

ANTIBODIES AND ASSAYS FOR DETERMINING CLOTTING FACTOR VIII ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/611,692 filed December 18, 2023, which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The content of the electronically submitted sequence listing in XML file (Name: 748429_SA9-493-1_ST26.xml; Size: 84,582 bytes; Date of Creation: December 18, 2023) is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Von Willebrand disease is characterized by the qualitative and quantitative deficiency in VWF. There are multiple subtypes of VWD (type 1, 2A, 2B, 2M, 2N, and 3). Type 2N and type 3 are rare recessive disorders resulting from the mutation in the FVIII binding region of VWF in type 2N and a complete lack of or very low levels of VWF in type 3. FVIII levels are substantially reduced in patients with type 2N and 3 VWD.

[0003] Inherited VWD is phenotypically heterogeneous and classified into 3 types. Type 1 and 3 reflect a partial or complete quantitative deficiency of VWF, respectively, while type 2 VWD reflects qualitative defects in VWF. Type 1 VWD is characterized by a mild to moderate reduction in plasma levels of VWF and is the most common (70-80%) form of VWD. The bleeding phenotype of type 1 VWD patients is generally milder than in type 2 and type 3 VWD. In type 2 VWD, 4 subtypes are recognized representing different qualitative VWF defects. Type 3 VWD is characterized by undetectable levels of VWF in plasma and very low plasma levels of FVIII (Mannucci PM. Blood. 2001;97(7):1915-9). The VWF gene, which largely determines VWF levels in circulation, is located at chromosome 12. A large variety of mutations or deletions in the VWF gene are associated with VWD (de Jong A. and Eikenboom J. Throm res. 2017;159:65-75).

[0004] Similar to patients with hemophilia A who have FVIII deficiency, arthropathy caused by joint bleeds has occasionally been described in patients with moderate and severe VWD. This is often associated with joint pain, lower health-related quality of life, and significantly more radiological and self-reported joint damage. This has led to the use of prophylaxis to prevent joint bleeds in some VWD patients experiencing this complication.

SUMMARY OF THE DISCLOSURE

[0005] The present disclosure provides, *inter alia*, anti-ELNN antibodies, as well as methods for assaying the level of Clotting Factor VIII (FVIII) activity level of certain chimeric proteins. Methods provided include methods for determining the level of FVIII activity in the blood or plasma of a human subject who has Willebrand disease (VWD) and to whom an ELNN- and/or VWF fragment-containing chimeric protein such as efanesoctocog alfa has been administered.

[0006] Certain aspects of the present disclosure are directed to a method of determining the level of exogenous Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising (i) separating the chimeric protein from a biological sample from the subject with an anti-ELNN antibody or antigen-binding fragment thereof to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample; (ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value; and (iii) reporting the assayed FVIII activity value or an approximated value thereof without correcting the assayed FVIII activity value or approximated value thereof.

[0007] In some embodiments, the chimeric protein was administered to the subject less than one week before the sample was taken from the subject. In some embodiments, the biological sample is a blood sample. In some embodiments, the biological sample is a plasma sample. In some embodiments, correcting the assayed FVIII activity value or approximated value thereof comprises reducing the value. In some embodiments, correcting the assayed FVIII activity value or approximated value thereof comprises reducing the value as if an overestimation of the FVIII activity needs to be accounted for. In some embodiments, an activated partial thromboplastin time (aPTT)-based one stage clotting assay is not used to assess the level of FVIII activity.

[0008] In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (LCDR) sequences, wherein LCDR1, LCDR2, and LCDR3 comprises SEQ ID NOs: 38, 39 and 40, respectively, and wherein the anti-ELNN antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining region (HCDR) sequences, wherein HCDR1, HCDR2, and HCDR3 comprises SEQ ID NOs: 32, 33 and 34, respectively. In some embodiments, the anti-ELNN antibody or an antigen binding fragment thereof comprises a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37. In some embodiments, the anti-ELNN antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising three heavy-

chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR).

[0009] In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 32 and a light chain variable region (LCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 37. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the antibody comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 36. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 31 and a light chain comprising the amino acid sequence of SEQ ID NO: 36.

[0010] In some embodiments, the antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the antibody comprises two heavy chains consisting of the amino acid sequence of SEQ ID NO:31, and two light chains consisting of the amino acid sequence of SEQ ID NO:36. In some embodiments, the antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37.

[0011] In some embodiments, the anti-ELNN antibody or antigen binding portion thereof comprises (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34.

[0012] In some embodiments, the ELNN polypeptide comprises at least three instances of the sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises at least 3 instances of the sequence: ESATPE (SEQ ID NO: 29). In some embodiments, the ELNN polypeptide comprises at least 4 instances of the following sequence: SATPE (SEQ ID NO: 28).

In some embodiments, the ELNN polypeptide comprises at least 5 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises at least 6 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, each of the at least 3, at least 4, at least 5, or at least 6 instances of SATPE (SEQ ID NO: 28) are as part of the following sequence: ESATPE (SEQ ID NO: 29). In some embodiments, each instance of SATPE (SEQ ID NO: 28) and/or ESATPE (SEQ ID NO: 29) in the sequence of the ELNN polypeptide is followed by at least 3 amino acids at the C-terminal end thereof. In some embodiments, the ELNN polypeptide comprises the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27. In some embodiments, wherein the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.

[0013] In some embodiments, the anti-ELNN antibody or binding portion thereof comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31, wherein the anti-ELNN antibody or binding portion thereof comprises a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36. In some embodiments, the anti-ELNN antibody or binding portion thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31, and wherein the anti-ELNN antibody or binding portion thereof a light chain comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the anti-ELNN antibody or binding portion thereof comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and wherein the anti-ELNN antibody or binding portion thereof comprises two light chains comprising the amino acid sequence of SEQ ID NO:36.

[0014] Certain aspects of the present disclosure are directed to a method of determining the level of exogenous Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising (i) separating the chimeric protein from a biological sample from the subject with an anti-ELNN antibody or antigen-binding fragment thereof to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample; (ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value; and (iii) reporting the assayed FVIII activity value or an approximated value thereof without correcting the assayed FVIII activity value or approximated value thereof, wherein the chimeric protein further comprises a von Willebrand Factor (VWF) fragment.

[0015] Certain aspects of the present disclosure are directed to a method for determining the level of Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von

Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises (a) a FVIII protein and (b) an ELNN polypeptide and/or a von Willebrand Factor (VWF) fragment, the method comprising (i) assaying the level of FVIII activity in the chimeric protein with an activated partial thromboplastin time (aPTT)-based one stage clotting assay to obtain an assayed FVIII activity value, wherein the aPTT-based one stage clotting assay does; (ii) reducing the assayed FVIII activity value to obtain an adjusted FVIII activity value; and (iii) reporting the adjusted FVIII activity value or an approximated value thereof.

[0016] In some embodiments, the chimeric protein comprises a FVIII protein and an ELNN polypeptide. In some embodiments, the ELNN polypeptide is inserted into the FVIII protein of the chimeric protein. In some embodiments, the FVIII activity value or the approximated value thereof is reported to the subject, a caregiver of the subject, or a medical professional. In some embodiments, the subject has a body mass index of ≥ 30 kg/m². In some embodiments, the subject is a female. In some embodiments, the subject is a male. In some embodiments, the subject is a human.

[0017] In some embodiments, the VWD is Type 2N VWD. In some embodiments, the VWD is Type 3 VWD.

[0018] Certain aspects of the present disclosure are directed to a method of monitoring the level of FVIII in the blood or plasma of a human subject who is receiving treatment for Willebrand disease (VWD), the method comprising performing a method for determining the level of FVIII activity disclosed herein at least once per year, e.g., at least once every 6 months or at least once per month. In some embodiments, the level of FVIII activity is determined at least once per year. In some embodiments, the level of FVIII activity is determined at least once every 9 months. In some embodiments, the level of FVIII activity is determined at least once every 6 months. In some embodiments, the level of FVIII activity is determined at least once per month.

[0019] In some embodiments, multiple doses of the chimeric protein are administered to the subject at a dosing interval. In some embodiments, at least one of the multiple doses is from about 10 IU/kg to about 30 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 25 IU/kg and the dosing interval is at least about 7 days.

[0020] Certain aspects of the present disclosure are directed to a method for determining the level of Clotting Factor VIII (FVIII) activity from a chimeric protein in a biological sample, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising (i) separating the chimeric protein from the biological sample from the subject with an anti-ELNN antibody to obtain a captured chimeric protein sample, wherein the biological sample is a blood

sample or a plasma sample; and (ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value.

[0021] In some embodiments, the chimeric protein is in a pharmaceutical composition comprising sucrose, histidine, arginine, calcium chloride, and polysorbate 80. In some embodiments, the chimeric protein is in a pharmaceutical composition comprising: (a) about 5% (w/v) sucrose; (b) about 10 mM histidine; (c) about 250 mM arginine; (d) about 5 mM calcium chloride; and (e) about 0.05% polysorbate 80, and a pH of about 7.0. In some embodiments, the pharmaceutical composition is lyophilized. In some embodiments, the lyophilized pharmaceutical composition is reconstituted with sterile water prior to administering to the subject.

[0022] In some embodiments, the chimeric protein comprises a VWF fragment. In some embodiments, the VWF fragment comprises a D'D3 fragment of VWF. In some embodiments, the VWF fragment consists of a D'D3 fragment of VWF. In some embodiments, the D'D3 fragment of VWF is mutated such that it cannot form disulfide binds with another D'D3 fragment of VWF. In some embodiments, the D' domain of the D'D3 fragment of VWF comprises the sequence of SEQ ID NO: 21. In some embodiments, the D3 domain of the D'D3 fragment of VWF comprises the sequence of SEQ ID NO: 23. In some embodiments, wherein the FVIII protein comprises at least a partial deletion of the wild-type FVIII B domain. In some embodiments, the chimeric protein comprises an ELNN polypeptide comprising the sequence of SEQ ID NO: 14. In some embodiments, the chimeric protein comprises an ELNN polypeptide comprising the sequence of SEQ ID NO: 24.

[0023] In some embodiments, the chimeric protein comprises a first polypeptide which comprises a FVIII protein and a first immunoglobulin ("Ig") constant region or a portion thereof, and a second polypeptide which comprises a VWF fragment and a second Ig constant region or a portion thereof. In some embodiments, the chimeric protein comprises (i) a first polypeptide comprising a FVIII protein, an ELNN polypeptide inserted within and replacing a portion of the B domain, and a first Fc region; and (ii) a second polypeptide comprising a VWF fragment, an additional ELNN polypeptide, an $\alpha 2$ linker, and a second Fc region. In some embodiments, the chimeric protein is a FVIII-ELNN-Fc/D'D3-ELNN-Fc heterodimer. In some embodiments, the first polypeptide chain comprises the amino acid sequence set forth as SEQ ID NO: 1 and the second polypeptide chain comprises the amino acid sequence set forth as SEQ ID NO: 2, and wherein the first polypeptide chain and the second polypeptide chain are covalently linked by two disulfide bonds between the Ig constant region domains of the first and second polypeptide chains.

[0024] In some embodiments, the chimeric protein is efanesoctocog alfa.

[0025] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, wherein the antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (CDR) sequences of SEQ ID NOs: 38, 39 and 40, and three heavy chain complementarity determining region (CDR) sequences of SEQ ID NOs: 32, 33 and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0026] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, wherein the antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (CDR) sequences of SEQ ID NOs: 38, 39 and 40, and three heavy chain complementarity determining region (CDR) sequences of SEQ ID NOs: 32, 33 and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).

[0027] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising three heavy-chain CDRs of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and three light-chain CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37 and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0028] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising three heavy-chain CDRs of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and three light-chain CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37 and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).

[0029] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region comprising three heavy-chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR), and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29). In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:32 and a light chain variable region (LCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:37. In

some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37. In some embodiments, the antibody comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the antibody comprises two heavy chains consisting of the amino acid sequence of SEQ ID NO:31, and two light chains consisting of the amino acid sequence of SEQ ID NO:36.

[0030] Certain aspects of the present disclosure are directed to a monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0031] Certain aspects of the present disclosure are directed to a monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 29).

[0032] Certain aspects of the present disclosure are directed to an antigen binding protein comprising (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises the sequence: ESATPE (SEQ ID NO: 28). In some embodiments, at least 3 instances of the sequence SATPE (SEQ ID NO: 28) are ESATPE (SEQ ID NO: 29). In some embodiments, each of the instances of the sequence SATPE (SEQ ID NO: 28) is ESATPE (SEQ ID NO: 29). In some

embodiments, the ELNN polypeptide comprises at least 4 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises at least 5 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises at least 6 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, each of the at least 3, at least 4, at least 5, or at least 6 instances of SATPE (SEQ ID NO: 28) are part of the following sequence: ESATPE (SEQ ID NO: 29). In some embodiments, each instance of SATPE (SEQ ID NO: 28) and/or ESATPE (SEQ ID NO: 29) in the sequence of the ELNN polypeptide is followed by at least 3 amino acids at the C-terminal end thereof. In some embodiments, the ELNN polypeptide comprises the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27.

[0033] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.

[0034] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment comprising a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36.

[0035] Certain aspects of the present disclosure are directed to an antibody or antigen-binding fragment comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36.

[0036] Certain aspects of the present disclosure are directed to an antibody comprising two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36.

[0037] In some embodiments, the antibody, antigen-binding fragment, or antigen binding protein is isolated antibody, antigen-binding fragment, or antigen binding protein.

[0038] Certain aspects of the present disclosure are directed to a polynucleotide or set of polynucleotides encoding any of the antibodies, antigen-binding fragments, or antigen binding proteins disclosed herein.

[0039] Certain aspects of the present disclosure are directed to a vector comprising the polynucleotide or set of polynucleotides encoding any of the antibodies, antigen-binding fragments, or antigen binding proteins disclosed herein.

[0040] Certain aspects of the present disclosure are directed to a host cell comprising a vector comprising the polynucleotide or set of polynucleotides encoding any of the antibodies, antigen-

binding fragments, or antigen binding proteins disclosed herein. In some embodiments, the host cell is a CHO cell or a HEK293 cell.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0041] FIG. 1 is a schematic representation of a rFVIII-Fc-VWF-ELNN heterodimer. Abbreviations are FVIII: factor VIII; VWF: von Willebrand Factor; A1, A2, A3, C1, C2: domains of FVIII; D'D3: domains of VWF; ELNN: extended length polypeptides; Fc: Fc region of immunoglobulin constant region.

[0042] FIG. 2 is a graphical representation of the design of the clinical study. Abbreviations are DDAVP: Desmopressin acetate (1-deamino-8-D-arginine vasopressin); FVIII: Factor VIII; IV: Intravenous; PK: Pharmacokinetics; VWF: von Willebrand factor.

[0043] FIG. 3 is a graphical representation of the mean and individual participant baseline-corrected FVIII activity-time profiles quantified by the (A) OSA and (B) CCS. Abbreviations are CCS: capture chromogenic assay; FVIII, factor VIII; h, hour; OSA, one-stage assay. Mean values are displayed with black filled circles. Participants with Type 2N VWD (Participants 2 and 3) are displayed with filled triangles and Xs, respectively. Participants with Type 3 VWD (Participants 1, 4, 5, and 6) displayed with open circles, open stars, open diamonds, and open squares, respectively.

[0044] FIG. 4 is a graphical display showing binding of the 4D9G3 antibody to 183 unique peptides. The x-axis indicates peptide number, and the y-axis represents ELISA signal intensity.

[0045] FIG. 5 is a graphical display showing binding of the 4D9G3 antibody to 183 unique peptides. 43 peptides containing the ESATPE epitope showed significant binding (black bars), 5 peptides contain only SATPE showed reduced binding (dark gray bars), and the remaining 135 peptides, which did not contain the epitope, showed very low binding (light gray bars). The x-axis indicates peptide number, while the y-axis represents ELISA signal intensity.

[0046] FIG. 6 is a graphical representation of the binding of the 43 peptides found to be significant binders. All peptides with equal or less than 2 aa on the C-terminus of the epitope showed inferior signal. On the other hand, if the epitope is on the very N-terminus, binding to the 4D9G3 antibody was not affected.

DETAILED DESCRIPTION

[0047] In the absence of exogenous VWF, the half-life of recombinant FVIII has been shown to be very short in certain VWD patients (VWD type 2N and 3) patients compared to hemophilia A patients, suggesting that regular FVIII products that depend on VWF cannot be readily used in

patients with severe VWD disease, even when the goal is that of correcting the FVIII defect only (e.g., for prophylaxis against bleeds or perioperative management). Efanesoctocog alfa temporarily replaces the missing FVIII needed for effective hemostasis in patients with a deficiency of FVIII. Efanesoctocog alfa is the first FVIII therapy that is engineered to circulate independently from VWF, thereby uncoupling FVIII from VWF clearance and extending half-life.

[0048] The present disclosure provides, *inter alia*, methods for assaying, determining, and monitoring the level of administered FVIII (e.g., FVIII of a chimeric protein such as efanesoctocog alfa) in patients who have VWD. In some embodiments, these methods comprise use of a chimeric protein such as efanesoctocog alfa.

[0049] Also provided are anti-ELNN antibodies and fragments thereof and methods using such antibodies and fragments. Non-limiting examples of such methods include binding ELNN polypeptides, capturing ELNN polypeptides (e.g., isolating ELNN polypeptide-containing polypeptides from a solution such as a sample), and labeling ELNN polypeptides (e.g., with anti-ELNN antibodies or fragments thereof that are conjugated to a fluorophore or other detectable molecule).

I. Definitions

[0050] 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.

[0051] 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).

[0052] 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). In some embodiments, the term indicates deviation from the indicated numerical value by $\pm 10\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, $\pm 1\%$, $\pm 0.9\%$, $\pm 0.8\%$, $\pm 0.7\%$, $\pm 0.6\%$, $\pm 0.5\%$, $\pm 0.4\%$, $\pm 0.3\%$, $\pm 0.2\%$, $\pm 0.1\%$, $\pm 0.05\%$, or $\pm 0.01\%$. In some embodiments, "about"

indicates deviation from the indicated numerical value by $\pm 10\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 5\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 4\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 3\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 2\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 1\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.9\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.8\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.7\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.6\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.5\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.4\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.3\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.1\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.05\%$. In some embodiments, "about" indicates deviation from the indicated numerical value by $\pm 0.01\%$.

[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.

[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] In some embodiments, a polynucleotide is an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA) or plasmid DNA (pDNA). Depending on context, a "polynucleotide" may be any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a larger polynucleotide, such as a genome or vector. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which is not in its natural milieu (*e.g.*, it is not within a wild-type cell or virus). For example, a recombinant polynucleotide encoding a Factor VIII

protein 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.

[0057] 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 some 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. It will be understood that, in instances where a polypeptide sequence is disclosed with a signal peptide, the form of the polypeptide sequence without the signal peptide is also disclosed.

[0058] 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, but does not refer to any particular amino acid length. 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, depending on context. 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.

[0059] 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.

[0060] 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 that retain at least one or more 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.

[0061] The term "VWF fragment" includes any VWF fragment that interacts with FVIII and retains at least one or more properties that are normally provided to FVIII by full-length VWF, e.g., preventing or reducing premature activation to FVIIIa, preventing or reducing premature proteolysis, preventing or reducing clearance, preventing or reducing association with phospholipid membranes that could lead to premature clearance, preventing or reducing binding to FVIII clearance receptors that can bind naked FVIII but not VWF-bound FVIII, and/or stabilizing FVIII (such as 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. In some embodiments, a VWF fragment is a fragment (which may be mutated) of full-length VWF that binds to a FVIII polypeptide such that the FVIII polypeptide has reduced binding to, or does not bind, full length VWF (e.g., endogenous VWF in a subject). In some embodiments, the VWF fragment is a human VWF fragment, or a mutant VWF fragment. In some embodiments, the VWF fragment comprises the D' and D3 domains of VWF. In some embodiments, the VWF fragment is a D'D3 fragment that is mutated to substitute one or two cysteines to alanines such that the fragment is not part of a dimer.

[0062] 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 some 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.

[0063] 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. Similarly, "sequence identity" between two polynucleotides is determined by comparing the nucleotide sequence of one polynucleotide to the sequence of a second polynucleotide. The terms "% identical", "% identity" or similar terms are intended to refer, in particular, to the percentage of nucleotides or amino acids (as applicable) which are identical in an optimal alignment between the sequences to be compared. Said percentage is purely statistical, and the differences between the two sequences may be but are not necessarily randomly distributed over the entire length of the sequences to be compared. Comparisons of two sequences are usually carried out by comparing the sequences, after optimal alignment, with respect to a segment or "window of comparison", in order to identify local regions of corresponding sequences. For example, the optimal alignment for a comparison may be carried out manually or with the aid of the local homology algorithm by Smith and Waterman, 1981, *Adv. App. Math.* 2, 482, with the aid of the local homology algorithm by Needleman and Wunsch, 1970, *J. Mol. Biol.* 48, 443, with the aid of the similarity search algorithm by Pearson and Lipman, 1988, *Proc. Natl Acad. Sci. USA* 88, 2444, or with the aid of computer programs using said algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.). In some embodiments, percent identity of two sequences is determined using the BLASTN or BLASTP algorithm, as available on the United States National Center for Biotechnology Information (NCBI) website (e.g., at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). In some embodiments, the algorithm parameters used for BLASTN algorithm on the NCBI website include: (i) Expect Threshold set to 10; (ii) Word Size set to 28; (iii) Max matches in a query range set to 0; (iv) Match/Mismatch Scores set to 1, -2; (v) Gap Costs set to Linear; and (vi) the filter for low complexity regions being used. In some embodiments, the algorithm parameters used for BLASTP algorithm on the NCBI website include: (i) Expect Threshold set to 10; (ii) Word Size set to 3; (iii) Max matches in a query range set to 0; (iv) Matrix set to BLOSUM62; (v) Gap Costs set to Existence: 11 Extension: 1; and (vi) conditional compositional score matrix adjustment. When discussed herein, whether any particular

polypeptide is, e.g., 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 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.

[0064] An amino acid corresponding to an amino acid in a VWF sequence or a FVIII sequence may be 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 a corresponding 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.

[0065] 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 (wild type) human FVIII (SEQ ID NO: 8) to which the insertion site corresponds, which is immediately C-terminal to the position of the insertion. For example, the phrase "comprises an ELNN polypeptide at an insertion site which corresponds to amino acid 1656 of SEQ ID NO: 8" indicates that the heterologous moiety is inserted immediately after the amino acid residue corresponding to amino acid residue 1656 of SEQ ID NO: 8 (without requiring amino acid residue 1657 of SEQ ID NO: 8 to be present).

[0066] The terms "inserted," "is inserted," "inserted into" or grammatically related terms, as used herein with respect to insertions of ELNN polypeptide into FVIII refers to the position of an ELNN polypeptide in a chimeric protein relative to the analogous position in native mature human FVIII (SEQ ID NO: 8). 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 protein was made. For example, in reference to a chimeric protein provided herein, the phrase "an ELNN polypeptide is inserted immediately downstream of residue 745 of the FVIII polypeptide" means that the chimeric protein comprises an ELNN

polypeptide immediately downstream of an amino acid residue which corresponds to amino acid residue 745 in native mature human FVIII, e.g., bounded by amino acids corresponding to amino acid residues 745 and 746 of native mature human FVIII (without requiring the presence of an amino acid residue corresponding to 746 of native mature human FVIII), and does not connote an order or method of production for which the chimeric protein was constructed.

[0067] As used herein, the terms "ELNN polypeptide" and "ELNN" are synonymous, and refer to extended length polypeptides comprising non-naturally occurring, substantially non-repetitive sequences (e.g., polypeptide motifs) 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. Such extended length polypeptides include unstructured hydrophilic polypeptides comprising repeating motifs of 6 natural amino acids (G, A, P, E, S, and/or T). In some embodiments, an ELNN polypeptide comprises multiple motifs of 6 natural amino acids (G, A, P, E, S, T), wherein the motifs are the same or comprise a combination of different motifs. ELNN polypeptides can confer certain desirable pharmacokinetic, physicochemical and pharmaceutical properties when linked to a VWF fragment or a FVIII sequence of the disclosure to create a chimeric protein. Such desirable properties include but are not limited to enhanced pharmacokinetic parameters and solubility characteristics. ELNN polypeptides are known in the art, and non-limiting descriptions relating to and examples of ELNN polypeptides referred to as XTEN polypeptides are available in Schellenberger et al., (2009) *Nat Biotechnol* 27(12):1186-90; Brandl et al., (2020) *Journal of Controlled Release* 327:186-197; and Radon et al., (2021) *Advanced Functional Materials* 31, 2101633 (pages 1-33), the entire contents of each of which are incorporated herein by reference.

