAGG ATCAG AACCT
 4351 GCTAC CTCAG GGTCA GAGAC ACCCG GATCT CCGGC AGGCT CACCA ACCTC
 4401 CACTG AGGAG GGCAC CAGCA CAGAA CCAAG CGAGG GCTCC GCACC CGGAA
 4451 CAAGC ACTGA ACCCA GTGAG GGTTC AGCAC CCGGC TCTGA GCCGG CCACA
 4501 AGTGG CAGTG AGACA CCCGG CACTT CAGAG AGTGC CACCC CCGAG AGTGG
 15 4551 CCCAG GCACT AGTAC CGAGC CCTCT GAAGG CAGTG CGCCA GATTC TGGCG
 4601 GTGGA GGTTC CGGTG GCGGG GGATC CGGTG GCGGG GGATC CGGTG GCGGG
 4651 GGATC CGGTG GCGGG GGATC CCTGG TCCCC CGGGG CAGCG GAGGC GACAA
 4701 AACTC ACACA TGCCC ACCGT GCCCA GCTCC AGAAC TCCTG GCGGG ACCGT
 4751 CAGTC TTCCT CTTCC CCCC AAACC CAAGG ACACC CTCAT GGCCT CCCGG
 20 4801 ACCCC TGAGG TCACA TCGT GGTGG TGGAC GTGAG CCACG AAGAC CCTGA
 4851 GGTCA AGTTC AACTG GTACG TGGAC GCGGT GGAGG TGCAT AATGC CAAGA
 4901 CAAAG CCGCG GGAGG AGCAG TACAA CAGCA CGTAC CGTGT GGTCA GCGTC
 4951 CTCAC CGTCC TGGCC CAGGA CTGGC TGAAT GGCAA GGAGT ACAAG TGCAA
 5001 GGTCT CCAAC AAAGC CCTCC CAGCC CCCAT CGAGA AAACC ATCTC CAAAG
 25 5051 CCAAA GGGCA GCCCC GAGAA CCACA GGTGT ACACC CTGCC CCCAT CCCGC
 5101 GATGA GCTGA CCAAG AACCA GGTC A CCTG ACCTG CCTGG TCAAA GGCTT
 5151 CTATC CCAGC GACAT CGCCG TGGAG TGGGA GAGCA ATGGG CAGCC GGAGA
 5201 ACAAC TACAA GACCA CGCCT CCCGT GTTGG ACTCC GACGG CTCCT TCTTC
 5251 CTCTA CAGCA AGCTC ACCGT GGACA AGAGC AGGTG GCAGC AGGGG AACGT
 30 5301 CTTCT CATGC TCCGT GATGC ATGAG GCTCT GCACA ACGCC TACAC GCAGA
 5351 AGAGC CTCTC CCTGT CTCCG GTTAA ATGA

VWF058 protein sequence (VWF034 with IHH mutation) (SEQ ID NO: 154)

35 1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNVTFDGSM
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 40 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 45 451 LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL
 651 NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 50 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGEQ YVLVQDYCGS

901 NPGTFRILVG NKGCSPSVK CKKRVTLVE GGEIELFDGE VNVKRPMKDE
 951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 1051 MKQTMVDSSC RILTSDVFQD CNKLVDPPEY LDVCIYDTCES SIGDCAAF
 5 1101 CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
 1151 PACQVTCQHP EPLACPVCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 1201 VAGRRFASGK KVTNLNPSDPE HCQICHCDVV NLTCEACQEP ISGTSESATP
 1251 ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
 1301 GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
 10 1351 GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
 1401 SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
 1451 ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
 1501 SGSETPGTSE SATPESGPGT STEPSEGSAP DSGGGGSGGG GSGGGGSGGG
 1551 GSGGGGSLVP RGSBGDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMASR
 15 1601 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
 1651 LTVLAQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR
 1701 DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSGDSFF
 1751 LYSKLTVDKS RWQQGNVFSC SVMHEALHNA YTQKSLSLSP GK*

20

FVIII 169 nucleotide sequence (SEQ ID NO: 155)

1 ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGC GA TTCTG
 51 CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 25 101 ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 151 CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 201 GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 251 GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 301 GATAC AGTGG TCATT AACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 30 351 TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 401 ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTC CTGGT
 451 GGAAG CCATA CATAT TCTG CTAGG TCCTG AAAGA GAATG GTCCA ATGGC
 501 CTCTG ACCCA CTGTG CCTTA CCTA TCATA TCTTT CTCAT GTGGA CCTGG
 551 TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 35 601 GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 651 TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAA GAAC
 701 CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
 751 CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
 801 CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 40 851 AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 901 CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 951 ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
 1001 ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
 1051 GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
 45 1101 TGATC TTAAT GATTC TGAAG TGGAT GTGGT CAGGT TTGAT GATGA CAACT
 1151 CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAAT
 1201 TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
 1251 AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
 1301 GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATA
 50 1351 ACAGA TGAAG CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
 1401 CTTGG GACCT TTAAT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
 1451 TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
 1501 GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT

1551 GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
 1601 TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
 1651 TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
 1701 TGGCC CTCTC CTCAT CTGCT ACAA GAATC TGTA AGAGG AAACC
 5 1751 AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
 1801 AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
 1851 AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
 1901 ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
 1951 CATGA GGTGG CATA TGGTA CATT TAAGC ATTGG AGCAC AGACT GACTT
 10 2001 CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAA ATGGT CTATG
 2051 AAGAC AACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCC
 2101 ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
 2151 GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
 2201 CTGGT GATTA TTACG AGGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
 15 2251 AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAAA ACGGC GCGCC
 2301 AGGTA CCTCA GAGTC TGCTA CCCCC GAGTC AGGGC CAGGA TCAGA GCCAG
 2351 CCACC TCCGG GTCTG AGACA CCGG GACTT CCGAG AGTGC CACCC CTGAG
 2401 TCCGG ACCCG GGTCC GAGCC CGCCA CTTCC GGCTC CGAAA CTCCC GGCAC
 2451 AAGCG AGAGC GCTAC CCCAG AGTCA GGACC AGGAA CATCT ACAGA GCCCT
 20 2501 CTGAA GGCTC CGCTC CAGGG TCCCC AGCCG GCAGT CCCAC TAGCA CCGAG
 2551 GAGGG AACCT CTGAA AGCGC CACAC CCGAA TCAGG GCCAG GGTCT GAGCC
 2601 TGCTA CCAGC GGCAG CGAGA CACCA GGCAC CTCTG AGTCC GCCAC ACCAG
 2651 AGTCC GGACC CGGAT CTCCC GCTGG GAGCC CCACC TCCAC TGAGG AGGGA
 2701 TCTCC TGCTG GCTCT CCAAC ATCTA CTGAG GAAGG TACCT CAACC GAGCC
 25 2751 ATCCG AGGGA TCAGC TCCCG GCACC TCAGA GTCGG CAACC CCGGA GTCTG
 2801 GACCC GGAAC TTCCG AAAGT GCCAC ACCAG AGTCC GTTCC CGGGA CTTCA
 2851 GAATC AGCAA CACCC GAGTC CGGCC CTGGG TCTGA ACCCG CCACA AGTGG
 2901 TAGTG AGACA CCAGG ATCAG AACCT GCTAC CTCAG GTTCA GAGAC ACCCG
 2951 GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
 30 3001 CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
 3051 AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCGG CACTT
 3101 CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
 3151 GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC
 3201 TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG
 35 3251 ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATT ATGAT
 3301 GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
 3351 TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
 3401 CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG
 3451 AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
 40 3501 CCGTG GAGAA CTAAG TGAAC ATTTG GGACT CCTGG GGCCA TATAT AAGAG
 3551 CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
 3601 CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
 3651 AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
 3701 ACTTT TGGA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
 45 3751 TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
 3801 CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC AACT GAACC
 3851 CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
 3901 ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGA AGAAA
 3951 CTGCA GGGCT CCTG CAATA TCCAG ATGGA AGATC CCACT TTAA AGAGA
 50 4001 ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
 4051 TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
 4101 CAGCA ATGAA AACAT CCATT CTATT CATTT CAGTG GACAT GTGTT CACTG
 4151 TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT

4201 GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
 4251 GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC AACTT TTTTC
 4301 TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
 4351 ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
 5 4401 AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
 4451 AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTG GCACC AATGA TTATT
 4501 CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
 4551 CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
 4601 ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
 10 4651 TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
 4701 CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTGC CATGG
 4751 AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
 4801 AGTAA AGCAA TATCA GATGC ACAGA TTAAT GCTTC ATCCT ACTTT ACCAA
 4851 TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
 15 4901 GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
 4951 GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
 5001 AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
 5051 GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
 5101 GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
 20 5151 CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
 5201 ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
 5251 TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
 5301 CGGAC CGTCA GTCTT CCTCT TCCCC CAAA ACCCA AGGAC ACCCT CATGA
 5351 TCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
 25 5401 GACCC TGAGG TCAAG TCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
 5451 TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
 5501 TCAGC GTCCT CACCG TCCTG CACCA GGAAT GGCTG AATGG CAAGG AGTAC
 5551 AAGTG CAAGG TCTCC AACA AGCCC TCCA GCCCC CATCG AGAAA ACCAT
 5601 CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
 30 5651 CATCC CGGGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
 5701 AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
 5751 GCCGG AGAAG AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
 5801 CCTTC TTCCT CTACA GCAAG CTCAG CGTGG ACAAG AGCAG GTGGC AGCAG
 5851 GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC CACTA
 35 5901 CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA

FVIII 169 protein sequence(SEQ ID NO: 70)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL GELPVDARFP
 40 51 PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMA SDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TPPEVHSIFL EGHTFLVRNH
 45 301 RQASLEISPI TFLTAQTLLM DLGQFLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGDLE LIIFKNQASR PYNIPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 50 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL

751 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 801 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 901 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 5 951 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1001 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
 1051 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1101 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
 1151 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
 10 1201 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1251 CKAWAYFSDV DLEKDVHSGI IGPLL VCHTN TLNPAHGRQV TVQEFALFFT
 1301 IFDETKSWYF TENMERNCR PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1351 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1401 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 15 1451 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
 1501 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 1551 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1601 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1651 VDFQKTMKVT GVTQGVKSL LTSYVKEFL ISSQDGHQW TLFFQNGKVK
 20 1701 VFQGNQDSFT PVVNSLDPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 1751 YDKTHTCPPC PAPELLGPGS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 1801 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 1851 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDEL TKNQVSLTCLV
 1901 KGFYPSDIAV EWESNGQPN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 25 1951 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

FVIII 263 nucleotide sequence (IHH triple mutant) (SEQ ID NO: 156)

1 ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGCGA TTCTG
 30 51 CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 101 ACTAT ATGCA AGGCG CGCCA ACATC AGAGA GCGCC ACCCC TGAAA GTGGT
 151 CCCGG GAGCG AGCCA GCCAC ATCTG GGTCG GAAAC GCCAG GCACA AGTGA
 201 GTCTG CAACT CCCGA GTCCG GACCT GGCTC CGAGC CTGCC ACTAG CGGCT
 251 CCGAG ACTCC GGGAA CTTCC GAGAG CGCTA CACCA GAAAG CGGAC CCGGA
 35 301 ACCAG TACCG AACCT AGCGA GGGCT CTGCT CCGGG CAGCC CAGCC GGCTC
 351 TCCTA CATCC ACGGA GGAGG GCACT TCCGA ATCCG CCACC CCGGA GTCAG
 401 GGCCA GGATC TGAAC CCGCT ACCTC AGGCA GTGAG ACGCC AGGAA CGAGC
 451 GAGTC CGCTA CACCG GAGAG TGGGC CAGGG AGCCC TGCTG GATCT CCTAC
 501 GTCCA CTGAG GAAGG GTCAC CAGCG GGCTC GCCCA CCAGC ACTGA AGAAG
 40 551 GTGCC TCGAG CAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 601 CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 651 GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 701 GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 751 GATAC AGTGG TCATT AACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 45 801 TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 851 ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
 901 GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 951 CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
 1001 TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 50 1051 GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 1101 TTTTG CTGTA TTTGA TGAAG GGAAG AGTTG GCACT CAGAA ACAA GAACT
 1151 CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
 1201 CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG

1251 CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 1301 AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 1351 CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 1401 ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
 5 1451 ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
 1501 GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
 1551 TGATC TTACT GATTG TGAAG TGGAT GTGGT CAGGT TTGAT GATGA CAACT
 1601 CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAAT
 1651 TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GACT ATGCT CCCTT
 10 1701 AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
 1751 GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAAC
 1801 ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
 1851 CTTGG GACCT TTACT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
 1901 TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
 15 1951 GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
 2001 GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
 2051 TGAAT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
 2101 TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
 2151 TGGCC CTCTC CTCAT CTGCT ACAA GAATC TGTAG ATCAA AGAGG AAACC
 20 2201 AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTC TCTGT ATTTG ATGAG
 2251 AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
 2301 AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
 2351 ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
 2401 CATGA GGTGG CATAAC TGGTA CATTC TAAGC ATTGG AGCAC AGACT GACTT
 25 2451 CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAA ATGGT CTATG
 2501 AAGAC AACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
 2551 ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
 2601 GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
 2651 CTGGT GATTA TTACG AGGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
 30 2701 AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAA ACGGC GCGCC
 2751 AGGTA CCTCA GAGTC TGCTA CCCCC GAGTC AGGGC CAGGA TCAGA GCCAG
 2801 CCACC TCCGG GTCTG AGACA CCGCG GACTT CCGAG AGTGC CACCC CTGAG
 2851 TCCGG ACCCG GGTCC GAGCC CGCCA CTTCC GGCTC CGAAA CTCCC GGCAC
 2901 AAGCG AGAGC GCTAC CCCAG AGTCA GGACC AGGAA CATCT ACAGA GCCCT
 35 2951 CTGAA GGCTC CGCTC CAGGG TCCCC AGCCG GCAGT CCCAC TAGCA CCGAG
 3001 GAGGG AACCT CTGAA AGCGC CACAC CCGAA TCAGG GCCAG GGTCT GAGCC
 3051 TGCTA CCAGC GGCAG CGAGA CACCA GGCAC CTCTG AGTCC GCCAC ACCAG
 3101 AGTCC GGACC CGGAT TCCCC GCTGG GAGCC CCACC TCCAC TGAGG AGGGA
 3151 TCTCC TGCTG GCTCT CCAAC ATCTA CTGAG GAAGG TACCT CAACC GAGCC
 40 3201 ATCCG AGGGA TCAGC TCCCG GCACC TCAGA GTCGG CAACC CCGGA GTCTG
 3251 GACCC GGAAC TTCCG AAAGT GCCAC ACCAG AGTCC GGTCC CGGGA CTTCA
 3301 GAATC AGCAA CACCC GAGTC CGGCC CTGGG TCTGA ACCCG CCACA AGTGG
 3351 TAGTG AGACA CCAGG ATCAG AACCT GCTAC CTCAG GTTCA GAGAC ACCCG
 3401 GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
 45 3451 CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
 3501 AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCCGG CACTT
 3551 CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
 3601 GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC
 3651 TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG
 50 3701 ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATTT ATGAT
 3751 GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
 3801 TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
 3851 CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG

3901 AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
 3951 CCGTG GAGAA CTAAG TGAAC ATTTG GGAAT CCTGG GGCCA TATAT AAGAG
 4001 CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
 4051 CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
 5 4101 AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
 4151 ACTTT TGGAA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
 4201 TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
 4251 CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC AACTT GAACC
 4301 CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
 10 4351 ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGAA AGAAA
 4401 CTGCA GGGCT CCTCG CAATA TCCAG ATGGA AGATC CCACT TTAA AGAGA
 4451 ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
 4501 TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
 4551 CAGCA ATGAA AACAT CCATT CTATT CATT T CAGTG GACAT GTGTT CACTG
 15 4601 TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT
 4651 GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
 4701 GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC AACTT TTTTC
 4751 TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
 4801 ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
 20 4851 AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
 4901 AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTG GCACC AATGA TTATT
 4951 CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
 5001 CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
 5051 ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
 25 5101 TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
 5151 CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCC CATGG
 5201 AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
 5251 AGTAA AGCAA TATCA GATGC ACAGA TTAAT GCTTC ATCCT ACTTT ACCAA
 5301 TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
 30 5351 GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
 5401 GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
 5451 AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
 5501 GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
 5551 GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
 35 5601 CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
 5651 ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
 5701 TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
 5751 CGGAC CGTCA GTCTT CCTCT TCCCC CAAA ACCCA AGGAC ACCCT CATGG
 5801 CCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
 40 5851 GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
 5901 TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
 5951 TCAGC GTCCT CACCG TCCTG GCCCA GGAAT GGCTG AATGG CAAGG AGTAC
 6001 AAGTG CAAGG TCTCC AACA AGCCC TCCA GCCCC CATCG AGAAA ACCAT
 6051 CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
 45 6101 CATCC CGCGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
 6151 AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
 6201 GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
 6251 CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
 6301 GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC GCCTA
 50 6351 CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA

FVIII 263 protein sequence(IHH triple mutant) (SEQ ID NO: 157)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP TSESATPESG
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101 TSTEPSEGS SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 151 ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 5 201 PRVPSFPPFN TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRSLPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 10 451 RQASLEISPI TFLTAQTLML DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601 TDETFKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR PYNIPHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 15 701 YYSSFVNMR DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEYEDTLTLF PFSGETVFMS
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSSESATPE
 20 951 SGPGSEPATSG SETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 1001 EGTSESATPE SGPGSEPATSG SETPGTSES ATPESGPGSP AGSPTSTEEG
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSSESATPE SGPSTSTEPS
 25 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPPQFK
 1301 KVVVFQFTDG SFTQPLRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
 1351 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1401 CKAWAYFSDV DLEKDVHSGI IGPLLCHTN TLNPAHGRQV TVQEFALFFT
 30 1451 IFDETKSWYF TENMERNCR PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1551 VFETVEMLP KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPGLMASGH
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 35 1701 SSGIKHNIFN PPIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1801 VDFQKTMKVT GVTQGVKSL LSTMYVKEFL ISSSQDGHQW TLFFQNGKVK
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 1901 YDKTHTCPPC PAPELLGGPS VFLFPKPKD TLMASRTPEV TCVVVDVSHE
 40 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL AQDWLNGKEY
 2001 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDEL TKNQVSLTCLV
 2051 KGFYPSDIAV EWESNGQPN NYKTTPVLD SDGSFFLYSK LTVDKSRWQQ
 2101 GNVFSCSVMH EALHNAYTQK SLSLSPGK*

45 **FVIII 282 nucleotide sequence(SEQ ID NO: 158)**

1 ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGCGA TTCTG
 51 CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 101 ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 50 151 CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 201 GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 251 GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 301 GATAC AGTGG TCATT AACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT

351 TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 401 ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
 451 GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 501 CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
 5 551 TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 601 GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 651 TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAA GAACT
 701 CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
 751 CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
 10 801 CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 851 AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 901 CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 951 ACTCT TGATG GACCT TGGAC AGTTT CTA CT GTTTT GTCAT ATCTC TTCCC
 1001 ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
 15 1051 GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
 1101 TGATC TTA CT GATTG TGAAG TGGAT GTGGT CAGGT TTGAT GATGA CAACT
 1151 CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAAT
 1201 TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
 1251 AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
 20 1301 GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATACT
 1351 ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
 1401 CTTGG GACCT TTA CT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
 1451 TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
 1501 GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
 25 1551 GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
 1601 TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
 1651 TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
 1701 TGGCC CTCTC CTCAT CTGCT ACAA GAATC TGTA AGATA AGAGG AAACC
 1751 AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
 30 1801 AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
 1851 AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
 1901 ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
 1951 CATGA GGTGG CATAG TGGTA CATTG TAAGC ATTGG AGCAC AGACT GACTT
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 35 2051 AAGAC AACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
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 2151 GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
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 40 2301 AACAT CAGAG AGCGC CACCC CTGAA AGTGG TCCCG GGAGC GAGCC AGCCA
 2351 CATCT GGGTC GGAAA CGCCA GGCAC AAGTG AGTCT GCAAC TCCCG AGTCC
 2401 GGACC TGGCT CCGAG CCTGC CACTA GCGGC TCCGA GACTC CGGGA ACTTC
 2451 CGAGA GCGCT ACACC AGAAA GCGGA CCCGG AACCA GTACC GAACC TAGCG
 2501 AGGGC TCTGC TCCGG GCAGC CCAGC CGGCT CTCCT ACATC CACGG AGGAG
 45 2551 GGCAC TTCCG AATCC GCCAC CCCGG AGTCA GGGCC AGGAT CTGAA CCCGC
 2601 TACCT CAGGC AGTGA GACGC CAGGA ACGAG CGAGT CCGCT ACACC GGAGA
 2651 GTGGG CCAGG GAGCC CTGCT GGATC TCCTA CGTCC ACTGA GGAAG GGTC
 2701 CCAGC GGGCT CGCCC ACCAG CACTG AAGAA GGTGC CTCGA GCCCA CCAGT
 2751 CTTGA AACGC CATCA AGCTG AAATA ACTCG TACTA CTCTT CAGTC AGATC
 50 2801 AAGAG GAAAT CGATT ATGAT GATAC CATAT CAGTT GAAAT GAAGA AGGAA
 2851 GATTT TGACA TTTAT GATGA GGATG AAAAT CAGAG CCCCC GCAGC TTTC
 2901 AAAGA AAACA CGACA CTATT TTATT GCTGC AGTGG AGAGG CTCTG GGATT
 2951 ATGGG ATGAG TAGCT CCCCC CATGT TCTAA GAAAC AGGGC TCAGA GTGGC

3001 AGTGT CCCTC AGTTC AAGAA AGTTG TTTTC CAGGA ATTTA CTGAT GGCTC
 3051 CTTTA CTCAG CCCTT ATACC GTGGA GAACT AAATG AACAT TTGGG ACTCC
 3101 TGGGG CCATA TATAA GAGCA GAAGT TGAAG ATAAT ATCAT GGTA CTTTC
 3151 AGAAA TCAGG CCTCT CGTCC CTATT CCTTC TATTC TAGCC TTATT TCTTA
 5 3201 TGAGG AAGAT CAGAG GCAAG GAGCA GAACC TAGAA AAAAC TTTGT CAAGC
 3251 CTAAT GAAAC CAAAA CTTAC TTTTG GAAAG TGCAA CATCA TATGG CACCC
 3301 ACTAA AGATG AGTTT GACTG CAAAG CCTGG GCTTA TTTCT CTGAT GTTGA
 3351 CCTGG AAAAA GATGT GCACT CAGGC CTGAT TGGAC CCCTT CTGGT CTGCC
 3401 AACT AACAC ACTGA ACCCT GCTCA TGGGA GACAA GTGAC AGTAC AGGAA
 10 3451 TTTGC TCTGT TTTTC ACCAT CTTTG ATGAG ACCAA AAGCT GGTAC TTCAC
 3501 TGAAA ATATG GAAAG AAAC TGCAGG GCTCC CTGCA ATATC CAGAT GGAAG
 3551 ATCCC ACTTT TAAAG AGAAT TATCG CTTCC ATGCA ATCAA TGGCT ACATA
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 15 3701 GTGGA CATGT GTTCA CTGTA CGAAA AAAAG AGGAG TATAA AATGG CACTG
 3751 TACAA TCTCT ATCCA GGTGT TTTTG AGACA GTGGA AATGT TACCA TCCAA
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 20 3951 ATATG GACAG TGGGC CCCAA AGCTG GCCAG ACTTC ATTAT TCCGG ATCAA
 4001 TCAAT GCCTG GAGCA CCAAG GAGCC CTTTT CTTGG ATCAA GGTGG ATCTG
 4051 TTGGC ACCAA TGATT ATTCA CGGCA TCAAG ACCCA GGGTG CCCGT CAGAA
 4101 GTTCT CCAGC CTCTA CATCT CTCAG TTTAT CATCA TGTAT AGTCT TGATG
 4151 GGAAG AAGTG GCAGA CTTAT CGAGG AAATT CCACT GGAAC CTAA TGGTC
 25 4201 TTCTT TGGCA ATGTG GATTC ATCTG GGATA AAACA CAATA TTTTT AACCC
 4251 TCCAA TTATT GCTCG ATACA TCCGT TTGCA CCCAA CTCAT TATAG CATT
 4301 GCAGC ACTCT TCGCA TGGAG TTGAT GGGCT GTGAT TTAAA TAGTT GCAGC
 4351 ATGCC ATTGG GAATG GAGAG TAAAG CAATA TCAGA TGCAC AGATT ACTGC
 4401 TTCAT CCTAC TTTAC CAATA TGTTT GCCAC CTGGT CTCCT TCAAA AGCTC
 30 4451 GACTT CACCT CCAAG GGAGG AGTAA TGCCT GGAGA CCTCA GGTGA ATAAT
 4501 CCAAA AGAGT GGCTG CAAGT GGAAT TCCAG AAGAC AATGA AAGTC ACAGG
 4551 AGTAA CTACT CAGGG AGTAA AATCT CTGCT TACCA GCATG TATGT GAAGG
 4601 AGTTT CTCAT CTCCA GCAGT CAAGA TGGCC ATCAG TGGAC TCTCT TTTTT
 4651 CAGAA TGGCA AAGTA AAGGT TTTTC AGGGA AATCA AGACT CCTTC ACACC
 35 4701 TGTGG TGAAC TCTCT AGACC CACCG TTAAT GACTC GCTAC CTTTC AATTC
 4751 ACCCC CAGAG TTGGG TGCAC CAGAT TGCCC TGAGG ATGGA GTTTC TGGGC
 4801 TGCGA GGCAC AGGAC CTCTA CGACA AAAC CACAC ATGCC CACCG TGCCC
 4851 AGCTC CAGAA CTCCT GGGCG GACCG TCAGT CTTCC TCTTC CCCCC AAAAC
 4901 CCAAG GACAC CCTCA TGATC TCCCG GACCC CTGAG GTCAC ATGCG TGGTG
 40 4951 GTGGA CGTGA GCCAC GAAGA CCCTG AGGTC AAGTT CAACT GGTAC GTGGA
 5001 CGGCG TGGAG GTGCA TAATG CCAAG ACAA GCCGC GGGAG GAGCA GTACA
 5051 ACAGC ACGTA CCGTG TGGTC AGCGT CCTCA CCGTC CTGCA CCAGG ACTGG
 5101 CTGAA TGGCA AGGAG TACAA GTGCA AGGTC TCCAA CAAAG CCCTC CCAGC
 5151 CCCC TCGAG AAAAC CATCT CCAAA GCCAA AGGGC AGCCC CGAGA ACCAC
 45 5201 AGGTG TACAC CCTGC CCCC TCCCG GGATG AGCTG ACCAA GAACC AGGTC
 5251 AGCCT GACCT GCCTG GTCAA AGGCT TCTAT CCCAG CGACA TCGCC GTGGA
 5301 GTGGG AGAGC AATGG GCAGC CGGAG ACAA CTACA AGACC ACGCC TCCCG
 5351 TGTTG GACTC CGACG GCTCC TTCTT CCTCT ACAGC AAGCT CACCG TGGAC
 5401 AAGAG CAGGT GGCAG CAGGG GAACG TCTTC TCATG CTCCG TGATG CATGA
 50 5451 GGCTC TGCAC AACCA CTACA CGCAG AAGAG CCTCT CCCTG TCTCC GGGTA
 5501 AATGA

