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BLOOD, vol. 130, no. Suppl. 1, 7 December 2017 (2017-12-07), 59TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ATLANTA, GA, USA; DECEMBER 09 -12, 2017, pages 3682, ISSN: 0006-4971(print)
VICKY MCDONALD: "Inherited bleeding disorders", 1 April 2017 (2017-04-01), XP055507424, Retrieved from the Internet

Fortsættes ...

<URL:<https://www.sciencedirect.com/science/article/pii/S1357303917300269/pdfft?md5=52b3ef379c66ae1c92737a7e5f2410ce&pid=1-s2.0-S1357303917300269-main.pdf>> [retrieved on 20180917]

DESCRIPTION

Description

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/546,999, filed on August 17, 2017, and U.S. Provisional Patent Application No. 62/530,024, filed on July 7, 2017.

BACKGROUND OF THE INVENTION

[0002] Coagulation diseases, such as von Willebrand Disease (VWD) generally result from a deficiency in the coagulation cascade. von Willebrand Disease (VWD) refers to the group of diseases caused by a deficiency of von Willebrand factor. Von Willebrand factor helps blood platelets clump together and stick to the blood vessel wall, which is necessary for normal blood clotting.

[0003] von Willebrand disease (VWD) is the most common inherited bleeding disorder, with an estimated prevalence rate of 1% (Veyradier A, et al., Medicine (Baltimore). 2016, 95(11):e3038). However, excluding milder forms of the disease, only about 1/10,000 patients actually require treatment. Current treatment for these coagulopathies includes a replacement therapy using pharmaceutical preparations comprising the normal coagulation factor.

[0004] VWF is a glycoprotein circulating in plasma as a series of multimers ranging in size from about 500 to 20,000 kD. The full length of cDNA of VWF has been cloned; the propolypeptide corresponds to amino acid residues 23 to 764 of the full length prepro-VWF (Eikenboom et al (1995) Haemophilia 1, 77-90). Multimeric forms of VWF are composed of 250 kD polypeptide subunits linked together by disulfide bonds. VWF mediates the initial platelet adhesion to the sub-endothelium of the damaged vessel wall, with the larger multimers exhibiting enhanced hemostatic activity. Multimerized VWF binds to the platelet surface glycoprotein Gp1b α , through an interaction in the A1 domain of VWF, facilitating platelet adhesion. Other sites on VWF mediate binding to the blood vessel wall. Thus, VWF forms a bridge between the platelet and the vessel wall that is essential to platelet adhesion and primary hemostasis under conditions of high shear stress. Normally, endothelial cells secrete large polymeric forms of VWF and those forms of VWF that have a lower molecular weight arise from proteolytic cleavage. The multimers of exceptionally large molecular masses are stored in the Weibel-Pallade bodies of the endothelial cells and liberated upon stimulation by agonists such as thrombin and histamine.

For patients with VWD, it is recommended that they be treated with von Willebrand factor (VWF) replacement given the need for prolonged hemostasis, particularly in major surgery (Mannucci PM and Franchini M., Haemophilia, 2017, 23(2): 182-187; National Institutes of Health. National Heart, Lung, and Blood Institute. The Diagnosis, Evaluation, and Management of von Willebrand Disease NIH Publication No. 08-5832; December, 2007). Plasma-derived VWF therapies contain factor VIII (FVIII) and have the potential for FVIII accumulation with repeated dosing. VONVENDI[®] (von Willebrand factor [recombinant], Shire, Westlake Village, CA) is the first and only recombinant VWF (rVWF) concentrate (Turecek PL, et al. Hamostaseologie. 2009;29(suppl 1):532-38; Mannucci PM, et al. Blood, 2013;122(5):648-657; Gill JC, et al. Blood, 2015;126(17):2038-2046). EP 1 593 390 refers to a treatment with plasma-derived VWF and does not disclose the present specific dosage regimen.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides for recombinant von Willebrand Factor (rVWF) for use in methods of pre-treatment for a patient with severe von Willebrand disease prior to a surgical procedure by administering 20-60 IU/kg recombinant von Willebrand Factor (rVWF) to the patient between 12 hours and 24 hours, e.g., 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 12 hours and 24 hours, 14 hours and 24 hours, 16 and 24 hours, 18 hours and 24 hours, or 20 hours and 24 hours prior to the surgical procedure, and not administering Factor VIII (FVIII) with the rVWF prior to the surgical procedure. In some embodiments, the rVWF for use in the method of pre-treating further comprises administering to the subject 5-90 IU/kg rVWF 1 hour prior to surgery. In some embodiments, the subject is administered 70-200 IU rVWF after the surgery, either with or without the pre-treatment described above. In some cases, the surgical procedure is selected from a group consisting of major surgery, minor surgery, and oral surgery.

[0006] In some embodiments, the subject is administered 35-60 IU/kg rVWF between 12 hours and 24 hours prior to a major surgical procedure. In other embodiments, the subject is administered 15-90 ILT/kg rVWF 1 hour prior to major surgical procedure. In another embodiment, the subject is administered 150-220 IU/kg rVWF after a major surgical procedure. In some instances, the subject undergoing a major surgical procedure is administered a total dosage of 220-320 ILT/kg. In some instances, when the surgical procedure is a major surgical procedure and the pre-treatment comprises administering at least two approximately equal doses of rVWF prior to the surgical procedure. By "approximately equal" as used herein refers to doses that have concentrations within 1-15%, 2-14%, 3-13%, 4-12%, 5-11%, 6-10%, 7-9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20% of each other.

[0007] In some embodiments, the subject is administered 50-60 IU/kg rVWF between 12 hours and 24 hours prior a minor surgical procedure. In other embodiments, the subject is administered 5-50 ILT/kg rVWF 1 hour prior to minor surgery. In another embodiment, the subject is administered 70-150 IU/kg rVWF after a minor surgical procedure. In some instances, the subject undergoing a minor surgical procedure is administered a total dosage of 100-220 IU/kg. In some instances, when the surgical procedure is a minor surgical procedure, the pre-treatment comprises administering at least two doses of rVWF prior to the surgical procedure, wherein the first dose is larger than the second dose.

[0008] In some embodiments, the subject is administered 20-40 ILT/kg rVWF between 12 hours and 24 hours prior to an oral surgical procedure. In other embodiments, the subject is administered 20-50 IU/kg rVWF 1 hour prior to the oral surgical procedure. In another embodiment, the subject is administered 10-50 IU/kg rVWF during the oral surgical procedure. In another embodiment, the subject is administered 70-150 IU/kg rVWF after an oral surgical procedure. In some instances, the subject undergoing an oral surgical procedure is administered a total dosage of 70-190 IU/kg. In some instances, when

the surgical procedure is an oral surgical procedure and the pre-treatment comprises administering at least two approximately equal doses of rVWF prior to the surgical procedure.

[0009] Other objects, advantages and embodiments of the invention will be apparent from the detailed description following.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

FIG. 1 shows overall hemostatic efficiency (primary endpoint) in the study patients.

FIG. 2 shows hemostatic efficiency (secondary endpoint) in the study patients.

FIG. 3 shows baseline demographics and clinical characteristics.

FIG. 4 shows PK parameters for VWF:RCo (n=11).

FIG. 5A and FIG. 5B shows mean VWF:RCo and Endogenous FVIII:C Levels in Response to rVWF 50±5 IU rVWF:RCo/kg in all Patients with VWD With PK data analyzed (n=11) (FIG. 5A), and the subset of patients with type 3 VWD (n=5) (FIG. 5B).

FIG. 6 shows VWF nucleic acid and amino acid sequences.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0011] The present invention provides rVWF for use in methods for pretreating a patient with severe von Willebrand disease prior to surgery by administering recombinant von Willebrand Factor (rVWF) to the patient 45-60 IG/kg rVWF without administering Factor VIII with the rVWF prior to the surgical procedure. In some cases, the surgical procedure is selected from a group consisting of major surgery, minor surgery, and oral surgery.