[0068] 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 immunoglobulin Fc domain. A fusion protein may be 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 protein 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.

[0069] With respect to amino acid and nucleotide sequences, 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. Depending on context, 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) at an insertion site of 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. In some embodiments, a first nucleotide sequence can be linked to a second nucleotide sequence by a phosphodiester bond or a linker. In some embodiments, 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" may also be indicated by a hyphen (-).

[0070] With respect to two polypeptides, the term "associated with" refers to one or more covalent or non-covalent bonds formed between a first polypeptide and a second polypeptide. 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 some embodiments, it means a covalent bond other than 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 many 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. In some embodiments, the one or more covalent bonds between the first amino acid chain and the second amino acid chain is two disulfide bonds. In some embodiments, the one or more covalent bonds between the first amino acid chain and the second amino acid chain is two disulfide bonds between a first Fc portion on the first amino acid chain and a second Fc portion on the second amino acid chain, wherein the two disulfide bonds occur in the hinge region of the two Fc portions.

[0071] 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. Other enzymatic cleavage sites are known in the art and described in elsewhere herein. In constructs that include more than one processing or cleavage site, it will be understood that such sites can be the same or different.

[0072] The composition, e.g., the chimeric protein, can be administered to a subject using methods known in the art. In some embodiments, the administration is intravenous. In some embodiments, the administration is subcutaneous. In some embodiments, the administration is self-administration. In some embodiments, a parent administers the chimeric protein to a child. In some embodiments, the chimeric protein is administered to a subject by a healthcare practitioner, such as a medical doctor, a medic, or a nurse.

[0073] In some embodiments, a single dose is administered to a subject. In some embodiments, multiple doses are administered 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.

[0074] In some embodiments, the composition, e.g., the chimeric protein, is delivered via a slow push IV injection of 8 ± 2 minutes. In some embodiments, the composition is delivered at a rate of administration determined by the subject's comfort level. In some embodiments, the composition is delivered via a slow push IV injection at a rate of administration determined by the subject's comfort level and according to the following vial injection rate recommendations: For subjects weighing ≤ 55 kg, the minimum injection duration per vial is 2 minutes per vial; for subjects weighing > 55 kg, the minimum injection duration per vial is 1 minute per vial.

[0075] As used herein, the term "dosing interval" refers to the amount of time that elapses between when a first dose of a composition and a subsequent dose of the same composition are administered to a subject. A dosing interval can refer to the time that elapses between multiple doses. For example, a dosing interval can be written as once a week, once every two weeks, etc.

[0076] A "pharmaceutical label" is a display of written matter upon a container or printed medium that comprises statements about the administration, efficacy, and/or safety of a therapeutic agent such as a chimeric protein or a pharmaceutical composition comprising a chimeric protein.

[0077] aPTT tests measure 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. In one embodiment, aPTT 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).

[0078] The aPTT assay may also be used for assessing the potency of a chimeric protein prior to administration to a patient or subject (Hubbard AR, et al. J Thromb Haemost 11: 988–9 (2013)). 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.

[0079] Aspects relating to the use of aPTT assays in connection with efanesoctocog alfa and hemophilia A (including with respect to different reagents) are described in Pipe *et al.*, “A global comparative field study to evaluate the factor VIII activity of efanesoctocog alfa by one-stage clotting and chromogenic substrate assays at clinical haemostasis laboratories”, *Haemophilia* 2023;1–10; DOI:10.1111/hae.14831, the entire contents of which are incorporated herein by reference.

[0080] 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 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 some embodiments, the chromogenic assay is the BIOPHEN FVIII:C assay (Hyphen Biomed, Neuville sur Oise, France)

[0081] The chromogenic assay may also be used for assessing the potency of a chimeric protein 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.

[0082] In some embodiments, the chromogenic assay is a capture chromogenic assay that uses an antibody (such as an anti-ELNN antibody) to separate exogenous FVIII (e.g., in the context of a chimeric protein, such as FVIII of efanesoctocog alfa) from endogenous FVIII in a sample (e.g., plasma), before assessing the level of the exogenous FVIII activity using a chromogenic assay. A non-limiting example of a capture chromogenic assay is described in Example 1. In some embodiments, the exogenous FVIII (such as a chimeric protein comprising FVIII, e.g., efanesoctocog alfa) is used as a standard (e.g., a calibration standard) for a capture chromogenic assay.

[0083] As used herein, a “captured chimeric protein sample” is a sample derived from a biological sample (such as blood or plasma) from a subject in which the ratio of chimeric protein to endogenous FVIII protein is greater than the ratio of chimeric protein to endogenous FVIII

protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is less than 10% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is less than 5% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is less than 1% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is less than 0.5% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is less than 0.1% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is less than 0.01% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured chimeric protein sample is undetectable by a Western Blot assay. In some embodiments, the biological sample is blood that has been obtained from a subject who has type 2N or type 3 VWD. In some embodiments, the biological sample is plasma that has been obtained from a subject who has type 2N or type 3 VWD.

[0084] Consistently, a “captured efanesoctocog alfa sample” is a sample derived from a biological sample (such as blood or plasma) from a subject in which the ratio of efanesoctocog alfa protein to endogenous FVIII protein is greater than the ratio efanesoctocog alfa protein to endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is less than 10% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is less than 5% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is less than 1% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is less than 0.5% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is less than 0.1% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is less than 0.01% the amount of the endogenous FVIII protein in the biological sample. In some embodiments, the amount of endogenous FVIII protein in the captured efanesoctocog alfa sample is undetectable by a Western Blot assay. In some embodiments, the biological sample is blood that has been

obtained from a subject who has type 2N or type 3 VWD. In some embodiments, the biological sample is plasma that has been obtained from a subject who has type 2N or type 3 VWD.

[0085] As used herein, an “approximated value” of a FVIII activity value (such as an assayed FVIII activity value or an adjusted FVIII activity value) is a value derived by approximating (e.g., rounding) the FVIII activity value (e.g., to the nearest integer (up or down), the nearest half of an integer, the nearest tenth of an integer, or the nearest hundredth of an integer), so long as the approximated value does not deviate from the FVIII activity value by more than 5 or $\pm 20\%$. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than 5. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than 1. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than $\pm 20\%$. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than $\pm 15\%$. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than $\pm 10\%$. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than $\pm 5\%$. In some embodiments, the approximated value does not deviate from the FVIII activity value by more than $\pm 5\%$. Unlimiting illustrative examples include the following: (i) the FVIII activity value is 14.455 IU/dL and the approximated value is 14.46 (e.g., approximated to the nearest hundredth with the number 5 being rounded up to 6), (ii) the FVIII activity value is 14.455 IU/dL and the approximated value is 14.5 (e.g., rounded to the nearest tenth with the number 4 being rounded up to 5); (iii) the FVIII activity value is 14.456 IU/dL and the approximated value is 14 (e.g., rounded to the nearest integer); (iv) the FVIII activity value is 14.456 IU/dL and the approximated value is 15 (e.g., rounded to the nearest 5th integer).

II. Chimeric Proteins

[0086] In an aspect, the present disclosure is directed to detecting levels of a chimeric protein. In some embodiments, the chimeric protein comprises a FVIII protein and an ELNN polypeptide. In some embodiments, the ELNN polypeptide is inserted into the FVIII protein of the chimeric protein. In some embodiments, the chimeric protein was administered to the subject less than one week before a sample was taken from the subject. In some embodiments, the chimeric protein further comprises a von Willebrand Factor (VWF) fragment. In some embodiments, a chimeric protein comprises a first polypeptide which comprises a Factor VIII (“FVIII”) protein or a portion thereof and a first immunoglobulin (“Ig”) constant region or a portion thereof, and a second polypeptide which comprises a von Willebrand Factor (“VWF”) fragment and a second Ig constant region or a portion thereof. In some embodiments, the chimeric protein comprises (i) a first polypeptide comprising a FVIII polypeptide, an ELNN polypeptide inserted within the B domain of

the FVIII protein (e.g. replacing a portion of the B domain, such as the majority of the B domain), and a first Fc region; and (ii) a second polypeptide comprising a VWF fragment, a second ELNN polypeptide sequence, an a2 linker, and a second Fc region. In some embodiments, the chimeric protein disclosed herein is a FVIII-ELNN-Fc/D'D3-ELNN-Fc heterodimer. A graphical representation of an rFVIII-Fc-VWF-ELNN heterodimer is shown in FIG. 1.

[0087] In some embodiments, the chimeric protein is efanesoctocog alfa. Efanesoctocog alfa, also known as "BIVV001", "Efa", and "rFVIII-Fc-VWF-XTEN", is described in Chhabra et al. *Blood* 2020; 135(17): 1484-1496, Konkle et al., *N Engl J Med* 2020; 383:1018-1027, and the International Nonproprietary Names for Pharmaceutical Substances (INN) WHO Drug Information, 2019, Vol. 33, No. 4, p.828-30, the entire contents of each of which are hereby incorporated by reference in their entireties. Efanesoctocog alfa is an exemplary FVIII-ELNN-Fc/D'D3-ELNN-Fc heterodimer.

[0088] In some embodiments, the chimeric protein is a FVIII-ELNN-Fc/D'D3-ELNN-Fc heterodimer comprising (i) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 1 and (ii) a second polypeptide comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the chimeric protein comprises (i) a first polypeptide and (ii) a second polypeptide that are covalently linked via one or more disulfide bonds (e.g., two disulfide bonds). In some embodiments, the chimeric protein comprises a FVIII polypeptide encoded by the nucleic acid sequence of SEQ ID NO: 4. In some embodiments, the chimeric protein comprises a VWF fragment encoded by the nucleic acid sequence of SEQ ID NO: 6.

[0089] In some embodiments, the chimeric protein is efanesoctocog alfa.

III. Methods of Assaying, Determining, and Monitoring FVIII Levels

[0090] In an aspect, the present disclosure is directed to a method for determining the level of Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises (a) a FVIII protein and (b) an ELNN polypeptide and/or a von Willebrand Factor (VWF) fragment, the method comprising: (i) assaying the level of FVIII activity in the captured chimeric protein with an activated partial thromboplastin time (aPTT)-based one stage clotting assay to obtain an assayed FVIII activity value; (ii) reducing the assayed FVIII activity value to obtain an adjusted FVIII activity value; and (iii) reporting the adjusted FVIII activity value or an approximated value thereof. In some embodiments, the FVIII activity in the blood is determined. In some embodiments, the FVIII activity in the plasma is determined. In some embodiments, the aPTT-based one stage clotting assay does not use a reagent comprising ellagic

acid. In some embodiments, the aPTT-based one stage clotting assay uses a reagent comprising ellagic acid. In some embodiments, the aPTT-based one stage clotting assay uses cephalin. In some embodiments, the aPTT-based one stage clotting assay uses ellagic acid and cephalin. In some embodiments, the cephalin is brain cephalin. In some embodiments, the cephalin is rabbit brain cephalin. In some embodiments, the aPTT-based one stage clotting assay does not use a reagent comprising both ellagic acid and phosphatides. In some embodiments, the aPTT-based one stage clotting assay does not use a reagent comprising both ellagic acid and phospholipids comprising phosphatides but not phosphatidylserine. In some embodiments, the aPTT-based one stage clotting assay does not use the reagent Actin[®] FS.

[0091] In some embodiments, the assayed FVIII activity value is reduced by about 25-50%. In some embodiments, the assayed FVIII activity value is reduced by about 30-55%. In some embodiments, the assayed FVIII activity value is reduced by about 35-60%. In some embodiments, the assayed FVIII activity value is reduced by about 40-60%. In some embodiments, the assayed FVIII activity value is reduced by about 45-75%. In some embodiments, the assayed FVIII activity value is reduced by about 50-75%. In some embodiments, the assayed FVIII activity value is reduced by about 75%. In some embodiments, the assayed FVIII activity value is reduced by about 70%. In some embodiments, the assayed FVIII activity value is reduced by about 65%. In some embodiments, the assayed FVIII activity value is reduced by about 60%. In some embodiments, the assayed FVIII activity value is reduced by about 55%. In some embodiments, the assayed FVIII activity value is reduced by about 50%. In some embodiments, the assayed FVIII activity value is reduced by about 45%. In some embodiments, the assayed FVIII activity value is reduced by about 40%. In some embodiments, the assayed FVIII activity value is reduced by about 35%. In some embodiments, the assayed FVIII activity value is reduced by about 30%. In some embodiments, the assayed FVIII activity value is reduced by at least 25%.

[0092] In some embodiments, detecting the level of exogenous Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, comprises: (i) separating the chimeric protein from a biological sample from the subject with an anti-ELNN antibody or an antigen-binding fragment thereof to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample; (ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value; and (iii) reporting the assayed FVIII activity value or an approximated value thereof without correcting the assayed FVIII activity value or approximated value thereof. In some embodiments, the chimeric protein is used as a standard (e.g., a calibration standard) for the chromogenic assay. In some embodiments, correcting the

assayed FVIII activity value or approximated value thereof comprises reducing the value. In some embodiments, correcting the assayed FVIII activity value or approximated value thereof comprises reducing the value as if an overestimation of the FVIII activity needs to be accounted for. In some embodiments, an activated partial thromboplastin time (aPTT)-based one stage clotting assay is not used to assess the level of FVIII activity. In some embodiments, the chimeric protein is separated from the biological sample with an anti-ELNN antibody. In some embodiments, the chimeric protein is separated from the biological sample with an antigen-binding fragment of an anti-ELNN antibody. In some embodiments, the biological sample is a blood sample. In some embodiments, the biological sample is a plasma sample.

[0093] In one aspect, the present disclosure is directed to a method for determining the level of Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises (a) a FVIII protein and (b) an ELNN polypeptide and/or a von Willebrand Factor (VWF) fragment, the method comprising: (i) assaying the level of FVIII activity in the captured chimeric protein with an activated partial thromboplastin time (aPTT)-based one stage clotting assay to obtain an assayed FVIII activity value; (ii) reducing the assayed FVIII activity value to obtain an adjusted FVIII activity value; and (iii) reporting the adjusted FVIII activity value or an approximated value thereof. In some embodiments, FVIII activity value or the approximated value thereof is reported to the subject, a caregiver of the subject, or a medical professional. In some embodiments, the aPTT-based one stage clotting assay does not use a reagent comprising ellagic acid. In some embodiments, the aPTT-based one stage clotting assay uses a reagent comprising ellagic acid. In some embodiments, the aPTT-based one stage clotting assay uses cephalin. In some embodiments, the aPTT-based one stage clotting assay uses ellagic acid and cephalin. In some embodiments, the cephalin is brain cephalin. In some embodiments, the cephalin is rabbit brain cephalin. In some embodiments, the aPTT-based one stage clotting assay does not use a reagent comprising both ellagic acid and phosphatides. In some embodiments, the aPTT-based one stage clotting assay does not use a reagent comprising both ellagic acid and phospholipids comprising phosphatides but not phosphatidylserine. In some embodiments, the aPTT-based one stage clotting assay does not use the reagent Actin® FS.

[0094] In some embodiments, the assayed FVIII activity value is reduced by about 25-50%. In some embodiments, the assayed FVIII activity value is reduced by about 50%. In some embodiments, the assayed FVIII activity value is reduced by at least 25%.

[0095] In some embodiments, the subject has a body mass index of ≥ 30 kg/m². In some embodiments, the subject is a female. In some embodiments, the subject is a male. In some embodiments, the subject is human.

[0096] In one aspect, the present disclosure is directed to a method of monitoring the level of FVIII in the blood or plasma of a human subject who is receiving treatment for Willebrand disease (VWD), the method comprising performing any of the methods disclosed herein at least once per year, e.g., at least once every 6 months or at least once per month. In some embodiments, the level of FVIII activity is determined at least once per year. In some embodiments, the level of FVIII activity is determined at least once every 9 months. In some embodiments, the level of FVIII activity is determined at least once every 6 months. In some embodiments, the level of FVIII activity is determined at least once per month. In some embodiments, the methods disclosed herein can be performed twice a month, three times a month, 4 times a month, one time a week, twice a week, three times a week, four times a week, five times a week, six times a week, or daily. In some embodiments, the methods disclosed herein can be performed once every two months, once every three months, once every four months, once every five months, once every six months or longer. In some embodiments, multiple doses of the chimeric protein are administered to the subject at a dosing interval. In some embodiments, the dosing interval is one day, two days, three days, four days, five days, six days, or 7 days. In some embodiments, the dosing interval is 7 days or longer.

[0097] In some embodiments, the subject has VWD, wherein the VWD is Type 2N VWD. In some embodiments, the subject has VWD, wherein the VWD is Type 3 VWD.

[0098] In some embodiments, multiple doses of the chimeric protein are administered. In some embodiments, each of the multiple doses is between about 10IU/kg and 100 IU/kg. In some embodiments, each of the multiple doses is 25 IU/kg. In some embodiments, each of the multiple doses is 50 IU/kg.

IV. Anti-ELNN Antibodies

[0099] In an aspect, the present disclosure is directed to an anti-ELNN antibody or antigen-binding fragment thereof. In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (CDR) sequences of SEQ ID NOs: 38, 39 and 40, and three heavy chain complementarity determining region (CDR) sequences of SEQ ID NOs: 32, 33 and 34. In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and three

light-chain CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37, wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28). In an aspect, the present disclosure is directed to an anti-ELNN antibody or antigen-binding fragment thereof. In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (CDR) sequences of SEQ ID NOs: 38, 39 and 40, and three heavy chain complementarity determining region (CDR) sequences of SEQ ID NOs: 32, 33 and 34. In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and three light-chain CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37, wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29). In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region comprising three heavy-chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR), and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region comprising three heavy-chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR), and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).

[0100] In some embodiments, the CDRs are identified by the Kabat definition, the IMGT definition, the Chothia definition, the AbM definition and/or the contact definition.

[0101] In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:32 and a light chain variable region (LCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:37. In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37. In some embodiments, the anti-ELNN antibody comprises a heavy chain comprising an amino acid sequence that is at least 95%

identical to SEQ ID NO:31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36.

[0102] In some embodiments, the anti-ELNN antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the anti-ELNN antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36. In some embodiments, the anti-ELNN antibody comprises two heavy chains consisting of the amino acid sequence of SEQ ID NO:31, and two light chains consisting of the amino acid sequence of SEQ ID NO:36. In some embodiments, the anti-ELNN antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the anti-ELNN antibody comprises two heavy chains consisting of the amino acid sequence of SEQ ID NO:31, and two light chains consisting of the amino acid sequence of SEQ ID NO:36. In some embodiments, the anti-ELNN antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).

[0103] In some embodiments, the anti-ELNN antibody or antigen binding portion thereof comprises (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34, wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the anti-ELNN antibody or antigen binding portion thereof comprises (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34, wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).

[0104] In some embodiments, the ELNN polypeptide comprises the sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises the sequence: ESATPE (SEQ ID NO: 29). In some embodiments, the ELNN polypeptide comprises at least 2 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises at least 5 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, the ELNN polypeptide comprises at least 6 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0105] In some embodiments, the ELNN polypeptide comprises at least 3 instances, at least 4 instances, at least 5 instances, or at least 6 instances of the following sequence: SATPE (SEQ ID NO: 28), wherein each of the at least 3, at least 4, at least 5, or at least 6 instances of SATPE (SEQ ID NO: 28) are part of the following sequence: ESATPE (SEQ ID NO: 29). In some embodiments, each instance of SATPE (SEQ ID NO: 28) and/or ESATPE (SEQ ID NO: 29) in the sequence of the ELNN polypeptide is followed by at least 3 amino acids at the C-terminal end thereof.

[0106] In some embodiments, the ELNN polypeptide comprises the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27. In some embodiments, the ELNN polypeptide comprises a von Willebrand Factor fragment.

[0107] In some embodiments, the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 36. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 31 and a light chain comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, the anti-ELNN antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO: 31, and two light chains comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, the anti-ELNN antibody, antigen-binding fragment, or antigen binding protein, which is an isolated antibody, antigen-binding fragment, or antigen binding protein.

[0108] In some embodiments, the anti-ELNN antibody is in a mouse IgG1 backbone. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 42, and a light chain variable region comprising an amino acid sequence that is at least 95%

identical to SEQ ID NO: 47. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence that is identical to SEQ ID NO: 42, and a light chain variable region comprising an amino acid sequence that is identical to SEQ ID NO: 47. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 41, and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 46. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain comprising an amino acid sequence that is identical to SEQ ID NO: 41, and a light chain comprising an amino acid sequence that is identical to SEQ ID NO: 46. In some embodiments, the anti-ELNN antibody comprises two heavy chains comprising amino acid sequences at least 95% identical to SEQ ID NO: 41, and two light chains comprising amino acid sequences at least 95% identical to SEQ ID NO: 46. In some embodiments, the anti-ELNN antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO: 41, and two light chains comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, the anti-ELNN antibody comprises a heavy chain, wherein the heavy chain comprises three complementary determining regions (CDR), wherein HCDR1 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 43, wherein HCDR2 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 44, wherein HCDR3 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 45. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 43, HCDR2 comprises the amino acid sequence of SEQ ID NO: 44, and HCDR3 comprises the amino acid sequence of SEQ ID NO: 45. In some embodiments, the anti-ELNN antibody comprises a light chain, wherein the light chain comprises three complementary determining regions (CDR), wherein LCDR1 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 48, wherein LCDR2 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 49, wherein LCDR3 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 50. In some embodiments, LCDR1 comprises the amino acid sequence of SEQ ID NO: 48, LCDR2 comprises the amino acid sequence of SEQ ID NO: 49, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 50.

[0109] In some embodiments, the anti-ELNN antibody is in a mouse IgG2a backbone. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 53, and a light chain variable region comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 57. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain variable region comprising an amino acid sequence that is identical to SEQ ID NO: 53, and a light chain comprising an amino acid sequence that is identical

to SEQ ID NO: 57. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 51, and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 52. In some embodiments, the anti-ELNN antibody or antigen-binding fragment comprises a heavy chain comprising an amino acid sequence that is identical to SEQ ID NO: 51, and a light chain comprising an amino acid sequence that is identical to SEQ ID NO: 52. In some embodiments, the anti-ELNN antibody comprises two heavy chains comprising amino acid sequences at least 95% identical to SEQ ID NO: 51, and two light chains comprising amino acid sequences at least 95% identical to SEQ ID NO: 52. In some embodiments, the anti-ELNN antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO: 51, and two light chains comprising the amino acid sequence of SEQ ID NO: 52. In some embodiments, the anti-ELNN antibody comprises a heavy chain, wherein the heavy chain comprises three complementary determining regions (CDR), wherein HCDR1 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 54, wherein HCDR2 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 55, wherein HCDR3 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 56. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 54, HCDR2 comprises the amino acid sequence of SEQ ID NO: 55, and HCDR3 comprises the amino acid sequence of SEQ ID NO: 56. In some embodiments, the anti-ELNN antibody comprises a light chain, wherein the light chain comprises three complementary determining regions (CDR), wherein LCDR1 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 58, wherein LCDR2 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 59 (KVS), wherein LCDR3 comprises an amino acid sequence at least 95% identical to SEQ ID NO: 60. In some embodiments, LCDR1 comprises the amino acid sequence of SEQ ID NO: 58, LCDR2 comprises the amino acid sequence of SEQ ID NO: 59 (KVS), and LCDR3 comprises the amino acid sequence of SEQ ID NO: 60.

[0110] In some embodiments, an anti-ELNN antibody binds to ELNN polypeptides that comprise at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28). In some embodiments, an anti-ELNN antibody binds to ELNN polypeptides that comprise at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29). Non-limiting examples of such ELNNs include ELNNs comprising the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27. In some embodiments, 2 or more of the instances are separated by one or more amino acids within the ELNN polypeptide's sequence. In some embodiments, all of the instances are separated by one or more amino acids within the ELNN polypeptide's sequence. In some embodiments, 2 or more instances are separated by three or more amino acids within the ELNN polypeptide's sequence.

In some embodiments, all of the instances are separated by three or more amino acids within the ELNN polypeptide's sequence.

[0111] In some embodiments, an anti-ELNN antibody binds to ELNN polypeptides that comprise at least 3 instances of the sequence ESATPE (SEQ ID NO: 29) but does not bind to ELNN polypeptides that comprise at only 1 instance of the sequence ESATPE (SEQ ID NO: 29).