FVIII 282 protein sequence(SEQ ID NO: 159)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL GELPVDARFP
 51 PRVPKSF PFN TSVVYKKT LF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR EKEDDKVFPG
 5 151 GSHTYVWQVL KENGP MASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 10 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 15 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDY YED SYEDISAYLL
 751 SKNNAIEPRS FSQNGAPTSE SATPESGPGS EPATSGSETP GTSSESATPES
 801 GPGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPGS PAGSPTSTEE
 851 GTSSESATPES GPGSEPATSG SETPGTSESA TPESGPGSPA GSPTSTEEGS
 20 901 PAGSPTSTEE GASSPPVLKR HQAEITRTTL QSDQEEIDYD DTISVEMKKE
 951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
 1001 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 1051 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQH HMAP
 1101 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
 25 1151 FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
 1201 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1251 YNLYPGVFET VEMPLSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1301 GMASGHIRDF QITASGQY GQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1351 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 30 1401 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 1451 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1501 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1551 QNGKVKVFQ NQDSFTP VVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG
 1601 CEAQDLYDKT HTCPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 35 1651 VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW
 1701 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 1751 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
 1801 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

40

FVIII 283 nucleotide sequence (FVIII 169 with IHH triple mutation) (SEQ ID NO: 160)

1 ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGCGA TTCTG
 51 CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 45 101 ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 151 CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
 201 GACTC TGT TT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 251 GGCCA CCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 301 GATAC AGTGG TCATT AACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 50 351 TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 401 ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
 451 GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 501 CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG

551 TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 601 GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 651 TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAA GAACT
 701 CTTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
 5 751 CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
 801 CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 851 AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 901 CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 951 ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
 10 1001 ACCAA CATGA TGGCA TGGAA GCTTA TGTCA AAGTA GACAG CTGTC CAGAG
 1051 GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
 1101 TGATC TTAAT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
 1151 CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAATC
 1201 TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
 15 1251 AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
 1301 GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAAC
 1351 ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
 1401 CTTGG GACCT TTAAT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
 1451 TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
 20 1501 GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
 1551 GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
 1601 TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
 1651 TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
 1701 TGGCC CTCTC CTCAT CTGCT ACAA GAATC TGTAAG ATCAA AGAGG AAACC
 25 1751 AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
 1801 AACCG AAGCT GGTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
 1851 AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC
 1901 ACAGC ATCAA TGGCT ATGTT TTTGA TAGTT TGCAG TTGTC AGTTT GTTTG
 1951 CATGA GGTGG CATAAC TGGTA CATTG TAAGC ATTGG AGCAC AGACT GACTT
 30 2001 CCTTT CTGTC TTCTT CTCTG GATAT ACCTT CAAAC ACAA ATGGT CTATG
 2051 AAGAC AACT CACCC TATTC CCATT CTCAG GAGAA ACTGT CTTCA TGTCG
 2101 ATGGA AAACC CAGGT CTATG GATTC TGGGG TGCCA CAACT CAGAC TTTCG
 2151 GAACA GAGGC ATGAC CGCCT TACTG AAGGT TTCTA GTTGT GACAA GAACA
 2201 CTGGT GATTA TTACG AGGAC AGTTA TGAAG ATATT TCAGC ATACT TGCTG
 35 2251 AGTAA AAACA ATGCC ATTGA ACCAA GAAGC TTCTC TCAAA ACGGC GCGCC
 2301 AGGTA CCTCA GAGTC TGCTA CCCCC GAGTC AGGGC CAGGA TCAGA GCCAG
 2351 CCACC TCCGG GTCTG AGACA CCGGG GACTT CCGAG AGTGC CACCC CTGAG
 2401 TCCGG ACCCG GGTCC GAGCC CGCCA CTTCC GGCTC CGAAA CTCCC GGCAC
 2451 AAGCG AGAGC GCTAC CCCAG AGTCA GGACC AGGAA CATCT ACAGA GCCCT
 40 2501 CTGAA GGCTC CGCTC CAGGG TCCCC AGCCG GCAGT CCCAC TAGCA CCGAG
 2551 GAGGG AACCT CTGAA AGCGC CACAC CCGAA TCAGG GCCAG GGTCT GAGCC
 2601 TGCTA CCAGC GGCAG CGAGA CACCA GGCAC CTCTG AGTCC GCCAC ACCAG
 2651 AGTCC GGACC CGGAT TCCCC GCTGG GAGCC CCACC TCCAC TGAGG AGGGA
 2701 TCTCC TGCTG GCTCT CCAAC ATCTA CTGAG GAAGG TACCT CAACC GAGCC
 45 2751 ATCCG AGGGA TCAGC TCCCC GCACC TCAGA GTCGG CAACC CCGGA GTCTG
 2801 GACCC GGAAC TTCCG AAAGT GCCAC ACCAG AGTCC GGTCC CGGGA CTTCA
 2851 GAATC AGCAA CACCC GAGTC CGGCC CTGGG TCTGA ACCCG CCACA AGTGG
 2901 TAGTG AGACA CCAGG ATCAG AACCT GCTAC CTCAG GGTCA GAGAC ACCCG
 2951 GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
 50 3001 CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
 3051 AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCGGG CACTT
 3101 CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
 3151 GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC

3201 TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG
 3251 ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATTG ATGAT
 3301 GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
 3351 TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
 5 3401 CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG
 3451 AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
 3501 CCGTG GAGAA CTAAG TGAAC ATTTG GGAAT CCTGG GGCCA TATAT AAGAG
 3551 CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
 3601 CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
 10 3651 AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
 3701 ACTTT TGGAA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
 3751 TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
 3801 CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC AACTT GAACC
 3851 CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
 15 3901 ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGAA AGAAA
 3951 CTGCA GGGCT CCCTG CAATA TCCAG ATGGA AGATC CCACT TTAA AGAGA
 4001 ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
 4051 TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
 4101 CAGCA ATGAA AACAT CCATT CTATT CATTG CAGTG GACAT GTGTT CACTG
 20 4151 TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT
 4201 GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
 4251 GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC AACTT TTTTC
 4301 TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
 4351 ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
 25 4401 AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
 4451 AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTG GCACC AATGA TTATT
 4501 CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
 4551 CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
 4601 ATCGA GGAAG TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
 30 4651 TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
 4701 CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCC CATGG
 4751 AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
 4801 AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
 4851 TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
 35 4901 GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
 4951 GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
 5001 AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
 5051 GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
 5101 GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
 40 5151 CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
 5201 ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
 5251 TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
 5301 CGGAC CGTCA GTCTT CCTCT TCCCC CAAA ACCCA AGGAC ACCCT CATGG
 5351 CCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
 45 5401 GACCC TGAGG TCAAG TCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
 5451 TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
 5501 TCAGC GTCCT CACCG TCCTG GCCCA GGAAT GGCTG AATGG CAAGG AGTAC
 5551 AAGTG CAAGG TCTCC ACAA AGCCC TCCA GCCCC CATCG AGAAA ACCAT
 5601 CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
 50 5651 CATCC CGGGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
 5701 AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
 5751 GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
 5801 CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG

5851 GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC GCCTA
 5901 CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA

5

FVIII 283 protein sequence (FVIII 169 with IHH triple mutation) (SEQ ID NO: 161)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL GELPVDARFP
 10 51 PRVPKSFPFN TSVVYKKTLLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKA SEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 15 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAEEEE DWDYAPLVA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 20 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDY YED SYEDISAYLL
 751 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 25 801 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 901 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 951 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1001 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
 30 1051 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1101 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSV PQFK
 1151 KVVVFQFTDG SFTQPLRYGE LNEHLG LLGP YIRAEVEDNI MVTFRNQASR
 1201 PYVFGYSSLIS YEEDQRQGA EPRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1251 CKAWAYFSDV DLEKDVHSG LIGPLL VCHTN TLNPAHGRQV TVQEALFFT
 35 1301 IFDETKSWYF TENMERN CRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1351 LVMAQDQ RIR WYLLSMGSNE NIHSIHFS GH VFTVRKKEEY KMALYNLYPG
 1401 VFETVEM LPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 1451 IRDFQITASG QYGQWAPKLA RLHYS GSINA WSTKEPFSWI KVDLLAPMII
 1501 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 40 1551 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1601 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1651 VDFQKTMKVT GVT TQGVKSL L TSMYVKEFL ISSSQDGHQW TLFFQNGKVK
 1701 VFQGNQDSFT PVVNSLD PPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 1751 YDKTHTCPPC PAPELLG GPS VFLFPPKPKD TLMASRTPEV TCVVVDVSHE
 45 1801 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL AQDWLNGKEY
 1851 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDEL TKNQVSLTCLV
 1901 KGFYPSDIAV EWESNGQ PEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 1951 GNVFSCSVMH EALHNAYTQK SLSLSPGK*

pSYNFVIII 010 nucleotide sequence-(Dual chain FVIII Fc) (SEQ ID NO: 162)

1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
 51 CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGA A CTGTCATGGG

101 ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCCCT
 151 CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
 201 GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
 251 GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 5 301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT
 351 TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG
 401 ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT CTTCCCTGGT
 451 GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG GTCCAATGGC
 501 CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT GTGGACCTGG
 10 551 TAAAAGACTT GAATTGAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT
 651 TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT
 701 CCTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG GCCTAAAATG
 751 CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC TGATTGGATG
 15 801 CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAAATGGGC ACCACTCCTG
 851 AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
 901 CGCCAGGCGT CTTTGGAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC
 951 ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCTAT ATCTCTTCCC
 1001 ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG CTGTCCAGAG
 20 1051 GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG ACTATGATGA
 1101 TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT GATGACAACT
 1151 CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
 1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT
 1251 AGTCCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG
 25 1301 GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT TATGGCATAAC
 1351 ACAGATGAAA CCTTTAAGAC TCGTGAAGCT ATTCAGCATG AATCAGGAAT
 1401 CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG TTGATTATAT
 1451 TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
 1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT
 30 1551 GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG
 1601 TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG CCGACCCGC
 1651 TATTACTCTA GTTTCGTTAA TATGGAGAGA GATCTAGCTT CAGGACTCAT
 1701 TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA AGAGGAAACC
 1751 AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
 35 1801 AACC GAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC
 1851 AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC
 1901 ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC AGTTTGTGTTG
 1951 CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
 2001 CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA ATGGTCTATG
 40 2051 AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTTATGTCTG
 2101 ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACCT CAGACTTTTCG
 2151 GAACAGAGGC ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA
 2201 CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTTCAGC ATACTTGCTG
 2251 AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCTCTCAA ACCCACCAGT
 45 2301 CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT CAGTCAGATC
 2351 AAGAGGAAAT TGACTATGAT GATACCATAT CAGTTGAAAT GAAGAAGGAA
 2401 GATTTTGACA TTTATGATGA GGATGAAAAT CAGAGCCCCC GCAGCTTTCA
 2451 AAAGAAAACA CGACACTATT TTATTGCTGC AGTGGAGAGG CTCTGGGATT
 2501 ATGGGATGAG TAGCTCCCCA CATGTTCTAA GAAACAGGGC TCAGAGTGGC
 50 2551 AGTGTCCCTC AGTTCAAGAA AGTTGTTTTC CAGGAATTTA CTGATGGCTC
 2601 CTTTACTCAG CCCTTATACC GTGGAGAACT AAATGAACAT TTGGGACTCC
 2651 TGGGGCCATA TATAAGAGCA GAAGTTGAAG ATAATATCAT GGTAACCTTC
 2701 AGAAATCAGG CCTCTCGTCC CTATTCCTTC TATTCTAGCC TTATTTCTTA

2751 TGAGGAAGAT CAGAGGCAAG GAGCAGAACC TAGAAAAAAC TTTGTCAAGC
 2801 CTAATGAAAC CAAAACCTTAC TTTTGGAAG TGCAACATCA TATGGCACCC
 2851 ACTAAAGATG AGTTTGACTG CAAAGCCTGG GCTTATTTCT CTGATGTTGA
 2901 CCTGGAAAAA GATGTGCACT CAGGCCTGAT TGGACCCCTT CTGGTCTGCC
 5 2951 AACTAACAC ACTGAACCCT GCTCATGGGA GACAAGTGAC AGTACAGGAA
 3001 TTTGCTCTGT TTTTCACCAT CTTTGATGAG ACCAAAAGCT GGTACTTCAC
 3051 TGAAAATATG GAAAGAACT GCAGGGCTCC CTGCAATATC CAGATGGAAG
 3101 ATCCCACCTT TAAAGAGAAT TATCGCTTCC ATGCAATCAA TGGCTACATA
 3151 ATGGATACAC TACCTGGCTT AGTAATGGCT CAGGATCAAA GGATTCTGATG
 10 3201 GTATCTGCTC AGCATGGGCA GCAATGAAAA CATCCATTCT ATTCATTTCA
 3251 GTGGACATGT GTTCACTGTA CGAAAAAAG AGGAGTATAA AATGGCACTG
 3301 TACAATCTCT ATCCAGGTGT TTTTGAGACA GTGGAAATGT TACCATCCAA
 3351 AGCTGGAATT TGGCGGGTGG AATGCCTTAT TGGCGAGCAT CTACATGCTG
 3401 GGATGAGCAC ACTTTTCTG GTGTACAGCA ATAAGTGTCA GACTCCCCTG
 15 3451 GGAATGGCTT CTGGACACAT TAGAGATTTT CAGATTACAG CTTTCCAGGACA
 3501 ATATGGACAG TGGGCCCCAA AGCTGGCCAG ACTTCATTAT TCCGGATCAA
 3551 TCAATGCCTG GAGACCAAG GAGCCCTTTT CTTGGATCAA GGTGGATCTG
 3601 TTGGCACCAA TGATTATTCA CGGCATCAAG ACCCAGGGTG CCCGTCAGAA
 3651 GTTCTCCAGC CTCTACATCT CTCAGTTTAT CATCATGTAT AGTCTTGATG
 20 3701 GGAAGAAGTG GCAGACTTAT CGAGGAAATT CCACTGGAAC CTTAATGGTC
 3751 TTCTTTGGCA ATGTGGATTG ATCTGGGATA AAACACAATA TTTTAAACCC
 3801 TCCAATTATT GCTCGATACA TCCGTTTGCA CCAACTCAT TATAGCATTC
 3851 GCAGCACTCT TCGCATGGAG TTGATGGGCT GTGATTTAAA TAGTTGCAGC
 3901 ATGCCATTGG GAATGGAGAG TAAAGCAATA TCAGATGCAC AGATTACTGC
 25 3951 TTCATCTAC TTTACCAATA TGTTTGCCAC CTGGTCTCCT TCAAAAGCTC
 4001 GACTTCACCT CCAAGGGAGG AGTAATGCCT GGAGACCTCA GGTGAATAAT
 4051 CAAAAGAGT GGCTGCAAGT GGAATCCAG AAGACAATGA AAGTCACAGG
 4101 AGTAATACT CAGGGAGTAA AATCTCTGCT TACCAGCATG TATGTGAAGG
 4151 AGTTCTCAT CTCCAGCAGT CAAGATGGCC ATCAGTGGAC TCTCTTTTTT
 30 4201 CAGAATGGCA AAGTAAAGGT TTTTCAGGGA AATCAAGACT CTTTACACCC
 4251 TGTGGTGAAC TCTCTAGACC CACCGTTACT GACTCGCTAC CTTTGAATTC
 4301 ACCCCAGAG TTGGGTGCAC CAGATTGCCC TGAGGATGGA GGTTCGGGC
 4351 TCGGAGGCAC AGGACCTCTA CGACAAAAC CACACATGCC CACCGTGCCC
 4401 AGCTCCAGAA CTCCTGGGCG GACCGTCAGT CTTCTCTTC CCCCCAAAAC
 35 4451 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
 4501 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
 4551 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
 4601 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
 4651 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
 40 4701 CCCCATCGAG AAAACCATCT CCAAGCCAA AGGGCAGCCC CGAGAACCAC
 4751 AGGTGTACAC CCTGCCCCCA TCCCGGGATG AGCTGACCAA GAACCAGGTC
 4801 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
 4851 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCC
 4901 TGTTGGACTC CGACGGCTCC TTCTTCTCT ACAGCAAGCT CACCGTGGAC
 45 4951 AAGAGCAGGT GGCAGCAGGG GAACGTCTC TCATGCTCCG TGATGCATGA
 5001 GGCTCTGCAC AACCCTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGTA
 5051 AATGA

pSYNFVIII 010 protein sequence-(Dual chain FVIIIc) (SEQ ID NO: 163)

50 1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL GELPVDARFP
 51 PRVPKSPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFP

151 GSHTYVWQVL KENGPMA SDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 5 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 10 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MUYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD DTISVEMKKE
 801 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
 15 851 SVPQFKKVV FQEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQH HMAP
 951 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
 1001 FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
 1051 MDTLPGLVMA QDQIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 20 1101 YNLYPGVFET VEMPLSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1151 GMASGHIRDF QITASGQYGG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1201 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1251 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 1301 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 25 1351 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1401 QNGKVKVFQ NQDSFTPVVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG
 1451 CEAQDLYDKT HTCPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 1501 VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW
 1551 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 30 1601 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDS DGS FFLYSKLTVD
 1651 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

FVIII 195 protein sequence (dual chain FVIII Fc with two 144 AE XTENs at amino acid 1656 and 1900) (SEQ ID NO: 73)

35 1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL GELPVDARFP
 51 PRVPKSFPFN TSVVYKKT LF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMA SDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 40 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 45 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MUYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 50 751 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QGAPGTPGSG TASSSPGASP
 801 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 851 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA

901 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
 951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
 1001 SVPQFKKVV FQEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 1051 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQHHPMAP
 5 1101 TKDEFDCKAW AYFSDVDLEK DVHSLGIGPL LVCHTNLTNP AHGRQVTVQE
 1151 FALFFTIFDE TKSIFYFTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
 1201 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSA PG
 1251 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
 1301 ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
 10 1351 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1401 YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL VYSNKQQTPL
 1451 GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1501 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1551 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 15 1601 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1651 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1701 QNGKVKVFQ NQDSFTPVVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG
 1751 CEAQDLYDKT HTCPPCAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 1801 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
 20 1851 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 1901 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGGS FFLYSKLTVD
 1951 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

25 **pSYN-FVIII-173 mature Protein sequencing (SEQ ID NO: 72):**

1 ATRRYYLGA VELS WDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKCTL
 51 FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVS LHA
 101 VGVSYWKASE GA EYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
 151 PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
 30 201 VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
 251 KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
 301 MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
 351 TDSEMDVVR FDDD NPSFIQ IRSAKKHPK TWVHYIAAEE EDWDYAPLVL
 401 APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDFTFKTRE AIQHESGILG
 35 451 PLYGEV GDTLLIIFKNQAS RPYNIYPHGI TDVRPLYSR LPKG VKHLKD
 501 FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSFNME RDLASGLIGP
 551 LLICYKESVD QRGNQIMSDK RNVLFSVFD ENRSWYLTEN IQRFLPNPAG
 601 VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
 651 VFFSGYTFKH KVMYEDTLTL PFPSGETVFM SMENPGLWIL GCHNSDFRNR
 40 701 GMTALLKVSS CDKNTGDYEE DSYEDISAYL LSKNNAIEPR SFSQNGAPGT
 751 SESATPESGP GSEPATSGSE TPGTSESATP ESGPGSEPA TSGSETPGTSE
 801 SATPESGPGT STEPSEGSA PGPAGSPTST EEGTSESATP ESGPGSEPA T
 851 SGSETPGTSE SATPESGPGS PAGSPTSTEE GSPAGSPTST EEGTSTEPSE
 901 GSAPGTSESA TPESGPGTSE SATPESGPGT SESATPESGP GSEPATSGSE
 45 951 TPGSEPA TSG SETPGSPAGS PTSTEEGTST EPSEGSA PGT STEPSEGSA P
 1001 GSEPATSGSE TPGTSESATP ESGPGTSTEP SEGSA PASS PVLKRHQREI
 1051 TRTTLQSDQE EIDYDDTISV EMKEDFDIY DEDENQSPRS FQKKTRHYFI
 1101 AAVERLWDYG MSSPHVLNR RAQSGSVPQF KKVV FQEFTD GSFTQPLYRG
 1151 ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYSFYSLI SYEEDQRQGA
 50 1201 EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF DCKAWAYFSD VDLEKDVHSG
 1251 LIGPLLVCHT NTLNPAHGRQ VTVQE FALFF TIFDETKSWY FTENMERNCR
 1301 APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI RWYLLSMGSN
 1351 ENIHSIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP SKAGIWRVEC

1401 LIGEHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS GQYGQWAPKL
 1451 ARLHYSGSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR QKFSSLYISQ
 1501 FIIMYSLDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNIF NPPIARYIR
 1551 LHPHTYSIRS TLRMELMGCD LNSCSMPLGM ESKAISDAQI TASSYFTNMF
 5 1601 ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV TGVTTQGVKS
 1651 LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV KVFQGNQDSF TPVVNSLDPP
 1701 LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LYDKTHTCPP CPAPELLGGP
 1751 SVFLFPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK
 1801 TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK
 10 1851 AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE
 1901 NNYKTPPV L DSDGSFFLYS KLTVDKSRWQ QGNVFSCSV HEALHNHYTQ
 1951 KSLSLSPGK

FVIII 196 protein sequence (dual chain FVIII Fc with three 144 AE XTENs at amino acid 26, 1656 and 1900) (SEQ ID NO: 74)

15 1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL GELPVGAPGS
 51 SPSASTGTGP GSSPSASTGT GPGASPGTSS TGSPGASPGT SSTGSPGSST
 101 PSGATGSPGS SPSASTGTGP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 151 TASSSPGSST PSGATGSPGS STPSGATGSP GASPGTSSTG SPASSDARFP
 20 201 PRVPKSF PFN TSVVYKKT LF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR EKEDDKVFPG
 301 GSHTYVWQVL KENGP MASDP LCLTYSYL SH VDLVKDLNSG LIGALLVCRE
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRS LPLIGCHRK SVYWHVIGMG TPEVHSIFL EGHTFLVRNH
 25 451 RQASLEISPI TFLTAQTLLM DLGQFLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 30 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEYEDTLTLF PFSGETVFMS
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901 SKNNAIEPRS FSQNPPVLKR HQREITR TTL QGAPGTPGSG TASSSPGASP
 35 951 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 1001 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA
 1051 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
 1101 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
 1151 SVPQFKK VVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 40 1201 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQH HMAP
 1251 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
 1301 FALFFTIFDE TKS WYFTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
 1351 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG
 1401 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
 45 1451 ESATPESGPG TSTEPSEGA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
 1501 MDTLPLGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1551 YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1601 GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1651 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 50 1701 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 1751 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1801 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1851 QNGKVKVFQG NQDSFTPVVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG

1901 CEAQDLYDKT HTCPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 1951 VDVSHEDPEV KFNWYVDGVE VHNATKTPRE EQYNSTYRVV SVLTVLHQDW
 2001 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 2051 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS FFLYSKLTVD
 5 2101 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

**FVIII 199 protein sequence (single chain FVIIIc with three 144 AE XTENS
 at amino acid 1656 and 1900) (SEQ ID NO: 75)**

10 1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL GELPVDARFP
 51 PRVPKSF PFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKA SEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 15 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIPHGIT
 20 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYDYE SYEDISAYLL
 25 751 SKNNAIEPRS FSQNPVLKR HQAEITRTTL QGAPGTPGSG TASSSPGASP
 801 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 851 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST
 901 TGSPGSSPTS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
 951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
 30 1001 SVPQFKKVV FQEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 1051 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQH HMAP
 1101 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
 1151 FALFFTIFDE TKSIFYTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
 1201 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSA PG
 35 1251 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
 1301 ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
 1351 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1401 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1451 GMASGHIRDF QITASGQYGO WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 40 1501 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1551 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 1601 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1651 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1701 QNGKVKVFQO NQDSFTPVVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG
 45 1751 CEAQDLYDKT HTCPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 1801 VDVSHEDPEV KFNWYVDGVE VHNATKTPRE EQYNSTYRVV SVLTVLHQDW
 1851 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 1901 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS FFLYSKLTVD
 1951 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

FVIII 201 protein sequence (single chain FVIIIc with three 144 AE XTENS

at amino acid 26, 1656 & 1900) (SEQ ID NO: 76)

1 MQIELSTCFF LCLLRFCFSA TRYYLGAVE LSWDYMQSDL GELPVGAPGS
 51 SPSASTGTGP GSSPSASTGT GPGASPGTSS TGSPGASPGT SSTGSPGSST
 5 101 PSGATGSPGS SPSASTGTGP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 151 TASSSPGSST PSGATGSPGS STPSGATGSP GASPGTSSTG SPASSDARFP
 201 PRVPKSFPFN TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 10 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRS LPLIGCHRK SVYWHVIGMG TPEVHSIFL EGHTFLVRNH
 451 RQASLEISPI TFLTAQTLLM DLGQFLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 15 601 TDETFKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR PYNIPHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEYEDTLTLF PFSGETVFMS
 20 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901 SKNNAIEPRS FSQNPPVLKR HQAEITRTTL QGAPGTPGSG TASSSPGASP
 951 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST GTGPGTPGSG
 1001 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSTPSGA
 1051 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD DTISVEMKKE
 25 1101 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
 1151 SVPQFKKVV FQEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 1201 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQHHPMAP
 1251 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
 1301 FALFFTIFDE TKSIFYTENM ERNCRGAPTS ESATPESGPG SEPATSGSET
 30 1351 PGTSESATPE SGPSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG
 1401 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG SPTSTEEGTS
 1451 ESATPESGPG TSTEPSEGA PGASSAPCNI QMEDPTFKEN YRFHAINGYI
 1501 MDTLPLVMA QDQIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1551 YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPD
 35 1601 GMASGHIRDF QITASGQYQY WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1651 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1701 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 1751 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1801 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 40 1851 QNGKVKVFQ NQDSFTPVVN SLDPLLTRY LRIHPQSWVH QIALRMEVLG
 1901 CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 1951 VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW
 2001 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 2051 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGGS FFLYSKLTVD
 45 2101 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

FVIII 203 protein sequence (single chain FVIIIc with two AE XTENS; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 1900) (SEQ ID NO: 77)

50 1 MQIELSTCFF LCLLRFCFSA TRYYLGAVE LSWDYMQSDL GELPVDARFP
 51 PRVPKSFPFN TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE

201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 5 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 10 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 751 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSSESATPE
 801 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 15 901 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 951 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1001 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSSESATPE SGPSTSTEPS
 1051 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1101 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVQPKF
 20 1151 KVVVFQFTDG SFTQPL YRGE LNEHLG LLGP YIRAEVEDNI MVTFRNQASR
 1201 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1251 CKAWAYFSDV DLEKDVHSG LIGPLL VCHTN TLNPAHGRQV TVQEFALFFT
 1301 IFDETKSWYF TENMERNCRG APTSESATPE SGPGSEPATS GSETPGTSES
 1351 ATPESGPGSE PATSGSETPG TSESATPESG PGTSTEPSEG SAPGTSESAT
 25 1401 PESGPGSPAG SPTSTEEGSP AGSPTSTEEG SPAGSPTSTE EGTSESATPE
 1451 SGPSTSTEPS EGSAPGASSA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1551 VFETVEMLP KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
 30 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1801 VDFQKTKVLT GVTTQGVKSL L TSMYVKEFL ISSQDGHQW TLFFQNGKVK
 1851 VFQGNQDSFT PVVNSLDPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 35 1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 2001 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDEL TKNQVSLTCLV
 2051 KGFYPSDIAV EWESNGQPN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 2101 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

FVIII 204 protein sequence (single chain FVIII Fc with two AE XTENs; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 403) (SEQ ID NO: 78)

45 1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL GELPVDARFP
 51 PRVPKSPFN TSVVYKKT LF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 50 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDGAPTSTEP SEGSA PGSPA GSPTSTEEGT