Definitions

[0012] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies and reference to "a host cell" includes reference to one or more host cells and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0013] Before the invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0014] The term "pre-propeptide VWF," "prepro-VWF" or "pro-VWF" refers to a non-mature VWF polypeptide comprising a signal peptide of about 22 amino acid residues, a VWF propeptide of about 741 amino acid residues, and a mature VWF subunit of about 2050 amino acid residues. Pro-VWF subunits can dimerize through disulfide bonds near their carboxyl termini in the endoplasmic reticulum to form tail-to tail dimers which are then transported to the Golgi. In the Golgi, additional head-to-head disulfide bonds are formed near the amino-termini of the subunits, thereby forming multimers. Proteolytic cleavage of the VWF propeptide occurs via the processing protease furin, thus producing a mature VWF/VWF-PP complex. When "r" is included prior to the VWF designation, this refers to the recombinant version. In some embodiments, the methods described herein apply to recombinant VWF (rVWF).

[0015] The term "VWF complex" or "mat-VWF/VWF-PP complex" refers to a non-covalently linked heterodimeric structure comprising a mature VWF subunit and VWF propeptide. The VWF complex can be generated as a product of furin cleavage between the propeptide portion and mature VWF portion of the pre-propeptide VWF. When "r" is included prior to the VWF designation, this refers to the recombinant version. In some embodiments, the rVWF for the use described herein apply to recombinant VWF (rVWF). As used herein, "rVWF" refers to recombinant VWF.

[0016] As used herein, "rFVIII" refers to recombinant FVIII.

[0017] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0018] As used herein, "recombinant VWF" includes VWF obtained via recombinant DNA technology. In certain embodiments, VWF proteins for the use of the invention can comprise a construct, for example, prepared as in WO 1986/06096 published on Oct. 23, 1986 and U.S. patent application Ser. No. 07/559,509, filed on Jul. 23, 1990, in the name of Ginsburg et al. The VWF for the of the present invention can include all potential forms, including the monomeric and multimeric forms. It should also be understood that the use of the present invention encompasses different forms of VWF to be used in combination. For example, the VWF for the use of the present invention may include different multimers, different derivatives and both biologically active derivatives and derivatives not biologically active.

[0019] In the context of the use of the present invention, the recombinant VWF embraces any member of the VWF family from, for example, a mammal such as a primate, human, monkey, rabbit, pig, rodent, mouse, rat, hamster, gerbil, canine, feline, and biologically active derivatives thereof. Mutant and variant VWF proteins having activity are also embraced, as are functional fragments and fusion proteins of the VWF proteins. Furthermore, the VWF for the use of the invention may further comprise tags that facilitate purification, detection, or both. The VWF described herein may further be modified with a therapeutic moiety or a moiety suitable imaging in vitro or in vivo.

[0020] As used herein, "plasma-derived VWF (pdVWF)" includes all forms of the protein found in blood including the mature VWF obtained from a mammal having the property of in vivo-stabilizing, e.g. binding, of at least one FVIII molecule.

[0021] The term "highly multimeric VWF" or "high molecular weight VWF" refers to VWF comprising at least 10 subunits, or 12, 14, or 16 subunits, to about 20, 22, 24 or 26 subunits or more. The term "subunit" refers to a monomer of VWF. As is known in the art, it is generally dimers of VWF that polymerize to form the larger order multimers (see Turecek et al., *Semin. Thromb. Hemost.* 2010, 36(5): 510-521).

[0022] As used herein, the term "factor VIII" or "FVIII" refers to any form of factor VIII molecule with the typical characteristics of blood coagulation factor VIII, whether endogenous to a patient, derived from blood plasma, or produced through the use of recombinant DNA techniques, and including all modified forms of factor VIII. Factor VIII (FVIII) exists naturally and in therapeutic preparations as a heterogeneous distribution of polypeptides arising from a single gene product (see, e.g., Andersson et al., *Proc. Natl. Acad. Sci. USA*, 83:2979-2983 (1986)). Commercially available examples of therapeutic preparations containing Factor VIII include those sold under the trade names of HEMOFIL M, ADVATE, and RECOMBINATE (available from Baxter Healthcare Corporation, Deerfield, Ill., U.S.A.).

[0023] As used herein, "plasma FVIII activity" and "in vivo FVIII activity" are used interchangeably. The in vivo FVIII activity measured using standard assays may be endogenous FVIII activity, the activity of a therapeutically administered FVIII (recombinant or plasma derived), or both endogenous and administered FVIII activity. Similarly, "plasma FVIII" refers to endogenous FVIII or administered recombinant or plasma derived FVIII.

[0024] As used herein "von Willebrand Disease" refers to the group of diseases caused by a deficiency of von Willebrand factor. Von Willebrand factor helps blood platelets clump together and stick to the blood vessel wall, which is necessary for normal blood clotting. As described in further detail herein, there are several types of Von Willebrand disease including type 1, 2A, 2B, 2M and 3.

[0025] The terms "isolated," "purified," or "biologically pure" refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. VWF is the predominant species present in a preparation is substantially purified. The term "purified" in some embodiments denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. In other embodiments, it means that the nucleic acid or protein is at least 50% pure, more preferably at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more pure. "Purify" or "purification" in other embodiments means removing at least one contaminant from the composition to be purified. In this sense, purification does not require that the purified compound be homogenous, e.g., 100% pure.

[0026] As used herein, "administering" (and all grammatical equivalents) includes intravenous administration, intramuscular administration, subcutaneous administration, oral administration, administration as a suppository, topical contact, intraperitoneal, intralesional, or intranasal administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route including parenteral, and transmucosal (e.g., oral, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc.

[0027] The terms "therapeutically effective amount or dose" or "therapeutically sufficient amount or dose" or "effective or sufficient amount or dose" refer to a dose that produces therapeutic effects for which it is administered. For example, a therapeutically effective amount of a drug useful for treating hemophilia can be the amount that is capable of preventing or relieving one or more symptoms associated with hemophilia. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and Remington: *The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0028] As used herein, the terms "patient" and "subject" are used interchangeably and refer to a mammal (preferably human) that has a disease or has the potential of contracting a disease.

[0029] As used herein, the term "about" denotes an approximate range of plus or minus 10% from a specified value. For instance, the language "about 20%" encompasses a range of 18-22%.

[0030] As used herein, the term "half-life" refers to the period of time it takes for the amount of a substance undergoing decay (or clearance from a sample or from a patient) to decrease by half.

I. Recombinant von Willebrand Factor (rVWF)

[0031] The present invention utilizes compositions comprising von Willebrand Factor (rVWF) for pretreatment of subject with severe VWD who are undergoing a surgical procedure, such as, but not limited to, major surgery, minor surgery, or oral surgery as further defined in the claims.

[0032] In certain embodiments, VWF proteins for the use of the invention may comprise a construct, for example, prepared as in WO 1986/06096 published on Oct. 23, 1986 and U.S. patent application Ser. No. 07/559,509, filed on Jul. 23, 1990, in the name of Ginsburg et al. The VWF useful for the present invention includes all potential forms, including the monomeric and multimeric forms. One particularly useful form of VWF are homo-multimers of at least two VWFs. The VWF proteins may be either a biologically active derivative, or when to be used solely as a stabilizer for FVIII the VWF may be of a form not biologically active. It should also be understood that the present invention encompasses different forms of VWF to be used in combination. For example, a composition useful for the present invention may include different multimers, different derivatives and both biologically active derivatives and derivatives not biologically active.