[0112] In some embodiments, antibodies and antigen-binding fragments thereof may comprise antibody sequences of different origins, comprising mouse, rat, rabbit, donkey, human, and/or camelid immunoglobulin sequences. In some embodiments, an antibody is fully human, humanized, or chimeric. In some embodiments, an antibody or antigen-binding fragment thereof is conjugated or linked to a tag or other functional moiety, *e.g.* a toxin, label, fluorophore, radiochemical, etc. In some embodiments an antibody or antigen-binding fragment thereof is conjugated or linked to biotin. In some embodiments, an antibody or antigen-binding fragment thereof is conjugated or linked to streptavidin.

V. Pharmaceutical Compositions

[0113] In an aspect, the present disclosure is directed to pharmaceutical compositions of a chimeric protein, *e.g.*, efanesoctocog alfa.

[0114] In some embodiments, the present disclosure is directed to a pharmaceutical composition comprising: efanesoctocog alfa, L-histidine, L-arginine hydrochloride, sucrose, calcium chloride, and polysorbate 80. In some embodiments, the pharmaceutical composition comprises 250, 500, 1000, 2000, 3000 or 4000 IU of efanesoctocog alfa. In some embodiments, the pharmaceutical composition comprises 1000 IU of efanesoctocog alfa. In some embodiments, the pharmaceutical composition comprises efanesoctocog alfa, about 10 mM L-histidine, about 250 mM Arginine-HCl, about 5mM CaCl₂, about 5% (w/v) sucrose, and about 0.05% (w/v) polysorbate 80. In some embodiments, the pharmaceutical composition comprises efanesoctocog alfa, 10 mM L-histidine, 250 mM Arginine-HCl, 5mM CaCl₂, 5% (w/v) sucrose, and 0.05% (w/v) polysorbate 80. In some embodiments, the pharmaceutical composition has a pH of about 7.0. In some embodiments, the pharmaceutical composition has a pH of 6.8 to 7.2. In some embodiments, the pharmaceutical composition has a pH of 6.8. In some embodiments, the pharmaceutical composition has a pH of 7.0.

[0115] In some embodiments, the chimeric protein is in a pharmaceutical composition that comprises about 250 IU, 500 IU, 1000 IU, 2000 IU, 3000 IU, or 4,000 IU of the chimeric protein. In some embodiments, the chimeric protein is efanesoctocog alfa. In some embodiments, the efanesoctocog alfa is in a pharmaceutical composition that comprises: (a) 45 mg/mL to 60 mg/mL

sucrose; (b) 1.5 mg/mL to 2.0 mg/mL L-histidine; (c) 40 mg/mL to 60 mg/mL L-arginine; (d) 0.5 mg/mL to 0.9 mg/mL calcium chloride; and (e) 0.4 mg/mL to 0.7 mg/mL polysorbate 80. In some embodiments, the efanesoctocog alfa is in a pharmaceutical composition that comprises: (a) about 50 mg/ml sucrose; (b) about 1.55 mg/ml L-histidine; (c) about 52.67 mg/ml L-arginine-HCl; (d) about 0.73 mg/ml calcium chloride dihydrate; and (e) about 0.50 mg/ml polysorbate 80. In some embodiments, the efanesoctocog alfa is in a pharmaceutical composition that comprises: (a) about 56.12 mg/ml sucrose; (b) about 1.74 mg/ml L-histidine; (c) about 59.11 mg/ml L-arginine-HCl; (d) about 0.62 mg/ml calcium chloride; and (e) about 0.56 mg/ml polysorbate 80. In some embodiments, the efanesoctocog alfa is in a pharmaceutical composition that comprises: (a) 5% (w/v) to 7.5% (w/v) sucrose; (b) 7.5 mM to 12.5 mM histidine; (c) 225 mM to about 300 mM arginine; (d) 5 mM to 6 mM calcium chloride; and (e) 0.01% (w/v) to about 0.075% (w/v) polysorbate 80.

[0116] In some embodiments, the lyophilized pharmaceutical composition is in a lyophilized cake. In some embodiments, the lyophilized cake is white. In some embodiments, the lyophilized cake is less than Y4 in the European Pharmacopoeia color scale. See Degree of Coloration of Liquids (Method 2.2.2), European Pharmacopoeia, 10th Ed. (2021).

[0117] In some embodiments, the pharmaceutical composition disclosed herein is a lyophilized pharmaceutical composition (e.g., powder). In some embodiments, the pharmaceutical composition is supplied in a sterile vial that requires reconstitution with sterile water.

[0118] In some embodiments, the lyophilized pharmaceutical composition and sterile water are combined to produce an injectable solution. In some embodiments, the lyophilized pharmaceutical composition is combined with about 2 mL to about 5 mL of sterile water. In some embodiments, the lyophilized pharmaceutical composition is combined with about 3 mL of sterile water. In some embodiments, the lyophilized pharmaceutical composition is combined with 3 mL of sterile water. In some embodiments, the sterile water is USP grade sterile water. In some embodiments, the sterile water is USP grade sterile water for injection. In some embodiments, the sterile water is pyrogen-free or nonpyrogenic. In some embodiments, the sterile water does not contain a bacteriostatic or antimicrobial agent. In some embodiments, the sterile water contains a bacteriostatic or antimicrobial agent. In some embodiments, the sterile water is sterilized using a filter. In some embodiments, the sterile water is sterilized using a 0.1µm filter. In some embodiments, the sterile water is distilled water. In some embodiments, the sterile water is sterile, nonpyrogenic, distilled water, hypotonic, with an osmolarity of zero mOsmol/L, and does not contain a bacteriostatic or antimicrobial agent.

[0119] In some embodiments, the chimeric protein is provided as part of a kit with a pharmaceutical label, wherein the pharmaceutical label comprises information that is sufficient to enable a human subject, a caregiver, or a medical practitioner to practice a method of administering a chimeric protein as described herein.

[0120] 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: A Phase 1, Open-Label Study to Assess the Pharmacokinetics, and Safety and Tolerability of a Single Intravenous Injection of efanesoctocog alfa in Adults with type 2N and 3 von Willebrand disease (VWD)

[0121] Extended half-life recombinant FVIII (rFVIII) products have reduced the frequency of FVIII administration for prophylaxis in hemophilia patients. Next-generation extended half-life rFVIII products that prevent and control bleeding episodes for longer periods of time could result in less frequent administration, which in turn could improve quality of life for VWD patients.

[0122] Efanesoctocog alfa, also known as “BIVV001”, “efanesoctocogum alfa”, “Efa”, and “rFVIII-Fc-VWF-XTEN”, is a high-sustained blood clotting factor VIII (FVIII), engineered to have an extended half-life that is independent of VWF. Preclinical and clinical experience indicate that efanesoctocog alfa has an extended half-life, irrespective of VWF level, and maintains high sustained FVIII activity levels.

[0123] This study was a Phase 1, multicenter, open-label, single-arm, single-dose, non-randomized study in adult participants with type 2N (Normandy) or type 3 VWD. The rationale to conduct the Phase 1 study in patients with type 2N and type 3 VWD, in whom FVIII levels are substantially reduced, is to characterize the pharmacokinetics (PK) of efanesoctocog alfa, which should be independent from FVIII binding capacity and VWF levels in patients with type 2N and type 3 VWD. This study was also conducted to assess the safety and tolerability of efanesoctocog alfa in these populations.

A. Study Design and Population

[0124] The object of the present study was to assess the safety, tolerability, and FVIII activity (e.g. PK) of a single dose of efanesoctocog alfa. After screening for up to 28 days, each participant was administered a single 25 IU/kg dose of efanesoctocog alfa. This was followed by a safety observation period, which included PK sampling up to 10 days post-dose, a visit at day 15 post-

dose, and the end of study at 28 days post-dose. A graphical representation of the study design is presented as **FIG. 2**.

[0125] The VWD patient population for this study was defined as having reduced levels of functioning VWF, thereby leading to reduced levels of FVIII activity. Participants were assessed as part of the screening criteria and those with endogenous FVIII activity equal to or less than 20% (<20 IU/dL) were included in this study.

[0126] Inclusion Criteria

[0127] 01. The patient population could include adult male and/or female patients between 18 and 65 years of age with type 2N or 3 VWD. 02. Each participant was diagnosed with hereditary type 3 VWD (≤ 3 IU/dL VWF: Ag) or type 2N VWD (FVIII:C/VWF: Ag <0.6 and severely decreased VWF:FVIII:B) as documented in historical medical records; or a documented genotype known to produce VWD type 3 or 2N VWD. 03. Type 3 VWD participants were included if they had a medical history of at least 25 exposure days to VWF-containing coagulation factor concentrates. 04. Contraceptive use by men or women is consistent with local regulations regarding the methods of contraception for those participating in clinical studies. a) Male participants - No contraceptive measures required for this study. b) Female participants - A female participant is eligible to participate if she is not pregnant or breastfeeding, and at least one of the following conditions applies: - Is not a WOCBP, defined as: underwent surgical sterilization OR no menses (amenorrhea) for 12 months without an alternative medical cause. In the absence of 12 months of amenorrhea, status of not WOCBP needs confirmation with more than one FSH measurement (>40 IU/L or mIU/mL), or - Is a WOCBP and using an acceptable contraceptive method namely, double contraception algorithm to be adapted to country specifications; For most European countries: The accepted double contraception methods include the use of intrauterine device or hormonal contraception in addition to one of the following contraceptive options: (1) condom; (2) diaphragm or cervical/vault cap; (3) spermicide], during the intervention period (at a minimum until the last day of the study). A WOCBP must have a negative highly sensitive pregnancy test before the dose administration of study intervention. A serum pregnancy test should be performed at screening with additional pregnancy tests just prior to efanesoctocog alfa administration and at end of study visit. For time points other than screening, serum or urine pregnancy testing may be performed at the discretion of the investigator. The investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy. 05. Type 2N VWD participants are included if the use of desmopressin acetate (1-deamino-8-D-arginine vasopressin) (DDAVP) is deemed insufficient or contraindicated, as assessed by the investigator, or if they have required prior use of VWF- and FVIII- containing coagulation factor concentrates.

[0128] Exclusion Criteria

[0129] Participants were excluded from the study if they met any one of the following criteria:

01. Hereditary or acquired coagulation disorder other than VWD (including qualitative and quantitative platelet disorders, and thrombocytopenia <100,000 cells/uL at Screening). 02. The participant has a FVIII activity level >20 IU/dL (>20%) at Screening. 03. History or presence of a VWF inhibitor or clinical suspicion of a VWF inhibitor. 04. History of a positive FVIII inhibitor test, defined as ≥ 0.6 BU/mL (by Nijmegen-modified Bethesda assay) or a clinical suspicion of a FVIII inhibitor. 05. Positive FVIII inhibitor test, defined as ≥ 0.6 BU/mL, at Screening. 06. History of hypersensitivity or anaphylaxis associated with any FVIII- or VWF-containing product. 07. Current serious bacterial, viral or fungal infection (other than chronic viral hepatitis or HIV). 08. Abnormal renal function, defined as a serum creatinine level >2.0 mg/dL at Screening. 09. Serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5 x upper limit of normal (ULN) at Screening. 10. Serum total bilirubin >3 x ULN at Screening. 11. History of an unprovoked venous thromboembolism, myocardial infarction within 12 months prior to Screening, or occlusive cerebral vascular accident within 12 months prior to Screening. 12. VWD-associated gastrointestinal angiodysplasia related bleeding within 6 months prior to Baseline. 13. HIV-positive with an absolute CD4 count <200 cells/mm³ or a viral load of >400 copies/mL within 6 months prior to baseline documented in medical records. 14. Previous participation in this study; participants who fail Screening may re-screen. 15. Presence of clinically relevant disease or any condition that, in the opinion of the investigator or sponsor, would make the participant unsuitable for enrollment. 16. The participant has received or anticipates receiving systemic immunosuppressive or immunomodulatory treatment within 12 weeks prior to baseline. 17. The participant requires the use of acetylsalicylic acid, non-NSAID anti-platelets, and NSAIDs above the maximum dose. 18. Any participant who, in the judgment of the investigator, is likely to be noncompliant during the study, or unable to cooperate because of a language problem or poor mental development. 19. Any participant enrolled in or having participated in any other clinical study involving an investigational medicinal product or in any other type of medical research and is still in the exclusion period according to applicable regulations. 20. Any participant who cannot be contacted in case of emergency. 21. Any participant who is the investigator or any sub-investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in conducting the study, or is an immediate family member of such individuals. 22. The participant is accommodated in an institution because of regulatory or legal order; prisoners or participants who are legally institutionalized. 23. The participant has undergone or is anticipated to undergo major surgery within 30 days of baseline. For the purpose of this Example 1, 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 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). 24. Patients currently on a prophylaxis regimen for the treatment of VWD that, in the investigator's opinion, would preclude participation in the study due to the possible increased risk of bleeding associated with the requirement to withhold prophylaxis during the study.

[0130] For statistical analysis, participants were analyzed according to the intervention received and classified as a member of one or more of the following sub-populations: (1) Safety population, which includes all enrolled participants who receive at least 1 dose of efanesoctocog alfa; (2) Pharmacokinetic population, which includes all enrolled and treated participants (safety population) with adequate blood samples enabling determination of at least one PK parameter; and/or (3) Anti-drug antibody (ADA) population, which includes all enrolled participants treated with efanesoctocog alfa with at least one post-baseline ADA result (positive, negative or inconclusive).

[0131] Primary Objectives and Endpoints

[0132] The primary objective of the study was to characterize the pharmacokinetics (PK) of efanesoctocog alfa after a single intravenous (IV) administration, as assessed by FVIII activity determined by the 1-stage activated partial thromboplastin time (aPPT) clotting assay, as well as the efanesoctocog alfa capture chromogenic Coatest FVIII activity assay. Details regarding these assays are provided elsewhere herein.

[0133] Primary endpoints were PK parameters, assessed as FVIII activity by two assay methods, which includes but not limited to the following: maximum activity (C_{max}); terminal half-life (t_{1/2z}); clearance (CL); volume of distribution at steady state (V_{ss}); area under the activity time curve extrapolated to infinity (AUC_∞); mean residence time (MRT); and incremental recovery (IR). A list of pharmacokinetic parameters and definitions is provided in **Table 3**.

[0134] The secondary objective of the study was to assess the safety and tolerability of a single IV dose of efanesoctocog alfa in adults with type 2N and 3 VWD.

[0135] Secondary endpoints were the occurrence of adverse events (AEs) and serious adverse events (SAEs); 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.

[0136] The exploratory objective was to perform additional assessments of immunogenicity. Exploratory endpoints were the occurrence of anti-drug antibodies (ADAs).

B. Dosing

[0137] Participants received a single IV dose of efanesoctocog alfa, with an injection duration of 8 ± 2 minutes. The dose of drug per administration was 25 IU/Kg. A single dose of efanesoctocog alfa at 25 IU/kg was selected to provide a robust PK profile while maintaining FVIII activity levels within normal physiological range. Administration occurred during the morning.

[0138] Formulation

[0139] Drug product was supplied as a lyophilized powder in a sterile vial requiring reconstitution with Sterile Water for Injection (diluent). For this study, each vial of drug product includes 1000 IU of efanesoctocog alfa along with excipients comprising L-histidine, L-arginine hydrochloride, sucrose, calcium chloride dihydrate, and polysorbate 80.

[0140] **Safety Assessment**

[0141] Drug product tolerability investigations at baseline and during the study are presented in Table 1.2. Non-limiting examples of these investigations include the following:

[0142] 1. Physical examination: assessment of the cardiovascular, respiratory, gastrointestinal, neurological, dermatological, and musculoskeletal systems.

[0143] 2. Vital signs: blood pressure, pulse rate, respiratory rate, and body temperature ($^{\circ}\text{C}$).

[0144] 3. Laboratory tests: a list of laboratory assessments is provided in **Table 1**.

[0145] 4. Serology tests: Hepatitis B antigen, Hepatitis C antibodies, anti-HIV1 and anti-HIV2 antibodies.

[0146] 5. β -HCG or FSH measurement (if applicable, for female participants only)

[0147] 6. Inhibitor development: Blood samples were collected per the schedule in **Table 1.2** for the detection of inhibitors. Samples were also collected at the time of any clinical event deemed relevant to inhibitor testing. Inhibitor development was assessed at a central laboratory by the Nijmegen-modified Bethesda assay and is defined as an initial test result of ≥ 0.6 BU/mL confirmed by a second test result from an independent blood sample collected within 2 to 4 weeks of the first positive sample. Initial samples (not for confirmation) were obtained at Screening, Day 1 (pre-dose), Day 15, and Day 29.

7. Anti-drug antibodies (ADA): Blood samples will be collected per the schedule in Section 1.2 for the detection and analysis of anti-efanesoctocog alfa antibodies. Samples may also be collected at the time of any clinical event deemed relevant to anti-efanesoctocog alfa antibody testing. Testing for potential antibody formation will be performed at a central laboratory using a validated

efanesoctocog alfa-specific ADA assay. Confirmed positive samples will be further characterized for antibodies specific to FVIII, Fc, ELNN (XTEN), or D'D3.

Table 1: Laboratory assessments

Hematology	Clinical chemistry	Von Willebrand (VWF) comprehensive panel
Platelet count	BUN	VWF ristocetin cofactor activity
RBC count	Creatinine	VWF antigen
Hemoglobin	Glucose (non-fasting)	
Hematocrit	Potassium	
WBC count with differential:	Sodium	
Neutrophils	Chloride	
Lymphocytes	AST	
Monocytes	ALT	
Eosinophils	ALP	
Basophils	GGT	
	Total and direct bilirubin	
	Total protein	
Immunogenicity	Coagulation parameters	Other screening test
Nijmegen-modified Bethesda inhibitor assay ADA ADA subtype (FVIII, Fc, D'D3 or XTEN)	FVIII activity	HIV (HIV-1 antibodies, HIV-2 antibodies, and HIV-1 p24 antigen), HBV (HBV surface antigen, anti-HBV surface antibody and anti-HBV core antibody) and HCV (anti-HCV antibodies) for participants who have been historically negative
		β-HCG or FSH

[0148] All tests and the schedule of assessments over the course of the study are presented in **Table 1.2.**

[0149] Concomitant Medication: The use of concomitant medication should not be allowed during the study unless specified in the inclusion criteria or study procedures or medically required. However, if a specific treatment is required for any reason, an accurate record must be entered in the eCRF, including the name of the medication (INN), daily dosage, and duration of use. Permitted concomitant therapy: Participants taking medication routinely for a pre-existing condition should be on a treatment regimen that has been stable for at least 3 weeks prior to enrollment, and dosage changes should not be anticipated during the observation period for this study. Pre-study stable NSAID doses below the maximum dose specified in the local prescribing information at the time of enrollment are permitted. All concurrent prescription and nonprescription medications including over-the counter and alternative preparations (including herbal remedies,

vitamins, and health food supplements) should be recorded at Screening and throughout the treatment and follow-up periods.

[0150] Prohibited concomitant therapy: No premedication for pain or pyrexia relief is to be given for administration of efanesoctocog alfa. Should pre-medications be contemplated, this will be discussed on a case-by-case basis with the Sanofi Study Medical Monitor or designee and before administration.

[0151] Medications prohibited during the study include: 1. Acetylsalicylic acid (ASA) or non-NSAID anti-platelet therapies 2. NSAIDs at doses above the maximum dose specified in local prescribing information 3. Systemic treatment with chemotherapy and/or other immunosuppressive drugs (except for the treatment of Hepatitis C virus [HCV] or HIV). The use of systemic steroids for the treatment of acute respiratory illness (eg, asthma), acute allergic episodes, or otherwise life-threatening episodes is allowed. Treatment in these circumstances should not exceed a 14-day duration. Local, topical, and/or inhaled steroid use is permitted. 4. Any other FVIII- or VWF-containing concentrate or DDAVP from at least 96 hours prior to BIVV001 dosing until blood samples for Day 15 laboratory assessments are collected. 5. Anticoagulant agents.

[0152] Adverse Events

[0153] Adverse events (AEs), reported by the participant or observed by the investigator, will be recorded. In this study, AEs were collected starting from signature of the informed consent form (ICF) through the end of the 28-day safety observation period. Laboratory assessments related to safety were collected as outlined in Table 1.2 and include evaluation for inhibitor development via a central laboratory using the Nijmegen-Modified Bethesda Assay at 14 and 28 days following administration of efanesoctocog alfa. The 14- and 28-day post-dose timepoints were selected based on published information on immunological responses (Shankar et al. AAPS J. 2014;16(4):658-73).

[0154] In this study, an adverse event (AE) is defined as any untoward medical occurrence in a participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a pharmaceutical product, whether or not related to the pharmaceutical product. Determination of whether an abnormal laboratory value and/or vital sign result meets the definition of an AE will be made by the investigator. Abnormal results are not considered AEs unless one or more of the following criteria are met: (1) symptomatic, (2) requiring either corrective treatment

or consultation, (3) leading to IMP discontinuation or modification of dosing, (4) fulfilling a seriousness criterion, and/or (5) defined as an adverse event of special interest (AESI).

[0155] Bleeding episodes in this patient population were not considered AEs; however, the concomitant events associated with a bleeding episode were reported as AEs as appropriate (e.g., an elbow fracture). Bleeding episodes that meet the criteria for a serious adverse event (SAE) were be reported as a SAE as appropriate.

[0156] In this study, a serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose results in death, or is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect, or is a medically important event.

[0157] In this study, an adverse event of special interest (AESI) is defined as an adverse event (serious or nonserious) of scientific and medical concern, specific to the IMP or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate. AESI may require further investigation in order to characterize and understand them. Examples of AESI include development of an inhibitor as defined herein and a Grade 3 or higher allergic reaction.

Table 1.2: Study Period Flow Chart and Schedule

		PK sampling and safety observation period																
		PK sampling																
Visit	Screening visits	Visit 1								Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10 EOS
Day	D -28 to D -1	D1								D2	D3	D4	D5	D6	D8	D11	D15	D29
Time in relation to dosing (hour/minute)	Screening	Pre-dose	Dosing	10 min	30 min	1H	3H	6H	9H	24H	48H	72H	96H	120H	168H	240H	336H	672H
Descriptive time window for blood sampling				±2 min	±5 min	±10 min	±10 min	±10 min	±10 min	±1 hr	±2 hr	±2 hr	±2 hr	±2 hr	±2 hr	±2 hr	±1 day	±1 day
Informed consent ^{1a}	X																	
Visits to clinical sites ^{1b}	X	X								X	X	X	X	X	X	X	X	X
Inclusion/ Exclusion criteria	X	X ²																
Demographics ^{2d}	X	X ²																
Medical/ Surgical history	X																	
Washout ²	X	X																
HBV, HCV, HIV status ³	X																	
FVIII activity (1-stage clotting assay) ^{4a}	X																	

		PK sampling and safety observation period														
Visit	Screening visits	PK sampling														
		Visit 1								Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Day	D -28 to D -1	D1								D2	D3	D4	D5	D6	D8	D11
Time in relation to dosing (hour/minute)	Screening	Pre-dose	Dosing	10 min	30 min	1H	3H	6H	9H	24H	48H	72H	96H	120H	168H	240H
Prior and concomitant medications	<		-	-	-	-	-	-	-	-	-	-	-	-	-	>
Inclusion		X														
Study Intervention administration																
Administration: EMVAGGIN 25 IU/kg ²			X													
Safety																
Physical examination ¹	X	X								X						X
Vital signs ²	X	X		X	X					X						X
Haematology/ clinical chemistry ^{3a}	X	X														X
Pregnancy test (if applicable) ⁴	X	X														X
Serum FSH (if applicable) ⁵	X															

		PK sampling and safety observation period																
		PK sampling																
Visit	Screening visits	Visit 1								Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10 EOS
Day	D -28 to D -1	D1								D2	D3	D4	D5	D6	D8	D11	D15	D29
Time in relation to dosing (hour/minute)	Screening	Pre-dose	Dosing	10 min	30 min	1H	3H	6H	9H	24H	48H	72H	96H	120H	168H	240H	336H	672H
Nijmegen-modified Bethesda FVIII inhibitor assay ^a	X	X															X	X
Anti rFVIII:VWF:XTEN antibody ^a	X	X															X	X
von Willebrand comprehensive panel ^a	X	X															X	X
Adverse event collection ^a	<		-	-	-	-	-		-	-	-	-	-	-	-	-		>
Pharmacokinetics																		
PK: FVIII activity (1-stage clotting assay and capture chromatogenic assay) ^a		P00		P01	P02	P03	P04	P06	P08	P07	P08	P09	P10	P11	P12	P13		

a Informed consent from the participant MUST be obtained prior to any study-related procedures, including washout of current therapy specifically for entry into the study.

b Visits at the site at screening (may have more than one visit to complete screening activities), D1 for predose activities and dosing, PK sampling as depicted, D15 for safety, and D29 for EOS activities.