451 STEPSEGSAP GTSTEPSEGS APGTSESATP ESGPGTSTEP SEGSAPGTSE
 501 SATPESGPGS EPATSGSETP GTSTEPSEGS APGTSTEPSE GSAPGTSESA
 551 TPESGPGTSE SATPESGPGA SSDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 5 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MUYEDTLTLF PFSGETVFMS
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 10 901 SKNNAIEPRS FSQNGAPGTS ESATPSGPG SEPATSGSET PGTSESATPE
 951 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 1001 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 1101 ESATPSGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 15 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPOFK
 1301 KVVVFQFTDG SFTQPL YRGE LNEHLG LLGP YIRAEVEDNI MVTFRNQASR
 1351 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 20 1401 CKAWAYFSDV DLEKDVHSGI IGPLL VCHTN TLNPAHGRQV TVQEFALFFT
 1451 IFDETKSWYF TENMERN CRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1551 VFETVEMLP S KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 1601 IRDFQITASG QYGQWAPKLA RLHYS GSINA WSTKEPFSWI KVDLLAPMII
 25 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1801 VDFQKTMKVT GVTTQGVKSL L TSMYVKEFL ISSSQDGHQW TLFFQNGKVK
 1851 VFQGNQDSFT PVVNSLDPL L TRYLRHPQ SWVHQIALRM EVLGCEAQDL
 30 1901 YDKTHTCPCP PPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 2001 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
 2051 KGFYPSDIAV EWESNGQPN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
 2101 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

FVIII 205 protein sequence (single chain FVIIIc with two AE XTENs; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 18) (SEQ ID NO: 79)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQGAP TSESATPESG
 40 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPSGPG
 101 TSTEPSEGS A PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151 ESATPSGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 201 PRVPKSFPFN TSVVYKKT LF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR EKEDDKVFPG
 45 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 50 551 WVHYIAAEEE DWDYAPLVA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIYPHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE

751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MUYEDTLTLF PFSGETVFM
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 5 951 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 1001 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPSTSTEPS
 10 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVQFK
 1301 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
 1351 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1401 CKAWAYFSDV DLEKDVHSGI IGPLLCHTN TLNPAHGRQV TVQEFALFFT
 15 1451 IFDETKSWYF TENMERNCR PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1551 VFETVEMLP KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 20 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1801 VDFQKTMKVT GVTQGVKSL LTSYVKEFL ISSSQDGHQW TLFFQNGKVK
 1851 VFQGNQDSFT PVVNSLDPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 25 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 2001 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDEL TKNQVSLTCLV
 2051 KGFYPSDIAV EWESNGQPN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQ
 2101 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

30

pSYN FVIII 266 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18 and 288 AE XTEN in B-domain) SEQ ID NO: 80)

35 1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQGAP GSPAGSPTST
 51 EGTSESATP ESGPGSEPA TSGSETPASS DLGELPVDAR FPPRPVKSFP
 101 FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIAE VYDTVVITLK
 151 NMASHPVSLH AVGVSYWKAS EGA EYDDQTS QREKEDDKVF PGGSHTYVWQ
 201 VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC REGSLAKEKT
 40 251 QTLHKFILLF AVFDEGKSWH SETKNSLMQD RDAASARAWP KMHTVNGYVN
 301 RSLPGLIGCH RKS VYWHVIG MGTTPEVHSI FLEGHTFLVR NHRQASLEIS
 351 PITFLTAQTL LMDLGQFLF CHISSHQHDG MEAYVKVDSC PEEPQLRMKN
 401 NEEAEDYDDD LTDSEMDVVR FDDDNPSFI QIRSVAKKHP KTWVHYIAAE
 451 EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM AYTDFTFKTR
 45 501 EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIPHG ITDVRPLYSR
 551 RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL TRYYSFVNM
 601 ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVLFSVF DENRSWYLTE
 651 NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFDLQLSV CLHEVAYWYI
 701 LSGAQTDFL SVFFSGYTFK HKMUYEDTLT LFPFSGETVF MSMENPGLWI
 50 751 LGCHNSDFRN RGMTALLKVS SCDKNTGDY ED SYEDISAY LLSKNNAIEP
 801 RSFSQNGAPG TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA
 851 TSGSETPGTS ESATPESGPG TSTEPSEGA PGSPAGSPTS TEEGTSESAT
 901 PESGPGSEPA TSGSETPGTS ESATPESGPG SPAGSPTSTE EGSPAGSPTS

951 TEEGTSTEPS EGSAPGTSES ATPESGPGTS ESATPESGPG TSESATPESG
 1001 PGSEPATSGS ETPGSEPATSGS ETPGSEPATSGS SPTSTEEGTS TEPSEGSAPG
 1051 TSTEPSEGSAPG PGSEPATSGS ETPGSEPATSGS PESGPGTSTE PSEGSAPASS
 1101 PPVLKRHQAE ITRTTLQSDQ EEIDYDDTIS VEMKKEDFDI YDEDENQSPR
 5 1151 SFQKKTRHYF IAAVERLWDY GMSSSPHVLNR AQSGSVLPQ FKKVVFQEF
 1201 DGSFTQPLYR GELNEHLGGL GPYIRAEVED NIMVTFRNQA SRPYSFYSSL
 1251 ISYEEDQRQG AEPRKNFVKP NETKTYFWKV QHHMAPTKDE FDCKAWAYFS
 1301 DVDLEKDVHS GLIGPLL VCH TNTLNPAHGR QVTVQEFALF FTIFDETKSW
 1351 YFTENMERNR RAPCNIQMED PTFKENYRFH AINGYIMDTL PGLVMAQDQR
 10 1401 IRWYLLSMGS NENIHSIHFS GHVFTVRKKE EYKMALYNLY PGVFETVEML
 1451 PSKAGIWRVE CLIGELHAG MSTLFLVYSN KCQTPLGMAS GHIRDFQITA
 1501 SGQYGQWAPK LARLHYSGLI NAWSTKEPFS WIKVDLLAPM IIHGIKTQGA
 1551 RQKFSSLYIS QFIIMYSLDG KKWQTYRGNS TGTLMVFFGN VDSSGIKHNI
 1601 FNPPIIARYI RLHPHTHSIR STLRMELMGC DLNSCSMPLG MESKAISDAQ
 15 1651 ITASSYFTNM FATWSPSKAR LHLQGRSNAW RPQVNNPKKEW LQVDFQKTMK
 1701 VTGVTQGVK SLLTSMYVKE FLISSQDGH QWTLLFFQNGK VKVFQGNQDS
 1751 FTPVVNSLDP PLLTRYLRH PQSWVHQIAL RMEVLGCEAQ DLYDKTHTCP
 1801 PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW
 1851 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLNQDQWLNGK EYKCKVSNKA
 20 1901 LPAPIETIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTCLVKGFYPSDI
 1951 AVEWESNGQP ENNYKTTTPV LDDSGSFLY SKLTVDKSRW QQGNVFSCSV
 2001 MHEALHNHYT QKSLSLSPGK *

25 **pSYN FVIII 267 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18 and 288
 AE XTEN in B-domain) SEQ ID NO: 81)**

1 MQIELSTCFF LCLLRFCFSA TRYYLGAVE LSWDYMQGAP TSESATPESG
 51 PGSEPATSGS ETPGSEPATSGS PESGPGSEPA TSGSETPGTS ESATPESGPG
 30 101 TSTEPSEGSAPG PGASSDLGE LPVDARFPPR VPKSFPFNTS VVYKKTFLVE
 151 FTDHLFNI AK PRPPWMGLLG PTIQAEVYDT VVITLKNMAS HPVSLHAVGV
 201 SYWKASEGAE YDDQTSQREK EDDKVFPFGS HTYVWQVLKE NGPMASDPLC
 251 LTYSYLSHVD LVKDLNSGLI GALLVCREGS LAKEKTQTLH KFIILLFAVFD
 301 EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP GLIGCHRKSV
 35 351 YWHVIGMGTT PEVHSIFLEG HTFLVRNHRQ ASLEISPITF LTAQTLLMDL
 401 GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA EDYDDDLTDS
 451 EMDVVRFD DD NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW DYAPLV LAPD
 501 DRSYKSQYLN NGPQRIGRKY KKVRFMAYTD ETFTKREAIQ HESGILGPLL
 551 YGEVGD TLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK GVKHLKDFPI
 40 601 LPGEIFKYKW TVTVEDGPTK SDPRCLTRY Y SSFVNMERDL ASGLIGPLLI
 651 CYKESVDQRG NQIMSDKRN V ILFSVFDENR SWYLTENIQR FLPNPAGVQL
 701 EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG AQTDFLSVFF
 751 SGYTFKHK MV YEDTLTLFPF SGETVFMSME NPGLWILGCH NSDFRNRGMT
 801 ALLKVSSCDK NTGDYYEDSY EDISAYLLSK NNAIEPRSF S QNGAPGTSES
 45 851 ATPESGPGSE PATSGSETPG TSESATPESG PGSEPATSGS ETPGSEPAT
 901 PESGPGTSTE PSEGSAPGSP AGSPTSTEEG TSESATPESG PGSEPATSGS
 951 ETPGSEPATSGS PESGPGSPAG SPTSTEEGSP AGSPTSTEEG TSTEPSEGSAPG
 1001 PGTSEPATSGS SGPSTSEPATSGS ATPESGPGSE PATSGSETPG
 1051 SEPATSGSET PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE PSEGSAPGSE
 50 1101 PATSGSETPG TSESATPESG PGTSTEPSEG SAPASSPPVL KRHQAEITRT
 1151 TLQSDQEEID YDDTISVEMK KEDFDIYDED ENQSPRSFQK KTRHYFIAAV
 1201 ERLWDYGMSS SPHVLNRNAQ SGSPVQFQKV VFQFTDGSF TQPLYRGELN
 1251 EHLGLLGPYI RAEVEDNIMV TFRNQASRPY SFYSSLISYE EDQRQGAEP

1301 KNFVKPNETK TYFWKVQHMH APTKDEFDCK AWAYFSDVDL EKDVHSGGLIG
 1351 PLLVCHTNLT NPAHGRQVTV QEFALFFTIF DETKSWYFTE NMERNCRAPC
 1401 NIQMEDPTFK ENYRFHAING YIMDTLPGLV MAQDQIRWY LLSMGSNENI
 1451 HSIHFSGHVF TVRKKEEYKM ALYNLYPGVF ETVEMLPSKA GIWRVECLIG
 5 1501 EHLHAGMSTL FLVYSNKCQT PLGMASGHIR DFQITASGQY GQWAPKLARL
 1551 HYSGSINAWS TKEPFSWIKV DLLAPMIIHG IKTQGARQKF SSLYISQFII
 1601 MYSLDGKKWQ TYRGNSTGTL MVFFGNVDSS GIKHNIFNPP IIARYIRLHP
 1651 THYSIRSTLR MELMGCDLNS CSMPLGMESK AISDAQITAS SYFTNMFATW
 1701 SPSKARLHLQ GRSNAWRPQV NNPKEWLQVD FQKTMKVTGV TTQGVKSLLT
 10 1751 SMYVKEFLIS SSQDGHQWTL FFQNGKVKVF QGNQDSFTPV VNSLDPPLLT
 1801 RYLRIHPQSW VHQIALRMEV LGCEAQDLYD KTHTCPPEPA PELLGGPSVF
 1851 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
 1901 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG
 1951 QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
 15 2001 KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
 2051 SLSPGK*

pSYN FVIII 268 protein sequence (FVIII Fc with 144 AE-XTEN at amino acid 18) SEQ ID NO: 82)

20 1 MQIELSTCFF LCLLRFCFSA TRYYLGAWE LSWDYMQGAP TSESATPESG
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101 TSTEPSEGA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151 ESATPESGPG SPAGSPTS EGSPTSPTS TEEGASSSDL GELPVDARFP
 25 201 PRVPKSFPFN TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 301 GSHTYVWQVL KENGPMSADP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRSLPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 30 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551 WWHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601 TDETFKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR PYNIPYHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 35 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFMS
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
 901 SKNNAIEPRS FSQNPVLKR HQAEITRTTL QSDQEEIDYD DTISVEMKKE
 40 951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LDYGMSSSP HVLNRNAQSG
 1001 SVPQFKKVV FQFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 1051 RNQASRPYSF YSSLISYED QRQGAEPKRN FVKPNETKTY FWKVQHMAP
 1101 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNLTNP AHGRQVTVQE
 1151 FALFFTIFDE TKSIFYTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
 45 1201 MDTLPGLVMA QDQIRRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
 1251 YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1301 GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1351 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1401 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 50 1451 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1501 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1551 QNGKVKVFQ NQDSFTPVVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG
 1601 CEAQDLYDKT HTCPPEPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV

1651 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
 1701 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 1751 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS FFLYSKLTVD
 1801 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

5

pSYN FVIII 269 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18) SEQ ID NO: 83)

10 1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP TSESATPESG
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101 TSTEPSEGS PGASSSDLGE LPVDARFPPR VPKSFPFNTS VVYKKTFLFE
 151 FTDHLFNI AK PRPPWMGLLG PTIQAEVYDT VVITLKNMAS HPVSLHAVGV
 201 SYWKASEGAE YDDQTSQREK EDDKVFP GGS HTYVWQVLKE NGPMASDPLC
 15 251 LTYSYLSHVD LVKDLNSGLI GALLVCREGS LAKEKTQTLH KFI LLFAVFD
 301 EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP GLIGCHRKSV
 351 YWHVIGMGTT PEVHSIFLEG HTFLVRNHRQ ASLEISPITF LTAQTLLMDL
 401 GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA EDYDDDLTDS
 451 EMDVVRFD DD NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW DYAPLV LAPD
 20 501 DRSYKSQYLN NGPQRIGRKY KKVRFMAYTD ETFKTREAIQ HESGILGPLL
 551 YGEVGD TLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK GVKHLKDFPI
 601 LPGEIFKYKW TVTVEDGPTK SDPRCLTRY Y SSFVNMERDL ASGLIGPLLI
 651 CYKESVDQRG NQIMSDKRVN ILFSVFDENR SWYL TENIQR FLPNPAGVQL
 701 EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG AQTD FL SVFF
 25 751 SGYTFKH KMV YEDTLTLFPF SGETVFMSME NPGLWILGCH NSDFRNRGMT
 801 ALLKVSSCDK NTGDY YEDSY EDISAYLLSK NNAIEPR SFS QNPPVLKR HQ
 851 AEITRTTLQS DQEEIDYDDT ISVEMKKEDF DIYDE DENQS PRSFQK KTRH
 901 YFIAAVERLW DYGMSSSPHV LRNRAQSGSV PQFKKVVFQE FTDGSFTQPL
 951 YRGELNEHLG LLGPYIRAEV EDNIMVTFRN QASRPYSFYS SLISYEEDQR
 30 1001 QGAEP RKNFV KPNETKTYFW KVQH HMAPTK DEFDC KAWAY FSDVDLEKDV
 1051 HSLGIGPLL V CHTNTLNPAH GRQVTVQEFA LFFTIFDETK SWYFTENMER
 1101 NCRAPCN IQM EDPTFKENYR FHAINGYIMD TLPGLVMAQD QRIRWYLLSM
 1151 GSNENIHSIH FSGHVFTVRK KEEYK MALYN LYPGVFETVE MLPSKAGIWR
 1201 VECLIGEHLH AGMSTLFLVY SNKCQTPLGM ASGHIRDFQI TASGQYQQWA
 35 1251 PKLARLHYS G SINAWSTKEP FSWIKVDLLA PMIIHG IKTQ GARQKFSSLY
 1301 ISQFIIMYS L DGKKWQTYRG NSTGTLMVFF GNVDSSGIKH NIFNPPIAR
 1351 YIRLHPHYS IRSTLRMELM GCDLNSCSMP LGMESKAISD AQITASSYFT
 1401 NMFATWSPSK ARLHLQGRSN AWRPQVNNPK EWLQVDFQKT MKVTGVTTQG
 1451 VKSLLTSMYV KEFLISSSQD GHQWTLFFQN GKVKVFQGNQ DSFTPVVNSL
 40 1501 DPPLLTRYLR IHPQSWVHQI ALRMEVLGCE AQDLYDKTHT CPPCPAPELL
 1551 GGPSVFLFP P KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
 1601 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
 1651 ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG
 1701 QPENNYKTTP PVLDSGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH
 45 1751 YTQKSLSLSP GK*

pSYNFVIII 271 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18) SEQ ID NO: 84)

50 1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP GSPAGSPTST
 51 EEGTSESATP ESGPGSEPAT SGSETPASSS DLGELPVDAR FPPRVPKSFP
 101 FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIQAE VYD TVVITLK

151 NMASHPVSLH AVGVSYWKAS EGAEYDDQTS QREKEDDKVF PGGSHTYVWQ
 201 VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC REGSLAKEKT
 251 QTLHKFILLF AVFDEGKSWH SETKNSLMQD RDAASARAWP KMHTVNGYVN
 301 RSLPGLIGCH RKSVMYWHVIG MGTTPPEVHSI FLEGHTFLVR NHRQASLEIS
 5 351 PITFLTAQTL LMDLGQFLLF CHISSHQHDG MEAYVKVDSC PEEPQLRMKN
 401 NEEAEDYDDD LTDSEMDVVR FDDDNPSFI QIRSVAKKHP KTWVHYIAAE
 451 EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM AYTDETFKTR
 501 EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIYPHG ITDVRPLYSR
 551 RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL TRYSSSFVNM
 10 601 ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVLFSVF DENRSWYLTE
 651 NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFDLSQLSV CLHEVAYWYI
 701 LSIGAQTDLF SVFFSGYTFK HKMVYEDTLT LFPFSGETVF MSMENPGLWI
 751 LGCHNSDFRN RGMTALLKVS SCDKNTGDYY EDSYEDISAY LLSKNNAIEP
 801 RSFSQNPPVL KRHQAEITRT TLQSDQEEID YDDTISVEMK KEDFDIYDED
 15 851 ENQSPRSFQK KTRHYFIAAV ERLWDYGMSS SPHVLNRNAQ SGSVPQFKKV
 901 VFQFTDGSF TQPLYRGELN EHLGLLGPYI RAEVEDNIMV TFRNQASRPY
 951 SFYSSLISYE EDQRQGAEPK KNFVKPNETK TYFWKVQHMM APTKDEFDCK
 1001 AWAYFSDVDL EKD VHSGLIG PLLVCHTNTL NPAHGRQVTV QEFALFFTIF
 1051 DETKSWYFTE NMERNCRAPC NIQMEDPTFK ENYRFHAING YIMDTLPGLV
 20 1101 MAQDQRIRWY LLSMGSNENI HSIHFSGHVF TVRKKEEYKM ALYNLYPGVF
 1151 ETVEMLPSKA GIWRVECLIG EHLHAGMSTL FLVYSNKCQT PLGMASGHIR
 1201 DFQITASGQY GQWAPKLARL HYSGSINAW S TKEPFSWIKV DLLAPMIHIG
 1251 IKTQGARQKF SSLYISQFII MYSLDGKKWQ TYRGNSTGTL MVFFGNVDSS
 1301 GIKHNIFNPP IIARYIRLHP THYSIRSTLR MELMGCDLNS CSMPLGMESK
 25 1351 AISDAQITAS SYFTNMFATW SPSKARLHLQ GRSNAWRPQV NNPKEWLQVD
 1401 FQKTMKVTGV TTQGVKSLT SMYVKEFLIS SSQDGHQWTL FFQNGKVKVF
 1451 QGNQDSFTPV VNSLDPPLT RYLRIHPQSW VHQIALRMEV LGCEAQDLYD
 1501 KTHTCPPCPA PELLGGPSVF LFPKPKDTL MISRTPEVTC VVVDVSHEDP
 1551 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC
 30 1601 KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG
 1651 FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN
 1701 VFSCSVMEHA LHNHYTQKSL SLSPGK*

35 **pSYN FVIII protein sequence 272 (FVIII with 144 AE XTEN at amino acid 18 and 244 AE XTEN in B-domain- no Fc) SEQ ID NO: 85)**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP TSESATPESG
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 40 101 TSTEPSEGS PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151 ESATPESGPG SPAGSPTS EGSPTSPTS TEEGASSDL GELPVDARFP
 201 PRVPKSFPFN TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVSYWKA SEG AEYDDQTSQR EKEDDKVFPG
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 45 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNPSFIQI RSVAKKHPKT
 551 WVHYIAAEED DWYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 50 601 TDETFKTREA IQHESGILGP LLYGEVGD TL IIFKNQASR PYNIYPHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701 YYSSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL

801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 951 SGPGESEPTS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 5 1001 EGTSESATPE SGPGESEPTS GSETPGTSES ATPESGPGSP AGSPTSTEEG
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
 10 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVQPK
 1301 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
 1351 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1401 CKAWAYFSDV DLEKDVHSLG IGPLLCHTN TLNPAHGRQV TVQEFALFFT
 1451 IFDETKSWYF TENMERNCR PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 15 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1551 VFETVEMLP KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 1701 SSGIKHNIFN PPIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 20 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1801 VDFQKTMKVT GVTQGVKSL LTSYVKEFL ISSSQDGHQW TLFFQNGKVK
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 1901 Y*

25

pSYN-FVIII-161 protein sequence (SEQ ID NO: 69) (FVIII sequence amino acid position 1-1457; underlined region represents Fc region; curvy underline represents cleavable linker in between first Fc and VWF fragment; double underlined region represents VWF fragment; bold region represents cleavable linker in between VWF fragment and Fc).

30

1 MQIELSTCFF LCLLRFCFSA TRYYLGAVE LSWDYMQSDL GELPVDARFP
 51 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKA SEG AEYDDQTSQR EKEDDKVFPG
 35 151 GSHTYVWQVL KENGPMSADP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 40 401 WVHYIAAEEE DWDYAPLVA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYNIPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 45 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751 SKNNAIEPRS FSQNPVLRK HQREITRTTL QSDQEEIDYD DTISVEMKKE
 801 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRQAQSG
 851 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 50 901 RNQASRPYSF YSSLISYEED QRGAEPKRN FVKPNETKTY FWKVQH HMAP
 951 TKDEFDCKAW AYFSDVDLEK DVHSLGIGPL LVCHTN TLNP AHGRQVTVQE
 1001 FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
 1051 MDTLPGLVMA QDQIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL

1101 YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
 1151 GMASGHIRD F QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
 1201 LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
 1251 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
 5 1301 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
 1351 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS QDGHQWTLFF
 1401 QNGKVKVFQ NQDSFTPVVN SLDPPLTRY LRIHPQSWVH QIALRMEVLG
 1451 CEAQDLYDKT HTCPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
 1501 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHODW
 10 1551 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
 1601 SLTCLVKGFY PSDIAVEWES NGOPENNYKT TPPVLDSDGS FFLYSKLTVD
 1651 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGKRRRRSG GGGSGGGGSG
 1701 GGGSGGGGSG GGGSGGGGSR KRRKRSLSR PPMVKLVCPA DNLRAEGLEC
 1751 TKTCQNYDLE CMSMGCVSGC LCPPGMVRHE NRCVALERCP CFHQGKEYAP
 15 1801 GETVKIGCNT CVCRRDRKWN TCVCDATCS TIGMAHYLTF DGLKYLFPGE
 1851 COYVLVDYCG SNPGTFRIL VGNKGCSPHS VKCKKRVTL VEGGEIELFD
 1901 GEVNVKRPMP DETHFEVVE GRYIILLGK ALSVWDRHL SISVVLKQTY
 1951 QEKVCGLCGN FDGIONNDLT SSNLQVEEDP VDFGNSWKVS SOCADTRKVP
 2001 LDSSPATCHN NIMKQTMVDS SCRILTSDFV QDCNKLVDPY PYLDVCIYDT
 20 2051 CSCESIGDCA AFCDTIAAYA HVCAQHGVV TWRTATLCPQ SCEERNLREN
 2101 GYEAERYNS CAPACQVTCQ HPEPLACPVO CVEGCHAHCP PGKILDELLO
 2151 TCVDPEDCPV CEVAGRRFAS GKKVTLNPSD PEHCQICHCD VVNLTCACQ
 2201 EPISGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE
 2251 TPGTSESATP ESGPGTSTEP SEGSA PGSPA GSPTSTEEGT SESATPESGP
 25 2301 GSEPATSGSE TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGT
 2351 STEPSEGSAP GTSESATPES GPGTSESATP ESGPGTSESA TPESGPGSEP
 2401 ATSGSETPGS EPATSGSETP GSPAGSPTST EGTSTEPSE GSAPGTSTEP
 2451 SEGSAPGSEP ATSGSETPGT SESATPESGP GTSTEPSEGS APDSGGGGSG
 2501 GGGSGGGGSG GGGSGGGGSL VPRGSGGDKT HTCPCPAPE LLGGPSVFLF
 30 2551 PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
 2601 EQYNSTYRVV SVLTVLHODW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
 2651 REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGOPENNYKT
 2701 TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 2751 SPGK

pSYN-FVIII-170 protein sequence (SEQ ID NO: 71)

1 SLSCRPPMVK LVCPADNLRA EGLECTKTCQ NYDLECMSMG CVSGCLCPPG
 51 MVRHENRCVA LERPCFHHQG KEYAPGETVK IGCNTCVCRD RKWNCTDHVC
 40 101 DATCSTIGMA HYLTFDGLKY LFPGEQYVL VQDYCGSNPG TFRILVGNKG
 151 CSHPSVKCKK RVTILVEGGE IELFDGEVNV KRPMKDETHF EVVESGRYII
 201 LLLGKALSIV WDRHLSISVV LKQTYQEKVC GLCGNFDGIQ NNDLTSSNLQ
 251 VEEDPVDFGN SWKVSSQCAD TRKVPLDSSP ATCHNNIMKQ TMVDSSCRIL
 301 TSDVFQDCNK LVDPEPYLDV CIYDTCSCES IGDCAAFCDT IAAYAHVCAQ
 45 351 HGKVVTWRTA TLCPQSCEER NRENGYEA WRYNSCAPAC QVTCQHPEPL
 401 ACPVQCVEGC HAHCPPGKIL DELLQTCVDP EDCPVCEVAG RRFASGKKVT
 451 LNPSDPEHCQ ICHCDVNL TCEACQEPISG TSESATPESG PGSEPATSGS
 501 ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGSA
 551 PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 50 601 SPAGSPTSTE EGSPAGSPTS TEEGTSTEPS EGSAPGTSES ATPESGPGTS
 651 ESATPESGPG TSESATPESG PGSEPATSGS ETPGSEPATS GSETPGSPAG
 701 SPTSTEEGTS TEPSEGSAPG TSTEPSEGSA PGSEPATSGS ETPGTSESAT
 751 PESGPGTSTE PSEGSAPDSG GGGSGGGGSG GGGSGGGGSG GGGSLVPRGS