[0033] In primary hemostasis VWF serves as a bridge between platelets and specific components of the extracellular matrix, such as collagen. The biological activity of VWF in this process can be measured by different *in vitro* assays (Turecek et al., *Semin. Thromb. Hemost.* 28: 149-160, 2002). The ristocetin cofactor assay is based on the agglutination of fresh or formalin-fixed platelets induced by the antibiotic ristocetin in the presence of VWF.

[0034] The degree of platelet agglutination depends on the VWF concentration and can be measured by the turbidimetric method, *e.g.* by use of an aggregometer (Weiss et al., *J. Clin. Invest.* 52: 2708-2716, 1973; Macfarlane et al., *Thromb. Diath. Haemorrh.* 34: 306-308, 1975). The second method is the collagen binding assay, which is based on ELISA technology (Brown et Bosak, *Thromb. Res.* 43: 303-311, 1986; Favalaro, *Thromb. Haemost.* 83: 127-135, 2000). A microtiter plate is coated with type I or III collagen. Then the VWF is bound to the collagen surface and subsequently detected with an enzyme-labeled polyclonal antibody. The last step is the substrate reaction, which can be photometrically monitored with an ELISA reader. As provided herein, the specific Ristocetin Cofactor activity of the VWF (VWF:RCO) for the use of the present invention is generally described in terms of mU/ μ g of VWF, as measured using *in vitro* assays.

[0035] An advantage of the rVWF compositions for the use of the present invention over pdVWF is that rVWF exhibits a higher specific activity than pdVWF. In some embodiments, the rVWF for the use of the invention has a specific activity of at least about 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, 57.5, 60, 62.5, 65, 67.5, 70, 72.5, 75, 77.5, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150 or more mU/ μ g.

[0036] The rVWF for the use of the present invention is highly multimeric comprising about 10 to about 40 subunits. In further embodiments, the multimeric rVWF produced using methods of the present specification comprise about 10-30, 12-28, 14-26, 16-24, 18-22, 20-21 subunits. In further embodiments, the rVWF is present in multimers varying in size from dimers to multimers of over 40 subunits (>10 million Daltons). The largest multimers provide multiple binding sites that can interact with both platelet receptors and subendothelial matrix sites of injury, and are the most hemostatically active form of VWF. Application of ADAMTS13 will cleave the ultra-large rVWF multimers over time, but during production (generally through expression in cell culture), rVWF compositions for the use of the present invention are generally not exposed to ADAMTS13 and retain their highly multimeric structure.

[0037] In one embodiment, a rVWF composition as used herein has a distribution of rVWF oligomers characterized in that 95% of the oligomers have between 6 subunits and 20 subunits. In other embodiments, the a rVWF composition has a distribution of rVWF oligomers characterized in that 95% of the oligomers have a range of subunits selected from variations 458 to 641 found in Table 2 of WO 2012/171031.

[0038] In one embodiment, a rVWF composition can be characterized according to the percentage of rVWF molecules that are present in a particular higher order rVWF multimer or larger multimer. For example, in one embodiment, at least 20% of rVWF molecules in a rVWF composition used in the methods described herein are present in an oligomeric complex of at least 10 subunits. In another embodiment, at least 20% of rVWF molecules in a rVWF composition used as described herein are present in an oligomeric complex of at least 12 subunits. In yet other embodiments, a rVWF composition used as provided herein has a minimal percentage (*e.g.*, has at least X %) of rVWF molecules present in a particular higher-order rVWF multimer or larger multimer (*e.g.*, a multimer of at least Y subunits) according to any one of variations 134 to 457 found in Table 3 to Table 5.

[0039] In accordance with the above, the rVWF composition administered to the subject (with or without FVIII) generally comprises a significant percentage of high molecular weight (HMW) rVWF multimers. In further embodiments, the HMW rVWF multimer composition comprises at least 10%-80% rVWF decamers or higher order multimers. In further embodiments, the composition comprises about 10-95%, 20-90%, 30-85%, 40-80%, 50-75%, 60-70% decamers or higher order multimers. In further embodiments, the HMW rVWF multimer composition comprises at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% decamers or higher order multimers.

[0040] Assessment of the number and percentage of rVWF multimers can be conducted using methods known in the art, including without limitation methods using electrophoresis and size exclusion chromatography methods to separate VWF multimers by size, for example as discussed by Cumming et al. (*J Clin Pathol.* 1993 May; 46(5): 470-473. Such techniques may further include immunoblotting techniques (such as Western Blot), in which the gel is immunoblotted with a radiolabeled antibody against VWF followed by chemiluminescent detection (see for example Wen et al., (1993), *J. Clin. Lab. Anal.*, 7: 317-323. Further assays for VWF include VWF:Antigen (VWF:Ag), VWF:Ristocetin Cofactor (VWF:RCof), and VWF:Collagen Binding Activity assay (VWF:CBA), which are often used for diagnosis and classification of Von Willebrand Disease. (see for example Favalaro et al., *Pathology*, 1997, 29(4): 341-456.

[0041] In further embodiments, higher order rVWF multimers for the use of the invention are stable for about 1 to about 90 hours post-administration. In still further embodiments, the higher order rVWF multimers are stable for about 5-80, 10-70, 15-60, 20-50, 25-40, 30-35 hours post-administration. In yet further embodiments, the higher order rVWF multimers are stable for at least 3, 6, 12, 18, 24, 36, 48, 72 hours post-administration. In certain embodiments the stability of the rVWF multimers is assessed *in vitro*.

[0042] In one embodiment, higher order rVWF multimers used in the compositions and methods provided herein have a half-life of at least 12 hour post administration. In another embodiment, the higher order rVWF multimers have a half-life of at least 24 hour post administration. In yet other embodiments, the higher order rVWF multimers have a half-life selected from variations 642 to 1045 found in Table 6 of WO 2012/171031.

[0043] In specific aspects, the rVWF (recombinant or plasma derived) used in accordance with the present invention are not modified with any conjugation, post-translation or covalent modifications. In particular embodiments, the rVWF for the use of the present invention is not modified with a water soluble polymer, including without limitation, a polyethylene glycol (PEG), a polypropylene glycol, a polyoxyalkylene, a polysialic acid, hydroxyl ethyl starch, a poly-carbohydrate moiety, and the like.

[0044] In other aspects, the rVWF (recombinant or plasma derived) used in accordance with the present invention is modified through conjugation, post-translation modification, or covalent modification, including modifications of the N- or C-terminal residues as well as modifications of selected side chains, for example, at free sulfhydryl-groups, primary amines, and hydroxyl-groups. In one embodiment, a water soluble polymer is linked to the protein (directly or via a linker) by a lysine group or other primary amine. In one embodiment, the rVWF proteins for the use of the present invention may be modified by conjugation of a water soluble polymer, including without limitation, a polyethylene glycol (PEG), a polypropylene glycol, a polyoxyalkylene, a polysialic acid, hydroxyl ethyl starch, a poly-carbohydrate moiety, and the like.

[0045] Water soluble polymers that may be used to modify the rVWF and/or FVIII include linear and branched structures. The conjugated polymers may be attached directly to the coagulation proteins for the use of the invention, or alternatively may be attached through a linking moiety. Non-limiting examples of

protein conjugation with water soluble polymers can be found in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192, and 4,179,337, as well as in Abuchowski and Davis "Enzymes as Drugs," Holcenberg and Roberts, Eds., pp. 367-383, John Wiley and Sons, New York (1981), and Hermanson G., Bioconjugate Techniques 2nd Ed., Academic Press, Inc. 2008.