Unscheduled visit may occur at the discretion of the investigator.

c Re-confirm inclusion and exclusion criteria prior to inclusion/enrollment.

d Demographics include Age (years), Height (cm), Body weight (kg), Body mass index (Kg/m²), Race/ethnicity, Gender, Date of birth (year only), as permitted by local regulations.

e Body weight only

f Washout of VWF- or FVIII-containing concentrates (eg, Vonvendi [rVWF] or Humate P. [VWF/FVIII complex]) and prohibited use of DDAVP for at least 96 h prior to Screening labs and/or prior to BIVV001 dosing.

g For participants who have been historically considered negative, assess HIV, HBV, and HCV status.

h Measured to assess eligibility.

i BIVV001 will be administered IV, with an injection duration of 8 ±2 minutes.

j Hematology parameters include red blood cell (RBC) count, white blood cell (WBC) count and differential, platelet count, hemoglobin (Hgb), and hematocrit (Hct). Samples will be obtained at screening,

Day 1 (pre-dose), Day 15, and Day 29.

m Clinical chemistry parameters include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), bilirubin, blood urea nitrogen (BUN), creatinine, glucose, total protein, sodium, potassium, and chloride; Samples will be obtained at screening, Day 1 (pre-dose prior to dosing), Day 15, and Day 29.

n A woman of child-bearing potential (WOCBP) must have a negative highly sensitive pregnancy test before the dose administration; a serum pregnancy test should be performed at screening. For all other time points urine beta-HCG test is appropriate to use in the office, unless the investigator selects another highly sensitive test. Pregnancy test will also be obtained at D29. The participant must be excluded from further participation if the pregnancy result at screening or pre-dose is positive.

o Serum FSH is to confirm that a female participant is not WOCBP [defined as: participant underwent surgical sterilization OR no menses (amenorrhea) for 12 months without an alternative medical cause] and therefore, does not require contraception or pregnancy testing (see inclusion criterion 5). In the absence of 12 months of amenorrhea, status of not WOCBP needs confirmation with more than one FSH measurement (>40 IU/L or mIU/mL).

p All inhibitor assays, including the assay for the Screening Visit and any confirmatory assays, will be performed by the central laboratory using the Nijmegen-modified Bethesda assay. If a Nijmegen-modified Bethesda assay result returns as ≥0.6 BU/mL, a separate sample must be collected and tested for confirmation of inhibitor development within 2 to 4 weeks of the original sample; initial samples (not for confirmation) will be obtained at Screening, Day 1 (pre-dose), Day 15, and Day 29.

q Testing for potential antibody formation will be performed at a central laboratory using a validated rFVIII-Fc-VWF-ELNN-specific ADA assay. Confirmed positive samples will be further characterized for antibodies specific to Fc, FVIII, D'D3, or ELNN; samples will be obtained at Screening, Day 1 (pre-dose), Day 15, and Day 29.

r Only for type 2N participants: von Willebrand comprehensive panel (which includes assessments of von Willebrand Factor [VWF] ristocetin cofactor activity, and VWF antigen). Samples will be obtained at screening, Day 1 (pre-dose), Day 15, and Day 29.

s Adverse events and SAEs occurring after signing of the ICF and until the End of Study visit will be recorded in the eCRF.

t BIVV001 dose will be administered via slow, manual IV injections over a duration of 8 ± 2 minutes. Samples will be collected for 2 bioanalytical assays: (1) 1-stage activated partial thromboplastin time (aPTT)-based FVIII activity assay and (2) BIVV001 capture chromogenic Coatest FVIII activity assay; PK samples will be collected according to scheme as described here. The predose sample should be taken within 30 minutes prior to the BIVV001 injection. PK sampling time points are measured relative to the start of injection. Infusion start time and end time should be recorded in eCRF.

Abbreviations: ADA: Antidrug antibodies; AE: Adverse event; D: Day; DDAVP: Desmopressin acetate (1-deamino-8-D-arginine vasopressin); eCRF: electronic Case Report Form; EOS: End of study; FSH: Follicle stimulating Hormone; FVIII: Blood clotting factor III; HCG: Human chorionic gonadotropin; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; IMP: Investigational medicinal product; IV: Intravenous; SAEs: Serious adverse events; WOCBP: Women of childbearing potential.

C. Pharmacokinetic Assessment

[0158] Pharmacokinetic (PK) assessments were conducted on participant samples collected over the course of the study. The sampling times for blood collection were 10 minutes pre-dose, followed by 10 min, 30 min, 1H, 3H, 6H, 9H, 24H, 48H, 72H, 96H, 120H, 168H, 240H, 336H, and 672H post-dose (see Table 1.2). Blood samples were taken at a volume of 3 mL each, and each participant provided 14 total samples for each of the two bioanalytical methods used in the study.

[0159] Two PK bioanalytical methods were used in this study: (1) 1-stage aPTT-based FVIII activity assay and (2) efanesoctocog alfa capture chromogenic Coatest FVIII activity assay. The first assay to measure FVIII activity of efanesoctocog alfa uses a modification of the aPTT using Dade Actin FSL activated PTT reagent (1-stage aPTT) on the BCS XP analyzer (Siemens Healthcare Diagnostics). The assay was validated using plasma standard that has been calibrated against the WHO (World Health Organization) 6th International Standard for FVIII. The 1-stage aPTT assay is able to measure both native and efanesoctocog alfa FVIII activity. The LLOQ of the assay is at 1% of normal FVIII activity (0.010 IU/mL).

[0160] The second method for determining efanesoctocog alfa activity in plasma is a modified chromogenic Coatest FVIII activity assay. In this assay, the analyte (efanesoctocog alfa) is first captured using an anti-ELNN antibody. The activity of captured efanesoctocog alfa is then determined with Coatest FVIII chromogenic assay, which is provided in the form of a chromogenic substrate assay that quantifies the activated FVIII (FVIIIa)-mediated conversion of factor X (FX) to activated FX (FXa) in the presence of trace amounts of thrombin (for FVIII activation) and excess activated factor IX (FIXa), FX, Ca⁺⁺, and phospholipids. Formation of FXa is measured by turnover of a chromogenic FXa peptide substrate. The LLOQ of the assay is at 0.4% of normal FVIII activity (0.004 IU/mL, or 4 mIU/mL). The assay was validated using efanesoctocog alfa self-standard.

[0161] Pharmacokinetic parameters

[0162] PK assessments were based on FVIII activity levels determined by 1-stage clotting assay and capture chromogenic assay performed at all scheduled visits (see Table 1.2). The PK of the single dose of efanesoctocog alfa was determined via the estimation of PK parameters, assessed as FVIII activity by 2 assay methods, which included the parameters shown in Table 3.

Table 3. List of pharmacokinetic parameters and definitions

Parameters	Drug/Analyte	Matrix	Definition/Calculation
C _{max}	BIVV001	Plasma	Maximum plasma concentration observed
T _{1/2z}	BIVV001	Plasma	Terminal half-life associated with the terminal shape (λ_z) determined according to the following equation: $t_{1/2z} = \frac{0.693}{\lambda_z}$ Where λ_z is the slope of the regression line of the terminal phase of the plasma concentration verses time curve, in semi-logarithmic scale. Half-life is calculated by taking the regression of at least 3 points.
CL	BIVV001	Plasma	Total body clearance of a drug from the plasma calculated using the following equation: $CL = \frac{Dose}{AUC}$
V _{ss}	BIVV001	Plasma	Volume of distribution at steady state based on the last observed concentration defined for IV bolus and infusion is $V_{ss} = MRT_{inf} * CL$
AUC _∞	BIVV001	Plasma	Area under the plasma concentration verses time curve extrapolated to infinity according to the following equation: $AUC_{\infty} = AUC_{last} + \frac{C_{last}}{\lambda_z}$ Values with percentage of extrapolation > 30% will not be reported.
MRT _{inf}	BIVV001	Plasma	Mean residence time (MRT) extrapolated to infinity $MRT_{inf} = \frac{AUMC_{INF}}{AUC_{INF}} - \frac{T1}{2}$ Where T1 represents infusion duration.
IR	BIVV001	Plasma	Incremental recovery of the drug from the plasma calculated using the following equation: $IR = \frac{[C_{max} - PreDose]}{Dose}$

Pharmacokinetic Results

[0163] Patients with type 2N (Normandy) or type 3 von Willebrand disease (VWD) exhibit substantially reduced FVIII levels. Hence the rationale for the study was to characterize the pharmacokinetics (PK) of efanesoctocog alfa, which should be independent of the FVIII binding capacity of VWF and the patient's VWF levels within these subgroups. In addition, the safety and tolerability of efanesoctocog alfa was assessed. Additional details regarding the study and its design are provided above.

[0164] Participant Disposition

[0165] Six participants were enrolled and completed the study (**Table 4**). Five participants were female (83%), 2 participants had Type 2N VWD (33%), and 4 participants had Type 3 VWD (67%). In the 2 participants with Type 2N VWD, VWF:Ag levels at baseline were 38% and 159%, with VWF ristocetin cofactor (VWF:RCO) activity being 15% and 145%, respectively. Individuals with Type 2N VWD had FVIII activity levels of approximately 3% and 7% by OSA; and the FVIII activity was approximately 1%, 6%, 7% and 15% by OSA among those with Type 3 disease. Participants had a median (range) age of 47 (38–55) years and at diagnosis of VWD had a median (range) age of 7 (1–41) years. Fifty percent of participants (n=3) had a history of joint bleeding and no participant had a history of bleeding related to gastrointestinal angiodysplasia. Participants had a median (range) of 3 (0–4) bleeding episodes in the 12 months prior to screening. Four participants (67%) with Type 3 VWD had previously received prophylaxis against bleeding. The most common medical conditions reported among participants were hepatitis C (n=5; 83%), tooth avulsion (n=4; 67%), and epistaxis (n=3; 50%); all other medical histories were reported for ≤2 participants (33%) each.

Table 4. Participant demographics and baseline characteristics

	Efanesoctocog alfa 25 IU/kg (N=6)
Age (years)	
Mean (SD)	46.5 (6.6)
Median (range)	47 (38–55)
Sex, n (%)	
Male	1 (16.7)
Female	5 (83.3)
Ethnicity, n (%)	
Hispanic or Latino	2 (33.3)
Not Hispanic or Latino	4 (66.7)
Baseline weight (kg)^a	
Mean (SD)	90.4 (18.3)
Median (range)	87.0 (73.0–117.3)
Baseline BMI (kg/m²)^a, n (%)	
25 to <30	2 (33.3)
≥30	4 (66.7)
Type of VWD, n (%)	
Type 2N	2 (33.3)
Type 3	4 (66.7)
Genotype	
Type 3	
Intron 2, C.55+1G>T homozygote	1 (16.7)
Exon 46, C.7761_7762INS11 (V2588EFSX12) heterozygote + unknown mutation	1 (16.7)
P.Leu414AlaFsX15	1 (16.7)
C.6236C>G (P.Ser2079X)	1 (16.7)
Type 2N	
C.2446C>T homozygote (R816W)	1 (16.7)
C.1071C>A (Y357X)+C.3178T>C (1060R)	1 (16.7)
Age at diagnosis of VWD (years)	

Mean (SD)	12.8 (15.3)
Median (range)	7 (1–41)
Total number of bleeding episodes in the 12 months prior to screening	
Mean (SD)	2.3 (1.9)
Median (range)	3 (0–4)
History of bleeding related to gastrointestinal dysplasia, n (%)	
Yes	0
No	6 (100)
History of joint bleeding, n (%)	
Yes	3 (50.0)
No	3 (50.0)
History of prophylaxis against bleeding episodes, n (%)	
Yes	4 (66.7)
No	2 (33.3)

BMI, body mass index; SD, standard deviation; VWD, von Willebrand disease.

^aWeight and BMI at baseline defined as the last available assessment before efanesoctocog alfa administration on Day 1.

[0166] Five participants (83%) received ≥ 1 concomitant medication, other than bleeding medications, during the study (**Table 5**). Half of the participants (n=3) received concomitant analgesic medication; all other concomitant medication was reported for ≤ 2 participants (33%) each. Three participants received concomitant bleeding medication during the study (not during the proscribed PK period), including tranexamic acid (n=2), VWF (n=1), and vonicog alfa (n=1) (**Table 5**).

Table 5. Concomitant medications^a

	Efanesoctocog alfa 25 IU/kg (N=6)
Standardised medication name, n (%)	
Any concomitant bleeding therapies medications	3 (50.0)
Tranexamic acid	2 (33.3)
von Willebrand factor	1 (16.7)
Vonicog alfa	1 (16.7)
Anatomic class and therapeutic class, n (%)	
Any other concomitant medications	5 (83.3)
Nervous system	3 (50.0)
Analgesics	3 (50.0)
Antiepileptics	1 (16.7)
Psychoanaleptics	1 (16.7)

Psycholeptics	1 (16.7)
Dermatologicals	1 (16.7)
Antipruritics, including antihistamines, anaesthetics, etc.	1 (16.7)
Other dermatological preparations	1 (16.7)
Genitourinary system and sex hormones	2 (33.3)
Sex hormones and modulators of the genital system	2 (33.3)
Other gynaecologicals	1 (16.7)
Alimentary tract and metabolism	1 (16.7)
Drugs for acid-related disorders	1 (16.7)
Anti-infectives for systemic use	1 (16.7)
Vaccines	1 (16.7)
Cardiovascular system	1 (16.7)
Beta blocking agents	1 (16.7)
Musculoskeletal system	1 (16.7)
Muscle relaxants	1 (16.7)
Respiratory system	1 (16.7)
Antihistamines for systemic use	1 (16.7)
Nasal preparations	1 (16.7)
Sensory organs	1 (16.7)
Ophthalmologicals	1 (16.7)

TEAE, treatment-emergent adverse event.

^aConcomitant medications are those the participant used at any time during the TEAE period (from efanesoctocog alfa administration up to and including the end of study visit).

[0167] Pharmacokinetic Results

[0168] Plasma FVIII activity was measured by 2 bioanalytical methods. The first method (1) was a 1-stage aPTT-based FVIII activity assay using Actin-FSL reagent (referred to as '1-stage clotting assay'). The 1-stage aPTT assay was calibrated using World Health Organization (WHO) 6th international FVIII standard and its lower limit of quantification (LLOQ) value was 1% FVIII activity (1 IU/dL). The second method (2) was an efanesoctocog alfa capture chromogenic Coatest FVIII activity assay (a non-limiting example of a capture chromogenic assay). In the capture chromogenic assay, an anti-ELNN antibody was used to capture efanesoctocog alfa and the

activity of the captured efanesoctocog alfa was then tested using a chromogenic assay. The anti-ELNN antibody comprised two heavy chains comprising the amino acid sequence of SEQ ID NO:31 and two light chains comprising the amino acid sequence of SEQ ID NO:36. The 1-stage aPTT assay measures the activity of both endogenous FVIII and efanesoctocog alfa, while the capture chromogenic assay measures only efanesoctocog alfa activity.

[0169] As the capture chromogenic assay could not detect WHO plasma FVIII standard, it was calibrated using efanesoctocog alfa (i.e., self or product specific standard). The LLOQ of capture chromogenic assay was 0.4% (0.4 IU/dL) FVIII activity. Baseline-corrected FVIII activity levels and actual collection time were used to calculate the PK parameters by noncompartmental analysis.

[0170] *Exemplary Capture Chromogenic Assay*

[0171] A description and procedure for the capture chromogenic assay used in this Example 1 is as follows:

[0172] This is a chromogenic assay where the analyte (efanesoctocog alfa) is captured using an antibody. A biotinylated anti-ELNN antibody is coated onto a microplate. Samples containing efanesoctocog alfa are added to the precoated microwells. After washing away unbound substances, the activity of captured efanesoctocog alfa is determined with coatest factor VIII activity assay. Briefly, the factor VIII activity assay is a chromogenic assay where factor Xa generation is measured. Factor X is activated to factor Xa by factor IXa in the presence of calcium and phospholipids. This reaction is greatly stimulated by factor VIII. By using optimal amounts of Ca²⁺ and phospholipids and an excess of factors IXa and X, the rate of activation of factor X to factor Xa is solely dependent on the amount of factor VIII. Thus, Factor Xa generation is proportional to FVIII activity. Factor Xa generation is measured using chromogenic substrate S-2765, which when hydrolyzed by Factor Xa liberates chromophoric group, pNA, that is measured photometrically at 405 nm.

[0173] Abbreviations:

BSA: Bovine Serum Albumin

CV: Coefficient of Variance

HPC: High Positive Control

LLOQ: Lower Limit Of Quantification

LPC: Low Positive Control

MPC: Medium Positive Control

PBS: Phosphate Buffered Saline

QC: Quality Control

ULOQ: Upper Limit Of Quantification

[0174] Procedure:

1. Make Capture Antibody Working Solution (Concentration -1.2mg/ml, Final Dilution- 2µg/mL).
-Add 20 µl of biotinylated anti-ELNN antibody to 12ml of 1X PBS, in order to obtain a final concentration of approximately 2µg/ml. Mix by inversion.
2. Wash Neutravidin low bind clear plate with washing buffer.
3. COATING: Add 100µl of capture antibody working solution per well of a Neutravidin low bind clear plate. Seal plate and incubate at ambient room temperature for 45±10 min.
4. Prepare efanesoctocog alfa intermediate Stocks/Standards/QCs as indicated in Reagent Table 1, 2, 3, and 4.
5. Make 10-fold dilution of Standards/QCs/Samples:
-Add 30µl of Standards /QCs/Samples to 270µl of low salt binding buffer. Mix well.
6. Wash plate with washing buffer.
7. Loading: transfer 100µl of the 10-fold diluted Standards/QCs/Samples in duplicate in the coated plate. Seal plate and incubate at ambient room temperature for 35±5 min.
8. During incubation and prior to washing (step 10), prepare 1X Buffer
-Add 0.5 ml of Buffer Stock Solution (10x concentrated) to 4.5 ml of Nerl water.
9. Prepare FIXa/FX/Phospholipids (FFP) solution 5 minutes prior to use
-Add 2 ml of Phospholipid to 10 ml of FIXa/FX mix.
10. Wash plate twice with washing buffer.
11. Add 25µl/well of 1x buffer stock solution.
12. Add 50µl/well of the FIXa/FX/ Phospholipid solution (FFP) and tap the plate to mix. Incubate at 37°C in dry incubator for 5min.
13. Add 25µl/well of CaCl₂, tap the plate to mix. Incubate at 37°C in dry incubator for 5 min.
14. Add 50µl/well of FXa substrate, tap the plate to mix. Incubate at 37°C in dry incubator for 10min.
15. Stopping: Add 25µl/well of 20% acetic acid solution to stop the colorimetric reaction in the well.

[0175] Reagents and Equipment:

- Efanesoctocog alfa (Activity - 4000 IU Vial), received in lyophilized condition shipped at 2-8 °C and stored at -80°C until use. Efanesoctocog alfa was reconstituted in 1ml vehicle solution (10 mM Histidine, 250 mM Arginine HCl, 5% w/v sucrose, 5 mM Calcium Chloride, 0.05% PS80), aliquoted and stored at -80°C.

- Biotinylated Anti-ELNN antibody (Concentration (1.2mg/ml)
- BioTek plate Reader (SN 200701)
- Single channel Rainin Pipettes (LTS20: 2-20µl, LTS200: 20-200µl, and LTS1000: 100-1000µl)
- Multichannel pipettes (12 channel 20-200µl)
- Neutravidin Low Bind Clear Plate (Thermo Scientific, Cat#15129)
- Microtiter tube 1.2ml (Fisher Scientific, Cat#02-681-376)
- Acetic Acid 99.7%+ A.C.S. reagent (Sigma, Cat#242853)
- 1X PBS pH7.4 without Calcium & magnesium
- Sodium Chloride (Sigma, Cat#S9888)
- Tween-20 (Bio-Rad Laboratory, M2595)
- Bovine Serum Albumin (Sigma, Cat#A4503)
- Chromogenix Coatest SP FVIII Kit (Diapharma, Cat# 82408663)
- Pooled Human Normal Plasma: (Precision BioLogic, Cat# CCN-20)

[0176] Reagent Preparation:

[0177] Efanesoctocog alfa: was reconstituted in 1ml vehicle solution (10 mM Histidine, 250 mM Arginine HCl, 5% w/v sucrose, 5 mM Calcium Chloride, 0.05% PS80), aliquoted and stored at -80°C. On the day of assay, one vial of efanesoctocog alfa was thawed quickly in a 37°C water bath.

[0178] Biotinylated Anti-ELNN antibody (Concentration - 1.2mg/ml): The test solution (1 ml per one Eppendorf tube, total 5 vials) was received in dry ice and stored at -80°C upon receipt. In order to prevent multiple freeze-thaw cycles it was quickly thawed in a 37°C water bath, aliquoted to and stored at -80°C until use. On the day of assay, one vial of biotinylated anti-ELNN antibody was thawed quickly in a 37°C water bath. 20 µl of biotinylated anti ELNN antibody was added to 12 ml of 1X PBS in order to obtain a final concentration of approximately 2µg/ml.

[0179] Wash Buffer: 1X PBS, 0.05% Tween-20, pH 7.4

[0180] Low Salt Binding Buffer (1X PBS, 0.05 % Tween-20, pH 7.4, 0.25% BSA, 200mM NaCl)

[0181] 20% Acetic Acid

[0182] FXa substrate: Reconstitute a vial of S-2765 with 12.0 mL of Nerl water.

[0183] Factor IXa + factor X: Reconstitute the vial of Factor IXa + Factor X with 10 ml of cold Nerl water.

[0184] Prepare efanesoctocog alfa intermediate Stocks in normal human citrated plasma as indicated in **Reagent Table 1**.

Reagent Table 1

Stock Identification	To make intermediate stock			Human Plasma Vol (µL)	Target Conc. (mIU/mL)
	Stock ID	Stock Conc. (mIU/mL)	Volume (µL)		
Stock 1	BIVV001	4000000	5	1995	10000
Stock 2	Stock 1	10000	30	570	500
Stock 3	Stock 2	500	50	450	50

[0185] Prepare efanesoctocog Standards (STD) normal human citrated plasma as indicated in **Reagent Table 2**.

Reagent Table 2

Standard Identification	To make Standards (STD) solution			Human Plasma Vol (µL)	Target Conc. (mIU/mL)
	Stock ID	Stock conc. (mIU/mL)	Volume (µL)		
Standard 0	NA	0	0	500	0
Standard 1*	Stock 3	50	20	480	2
Standard 2 (LLOQ)	Stock 3	50	40	460	4
Standard 3	Stock 2	500	10	490	10
Standard 4	Stock 2	500	20	480	20
Standard 5	Stock 2	500	30	470	30
Standard 6	Stock 2	500	44	456	44
Standard 7	Stock 2	500	60	440	60
Standard 8	Stock 2	500	92	408	92
Standard 9	Stock 2	500	120	380	120
Standard 10	Stock 2	500	200	300	200

* accessory standards = they are used to define the lower and higher ends of the calibration curve.

[0186] Prepare efanesoctocog alfa Quality Controls (LPC, MPC, HPC) in human normal plasma as indicated in **Reagent Table 3**.

Reagent Table 3

Quality Controls	To make Quality Controls (QC) solution			Human Plasma Vol (µL)	Target Conc. (mIU/mL)
	Stock ID	Stock conc. (mIU/mL)	Volume (µL)		
LPC	Stock 3	50	120	380	12
MPC	Stock 2	500	25	475	25
HPC	Stock 2	500	50	450	50

[0187] Prepare efanesoctocog alfa Quality Controls (QC) in human FVIII deficient plasma as indicated in **Reagent Table 4**.

Reagent Table 4

QC identification	QC Stock			FVIII (-) Plasma Vol (μL)	Target Conc. (mIU/mL)
	Stock ID	Conc.(mIU/mL)	Vol (μL)		
QC stock1 in F8(-)	BIVV001	4000000	5	1995	10000
QC stock2 in F8(-)	QC stock1 in F8(-)	10000	10	190	500
QC stock3 in F8(-)	QC stock2 in F8(-)	500	50	450	50
LPC in F8(-)	QC stock3 in F8(-)	50	120	380	12
MPC in F8(-)	QC stock2 in F8(-)	500	25	475	25
HPC in F8(-)	QC stock2 in F8(-)	500	50	450	50

[0188] *Plasma baseline-corrected factor VIII activity levels*

[0189] Mean plasma baseline-corrected FVIII activity level-time profiles, quantified by the 1-stage clotting assay and capture chromogenic assay are presented in Table 6.