801 GGASATRRYY LGAVELSWDY MQSDLGELPV DARFPPRVPK SFPFNTSVVY
 851 KKTLEFVEFTD HLFNIAKPRP PWMGLLGPTI QAEVYDVTVI TLKNMASHPV
 901 SLHAVGVSYW KASEGAEYDD QTSQREKEDD KVFPGGSHY VWQVLKENG
 951 MASDPLCLTY SYLSHVDLVK DLNSGLIGAL LVCREGSLAK EKTQTLHKFI
 5 1001 LLFAVFDEGK SWHSETKNSL MQDRDAASAR AWPKMHTVNG YVNRSLPGLI
 1051 GCHRKSVYWH VIGMGTTPEV HSIFLEGHTF LVRNHRQASL EISPITFLTA
 1101 QTLLMDLGQF LLFCHISSHQ HDGMEAYVKV DSCPEEPQLR MKNNEEAEDY
 1151 DDDLTDSEMD VVRFDDDNSP SFIQIRSVAK KHPKTWVHYI AAEEDWDYA
 1201 PLVLAPDDRS YKSQYLNNGP QRIGRKYKKV RFMAYTDETF KTREAIQHE
 10 1251 GILGPLLYGE VGDTHLIFK NQASRPYNIY PHGITDVRPL YSRRLPGVK
 1301 HLDKDFILPG EIFKYKWTVT VEDGPTKSDP RCLTRYYSF VNMERDLASG
 1351 LIGPLLCYK ESVDQGRNQI MSDKRNVLV SVFDENRSWY LTENIQRFLP
 1401 NPAGVQLED PEFQASNIMHS INGYVFDSLQ LSVCLHEVAY WYILSIGAQT
 1451 DFLSVFFSGY TFKHKMVEYD TLTLFPFSGE TVFMSMENPG LWILGCHNSD
 15 1501 FRNRGMTALL KVSSCDKNTG DYYEDSYEDI SAYLLSKNNA IEPRSFSQNP
 1551 PVLKRHQREI TRTTLQSDQE EIDYDDTISV EMKKEDFDIY DEDENQSPRS
 1601 FQKTRHYFI AAVERLWDYG MSSSPHVLN RAQSGSVQF KKVVFQEFTD
 1651 GSFTQPLYRG ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYSFYSSLI
 1701 SYEEDQRQGA EPRKNFVKPN ETCTYFWKVQ HHMAPTKDEF DCKAWAYFSD
 20 1751 VDLEKDVHSG LIGPLLVCHT NTLNPAHGRQ VTVQEFALFF TIFDETKSWY
 1801 FTENMERNCR APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI
 1851 RWYLLSMGSN ENHSIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP
 1901 SKAGIWRVEC LIGEHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS
 1951 GQYGQWAPKL ARLHYSGSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR
 25 2001 QKFSSLYISQ FIIMYSLDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNI
 2051 NPPIIARYIR LHPHTYSIRS TLRMELMGCD LNSCSMPLGM ESKAISDAQI
 2101 TASSYFTNMF ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV
 2151 TGVTTQGVKS LLTSMYVKEF LISSQDGHQ WTLFFQNGKV KVFQGNQDSF
 2201 TPVVNSLDPP LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LY
 30

pSYN FVIII 310 nucleotide sequence (encoding FVIII with complete B-domain deletion except 2 amino acid residues and 288 AE-XTEN inserted after aa 742) (SEQ ID NO:170)

35 1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
 51 CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCACTGGAA CTGTCATGGG
 101 ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCTT
 151 CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
 201 GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
 40 251 GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAATCT
 351 TCATGCTGTT GGTGTATCCT ACTGGAAGC TTCTGAGGGA GCTGAATATG
 401 ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT CTTCCCTGGT
 451 GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG GTCCAATGGC
 45 501 CTCTGACCCA CTGTGCCTTA CTTACTCATA TCTTTCTCAT GTGGACCTGG
 551 TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT
 651 TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAAT
 701 CCTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG GCCTAAAATG
 50 751 CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC TGATTGGATG
 801 CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC ACCACTCCTG
 851 AAGTGACTC AATATTCCTC GAAGGTCACA CATTTCCTTG GAGGAACCAT
 901 CGCCAGGCGT CTTTGGAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC

951 ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC
 1001 ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG CTGTCCAGAG
 1051 GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG ACTATGATGA
 1101 TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT GATGACAACT
 5 1151 CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT
 1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT
 1251 AGTCCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG
 1301 GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT TATGGCATACT
 1351 ACAGATGAAA CCTTTAAGAC TCGTGAAGCT ATTCAGCATG AATCAGGAAT
 10 1401 CTTGGGACCT TTACTTTATG GGAAGTTGG AGACACACTG TTGATTATAT
 1451 TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
 1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAAGGTG TAAAACATTT
 1551 GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG
 1601 TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG CCTGACCCGC
 15 1651 TATTACTCTA GTTTCGTAA TATGGAGAGA GATCTAGCTT CAGGACTCAT
 1701 TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA AGAGGAAACC
 1751 AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
 1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC
 1851 AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC
 20 1901 ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC AGTTTGTTTG
 1951 CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
 2001 CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA ATGGTCTATG
 2051 AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTGC
 2101 ATGGAACACC CAGGTCTATG GATTCTGGGG TGCCACAACCT CAGACTTTTCG
 25 2151 GAACAGAGGC ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA
 2201 CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTTCAGC ATACTTGCTG
 2251 AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCGGTACCT CAGAGTCTGC
 2301 TACCCCCGAG TCAGGGCCAG GATCAGAGCC AGCCACCTCC GGGTCTGAGA
 2351 CACCCGGGAC TTCCGAGAGT GCCACCCCTG AGTCCGGACC CGGGTCCGAG
 30 2401 CCCGCCACTT CCGGCTCCGA AACTCCCGGC ACAAGCGAGA GCGCTACCCC
 2451 AGAGTCAGGA CCAGGAACAT CTACAGAGCC CTCTGAAGGC TCCGCTCCAG
 2501 GGTCCCCAGC CGGCAGTCCC ACTAGCACCG AGGAGGGAAC CTCTGAAAGC
 2551 GCCACACCCG AATCAGGGCC AGGGTCTGAG CCTGCTACCA GCGGCAGCGA
 2601 GACACCAGGC ACCTCTGAGT CCGCCACACC AGAGTCCGGA CCCGATCTC
 35 2651 CCGCTGGGAG CCCCACCTCC ACTGAGGAGG GATCTCCTGC TGGCTCTCCA
 2701 ACATCTACTG AGGAAGGTAC CTCAACCGAG CCATCCGAGG GATCAGCTCC
 2751 CGGCACCTCA GAGTCGGCAA CCCCAGGAGT TGGACCCGGA ACTTCCGAAA
 2801 GTGCCACACC AGAGTCCGGT CCCGGGACTT CAGAATCAGC AACACCCGAG
 2851 TCCGGCCCTG GGTCTGAACC CGCCACAAGT GGTAGTGAGA CACCAGGATC
 40 2901 AGAACCTGCT ACCTCAGGGT CAGAGACACC CGGATCTCCG GCAGGCTCAC
 2951 CAACCTCCAC TGAGGAGGGC ACCAGCACAG AACCAAGCGA GGGCTCCGCA
 3001 CCCGGAACAA GCACTGAACC CAGTGAGGGT TCAGCACCCG GCTCTGAGCC
 3051 GGCCACAAGT GGCAGTGAGA CACCCGGCAC TTCAGAGAGT GCCACCCCCG
 3101 AGAGTGGCCC AGGCACTAGT ACCGAGCCCT CTGAAGGCAG TGCGCCAGCC
 45 3151 TCGAGCGAAA TAACTCGTAC TACTCTTCAG TCAGATCAAG AGGAAATCGA
 3201 TTATGATGAT ACCATATCAG TTGAAATGAA GAAGGAAGAT TTTGACATTT
 3251 ATGATGAGGA TGAAAATCAG AGCCCCCGCA GCTTTCAAAA GAAAACACGA
 3301 CACTATTTTA TTGCTGCAGT GGAGAGGCTC TGGGATTATG GGATGAGTAG
 3351 CTCCCCACAT GTTCTAAGAA ACAGGGCTCA GAGTGGCAGT GTCCCTCAGT
 50 3401 TCAAGAAAGT TGTTTTCCAG GAATTTACTG ATGGCTCCTT TACTCAGCCC
 3451 TTATACCGTG GAGAACTAAA TGAACATTTG GGAATCCTGG GGCCATATAT
 3501 AAGAGCAGAA GTTGAAGATA ATATCATGGT AACTTTCAGA AATCAGGCCT
 3551 CTCGTCCCTA TTCCTTCTAT TCTAGCCTTA TTTCTTATGA GGAAGATCAG

3601 AGGCAAGGAG CAGAACCTAG AAAAACTTT GTCAAGCCTA ATGAAACCAA
 3651 AACTTACTTT TGGAAAGTGC AACATCATAT GGCACCCACT AAAGATGAGT
 3701 TTGACTGCAA AGCCTGGGCT TATTTCTCTG ATGTTGACCT GGAAAAAGAT
 3751 GTGCACTCAG GCCTGATTGG ACCCCTTCTG GTCTGCCACA CTAACACACT
 5 3801 GAACCCTGCT CATGGGAGAC AAGTGACAGT ACAGGAATTT GCTCTGTTTT
 3851 TCACCATCTT TGATGAGACC AAAAGCTGGT ACTTCACTGA AAATATGGAA
 3901 AGAAACTGCA GGGCTCCCTG CAATATCCAG ATGGAAGATC CCACTTTTAA
 3951 AGAGAATTAT CGCTTCCATG CAATCAATGG CTACATAATG GATACACTAC
 4001 CTGGCTTAGT AATGGCTCAG GATCAAAGGA TTCGATGGTA TCTGCTCAGC
 10 4051 ATGGGCAGCA ATGAAAACAT CCATTCTATT CATTTCACTG GACATGTGTT
 4101 CACTGTACGA AAAAAAGAGG AGTATAAAAT GGCACGTAC AATCTCTATC
 4151 CAGGTGTTTT TGAGACAGTG GAAATGTTAC CATCCAAAGC TGGAATTTGG
 4201 CGGGTGGAAT GCCTTATTGG CGAGCATCTA CATGCTGGGA TGAGCACACT
 4251 TTTTCTGGTG TACAGCAATA AGTGTCAGAC TCCCCTGGGA ATGGCTTCTG
 15 4301 GACACATTAG AGATTTTTCAG ATTACAGCTT CAGGACAATA TGGACAGTGG
 4351 GCCCCAAAGC TGGCCAGACT TCATTATTCC GGATCAATCA ATGCCTGGAG
 4401 CACCAAGGAG CCCTTTTCTT GGATCAAGGT GGATCTGTTG GCACCAATGA
 4451 TTATTCACGG CATCAAGACC CAGGGTGCCC GTCAGAAAGT CTCCAGCCTC
 4501 TACATCTCTC AGTTTATCAT CATGTATAGT CTTGATGGGA AGAAGTGGCA
 20 4551 GACTTATCGA GGAAATTCCA CTGGAACCTT AATGGTCTT TTTGGCAATG
 4601 TGGATTCATC TGGGATAAAA CACAATATTT TTAACCCTCC AATTATTGCT
 4651 CGATACATCC GTTTGCACCC AACTCATTAT AGCATTTCGA GCACTCTTCG
 4701 CATGGAGTTG ATGGGCTGTG ATTTAAATAG TTGCAGCATG CCATTGGGAA
 4751 TGGAGAGTAA AGCAATATCA GATGCACAGA TTAAGTCTT ATCCTACTTT
 25 4801 ACCAATATGT TTGCCACCTG GTCTCCTTCA AAAGCTCGAC TTCACCTCCA
 4851 AGGGAGGAGT AATGCCTGGA GACCTCAGGT GAATAATCCA AAAGAGTGGC
 4901 TGCAAGTGGA CTTCCAGAAG ACAATGAAAG TCACAGGAGT AACTACTCAG
 4951 GGAGTAAAAT CTCTGCTTAC CAGCATGTAT GTGAAGGAGT TCCTCATCTC
 5001 CAGCAGTCAA GATGGCCATC AGTGGACTCT CTTTTTTCAG AATGGCAAAG
 30 5051 TAAAGGTTTT TCAGGGAAAT CAAGACTCCT TCACACCTGT GGTGAAGTCT
 5101 CTAGACCCAC CGTTACTGAC TCGTACCTT CGAATTCACC CCCAGAGTTG
 5151 GGTGCACCA ATTGCCCTGA GGATGGAGGT TCTGGGCTGC GAGGCACAGG
 5201 ACCTCTACGA CAAAACTCAC ACATGCCCAC CGTGCCACG TCCAGACATC
 5251 CTGGGCGGAC CGTCAGTCTT CCTCTTCCCC CAAAACCCA AGGACACCTT
 35 5301 CATGATCTCC CGGACCCCTG AGGTACATG CGTGGTGGTG GACGTGAGCC
 5351 ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
 5401 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG
 5451 TGTGGTCAGC GTCCTACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG
 5501 AGTACAAGTG CAAGGTCTCC AACAAAGCCC TCCAGCCCC CATCGAGAAA
 40 5551 ACCATCTCCA AAGCCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT
 5601 GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC CTGACCTGCC
 5651 TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
 5701 GGGCAGCCGG AGAACAATA CAAGACCACG CCTCCCGTGT TGGACTCCGA
 5751 CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC
 45 5801 AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC
 5851 CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA

pSYN FVIII 310 protein sequence (FVIII with complete B-domain deletion except 2 amino acid residues and 288 AE-XTEN inserted after aa 742) (SEQ ID NO:171)

50

1 ATRRYYLGAVALSWDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKKTLL
 51 FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLHA

101 VGVSYWKASE GAEYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
 151 PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
 201 VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
 251 KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
 5 301 MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
 351 TDSEMDVVRF DDDNSPSFIQ IRSVAKKHPK TWVHYIAAEE EDWDYAPLVL
 401 APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTRE AIQHESGILG
 451 PLYGEVGD TLLIFKNQAS RPYNIYPHGI TDVRPLYSRRLPKGVKHLKD
 501 FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSFVNME RDLASGLIGP
 10 551 LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYL TEN IQRFLPNPAG
 601 VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
 651 VFFSGYTFKH KVMYEDTLTL PFPSGETVFM SMENPGLWIL GCHNSDFRNR
 701 GMTALLKVSS CDKNTGDYEE DSYEDISAYL LSKNNAIEPR SFGTSESATP
 751 ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
 15 801 GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
 851 GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
 901 SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
 951 ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
 1001 SGSETPGTSE SATPESGPGT STEPSEGSAP ASSEITRTTL QSDQEEIDYD
 20 1051 DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
 1101 HVLRNRAQSG SVPQFKKVV FQEFTDGSFTQ PLYRGELNEH LGLLGPYIRA
 1151 EVEDNIMVTF RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY
 1201 FWKVQHMAP TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP
 1251 AHGRQVTVQE FALFFTIFDE TKSIFYTENM ERNCRAPCNI QMEDPTFKEN
 25 1301 YRFHAINGYI MDTLPLGLVMA QDQIRRWYLL SMGSNENIHS IHFSGHVFTV
 1351 RKKEEYKMAL YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL
 1401 VYSNKCQTPL GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK
 1451 EPFSWIKVDL LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY
 1501 RGNSTGTLMV FFGNVDSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME
 30 1551 LMGC DLNSCS MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR
 1601 SNAWRPQVNN PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISS
 1651 QDGHQWTLFF QNGKVKVFQ QNDSFTPVVN SLDPPLTRY LRIHPQSWVH
 1701 QIALRMEVLG CEAQDLYDKT HTCPCPAPE LLGGPSVFLF PPKPKDTLMI
 1751 SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
 35 1801 SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
 1851 SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS
 1901 FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

40 **pSYN FVIII 312 nucleotide sequence (encoding FVIII with complete B-domain deletion except 5 amino acid residues and 288 AE-XTEN inserted after aa 745- B5 version) (SEQ ID NO:172)**

1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
 51 CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG
 45 101 ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCTT
 151 CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
 201 GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
 251 GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
 301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT
 50 351 TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG
 401 ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT CTTCCTGGT
 451 GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG GTCCAATGGC
 501 CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT GTGGACCTGG

551 TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT
 651 TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAAGAACT
 701 CTTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG GCCTAAAATG
 5 751 CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC TGATTGGATG
 801 CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC ACCACTCCTG
 851 AAGTGCCTC AATATTCCTC GAAGGTCACA CATTTCCTGT GAGGAACCAT
 901 CGCCAGGCGT CTTTGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC
 951 ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCTAT ATCTCTTCCC
 10 1001 ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG CTGTCCAGAG
 1051 GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG ACTATGATGA
 1101 TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT GATGACAACT
 1151 CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAAC
 1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT
 15 1251 AGTCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG
 1301 GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT TATGGCATACT
 1351 ACAGATGAAA CCTTAAAGAC TCGTGAAGCT ATTCAGCATG AATCAGGAAT
 1401 CTTGGGACCT TTACTTTATG GGAAGTTGG AGACACACTG TTGATTATAT
 1451 TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA CGGAATCACT
 20 1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CAAAAGGTG TAAAACATTT
 1551 GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG
 1601 TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG CCTGACCCGC
 1651 TATTACTCTA GTTTCGTAA TATGGAGAGA GATCTAGCTT CAGGACTCAT
 1701 TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA AGAGGAAACC
 25 1751 AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
 1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCAATCC
 1851 AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC
 1901 ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC AGTTTGTGTTG
 1951 CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
 30 2001 CTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA ATGGTCTATG
 2051 AAGACACACT CACCCTATTC CCATTCTCAG GAGAACTGT CTTTCATGTG
 2101 ATGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAAC CAGACTTTCG
 2151 GAACGAGGC ATGACCGCCT TACTGAAGT TTCTAGTTGT GACAAGAAC
 2201 CTGGTGATTA TTACGAGGAG AGTTATGAAG ATATTTTCAGC ATACTTGCTG
 35 2251 AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCTCTCAA ACGGTACCTC
 2301 AGAGTCTGCT ACCCCGAGT CAGGGCCAGG ATCAGAGCCA GCCACCTCCG
 2351 GGTCTGAGAC ACCCGGGACT TCCGAGAGTG CCACCCCTGA GTCCGGACCC
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 2451 CGCTACCCCA GAGTCAGGAC CAGGAACATC TACAGAGCCC TCTGAAGGCT
 40 2501 CCGCTCCAGG GTCCCCAGCC GGCAGTCCCA CTAGCACCGA GGAGGGAACC
 2551 TCTGAAAGCG CCACACCCGA ATCAGGGCCA GGGTCTGAGC CTGCTACCAG
 2601 CGGCAGCGAG ACACCAGGCA CCTCTGAGTC CGCCACACCA GAGTCCGGAC
 2651 CCGGATCTCC CGCTGGGAGC CCCACCTCCA CTGAGGAGGG ATCTCCTGCT
 2701 GGCTCTCAA CATCTACTGA GGAAGGTACC TCAACCGAGC CATCCGAGGG
 45 2751 ATCAGTCCC GGCACCTCAG AGTCGGCAAC CCCGGAGTCT GGACCCGGAA
 2801 CTTCCGAAAG TGCCACACCA GAGTCCGGTC CCGGGACTTC AGAATCAGCA
 2851 ACACCCGAGT CCGGCCCTGG GTCTGAACCC GCCACAAGTG GTAGTGAGAC
 2901 ACCAGGATCA GAACCTGCTA CCTCAGGGTC AGAGACACCC GGATCTCCGG
 2951 CAGGCTCACC AACCTCCACT GAGGAGGGCA CCAGCACAGA ACCAAGCGAG
 50 3001 GGCTCCGCAC CCGGAACAAG CACTGAACCC AGTGAGGGTT CAGCACCCGG
 3051 CTCTGAGCCG GCCACAAGTG GCAGTGAGAC ACCCGGCACT TCAGAGAGTG
 3101 CCACCCCGCA GAGTGGCCCA GGCAGTAGTA CCGAGCCCTC TGAAGGCAGT
 3151 GCGCCAGCCT CGAGCGAAAT AACTCGTACT ACTCTTCAGT CAGATCAAGA

3201 GGAAATCGAT TATGATGATA CCATATCAGT TGAAATGAAG AAGGAAGATT
 3251 TTGACATTTA TGATGAGGAT GAAAATCAGA GCCCCGCAG CTTTCAAAAG
 3301 AAAACACGAC ACTATTTTAT TGCTGCAGTG GAGAGGCTCT GGGATTATGG
 3351 GATGAGTAGC TCCCCACATG TTCTAAGAAA CAGGGCTCAG AGTGGCAGTG
 5 3401 TCCCTCAGTT CAAGAAAGTT GTTTTCCAGG AATTTACTGA TGGCTCCTTT
 3451 ACTCAGCCCT TATACCGTGG AGAACTAAAT GAACATTTGG GACTCCTGGG
 3501 GCCATATATA AGAGCAGAAG TTGAAGATAA TATCATGGTA ACTTTCAGAA
 3551 ATCAGGCCTC TCGTCCCTAT TCCTTCTATT CTAGCCTTAT TTCTTATGAG
 3601 GAAGATCAGA GGCAAGGAGC AGAACCTAGA AAAAAGCTTTG TCAAGCCTAA
 10 3651 TGAAACCAAA ACTTACTTTT GGAAAGTGCA ACATCATATG GCACCCACTA
 3701 AAGATGAGTT TGACTGCAAA GCCTGGGCTT ATTTCTCTGA TGTGACCTG
 3751 GAAAAAGATG TGCACCTCAGG CCTGATTGGA CCCCTTCTGG TCTGCCACAC
 3801 TAACACACTG AACCCTGCTC ATGGGAGACA AGTGACAGTA CAGGAATTTG
 3851 CTCTGTTTTT CACCATCTTT GATGAGACCA AAAGCTGGTA CTTCACTGAA
 15 3901 AATATGGAAG GAAACTGCAG GGCTCCCTGC AATATCCAGA TGGAAGATCC
 3951 CACTTTTAAA GAGAATTATC GCTTCCATGC AATCAATGGC TACATAATGG
 4001 ATACACTACC TGGCTTAGTA ATGGCTCAGG ATCAAAGGAT TCGATGGTAT
 4051 CTGCTCAGCA TGGGCAGCAA TGAAAACATC CATTCTATTC ATTTCACTGG
 4101 ACATGTGTTT ACTGTACGAA AAAAAGAGGA GTATAAAATG GCACTGTACA
 20 4151 ATCTCTATCC AGGTGTTTTT GAGACAGTGG AAATGTTACC ATCCAAAGCT
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 4301 TGGCTTCTGG ACACATTAGA GATTTTCAGA TTACAGCTTC AGGACAATAT
 4351 GGACAGTGGG CCCCAGAGCT GGCCAGACTT CATTATTCCG GATCAATCAA
 25 4401 TGCCTGGAGC ACCAAGGAGC CTTTTTCTTG GATCAAGGTG GATCTGTTGG
 4451 CACCAATGAT TATTCACGGC ATCAAGACCC AGGGTGCCCG TCAGAAAGTTC
 4501 TCCAGCCTCT ACATCTCTCA GTTTATCATC ATGTATAGTC TTGATGGGAA
 4551 GAAGTGGCAG ACTTATCGAG GAAATTCCAC TGGAACCTTA ATGGTCTTCT
 4601 TTGGCAATGT GGATTCATCT GGGATAAAAC ACAATATTTT TAACCCCTCA
 30 4651 ATTATTGCTC GATACATCCG TTTGCACCCA ACTCATTATA GCATTGCGAG
 4701 CACTCTTCGC ATGGAGTTGA TGGGCTGTGA TTTAAATAGT TGCAGCATGC
 4751 CATTGGGAAT GGAGAGTAAA GCAATATCAG ATGCACAGAT TACTGCTTCA
 4801 TCCTACTTTA CCAATATGTT TGCCACCTGG TCTCCTTCAA AAGCTCGACT
 4851 TCACCTCCAA GGGAGGAGTA ATGCCTGGAG ACCTCAGGTG AATAATCCAA
 35 4901 AAGAGTGGCT GCAAGTGGAC TTCCAGAAGA CAATGAAAGT CACAGGAGTA
 4951 ACTACTCAGG GAGTAAAATC TCTGCTTACC AGCATGTATG TGAAGGAGTT
 5001 CCTCATCTCC AGCAGTCAAG ATGGCCATCA GTGGACTCTC TTTTTTCAGA
 5051 ATGGCAAAGT AAAGGTTTTT CAGGGAAATC AAGACTCCTT CACACCTGTG
 5101 GTGAACTCTC TAGACCCACC GTTACTGACT CGCTACCTTC GAATTCACCC
 40 5151 CCAGAGTTGG GTGCACCAGA TTGCCCTGAG GATGGAGGTT CTGGGCTGCG
 5201 AGGCACAGGA CCTCTACGAC AAAACTCACA CATGCCCACC GTGCCAGCT
 5251 CCAGAACTCC TGGGCGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
 5301 GGACACCCTC ATGATCTCCC GGACCCCTGA GGTACATGC GTGGTGCTGG
 5351 ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC
 45 5401 GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG
 5451 CACGTACCGT GTGGTCAGCG TCCTACCGT CCTGCACCAG GACTGGCTGA
 5501 ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
 5551 ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT
 5601 GTACACCCTG CCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC
 50 5651 TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
 5701 GAGAGCAATG GGCAGCCGGA GAACAACCTAC AAGACCACGC CTCCCGTGTT
 5751 GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA
 5801 GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT

5851 CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG

pSYN FVIII 312 protein sequence (FVIII with complete B-domain deletion except 5 amino acid residues and 288 AE-XTEN inserted after aa 745- B5 version) (SEQ ID NO:173)

5
1 ATRRYYLGA V ELSWDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKCTL
51 FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLHA
101 VGVSYW KASE GA EYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
10 151 PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
201 VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
251 KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
301 MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
351 TDSEMDVVR F DDDNSPSFIQ IRVAKKH PK TWVHYIAAEE EDWDYAPLVL
15 401 APDDRSYK SQ YLNNGPQRIG RKYKKVRFMA YDDETFKTRE AIQHESGILG
451 PLYGEV GDT LLIIFKNQAS RPYNIYPHGI TDVRPLYSR R LPKGVKHLKD
501 FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSFVNME RDLASGLIGP
551 LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG
601 VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
20 651 VFFSGYTFKH KMYVEDTLTL PPFSGETVFM SMENPGLWIL GCHNSDFRNR
701 GMTALLKVSS CDKNTGDYEE DSYEDISAYL LSKNNAIEPR SFSQNGTSES
751 ATPESGPGSE PATSGSETPG TSESATPESG PGSEPATSGS ETPGTSESAT
801 PESGPGTSTE PSEGSA PGSP AGSPTSTEEG TSESATPESG PGSEPATSGS
851 ETPGTSESAT PESGPGSPAG SPTSTEEGSP AGSPTSTEEG TSTEPSEGS
25 901 PGTSESATPE SGPGTSESAT PESGPGTSES ATPESGPGSE PATSGSETPG
951 SEPATSGSET PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE PSEGSA PGSE
1001 PATSGSETPG TSESATPESG PGTSTEPSEG SAPASSEITR TTLQSDQEEI
1051 DYDDTISVEM KKEDFDIYDE DENQSPRSFQ KKTRHYFIAA VERLWDYGM S
1101 SSPHLVRNRA QSGSV PQFKK VVFQEFTDGS FTQPLYRGEL NEHLGLLGPY
30 1151 IRAEVEDNIM VTFRNQASRP YSFYSSLISY EEDQRQGAEP RKNFVKPNET
1201 KTYFWKVQHH MAPTKDEFDC KAWAYFSDVD LEKDVHSGLI GPLLVCHTNT
1251 LNPAHGRQVT VQEFALFFTI FDETKSWYFT ENMERNCRAP CNIQMEDPTF
1301 KENYRFHAIN GYIMDTLPGL VMAQDQRIRW YLLSMGSNEN IHSIHFSGHV
1351 FTVRKKEEYK MALYNLYPGV FETVEMLPSK AGIWRVECLI GEHLHAGMST
35 1401 LFLVYSNKCQ TPLGMASGHI RDFQITASGQ YGQWAPKLAR LHYSGSINAW
1451 STKEPFSWIK VDLLAPMIH GIKTQGARQK FSSLYISQFI IMYSLDGKKW
1501 QTYRGNSTGT LMVFFGNVDS SGIKHNIFNP PIARYIRLH PTHYSIRSTL
1551 RMELMGCDLN SCSMPLGMES KAISDAQITA SSYFTNMFAT WSPSKARLHL
1601 QGRSNAWRPQ VNNPKEWLQV DFQKTMKVTG VTTQGVKSLL TSMYVKEFLI
40 1651 SSSQDGHQWT LFFQNGKVKV FQGNQDSFTP VVNSLDPPLL TRYLRHPQS
1701 WVHQIALRME VLGCEAQDLY DKTHTCPPCP APELLGGPSV FLFPPKPKDT
1751 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
1801 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
1851 LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLD S
45 1901 DGSFFLYSKL TVDKSRWQQG NVFSCSV MHE ALHNHYTQKS LSLSPGK*

pSYN VWF059 nucleotide sequence (encoding VWF D'D3-Fc with acidic region 2 (a2) thrombin site in the linker) (SEQ ID NO: 196)