[0046] Protein conjugation may be performed by a number of well-known techniques in the art, for example, see Hermanson G., Bioconjugate Techniques 2nd Ed., Academic Press, Inc. 2008. Examples include linkage through the peptide bond between a carboxyl group on one of either the coagulation protein or water-soluble polymer moiety and an amine group of the other, or an ester linkage between a carboxyl group of one and a hydroxyl group of the other. Another linkage by which a coagulation protein for the use of the invention could be conjugated to a water-soluble polymer compound is via a Schiff base, between a free amino group on the polymer moiety being reacted with an aldehyde group formed at the non-reducing end of the polymer by periodate oxidation (Jennings and Lugowski, J. Immunol. 1981; 127:1011-8; Fernandes and Gregonradis, Biochim Biophys Acta. 1997; 1341; 26-34). The generated Schiff Base can be stabilized by specific reduction with NaCNBH₃ to form a secondary amine. An alternative approach is the generation of terminal free amino groups on the polymer by reductive amination with NH₄Cl after prior oxidation. Bifunctional reagents can be used for linking two amino or two hydroxyl groups. For example a polymer containing an amino group can be coupled to an amino group of the coagulation protein with reagents like BS3 (Bis(sulfosuccinimidyl)suberate/Pierce, Rockford, Ill.). In addition heterobifunctional cross linking reagents like Sulfo-EMCS (N-epsilon-Maleimidocaproyloxy) sulfosuccinimide ester/Pierce) can be used for instance to link amine and thiol groups. In other embodiments, an aldehyde reactive group, such as PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, and PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, succinimidyl active esters, activated dithiocarbonate P.E.G., 2,4,5-trichlorophenylchloroformate and P-nitrophenylchloroformate activated P.E.G., may be used in the conjugation of a coagulation protein.

[0047] In some aspects, the rVWF for the use of the present invention has been matured in vitro with furin. In further embodiments, the furin is recombinant furin.

[0048] In further aspects, the rVWF for the use of the present invention are produced by expression in a mammalian cell culture using methods known in the art. In particular embodiments, the mammalian culture comprises CHO cells. In an exemplary embodiment, the rVWF for the use of the invention comprises rVWF protein isolated from a CHO cell expression system. In a further embodiment, the propeptide removal is mediated in vitro through exposure of the pro-VWF to furin--in a still further embodiment, the Furin used for propeptide removal is recombinant furin. In as yet further embodiment, fully glycosylated/ABO blood group glycans are absent.

[0049] In yet further embodiments, the rVWF for the use of the present invention by expression in a suitable eukaryotic host system. Examples of eukaryotic cells include, without limitation, mammalian cells, such as CHO, COS, HEK 293, BHK, SK-Hep, and HepG2; insect cells, e.g., SF9 cells, SF21 cells, S2 cells, and High Five cells; and yeast cells, e.g., Saccharomyces or Schizosaccharomyces cells. In one embodiment, the VWF can be expressed in yeast cells, insect cells, avian cells, mammalian cells, and the like. For example, in a human cell line, a hamster cell line, or a murine cell line. In one particular embodiment, the cell line is a CHO, BHK, or HEK cell line. Typically, mammalian cells, e.g., CHO cell from a continuous cell line, can be used to express the VWF for the use of the present invention.

[0050] In certain embodiments, the nucleic acid sequence comprising a sequence coding for VWF can be a vector. The vector can be delivered by a virus or can be a plasmid. The nucleic acid sequence coding for the protein can be a specific gene or a biologically functional part thereof. In one embodiment, the protein is at least a biologically active part of VWF. A wide variety of vectors can be used for the expression of the VWF and can be selected from eukaryotic expression vectors. Examples of vectors for eukaryotic expression include: (i) for expression in yeast, vectors such as pAO, pPIC, pYES, pMET, using promoters such as AOX1, GAP, GAL1, AUG1, etc.; (ii) for expression in insect cells, vectors such as pMT, pAc5, pIB, pMIB, pBAC, etc., using promoters such as PH, p10, MT, Ac5, OplE2, gp64, polh, etc., and (iii) for expression in mammalian cells, vectors such as pSVL, pCMV, pRc/RSV, pCDNA3, pBPV, etc., and vectors derived from viral systems such as vaccinia virus, adeno-associated viruses, herpes viruses, retroviruses, etc., using promoters such as CMV, SV40, EF-1, UbC, RSV, ADV, BPV, and β -actin.

[0051] In some embodiments of the present invention, the nucleic acid sequence further comprises other sequences suitable for a controlled expression of a protein such as promoter sequences, enhancers, TATA boxes, transcription initiation sites, polylinkers, restriction sites, poly-A-sequences, protein processing sequences, selection markers, and the like which are generally known to a person of ordinary skill in the art.

[0052] In certain embodiments, the cell-culture methods of the specification may comprise the use of a microcarrier. In some embodiments, the cell-cultures of the embodiments can be performed in large bioreactors under conditions suitable for providing high volume-specific culture surface areas to achieve high cell densities and protein expression. One means for providing such growth conditions is to use microcarriers for cell-culture in stirred tank bioreactors. The concept of cell-growth on microcarriers was first described by van Wèzel (van Wèzel, A. L., Nature 216:64-5 (1967)) and allows for cell attachment on the surface of small solid particles suspended in the growth medium. These methods provide for high surface-to-volume ratios and thus allow for efficient nutrient utilization. Furthermore, for expression of secreted proteins in eukaryotic cell lines, the increased surface-to-volume ratio allows for higher levels of secretion and thus higher protein yields in the supernatant of the culture. Finally, these methods allow for the easy scale-up of eukaryotic expression cultures.

[0053] The cells expressing VWF can be bound to a spherical or a porous microcarrier during cell culture growth. The microcarrier can be a microcarrier selected from the group of microcarriers based on dextran, collagen, plastic, gelatine and cellulose and others as described in Butler (1988. In: Spier & Griffiths, Animal Cell Biotechnology 3:283-303). It is also possible to grow the cells to a biomass on spherical microcarriers and subculture the cells when they have reached final fermenter biomass and prior to production of the expressed protein on a porous microcarrier or vice versa. Suitable spherical microcarriers can include smooth surface microcarriers, such as Cytodex™ 1, Cytodex™ 2, and Cytode™ 3 (GE Healthcare) and macroporous microcarriers such as Cytopore™ 1, Cytopore™ 2, Cytoline™ 1, and Cytoline™ 2 (GE Healthcare).

[0054] In certain embodiments, rVWF is expressed in cells cultured in cell culture media that produces high molecular weight rVWF. The terms "cell culture solution," "cell culture medium or media," and "cell culture supernatant" refer to aspects of cell culture processes generally well known in the art. In the context of the present invention, a cell culture solution can include cell culture media and cell culture supernatant. The cell culture media are externally added to the cell culture solution, optionally together with supplements, to provide nutrients and other components for culturing the cells expressing VWF. The cell culture supernatant refers to a cell culture solution comprising the nutrients and other components from the cell culture medium as well as products released, metabolized, and/or excreted from the cells during culture. In further embodiments, the media can be animal protein-free and chemically defined. Methods of preparing animal protein-free and chemically defined culture media are known in the art, for example in US 2008/0009040 and US 2007/0212770. "Protein free" and related terms refers to protein that is from a source exogenous to or other than the cells in the culture, which naturally

shed proteins during growth. In another embodiment, the culture medium is polypeptide free. In another embodiment, the culture medium is serum free. In another embodiment the culture medium is animal protein free. In another embodiment the culture medium is animal component free. In another embodiment, the culture medium contains protein, *e.g.*, animal protein from serum such as fetal calf serum. In another embodiment, the culture has recombinant proteins exogenously added. In another embodiment, the proteins are from a certified pathogen free animal. The term "chemically defined" as used herein shall mean, that the medium does not comprise any undefined supplements, such as, for example, extracts of animal components, organs, glands, plants, or yeast. Accordingly, each component of a chemically defined medium is accurately defined. In a preferred embodiment, the media are animal-component free and protein free.