[0190] Prior to receiving efanesoctocog alfa, mean (range) baseline endogenous FVIII activity level with the OSA was 6.7 IU/dL (1.3 IU/dL–14.6 IU/dL) and was under the LLOQ with the CCS. As quantified by both the OSA and CCS, a single dose of efanesoctocog alfa (25 IU/kg) maintained mean baseline-corrected FVIII activity level >1 IU/dL up to 10 days post-dose (**Figure 3 and Table 6**). Mean baseline-corrected FVIII activities were maintained >40 IU/dL for nearly 3 days post-dose (38.5 IU/dL at 72 hours) and >10 IU/dL up to 5 days post-dose when quantified with the OSA (**Figure 3A and Table 6**). When quantified by the CCS, mean baseline-corrected FVIII activity levels were maintained >40 IU/dL up to 1-day post-dose and >10 IU/dL up to 4 days post-dose (**Figure 3B and Table 6**).

Table 6. Mean plasma baseline-corrected FVIII activity levels over time quantified by the one-stage clotting assay or capture chromogenic assay following a single dose of efanesoctocog alfa (25 IU/kg)

Nominal time post-dose (hour)	One-stage assay (N=6)	Capture chromogenic assay (N=6)
0	<LLOQ	<LLOQ

0.17	90.8 ^a	57.3 ^a
0.5	89.4 ^a	55.8 ^a
1	97.6	57.0
3	101.0	60.6
6	97.2 ^b	59.5 ^b
9	87.7 ^a	53.6 ^a
24	74.5	43.3
48	54.8	27.1
72	38.5	16.1
96	25.1	11.9
120	14.2	7.53
168	6.00	3.78
240	1.82	1.45

FVIII, factor VIII; LLOQ, lower limit of quantitation.

^an=5. ^bn=4.

[0191] As quantified by the 1-stage clotting assay, efanesoctocog alfa maintained a mean baseline-corrected FVIII activity level of >40 IU/dL (normal to near normal level) up to nearly 72 hours (3 days) postdose (38.5 IU/dL at 72 hours), >10 IU/dL up to 120 hours (5 days) postdose, and >1 IU/dL up to the last sampling time point of 240 hours (10 days) postdose.

[0192] As quantified by the capture chromogenic assay, efanesoctocog alfa maintained a mean baseline-corrected FVIII activity level of >40 IU/dL (normal to near normal level) up to 24 hours (1 day) postdose, >10 IU/dL up to 96 hours (4 days) postdose, and >1 IU/dL up to the last sampling time point of 240 hours (10 days) postdose.

[0193] *Pharmacokinetic parameters for baseline-corrected factor VIII activity*

[0194] A summary of plasma PK parameters for baseline-corrected FVIII activity is presented in **Table 7**.

[0195] Following a single dose of efanesoctocog alfa (25 IU/kg) the median (range) time to maximum (t_{max}) of the baseline-corrected FVIII activity level was 1.09 (0.17–2.95) hours and 2.96 (0.50–6.00) hours, for the OSA and CCS, respectively (**Table 7**). Mean (SD) t_{1/2z} values were 39.1 (8.70) hours and 49.0 (10.2) hours, for the OSA and CCS, respectively. For the OSA, mean (SD) C_{max}, AUC, and IR were 111 (30.9) IU/dL, 6910 (1550) IU·h/dL, and 4.41 (1.24)

(IU/dL)/(IU/kg), respectively. Corresponding values for the CCS were 63.8 (14.8) IU/dL, 3730 (419) IU·h/dL, and 2.55 (0.596) (IU/dL)/(IU/kg). Between-participant variation in IR, $C_{\max}$, and AUC were low, with coefficient of variation values ranging from 11% to 28%.

Table 7: Mean $\pm$ SD (geometric mean) [CV%] pharmacokinetic parameters following a 25 IU/kg dose of efanesoctocog alfa in participants with type 2N or 3 von Willebrand disease

PK Parameters	1-Stage Clotting Assay	Capture Chromogenic Assay
N	6	6
$C_{\max}$ (IU/dL)	111 $\pm$ 30.9 (107) [28]	63.8 $\pm$ 14.8 (62.5) [23]
$t_{\max}^a$ (h)	1.09 (0.17, 2.95)	2.96 (0.50, 6.00)
IR ((IU/dL)/(IU/kg))	4.41 $\pm$ 1.24 (4.27) [28]	2.55 $\pm$ 0.596 (2.49) [23]
AUC _{last} (IU h/dL)	6620 $\pm$ 1630 (6440) [25]	3620 $\pm$ 387 (3600) [11]
AUC (IU h/dL)	6910 $\pm$ 1550 (6760) [22]	3730 $\pm$ 419 (3710) [11]
$t_{1/2z}$ (h)	39.1 $\pm$ 8.70 (38.2) [22]	49.0 $\pm$ 10.2 (48.3) [21]
MRT _{inf} (h)	59.9 $\pm$ 11.7 (59.0) [20]	61.3 $\pm$ 9.74 (60.6) [16]
CL (mL/(h·kg))	0.380 $\pm$ 0.0910 (0.371) [24]	0.681 $\pm$ 0.0926 (0.677) [14]
V_{ss} (mL/kg)	22.1 $\pm$ 3.50 (21.9) [16]	41.2 $\pm$ 6.78 (41.0) [16]

[CV] = Coefficient of variation; a = Median (Min, Max); AUC, area under the activity time curve; CL, clearance; $C_{\max}$, maximum activity; CV%, coefficient of variation; h, hour; IR, incremental recovery; MRT, mean residence time; PK, pharmacokinetic; SD, standard deviation; $t_{1/2z}$, terminal half-life; $t_{\max}$, time to $C_{\max}$; V_{ss} , volume of distribution at steady state; VWD, von Willebrand disease.

[0196] Summary – Pharmacokinetic Results

[0197] This Phase 1 study of participants with Type 2N and Type 3 VWD demonstrated that a single dose of efanesoctocog alfa (25 IU/kg) maintains FVIII activity levels >40 IU/dL (normal to near-normal levels) for nearly 3 days post-dose when assessed with the 1-stage clotting assay, and >40 IU/dL for up to 1-day post-dose when assessed by the capture chromogenic assay. Mean half-life values for baseline-corrected FVIII activity quantified by the 1-stage clotting assay and capture chromogenic assay were 39.1 and 49.0 hours, respectively.

[0198] The PK profile obtained using the capture chromogenic assay in this study of participants with Type 2N and Type 3 VWD is comparable to the profile observed in participants with severe haemophilia A in a Phase 1/2a study following a single 25 IU/kg dose.

[0199] In the haemophilia Phase 1/2a study, mean FVIII activity levels assessed by the OSA were 17 IU/dL at 4 days and >1 IU/dL for up to 10 days post-dose. There were no prominent

differences in PK results between patients with type 2N and type 3 VWD, though it should be noted that patient numbers for this comparison were low.

[0200] The data obtained from the 1-stage clotting assay appeared to be higher than those obtained from the capture chromogenic assay. The reason for this discrepancy is not entirely clear, however, in this patient population the capture chromogenic assay results may be most accurate as interference from endogenous FVIII is avoided by use of a self-standard.

Safety and Tolerability Results

[0201] A single dose of efanesoctocog alfa (25 IU/kg) was well-tolerated, inhibitor development to FVIII was not detected, and there were no reports of serious allergic reaction, anaphylaxis, or vascular thrombotic events. Three participants (50%) experienced a total of 8 treatment-emergent AEs (TEAEs) during the study (**Tables 8 and 9**). TEAEs reported included 5 events of headache in 2 participants, and 1 event each of gastrointestinal motility disorder, nausea, and arthropod bite (**Table 9**). All TEAEs were assessed by the investigator as non-serious, mild, and not related to efanesoctocog alfa treatment. Of the 8 TEAEs, 6 recovered/resolved, and 2 (gastrointestinal motility disorder and nausea) did not. No deaths, serious or severe TEAEs, AESIs, TEAEs leading to permanent discontinuation of study intervention or withdrawal from the study were reported (**Table 8**). There were no clinically meaningful patterns identified in clinical laboratory parameters, vital signs, or physical examinations throughout the study. No treatment-emergent ADAs, either treatment-induced or treatment-boosted, were identified. For the 2 participants with Type 2N VWD, 1 participant's VWF levels were within the normal range for all assessments and 1 participant had VWF levels below the normal range at all assessments (including prior to efanesoctocog dosing). Following a single dose of efanesoctocog alfa (25 IU/kg), there was no change in either VWF antigen or activity levels.

Table 8. Overview of treatment-emergent adverse events^a

	Efanesoctocog alfa (25 IU/kg) N=6
Participants with any TEAE, n (%)	3 (50.0)
Participants with any severe TEAE, n (%)	0
Participants with any treatment-emergent SAE, n (%)	0
Participants with any TEAE leading to permanent study discontinuation, n (%)	0
Participants with any treatment-emergent AESI^b, n (%)	0

AESI, adverse event of special interest; TEAE, treatment-emergent adverse event; SAE, serious adverse event.

^aAn AE is considered treatment-emergent if it occurred any time during the TEAE period (from efanesoctocog alfa administration up to and including the end of study visit). ^bAESIs included development of either an inhibitor, a Grade ≥ 3 allergic reaction, or an embolic or thrombotic event (except for injection site thrombophlebitis), among others.

Table 9. Number of participants with TEAE by primary system organ class and preferred term^a

	Efanesoctocog alfa (25 IU/kg) N=6	
	n (%)	Events, n
Any event	3 (50.0)	8
Nervous system disorders	2 (33.3)	5
Headache	2 (33.3)	5
Gastrointestinal disorders	1 (16.7)	2
Gastrointestinal motility disorder	1 (16.7)	1
Nausea	1 (16.7)	1
Injury, poisoning, and procedural complications	1 (16.7)	1
Arthropod bite	1 (16.7)	1

AE, adverse event; TEAE, treatment-emergent adverse event.

^aAn AE is considered treatment-emergent if it occurred any time during the TEAE period (from efanesoctocog alfa administration up to and including the end of study visit).

[0202] Factor VIII Inhibitor Development

[0203] Factor VIII inhibitor development was not detected during this study.

[0204] Antidrug Antibodies

[0205] No treatment-emergent ADA response against efanesoctocog alfa was observed as no participant had treatment-induced or treatment-boosted ADAs.

[0206] Summary – Safety and Tolerability

[0207] In this study, a single dose of efanesoctocog alfa (25 IU/kg) was well-tolerated in VWD patients. No participants developed inhibitors to FVIII or anti-drug antibodies (ADA) and there were no reports of SAEs, serious allergic reactions, anaphylaxis, or thrombotic events. These safety results are also consistent with Phase 1/2a single and repeat dose studies, and Phase 3 XTEND-1 and XTEND-Kids studies in haemophilia A. In these studies of patients with haemophilia A, efanesoctocog alfa was well-tolerated and FVIII inhibitor development was not detected. The results from this study demonstrate that a single dose of efanesoctocog alfa (25 IU/kg) is well-tolerated and maintains high FVIII activity levels for a prolonged period in patients with Type 2N or Type 3 VWD.

Example 2: Characterization of an anti-ELNN antibody.

[0208] Anti-ELNN antibodies are needed. However, the properties of ELNNs minimize the potential for immunogenicity, as their lack of stable tertiary structure disfavors antibody binding and the absence of hydrophobic, aromatic and positively charged residues that serve as anchor residues for peptide MHC II binding reduces the potential for T cell epitopes.

[0209] This example relates to an anti-ELNN antibody (termed the “4D9G3 antibody”), which is a mouse IgG₁ monoclonal antibody with a kappa light chain. The anti-ELNN antibody is produced from the mouse hybridoma clone 4D9G3.

[0210] The 4D9G3 antibody can be recombinantly expressed in host cells (e.g., CHO cells that are well-known in the art) and purified using well known procedures for antibody purification, including but not limited to processes comprising protein A chromatography.

[0211] To map the epitope of the 4D9G3 antibody, 183 unique peptides were designed based on ELNN_AE864 (SEQ ID NO: 26) (1 amino acid offset).

[0212] The 4D9G3 antibody recognizes the amino acid sequence (E)SATPE, which is within one of the four building blocks of ELNN_AE864. ELNN_AE864 contains 23 copies and ELNN_144 contains 3 copies (see FIG. 4). Of all 183 unique peptides, 43 peptides containing the ESATPE epitope showed significant binding while 5 peptides containing only SATPE showed reduced binding. The remaining 135 peptides, which did not contain the epitope, showed very low binding. (FIG. 5).

[0213] Within the 43 peptides containing the epitope ESATPE, there is also variability in binding. By examining the corresponding peptide sequence, it appears that all peptides with less than or equal to 2 amino acids on the C-terminus of the epitope showed inferior signal. On the other hand, if the epitope is on the very N-terminus, binding to the 4D9G3 antibody was not affected (FIG. 6).

[0214] The epitope of the antibody is (E)SATPE.

[0215] Non-limiting uses of the 4D9G3 antibody and antibodies derived therefrom include:

- a. Use as a capture antibody, e.g., in ELISA or in capture chromatography assays
- b. Biotin-4D9G3 can be used as detection antibody in ELISA
- c. ELNNs that contain multiple copies of the epitope can be quantified by ELISA using 4D9G3 for capture and biotin-4D9G3 for detection

Example 3. Transfer of 4D9G3 CDRs into alternative IgG backbones

[0216] CDRs, variable regions, and/or other parts of the 4D9G3 antibody can be moved into different antibody backbones. In a non-limiting example, certain sequences from the 4D9G3 antibody (which is in a mouse IgG1 background) can be moved into a mouse IgG2a backbone. The IgG2a backbone has advantages with respect to protein A purification, as well as potential advantages with respect to stability. In another non-limiting example, sequences of the 4D9G3 antibody were moved into a human IgG1 antibody backbone, which was the antibody used in Example 1. The sequences of this antibody are disclosed elsewhere in the application (see Table C).

VI. Embodiments of the Disclosure

[0217] The present disclosure includes (and is not limited to) the following exemplary embodiments (E):

[0218] E1. A method for determining the level of exogenous Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising

(i) separating the chimeric protein from a biological sample from the subject with an anti-ELNN antibody or antigen-binding fragment thereof to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample;

(ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value; and

(iii) reporting the assayed FVIII activity value or an approximated value thereof without correcting the assayed FVIII activity value or approximated value thereof.

[0219] E2. The method of E1, wherein the chimeric protein was administered to the subject less than one week before the sample was taken from the subject.

[0220] E3. The method of E1 or E2, wherein the biological sample is a blood sample.

[0221] E4. The method of E1 or E2, wherein the biological sample is a plasma sample.

[0222] E5. The method of any one of E1 to E4, wherein correcting the assayed FVIII activity value or approximated value thereof comprises reducing the value.

[0223] E6. The method of any one of E1 to E5, wherein correcting the assayed FVIII activity value or approximated value thereof comprises reducing the value, as if an overestimation of the FVIII activity needs to be accounted for.

[0224] E7. The method of any one of E1 to E6, wherein an activated partial thromboplastin time (aPTT)-based one stage clotting assay is not used to assess the level of FVIII activity.

[0225] E8. The method of any one of E1 to E7, wherein the anti-ELNN antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (LCDR) sequences, wherein LCDR1, LCDR2, and LCDR3 comprises SEQ ID NOs: 38, 39 and 40, respectively, and wherein the anti-ELNN antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining region (HCDR) sequences, wherein HCDR1, HCDR2, and HCDR3 comprises SEQ ID NOs: 32, 33 and 34, respectively.

[0226] E9. The method of any one of E1 to E8, wherein the anti-ELNN antibody or an antigen binding fragment thereof comprises a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37.

[0227] E10. The method of any one of E1 to E9, wherein the anti-ELNN antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising three heavy-chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR).

[0228] E11. The method of any one of E1 to E10, wherein the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:32 and a light chain variable region (LCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:37.

[0229] E12. The method of any one of E1 to E11, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.

[0230] E13. The method of any one of E1 to E12, wherein the antibody comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36.

[0231] E14. The method of any one of E1 to E13, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36.

[0232] E15. The method of any one of E1 to E14, wherein the antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36.

[0233] E16. The method of any one of E1 to E15, wherein the antibody comprises two heavy chains consisting of the amino acid sequence of SEQ ID NO:31, and two light chains consisting of the amino acid sequence of SEQ ID NO:36.

[0234] E17. The method of any one of E1 to E16, wherein the antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37.

[0235] E18. The method of any one of E1 to E8, wherein the anti-ELNN antibody or antigen binding portion thereof comprises (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34.

[0236] E19. The method of any one of E1 to E18, wherein the ELNN polypeptide comprises at least three instances of the sequence: SATPE (SEQ ID NO: 28).

[0237] E20. The method of any one of E1 to E19, wherein the ELNN polypeptide comprises at least 3 instances of the sequence: ESATPE (SEQ ID NO: 29).

[0238] E21. The method of any one of E1 to E20, wherein the ELNN polypeptide comprises at least 4 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0239] E22. The method of any one of E1 to E21, wherein the ELNN polypeptide comprises at least 5 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0240] E23. The method of any one of E1 to E22, and wherein the ELNN polypeptide comprises at least 6 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0241] E24. The method of E21 to E23, wherein each of the at least 3, at least 4, at least 5, or at least 6 instances of SATPE (SEQ ID NO: 28) are as part of the following sequence: ESATPE (SEQ ID NO: 29).

[0242] E25. The method of E21 to E24, wherein each instance of SATPE (SEQ ID NO: 28) and/or ESATPE (SEQ ID NO: 29) in the sequence of the ELNN polypeptide is followed by at least 3 amino acids at the C-terminal end thereof.

[0243] E26. The method of any one of E1 to E25, wherein the ELNN polypeptide comprises the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27.

[0244] E27. The method of any one of E18 to E26, wherein the anti-ELNN antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.

[0245] E28. The method of any one of E18 to E27, wherein the anti-ELNN antibody or binding portion thereof comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31, wherein the anti-ELNN antibody or binding portion thereof comprises a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36.

[0246] E29. The method of any one of E18 to E27, wherein the anti-ELNN antibody or binding portion thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31, and wherein the anti-ELNN antibody or binding portion thereof a light chain comprising the amino acid sequence of SEQ ID NO:36.

[0247] E30. The method of E29, wherein the anti-ELNN antibody or binding portion thereof comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and wherein the anti-ELNN antibody or binding portion thereof comprises two light chains comprising the amino acid sequence of SEQ ID NO:36.

[0248] E31. The method of any one of E1 to E30, wherein the chimeric protein further comprises a von Willebrand Factor (VWF) fragment.

[0249] E32. A method for determining the level of Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises (a) a FVIII protein and (b) an ELNN polypeptide and/or a von Willebrand Factor (VWF) fragment, the method comprising

(i) assaying the level of FVIII activity in the chimeric protein with an activated partial thromboplastin time (aPTT)-based one stage clotting assay to obtain an assayed FVIII activity value, wherein the aPTT-based one stage clotting assay does;

(ii) reducing the assayed FVIII activity value to obtain an adjusted FVIII activity value;
and

(iii) reporting the adjusted FVIII activity value or an approximated value thereof.

[0250] E33. The method of E32, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide.

[0251] E34. The method of any one of E32 to E33, wherein the ELNN polypeptide is inserted into the FVIII protein of the chimeric protein.

[0252] E35. The method of any one of E32 to E34, wherein the FVIII activity value or the approximated value thereof is reported to the subject, a caregiver of the subject, or a medical professional.

[0253] E36. The method of any one of E32 to E35, wherein the FVIII activity value or the approximated value thereof is reported to the subject.

[0254] E37. The method any one of E32 to E36, wherein the subject has a body mass index of ≥ 30 kg/m².

[0255] E38. The method any one of E32 to E37, wherein the subject is a female.

[0256] E39. The method any one of E32 to E37, wherein the subject is a male.

[0257] E40. The method any one of E32 to E39, wherein the subject is human.

[0258] E41. The method any one of E1 to E40, wherein the VWD is Type 2N VWD.

[0259] E42. The method any one of E1 to E40, wherein the VWD is Type 3 VWD.

[0260] E43. A method for determining the level of Clotting Factor VIII (FVIII) activity from a chimeric protein in a biological sample, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising

(i) separating the chimeric protein from the biological sample from the subject with an anti-ELNN antibody to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample; and

(ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value.

[0261] E44. The method of any one of E1 to E43, wherein the chimeric protein is in a pharmaceutical composition comprising sucrose, histidine, arginine, calcium chloride, and polysorbate 80.

[0262] E45. The method of any one of E1 to 44, wherein the chimeric protein is in a pharmaceutical composition comprising:

- (a) about 5% (w/v) sucrose;
 - (b) about 10 mM histidine;
 - (c) about 250 mM arginine;
 - (d) about 5 mM calcium chloride; and
 - (e) about 0.05% polysorbate 80,
- and a pH of about 7.0.

[0263] E46. The method of E50 or E51, wherein the pharmaceutical composition is lyophilized.

[0264] E47. The method of E52, wherein the lyophilized pharmaceutical composition is reconstituted with sterile water prior to administering to the subject.

[0265] E48. The method of any one of E1 to E53, wherein the chimeric protein comprises a VWF fragment.

[0266] E49. The method of E54, wherein the VWF fragment comprises a D'D3 fragment of VWF.

[0267] E50. The method of E55, wherein the VWF fragment consists of a D'D3 fragment of VWF.

[0268] E51. The method of any one of E55 to E56, wherein the D'D3 fragment of VWF is mutated such that it cannot form disulfide binds with another D'D3 fragment of VWF.

[0269] E52. The method of any one of E55 to E57, wherein the D' domain of the D'D3 fragment of VWF comprises the sequence of SEQ ID NO: 21.

[0270] E53. The method of any one of E55 to E58, wherein the D3 domain of the D'D3 fragment of VWF comprises the sequence of SEQ ID NO: 23.

[0271] E54. The method of any one of E1 to E59, wherein the FVIII protein comprises at least a partial deletion of the wild-type FVIII B domain.

[0272] E55. The method of any one of E1 to E60, wherein the chimeric protein comprises an ELNN polypeptide comprising the sequence of SEQ ID NO: 14.

[0273] E56. The method of any one of E1 to E60, wherein the chimeric protein comprises an ELNN polypeptide comprising the sequence of SEQ ID NO: 24.

[0274] E57. The method of any one of E1 to E62, wherein the chimeric protein comprises a first polypeptide which comprises a FVIII protein and a first immunoglobulin ("Ig") constant region or a portion thereof, and a second polypeptide which comprises a VWF fragment and a second Ig constant region or a portion thereof.

[0275] E58. The method of E63, wherein the chimeric protein comprises (i) a first polypeptide comprising a FVIII protein, an ELNN polypeptide inserted within and replacing a portion of the B domain, and a first Fc region; and (ii) a second polypeptide comprising a VWF fragment, an additional ELNN polypeptide, an a2 linker, and a second Fc region.

[0276] E59. The method of E64, wherein the chimeric protein is a FVIII-ELNN-Fc/D'D3-ELNN-Fc heterodimer.

[0277] E60. The method of any one of E63 to E65, wherein the first polypeptide chain comprises the amino acid sequence set forth as SEQ ID NO: 1 and the second polypeptide chain comprises the amino acid sequence set forth as SEQ ID NO: 2, and wherein the first polypeptide chain and the second polypeptide chain are covalently linked by two disulfide bonds between the Ig constant region domains of the first and second polypeptide chains.

[0278] E61. The method of any one of E1 to E66, wherein the chimeric protein is efanesoctocog alfa.

[0279] E62. A method of monitoring the level of FVIII in the blood or plasma of a human subject who is receiving treatment for Willebrand disease (VWD), the method comprising performing the method of any one of E1 to E42 or E49 to E67 at least once per year.

[0280] E63. The method of E43, comprising performing the method of any one of E1 to E42 at least once every six months.

[0281] E64. The method of E44, comprising performing the method of any one of E1 to E42 at least once per month.

[0282] E65. The method of any one of E1 to E45, wherein multiple doses of the chimeric protein are administered to the subject at a dosing interval.

[0283] E66. The method of E46, wherein at least one of the multiple doses is from about 10 IU/kg to about 30 IU/kg and the dosing interval is at least about 7 days.

[0284] E67. The method of E46, wherein at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 7 days.