50
1 ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
51 GCCAGGGACC CTTTGTGCAG AAGGAAC TCG CGGCAGGTCA TCCACGGCCC
101 GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG

151 TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA
 201 ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
 251 TCTCCGTGTA TCTTGGGGAA TTTTGTGACA TCCATTTGTT TGTCAATGGT
 301 ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
 5 351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT
 401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
 451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
 501 CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTG ACCTCGGACC
 551 CTTATGACTT TGCCAACCTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
 10 601 GAACGGGCAT CTCCTCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
 651 GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
 701 TTGCCCGCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
 751 GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC
 801 CCTCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
 15 851 GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
 901 TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
 951 CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG
 1001 GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
 1051 GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
 20 1101 CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
 1151 GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
 1201 AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA
 1251 TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
 1301 ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC
 25 1351 CTGCACAACA GCCTTGTAAG ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
 1401 TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC
 1451 ATACAGTGAC GGCCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG
 1501 GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
 1551 CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
 30 1601 ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGAATTCGGG
 1651 AACGCCTGGA AGCTGCACGG GGAATGCCAG GACCTGCAGA AGCAGCACAG
 1701 CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
 1751 GCGCGTCTCT GACGTCCCCC ACATTCGAGG CCTGCCATCG TGCCGTGACG
 1801 CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA
 35 1851 CGGCCGCGAG TGCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
 1901 CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
 1951 AACTGCCCCA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
 2001 GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
 2051 TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
 40 2101 TGCGTGCCCA AGGCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
 2151 GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
 2201 GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC
 2251 GCTGTCTTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
 2301 TCGGCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAAC CTGCGGGCTG
 45 2351 AAGGGCTCGA GTGTACAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
 2401 AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
 2451 TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC CATCAGGGCA
 2501 AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC
 2551 TGTCGGGACC GGAAGTGGA CTGCACAGAC CATGTGTGTG ATGCCACGTG
 50 2601 CTCCACGATC GGCATGGCCC ACTACCTCAC CTTGACGGG CTCAAATACC
 2651 TGTTCCCCCG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
 2701 AACCCTGGGA CTTTTCGGAT CTAAGTGGG AATAAGGGAT GCAGCCACCC
 2751 CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA

2801 TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
 2851 ACTCACTTTG AGGTGGTGGG GTCTGGCCGG TACATCATTC TGCTGCTGGG
 2901 CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
 2951 TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
 5 3001 GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
 3051 CCCTGTGGAC TTTGGGAACT CCTGGAAAGT GAGCTCGCAG TGTGCTGACA
 3101 CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC
 3151 ATGAAGCAGA CGATGGTGGG TTCCTCCTGT AGAATCCTTA CCAGTGACGT
 3201 CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
 10 3251 GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTC
 3301 TGCGACACCA TTGCTGCCTA TGCCCACGTG TGTGCCCAGC ATGGCAAGGT
 3351 GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC GAGGAGAGGA
 3401 ATCTCCGGGA GAACGGGTAT GAGGCTGAGT GGCCTATAA CAGCTGTGCA
 3451 CCTGCCTGTC AAGTCACGTG TCAGCACCTT GAGCCACTGG CCTGCCCTGT
 15 3501 GCAGTGTGTG GAGGGCTGCC ATGCCACTG CCCTCCAGGG AAAATCCTGG
 3551 ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
 3601 GTGGCTGGCC GCGTTTTGTC CTCAGGAAAG AAAGTCACCT TGAATCCAG
 3651 TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC AACCTCACCT
 3701 GTGAAGCCTG CCAGGAGCCG ATATCGGGCG CGCCAACATC AGAGAGCGCC
 20 3751 ACCCTGAAA GTGGTCCCGG GAGCGAGCCA GCCACATCTG GGTCGGAAAC
 3801 GCCAGGCACA AGTGAGTCTG CAACTCCCGA GTCCGGACCT GGCTCCGAGC
 3851 CTGCCACTAG CGGCTCCGAG ACTCCGGGAA CTCCGAGAG CGCTACACCA
 3901 GAAAGCGGAC CCGGAACCAG TACCGAACCT AGCGAGGGCT CTGCTCCGGG
 3951 CAGCCCAGCC GGCTCTCCTA CATCCACGGA GGAGGGCACT TCCGAATCCG
 25 4001 CCACCCCGGA GTCAGGGCCA GGATCTGAAC CCGCTACCTC AGGCAGTGAG
 4051 ACGCCAGGAA CGAGCGAGTC CGCTACACCG GAGAGTGGGC CAGGGAGCCC
 4101 TGCTGGATCT CTAAGTCCA CTGAGGAAGG GTCACCAGCG GGCTCGCCCA
 4151 CCAGCACTGA AGAAGGTGCC TCGATATCTG ACAAGAACAC TGGTGATTAT
 4201 TACGAGGACA GTTATGAAGA TATTTACGCA TACTTGCTGA GTAAAAACAA
 30 4251 TGCCATTGAA CCAAGAAGCT TCTCTGACAA AACTCACACA TGCCCACCGT
 4301 GCCCAGCTCC AGAACTCCTG GGCGGACCGT CAGTCTTCCT CTTCCCCCA
 4351 AAACCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT
 4401 GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG
 4451 TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG
 35 4501 TACAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA
 4551 CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC
 4601 CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
 4651 CCACAGGTGT ACACCCTGCC CCCATCCCGG GATGAGCTGA CCAAGAACCA
 4701 GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG
 40 4751 TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT
 4801 CCCGTGTTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT
 4851 GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC
 4901 ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
 4951 GGTAAATGA

pSYN VWF059 protein sequence (VWF D'D3-Fc with a2 region of FVIII thrombin site in the linker) - bold underlined area shows a2 region (SEQ ID NO: 197)

1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNFTFDGSM
 50 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQKRS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201 ERASPPSSSS NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC

251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTTEPC
 351 VHSBKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 5 451 LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
 501 DWDGRGRLLV KLSPPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPREDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
 651 NCPKGQVYLQ CGTPCNLTCT SLSYPDEECN EACLEGCFCP PGLYMDERGD
 10 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGEQ YVLVQDYCGS
 901 NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMDKE
 15 951 THFEVVEGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 1051 MKQTMVDSSC RILTSDVFQD CNKLVDPPEY LDVCIYDTCS CESIGDCAAF
 1101 CDTIAAYAHV CAQHGKVVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
 1151 PACQVTCQHP EPLACPVCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 20 1201 VAGRFRASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGAPTESA
 1251 TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE TPGTSESATP
 1301 ESGPGTSTEP SEGSA PGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
 1351 TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA **SISDKNTGDY**
 1401 **YEDSYEDISA YLLSKNNAIE PRFS**SDKTHT CPPCPAPELL GGPSVFLFPP
 25 1451 KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
 1501 YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIKT ISKAKGQPRE
 1551 PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 1601 PVLDSGDSFF LYSKLTVDKS RWQQGNVFSV SVMHEALHNH YTQKSLSLSP
 1651 GK*

pSYN VWF062 nucleotide sequence (encoding VWF D'D3-Fc with no thrombin site in the linker) (SEQ ID NO: 198)

1 ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
 35 51 GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC
 101 GATGCAGCCT TTTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG
 151 TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA
 201 ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
 251 TCTCCGTGTA TCTTGGGGAA TTTTGTGACA TCCATTTGTT TGTCAATGGT
 40 301 ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
 351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT
 401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
 451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
 501 CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
 45 551 CTTATGACTT TGCCAACTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
 601 GAACGGGCAT CTCCTCCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
 651 GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
 701 TTGCCCGCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
 751 GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC
 50 801 CCTCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
 851 GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
 901 TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
 951 CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG

1001 GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
 1051 GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
 1101 CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
 1151 GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
 5 1201 AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCCGGA
 1251 TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
 1301 ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC
 1351 CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
 1401 TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC
 10 1451 ATACAGTGAC GGCCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG
 1501 GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
 1551 CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
 1601 ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGACTTCGGG
 1651 AACGCCTGGA AGCTGCACGG GGACTGCCAG GACCTGCAGA AGCAGCACAG
 15 1701 CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
 1751 GCGCGGTCTT GACGTCCCCC ACATTCGAGG CCTGCCATCG TGCCGTCAGC
 1801 CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA
 1851 CGGCCGCGAG TGCCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
 1901 CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
 20 1951 AACTGCCCCA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
 2001 GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
 2051 TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
 2101 TCGGTGCCCC AGGCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
 2151 GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
 25 2201 GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC
 2251 GCTGTCTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
 2301 TCGGCCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAACT GTGCGGGCTG
 2351 AAGGGCTCGA GTGTACAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
 2401 AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
 30 2451 TGAGAACAGA TGTGTGGCCC TGGAAGGTG TCCCTGCTTC CATCAGGGCA
 2501 AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC
 2551 TGTGCGGACC GGAAGTGGAA CTGCACAGAC CATGTGTGTG ATGCCACGTG
 2601 CTCCAGGATC GGCATGGCCC ACTACCTCAC CTTGACGGG CTCAAATACC
 2651 TGTTCCCCGG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
 35 2701 AACCCTGGGA CCTTTCGGAT CTAGTGGGG AATAAGGGAT GCAGCCACCC
 2751 CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
 2801 TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
 2851 ACTCACTTTG AGGTGGTGGG GTCTGGCCGG TACATCATTC TGCTGCTGGG
 2901 CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
 40 2951 TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
 3001 GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
 3051 CCCTGTGGAC TTTGGGAACT CCTGGAAAGT GAGCTCGCAG TGTGCTGACA
 3101 CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC
 3151 ATGAAGCAGA CGATGGTGGG TTCCTCCTGT AGAATCCTTA CCAGTGACGT
 45 3201 CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
 3251 GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTC
 3301 TGCGACACCA TTGCTGCCTA TGCCCACGTG TGTGCCCAGC ATGGCAAGGT
 3351 GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC GAGGAGAGGA
 3401 ATCTCCGGGA GAACGGGTAT GAGGCTGAGT GGCGCTATAA CAGCTGTGCA
 50 3451 CCTGCCTGTC AAGTCACGTG TCAGCACCTT GAGCCACTGG CCTGCCCTGT
 3501 GCAGTGTGTG GAGGGCTGCC ATGCCCACTG CCCTCCAGGG AAAATCCTGG
 3551 ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
 3601 GTGGCTGGCC GGCGTTTTGC CTCAGGAAAG AAAGTCACCT TGAATCCAG

3651 TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC AACCTCACCT
 3701 GTGAAGCCTG CCAGGAGCCG ATATCGGGCG CGCCAACATC AGAGAGCGCC
 3751 ACCCCTGAAA GTGGTCCCGG GAGCGAGCCA GCCACATCTG GGTCGGAAAC
 3801 GCCAGGCACA AGTGAGTCTG CAACTCCCGA GTCCGGACCT GGCTCCGAGC
 5 3851 CTGCCACTAG CGGCTCCGAG ACTCCGGGAA CTTCCGAGAG CGCTACACCA
 3901 GAAAGCGGAC CCGGAACCAG TACCGAACCT AGCGAGGGCT CTGCTCCGGG
 3951 CAGCCCAGCC GGCTCTCCTA CATCCACGGA GGAGGGCACT TCCGAATCCG
 4001 CCACCCCGGA GTCAGGGCCA GGATCTGAAC CCGCTACCTC AGGCAGTGAG
 4051 ACGCCAGGAA CGAGCGAGTC CGCTACACCG GAGAGTGGG CAGGGAGCCC
 10 4101 TGCTGGATCT CCTACGTCCA CTGAGGAAGG GTCACCAGCG GGCTCGCCCA
 4151 CCAGCACTGA AGAAGGTGCC TCGAGCGACA AAACCTACAC ATGCCACCG
 4201 TGCCCAGCTC CAGAACTCCT GGGCGGACCG TCAGTCTTCC TCTTCCCCC
 4251 AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG
 4301 TGGTGGTGGA CGTGAGCCAC GAAGACCCTG AGGTCAAGTT CAACTGGTAC
 15 4351 GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA
 4401 GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG
 4451 ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC
 4501 CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA
 4551 ACCACAGGTG TACACCCTGC CCCCATCCCG GGATGAGCTG ACCAAGAACC
 20 4601 AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
 4651 GTGGAGTGGG AGAGCAATGG GCAGCCGAG AACAACTACA AGACCACGCC
 4701 TCCCGTGTTG GACTCCGACG GTCCTTCTT CCTCTACAGC AAGCTCACCG
 4751 TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
 4801 CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
 25 4851 GGGTAAATGA

pSYN VWF062 protein sequence (VWF D'D3-Fc with no thrombin site in the linker) (SEQ ID NO: 199)

30 1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNVTDFGSM
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DSGSNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 35 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251 EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLEDEG LCVESTECPC
 351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
 40 451 LHNSLVKLKH GAGVAMDQD IQLPLKGD LRIQHTVTASV RLSYGEDLQM
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
 651 NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 45 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901 NPGTFRILVG NKGCSHP SVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
 50 951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 1051 MKQTMVDSSC RILTSDFVQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
 1101 CDTIAAYAHV CAQHKGKVVW RTATLCPQSC EERNLRENGY EAEWRYNSCA

1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGAPTSESA
 1251 TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE TPGTSESATP
 1301 ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
 5 1351 TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA SSDKTHTCP
 1401 CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
 1451 VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
 1501 PAPIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
 1551 VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
 10 1601 HEALHNHYTQ KSLSLSPGK*

pSYN VWF073 nucleotide sequence- (encoding VWFD1D2D'D3- 144 AE XTEN- FVIII truncated a2 thrombin site-Fc) (SEQ ID NO:174)

15 1 ATGATTCCTG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
 51 GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC
 101 GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG
 151 TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA
 20 201 ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
 251 TCTCCGTGTA TCTTGGGGAA TTTTGTGACA TCCATTTGTT TGTCAATGGT
 301 ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
 351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT
 401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
 25 451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
 501 CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
 551 CTTATGACTT TGCCAACTCA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
 601 GAACGGGCAT CTCCTCCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
 651 GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
 30 701 TTGCCCCTG CCACCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
 751 GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC
 801 CCTCTGGAG TACGCCCGGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
 851 GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
 901 TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
 35 951 CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG
 1001 GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
 1051 GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
 1101 CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
 1151 GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
 40 1201 AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA
 1251 TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
 1301 ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCTGGC
 1351 CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
 1401 TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC
 45 1451 ATACAGTGAC GGCTCCGTG CGCCTCAGCT ACGGGGAGGA CCTGCAGATG
 1501 GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
 1551 CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
 1601 ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGAATTCCGG
 1651 AACGCCTGGA AGCTGCACGG GGAAGTCCAG GACCTGCAGA AGCAGCACAG
 50 1701 CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
 1751 GCGCGGTCTT GACGTCCCCC ACATTCGAGG CCTGCCATCG TGCCGTACAG
 1801 CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA

1851 CGGCCGCGAG TGCCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
 1901 CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
 1951 AACTGCCCCA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
 2001 GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
 5 2051 TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
 2101 TGCGTGCCCA AGGCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
 2151 GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
 2201 GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC
 2251 GCTGTCTCA GCAGTCCCCT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
 10 2301 TCGGCCCCCC ATGGTCAAGC TGGTGTGTCC CGCTGACAAC CTGCGGGCTG
 2351 AAGGGCTCGA GTGTACAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
 2401 AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
 2451 TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC CATCAGGGCA
 2501 AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC
 15 2551 TGTCGGGACC GGAAGTGGA CTGCACAGAC CATGTGTGTG ATGCCACGTG
 2601 CTCCACGATC GGCATGGCCC ACTACCTCAC CTTGACGGG CTCAAATACC
 2651 TGTTCCCCCG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
 2701 AACCCTGGGA CCTTTCGGAT CTAGTGGGG AATAAGGGAT GCAGCCACCC
 2751 CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
 20 2801 TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
 2851 ACTCACTTTG AGGTGGTGA GTCTGGCCGG TACATCATTC TGCTGCTGGG
 2901 CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
 2951 TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
 3001 GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
 25 3051 CCCTGTGGAC TTTGGGAACT CCTGGAAGT GAGCTCGCAG TGTGCTGACA
 3101 CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC
 3151 ATGAAGCAGA CGATGGTGGA TTCCTCCTGT AGAATCCTTA CCAGTGACGT
 3201 CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
 3251 GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTC
 30 3301 TGCGACACCA TTGCTGCCTA TGCCACGTG TGTGCCAGC ATGGCAAGGT
 3351 GGTGACCTGG AGGACGGCCA CATTGTGCC CCAGAGCTGC GAGGAGAGGA
 3401 ATCTCCGGGA GAACGGGTAT GAGGCTGAGT GGCGCTATAA CAGTGTGCA
 3451 CCTGCCTGTC AAGTCACCTG TCAGACCCT GAGCCACTGG CTGCCCTGT
 3501 GCAGTGTGTG GAGGGCTGCC ATGCCACTG CCCTCCAGGG AAAATCCTGG
 35 3551 ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
 3601 GTGGCTGGCC GGCGTTTTGC CTCAGGAAAG AAAGTCACCT TGAATCCAG
 3651 TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC AACCTCACCT
 3701 GTGAAGCCTG CCAGGAGCCG GGCGCGCCAA CATCAGAGAG CGCCACCCCT
 3751 GAAAGTGGTC CCGGAGCGA GCCAGCCACA TCTGGGTCGG AAACGCCAGG
 40 3801 CACAAGTGAG TCTGCAACTC CCGAGTCCGG ACCTGGCTCC GAGCCTGCCA
 3851 CTAGCGGCTC CGAGACTCCG GGAACCTCCG AGAGCGCTAC ACCAGAAAGC
 3901 GGACCCGGAA CCAGTACCGA ACCTAGCGAG GGCTCTGCTC CGGGCAGCCC
 3951 AGCCGGCTCT CCTACATCCA CGGAGGAGGG CACTTCCGAA TCCGCCACCC
 4001 CGGAGTCAGG GCCAGGATCT GAACCCGCTA CCTCAGGCAG TGAGACGCCA
 45 4051 GGAACGAGCG AGTCCGCTAC ACCGGAGAGT GGGCCAGGGA GCCCTGCTGG
 4101 ATCTCCTACG TCCACTGAGG AAGGGTCACC AGCGGGCTCG CCCACCAGCA
 4151 CTGAAGAAGG TGCCTCGAGC GGCGGTGGAG GATCCGGTGG CGGGGGATCC
 4201 GGTGGCGGGG GATCCGGTGG CGGGGGATCC GGTGGCGGGG GATCCGGTGG
 4251 CGGGGGATCC ATTGAACCAA GAAGCTTCTC TGGCAGCGGA GGCGACAAAA
 50 4301 CTCACACATG CCCACCGTGC CCAGCTCCAG AACTCCTGGG CGGACCGTCA
 4351 GTCTTCTCT TCCCCCAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC
 4401 CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG
 4451 TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA

4501 AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT
 4551 CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG
 4601 TCTCCAACAA AGCCCTCCCA GCCCCATCG AGAAAACCAT CTCCAAAGCC
 4651 AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA
 5 4701 TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT
 4751 ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC
 4801 AACTACAAGA CCACGCCTCC CGTGTTGGAC TCCGACGGCT CCTTCTTCCT
 4851 CTACAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT
 4901 TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
 10 4951 AGCCTCTCCC TGTCTCCGG TAAATGA

pSYN VWF073 protein sequence- (VWFD1D2D'D3- 144 AE XTEN- truncated a2 thrombin site-Fc) (SEQ ID NO:175)

15 1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQ RVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 20 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTCPC
 351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
 401 NRYFTFSGIC QYLLARDCQD HSFIVIVETV QCADDRDAVC TRSVTVRLPG
 25 451 LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTSPG LAEPRVEDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL
 651 NCPKGQVYLQ CGTPCNLT CR SLSYPDEECN EACLEGCFCP PGLYMDERGD
 30 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK TCQNYDLECM
 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
 901 NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
 35 951 THFEVVESGR YIILLGKAL SVVWDRHL SI SVVLKQTYQE KVCGLCGNFD
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 1051 MKQTMVDSSC RILTSDFVQD CNKLVDP EY LDVCIYDTCS CESIGDCAAF
 1101 CDTIAAYAHV CAQH GK VVTW RTATLCPQSC EERNLRENGY EAEWRYNSCA
 1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 40 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP GAPTS SATP
 1251 ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
 1301 GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
 1351 GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGASS GGGGSGGGGS
 1401 GGGGSGGGGS GGGGSGGGGS IEPRSFSGSG GDKTHTCPPC PAPELLGGPS
 45 1451 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
 1501 KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
 1551 KGQPREPQVY TLPPSRDEL T KNQVSLTCLV KGFYPSDIAV EWESNGQPEN
 1601 NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK
 1651 SLSLSPGK*

Table 18: Exemplary Chimeric Polypeptide Sequences

FVIII-XTEN-Fc: SEQ ID NO: 201
TRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTFLFVEFTDHLFNIAKP RPPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAHEYDDQTSQREKED DKVFPGGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKE KTQTLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGC HRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHIS SHQHDGMEAYVKVDSCEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNPSFIQIRSV KKHPKTWVHYIAAEEEDWDYAPLV LAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETF KTREAIQHESGILGPLYGEVGDTLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDF PILPGEIFKYKWTVTVEDGPTKSDPRCLTRYSSYFVNMERDLASGLIGPLLCYKESVDQGRNQ IMSDKRNVILFSVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSV CLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMYEDTLTLFPFSGETVFMMSMENPGLWILGC HNSDFRNRGMTALLKVSSCDKNTGDYEDSYEDISAYLLSKNNAIEPRSFQNGTSESATPES GPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTE GSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTE EGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPAT SGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPG TSTEPSEGSAPASSEITRITLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFI AAVERLWDYGMSSSPHVLNRNAQSGSVPPQFKKVVVFQFTDGSFTQPLYRGELNEHLGLLGPY IRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPKRFVKNPNETKTYFWKVQHMAP TKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLCHTNTLNPAHGRQVTVQEFALFFTIFDETKS WYFTENMERNCRAPCNQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQIRWYLLSMGS NENIHSIHFSGHVFTVRKKKEEYKMALYNLYPGVFETVEMLPKAGIWRVECLIGEHLHAGMST LFLVYSNKCQTPLGMASGHIRDFQITASGQYQGWAPKLARLHYSGSINAWSTKEPFSWIKVDL LAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNF NPPIIARYIRLHPHTYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSP SKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTQGVKSLTSMYVKEFLISSSQ DGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRHPQSWVHQIALRMEVLGCEA QDLYDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPG
VWF(D'D3)-XTEN-a2 Linker-Fc: SEQ ID NO: 202
SLSCRPPMVKLVCADNLRAEGLECTKTCQNYDLECMMSMGCVSGCLCPPGMVRHENRCVAL ERPCFHHQKEYAPGETVKIGCNTCVCRDRKWNCTDHVCDATCSTIGMAHYLTFDGLKYLFP GECQYVLVQDYCGSNPGTFRILVGNGC SHPSVKCKKRVTLVEGGEIELFDGEVNVKRPMK DETHFEVVESGRYIILLGKALS VVWDRHLSISVVLKQTYQEKVCGLCGNFDGIQNNDLTSSN LQVEEDPVDFGNSWKVSSQCADTRKVPLDSSPATCHNNIMKQTMVDSSCRILTSDVFQDCNK LVDPEPYLDVCIYDTCSCESIGDCAAFCDTIAAYAHVCAQH GKVVVTWRTATLCPQSCEERNLR ENGYEAEWRYN SCAPACQVTCQHPEPLACPVQCVEGCHAHCPPGKILDELLQTCVDPEDCPV CEVAGRRFASGKKVTLNPSDPEHCQICHCDVNLNLTCEACQEPGTSESATPESGPGSEPATSGSE TPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSES ATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGASSDKNTGDY YEDSYEDISAYLLSKNNAIEPRSFSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPG

Amino acid sequence of FVIII protein (312A): SEQ ID NO: 203

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAVE	LSWDYMQSDL	GELPVDARFP
51	PRVPKSFPPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG
151	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
201	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
251	HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
401	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFTKTR	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
501	DVRPLYSRRL	PKGKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
601	NRSWYLTEI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
651	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
701	MENPGLWLIG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYED	SYEDISAYLL
751	SKNNAIEPRS	FSQNGTSESA	TPESGPGSEP	ATSGSETPGT	SESATPESGP
801	GSEPATSGSE	TPGTSESATP	ESGPGTSTEP	SEGSAPGSPA	GSPTSTEEGT
851	SESATPESGP	GSEPATSGSE	TPGTSESATP	ESGPGSPAGS	PTSTEEGSPA
901	GSPTSTEEGT	STEPSEGSAP	GTSESATPES	GPGTSESATP	ESGPGTSESA
951	TPESGPGSEP	ATSGSETPGS	EPATSGSETP	GSPAGSPTST	EEGTSTEPSE
1001	GSAPGTSTEP	SEGSAPGSEP	ATSGSETPGT	SESATPESGP	GTSTEPSEGS
1051	APASSEITRT	TLQSDQEEID	YDDTISVEMK	KEDFDIYDED	ENQSPRSFQK
1101	KTRHYFIAAV	ERLWDYGMSS	SPHVLNRNAQ	SGSVPOFKKV	VFQEFDTGSGF
1151	TQPLYRGELN	EHLGLLGPYI	RAEVEDNIMV	TFRNQASRPY	SFYSSLISYE
1201	EDQRQGAEP	KNFVKPNETK	TYFWKVQHMM	APTKDEFDCK	AWAYFSDVDL
1251	EKDVHSLIG	PLLVCHTNLT	NPAHGRQVTV	QEFALFFTIF	DETKSWYFTE
1301	NMERNCRAPC	NIQMEDPTFK	ENYRFHAING	YIMDTLPGLV	MAQDQIRRWY
1351	LLSMGSNENI	HSIHFSGHVF	TVRKKEEYKM	ALYNLYPGVF	ETVEMLPSCA
1401	GIWRVECLIG	EHLHAGMSTL	FLVYSNKCQT	PLGMASGHIR	DFQITASGQY
1451	GQWAPKLARL	HYSGSINAWS	TKEPFSWIKV	DLLAPMIIHG	IKTQGARQKF
1501	SSLYISQFII	MYSLDGKKWQ	TYRGNSTGTL	MVFFGNVDSS	GIKHNI FNPP
1551	IIARYIRLHP	THYSIRSTLR	MELMGCDLNS	CSMPLGMESK	AISDAQITAS
1601	SYFTNMFATW	SPSKARLHLQ	GRSNAWRPQV	NNPKEWLQVD	FQKTMKVTGV
1651	TTQGVKSLLT	SMYVKEFLIS	SSQDGHQWTL	FFQNGKVVKF	QGNQDSFTPV
1701	VNSLDPPLLT	RYLRIHPQSW	VHQIALRMEV	LGCEAQDLYD	KTHTCPPCPA
1751	PELLGGPSVF	LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG
1801	VEVHNAKTKP	REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP
1851	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG	FYPSDIAVEW
1901	ESNGQPENNY	KTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	VFSCSVMEHA
1951	LHNHYTQKSL	SLSPG*			