[0055] In further embodiments, subsequent to purification from a mammalian cell culture, rFVIII is reconstituted prior to administration. In still further embodiments, the rVWF is treated with furin prior to or subsequent to reconstitution. In further embodiments, the Furin is recombinant furin. In still further embodiments, the rVWF for the use of the invention is not exposed to ADAMTS13, with the result that ultra large (*i.e.*, comprising 10 or more subunits) are present in rVWF compositions for the use of the invention.

[0056] In specific aspects, the rVWF used in the present invention is contained in a formulation containing a buffer, a sugar and/or a sugar alcohol (including without limitation trehalose and mannitol), a stabilizer (such as glycine), and a surfactant (such as polysorbate 80). In further embodiments, for formulations containing rFVIII, the formulation may further include sodium, histidine, calcium, and glutathione.

[0057] In one aspect, the formulations comprising rVWF is lyophilized prior to administration. Lyophilization is carried out using techniques common in the art and should be optimized for the composition being developed [Tang et al., Pharm Res. 21:191-200. (2004) and Chang et al., Pharm Res. 13:243-9 (1996)].

[0058] Methods of preparing pharmaceutical formulations can include one or more of the following steps: adding a stabilizing agent as described herein to said mixture prior to lyophilizing, adding at least one agent selected from a bulking agent, an osmolarity regulating agent, and a surfactant, each of which as described herein, to said mixture prior to lyophilization. A lyophilized formulation is, in one aspect, at least comprised of one or more of a buffer, a bulking agent, and a stabilizer. In this aspect, the utility of a surfactant is evaluated and selected in cases where aggregation during the lyophilization step or during reconstitution becomes an issue. An appropriate buffering agent is included to maintain the formulation within stable zones of pH during lyophilization.

[0059] The standard reconstitution practice for lyophilized material is to add back a volume of pure water or sterile water for injection (WFI) (typically equivalent to the volume removed during lyophilization), although dilute solutions of antibacterial agents are sometimes used in the production of pharmaceuticals for parenteral administration [Chen, Drug Development and Industrial Pharmacy, 18:1311-1354 (1992)]. Accordingly, methods are provided for preparation of reconstituted recombinant VWF compositions comprising the step of adding a diluent to a lyophilized recombinant VWF composition for the use of the invention.

[0060] The lyophilized material may be reconstituted as an aqueous solution. A variety of aqueous carriers, *e.g.*, sterile water for injection, water with preservatives for multi dose use, or water with appropriate amounts of surfactants (for example, an aqueous suspension that contains the active compound in admixture with excipients suitable for the manufacture of aqueous suspensions). In various aspects, such excipients are suspending agents, for example and without limitation, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents are a naturally-occurring phosphatide, for example and without limitation, lecithin, or condensation products of an alkylene oxide with fatty acids, for example and without limitation, polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example and without limitation, heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example and without limitation, polyethylene sorbitan monooleate. In various aspects, the aqueous suspensions also contain one or more preservatives, for example and without limitation, ethyl, or n-propyl, p-hydroxybenzoate.

[0061] In certain embodiments, compositions for the use of the present invention are liquid formulations for administration with the use of a syringe or other storage vessel. In further embodiments, these liquid formulations are produced from lyophilized material described herein reconstituted as an aqueous solution.

[0062] In a further aspect, the compositions for the use of the invention further comprise one or more pharmaceutically acceptable carriers. The phrases "pharmaceutically" or "pharmacologically" acceptable refer to molecular entities and compositions that are stable, inhibit protein degradation such as aggregation and cleavage products, and in addition do not produce allergic, or other adverse reactions when administered using routes well-known in the art, as described below. "Pharmaceutically acceptable carriers" include any and all clinically useful solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like, including those agents disclosed above.

II. Production of Recombinant VWF

[0063] The free mature recombinant von Willebrand Factor (rVWF) for the use of the present invention can be produced recombinantly. One skilled in the art recognizes useful methods for expressing a recombinant protein in a host cell. In some instances, the method includes expressing a nucleic acid sequence encoding rVWF in a host cell such as a CHO cell and culturing the resulting host cell under certain conditions to produce rVWF, prepro-VWF, pro-VWF, and the like.

[0064] In certain embodiments, the nucleic acid sequence comprising a sequence coding for VWF can be an expression vector. The vector can be delivered by a virus or can be a plasmid. The nucleic acid sequence coding for the protein can be a specific gene or a biologically functional part thereof. In one embodiment, the protein is at least a biologically active part of VWF. The nucleic acid sequence can further comprise other sequences suitable for a controlled expression of a protein such as promoter sequences, enhancers, TATA boxes, transcription initiation sites, polylinkers, restriction sites, poly-A-sequences, protein processing sequences, selection markers, and the like which are generally known to a person of ordinary skill in the art.

[0065] A wide variety of vectors can be used for the expression of the VWF and can be selected from eukaryotic expression vectors. Examples of vectors for eukaryotic expression include: (i) for expression in yeast, vectors such as pAO, pPIC, pYES, pMET, using promoters such as AOX1, GAP, GAL1, AUG1, etc.; (ii) for expression in insect cells, vectors such as pMT, pAc5, pIB, pMIB, pBAC, etc., using promoters such as PH, p10, MT, Ac5, OplE2, gp64, polh, etc., and (iii) for expression in mammalian cells, vectors such as pSVL, pCMV, pRc/RSV, pCDNA3, pBPV, etc., and vectors derived from viral systems such as

vaccinia virus, adeno-associated viruses, herpes viruses, retroviruses, etc., using promoters such as CMV, SV40, EF-1, UbC, RSV, ADV, BPV, and β -actin.

[0066] In some aspects, the rVWF for the use of the present invention is produced by expression in a mammalian cell culture using methods known in the art. In particular embodiments, the mammalian culture comprises CHO cells. In further embodiments, the rVWF is co-expressed with recombinant Factor VIII (rFVIII) in the same culture. In such embodiments, the rVWF and the rFVIII are purified together (co-purified) or separately using methods known in the art. In other embodiments, the rVWF is expressed in a culture that does not contain rFVIII.

[0067] In some embodiments, rVWF is expressed and isolated from a suitable eukaryotic host system. Examples of eukaryotic cells include, without limitation, mammalian cells, such as CHO, COS, HEK 293, BHK, SK-Hep, and HepG2; insect cells, e.g., SF9 cells, SF21 cells, S2 cells, and High Five cells; and yeast cells, e.g., *Saccharomyces* or *Schizosaccharomyces* cells. In one embodiment, the VWF can be expressed in yeast cells, insect cells, avian cells, mammalian cells, and the like. For example, in a human cell line, a hamster cell line, or a murine cell line. In one particular embodiment, the cell line is a CHO, BHK, or HEK cell line. Typically, mammalian cells, e.g., CHO cell from a continuous cell line, can be used to express the VWF for the use of the present invention. In certain instances, VWF protein is expressed and isolated from a CHO cell expression system.

[0068] VWF can be produced in a cell culture system or according to any cell culture method recognized by those in the art. In some embodiments, the cell cultures can be performed in large bioreactors under conditions suitable for providing high volume-specific culture surface areas to achieve high cell densities and protein expression. One means for providing such growth conditions is to use microcarriers for cell-culture in stirred tank bioreactors. The concept of cell-growth on microcarriers was first described by van Wezel (van Wezel, A. L., *Nature*, 1967, 216:64-5) and allows for cell attachment on the surface of small solid particles suspended in the growth medium. These methods provide for high surface-to-volume ratios and thus allow for efficient nutrient utilization. Furthermore, for expression of secreted proteins in eukaryotic cell lines, the increased surface-to-volume ratio allows for higher levels of secretion and thus higher protein yields in the supernatant of the culture. Finally, these methods allow for the easy scale-up of eukaryotic expression cultures.