[0285] E68. An antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, wherein the antibody or antigen-binding fragment thereof comprises three light chain

complementarity determining region (CDR) sequences of SEQ ID NOs: 38, 39 and 40, and three heavy chain complementarity determining region (CDR) sequences of SEQ ID NOs: 32, 33 and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0286] E69. An antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising three heavy-chain CDRs of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and three light-chain CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37 and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0287] E70. An antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region comprising three heavy-chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR), and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).

[0288] E71. The antibody or antigen-binding fragment of any one of claims 68 to 70, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:32 and a light chain variable region (LCVR) comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:37.

[0289] E72. The antibody or antigen-binding fragment of any one of claims 68 to 71, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.

[0290] E73. The antibody or antigen-binding fragment of any one of E68 to E72, wherein the antibody comprises a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36.

[0291] E74. The antibody or antigen-binding fragment of any one of E68 to E73, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36.

[0292] E75. The antibody of any one of E68 to E74, wherein the antibody comprises two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36.

[0293] E76. The antibody of any one of E68 to E75, wherein the antibody comprises two heavy chains consisting of the amino acid sequence of SEQ ID NO:31, and two light chains consisting of the amino acid sequence of SEQ ID NO:36.

[0294] E77. A monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0295] E78. An antigen binding protein comprising (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0296] E79. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E78, wherein the ELNN polypeptide comprises the sequence: ESATPE (SEQ ID NO: 29).

[0297] E80. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E79, wherein each of the instances of the sequence SATPE (SEQ ID NO: 28) is ESATPE (SEQ ID NO: 29).

[0298] E81. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E79, wherein the ELNN polypeptide comprises at least 4 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0299] E82. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E79, and wherein the ELNN polypeptide comprises at least 5 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0300] E83. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E79, and wherein the ELNN polypeptide comprises at least 6 instances of the following sequence: SATPE (SEQ ID NO: 28).

[0301] E84. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E83, wherein each of the at least 3, at least 4, at least 5, or at least 6 instances of SATPE (SEQ ID NO: 28) are part of the following sequence: ESATPE (SEQ ID NO: 29).

[0302] E85. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E84, wherein each instance of SATPE (SEQ ID NO: 28) and/or ESATPE (SEQ ID NO: 29) in the sequence of the ELNN polypeptide is followed by at least 3 amino acids at the C-terminal end thereof.

[0303] E86. The antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E85, wherein the ELNN polypeptide comprises the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27.

[0304] E87. The antibody or antigen-binding fragment of any of the preceding claims, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.

[0305] E88. An antibody or antigen-binding fragment comprising a heavy chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31 and a light chain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36.

[0306] E89. An antibody or antigen-binding fragment comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36.

[0307] E90. An antibody comprising two heavy chains comprising the amino acid sequence of SEQ ID NO:31, and two light chains comprising the amino acid sequence of SEQ ID NO:36.

[0308] E91. The antibody, antigen-binding fragment, or antigen binding protein of any one of E66 to E88, which is an isolated antibody, antigen-binding fragment, or antigen binding protein.

[0309] E92. A polynucleotide or set of polynucleotides encoding the antibody, antigen-binding fragment, or antigen binding protein of any one of E68 to E91.

[0310] E93. A vector comprising the polynucleotide or set of polynucleotides of E92.

[0311] E94. A host cell comprising the vector of E93.

[0312] E95. The host cell comprising of E94, which is a CHO or HEK293 cell.

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SEQUENCES

Table A: Exemplary chimeric protein sequences

A-FVIII(ELNN)-Fc: SEQ ID NO: 1						
ATRRYYLGAV	ELSDWYMQSD	LGELPVDARF	PPRVKSPFP	NTSVVYKCTL	FVEFTDHLFN	60
IAKPRPPWMG	LLGPTIAQEV	YDTVVITLKN	MASHPVSLHA	GVGSYWKASE	GAEYDDQTSQ	120
REKEDDKVFP	GGSHTYVWQV	LKENGPMASD	PLCLTYSYLS	HVDLVKDLNS	GLIGALLVCR	180
EGSLAKEKTQ	TLHKFILLFA	VFDEGKSWHS	ETKNSLMQDR	DAASARAWPK	MHTVNGYVNR	240
SLPGLIGCHR	KSVYWHVIGM	GTTPEVHSIF	LEGHTFLVRN	HRQASLEISP	ITFLTAQTLT	300
MDLQGFLFC	HISSHQHDGM	EAYVKVDSCP	EEPQLRMKN	EEAEDYDDL	TDSEMDVVR	360
DDDNPSFSIQ	IRSVAKKHPK	TWVHYIAAEE	EDWDYAPLVL	APDRSYKSQ	YLNNGPQRIG	420
RKYKKVRFMA	YTDETFKTRE	AIQHESGILG	PLLYGEVGD	LLIIFKNQAS	RPYNIYPHGI	480
TDVRPLYSRR	LPKGVKHLKD	FPILPGEIFK	YKWTVTVEDG	PTKSDPRCLT	RYYSFVNME	540
RDLASGLIGP	LLICYKESVD	QRGNQIMSDK	RNVILFSVFD	ENRSWYLTEN	IQRFLPNPAG	600
VQLEDPEFQA	SNIMHSINGY	VFDSLQLSVC	LHEVAYWYIL	SIGAQTDFLS	VFFSGYTFKH	660
KMYVEDTLTL	PPFSGETVFM	SMENPGLWIL	GCHNSDFRNR	GMTALLKVSS	CDKNTGDYVE	720
DSYEDISAYL	LSKNNAIEPR	SFSQNGTSES	ATPESGPGSE	PATSGSETPG	TSESATPESG	780
PGSEPATSGS	ETPGTSESAT	PESGPGTSTE	PSEGSAPGSP	AGSPTSTEEG	TSESATPESG	840
PGSEPATSGS	ETPGTSESAT	PESGPGSPAG	SPTSTEEGSP	AGSPTSTEEG	TSTEPSEGS	900
PGTSESATPE	SGPGTSESAT	PESGPGTSES	ATPESGPGSE	PATSGSETPG	SEPATSGSET	960
PGSPAGSPTS	TEEGTSTEPS	EGSAPGTSTE	PSEGSAPGSE	PATSGSETPG	TSESATPESG	1020
PGTSTEPSEG	SAPASSEITR	TTLQSDQEEI	DYDDTISVEM	KKEDFDIYDE	DENQSPRSFQ	1080
KKTRHYFIAA	VERLWDYGMS	SSPHVLNRRA	QSGSVPPQFK	VVFQEFTDGS	FTQPLYRGEL	1140
NEHLGLLPHY	IRAEVEDNIM	VTFRNQASRP	YSFYSSLISY	EEDQRQGAEP	RKNFVKPNET	1200
KTYFWKQVHH	MAPTKDEFDC	KAWAYFSDVD	LEKDVHSGLI	GPTLVCHTNT	LNPAHGRQVT	1260
VQEFALFFTI	FDETKSWYFT	ENMERNCRAP	CNIQMEDPTF	KENYRFHAIN	GYIMDTLPLG	1320
VMAQQQIRIW	YLLSMGSEN	IHSIHFSGHV	FTVRKKEEYK	MALYNLYPGV	FETVEMLPSK	1380
AGIWRVECLI	GEHLHAGMST	LFLVYSNKCQ	TPLGMASGHI	RDFQITASGQ	YGQWAPKLAR	1440
LHYSGSINAW	STKEPFSWIK	VDLLAPMIIH	GIKTQGARQK	FSSLYISQFI	IMYSLDGKKW	1500
QTYRGNSTGT	LMVFFGNVDS	SGIKHNIFNP	PIIARYIRLH	PTHYSIRSTL	RMLMGCDLN	1560
SCSMPLGMES	KAISDAQITA	SSYFTNMFAT	WSPSKARLHL	QGRSNAWRPQ	VNNPKEWLQV	1620
DFQKTMKVGT	VTTQGVKSL	LSMYVKEFLI	SSSQDGHQWT	LFFQNGKVKV	FQGNQDSFTP	1680
VVNSLDPPLL	TRYLRIHPQS	WVHQIALRME	VLGCEAQDLY	DKTHTCPCPC	APELLGGGPSV	1740
FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY	1800
RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPVYVT	LPPSRDELTK	1860
NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTTPPVLD	DGSFFLYSKL	TVDSKRWQQG	1920
NVFSQVMHE	ALHNHYTQKS	LSLSPG				
VWF(D'D3)-ELNN-a2 Linker-Fc: SEQ ID NO: 2						
SLSCRPPMVK	LVCPADNLRA	EGLECTKTCQ	NYDLECMMSG	CVSGCLCPPG	MVRHENRCVA	60
LERCPCFHQG	KEYAPGETVK	IGCNTCVCRD	RKWNCTDHVC	DATCSTIGMA	HYLTFDGLKY	120
LFPGCEQVYL	VQDYCGSNPG	TFRILVGNKG	CSHPSVKCKK	RVTLIVEGGE	IELFDGEVNV	180
KRPMKDETHF	EVWESGRYII	LLLGKALSVV	WDRHLSISVV	LKQTYQEKVC	GLCGNFDGIQ	240
NNDLTSSNLQ	VEEDPVDFGN	SWKVSSQCAD	TRKVPDLSSP	ATCHNNIMKQ	TMVDSSCRIL	300
TSDFVQDCNK	LVDPEPYLDV	CIYDTCSCES	IGDCAAFCDT	IAAYAHVCAQ	HGKVVTWRTA	360
TLCPCQSEER	NLRENGYEA	WRYNSCAPAC	QVTCQHPEPL	ACPVCQVEGC	HAHCPPGKIT	420
DELLQTCVDP	EDCPVCEVAG	RRFASGKKVT	LNPSDPEHCQ	ICHCDVNLNT	CEACQEPGTS	480
ESATPESGPG	SEPATSGSET	PGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	540
TEPSEGSAPG	SPAGSPTSTE	EGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGSP	600
AGSPTSTEEG	SPAGSPTSTE	EGASSDKNTG	DYVEDSYEDI	SAYLLSKNNA	IEPRSFSDKT	660
HTCPCCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV	VDVSHEDPEV	KFNWYVDGVE	720
VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW	LNGEYKCKCV	SNKALPAPIE	KTISKAKGQP	780
REPQVYTLPP	SRDELTKNQV	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	840
FFLYSKLTVD	KSRWQQGNVF	SCSVMHEALH	NHYTQKSLSL	SPG		883
Amino acid sequence of FVIII protein (312A) - includes signal peptide: SEQ ID NO: 3						
1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGA	VE	ELSDYMQSD	LGELPVDARFP
51	PRVPKSFPPN	TSVVYKTKLF	VEFTDHLFNI	AKPRPPWMGL	LGPTIAQEVY	
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPFG	
151	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE	
201	GLSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM	
251	HTVNGYVNR	LPGLIGCHRK	SVYWHVIGM	TTPEVHSIFL	EGHTFLVRNH	

301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
401	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFKTRFA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
501	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YYSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFVVFDE
601	NRSWYLTENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
651	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMFS
701	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYDED	SYEDISAYLL
751	SKNNAIEPRS	FSQNGTSESA	TPESGPGSEP	ATSGSETPGT	SESATPESGP
801	GSEPATSGSE	TPGTSESATP	ESGPGTSTEP	SEGSAPGSPA	GSPTSTEEGT
851	SESATPESGP	GSEPATSGSE	TPGTSESATP	ESGPGSPAGS	PTSTEEGSPA
901	GSPTSTEEGT	STEPSEGSAP	GTSESATPES	PGTSESATP	ESGPGTSESA
951	TPESGPGSEP	ATSGSETPGS	EPATSGSETP	GSPAGSPSTST	EEGTSTEPSE
1001	GSAPGTSTEP	SEGSAPGSEP	ATSGSETPGT	SESATPESGP	GTSTEPSEGS
1051	APASSEITRT	TLQSDQEEID	YDDTISVEMK	KEDFDIYDED	ENQSPRSFQK
1101	KTRHYFIAAV	ERLWDYGMSS	SPHVLNRNAQ	SGSVQPQKKV	VFQFTDGSF
1151	TQPLYRGELN	EHLGLLGPYI	RAEVEDNIMV	TFRNQASRPY	SFYSSLISYE
1201	EDQRQGAEP	KNFVKPNETK	TYFWKVQHMM	APTKDEFDCK	AWAYFSDVDL
1251	EKDVHSGLIG	PLLVCNTNTL	NPAHGRQVTV	QEFALFFTIF	DETKSWYFTE
1301	NMERNCRAPC	NIQMEDPTFK	ENYRFHAING	YIMDTLPGLV	MAQDQIRRWY
1351	LLSMGSENENI	HSIHFSGHVF	TVRKKEEYKM	ALYNLYPGVF	ETVEMLPSPA
1401	GIWRVECLIG	EHLHAGMSTL	FLVYSNKCQT	PLGMASGHIR	DFQITASGGY
1451	GQWAPKLARL	HYSGSINAWS	TKEPFSWIKV	DLLAPMIHIG	IKTQGARQKF
1501	SSLYISQFII	MYSLDGKKWQ	TYRGNSTGTL	MVFFGNVDSS	GIKHNIFFNPP
1551	IIARYIRLHP	THYSIRSTLR	MELMGCDLNS	CSMPLGMESK	AISDAQITAS
1601	SYFTNMFAFW	SPSKARLHLQ	GRSNAWRPQV	NNPKEWLQVD	FQKTMKVTVG
1651	TTQGVKSLLT	SMYVKEFLIS	SSQDGHQWTL	FFQNGKVXVF	QGNQDSFTPV
1701	VNSLDPPLLT	RYLRIHPQSW	VHQIALRMEV	LGCEAQDLYD	KTHTCPPCPA
1751	PELLGGPSVF	LFPKPXKDTL	MISRTPTEVC	VVDVSHEDP	EVKFNWYVDG
1801	VEVHNAKTKP	REEQYNSTYR	VVSVLTVLHQ	DWLNKGKEYKC	KVSNKALPAP
1851	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG	FYPSDIAVEW
1901	ESNGQPENNY	KTPPVVLDS	GSFFLYSKLT	VDSRWQQGN	VFSGSCVMHEA
1951	LHNHYTQKSL	SLSPG			

Nucleotide sequence encoding FVIII 312A: SEQ ID NO: 4

1	ATGCAAAATAG	AGCTCTCCAC	CTGCTTCTTT	CTGTGCCTTT	TGCGATTCTG
51	CTTTAGTGCC	ACCAGAAGAT	ACTACCTGGG	TGCAGTGGAA	CTGTCATGGG
101	ACTATATGCA	AAGTGATCTC	GGTGAGCTGC	CTGTGGACGC	AAGATTTCTC
151	CCTAGAGTGC	CAAAATCTTT	TCCATTCAAC	ACCTCAGTCG	TGTACAAAAA
201	GACTCTGTTT	GTAGAATTCA	CGGATCACCT	TTTCAACATC	GCTAAGCCAA
251	GGCCACCCCTG	GATGGGTCTG	CTAGGTCCTA	CCATCCAGGC	TGAGGTTTAT
301	GATACAGTGG	TCATTACACT	TAAGAACATG	GCTTCCCATC	CTGTCACTCT
351	TCATGCTGTT	GGTGATCCT	ACTGGAAGC	TTCTGAGGGA	GCTGAATATG
401	ATGATCAGAC	CAGTCAAAGG	GAGAAAGAA	ATGATAAAGT	CTTCCCTGGT
451	GGAAGCCATA	CATATGTCTG	GCAGGTCCTG	AAAGAGAAAT	GTCCAATGGC
501	CTCTGACCCA	CTGTGCCTTA	CCTACTCATA	TCTTTCTCAT	GTGGACCTGG
551	TAAAAGACTT	GAATTCAGGC	CTCATTGGAG	CCCTACTAGT	ATGTAGAGAA
601	GGGAGTCTGG	CCAAGGAAAA	GACACAGACC	TTGCACAAAT	TTATACTACT
651	TTTTGCTGTA	TTTGATGAAG	GGAAAAGTTG	GCACTCAGAA	ACAAAAGAACT
701	CCTTGATGCA	GGATAGGGAT	GCTGCATCTG	CTCGGGCCTG	GCCTAAAAATG
751	CACACAGTCA	ATGTTATGT	AAACAGGTCT	CTGCCAGGTC	TGATTGGATG
801	CCACAGGAAA	TCAGTCTATT	GGCATGTGAT	TGGAATGGGC	ACCACTCCTG
851	AAGTGCACCT	AATATTCCTC	GAAGGTCACA	CATTTCCTGT	GAGGAACCAT
901	CGCCAGGCGT	CCTTGGAAAT	CTCGCCAATA	ACTTTCCTTA	CTGCTCAAAC
951	ACTCTTGATG	GACCTTGGAC	AGTTTCTACT	GTTTTGTGAT	ATCTCTTCCC
1001	ACCAACATGA	TGGCATGGAA	GCTTATGTCA	AAGTAGACAG	CTGTCCAGAG
1051	GAACCCCAAC	TACGAATGAA	AAATAATGAA	GAAGCGGAAG	ACTATGATGA
1101	TGATCTTACT	GATTCTGAAA	TGGATGTGGT	CAGGTTTGAT	GATGACAACT
1151	CTCCTTCCTT	TATCCAAATT	CGCTCAGTTG	CCAAGAAAGCA	TCCTAAAACT
1201	TGGGTACATT	ACATTGTCTG	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT
1251	AGTCTTCGCC	CCCGATGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG
1301	GCCCTCAGCG	GATTGGTAGG	AAGTACAAAA	AAGTCCGATT	TATGGCATA
1351	ACAGATGAAA	CCTTTAAGAC	TCGTGAAGCT	ATTCAGCATG	AATCAGGAAT
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1451	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCTCA	CGGAATCACT
1501	GATGTCCTGC	CTTTGTATT	AAGGAGATTA	CCAAAAGGTG	TAAAAATTT
1551	GAAGGATTTT	CCAATTCTGC	CAGGAGAAAT	ATTCAAATAT	AAATGGACAG

1601 TGA CTGTAGA 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
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1951 CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC AGACTGACTT
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2201 CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTGAGC ATACTTGCTG
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2851 ACACCCGAGT CCGGCCCTGG GTCTGAACCC GCCACAAGTG GTAGTGAGAC
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2951 CAGGCTCACC AACCTCACT GAGGAGGGCA CCAGCACAGA ACCAAGCGAG
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4151 ATCTCTATCC AGGTGTTTTT GAGACAGTGG AAATGTTACC ATCCAAGCT
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5051 ATGGCAAAAG AAAGTTTTT CAGGGAATC AAGACTCCTT CACACCTGTG
5101 GTGAACCTCT TAGACCCACC GTTACTGACT CGTACCTTC GAATTCACCC

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5151 CCAGAGTTGG GTGACCAGA TTGCCCTGAG GATGGAGGTT CTGGGCTGCG
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5801 GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
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Amino acid sequence of VWF059A protein - includes signal peptide and D1D2 region: SEQ ID NO: 5

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1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNVTFDGSM
51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
101 TVTQGDQKRV MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSNGFQVLL
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201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWDHSA CSPVCPAGME
301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
351 VHSGRYPPG TSLSRDNTC ICRNSQWICS NEECPGECVL TQGSFHSFSD
401 NRYFTFSGIC QYLLARDQDQ HSFISIVETV QCADDRDAVC TRSVTVRLPG
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501 DWQDGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTSPG LAEPRVDFG
551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCLE
651 NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
701 CVPKAQPCPY YDGEIFQPED IFSDHHTMCT CEDGFMHCTM SGVPGSLLPD
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801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
851 CRDRKWNCTD HVCATCSTI GMAHYLTFDG LKYLFPGEQY YVLVQDYCGS
901 NPGTFRILVG NKGCSHPSVK CKKRVTLIVE GGEIELFDGE VNVKRPMDKE
951 THFEVVESEGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVGCLCGNFD
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1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
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1351 SESATPESGP GSPAGSPTST EEGSPAGSPT STEEGASDCK NTGDYEDSY
1401 EDISAYLLSK NNAIEPRFS DKHTTCPPCP APELLGGPSV FLFPPKPKDT
1451 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
1501 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKISKAK GQPREPQVYT
1551 LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS
1601 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPG

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Nucleotide sequence encoding VWF059A protein: SEQ ID NO: 6

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351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GTGAGGCGCT
401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
501 CTTTGCTGAA GATGACTTTA TGACCCAAGA AGGGACCTTG ACCTCGGACC
551 CTTATGACTT TGCCAACCTA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
601 GAACGGGCAT CTCCTCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT

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651	GCAGAAGGGC	CTGTGGGAGC	AGTGCCAGCT	TCTGAAGAGC	ACCTCGGTGT
701	TTGCCCGCTG	CCACCCTCTG	GTGGACCCCG	AGCCTTTTGT	GGCCCTGTGT
751	GAGAAGACTT	TGTGTGAGTG	TGCTGGGGGG	CTGGAGTGCG	CCTGCCCTGC
801	CCTCCTGGAG	TACGCCCGGA	CCTGTGCCCA	GGAGGGAATG	GTGCTGTACG
851	GCTGGACCGA	CCACAGCGCG	TGCAGCCAG	TGTGCCCTGC	TGGTATGGAG
901	TATAGGCAGT	GTGTGTCCCC	TTGCGCCAGG	ACCTGCCAGA	GCCTGCACAT
951	CAATGAAATG	TGTCAAGGAGC	GATGCGTGGA	TGGCTGCAGC	TGCCCTCAGG
1001	GACAGCTCCT	GGATGAAGGC	CTCTGCGTGG	AGAGCACCAG	GTGTCCCTGC
1051	GTGCATTCCG	GAAAGCGCTA	CCCTCCCGGC	ACCTCCCTCT	CTCGAGACTG
1101	CAACACCTGC	ATTTGCCGAA	ACAGCCAGTG	GATCTGCAGC	AATGAAGAAT
1151	GTCCAGGGGA	GTGCCCTTGT	ACTGGTCAAT	CCCACCTCAA	GAGCTTTGAC
1201	AACAGATACT	TCACCTTCAG	TGGGATCTGC	CAGTACCTGC	TGGCCCGGGA
1251	TTGCCAGGAC	CACCTCCTTCT	CCATTGTGAT	TGAGACTGTG	CAGTGTGCTG
1301	ATGACCGCGA	CGCTGTGTGC	ACCCGCTCCG	TCACCGTCCG	GCTGCCCTGGC
1351	CTGCACAACA	GCCTTGTGAA	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
1601	ACTTCTTTAC	CCCCTCTGGG	CTGGCGGAGC	CCCGGGTGGG	GGACTTCGGG
1651	AACGCTGGGA	AGCTGCACGG	GGACTGCCAG	GACCTGCAGA	AGCAGCACAG
1701	CGATCCCTGC	GCCCTCAACC	CGCGCATGAC	CAGGTTCTCC	GAGGAGCGGT
1751	GCGCGGTCTT	GACGTCCCCC	ACATTGAGG	CCTGCCATCG	TGCCGTACAG
1801	CCGCTGCCCT	ACCTGCGGAA	CTGCCGCTAC	GACGTGTGCT	CCTGCTCGGA
1851	CGGCCGCGAG	TGCTGTGCG	GCGCCCTGGC	CAGCTATGCC	GCGGCCCTGC
1901	CGGGGAGAGG	CGTGCCTGTC	GCGTGGCGCG	AGCCAGGCCG	CTGTGAGCTG
1951	AACTGCCCGA	AAGGCCAGGT	GTACCTGCAG	TGCGGGACCC	CCTGCAACCT
2001	GACCTGCCGC	TCTCTCTCTT	ACCCGGATGA	GGAAATGCAAT	GAGGCCCTGC
2051	TGGAGGGCTG	CTTCTGCCCC	CCAGGGCTCT	ACATGGATGA	GAGGGGGGAC
2101	TGCGTGCCCA	AGGCCCAAGT	CCCCTGTTAC	TATGACGGTG	AGATCTTTCA
2151	GCCAGAAGAC	ATCTTCTCAG	ACCATCACAC	CATGTGCTAC	TGTGAGGATG
2201	GCTTACATGA	CTGTACCATG	AGTGGAGTCC	CCGGAAGCTT	GCTGCCCTGAC
2251	GCTGTCTTCA	GCAAGTCCCT	GTCTCATCGC	AGCAAAAAGG	GCCTATCCTG
2301	TCGGCCCCCC	ATGGTCAAGC	TGGTGTGTCC	CGCTGACAAC	CTGCGGECTG
2351	AAGGGCTCGA	GTGTACCAAA	ACGTGCCAGA	ACTATGACCT	GGAGTGATG
2401	AGCATGGGCT	GTGTCTCTGG	CTGCCCTTGC	CCCCGGGGCA	TGGTCCGGCA
2451	TGAGAAACAG	TGTGTGGCCC	TGGAAGAGTG	TCCCTGCTTC	CATCAGGGCA
2501	AGGAGTATGC	CCCTGGAGAA	ACAGTGAAGA	TTGGCTGCAA	CACTTGTGTC
2551	TGTCGGGACC	GGAAGTGGAA	CTGCACAGAC	CATGTGTGTG	ATGCCACGTG
2601	CTCCACGATC	GGCATGGCCC	ACTACCTCAC	CTTCGACGGG	CTCAAAATACC
2651	TGTTCCCGCG	GGAGTGCCAG	TACGTTCTGG	TGCAGGATTA	CTGCGGCAGT
2701	AACCTTGGGA	CCTTTCGGAT	CCTAGTGGGG	AATAAGGGAT	GCAGCCACCC
2751	CTCAGTGAAA	TGCAAGAAAC	GGGTCAACAT	CCTGGTGGAG	GGAGGAGAGA
2801	TTGAGCTGTT	TGACGGGGAG	GTGAATGTGA	AGAGGCCCAT	GAAGGATGAG
2851	ACTACATTTG	AGGTGGTGGG	GTCCTGGCGG	TACATCATTC	TGCTGTGGGG
2901	CAAGACCCCT	TCCGTGGTCT	GGGACCGCCA	CCTGAGCATC	TCCGTGCTCC
2951	TGAAGCAGAC	ATACCAGGAG	AAAGTGTGTG	GCCTGTGTGG	GAATTTTGAT
3001	GGCATCCAGA	ACAATGACCT	CACCAGCAGC	AACTCCCAAG	TGGAGGAAGA
3051	CCCTGTGGAC	TTTGGGAAC	CCTGGAAGT	GAGCTCGCAG	TGTGCTGACA
3101	CCAGAAAAGT	GCCTCTGGAC	TCATCCCTGT	CCACCTGCCA	TAACAACATC
3151	ATGAAGCAGA	CGATGGTGGG	TTCTCTCTGT	AGAATCCTTA	CCAGTGACGT
3201	CTTCCAGGAC	TGCAACAAGC	TGGTGGACCC	CGAGCCATAT	CTGGATGTCT
3251	GCATTTACGA	CACCTGTCTC	TGTGAGTCCA	TTGGGGACTG	CGCCGCAATC
3301	TGCGACACCA	TTGCTGCCTA	TGCCACGCTG	TGTGCCCAGC	ATGGCAAGGT
3351	GGTGACCTGG	AGGACGGCCA	CATTGTGCCC	CCAGAGCTGC	GAGGAGAGGA
3401	ATCTCCGGGA	GAACGGGTAT	GAGGCTGAGT	GGCGCTATAA	CAGCTGTGCA
3451	CCTGCCCTGT	AAGTCACGTG	TCAGCACCTT	GAGCCACTGG	CCTGCCCTGT
3501	GCAGTGTGTG	GAGGGCTGCC	ATGCCCACTG	CCTCCAGGGG	AAAATCCTGG
3551	ATGAGCTTTT	GCAGACCTGC	GTTGACCTTG	AAGACTGTCC	AGTGTGTGAG
3601	GTGGCTGGCC	GGCGTTTTGC	CTCAGGAAAG	AAAGTCACCT	TGAATCCCGA
3651	TGACCTTGAG	CACCTGCCAG	TTTGCCACTG	TGATGTTGTC	AACCTCACCT
3701	GTGAAGCCTG	CCAGGAGCCG	GGTACATCAG	AGAGCGCCAC	CCCTGAAAAGT
3751	GGTCCCGGGA	GCGAGCCAGC	CACATCTGGG	TCGGAACCGC	CAGGCACATC
3801	CGAGTCTGCA	ACTCCCGAGT	CCGGACCTGG	CTCCGAGCCT	GCCACTAGCG
3851	GCTCCGAGAC	TCCGGGAAC	TCCGAGAGCG	CTACACCAGA	AAGCGGACCC
3901	GGAAACAGTA	CCGAACCTAG	CGAGGGCTCT	GCTCCGGGCA	GCCAGCCGGG
3951	CTCTCTTACA	TCCACGGAGG	AGGGCACTTC	CGAATCCGCC	ACCCCGGAGT
4001	CAGGGCCAGG	ATCTGAACCC	GCTACCTCAG	GCAGTGAGAC	GCCAGGAACG
4051	AGCGAGTCCG	CTACACCGGA	GAGTGGGCCA	GGGAGCCCTG	CTGATCTCC
4101	TACGTCCACT	GAGGAAGGGT	CACCAGCGGG	CTCGCCACCC	AGCACTGAAG
4151	AAGGTGCCCT	GTCTGACAAG	AACACTGGTG	ATTATTACGA	GGACAGTTAT
4201	GAAGATATTT	CAGCATATTT	GCTGAGTAAA	AACAATGCCA	TTGAACCAAG

4251	AAGCTTCTCT	GACAAAAC	ACACATGCC	ACCGTGCCA	GCTCCAGAA	
4301	TCCTGGGCGG	ACCGTCAGTC	TTCCTCTTCC	CCCCAAAAC	CAAGGACAC	
4351	CTCATGATCT	CCCGGACCCC	TGAGGTCACA	TGCGTGGTGG	TGGACGTGAG	
4401	CCACGAAGAC	CCTGAGGTCA	AGTTCAACTG	GTATGTGGAC	GGCGTGGAA	
4451	TGCATAATGC	CAAGACAAAG	CCCGGGGAGG	AGCAGTACAA	CAGCAGCTAG	
4501	CGTGTGGTCA	GCCTCCTCAC	CGTCTGCAC	CAAGACTGGC	TGAATGCGAA	
4551	GGAGTACAAG	TGCAAGGTCT	CCAACAAAGC	CCTCCAGCC	CCCATCGAGA	
4601	AAACCATCTC	CAAAAGCCAA	GGCGAGCCCC	GAGAACACAC	GGGTATACCC	
4651	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAAGTTA	GCCTGACCTG	
4701	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	
4751	ATGGGCGACC	GGAGAACAA	TACAAGACCA	CGCTCCCGT	GTTGGACTGC	
4801	GACGGCTCCT	TCTTCTCTTA	CTCCAAGCTC	ACCGTGGACA	AGAGCAGGTG	
4851	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	GCTCTGCACA	
4901	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTTG	A	

FVIII(312)(ELNN)-Fc: SEQ ID NO: 7							
TRRYYLGA	LSWDYMQSD	GELPVDARF	PRVPKSFPFN	TSVYVKTLF	VEFTDHLFNI	60	
AKPRPPMGL	LGPTIAQAEV	DTVTITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	120	
EKEDDKVFP	GSHTYVWQVL	KENGPMSADP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE	180	
GLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM	HTVNGYVNR	240	
LPGLIGCH	SVYWHVIGM	TTPEVHSIFL	EGHTFLVRNH	RQASLEISPI	TFLTAQTLLM	300	
DLGQFLFCH	ISSHQHDGME	AVYKVDSCPE	EPQLRMKNNE	EAEYDDDLT	DSEMDVVRFD	360	
DDNSPSFIQ	RSVAKKHPKT	WHYIAAE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	420	
KYKVRFMAY	YDTEFTKREA	IQHESGILGP	LLYGEVGD	LIIIFKNQASR	PYNIYPHGIT	480	
DVRPLYSRR	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR	YYSSFVNMER	540	
DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE	NRSWYLTENI	QRFLPNPAGV	600	
QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	660	
MYVEDTLTLF	PFGSETVFMS	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYIED	720	
SYEDISAYLL	SKNNAIEPRS	FSQNGTSESA	TPESGPGSEP	ATSGSETPGT	SESATPESGP	780	
GSEPATSGSE	TPGTSESATP	ESGPGTSTEP	SEGSAPGSPA	GSPTSTEEGT	SESATPESGP	840	
GSEPATSGSE	TPGTSESATP	ESGPGSPAGS	PTSTEEGSPA	GSPTSTEEGT	STEPSEGSAP	900	
GTSESATPES	GPGTSESATP	ESGPGTSESA	TPESGPGSEP	ATSGSETPGS	EPATSGSETP	960	
GSPAGSPSTST	EESTSTEPSE	GSAPGTSTEP	SEGSAPGSEP	ATSGSETPGT	SESATPESGP	1020	
GSTEPSEGS	APASSEITRT	TLQSDQEEID	YDDTISVEMK	KEDFDIYDED	ENQSPRSFQK	1080	
KTRHYFIAAV	ERLWDYGMSS	SPHVLNRNAQ	SGSVYPQKKV	VFQEFTDGSF	TQPLYRGELN	1140	
EHLGLLGPYI	RAEVEDNIMV	TFRNQASRPY	SFYSSLSISYE	EDQRQGAEP	RKNFVKPNETK	1200	
TYFWKQVHHM	APT	KDEFDCK	AWAYFSDVDL	EKDVHSGLIG	PLL	VCHTNLNPAGHRQVTV	1260
QEFA	LFFTFIDETKSWYFTE	NMERNCRAPC	NIQMEDPTFK	ENYRFHAING	YIMDTLPGLV	1320	
MAQDQRIRWY	LLSMG	SNENIHSIHFSGHVF	TVRKKEEYKM	ALYNLYPGVF	ETVEMLP	PSKA1380	
GLWRVECLIG	EHLHAGMSTL	FLVYSNKCQT	PLGMASGHIR	DFQITASGQY	GQWAPKLARL	1440	
HYSGSINAW	S	KEPFSWIKV	DLLAPMIIHG	IKTQGARQKF	SSLYISQFII	MYSLDGKKWQ	1500
TYRGNSTGTL	MVFFGNVDSS	GIKHNIFNPP	IIARYIRLHP	THYSIRSTLR	MELMGCDLNS	1560	
CSMPLGMESK	AISDAQITAS	SYFTNMFATW	SPSKARLHLQ	GRSNAWRPQV	NNPKEWLQVD	1620	
FQKTMKVTV	TTQGVKSLLT	SMYVKEFLIS	SSQDGHQWTL	FFQNGKV	KVVFQGNQDSFTPV	1680	
VNSLDPLLT	RYLR	IHPQSW	VHQIALRMEV	LGCEAQDLYD	KTHTCPPCPA	PELLGGPSVF	1740
LFPPKPKDTL	MISRTPEVTC	VWVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	1800	
VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	1860	
QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY	KTTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	1920	
VFSCSVMHEA	LHNHYTQKSL	SLSPG				1945	

Table B. Additional chimeric protein sequences

Description / SEQ ID NO.	Sequence
Mature native human FVIII	ATRRYYLGAV ELSDYMQSD LGELPVDARF PPRVPKSFPF NTSVYVKTL FVEFTDHLFN 60 IAKPRPPMWMG LLGPTIAQAEV YDVTVTITLKN MASHPVS
SEQ ID NO: 8	REKEDDKVFP GGSHTYVWQV LKENGPMASD PLCLTSYLS HVDLVKDLNS GLIGALLVCR 120 EGSLAKEKTQ TLHKFILLFA VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR 180 SLPGLIGCHR KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL 240 MDLGQFLFCH HISSHQHDGM EAYVKVDSCP EEPQLRMKN EEAEDYDDDL TDSEMDVVRFD 300 DDNSPSFIQ IRSVAKKHPK TWVHYIAEEE EDWDYAPLVL APDDRSYKSQ YLNNGPQRIG 360 RKYKVRFMAY YD
	ETFKTRE AIQHESGILG PLYGEVGD
	TLIIIFKNQAS RPYNIYPHGI 420
	TDVRLPLYSRR LPKGVKHLKD FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYSSSFVNME 480
	RDLASGLIGP LLICYKESVD RGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG 540
	VQLEDPEFQA SNIMHSINGY VFDSLQLSVCL LHEVAYWYIL SIGAQTDFLS VFSSGYTFKH 600

	KMVYEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR GMTALLKVSS CDKNTGDYYE 720
	DSYEDISAYL LSKNNAIEPR SFSQNSRHPS TRQKQFNATT IPENDIEKTD PWFahrTPMP 780
	KIQNVSSSDL LMLLRQSPPT HGLSLSDLQE AKYETFSDDP SPGAIDSNNS LSEMTHFRPQ 840
	LHHSQDMVFT PESGLQLRLN EKLGTAAATE LKKLDFKVSS TSNNLISTIP SDNLAAAGTDN 900
	TSSLGPPSMP VHYDSQLDTT LFGKKSSPLT ESGGPLSLSE ENNDSKLLS GLMNSQESSW 960
	GKNVSSSESG RLFGKGRAGH PALLTkdNAL FKVSISLLKT NKTSNNSATN RKTHIDGPSL 1020
	LIENSPPSVWQ NILESDTEFK KVTPLIHDRM LMDKNATALR LNHMSNKTTS SKNMEMVQKQ 1080
	KEGPIPPDAQ NPDMSFFKML FLPEsARWIQ RTHGKNSLNS GQGPSPKQLV SLGPEKSEVG 1140
	QNFLESEKNV VVGKGEFTK VGLKEMVFPs SRNFLTLNLD NLHENNTHNQ EKKIQEEIEK 1200
	KETLIQENVV LPQIHTVTGT KNFMKNLFL L STRQNVESGY DGAYAPVLQD FRSLNDSTNR 1260
	TKKHTAHFSK KGEEENLEGL GNQTKQIVEK YACTTRISPn TSQQNFVTQR SKRALKQFRL 1320
	PLEETELEKR IIVDDTSTQW SKNMKHLTPS TLTQIDYNEK EKGAITQSPL SDCLTRSHSI 1380
	PQANRSLPI AKVSSFPsIR PIYLTRVLfQ DNSSHLPAAS YRKkDSGVQE SSHFLQGAKK 1440
	NNLSLAILTL EMTGDQREVG SLGTSATNSV TYKKVENTVL PKPDLPKTSg KVELLPKVHI 1500
	YQKDLFPPT SNGSPGHLDL VEGSLLQGT E GAIKWNEANR PGKVPFLRVA TESSAKTPSK 1560
	LLDPLAWDNH YGTQIPKEEW KSQEKSEPT AFKKKDTILs LNACESNHAI AAINEGQNKp 1620
	EIEVTWAKQG RTERLCSQNP PVLKRHRQEI TRTTLQSDQE EIDYDDTISV EMKKEFDIY 1680
	DEDENQSPRS FQKkTRHYFI AAVERLWDYG MSSSPHVLrN RAQSGSVpQF KKVVFQFTD 1740
	GSFTQPLYRG ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYsFYSSLI SYEEDQRQGA 1800
	EPRKNFVKPN ETkTYFWKVQ HHMAPTKDEF DCKAWAYFSd VDLEKDVHSG LIGPLLCHT 1860
	NTLNPAGHRQ VTVQEFALFF TIFDEKSWY FTENMERNCR APCNIQMEDP TFKENYRFHA 1920
	INGYIMDTLP GLVMAQDQRI RWYLLSMGSN ENIHsIHfSG HVFTVRKKEE YKMALYNLYP 1980
	GVFETVEMLP SKAGIWRVEC LIGEHlHAGM STLFLVYSNK CQTPLGMAsg HIRDFQITAS 2040
	GQYQGWAPKL ARlHYSGsIN AWSTKEPFSW IKVDL LAPMI IHGIKTQGAR QKFSSLYISQ 2100
	FIIMYLDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNI F NPPIIARYIR LHPThYSIRs 2160
	TLRMELMGCD LNSCSMPLGM ESKAISDAQI TASSYFTNMf ATWSPSKARL HLQGRsNAWR 2220
	PQVNNPKEWL QVDFQKTMKV TGVTTQGVKS LLTSMYVKEF LISSSQGDGHQ WTLFFQNGKV 2280
	KVFQGNQDSF TPVNSLDLP LLTRYLRlHP QSWWHQIALR MEVLGCEAQD LY 2332
ELNN polypeptide AE288	GTSEsATPES GPGSEPATSG SETPGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 60
	GTSTEPSEGS APGSPAGSPT STEEGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 120
	GSPAGSPTST EEGSPAGSPT STEEGTSTEP SEGsAPGTSE SATPESGPGT SESATPESGP 180
SEQ ID NO: 9	GTSEsATPES GPGSEPATSG SETPGSEPAT SGSETPGSPA GSPTSTEEGT STEPSEGsAP 240
	GTSTEPSEGS APGSEPATSG SETPGTSESA TPESGPGTST EPSEGsAP 288
pSYN VwF059	TSTEEGASIS DKNTGDYYED SYEDISAYLL SKNNAIEPRs FSDKTH
SEQ ID NO: 10	
pSYN VwF059A	TSTEEGASSD KNTGDYYEDs YEDISAYLLs KNNAIePRsF SDKTH
SEQ ID NO: 11	
pSYN FVIII 312	ATRRYYLGAV ELsWDYMQSD LGELPVDARF PPRVPKSFPF NTSVvYKkTL FVEFTDHLfN 60
	IAKPRPPWMG LLGPTIQAEV YDTVWITLKN MASHPVSLHA VGVsYWKASE GAeyDDQTSQ 120
	REKEDDKVFP GGSHTYVWQV LKENGPMASD PLCLTYsYLS HVDLVKDLNS GLIGALLVCR 180
	EGSLAKEKTQ TLHKFILLFA VFDEGKSWhS ETkNSLMQDR DAASARAWPK MHTVNGVvNR 240
	SLPGLIGCHR KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTL L 300
	MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL TDSEMDVvRF 360
	DDDNsPSFIQ IRsVAKKHPK TWVHYIAAEE EDWDYAPLV L APDDRSYKsQ YLNNGPQRIG 420
	RKYKKVRfMA YDTEfKTR E AIQHESGILG PLLYGEVGD T LLIIFKNQAS RPYNIYPHGI 480
	TDVRPLYSRR LPKGVKHLKD FPILPGEIFK YKwTVTVEDG PTKSDPRCLT RYSSsFVNME 540
	RDLASGLIGP LLICyKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLtEN IQRFLPNPAG 600
	VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDfLS VFFSGYTfKH 660
	KMVYEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR GMTALLKVSS CDKNTGDYYE 720
	DSYEDISAYL LSKNNAIEPR SFSQNGTSES ATPESGPGSE PATSGSETPG TSESATPESG 780
	PGSEPATSGS ETPGTSESAT PESGPGTSTE PSEGsAPGSP AGSPTSTEEG TSESATPESG 840
	PGSEPATSGS ETPGTSESAT PESGPGSPAG SPTSTEEGSP AGSPTSTEEG TSTEPSEGSA 900
	PGTSESATPE SGPTGESAT PESGPGTSES ATPESGPGSE PATSGSETPG SEPATSGSET 960
	PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE PSEGsAPGSE PATSGSETPG TSESATPESG 1020
	PGTSTEPSEG sAPASSEITR TTlQSDQEEI DYDDTISVEM KKEFDIYDE DENQSPRSFQ 1080
	KKTRHYfIAA VERLWDYGMS SSPHVLrNRA QSGSVpQFKK VVFQFTDGS FTQPLYRGEL 1140
	NEHLGLLGPY IRAEVEDNIM VTFRNQASRP YsFYSSLI S EEDQRQGAEP RKNFVKPNET 1200
	KTYFWKVQHH MAPTKDEFDC KAWAYFSdVD LEKDVHSGLI GPLLvCHTNT LNPAGHRQVT 1260
	VQEFALFFTI FDEKTSWYFT ENMERNCRAP CNIQMEDPTF KENYRFHAIN GYIMDTLPGL 1320
	VMAQDQRIrW YLLSMGSNEN IHsIHfSGHV FTVRKKEEYK MALYNLYPGV FETVEMLPsK 1380
	AGIWRVECL I GEHLHAGMST LFLVYSNKcQ TPLGMASGH I RDFQITASGQ YGQWAPKLAR 1440
	LHYSGsINAW STKEPFSWIK VDL LAPMI IH GIKTQGARQK FSSLYISQFI IMYsLDGKkW 1500
	QTYRGNSTGT LMVFFGNVDS SGIKHNI FNP PIIARYIRLH PThYSIRsTL RMELMGCDLN 1560

	SCSMPLGMES KAISDAQITA SSVFTNMFAT WSPSKARLHL QGRSNAWRPQ VNNPKEWLQV 1620 DFQKTMKVTG VTTQGVKSLL TSMYVKEFLI SSSQDGHQWT LFFQNGKVKV FQGNQDSFTP 1680 VVNSLDPPLL TRYLRTHPQS WWHQIALRME VLGCEAQDLY DKHTHTCPPCP APELLGGPSV 1740 FLFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 1800 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPRFPQVYT LPPSRDELTK 1860 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG 1920 NVFSCSVMEH ALHNHYTQKS LSLSPGK	
ELNN polypeptide AE288_2	GSPAGSPTST EEGTSESATP ESGPGSEPAT SGSETPGTSE SATPESGPGT STEPSEGSAP 60 GTSTEPSEGS APGTSTEPSE GSAPGTSTEP SEGSAPTST EPSEGSAPGT STEPSEGSAP 120 GSPAGSPTST EEGTSTEPSE GSAPGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 180 GSEPATSGSE TPGTSESATP ESGPGTSTEP SEGSAPTSE SATPESGPGS PAGSPTSTEE 240 GSPAGSPTST EEGSPAGSPT STEEGTSESA TPESGPGTST EPSEGSAP 288	
ELNN polypeptide AE144_5A	TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG 60 TSTEPSEGA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG 120 SPAGSPTSTE EGSPAGSPTS TEEG	
a2 Linker of chimeric protein	DKNTGDYYED SYEDISAYLL SKNNAIEPRS FS 32	
Signal Peptide of FVIII	MQIELSTCFFLCLLRFCFS	
SEQ ID NO: 15		
FVIII fragment 1 of chimeric protein	ATRRYYLGAV ELSWDYMQSD LGELPVDARF PPRVPKSPFF NTSVVYKKTLL FVEFTDHLFN 60 IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLLHA VGVSYWKASE GAEDDQTSQ 120 REKEDDKVFP GGSHTYVWQV LKENGPMASD PLCLTYSYLS HVDLVKDLNS GLIGALLVCR 180 EGSLAKEKTQ TLHKFILLFA VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR 240 SLPLGIGCHR KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL 300 MDLGQFLLFC HISSHQHDGM EAYVKVDSKP EEPQLRMKN EEAEDYDDDL TDSEMDVVRF 360 DDDNPSFIFQ IRSVAKKHPK TWVHYIAAEE EDWDYAPLVL APDDRSYKSK YLNNGPQRIG 420 RKYKKVRFMA YTDFTFKTRE AIQHESGILG PLYGVEGDT LLIIIFKNQAS RPYNIYPHGI 480 TDVRPLYSRR LPKGVKHLKD FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYSSSFVNME 540 RDLASGLIGP LLICYKESVD QRGNQIMSDK RNVLFSVFD ENRSWYLTEN IQRFLPNPAG 600 VQLDEPFQFA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS VFFSGYTFKH 660 KMYVEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR GMTALLKVSS CDKNTGDYYE 720 DSYEDISAYL LSKNNAIEPR SFSQN	
SEQ ID NO: 17		
FVIII fragment 2 of chimeric protein	EITRTTLQSD QEEIDYDDTI SVEMKKEDFD IYDEDENQSP RSFQKKTRHY FIAAVERLWD 60 YGMSSSPHVL RNRAQSGSVP QFKKVVQEF TDGSFTQPLY RGELEHGL LGPYIRAEVE 120 DNIMVTFRNQ ASRPYSFYSS LISYEEDQRQ GAEPKKNFVK PNETKTYFWK VQHMMAPT KD 180 EFDCKAWAYF SDVDLEKDVH SGLIGPLLVLC HTNLTNPAHG RQVTVQEFAL FFTIFDETKS 240 WYFTENMERN CRAPCNIQME DPTFKENYRF HAINGYIMDT LPGLVMAQDQ RIRWYLLSMG 300 SNENIHSIHF SGHVFTVRKK EYKMALYNL YPGVFETVEM LPSKAGIWRV ECLIGEHLHA 360 GMSTLFLVYS NKQQTPLGMA SGHIRDFQIT ASGQYQGWAP KLARLHYSGS INAWSTKEPF 420 SWIKVDLLAP MIIHGKIQG ARQKFSSLYI SQFIIMYSLD GKKWQTYRGN STGTLMVFFG 480 NVDSSGIKHN IFNPPIIARY IRLHPHYSI RSTLRMELMG CDLNSCSMPL GMESKAISDA 540 QITASSYFTN MFATWSPSKA RLHLQGRSNA WRPQVNNPKE WLQVDFQKTM KVTGVTQGV 600 KSLLTSMYVK EFLISSQDQ HQWTLFFQNG KVKVFQGNQD SFTPVVNSLD PPLLTRYLRI 660 HPQSWWHQIA LRMEVLGCEA QDLY	
SEQ ID NO: 18		
VWF Signal Peptide	MIPARFAGVL LALALILPGT LC	
SEQ ID NO: 19		
VWF D1D2 domain of chimeric protein	AEGTRGRSST ARCSLFGSDF VNTFDGSMYS FAGYCSYLLA GGCQKRSFSI IGDFQNGKRV 60 SLSVYLGEFF DIHLFVNGTV TQGDQRVSMP YASKGLYLET EAGYKLSGE AYGFVARIDG 120 SGNFQVLLSD RYFNKTCGLC GNFNIFAEDD FMTQEGTLTS DPYDFANSWA LSSGEQWCR 180 ASPPSSSCNI SSGEMQKGLW EQCQLLKSTS VFARCHPLVD PEPFVALCEK TLCECAGGLE 240 CACPALLEYA RTCAQEGMVL YGWDHSHACS PVCAPAGMEYR QCVSPCARTC QSLHINEMCQ 300 ERCVDGCSCP EGQLLDEGLC VESTECPCVH SGKRYPPGTS LSRDCNTCIC RNSQWICSNE 360 ECPGECLVTG QSHFKSFDNR YFTFSGICQY LLARDCQDHS FSIVIETVQC ADDRDAVCTR 420	
SEQ ID NO: 20		