Nucleotide sequence encoding FVIII 312A: SEQ ID NO: 204

1	ATGCAAATAG	AGCTCTCCAC	CTGCTTCTTT	CTGTGCCTTT	TGCGATTCTG
51	CTTTAGTGCC	ACCAGAAGAT	ACTACCTGGG	TGCAGTGGAA	CTGTCATGGG
101	ACTATATGCA	AAGTGATCTC	GGTGAGCTGC	CTGTGGACGC	AAGATTTCTT
151	CCTAGAGTGC	CAAAATCTTT	TCCATTCAAC	ACCTCAGTCG	TGTACAAAAA

201	GACTCTGTTT	GTAGAATTCA	CGGATCACCT	TTTCAACATC	GCTAAGCCAA
251	GGCCACCCTG	GATGGGTCTG	CTAGGTCCTA	CCATCCAGGC	TGAGGTTTAT
301	GATACAGTGG	TCATTACACT	TAAGAACATG	GCTTCCCATC	CTGTCAGTCT
351	TCATGCTGTT	GGTGTATCCT	ACTGGAAAGC	TTCTGAGGGA	GCTGAATATG
401	ATGATCAGAC	CAGTCAAAGG	GAGAAAGAAG	ATGATAAAGT	CTTCCCTGGT
451	GGAAGCCATA	CATATGTCTG	GCAGGTCCTG	AAAGAGAATG	GTCCAATGGC
501	CTCTGACCCA	CTGTGCCTTA	CCTACTCATA	TCTTTCTCAT	GTGGACCTGG
551	TAAAAGACTT	GAATTCAGGC	CTCATTGGAG	CCCTACTAGT	ATGTAGAGAA
601	GGGAGTCTGG	CCAAGGAAAA	GACACAGACC	TTGCACAAAT	TTATACTACT
651	TTTTGCTGTA	TTTGATGAAG	GGAAAAGTTG	GCACTCAGAA	ACAAAGAACT
701	CCTTGATGCA	GGATAGGGAT	GCTGCATCTG	CTCGGGCCTG	GCCTAAAATG
751	CACACAGTCA	ATGGTTATGT	AAACAGGTCT	CTGCCAGGTC	TGATTGGATG
801	CCACAGGAAA	TCAGTCTATT	GGCATGTGAT	TGGAATGGGC	ACCACTCCTG
851	AAGTGCACTC	AATATTCCTC	GAAGGTCACA	CATTTCTTGT	GAGGAACCAT
901	CGCCAGGCGT	CCTTGGAAT	CTCGCCAATA	ACTTTCTTGA	CTGCTCAAAC
951	ACTCTTGATG	GACCTTGGAC	AGTTTCTACT	GTTTTGTCAT	ATCTCTTCCC
1001	ACCAACATGA	TGGCATGGAA	GCTTATGTCA	AAGTAGACAG	CTGTCCAGAG
1051	GAACCCCAAC	TACGAATGAA	AAATAATGAA	GAAGCGGAAG	ACTATGATGA
1101	TGATCTTACT	GATTCTGAAA	TGGATGTGGT	CAGGTTTGAT	GATGACAACT
1151	CTCCTTCCTT	TATCCAAATT	CGCTCAGTTG	CCAAGAAGCA	TCCTAAAAC
1201	TGGGTACATT	ACATTGCTGC	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT
1251	AGTCCTCGCC	CCCATGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG
1301	GCCCTCAGCG	GATTGGTAGG	AAGTACAAAA	AAGTCCGATT	TATGGCATA
1351	ACAGATGAAA	CCTTTAAGAC	TCGTGAAGCT	ATTCAGCATG	AATCAGGAAT
1401	CTTGGGACCT	TTACTTTATG	GGGAAGTTGG	AGACACACTG	TTGATTATAT
1451	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCCTCA	CGGAATCACT
1501	GATGTCCGTC	CTTTGTATTG	AAGGAGATTA	CCAAAAGGTG	TAAAACATTT
1551	GAAGGATTTT	CCAATTCTGC	CAGGAGAAAT	ATTCAAATAT	AAATGGACAG
1601	TGACTGTAGA	AGATGGGCCA	ACTAAATCAG	ATCCTCGGTG	CCTGACCCGC
1651	TATTACTCTA	GTTTCGTTAA	TATGGAGAGA	GATCTAGCTT	CAGGACTCAT
1701	TGGCCCTCTC	CTCATCTGCT	ACAAAGAATC	TGTAGATCAA	AGAGGAAACC
1751	AGATAATGTC	AGACAAGAGG	AATGTCATCC	TGTTTTCTGT	ATTTGATGAG
1801	AACCGAAGCT	GGTACCTCAC	AGAGAATATA	CAACGCTTTC	TCCCCAATCC
1851	AGCTGGAGTG	CAGCTTGAGG	ATCCAGAGTT	CCAAGCCTCC	AACATCATGC
1901	ACAGCATCAA	TGGCTATGTT	TTTGATAGTT	TGCAGTTGTC	AGTTTGTTTG
1951	CATGAGGTGG	CATACTGGTA	CATTCTAAGC	ATTGGAGCAC	AGACTGACTT
2001	CCTTTCTGTC	TTCTTCTCTG	GATATACCTT	CAAACACAAA	ATGGTCTATG
2051	AAGACACACT	CACCCTATTG	CCATTCTCAG	GAGAACTGT	CTTCATGTCTG
2101	ATGGAAAACC	CAGGTCTATG	GATTCTGGGG	TGCCACAAC	CAGACTTTCG
2151	GAACAGAGGC	ATGACCGCCT	TACTGAAGGT	TTCTAGTTGT	GACAAGAACA
2201	CTGGTGATTA	TTACGAGGAC	AGTTATGAAG	ATATTTTCTG	ATACTTGCTG
2251	AGTAAAAACA	ATGCCATTGA	ACCAAGAAGC	TTCTCTCAAA	ACGGTACCTC
2301	AGAGTCTGCT	ACCCCGAGT	CAGGGCCAGG	ATCAGAGCCA	GCCACCTCCG
2351	GGTCTGAGAC	ACCCGGGACT	TCCGAGAGTG	CCACCCCTGA	GTCCGGACCC
2401	GGGTCCGAGC	CCGCCACTTC	CGGCTCCGAA	ACTCCCGGCA	CAAGCGAGAG
2451	CGCTACCCCA	GAGTCAGGAC	CAGGAACATC	TACAGAGCCC	TCTGAAGGCT
2501	CCGCTCCAGG	GTCCCGAGCC	GGCAGTCCCA	CTAGCACCGA	GGAGGGAACC
2551	TCTGAAAGCG	CCACACCCGA	ATCAGGGCCA	GGGTCTGAGC	CTGCTACCAG
2601	CGGCAGCGAG	ACACCAGGCA	CCTCTGAGTC	CGCCACACCA	GAGTCCGGAC
2651	CCGGATCTCC	CGCTGGGAGC	CCCACCTCCA	CTGAGGAGGG	ATCTCCTGCT
2701	GGCTCTCCAA	CATCTACTGA	GGAAGGAACC	TCAACCGAGC	CATCCGAGGG
2751	ATCAGCTCCC	GGCACCTCAG	AGTCGGCAAC	CCCGGAGTCT	GGACCCGGAA
2801	CTTCCGAAAG	TGCCACACCA	GAGTCCGGTC	CCGGGACTTC	AGAATCAGCA
2851	ACACCCGAGT	CCGGCCCTGG	GTCTGAACCC	GCCACAAGTG	GTAGTGAGAC

2901	ACCAGGATCA	GAACCTGCTA	CCTCAGGGTC	AGAGACACCC	GGATCTCCGG
2951	CAGGCTCACC	AACCTCCACT	GAGGAGGGCA	CCAGCACAGA	ACCAAGCGAG
3001	GGCTCCGCAC	CCGGAACAAG	CACTGAACCC	AGTGAGGGTT	CAGCACCCGG
3051	CTCTGAGCCG	GCCACAAGTG	GCAGTGAGAC	ACCCGGCACT	TCAGAGAGTG
3101	CCACCCCGA	GAGTGGCCCA	GGCACTAGTA	CCGAGCCCTC	TGAAGGCAGT
3151	GCGCCAGCCT	CGAGCGAAAT	AACCTCGTACT	ACTCTTCAGT	CAGATCAAGA
3201	GGAAATTGAC	TATGATGATA	CCATATCAGT	TGAAATGAAG	AAGGAAGATT
3251	TTGACATTTA	TGATGAGGAT	GAAAATCAGA	GCCCCCGCAG	CTTTCAAAAG
3301	AAAACACGAC	ACTATTTTAT	TGCTGCAGTG	GAGAGGCTCT	GGGATTATGG
3351	GATGAGTAGC	TCCCCACATG	TTCTAAGAAA	CAGGGCTCAG	AGTGGCAGTG
3401	TCCCTCAGTT	CAAGAAAGTT	GTTTTCCAGG	AATTTACTGA	TGGCTCCTTT
3451	ACTCAGCCCT	TATACCGTGG	AGAACTAAAT	GAACATTTGG	GACTCCTGGG
3501	GCCATATATA	AGAGCAGAAG	TTGAAGATAA	TATCATGGTA	ACTTTCAGAA
3551	ATCAGGCCTC	TCGTCCCTAT	TCCTTCTATT	CTAGCCTTAT	TTCTTATGAG
3601	GAAGATCAGA	GGCAAGGAGC	AGAACCTAGA	AAAAACTTTG	TCAAGCCTAA
3651	TGAAACCAAA	ACTTACTTTT	GGAAAGTGCA	ACATCATATG	GCACCCACTA
3701	AAGATGAGTT	TGACTGCAAA	GCCTGGGCTT	ATTTCTCTGA	TGTTGACCTG
3751	GAAAAAGATG	TGCACTCAGG	CCTGATTGGA	CCCCTTCTGG	TCTGCCACAC
3801	TAACACACTG	AACCCTGCTC	ATGGGAGACA	AGTGACAGTA	CAGGAATTTG
3851	CTCTGTTTTT	CACCATCTTT	GATGAGACCA	AAAGCTGGTA	CTTCACTGAA
3901	AATATGGAAA	GAAACTGCAG	GGCTCCCTGC	AATATCCAGA	TGGAAGATCC
3951	CACTTTTAAA	GAGAATTATC	GCTTCCATGC	AATCAATGGC	TACATAATGG
4001	ATACACTACC	TGGCTTAGTA	ATGGCTCAGG	ATCAAAGGAT	TCGATGGTAT
4051	CTGCTCAGCA	TGGGCAGCAA	TGAAAACATC	CATTCTATTC	ATTTCACTGG
4101	ACATGTGTTT	ACTGTACGAA	AAAAAGAGGA	GTATAAAATG	GCACTGTACA
4151	ATCTCTATCC	AGGTGTTTTT	GAGACAGTGG	AAATGTTACC	ATCCAAAGCT
4201	GGAATTTGGC	GGGTGGAATG	CCTTATTGGC	GAGCATCTAC	ATGCTGGGAT
4251	GAGCACACTT	TTTCTGGTGT	ACAGCAATAA	GTGTCAGACT	CCCCTGGGAA
4301	TGGCTTCTGG	ACACATTAGA	GATTTTCAGA	TTACAGCTTC	AGGACAATAT
4351	GGACATGGG	CCCCAAGCT	GGCCAGACTT	CATTATTCCG	GATCAATCAA
4401	TGCCTGGAGC	ACCAAGGAGC	CGTTTTCTTG	GATCAAGGTG	GATCTGTTGG
4451	CACCAATGAT	TATTCACGGC	ATCAAGACCC	AGGGTGCCCG	TCAGAAGTTC
4501	TCCAGCCTCT	ACATCTCTCA	GTTTATCATC	ATGTATAGTC	TTGATGGGAA
4551	GAAGTGGCAG	ACTTATCGAG	GAAATTCCAC	TGGAACCTTA	ATGGTCTTCT
4601	TTGGCAATGT	GGATTCTATC	GGGATAAAAC	ACAATATTTT	TAACCCCTCA
4651	ATTATTGCTC	GATACATCCG	TTTGACCCCA	ACTCATTATA	GCATTGCGAG
4701	CACTCTTCGC	ATGGAGTTGA	TGGGCTGTGA	TTTAAATAGT	TGCAGCATGC
4751	CATTGGGAAT	GGAGAGTAAA	GCAATATCAG	ATGCACAGAT	TACTGCTTCA
4801	TCCTACTTTA	CCAATATGTT	TGCCACCTGG	TCTCCTTCAA	AAGCTCGACT
4851	TCACCTCCAA	GGGAGGAGTA	ATGCCTGGAG	ACCTCAGGTG	AATAATCCAA
4901	AAGAGTGGCT	GCAAGTGGAC	TTCCAGAAGA	CAATGAAAGT	CACAGGAGTA
4951	ACTACTCAGG	GAGTAAAATC	TCTGCTTACC	AGCATGTATG	TGAAGGAGTT
5001	CCTCATCTCC	AGCAGTCAAG	ATGGCCATCA	GTGGACTCTC	TTTTTTTCAGA
5051	ATGGCAAAGT	AAAGGTTTTT	CAGGGAAATC	AAGACTCCTT	CACACCTGTG
5101	GTGAACTCTC	TAGACCCACC	GTTACTGACT	CGCTACCTTC	GAATTACCCC
5151	CCAGAGTTGG	GTGCACCAGA	TTGCCCTGAG	GATGGAGGTT	CTGGGCTGCG
5201	AGGCACAGGA	CCTCTACGAC	AAAACCTACA	CATGCCACC	GTGCCAGCT
5251	CCAGAACTCC	TGGGCGGACC	GTCACTCTTC	CTCTTCCCCC	CAAAACCCAA
5301	GGACACCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGTGGTGG
5351	ACGTGAGCCA	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	TGTGGACGGC
5401	GTGGAAGTGC	ATAATGCCAA	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG
5451	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	CCTGCACCAA	GACTGGCTGA
5501	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	CCCAGCCCCC
5551	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGG	CAGCCCCGAG	AACCACAGGT

5601	GTACACCCTG	CCCCATCCC	GGGATGAGCT	GACCAAGAAC	CAAGTTAGCC
5651	TGACCTGCCT	GGTCAAAGGC	TTCTATCCCA	GCGACATCGC	CGTGGAGTGG
5701	GAGAGCAATG	GGCAGCCGGA	GAACAACTAC	AAGACCACGC	CTCCCGTGTT
5751	GGACTCCGAC	GGCTCCTTCT	TCCTCTACTC	CAAGCTCACC	GTGGACAAGA
5801	GCAGGTGGCA	GCAGGGGAAC	GTCTTCTCAT	GCTCCGTGAT	GCATGAGGCT
5851	CTGCACAACC	ACTACACGCA	GAAGAGCCTC	TCCCTGTCTC	CGGGTTGA

Amino acid sequence of VWF059A protein: SEQ ID NO: 205

1	MIPARFAGVL	LALALILPGT	LCAEGTRGRS	STARCSLFGS	DFVNTFDGSM
51	YSFAGYCSYL	LAGGCQKRSF	SIIGDFQNGK	RVSLSVYLGE	FFDIHLFVNG
101	TVTQGDQRV	MPYASKGLYL	ETEAGYYKLS	GEAYGFVARI	DGSGNFQVLL
151	SDRYFNKTCG	LCGNFNIFAE	DDFMTQEGTL	TSDPYDFANS	WALSSGEQWC
201	ERASPPSSSC	NISSGEMQKG	LWEQCQLLKS	TSVFARCHPL	VDPEPFVALC
251	EKTLCECAGG	LECACPALLE	YARTCAQEGM	VLYGWTDHSA	CSPVCPAGME
301	YRQCVSPCAR	TCQSLHINEM	CQERCVDGCS	CPEGQLLDEG	LCVESTECPC
351	VHSGKRYPPG	TSLSRDCNTC	ICRNSQWICS	NEECPGECLV	TGQSHFKSFD
401	NRYFTFSGIC	QYLLARDCQD	HSFSIVIETV	QCADDRDAVC	TRSVTVRLPG
451	LHNSLVKLKH	GAGVAMDGQD	IQLPLLKGD	RIQHTVTASV	RLSYGEDLQM
501	DWDGRGRLLV	KLSPVYAGKT	CGLCGNYNGN	QGDDFLTPSG	LAEPRVEDFG
551	NAWKHLHGDCQ	DLQKQHSDFC	ALNPRMTRFS	EEACAVLTSP	TFEACHRAVS
601	PLPYLRNCRY	DVCSCSDGRE	CLCGALASYA	AACAGRGVRV	AWREPGRCEL
651	NCPKGQVYLQ	CGTPCNLTCT	SLSYPDEECN	EACLEGCFCP	PGLYMDERGD
701	CVPKAQCPCY	YDGEIFQPED	IFSDHHTMCY	CEDGFMHCTM	SGVPGSLLPD
751	AVLSSPLSHR	SKRSLSCRPP	MVKLVCPADN	LRAEGLECTK	TCQNYDLECM
801	SMGCVSGCLC	PPGMVRHENR	CVALERCPCF	HQGKEYAPGE	TVKIGCNTCV
851	CRDRKWNCTD	HVCDATCSTI	GMAHYLTFDG	LKYLFPGECQ	YVLVQDYCGS
901	NPGTFRILVG	NKGCSHPSVK	CKKRVTLIVE	GGEIELFDGE	VNVKRPMDKE
951	THFEVVESEGR	YIILLGKAL	SVVWDRHLSI	SVVLKQTYQE	KVCGLCGNFD
1001	GIQNNDLTSS	NLQVEEDPVD	FGNSWKVSSQ	CADTRKVPLD	SSPATCHNNI
1051	MKQTMVDSSC	RILTSDFVQD	CNKLVDPPEY	LDVCIYDTCS	CESIGDCAAF
1101	CDTIAAYAHV	CAQHGVVVTW	RTATLCPQSC	EERNLRENGY	EAEWRYNSCA
1151	PACQVTCQHP	EPLACPVQCV	EGCHAHCPPG	KILDELLQTC	VDPEDCPVCE
1201	VAGRRFASGK	KVTLNPSDPE	HCQICHCDVV	NLTCEACQEP	GTSESATPES
1251	GPGSEPATSG	SETPGTSESA	TPESGPGSEP	ATSGSETPGT	SESATPESGP
1301	GTSTEPSEGS	APGSPAGSPT	STEEGTSESA	TPESGPGSEP	ATSGSETPGT
1351	SESATPESGP	GSPAGSPTST	EEGSPAGSPT	STEEGASSDK	NTGDYEDSY
1401	EDISAYLLSK	NNAIEPRFS	DKTHTCPPCP	APELLGGPSV	FLFPPKPKDT
1451	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY
1501	RVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT
1551	LPPSRDELTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTPPVVLD
1601	DGSFFLYSKL	TVDKSRWQQG	NVFSCVMHE	ALHNHYTQKS	LSSLSPG*

Nucleotide sequence encoding VWF059A protein: SEQ ID NO: 206

1	ATGATTCCTG	CCAGATTTCG	CGGGGTGCTG	CTTGCTCTGG	CCCTCATTTT
51	GCCAGGGACC	CTTTGTGCAG	AAGGAACCTG	CGGCAGGTCA	TCCACGGCCC
101	GATGCAGCCT	TTTCGGAAGT	GACTTCGTCA	ACACCTTTGA	TGGGAGCATG
151	TACAGCTTTG	CGGGATACTG	CAGTTACCTC	CTGGCAGGGG	GCTGCCAGAA
201	ACGCTCCTTC	TCGATTATTG	GGGACTTCCA	GAATGGCAAG	AGAGTGAGCC
251	TCTCCGTGTA	TCTTGGGGAA	TTTTTTGACA	TCCATTTGTT	TGTCAATGGT

301	ACCGTGACAC	AGGGGGACCA	AAGAGTCTCC	ATGCCCTATG	CCTCCAAAGG
351	GCTGTATCTA	GAAACTGAGG	CTGGGTACTA	CAAGCTGTCC	GGTGAGGCCT
401	ATGGCTTTGT	GGCCAGGATC	GATGGCAGCG	GCAACTTTCA	AGTCCTGCTG
451	TCAGACAGAT	ACTTCAACAA	GACCTGCGGG	CTGTGTGGCA	ACTTTAACAT
501	CTTTGCTGAA	GATGACTTTA	TGACCCAAGA	AGGGACCTTG	ACCTCGGACC
551	CTTATGACTT	TGCCAACTCA	TGGGCTCTGA	GCAGTGGAGA	ACAGTGGTGT
601	GAACGGGCAT	CTCCTCCCAG	CAGCTCATGC	AACATCTCCT	CTGGGGAAAT
651	GCAGAAGGGC	CTGTGGGAGC	AGTGCCAGCT	TCTGAAGAGC	ACCTCGGTGT
701	TTGCCCCTG	CCACCCTCTG	GTGGACCCCG	AGCCTTTTGT	GGCCCTGTGT
751	GAGAAGACTT	TGTGTGAGTG	TGCTGGGGGG	CTGGAGTGCG	CCTGCCCTGC
801	CCTCCTGGAG	TACGCCCGGA	CCTGTGCCCA	GGAGGGAATG	GTGCTGTACG
851	GCTGGACCGA	CCACAGCGCG	TGCAGCCCAG	TGTGCCCTGC	TGGTATGGAG
901	TATAGGCAGT	GTGTGTCCCC	TTGCGCCAGG	ACCTGCCAGA	GCCTGCACAT
951	CAATGAAATG	TGTCAGGAGC	GATGCGTGGA	TGGCTGCAGC	TGCCCTGAGG
1001	GACAGCTCCT	GGATGAAGGC	CTCTGCGTGG	AGAGCACCGA	GTGTCCCTGC
1051	GTGCATTCCG	GAAAGCGCTA	CCCTCCCAGG	ACCTCCCTCT	CTCGAGACTG
1101	CAACACCTGC	ATTTGCCGAA	ACAGCCAGTG	GATCTGCAGC	AATGAAGAAT
1151	GTCCAGGGGA	GTGCCTTGTC	ACTGGTCAAT	CCCACCTCAA	GAGCTTTGAC
1201	AACAGATACT	TCACCTTCAG	TGGGATCTGC	CAGTACCTGC	TGGCCCCGGA
1251	TTGCCAGGAC	CACTCCTTCT	CCATTGTCAT	TGAGACTGTC	CAGTGTGCTG
1301	ATGACCGCGA	CGCTGTGTGC	ACCCGCTCCG	TCACCGTCCG	GCTGCCTGGC
1351	CTGCACAACA	GCCTTGTGAA	ACTGAAGCAT	GGGGCAGGAG	TTGCCATGGA
1401	TGGCCAGGAC	ATCCAGCTCC	CCCTCCTGAA	AGGTGACCTC	CGCATCCAGC
1451	ATACAGTGAC	GGCCTCCGTG	CGCCTCAGCT	ACGGGGAGGA	CCTGCAGATG
1501	GACTGGGATG	GCCGCGGGAG	GTGCTGGTGG	AAGCTGTCCC	CCGTGCATGC
1551	CGGGAAGACC	TGCGGCCTGT	GTGGGAATTA	CAATGGCAAC	CAGGGCGACG
1601	ACTTCCTTAC	CCCCTCTGGG	CTGGCGGAGC	CCCGGGTGGA	GGACTTCGGG
1651	AACGCCTGGA	AGCTGCACGG	GGACTGCCAG	GACCTGCAGA	AGCAGCACAG
1701	CGATCCCTGC	GCCCTCAACC	CGCGCATGAC	CAGGTTCTCC	GAGGAGGCGT
1751	GCGCGGTCTT	GACGTCCCCC	ACATTGAGAG	CCTGCCATCG	TGCCGTCAGC
1801	CCGCTGCCCT	ACCTGCGGAA	CTGCCGCTAC	GACGTGTGCT	CCTGCTCGGA
1851	CGGCCGCGAG	TGCCTGTGCG	GCGCCCTGGC	CAGCTATGCC	GCGGCCTGCG
1901	CGGGGAGAGG	CGTGCGCGTC	GCGTGGCGCG	AGCCAGGCCG	CTGTGAGCTG
1951	AAC TGCCCGA	AAGGCCAGGT	GTACCTGCAG	TGCGGGACCC	CCTGCAACCT
2001	GACCTGCCGC	TCTCTCTCTT	ACCCGGATGA	GGAATGCAAT	GAGGCCTGCC
2051	TGGAGGGCTG	CTTCTGCCCC	CCAGGGCTCT	ACATGGATGA	GAGGGGGGAC
2101	TGCGTGCCCA	AGGCCAGTG	CCCCTGTTAC	TATGACGGTG	AGATCTTCCA
2151	GCCAGAAGAC	ATCTTCTCAG	ACCATCACAC	CATGTGCTAC	TGTGAGGATG
2201	GCTTCATGCA	CTGTACCATG	AGTGGAGTCC	CCGGAAGCTT	GCTGCCTGAC
2251	GCTGTCTCTA	GCAGTCCCCC	GTCTCATCGC	AGCAAAAGGA	GCCTATCCTG
2301	TCGGCCCCCC	ATGGTCAAGC	TGGTGTGTCC	CGCTGACAAC	CTGCGGGCTG
2351	AAGGGCTCGA	GTGTACCAA	ACGTGCCAGA	ACTATGACCT	GGAGTGCATG
2401	AGCATGGGCT	GTGTCTCTGG	CTGCCTCTGC	CCCCCGGGCA	TGGTCCGGCA
2451	TGAGAACAGA	TGTGTGGCCC	TGGAAAGGTG	TCCCTGCTTC	CATCAGGGCA
2501	AGGAGTATGC	CCCTGGAGAA	ACAGTGAAGA	TTGGCTGCAA	CACCTGTGTC
2551	TGTCGGGACC	GGAAGTGGA	CTGCACAGAC	CATGTGTGTG	ATGCCACGTG
2601	CTCCACGATC	GGCATGGCCC	ACTACCTCAC	CTTCGACGGG	CTCAAATACC
2651	TGTTCCCCCG	GGAGTGCCAG	TACGTTCTGG	TGCAGGATTA	CTGCGGCAGT
2701	AACCCTGGGA	CCTTTCGGAT	CCTAGTGGGG	AATAAGGGAT	GCAGCCACCC
2751	CTCAGTGAAA	TGCAAGAAAC	GGGTCAACAT	CCTGGTGGAG	GGAGGAGAGA
2801	TTGAGCTGTT	TGACGGGGAG	GTGAATGTGA	AGAGGCCCAT	GAAGGATGAG
2851	ACTCACTTTG	AGGTGGTGGA	GTCTGGCCGG	TACATCATTC	TGCTGTGGG
2901	CAAAGCCCTC	TCCGTGGTCT	GGGACCGCCA	CCTGAGCATC	TCCGTGGTCC
2951	TGAAGCAGAC	ATACCAGGAG	AAAGTGTGTG	GCCTGTGTGG	GAATTTTGAT