[0069] The cells expressing VWF can be bound to a spherical or a porous microcarrier during cell culture growth. The microcarrier can be a microcarrier selected from the group of microcarriers based on dextran, collagen, plastic, gelatine and cellulose and others as described in Butler (1988. In: Spier & Griffiths, *Animal Cell Biotechnology* 3:283-303). It is also possible to grow the cells to a biomass on spherical microcarriers and subculture the cells when they have reached final fermenter biomass and prior to production of the expressed protein on a porous microcarrier or vice versa. Suitable spherical microcarriers can include smooth surface microcarriers, such as Cytodex™ 1, Cytodex™ 2, and Cytodex™ 3 (GE Healthcare) and macroporous microcarriers such as Cytopore™ 1, Cytopore™ 2, Cytoline™ 1, and Cytoline™ 2 (GE Healthcare).

[0070] In a further embodiment, the VWF propeptide is cleaved from the non-mature VWF in vitro through exposure of the pro-VWF to furin. In some embodiments, the furin used for propeptide cleavage is recombinant furin.

[0071] In certain embodiments, rVWF is expressed in cells cultured in cell culture media that produces high molecular weight rVWF. The terms "cell culture solution," "cell culture medium or media," and "cell culture supernatant" refer to aspects of cell culture processes generally well known in the art. In the context of the present invention, a cell culture solution can include cell culture media and cell culture supernatant. The cell culture media are externally added to the cell culture solution, optionally together with supplements, to provide nutrients and other components for culturing the cells expressing VWF. The cell culture supernatant refers to a cell culture solution comprising the nutrients and other components from the cell culture medium as well as products released, metabolized, and/or excreted from the cells during culture. In further embodiments, the media can be animal protein-free and chemically defined. Methods of preparing animal protein-free and chemically defined culture media are known in the art, for example in US 2006/0094104, US 2007/0212770, and US 2008/0009040. "Protein free" and related terms refers to protein that is from a source exogenous to or other than the cells in the culture, which naturally shed proteins during growth. In another embodiment, the culture medium is polypeptide free. In another embodiment, the culture medium is serum free. In another embodiment the culture medium is animal protein free. In another embodiment the culture medium is animal component free. In another embodiment, the culture medium contains protein, e.g., animal protein from serum such as fetal calf serum. In another embodiment, the culture has recombinant proteins exogenously added. In another embodiment, the proteins are from a certified pathogen free animal. The term "chemically defined" as used herein shall mean, that the medium does not comprise any undefined supplements, such as, for example, extracts of animal components, organs, glands, plants, or yeast. Accordingly, each component of a chemically defined medium is accurately defined. In a preferred embodiment, the media are animal-component free and protein free.

[0072] In certain embodiments, the culture of cells expressing VWF can be maintained for at least about 7 days, or at least about 14 days, 21 days, 28 days, or at least about 5 weeks, 6 weeks, 7 weeks, or at least about 2 months, or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 months or longer. The cell density at which a cell-culture is maintained at for production of a recombinant VWF protein will depend upon the culture-conditions and medium used for protein expression. One of skill in the art will readily be able to determine the optimal cell density for a cell-culture producing an VWF. In one embodiment, the culture is maintained at a cell density of between about 0.5×10^6 and 4×10^7 cells/ml for an extended period of time. In other embodiments, the cell density is maintained at a concentration of between about 1.0×10^6 and about 1.0×10^7 cells/ml for an extended period of time. In other embodiments, the cell density is maintained at a concentration of between about 1.0×10^6 and about 4.0×10^6 cells/ml for an extended period of time. In other embodiments, the cell density is maintained at a concentration of between about 1.0×10^6 and about 4.0×10^6 cells/ml for an extended period of time. In yet other embodiments, the cell density may be maintained at a concentration between about 2.0×10^6 and about 4.0×10^6 , or between about 1.0×10^6 and about 2.5×10^6 , or between about 1.5×10^6 and about 3.5×10^6 , or any other similar range, for an extended period of time. After an appropriate time in cell culture, the rVWF can be isolated from the expression system using methods known in the art.

[0073] In a specific embodiment, the cell density of the continuous cell culture for production of rVWF is maintained at a concentration of no more than 2.5×10^6 cells/mL for an extended period. In other specific embodiments, the cell density is maintained at no more than 2.0×10^6 cells/mL, 1.5×10^6 cells/mL, 1.0×10^6 cells/mL, 0.5×10^6 cells/mL, or less. In one embodiment, the cell density is maintained at between 1.5×10^6 cells/mL and 2.5×10^6 cells/mL.

[0074] In one embodiment of the cell cultures described above, the cell culture solution comprises a medium supplement comprising copper. Such cell culture solutions are described, for example, in U.S. Patent No. 8,852,888 and U.S. Patent No. 9,409,971.

[0075] The polynucleotide and amino acid sequences of prepro-VWF are set out in SEQ ID NO:1 and SEQ ID NO:2, respectively, and are available at GenBank Accession Nos. NM_000552 (*Homo sapiens* von Willebrand factor (VWF) mRNA) and NP_000543, respectively. The amino acid sequence

corresponding to the mature VWF protein is set out in SEQ ID NO: 3 (corresponding to amino acids 764-2813 of the full length prepro-VWF amino acid sequence). In some embodiments, the VWF exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of SEQ ID NO:3. In some embodiments, the rVWF for the use of the invention exhibits at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity to the sequence of SEQ ID NO:3. See, for example, U.S. Patent No. 8,597,910, U.S. Patent Publication No. 2016/0129090, as well as FIG. 6.

[0076] One form of useful rVWF has at least the property of in vivo-stabilizing, *e.g.* binding, of at least one Factor VIII (FVIII) molecule and having optionally a glycosylation pattern which is pharmacologically acceptable. Specific examples thereof include VWF without the A2 domain thus resistant to proteolysis (Lankhof et al., *Thromb. Haemost.* 77: 1008-1013, 1997), and a VWF fragment from Val 449 to Asn 730 including the glycoprotein Ib-binding domain and binding sites for collagen and heparin (Pietu et al., *Biochem. Biophys. Res. Commun.* 164: 1339-1347, 1989). The determination of the ability of a VWF to stabilize at least one FVIII molecule is, in one aspect, carried out in VWF-deficient mammals according to methods known in the state in the art.

[0077] The rVWF for the use of the present invention can be produced by any method known in the art. One specific example is disclosed in WO86/06096 published on Oct. 23, 1986 and U.S. Patent Application No. 07/559,509, filed on Jul. 23, 1990. Thus, methods are known in the art for (i) the production of recombinant DNA by genetic engineering, *e.g.* via reverse transcription of RNA and/or amplification of DNA, (ii) introducing recombinant DNA into prokaryotic or eukaryotic cells by transfection, *e.g.* via electroporation or microinjection, (iii) cultivating the transformed cells, *e.g.* in a continuous or batchwise manner, (iv) expressing VWF, *e.g.* constitutively or upon induction, and (v) isolating the VWF, *e.g.* from the culture medium or by harvesting the transformed cells, in order to (vi) obtain purified rVWF, *e.g.* via anion exchange chromatography or affinity chromatography. A recombinant VWF is, in one aspect, made in transformed host cells using recombinant DNA techniques well known in the art. For instance, sequences coding for the polypeptide could be excised from DNA using suitable restriction enzymes. Alternatively, the DNA molecule is, in another aspect, synthesized using chemical synthesis techniques, such as the phosphoramidate method. Also, in still another aspect, a combination of these techniques is used.

[0078] The specification also provides vectors encoding polypeptides for the use of the invention, in an appropriate host. The vector comprises the polynucleotide that encodes the polypeptide operatively linked to appropriate expression control sequences. Methods of effecting this operative linking, either before or after the polynucleotide is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal binding sites, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation. The resulting vector having the polynucleotide therein is used to transform an appropriate host. This transformation may be performed using methods well known in the art.