	SVTVRLPGLH NSLVKLKHGA GVAMDGQDIQ LPLIKGDLRT QHTVTASVRL SYGEDLQMDW 480 DGRGRLLVKL SPVYAGKTCG LCGNYNGNQG DDFLTTPSGLA EPRVEDFGNA WKLHGDCQDL 540 QKQHSDPCAL NPRMTRFSEE ACAVLTSPTF EACHRAVSPL PYLRNCRYDV CSCSDGRECL 600 CGALASYAAA CAGRGVRVAV REPGRCELNC PKGQVYLQCG TPCNLTCRSL SYPDEECNEA 660 CLEGCFCPPG LYMDERGDCV PKAQCPCYD GEIFQPEDIF SDHHTMCYCE DGFHMCTMSG 720 VPGSLLPDAV LSSPLSHRSK R	
VWF D' domain of chimeric protein SEQ ID NO: 21	SLSCRPPMVK LVCPADNLRA EGLECTKTCQ NYDLECMSMG CVSGCLCPPG MVRHENRCVA 60 LERPCPFHQG KEYAPGETVK IGCNTCVCRD RKWNCTDHVC DAT 103	
VWF D3 domain of chimeric protein SEQ ID NO: 22	CSTIGMAHYL TFDGLKYLFP GECQYVLVQD YCGSNPGTFR ILVGNKGCSH PSVKCKKRV 60 ILVEGGEIEL FDGEVNVKRP MKDETHFEVV ESGRYIILL GKALSVVWDR HLSISVVLKQ 120 TYQEKVCGLC GNFDGIQND LTSSNLQVEE DPVDFGNSWK VSSQCADTRK VPLDSSPATC 180 HNNIMKQTMV DSSCRILTSD VFQDCNKLV D PEPYLDVCYI DTCSCESIGD CAAFCDTIAA 240 YAHVCAQH GK VVTWRTATLC PQSCEERNLR ENGVEAEWRY NSCAPACQVT CQHPEPLACP 300 VQCVEGCHAH CPPGKILDEL LQTCVDPEDC PVCEVAGRRF ASGKKVTLNP SDPEHCQICH 360 CDVVNLCEA CQEP 374	
Fc region SEQ ID NO: 23	DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD 60 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTIKAK 120 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPPVLD 180 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPG	
ELNN polypeptide AE288_3 SEQ ID NO: 24	GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 60 GTSTEPSEGS APGSPAGSPT STEEGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP 120 GSPAGSPTST EEGSPAGSPT STEEGTSTEP SEGSA PGTSE SATPESGPGT SESATPESGP 180 GTSESATPES GPGSEPATSG SETPGSEPAT SGSETPGSPA GSPTSTEEGT STEPSEGSAP 240 GTSTEPSEGS APGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPAS S 291	

Table C. Additional Sequences

Description / SEQ ID NO.	Sequence	
ELNN polypeptide AE576 SEQ ID NO: 25	GSPAGSPTST EEGTESATP ESGPGTSTEP SEGSA PGSPA GSPTSTEEGT STEPSEGSAP 60 GTSTEPSEGS APGTSESATP ESGPGSEPAT SGSETPGSEP ATSGSETPGS PAGSPTSTEE 120 GTSESATPES GPGTSTEPSE GSAPGTSTEP SEGSA PGSPA GSPTSTEEGT STEPSEGSAP 180 GTSTEPSEGS APGTSESATP ESGPGTSTEP SEGSA PGTSE SATPESGPGS EPATSGSETP 240 GTSTEPSEGS APGTSTEPSE GSAPGTSTEP TPESGPGTSE SATPESGPGS PAGSPTSTEE 300 GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPGT STEPSEGSAP 360 GTSTEPSEGS APGTSTEPSE GSAPGTSTEP SEGSA PGTST EPSEGSAPGS PAGSPTSTEE 420 GTSTEPSEGS APGTSESATP ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP 480 GTSESATPES GPGTSTEPSE GSAPGTSESA TPESGPGSPA GSPTSTEEGS PAGSPTSTEE 540 GSPAGSPTST EEGTESATP ESGPGTSTEP SEGSA P 576	
ELNN polypeptide AE864 SEQ ID NO: 26	GTSTEPSEGS APGTSESATP ESGPGTSTEP SEGSA PGTSE SATPESGPGS EPATSGSETP 240 GTSTEPSEGS APGTSTEPSE GSAPGTSESA TPESGPGTST EPSEGSAPGT STEPSEGSAP 300 GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPGT STEPSEGSAP 360 GTSTEPSEGS APGTSTEPSE GSAPGTSTEP SEGSA PGTST EPSEGSAPGS PAGSPTSTEE 420 GTSTEPSEGS APGTSESATP ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP 480 GTSESATPES GPGTSTEPSE GSAPGTSESA TPESGPGSPA GSPTSTEEGS PAGSPTSTEE 540 GSPAGSPTST EEGTESATP ESGPGTSTEP SEGSA PGTSE SATPESGPGS EPATSGSETP 600 GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPGS PAGSPTSTEE 660 GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGSPA GSPTSTEEGS PAGSPTSTEE 720 GTSTEPSEGS APGTSESATP ESGPGTSESA TPESGPGTSE SATPESGPGS EPATSGSETP 780 GSEPATSGSE TPGSPAGSPT STEEGTSTEP SEGSA PGTST EPSEGSAPGS EPATSGSETP 840 GTSESATPES GPGTSTEPSE GSAP 864	
ELNN polypeptide Binding Sequence SEQ ID NO: 27	SATPE	5

ELNN polypeptide Binding Sequence SEQ ID NO: 28	ESATPE 6
Nucleotide sequence of Anti-ELNN Heavy Chain SEQ ID NO: 29	gatgtgcacc tgggtggagtc tggacctgag ctggtgaagc ctggggcttc agtgaagata 60 tcctgcaagg ctcttggtta ttatttact gactacatca tgctctgggt gaagcagagc 120 catggaaaga gccttgagtg gattggaaat attcatcctt actctgatag tactaactac 180 aatctgaagt tcaagggcaa ggccacattg actgtagaca aatcttccag cacagcctac 240 atgaagctca acagtctgac atctgaggac tctgcagtct attactgtgc aagattaggc 300 ccggctatgg actactgggg tcaaggaacc tcagtcacgc tctctcagc tagcacgaag 360 ggccccagcg tgttccccct ggccccagc agcaagagca ccagcggcgg caccgccgcc 420 ctgggctgcc tgggtgaagga ctacttccc gaaccgggta cgggtgtctg gaactcaggc 480 gccctgacca gcggcgtgca cacttccc gctgtcctac agtctcagg actctactcc 540 ctccagcagc tgggtgaccgt gccctcagc agcttgggca cccagacctc catctgcaac 600 gtgaatcaca agcccagcaa cccaaggtg gacaagaaag ttgagcccaa atctgtgtgac 660 aagactcaca catgccacc gtgccagca cctgaactcc tggggggacc gtcagtcttc 720
Amino Acid sequence of Heavy chain of anti-ELNN Seq SEQ ID NO: 30	DVHIVESGPE LVKPGASVKI SCKASGYSFT DYIMLWKQS HGKSLIEWIGN IHPYSDSTNY 60 NLKFKGKATL TVDKSSSTAY MKLNSLTSED SAVVYCARLG PAMDYWGQGT SVTVSSASTK 120 GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS 180 LSSVTVTPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF 240 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR 300 VVSVLTVLHQ DWLNGKEYKC KVSNAKLPAP IEKTIKAKG QPREPQVYTL PPSRDELTKN 360 QVSLTCLVKG FYPSDIAVEW ESNQGPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN 420 VFSCSVMEHA LHNHYTKSL SLSPG 445
Heavy chain variable region of anti-ELNN antibody SEQ ID NO: 31	DVHIVESGPE LVKPGASVKI SCKASGYSFT DYIMLWKQS HGKSLIEWIGN IHPYSDSTNY 60 NLKFKGKATL TVDKSSSTAY MKLNSLTSED SAVVYCARLG PAMDYWGQGT SVTVSS 116
HCDR1 of anti-ELNN antibody SEQ ID NO: 32	DYIML 5
HCDR2 of anti-ELNN antibody SEQ ID NO: 33	NIHPYSDSTN YNLKFKG 17
HCDR3 of anti-ELNN antibody SEQ ID NO: 34	LGPAMDY 7
Nucleotide sequence of Anti-ELNN Light Chain SEQ ID NO: 35	gatgttgtga tgacccaac tccactctct ctgcctgtca gtcttggaga tcaagcctcc 60 atctcttgca gatctagtca gagccttgca catagtaagt gaaacacctt ttacattgg 120 tacctgcaga agacaggcca gtctccaag ctctgtatct acaagtttc caaccgattt 180 tctggggctc cagacagggt cagtggcagt ggatcaggga cagatttacc actcaagatc 240 agcagagtgg aggctgagga tctgggagtt tatttctgct ctcaaagtac atatgatcca 300 ttcacgttcg gctcggggac aaagttggaa ataaaacgta cgggtggctgc accatctgtc 360 ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg 420 ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa 480 tcgggtaact ccagggagag tgtcacagag caggacagca aggacagcac ctacagcctc 540 agcagcacc tgacgtgag caaagcagac tacgagaac acaaagtcta cgcctgcgaa 600 gtcaccatc agggcctgag ctgccccgtc acaaagagct tcaacagggg agagtgt 657
Amino Acid Sequence of Anti-ELNN Light Chain SEQ ID NO: 35	DVVMTQTPLS LPVSLGDQAS ISCRSSQSLA HSNNGNTYLHW YLQKTGQSPK LLIIYKVSNRF 60 SGVPDRFSGS GSGDFTLKI SRVEAEDLGV YFCSQSYDYP FTFGSGTKLE IKRTVAAPSV 120

	FIFPPSDEQL KSGTASVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDYSTYSL 180 SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC 219
Amino Acid sequence of Light Chain Variable Region of Anti-ELNN antibody SEQ ID NO: 37	DVVMQTPLS LPVSLGDQAS ISCRSSQSLA HSNNGTYLHW YLQKTGQSPK LLIYKVSNRF 60 SGVPDRFSGS GSGDTFTLKI SRVEAEDLGV YFCSQSTYDP FTFGSGTKLE IK 112
LCDR1 of anti-ELNN antibody SEQ ID NO: 38	RSSQSLAHSNGNTYLH
LCDR2 of anti-ELNN antibody SEQ ID NO: 39	KVSNRFS
LCDR3 of anti-ELNN antibody SEQ ID NO: 40	SQSTYDPFT
Amino Acid sequence of aELNN-mIgG1 Heavy Chain SEQ ID NO: 41	EIQLQQTGPE LVKPGASVKI SCKASGYSFT DYIMLWKQS HGKSLEWIGN IHPYSDSTNY 60 NLKFKGKATL TVDKSSSTAY MKLNSLTSED SAVYYCARLG PAMDYWGQGA SVTVSSAKTT 120 PPSVYPLAPG SAAQTNSMVT LGCLVKGYFP EPVTVTNSG SLSSGVHTFP AVLQSDLYTL 180 SSSVTVPSST WPSETVTCNV AHPASSTKVD KKIVPRDCGC KPCICTVPEV SSVFIFPPKP 240 KDVLTITLTP KVTCTVVDIS KDDPEVQFSW FVDDVEVHTA QTQPREEQFN STFRSVSELP 300 IMHQDWLNGK EFKCRVNSAA FPAPIEKTIS KTKGRPKAPQ VYTIPPPKEQ MAKDKVSLTC 360 MITDFFPEDI TVEWQWNGQP AENYKNTQPI MDTDGSYFVY SKLNVQKSNW EAGNTFTCSV 420 LHEGLHNHHT EKSLSHSPGK 440
Amino Acid Sequence of aELNN-mIgG1 Heavy Chain Variable Region SEQ ID NO: 42	EIQLQQTGPE LVKPGASVKI SCKASGYSFT DYIMLWKQS HGKSLEWIGN IHPYSDSTNY 60 NLKFKGKATL TVDKSSSTAY MKLNSLTSED SAVYYCARLG PAMDYWGQGA SVTVSS 116
Amino Acid Sequence of aELNN-mIgG1 HCDR1 SEQ ID NO: 43	GYSFTDYI 8
Amino Acid Sequence of aELNN-mIgG1 HCDR2 SEQ ID NO: 44	IHPYSDST 8
Amino Acid Sequence of aELNN-mIgG1 HCDR3 SEQ ID NO: 45	ARLGPAMDY 9
Amino Acid sequence of aELNN-mIgG1 Light Chain SEQ ID NO: 46	DVVMQTPLS LPVSLGDQAS ISCRSSQSLA HSNNGTYLHW YLQKTGQSPK LLIYKVSNRF 60 SGVPDRFSGS GSGDTFTLKI SRVEAEDLGV YFCSQSTYDP FTFGSGTKLE IKRADAAPT 120 SIFPPSSEQL TSGGASVCF LNNFYPKDIN VKWKIDGSER QNGVLNSWTD QDSKDYSTYS 180 SSTLTLTKE YERHNSYTCE ATHKTSTSPI VKSFNRNEC 219

Amino Acid sequence of aELNN-mIgG1 Light Chain Variable Region SEQ ID NO: 47	DVVMQTPLS LPVSLGDQAS ISCRSSQSLA HSNNGNTYLHW YLQKTGQSPK LLIYKVSNRF 60 SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTYDP FTFGSGTKLE IK 112
Amino Acid Sequence of aELNN-mIgG1 LCDR1 SEQ ID NO: 48	QSLAHSNGNT Y 11
Amino Acid Sequence of aELNN-mIgG1 LCDR2 SEQ ID NO: 49	KVS 3
Amino Acid Sequence of aELNN-mIgG1 LCDR3 SEQ ID NO: 50	SQSTYDPFT 9
Amino Acid Sequence of aELNN-mIgG2a Heavy Chain SEQ ID NO: 51	EIQLQQTGPE LVKPGASVKI SCKASGYSFT DYIMLWVKQS HGKSLEWIGN IHPYSDSTNY 60 NLKFKGKATL TVDKSSSTAY MKLNSLTSED SAVYYCARLG PAMDYWGQGA SVTVSSAKTT 120 APSVYPLAPV CGDTTGSSVT LGCLVKGYFP EPVTLTWSG SLSSGVHTFP AVLQSDLYTL 180 SSSVTVTSST WPSQSITCNV AHPASSTKVD KKIEPRGPTI KPCPPCKCPA PNLLGGPSVF 240 IFPPKIKDVL MISLSPIVTC VVVDVSEDDP DVQISWFEVNN VEVHTAQQT HREDYNSTLR 300 VVSALPIQHQ DWMSGKEFKC KVNNDLPAP IERTISKPKG SVRAPQVVVL PPPEEEMTKK 360 QVTLTCMVD FMPEDIYVEW TNNGKTELNY KNTPEVLDSO GSYFMYSKLR VEKKNWVERN 420 SYSCSVVHEG LHNHHTTKSF SRTPG 445
Amino Acid Sequence of aELNN-mIgG2a Light Chain SEQ ID NO: 52	DVVMQTPLS LPVSLGDQAS ISCRSSQSLA HSNNGNTYLHW YLQKTGQSPK LLIYKVSNRF 60 SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTYDP FTFGSGTKLE IKRADAAPT 120 SIFPPSSEQL TSGGASVCF LNNFYPKDIN VKWKIDGSER QNGVLNSWTD QDSKDYSTYS 180 SSTLTCLKDE YERHNSYTCE ATHKTSTSPI VKSFNRNEC 219
Amino Acid Sequence of aELNN-mIgG2a Heavy Chain Variable Region SEQ ID NO: 53	EIQLQQTGPE LVKPGASVKI SCKASGYSFT DYIMLWVKQS HGKSLEWIGN IHPYSDSTNY 60 NLKFKGKATL TVDKSSSTAY MKLNSLTSED SAVYYCARLG PAMDYWGQGA SVTVSS 116
Amino Acid Sequence of aELNN-mIgG2a HCDR1 SEQ ID NO: 54	GYSFTDYI 8
Amino Acid Sequence of aELNN-mIgG2a HCDR2 SEQ ID NO: 55	IHPYSDST 8
Amino Acid Sequence of aELNN-mIgG2a HCDR3 SEQ ID NO: 56	ARLGPAMDY 9
Amino Acid Sequence of aELNN-mIgG2a	DVVMQTPLS LPVSLGDQAS ISCRSSQSLA HSNNGNTYLHW YLQKTGQSPK LLIYKVSNRF 60

Light Chain Variable Region SEQ ID NO: 57	SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTYDP FTFGSGTKLE IK 112
Amino Acid Sequence of aELNN-mIgG2a LCDR1 SEQ ID NO: 58	QSLAHSNGNTY 11
Amino Acid Sequence of aELNN-mIgG2a LCDR2 SEQ ID NO: 59	KVS 3
Amino Acid Sequence of aELNN-mIgG2a LCDR3 SEQ ID NO: 60	SQSTYDPFT 9

[0333] 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 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.

[0334] 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.

[0335] All patents and publications cited herein are incorporated by reference herein in their entirety.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for determining the level of exogenous Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising
 - (i) separating the chimeric protein from a biological sample from the subject with an anti-ELNN antibody or antigen-binding fragment thereof to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample;
 - (ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value; and
 - (iii) reporting the assayed FVIII activity value or an approximated value thereof without correcting the assayed FVIII activity value or approximated value thereof.
2. The method of claim 1, wherein the chimeric protein was administered to the subject less than one week before the sample was taken from the subject.
3. The method of claim 1 or 2, wherein the biological sample is a plasma sample.
4. The method of any one of claims 1 to 3, wherein correcting the assayed FVIII activity value or approximated value thereof comprises reducing the value as if an overestimation of the FVIII activity needs to be accounted for.
5. The method of any one of claims 1 to 4, wherein the anti-ELNN antibody or an antigen binding fragment thereof comprises a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37.
6. The method of any one of claims 1 to 5, wherein the ELNN polypeptide comprises at least 3 instances of the sequence: ESATPE (SEQ ID NO: 29).
7. The method of any one of claims 1 to 6, wherein the ELNN polypeptide comprises the sequence of SEQ ID NO: 14, 24, 25, 26, and/or 27.

8. A method for determining the level of Clotting Factor VIII (FVIII) activity in the blood or plasma of a human subject who has von Willebrand disease (VWD) and to whom a chimeric protein has been administered, wherein the chimeric protein comprises (a) a FVIII protein and (b) an ELNN polypeptide and/or a von Willebrand Factor (VWF) fragment, the method comprising
- (i) assaying the level of FVIII activity in the chimeric protein with an activated partial thromboplastin time (aPTT)-based one stage clotting assay to obtain an assayed FVIII activity value, wherein the aPTT-based one stage clotting assay does;
 - (ii) reducing the assayed FVIII activity value to obtain an adjusted FVIII activity value; and
 - (iii) reporting the adjusted FVIII activity value or an approximated value thereof.
9. The method of claim 8, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide.
10. The method of any one of claims 8-9, wherein the ELNN polypeptide is inserted into the FVIII protein of the chimeric protein.
11. The method any one of claims 8-10, wherein the subject is human.
12. The method any one of claims 1 to 11, wherein the VWD is Type 2N VWD.
13. The method any one of claims 1 to 11, wherein the VWD is Type 3 VWD.
14. A method for determining the level of Clotting Factor VIII (FVIII) activity from a chimeric protein in a biological sample, wherein the chimeric protein comprises a FVIII protein and an ELNN polypeptide, the method comprising
- (i) separating the chimeric protein from the biological sample from the subject with an anti-ELNN antibody to obtain a captured chimeric protein sample, wherein the biological sample is a blood sample or a plasma sample; and
 - (ii) assaying the level of FVIII activity in the captured chimeric protein with a chromogenic assay to obtain an assayed FVIII activity value.
15. The method of any one of claims 1 to 14, wherein the chimeric protein comprises a VWF fragment.

16. The method of claim 15, wherein the VWF fragment comprises a D'D3 fragment of VWF.
17. The method of claim 16, wherein the VWF fragment consists of a D'D3 fragment of VWF.
18. The method of any one of claims 16-17, wherein the D'D3 fragment of VWF is mutated such that it cannot form disulfide binds with another D'D3 fragment of VWF.
19. The method of any one of claims 1 to 18, wherein the FVIII protein comprises at least a partial deletion of the wild-type FVIII B domain.
20. The method of any one of claims 1 to 19 wherein the chimeric protein is a FVIII-ELNN-Fc/D'D3-ELNN-Fc heterodimer.
21. The method of any one of claims 1 to 20, wherein the chimeric protein is efanesoctocog alfa.
22. A method of monitoring the level of FVIII in the blood or plasma of a human subject who is receiving treatment for Willebrand disease (VWD), the method comprising performing the method of any one of claims 1-21 at least once per year.
23. The method of any one of claims 1 to 22, wherein multiple doses of the chimeric protein are administered to the subject at a dosing interval.
24. The method of claim 23, wherein at least one of the multiple doses is from about 10 IU/kg to about 30 IU/kg and the dosing interval is at least about 7 days.
25. The method of claim 24, wherein at least one of the multiple doses is from about 25 IU/kg and the dosing interval is at least about 7 days.
26. An antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, wherein the antibody or antigen-binding fragment thereof comprises three light chain complementarity determining region (CDR) sequences of SEQ ID NOs: 38, 39 and 40, and three heavy chain complementarity determining region (CDR) sequences of SEQ ID NOs: 32, 33 and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).

27. An antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising three heavy-chain CDRs of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:32 and three light-chain CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:37 and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).
28. An antibody or antigen-binding fragment thereof, which binds an ELNN polypeptide, comprising a heavy chain variable region comprising three heavy-chain CDRs, and a light chain variable region comprising three light-chain CDRs, wherein said three heavy-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:32 (HCVR), and said three light-chain CDRs comprise the CDR1, CDR2 and CDR3 from SEQ ID NO:37 (LCVR), and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: ESATPE (SEQ ID NO: 29).
29. The antibody or antigen-binding fragment of any one of claims 66 to 69, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO:37.
30. A monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen binding fragment thereof binds to an ELNN polypeptide and comprises (i) a heavy chain variable region which is at least 98% identical to SEQ ID NO: 32 and (ii) a light chain variable region which is at least 98% identical to SEQ ID NO: 37, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).
31. An antigen binding protein comprising (i) a light chain variable domain comprising an amino acid sequence at least 90% identical to a light chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs 38, 39 and 40, and (ii) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to a heavy chain variable domain sequence comprising a CDR sequence selected from the group consisting of SEQ ID NOs: 32, 33, and 34, and wherein the ELNN polypeptide comprises at least 3 instances of the following sequence: SATPE (SEQ ID NO: 28).
32. An antibody or antigen-binding fragment comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and a light chain comprising the amino acid sequence of SEQ ID NO:36.

- 33. A polynucleotide or set of polynucleotides encoding the antibody, antigen-binding fragment, or antigen binding protein of any one of claims 26-32.
- 34. A vector comprising the polynucleotide or set of polynucleotides of claim 33.
- 35. A host cell comprising the vector of claim 34.
- 36. The host cell of claim 35, wherein the host cell is a CHO or HEK293 cell.