3001	GGCATCCAGA	ACAATGACCT	CACCAGCAGC	AACCTCCAAG	TGGAGGAAGA
3051	CCCTGTGGAC	TTTGGGAACT	CCTGGAAAGT	GAGCTCGCAG	TGTGCTGACA
3101	CCAGAAAAGT	GCCTCTGGAC	TCATCCCCTG	CCACCTGCCA	TAACAACATC
3151	ATGAAGCAGA	CGATGGTGA	TTCCTCCTGT	AGAATCCTTA	CCAGTGACGT
3201	CTTCCAGGAC	TGCAACAAGC	TGGTGGACCC	CGAGCCATAT	CTGGATGTCT
3251	GCATTTACGA	CACCTGCTCC	TGTGAGTCCA	TTGGGGACTG	CGCCGCATTC
3301	TGCGACACCA	TTGCTGCCTA	TGCCCACGTG	TGTGCCCAGC	ATGGCAAGGT
3351	GGTGACCTGG	AGGACGGCCA	CATTGTGCCC	CCAGAGCTGC	GAGGAGAGGA
3401	ATCTCCGGGA	GAACGGGTAT	GAGGCTGAGT	GGCGCTATAA	CAGCTGTGCA
3451	CCTGCCTGTC	AAGTCACGTG	TCAGCACCCCT	GAGCCACTGG	CCTGCCCTGT
3501	GCAGTGTGTG	GAGGGCTGCC	ATGCCCACTG	CCCTCCAGGG	AAAATCCTGG
3551	ATGAGCTTTT	GCAGACCTGC	GTTGACCCTG	AAGACTGTCC	AGTGTGTGAG
3601	GTGGCTGGCC	GGCGTTTTGC	CTCAGGAAAG	AAAGTCACCT	TGAATCCCAG
3651	TGACCCTGAG	CACTGCCAGA	TTTGCCACTG	TGATGTTGTC	AACCTCACCT
3701	GTGAAGCCTG	CCAGGAGCCG	GGTACATCAG	AGAGCGCCAC	CCCTGAAAGT
3751	GGTCCCAGGA	GCGAGCCAGC	CACATCTGGG	TCGAAACGC	CAGGCACATC
3801	CGAGTCTGCA	ACTCCCGAGT	CCGGACCTGG	CTCCGAGCCT	GCCACTAGCG
3851	GCTCCGAGAC	TCCGGGAACT	TCCGAGAGCG	CTACACCAGA	AAGCGGACCC
3901	GGAACCAGTA	CCGAACCTAG	CGAGGGCTCT	GCTCCGGGCA	GCCCAGCCGG
3951	CTCTCCTACA	TCCACGGAGG	AGGGCACTTC	CGAATCCGCC	ACCCCGGAGT
4001	CAGGGCCAGG	ATCTGAACCC	GCTACCTCAG	GCAGTGAGAC	GCCAGGAACG
4051	AGCGAGTCCG	CTACACCGGA	GAGTGGGCCA	GGGAGCCCTG	CTGGATCTCC
4101	TACGTCCACT	GAGGAAGGGT	CACCAGCGGG	CTCGCCCACC	AGCACTGAAG
4151	AAGGTGCCTC	GTCTGACAAG	AACACTGGTG	ATTATTACGA	GGACAGTTAT
4201	GAAGATATTT	CAGCATACTT	CTGAGTAAA	AACAATGCCA	TTGAACCAAG
4251	AAGCTTCTCT	GACAAAACCT	ACACATGCCC	ACCGTGCCCA	GCTCCAGAAC
4301	TCCTGGGCGG	ACCGTCAGTC	TTCCTCTTCC	CCCCAAAACC	CAAGGACACC
4351	CTCATGATCT	CCCGGACCCC	TGAGGTCACA	TGCGTGGTGG	TGGACGTGAG
4401	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	GTATGTGGAC	GGCGTGGAAG
4451	TGCATAATGC	CAAGACAAAG	CCGCGGGAGG	AGCAGTACAA	CAGCACGTAC
4501	CGTGTGGTCA	GCGTCCTCAC	CGTCCTGCAC	CAAGACTGGC	TGAATGGCAA
4551	GGAGTACAAG	TGCAAGGTCT	CCAACAAAGC	CCTCCCAGCC	CCCATCGAGA
4601	AAACCATCTC	CAAAGCCAAA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC
4651	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAAGTTA	GCCTGACCTG
4701	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA
4751	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT	GTTGGACTCC
4801	GACGGCTCCT	TCTTCCTCTA	CTCCAAGCTC	ACCGTGGACA	AGAGCAGGTG
4851	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA
4901	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTTG	A

A-FVIII(XTEN)-Fc: SEQ ID NO: 207

ATRRYYLGAVELSWDYMQSDLGELPVDARFPFPRVPSKSPFNSTSVVYKKTLFVEFTDHLFNIK
 PRPPWMGLLGPTIQAEVYDVTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKE
 DDKVFPGGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGLAK
 EKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGC
 HRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHIS
 SHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDNPSFQIRSV
 KKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETF
 KTREAIQHESGILGPLLYGEVGDLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDF
 PILPGEIFKYKWTVTVEDGPTKSDPRCLTRYSSSVNMERDLASGLIGPLLYCYKESVDQGRNQ
 IMSDKRNVILFSVF DENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQLSV
 CLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMYEDTLTLFPFSGETVFMSMENPGLWILGC
 HNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFSSQNGTSESATPESG

PGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGS
PTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEG
TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSG
SETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTS
TEPSEGSAPASSEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIA
AVERLWDYGMSSSPHVLNRNAQSGSVPPQFKKVVFQEFDTGSGFTQPLRYGELNEHLGLLGPYIR
AEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPKRFVVKPNETKTYFWKVQHMAPT
KDEFDCKAWAYFSDVDLEKDVHSLIGLPLLVCHTNLNPAGHRQVTVQEFALFFTIFDETKS
WYFTENMERNCRAPCNQMEDPTFKENYRFHAINGYIMDTLPLGLVMAQDQRIRWYLLSMGS
NENIHSIHFSGHVFTVRKKKEEYKMALYNLYPGVFETVEMLPKAGIWRVECLIGEHLHAGMST
LFLVYSNKCQTLGMAAGHIRDFQITASGQYQGWAPKLARLHYSGSINAWSTKEPFSWIKVDL
LAPMIIHGKIQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIF
NPPIIARYIRLHPHTYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSP
SKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTVGTGVTQGVKSLTSMYVKEFLISSSQ
DGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRHPQSWVHQIALRMEVLGCEA
QDLYDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREEQVYVTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPG

Table 19. Additional chimeric polypeptide sequences

Description / SEQ ID NO.	Sequence
AE288	GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 60
SEQ ID NO: 8	GTSTEPSEGS APGSPAGSPT STEEGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 120
	GSPAGSPTST EEGSPAGSPT STEEGTSTEP SEGSAAGTSE SATPESGPGT SESATPESGP 180
	GTSESATPES GPGSEPATSG SETPGSEPAT SGSETPGSPA GSPTSTEEGT STEPSEGSAP 240
	GTSTEPSEGS APGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAP 288
pSYN VWF059	TSTEEGASIS DKNTGDYED SYEDISAYLL SKNNAIEPRS FSDKTH
SEQ ID NO: 13	
pSYN VWF059A	TSTEEGASSD KNTGDYEDS YEDISAYLLS KNNAIEPRSF SDKTH
SEQ ID NO: 22	
pSYN FVIII 312	ATRRYYLGAV ELSWDYMQSD LGELPVDARF PPRVPKSEPF NTSVVYKCTL FVEFTDHLFN 60
SEQ ID NO: 173	IAKPRPPWVG LLGPTIQAEV YDTVVITLKN MASHPVSLHA VGVSYWKASE GAEYDDQTSQ 120
	REKEDDKVFP GGSHTYVWQV LKENGPMASD PLCLTYSYLS HVDLVKDLNS GLIGALLVCR 180
	EGSLAKEKTQ TLHKFILLFA VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR 240
	SLPGLIGCHR KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL 300
	MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKN EEAEDYDDDL TDSEMDVVRV 360
	DDDNPSFIFQ IRSVAKKHFK TWVHYIAAEE EDWDYAPLVL APDDRSYKSQ YLNNGPQRIG 420
	RKYKVRVMA YTDFTFKTRE AIQHESGILG PLYGEVGD TLLIFKNQAS RPYNIYPHGI 480
	TDVRPLYSRRLPKGVKHLKD FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYSSFNME 540
	RDLASGLIGP LLYCYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG 600
	VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS VFFSGYTFKH 660
	KMVYEDTTLTLPFSGETVFM SMENPGLWIL GCHNSDFRNR GMTALLKVSS CDKNTGDYEE 720
	DSYEDISAYL LSKNNAIEPR SFSQNGTSES ATPESGPGSE PATSGSETPG TSESATPESG 780

	PGSEPATSGS ETPGTSESAT PESGPGTSTE PSEGSAPGSP AGSPTSTEEG TSESATPESG 840
	PGSEPATSGS ETPGTSESAT PESGPGSPAG SPTSTEEGSP AGSPTSTEEG TSTEPSEGSAP 900
	PGTSESATPE SGPGTSESAT PESGPGTSES ATPESGPGSE PATSGSETPG SEPATSGSET 960
	PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE PSEGSAPGSE PATSGSETPG TSESATPESG 1020
	PGTSTEPSEG SAPASSEITR TTLQSDQEEI DYDDTISVEM KKEDFDIYDE DENQSPRSFQ 1080
	KKTRHYFIAA VERLWDYGMS SSPHVLRNRA QSGSVPPQFKK VVFQEFQDGS FTQPLYRGEL 1140
	NEHLGLLGPY IRAEVEDNIM VTFRNQASRP YSFYSSLSY EEDQRQGAEP RKNFVKPNET 1200
	KTYFWKVQHH MAPTKDEFDC KAWAYFSDVD LEKDVHSGLI GPLLVCHTNT LNPAGHRQVT 1260
	VQEFALFFTI FDETKSWYFT ENMERNCRAP CNIQMEDPTF KENYRFHAIN GYIMDTLPGL 1320
	VMAQDQIRIW YLLSMGSNEN IHSIHFSGHV FTVRKKEEYK MALYNLYPGV FETVEMLP SK 1380
	AGIWRVECLI GEHLHAGMST LFLVYSNKCQ TPLGMASGHI RDFQITASGQ YGQWAPKLAR 1440
	LHYSGSINAW STKEPFSWIK VDLLAPMIH GIKTQGARQK FSSLYISQFI IMYSLDGKKW 1500
	QTYRGNSTGT LMVFFGNVDS SGIKHNIFNP PIARYIRLH PTHYSIRSTL RMELMGCDLN 1560
	SCSMPLGMES KALSDAQITA SSYFTNMFAT WSPSKARLHL QGRSNARWPQ VNNPKEWLQV 1620
	DFQTMKVTVG VTTQGVKSLT TSMYVKEFLI SSSQDGHQWT LFFQNGKVKV FQGNQDSFTF 1680
	VVNSLDPPLL TRYLRHPQS WWHQIALRME VLGCEAQDLY DKTHTCPPCP APELLGGPSV 1740
	FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNATK PREEQYNSTY 1800
	RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK 1860
	NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTPPVLDS DGSFFLYSKL TVDKSRWQQG 1920
	NVFCSCVMHE ALHNHYTQKS LSLSPGK
XTEN_AE288_2	GSPAGSPTST EEGTSESATP ESGPGSEPAT SGSETPGTSE SATPESGPGT STEPSEGSAP 60
SEQ ID NO: 54	GTSTEPSEGS APGTSTEPSE GSAPGTSTEP SEGSAPGTST EPSEGSAPGT STEPSEGSAP 120
	GSPAGSPTST EEGTSTEPSE GSAPGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 180
	GSEPATSGSE TPGTSESATP ESGPGTSTEP SEGSAPGTSE SATPESGPGS PAGSPTSTEE 240
	GSPAGSPTST EEGSPAGSPT STEEGTSESA TPESGPGTST EPSEGSAP 288
XTEN AE144_5A	TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG 60
SEQ ID NO: 58	TSTEPSEGSAP PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG 120
	SPAGSPTSTE EGSPAGSPTS TEEG
a2 Linker of chimeric polypeptide	DKNTGDYYED SYEDISAYLL SKNNAIEPRS FS 32
SEQ ID NO: 88	
Signal Peptide of FVIII	MQIELSTCFFLCLLRFCFS
SEQ ID NO: 64	
FVIII fragment 1 of chimeric polypeptide	ATTRYYLGAV ELSWDYMQSD LGELPVDARF PPRVPKSFPF NTSVVYKCTL FVEFTDHLFN 60
SEQ ID NO: 215	IAKPRPPWMG LLGPTIQAEV YDVTVITLKN MASHPVSLHA VGVSYWKASE GA EYDDQTSQ 120
	REKEDDKVFP GGSHTYVWQV LKENGPMASD PLCLTYSYLS HVDLVKDLNS GLIGALLVCR 180
	EGSLAKEKTQ TLHKFILLFA VFDEGKSWHS ETKNLSMQDR DAASARAWPK MHTVNGYVNR 240
	SLPGLIGCHR KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL 300
	MDLGQFLLFC HISSHQHDGM EAYVKVDS CP EEPQLRMKN EEAEDYDDL TDSEMDVVR 360
	DDDNPSFIFQ IRSVAKKHPK TWVHYIAAE EDWDYAPLVL APDDRSYKSQ YLNNGPQRIG 420
	RKYKKVRFMA YTDFTFKTRE AIQHEGSLG PLYGEVGDY LLIIIFKNQAS RPYNIYPHGI 480
	TDVRPLYSRR LPKGVKHLKD FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYSSSFVNME 540
	RDLASGLIGP LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYL TEN IQRFLPNPAG 600
	VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS VFFSGYTFKH 660
	KMVEDTLTL FPFSGETVEM SMENPGLWIL GCHNSDFRNR GMTALLKVSS CDKNTGDYYE 720
	DSYEDISAYL LSKNNAIEPR SFSQN
FVIII fragment 2 of chimeric polypeptide	EITRRTLQSD QEEIDYDDTI SVEMKKEDFD IYDEDENQSP RSFQKKTRHY FIAAVERLWD 60
	YGMSSSPHVL RNRAQSGSVF QFKKVVFQEF TDGSFTQPLY RGELNEHLGL LGPYIRAEVE 120
	DNIMVTFRNQ ASRPYSFYSS LISYEEDQRQ GAEPRKNFVK PNETKTYFWK VQHHMAPTKD 180
	EFDC KAWAYF SDVDLEKDVH SGLIGPLLV C HTNTLNPAHG RQVTVQEFAL FFTIFDET KS 240

SEQ ID NO. 216	WYFTENMERN	CRAPCNIQME	DPTFKENYRF	HAINGYIMDT	LPGLVMAQDQ	RIRWYLLSMG	300
	SNENIHSIHF	SGHVFTVRKK	EEYKMALYNL	YPGVFETVEM	LPSKAGIWRV	ECLIGEHLHA	360
	GMSTLFLVYS	NKCQTPLGMA	SGHIRDFQIT	ASGOYQGWAP	KLARLHYSGS	INAWSTKEPF	420
	SWIKVDLLAP	MIHGIKTQG	ARQKFSSLYI	SQFIIMYSLD	GKKWQTYRGN	STGTLMVFFG	480
	NVDSSGIKHN	IFNPPIIARY	IRLHPHTHYSI	RSTLRMELMG	CDLNSCSMPL	GMESKAISDA	540
	QITASSYFTN	MFATWSPSKA	RLHLQGRSNA	WRPQVNNPKE	WLQVDFQKTM	KVTGVTQGV	600
	KSLTSMYVK	EFLISSSQDG	HQWTLFFQNG	KVKVFQGNQD	SFTPVVNSLD	PPLLTRYLRI	660
	HPQSWVHQIA	LRMEVLGCEA	QDLY				
VWF Signal Peptide	MIPARFAGVL	LALALILPGT	LC				
SEQ ID NO: 208							
VWF D1D2 domain of chimeric polypeptide	AEGTRGRSST	ARCSLFGSDF	VNTFDGSMYS	FAGYCSYLLA	GGCQKRSFSI	IGDFQNGKRV	60
	SLSVYLGEFF	DIHLFVNGTV	TQGDQRVSM	YASKGLYLET	EAGYYKLSGE	AYGFVARIDG	120
	SGNFQVLLSD	RYFNKTCGLC	GNFNIFAEDD	FMTQEGTLTS	DPYDFANWSA	LSSGEQWCER	180
	ASPPSSSCNI	SSGEMQKGLW	EQCQLLKSTS	VFARCHPLVD	PEPFVALCEK	TLCECAGGLE	240
	CACPALLEYA	RTCAQEGMVL	YGWTDHSACS	PVCPAGMEYR	QCVSPCARTC	QSLHINEMCQ	300
	ERCVDGSCSP	EGQLLDEGLC	VESTTEPCVH	SGKRYPPGTS	LSRDCNTCIC	RNSQWICSNE	360
	ECPGECCLVTG	QSHFKSFDNR	YFTFSGICQY	LLARDCQDHS	FSIVIETVQC	ADDRDAVCTR	420
	SVTVRLPGLH	NSLVKLKHGA	GVAMDGQDIQ	LPLLKGDRLI	QHTVTASVRL	SYGEDLQMDW	480
	DGRGRLLVKL	SPVYAGKTCG	LCGNYNNGQG	DDFLTPTSGLA	EPRVEDFGNA	WKLHGDQCQDL	540
SEQ ID NO: 209	QKQHSDDPCAL	NPRMTRFSEE	ACAVLTSPTF	EACHRAVSPL	PYLRNCRYDV	CSCSDGRECL	600
	CGALASYAAA	CAGRGVVRVAW	REPGRCELNC	PKGQVYLQCG	TPCNLTCSRSL	SYPDCECNEA	660
	CLEGCFCCPPG	LYMDERGDCV	PKAQCCPCYYD	GEIFQPEDIF	SDHHTMCYCE	DGFMHCTMSG	720
	VPGSLLPDAV	LSSPLSHRSK	R				
VWF D' domain of chimeric polypeptide	SLSCRPPMVK	LVCPADNLRA	EGLECTKTCQ	NYDLECMSMG	CVSGCLCPPG	MVRHENRCVA	60
	LERPCPCFHQ	KEYAPGETVK	IGCNTCVCRD	RKWNCTDHVC	DAT		103
SEQ ID NO: 210							
VWF D3 domain of chimeric polypeptide	CSTIGMAHYL	TFDGLKYLFP	GECQYVLVQD	YCGSNPGTFR	ILVGNKGCSH	PSVKCKKRV	60
	ILVEGGEIEL	FDGEVNVKRP	MKDETHFEVV	ESGRYIILL	GKALSVVWDR	HLISVVLKQ	120
	TYQEKVCGLC	GNFDGIQNN	LTSSNLQVEE	DPVDFGNSWK	VSSQCADTRK	VPLDSSPATC	180
	HNNIMKQTMV	DSSCRILTSD	VFQDCNKLVD	PEPYLDVCIY	DTCSCESIGD	CAAFCDTIAA	240
	YAHVCAQH GK	VVTWRTATLC	PQSCEERNLR	ENGVEAEWRY	NSCAPACQVT	CQHPEPLACP	300
	VQCVEGCHAH	CPPGKILDEL	LQTCVDPEDC	PVCEVAGRRF	ASGKKVTLNP	SDPEHCQICH	360
	CDVNVLTCEA	CQEP					374
Fc region	DKHTCPCPP	APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	60
	GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	120
	GQPREPQVYT	LPPSRDELTK	NQVSLTCLVK	GFYPSTDAVE	WESNGQPENN	YKTTTPPVLD	180
	DGSFFLYSKL	TVDKSRWQQG	NVFSCSVME	ALHNHYTQKS	LSLSPG		
SEQ ID NO: 217							
XTEN AE288_3	GTSESATPES	GPGESEATSG	SETPGTSESA	TPESGPGSEP	ATSGSETPGT	SESATPESGP	60
	GTSTEPSEGS	APGSPAGSPT	STEEGTSESA	TPESGPGSEP	ATSGSETPGT	SESATPESGP	120
	GSPAGSPTST	EEGSPAGSPT	STEEGTSTEP	SEGSAPGTSE	SATPESGPGT	SESATPESGP	180
	GTSESATPES	GPGESEATSG	SETPGTSESA	SGSETPGSPA	GSPTSTEEGT	STEPSEGSAP	240
	GTSTEPSEGS	APGSEATSG	SETPGTSESA	TPESGPGTST	EPSEGSAPAS	S	291
SEQ ID NO: 218							

[0387] The foregoing description of the specific embodiments will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations
5 and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

10 **[0388]** Other embodiments of the disclosure will be apparent to those skilled in the art from consideration of the specification and practice of the disclosure disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the disclosure being indicated by the following claims.

15 **[0389]** All patents and publications cited herein are incorporated by reference herein in their entirety.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating hemophilia A in a human subject in need thereof comprising administering to the subject multiple doses of a chimeric polypeptide comprising (i) a factor VIII (FVIII) polypeptide and (ii) a von Willebrand factor (VWF) fragment comprising a D' domain of VWF and a D3 domain of VWF at a dosing interval,
5 wherein at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg and the dosing interval is at least about 7 days.
2. The method of claim 1, wherein the FVIII polypeptide is associated with the VWF fragment by a covalent bond.
10
3. The method of claim 1 or 2, wherein the covalent bond is a peptide bond or a disulfide bond.
4. The method of any one of claims 1 to 3, wherein the FVIII polypeptide comprises a first half-life extending moiety.
5. The method of claim 4, wherein the first half-life extending moiety is fused to the C-terminus or the N-terminus of the FVIII polypeptide.
15
6. The method of claim 4, wherein the first half-life extending moiety is inserted within the FVIII polypeptide.
7. The method of claim 5 or 6, wherein the first half-life extending moiety is inserted within the B domain of the FVIII polypeptide.
8. The method of claim 7, wherein the first half-life extending moiety is inserted within the FVIII polypeptide immediately downstream of an amino acid corresponding to amino acid residue 745 of SEQ ID NO: 65.
20
9. The method of any one of claims 4 to 8, wherein the first half-life extending moiety is fused to the FVIII polypeptide by a linker.

10. The method of any one of claims 4 to 9, wherein the VWF fragment comprises a second half-life extending moiety.
11. The method of claim 10, wherein the second half-life extending moiety is fused to the C-terminus or the N-terminus of the VWF fragment.
- 5 12. The method of claim 10, wherein the second half-life extending moiety is inserted within the VWF fragment.
13. The method of claim 10 or 11, wherein the second half-life extending moiety is fused to the C-terminus of the VWF fragment.
- 10 14. The method of any one of claims 10 to 13, wherein the second half-life extending moiety is fused to the VWF fragment by a linker.
15. The method of any one of claims 4 to 14, wherein the first half-life extending moiety, the second half-life extending moiety, or both is selected from the group consisting of an albumin, an immunoglobulin Fc region, an XTEN sequence, the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, a PAS sequence, a HAP sequence, a transferrin, albumin-binding moieties, or any fragments, derivatives, variants, and any combination thereof.
- 15 16. The method of any one of claims 4 to 15, wherein the first half-life extending moiety comprises a first XTEN.
17. The method of claim 16, wherein the first XTEN is inserted within the FVIII polypeptide immediately downstream of an amino acid corresponding to amino acid residue 745 of SEQ ID NO: 65.
- 20 18. The method of any one of claims 10 to 17, wherein the second half-life extending moiety comprises a second XTEN.
19. The method of claim 18, wherein the second XTEN is fused to the C-terminus of the VWF fragment.
- 25

20. The method of any one of claims 1 to 19, wherein the FVIII polypeptide comprises a first immunoglobulin (Ig) constant region or a portion thereof.
21. The method of claim 20, wherein the first Ig constant region or the portion thereof is fused to the C-terminus or the N-terminus of the FVIII polypeptide.
- 5 22. The method of claim 20, wherein the first Ig constant region or the portion thereof is inserted within the FVIII polypeptide.
23. The method of claim 20 or 21, wherein the first Ig constant region or the portion thereof is fused to the C-terminus of the FVIII polypeptide.
- 10 24. The method of any one of claims 20 to 23, wherein the first Ig constant region or the portion thereof is fused to the FVIII polypeptide by a linker.
25. The method of any one of claims 20 to 24, wherein the first Ig constant region or the portion thereof comprises a first Fc domain or a portion thereof.
26. The method of any one of claims 1 to 25, wherein the VWF fragment comprises a second Ig constant region or a portion thereof.
- 15 27. The method of claim 26, wherein the second Ig constant region or the portion thereof is fused to the C-terminus or the N-terminus of the VWF fragment.
28. The method of claim 26, wherein the second Ig constant region or the portion thereof is inserted within the VWF fragment.
- 20 29. The method of claim 26 or 27, wherein the second Ig constant region or the portion thereof is fused to the C-terminus of the VWF fragment.
30. The method of any one of claims 26 to 29, wherein the second Ig constant region or the portion thereof is fused to the VWF fragment by a linker.
31. The method of claim 30, wherein the linker is a cleavable linker.
- 25 32. The method of any one of claims 26 to 31, wherein the second Ig constant region or the portion thereof comprises a second Fc domain or a portion thereof.

33. The method of claim 32, wherein the FVIII protein and the VWF fragment are associated with each other through a covalent bond between the first Fc domain and the second Fc domain.
34. The method of claim 33, wherein the FVIII protein and the VWF fragment are further associated with each other through a non-covalent interaction between the FVIII protein and the VWF fragment.
35. A method of treating hemophilia A in a human subject comprising administering to the subject in need thereof multiple doses of a chimeric polypeptide at a dosing interval, wherein the chimeric polypeptide comprises:
- (i) a FVIII protein comprising:
 - (a) a first FVIII polypeptide fragment comprising the amino acid sequence of SEQ ID NO: 215;
 - (b) a first XTEN sequence comprising the amino acid sequence of SEQ ID NO: 8 (AE288);
 - (c) a second FVIII polypeptide fragment comprising the amino acid sequence of SEQ ID NO: 216; and
 - (d) a first Fc region comprising the amino acid sequence of SEQ ID NO: 217;
 - (ii) a VWF protein comprising:
 - (a) a D' domain of VWF comprising the amino acid sequence of SEQ ID NO: 210;
 - (b) a D3 domain of VWF comprising the amino acid sequence of SEQ ID NO: 214;
 - (c) a second XTEN sequence comprising the amino acid sequence of SEQ ID NO: 58 (AE144_5A);
 - (d) an a2 linker comprising the amino acid sequence of SEQ ID NO: 88; and
 - (e) a second Fc region comprising the amino acid sequence of SEQ ID NO: 217;
- wherein the first Fc region is covalently linked to the second Fc region by a disulfide bond.

36. The method of claim 35, wherein the FVIII protein further comprises a FVIII signal peptide comprising the amino acid sequence of SEQ ID NO: 64.
- 5 37. The method of claim 35, wherein the VWF protein further comprises a VWF signal peptide comprising the amino acid sequence of SEQ ID NO: 208.
38. The method of claim 35, wherein the VWF protein further comprises a D1D2 domain of VWF comprising the amino acid sequence of SEQ ID NO: 209.
- 10 39. A method of treating hemophilia A in a human subject comprising administering to the subject in need thereof multiple doses of a chimeric polypeptide at a dosing interval, wherein the chimeric polypeptide comprises:
- 15 (i) a FVIII protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to SEQ ID NO: 173, SEQ ID NO: 201, SEQ ID NO: 203, or SEQ ID NO: 207; and
 - (ii) a VWF protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to SEQ ID NO: 197, SEQ ID NO: 202, or SEQ ID NO: 205.
- 20 40. A method of treating hemophilia A in a human subject comprising administering to the subject in need thereof multiple doses of a chimeric polypeptide at a dosing interval, wherein the chimeric polypeptide comprises:
- 25 (i) a FVIII protein comprising the amino acid sequence of SEQ ID NO: 203; and
 - (ii) a VWF protein comprising the amino acid sequence of SEQ ID NO: 205.
41. A method of treating hemophilia A in a human subject comprising administering to the subject in need thereof multiple doses of a chimeric polypeptide at a dosing interval, wherein the chimeric polypeptide comprises:
- 30 (i) a FVIII protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from FVIII-161 (SEQ ID NO: 69), FVIII-169 (SEQ ID NO: 70), FVIII-170 (SEQ ID NO: 71), FVIII-173 (SEQ

ID NO: 72); FVIII-195 (SEQ ID NO: 73); FVIII-196 (SEQ ID NO: 74), FVIII199 (SEQ ID NO: 75), FVIII-201 (SEQ ID NO: 76); FVIII-203 (SEQ ID NO: 77), FVIII-204 (SEQ ID NO: 78), FVIII-205 (SEQ ID NO: 79), FVIII-266 (SEQ ID NO: 80), FVIII-267 (SEQ ID NO: 81), FVIII-268 (SEQ ID NO: 82), FVIII-269 (SEQ ID NO: 83), FVIII-271 (SEQ ID NO: 84), FVIII-272 (SEQ ID NO: 85), FVIII-312 (SEQ ID NO: 173), or FVIII-312A (SEQ ID NO: 203); and

- (ii) a VWF protein comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from VWF031 (SEQ ID NO: 86), VWF034 (SEQ ID NO: 87), VWF059 (SEQ ID NO: 197), VWF059A (SEQ ID NO: 202), or VWF036.