[0079] Any of a large number of available and well-known host cells are used in the practice of this invention. The selection of a particular host is dependent upon a number of factors recognized by the art, including, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of transformation, ease of recovery of the peptides, expression characteristics, bio-safety and costs. A balance of these factors must be struck with the understanding that not all host cells are equally effective for the expression of a particular DNA sequence. Within these general guidelines, useful microbial host cells include, without limitation, bacteria, yeast and other fungi, insects, plants, mammalian (including human) cells in culture, or other hosts known in the art.

[0080] Transformed host cells are cultured under conventional fermentation conditions so that the desired compounds are expressed. Such fermentation conditions are well known in the art. Finally, the polypeptides are purified from culture media or the host cells themselves by methods well known in the art.

[0081] Depending on the host cell utilized to express a compound for the use of the invention, carbohydrate (oligosaccharide) groups are optionally attached to sites that are known to be glycosylation sites in proteins. Generally, O-linked oligosaccharides are attached to serine (Ser) or threonine (Thr) residues while N-linked oligosaccharides are attached to asparagine (Asn) residues when they are part of the sequence Asn-X-Ser/Thr, where X can be any amino acid except proline. X is preferably one of the 19 naturally occurring amino acids not counting proline. The structures of N-linked and O-linked oligosaccharides and the sugar residues found in each type are different. One type of sugar that is commonly found on both N-linked and O-linked oligosaccharides is N-acetylneuraminic acid (referred to as sialic acid). Sialic acid is usually the terminal residue of both N-linked and O-linked oligosaccharides and, by virtue of its negative charge, in one aspect, confers acidic properties to the glycosylated compound. Such site(s) may be incorporated in the linker of the compounds for the use of this invention and are preferably glycosylated by a cell during recombinant production of the polypeptide compounds (*e.g.*, in mammalian cells such as CHO, BHK, COS). In other aspects, such sites are glycosylated by synthetic or semi-synthetic procedures known in the art.

[0082] In some embodiments, sialylation (also referred to as sialylation), can be performed on the column as part of the purification procedures described herein (including the anion exchange, cation exchange, size exclusion, and/or immunoaffinity methods). In some embodiments, the sialylation results in increased stability of the rVWF as compared to rVWF that has not undergone sialylation. In some embodiments, the sialylation results in increased stability of the rVWF in blood circulation (for example, after administration to a subject) as compared to rVWF that has not undergone sialylation. In some embodiments, the increased stability of sialylated rVWF results in an increase of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more as compared to rVWF that has not undergone sialylation. In some embodiments, the sialylation results in increased half-life for the rVWF as compared to rVWF that has not undergone sialylation. In some embodiments, the sialylation results in increased half-life for the rVWF in blood circulation (for example, after administration to a subject) as compared to rVWF that has not undergone sialylation. In some embodiments, the increased half-life of sialylated rVWF results in an increase of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more as compared to rVWF that has not undergone sialylation. In some embodiments, the increased half-life of sialylated rVWF results in rVWF that is stable for 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, 24 hours or more in blood circulation (for example, after administration to a subject) as compared to rVWF that has not undergone sialylation. In some embodiments, sialylation increases the number of 2,3 sialylation and/or 2,6 sialylation. In some embodiments, sialylation is increased by the addition of 2,3 sialyltransferase and/or 2,6 sialyltransferase and CMP-NANA (Cytidine-5'-monophospho-N-acetylneuraminic acid sodium salt) as an additional buffer step. In some embodiments, sialylation is increased by the addition of 2,3 sialyltransferase and CMP-NANA (Cytidine-5'-monophospho-N-acetylneuraminic acid sodium salt) as an additional buffer step. In some embodiments, 2,3 sialylation is increased by the addition of 2,3 sialyltransferase and CMP-NANA (Cytidine-5'-monophospho-N-acetylneuraminic acid sodium salt) as an additional buffer step.

[0083] In some embodiments, 2,6 sialylation is increased by the addition of 2,6 sialyltransferase and CMP-NANA (Cytidine-5'-monophospho-N-acetylneuraminic acid sodium salt) as an additional buffer step. In some embodiments, 2,3 sialylation and/or 2,6 sialylation are increased by the addition of 2,3 sialyltransferase and/or 2,6 sialyltransferase and CMP-NANA (Cytidine-5'-monophospho-N-acetylneuraminic acid sodium salt) as an additional buffer step. In some embodiments, CMP-NANA is chemically or enzymatic modified to transfer modified sialic acid to potential free position. In some embodiments, sialylation is performed by loading rVWF onto the resin, washing with one or more buffers as described herein to deplete unwanted impurities, apply one or

more buffers containing sialyltransferase and CMP-NANA at conditions that allow additional sialylation, and washing with one or more buffers to deplete excess of the sialylation reagents, and eluting with one or more buffers the enhanced rVWF (e.g., the rVWF with increased sialylation). In some embodiments, the sialylation process is performed as part of a cation exchange method, an anion exchange method, a size exclusion method, or an immunoaffinity purification method, as described herein.

[0084] Alternatively, the compounds are made by synthetic methods using, for example, solid phase synthesis techniques. Suitable techniques are well known in the art, and include those described in Merrifield (1973), Chem. Polypeptides, pp. 335-61 (Katsoyannis and Panayotis eds.); Merrifield (1963), J. Am. Chem. Soc. 85: 2149; Davis et al. (1985), Biochem. Intl. 10: 394-414; Stewart and Young (1969), Solid Phase Peptide Synthesis; U.S. Pat. No. 3,941,763; Finn et al. (1976), The Proteins (3rd ed.) 2: 105-253; and Erickson et al. (1976), The Proteins (3rd ed.) 2: 257-527. Solid phase synthesis is the preferred technique of making individual peptides since it is the most cost-effective method of making small peptides.

[0085] Fragments, variants and analogs of VWF can be produced according to methods that are well-known in the art. Fragments of a polypeptide can be prepared using, without limitation, enzymatic cleavage (e.g., trypsin, chymotrypsin) and also using recombinant means to generate a polypeptide fragments having a specific amino acid sequence. Polypeptide fragments may be generated comprising a region of the protein having a particular activity, such as a multimerization domain or any other identifiable VWF domain known in the art.

[0086] Methods of making polypeptide analogs are also well-known. Amino acid sequence analogs of a polypeptide can be substitutional, insertional, addition or deletion analogs. Deletion analogs, including fragments of a polypeptide, lack one or more residues of the native protein which are not essential for function or immunogenic activity. Insertional analogs involve the addition of, e.g., amino acid(s) at a non-terminal point in the polypeptide. This analog may include, for example and without limitation, insertion of an immunoreactive epitope or simply a single residue. Addition analogs, including fragments of a polypeptide, include the addition of one or more amino acids at either or both termini of a protein and include, for example, fusion proteins. Combinations of the aforementioned analogs are also contemplated.

[0087] Substitutional analogs typically exchange one amino acid of the wild-type for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide without the complete loss of other functions or properties. In one aspect, substitutions are conservative substitutions. "Conservative amino acid substitution" is substitution of an amino acid with an amino acid having a side chain or a similar chemical character. Similar amino acids for making conservative substitutions include those having an acidic side chain (glutamic acid, aspartic acid); a basic side chain (arginine, lysine, histidine); a polar amide side chain (glutamine, asparagine); a hydrophobic, aliphatic side chain (leucine, isoleucine, valine, alanine, glycine); an aromatic side chain (phenylalanine, tryptophan, tyrosine); a small side chain (glycine, alanine, serine, threonine, methionine); or an aliphatic hydroxyl side chain (serine, threonine).

[0088] In one aspect, analogs are substantially homologous or substantially identical to the recombinant VWF from which they are derived. Analogs include those which retain at least some of the biological activity of the wild-type polypeptide, e.g. blood clotting activity.