42. The method of any one of claims 39-41, wherein at least one of the multiple doses is from about 15 IU/kg to about 100 IU/kg and the dosing interval is at least about 7 days.

43. The method of any one of claims 39-41, wherein at least one of the multiple doses is about 50 IU/kg or about 65 IU/kg and the dosing interval is at least about 5 days or about 7 days.

44. The method of any one of claims 1-41, wherein the multiple doses comprise at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, at least twelve doses, at least thirteen doses, at least fourteen doses, at least fifteen doses, at least sixteen doses, at least seventeen doses, at least eighteen doses, at least nineteen doses, at least twenty doses, or more.

45. The method of any one of claims 1-41, wherein the treatment of hemophilia A comprises controlling or decreasing the incidence or frequency of a bleeding episode in a human subject in need thereof.

46. The method of any one of claims 1-41, wherein the treatment of hemophilia A comprises preventing or treating a bleeding episode in a human subject in need thereof.

47. The method of any one of claims 1-41, wherein at least one of the multiple doses is from about 20 IU/kg to about 95 IU/kg, from about 20 IU/kg to about 90 IU/kg, from about 20 IU/kg to

about 85 IU/kg, from about 20 IU/kg to about 80 IU/kg, from about 20 IU/kg to about 75 IU/kg, from about 20 IU/kg to about 70 IU/kg, from about 20 IU/kg to about 65 IU/kg, from about 20 IU/kg to about 60 IU/kg, from about 20 IU/kg to about 55 IU/kg, from about 20 IU/kg to about 50 IU/kg, from about 20 IU/kg to about 45 IU/kg, from about 20 IU/kg to about 40 IU/kg, from about 20 IU/kg to about 35 IU/kg, from about 20 IU/kg to about 30 IU/kg, or from about 20 IU/kg to about 25 IU/kg.

48. The method of any one of claims 1-415, wherein at least one of the multiple doses is from about 20 IU/kg to about 100 IU/kg, from about 25 IU/kg to about 100 IU/kg, from about 30 IU/kg to about 100 IU/kg, from about 35 IU/kg to about 100 IU/kg, from about 40 IU/kg to about 100 IU/kg, from about 45 IU/kg to about 100 IU/kg, from about 50 IU/kg to about 100 IU/kg, from about 55 IU/kg to about 100 IU/kg, from about 60 IU/kg to about 100 IU/kg, from about 65 IU/kg to about 100 IU/kg, from about 70 IU/kg to about 100 IU/kg, from about 75 IU/kg to about 100 IU/kg, from about 80 IU/kg to about 100 IU/kg, from about 85 IU/kg to about 100 IU/kg, or from about 90 IU/kg to about 100 IU/kg.

49. The method of any one of claims 1-41, wherein at least one of the multiple doses is from about 20 IU/kg to about 80 IU/kg, from about 25 IU/kg to about 75 IU/kg, from about 30 IU/kg to about 70 IU/kg, from about 35 IU/kg to about 65 IU/kg, from about 40 IU/kg to about 60 IU/kg, or from about 45 IU/kg to about 55 IU/kg.

50. The method of any one of claims 1-41, wherein at least one of the multiple doses is from about 25 IU/kg to about 65 IU/kg.

51. The method of any one of claims 1-41, wherein at least one of the multiple doses is about 20 IU/kg, about 25 IU/kg, about 30 IU/kg, about 35 IU/kg, about 40 IU/kg, about 45 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, about 80 IU/kg, about 85 IU/kg, about 90 IU/kg, about 95 IU/kg, or about 100 IU/kg.

52. The method of any one of claims 1-41, wherein at least one of the multiple doses is about 25 IU/kg.

53. The method of any one of claims 1-41, wherein at least one of the multiple doses is about 65 IU/kg.

54. The method of any one of claims 1-41, wherein at least one of the multiple doses is about 80 IU/kg.
55. The method of any one of claims 1-41, wherein the dosing interval is at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, at least about 30 days, or at least about 31 days.
56. The method of any one of claims 1-41, wherein the frequency of the doses is at least once every week, at least once every 2 weeks, at least once every 3 weeks, or at least once every 4 weeks.
57. The method of any one of claims 1-41, wherein the frequency of the doses is at least once every week.
58. The method of any one of claims 1-41, wherein the frequency of the doses is at least once every two weeks.
59. The method of any one of claims 1-41, wherein the chimeric polypeptide is administered for prophylactic treatment.
60. The method of any one of claims 1-41, wherein the chimeric polypeptide is administered by a route selected from the group consisting of intravenous injection, intravenous infusion, subcutaneous administration, intramuscular administration, oral administration, nasal administration, and pulmonary administration.
61. The method of any one of claims 1-41, wherein the chimeric polypeptide after the administration results in a FVIII plasma activity level of at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10%.
62. The method of claim 61, wherein the FVIII plasma activity level is at least about 3%.

63. The method of claim 61, wherein the FVIII plasma activity level is at least about 5%.
64. The method of any one of claims 1 to 41, wherein the chimeric polypeptide after the administration results in a FVIII plasma activity level of at least about 1 IU/dL, at least about 2 IU/dL, at least about 3 IU/dL, at least about 4 IU/dL, at least about 5 IU/dL, at least about 6 IU/dL, at least about 7 IU/dL, at least about 8 IU/dL, at least about 9 IU/dL, or at least about 10 IU/dL.
65. The method of claim 64, wherein the FVIII plasma activity level is at least about 3 IU/dL.
66. The method of claim 64, wherein the FVIII plasma activity level is at least about 5 IU/dL.
67. The method of any one of claims 1 to 41, wherein the FVIII plasma activity level is at least about 10 IU/dL at least about 5 days after the administration of the chimeric polypeptide.
68. The method of any one of claims 1 to 41, wherein the FVIII plasma activity level is at least about 5 IU/dL at least about 7 days after the administration of the chimeric polypeptide.
69. The method of any one of claims 1 to 41, wherein the FVIII plasma activity level is at least about 3 IU/dL at least about 8 days after the administration of the chimeric polypeptide.
70. The method of any one of claims 1 to 41, wherein the FVIII plasma activity level is at least about 1 IU/dL at least about 10 days after the administration of the chimeric polypeptide.
71. The method of any one of claims 1-41, wherein at least one of the multiple doses is about 50 IU/kg.
72. The method of any one of claims 1-41, wherein the multiple doses are about 50 IU/kg, and the dosing interval is about 7 days.
73. The method of any one of claims 1-41, wherein the administration of the chimeric polypeptide induces no FVIII inhibitor after about 7 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 20 days, about 24 days, about 25 days, about 28 days, about 30 days, or about 35 days of the administration.
74. The method of any one of claims 1-41, wherein the administration of the chimeric polypeptide induces no FVIII inhibitor after about 28 days of the administration.

75. The method of any one of claims 1-41, wherein at least one of the multiple doses is from about 50 IU/kg to about 80 IU/kg.
76. The method of any one of claims 1-41, wherein at least one of the multiple doses is from about 50 IU/kg to about 65 IU/kg.
- 5 77. The method of any one of claims 1-41, wherein at least one of the multiple doses is from about 65 IU/kg to about 80 IU/kg.
78. The method of any one of claims 1-41, wherein the dosing interval is at least about 7 days.
79. The method of any one of claims 1-41, wherein the dosing interval is about 7 days to about 14 days.
- 10 80. The method of any one of claims 1-41, wherein the dosing interval is at least about 10 days.
81. The method of any one of claims 1-41, wherein the dosing interval is about 10 days to about 21 days.
82. The method of any one of claims 1-41, wherein the dosing interval is about 14 days to about 21 days.
- 15 83. The method of any one of claims 1-41, wherein the dosing interval is about 14 days.
84. The method of any one of claims 1-41, wherein the human subject is a female.
85. The method of any one of claims 1-41, wherein the human subject is a child.
86. The method of claim 85, wherein the human subject is a child less than or equal to about 12 years old, less than about 11 years old, less than about 10 years old, less than about 9 years
20 old, less than about 8 years old, less than about 7 years old, less than about 6 years old, less than about 5 years old, less than about 4 years old, less than about 3 years old, less than about 2 years old, or less than about 1 year old.
87. The method of any one of claims 1-41, wherein the administration induces immune tolerance to FVIII in the human subject.

88. The method of any one of claims 1-41, wherein the administration reduces an inhibitory immune response to FVIII in the human subject.
89. The method of claim 88, wherein the inhibitory immune response to FVIII comprises a high titer of anti-FVIII antibodies in the human subject.

FIG. 1

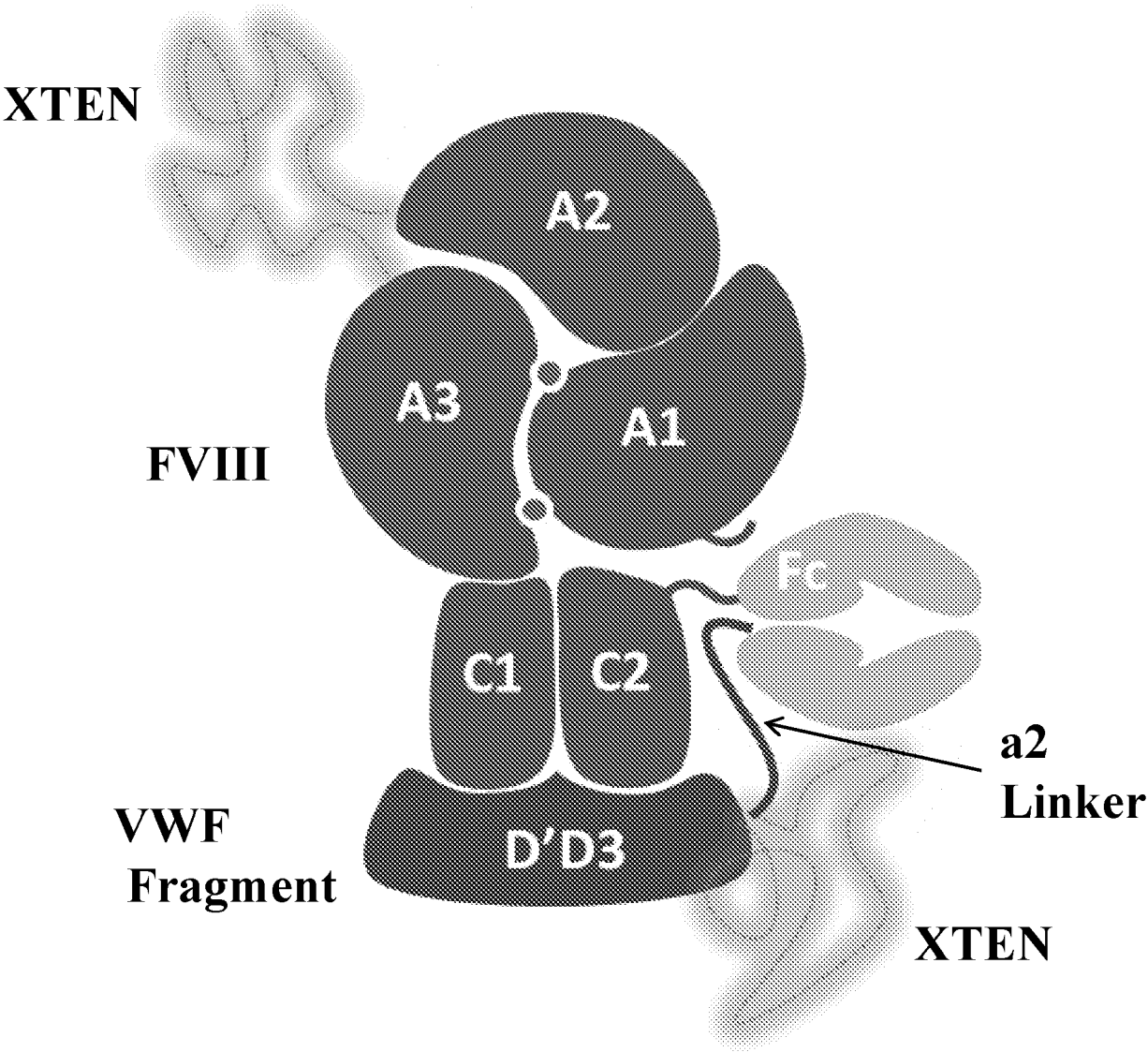


FIG. 2A

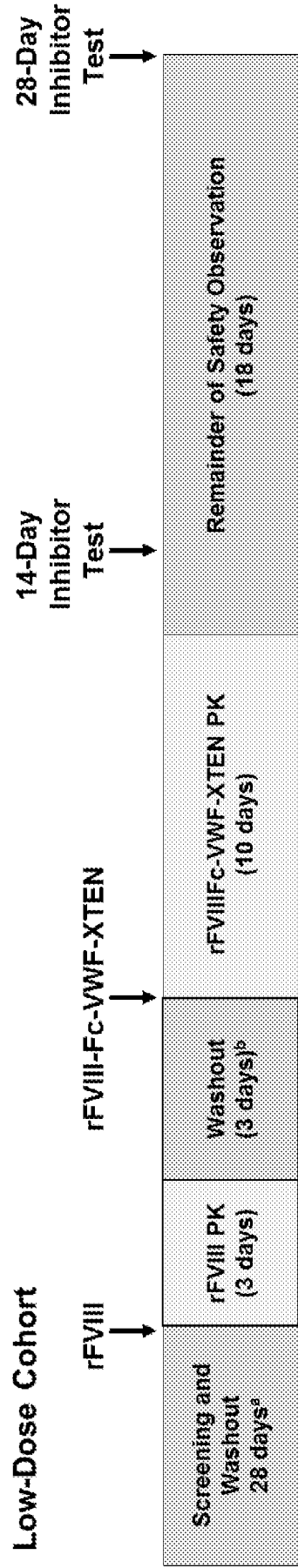


FIG. 2B

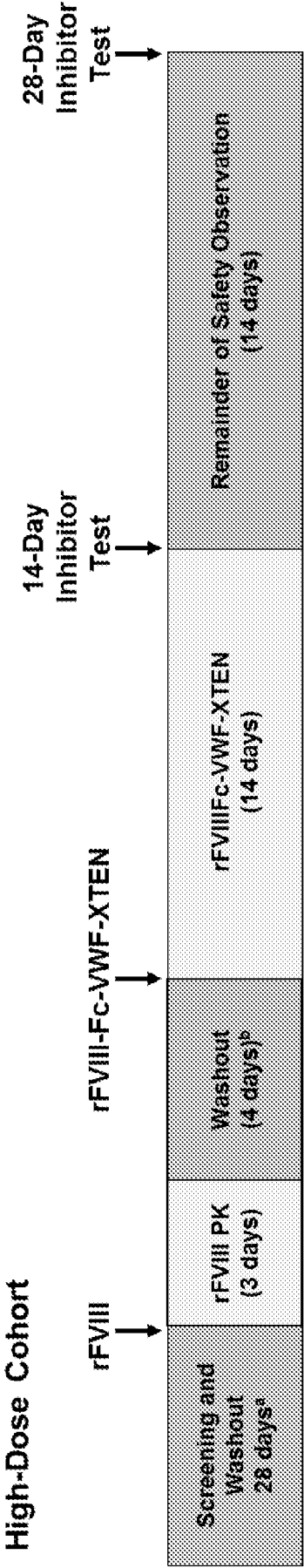


FIG. 3A

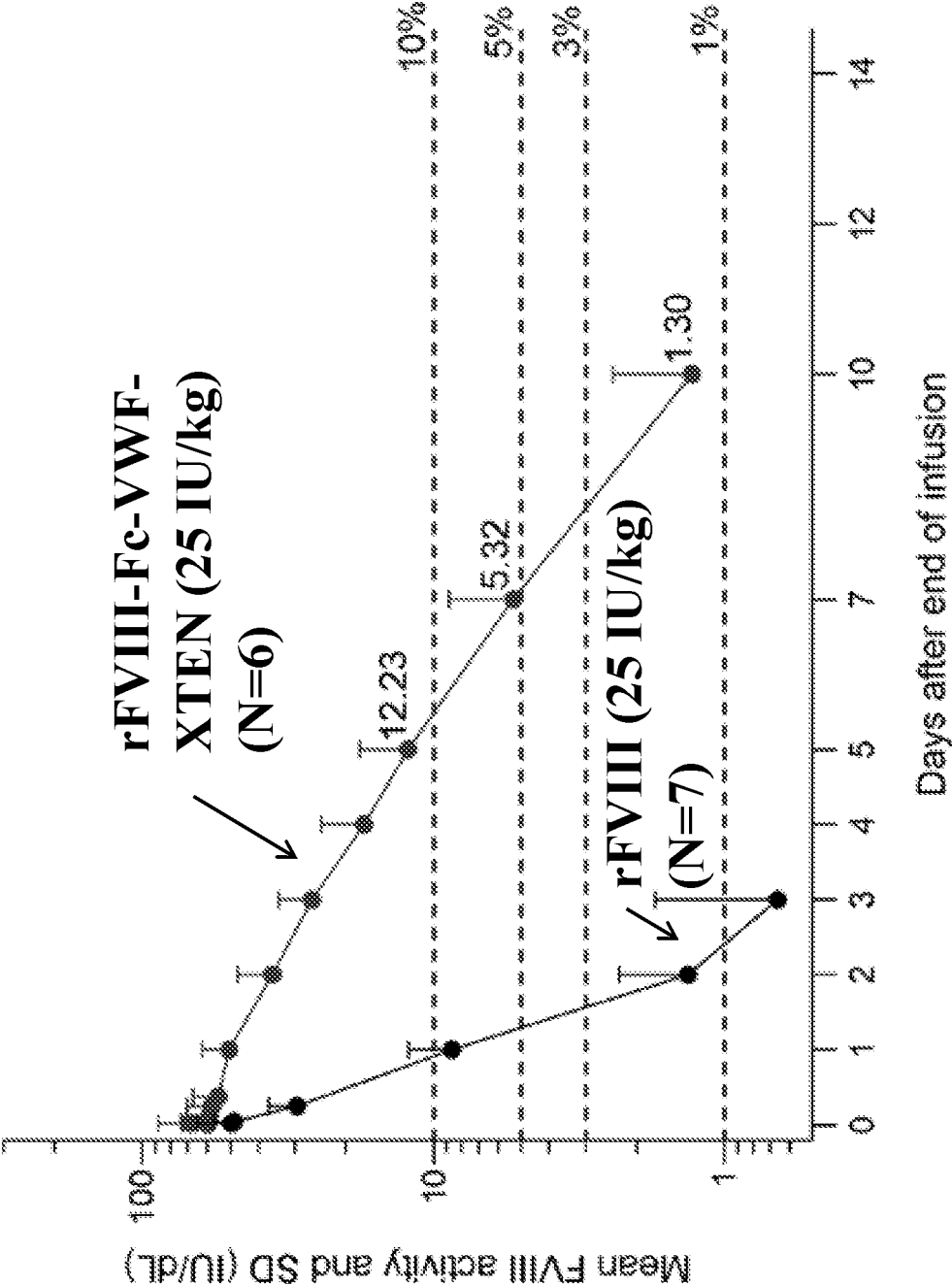


FIG. 3B

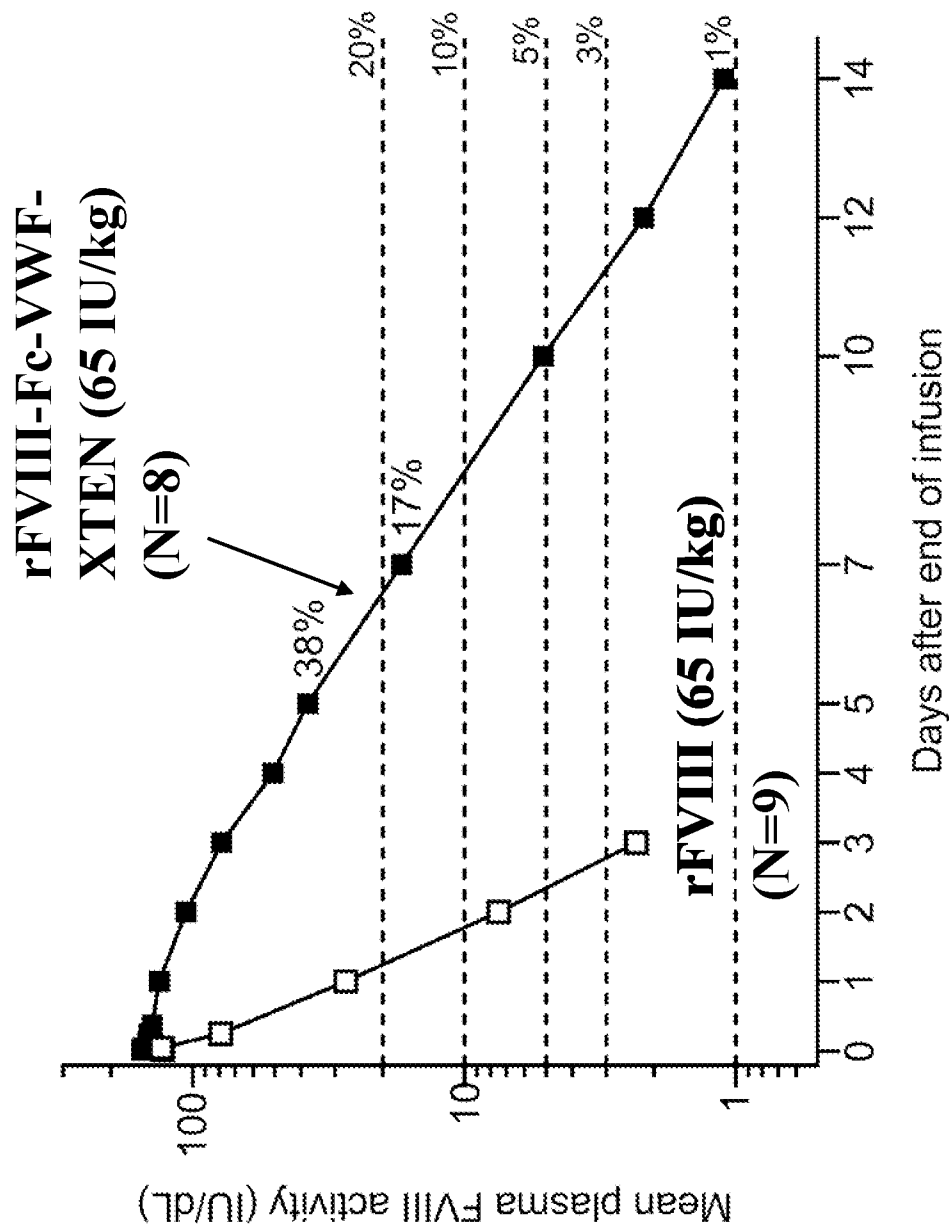
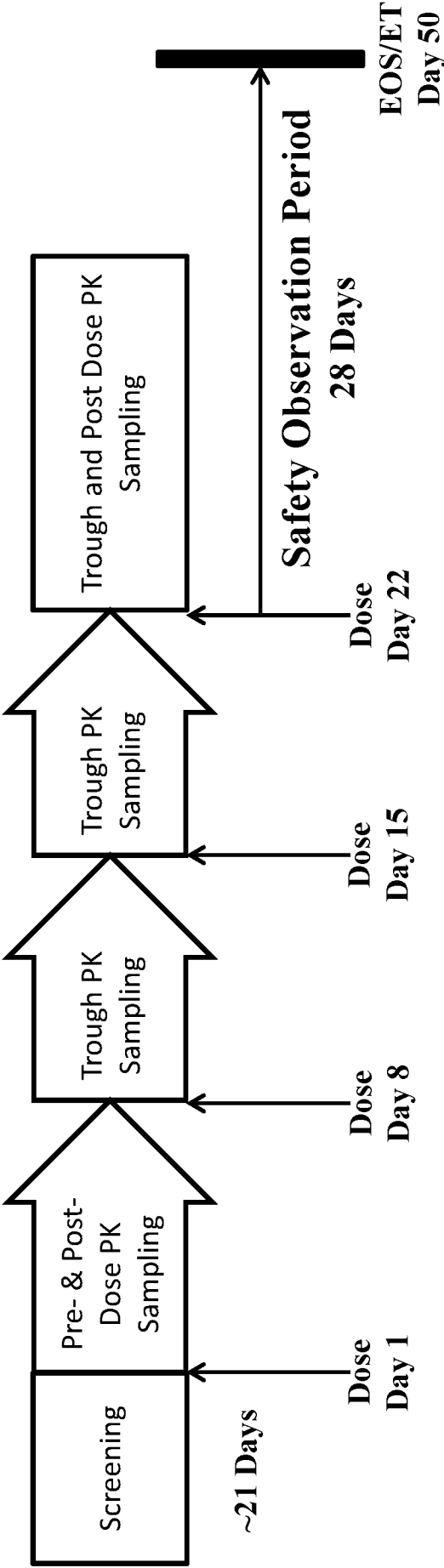


FIG. 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/032956

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/36 A61K38/37 A61P7/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/106052 A1 (BIOGEN MA INC [US]) 16 July 2015 (2015-07-16) cited in the application	1-89
Y	abstract page 1, paragraph 0002 - page 118, paragraph 0317 examples claims ----- -/--	1-89



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 September 2019

Date of mailing of the international search report

04/10/2019

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Authorized officer

Camilleri, Alain

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/032956

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/021423 A2 (BIOGEN IDEC INC [US]) 12 February 2015 (2015-02-12)	1-89
Y	abstract page 1, paragraph 0001 - page 3, paragraph 0013 page 8, paragraph 0036 - page 11, paragraph 0045 page 13, paragraph 0055 - page 17, paragraph 0071 page 35, paragraph 0135 - page 133, paragraph 0362 examples claims sequences	1-89
X	----- WO 2013/106787 A1 (BIOGEN IDEC INC [US]) 18 July 2013 (2013-07-18) cited in the application	1-89
Y	abstract page 1, paragraph 0001 - page 133, paragraph 0320 examples claims sequences 72, 73	1-89
X	----- PATARROYO-WHITE S ET AL: "OR413: A FVIII/VWF chimeric protein with VWF independent pharmacokinetic properties", JOURNAL OF THROMBOSIS AND HAEMOSTASIS; ABSTRACTS OF THE XXV CONGRESS OF THE INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS, JUNE 20-25, 2015, BLACKWELL PUBLISHING, OXFORD, GB, vol. 13, no. Suppl. 2, 1 June 2015 (2015-06-01), page 254, XP009186864, ISSN: 1538-7933, DOI: 10.1111/JTH.12993 [retrieved on 2015-06-03]	1-89
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Y	page 10	1-89
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/032956

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Douglas Drager ET AL: "Paper: Recombinant FVIIIIFc-VWF-XTEN Demonstrates Significant Bioavailability Following Subcutaneous Administration in Hemophilia A Mice", 7 December 2015 (2015-12-07), XP055621737, Retrieved from the Internet: URL:https://ash.confex.com/ash/2015/webpro gramscheduler/Paper85338.html [retrieved on 2019-09-12]	1-89
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Information on patent family members

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