[0089] Polypeptide variants contemplated include, without limitation, polypeptides chemically modified by such techniques as ubiquitination, glycosylation, including polysialation (or polysialylation), conjugation to therapeutic or diagnostic agents, labeling, covalent polymer attachment such as pegylation (derivatization with polyethylene glycol), introduction of non-hydrolyzable bonds, and insertion or substitution by chemical synthesis of amino acids such as omithine, which do not normally occur in human proteins. Variants retain the same or essentially the same binding properties of non-modified molecules for the use of the invention. Such chemical modification may include direct or indirect (e.g., via a linker) attachment of an agent to the VWF polypeptide. In the case of indirect attachment, it is contemplated that the linker may be hydrolyzable or non-hydrolyzable.

[0090] Preparing pegylated polypeptide analogs will in one aspect comprise the steps of (a) reacting the polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the binding construct polypeptide becomes attached to one or more PEG groups, and (b) obtaining the reaction product(s). In general, the optimal reaction conditions for the acylation reactions are determined based on known parameters and the desired result. For example, the larger the ratio of PEG: protein, the greater the percentage of poly-pegylated product. In some embodiments, the binding construct has a single PEG moiety at the N-terminus. Polyethylene glycol (PEG) may be attached to the blood clotting factor to, for example, provide a longer half-life in vivo. The PEG group may be of any convenient molecular weight and is linear or branched. The average molecular weight of the PEG ranges from about 2 kilodalton ("kD") to about 100 kDa, from about 5 kDa to about 50 kDa, or from about 5 kDa to about 10 kDa. In certain aspects, the PEG groups are attached to the blood clotting factor via acylation or reductive alkylation through a natural or engineered reactive group on the PEG moiety (e.g., an aldehyde, amino, thiol, or ester group) to a reactive group on the blood clotting factor (e.g., an aldehyde, amino, or ester group) or by any other technique known in the art.

[0091] Methods for preparing polysialylated polypeptide are described in United States Patent Publication 20060160948, Fernandes et Gregoriadis; Biochim. Biophys. Acta 1341: 26-34, 1997, and Saenko et al., Haemophilia 12:42-51, 2006. Briefly, a solution of colominic acid (CA) containing 0.1 M NaIO₄ is stirred in the dark at room temperature to oxidize the CA. The activated CA solution is dialyzed against, e.g., 0.05 M sodium phosphate buffer, pH 7.2 in the dark and this solution was added to a rVWF solution and incubated for 18 h at room temperature in the dark under gentle shaking. Free reagents are optionally be separated from the rVWF-polysialic acid conjugate by, for example, ultrafiltration/diafiltration. Conjugation of rVWF with polysialic acid is achieved using glutaraldehyde as cross-linking reagent (Migneault et al., Biotechniques 37: 790-796, 2004).

[0092] It is also contemplated in another aspect that prepro-VWF and pro-VWF polypeptides will provide a therapeutic benefit in the formulations for the use in the present invention. For example, US Patent No. 7,005,502 describes a pharmaceutical preparation comprising substantial amounts of pro-VWF that induces thrombin generation in vitro. In addition to recombinant, biologically active fragments, variants, or other analogs of the naturally-occurring mature VWF, the present invention contemplates the use of recombinant biologically active fragments, variants, or analogs of the prepro-VWF (set out in SEQ ID NO:2) or pro-VWF polypeptides (amino acid residues 23 to 764 of SEQ ID NO: 2) in the formulations described herein.

[0093] Polynucleotides encoding fragments, variants and analogs may be readily generated by a worker of skill to encode biologically active fragments, variants, or analogs of the naturally-occurring molecule that possess the same or similar biological activity to the naturally-occurring molecule. In various aspects, these polynucleotides are prepared using PCR techniques, digestion/ligation of DNA encoding molecule, and the like. Thus, one of skill in the art will be able to generate single base changes in the DNA strand to result in an altered codon and a missense mutation, using any method known in the art, including, but not limited to site-specific mutagenesis. As used herein, the phrase "moderately stringent hybridization conditions" means, for example, hybridization at 42 ° C in 50% formamide and washing at 60 ° C in 0.1 × SSC, 0.1% SDS. It is understood by those of skill in the art that variation in these conditions occurs based on the length and GC nucleotide base content of the sequences to be hybridized. Formulas standard in the art are appropriate for

determining exact hybridization conditions. See Sambrook et al., 9.47-9.51 in *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

A. VWF Multimers

[0094] Assessment of the number and percentage of rVWF multimers can be conducted using methods known in the art, including without limitation methods using electrophoresis and size exclusion chromatography methods to separate VWF multimers by size, for example as discussed by Cumming et al., (*J Clin Pathol.*, 1993 May; 46(5): 470-473. Such techniques may further include immunoblotting techniques (such as Western Blot), in which the gel is immunoblotted with a radiolabeled antibody against VWF followed by chemiluminescent detection (see, for example, Wen et al., *J. Clin. Lab. Anal.*, 1993, 7: 317-323. Further assays for VWF include VWF:Antigen (VWF:Ag), VWF:Ristocetin Cofactor (VWF:RCof), and VWF:Collagen Binding Activity assay (VWF:CBA), which are often used for diagnosis and classification of Von Willebrand Disease (see, for example, Favalaro et al., *Pathology*, 1997, 29(4): 341-456; Sadler, JE, *Annu Rev Biochem*, 1998, 67:395-424; and Turecek et al., *Semin Thromb Hemost*, 2010, 36:510-521. In some embodiments, the rVWF obtained using the present methods includes any multimer pattern present in the loading sample of the rVWF. In some embodiments, the rVWF obtained using the present methods includes physiologically occurring multimer patterns as well as ultralarge VWF-multimer patterns.

b. VWF Assays

[0095] In primary hemostasis VWF serves as a bridge between platelets and specific components of the extracellular matrix, such as collagen. The biological activity of VWF in this process can be measured by different in vitro assays (Turecek et al., *Semin Thromb Hemost*, 2010, 36: 510-521).

[0096] The VWF:Ristocetin Cofactor (VWF:RCof) assay is based on the agglutination of fresh or formalin-fixed platelets induced by the antibiotic ristocetin in the presence of VWF. The degree of platelet agglutination depends on the VWF concentration and can be measured by the turbidimetric method, e.g., by use of an aggregometer (Weiss et al., *J. Clin. Invest.*, 1973, 52: 2708-2716; Macfarlane et al., *Thromb. Diath. Haemorrh.*, 1975, 34: 306-308). As provided herein, the specific ristocetin cofactor activity of the VWF (VWF:RCo) for the use of the present invention is generally described in terms of mU/ μ g of VWF, as measured using in vitro assays.

[0097] In some embodiments, the rVWF purified according to the methods of the present specification has a specific activity of at least about 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, 45, 47.5, 50, 52.5, 55, 57.5, 60, 62.5, 65, 67.5, 70, 72.5, 75, 77.5, 80, 82.5, 85, 87.5, 90, 92.5, 95, 97.5, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150 or more mU/ μ g. In some embodiments, rVWF used in the methods described herein has a specific activity of from 20 mU/ μ g to 150 mU/ μ g. In some embodiments, the rVWF has a specific activity of from 30 mU/ μ g to 120 mU/ μ g. In some embodiments, the rVWF has a specific activity from 40 mU/ μ g to 90 mU/ μ g. In some embodiments, the rVWF has a specific activity selected from variations 1 to 133 found in Table 3, below.

Table 3. Exemplary embodiments for the specific activity of rVWF found in the compositions and used in the methods provided herein.

(mU/ μ g)		(mU/ μ g)		(mU/ μ g)		(mU/ μ g)	
20	Var. 1	110	Var. 35	40-150	Var. 68	70-120	Var. 101
22.5	Var. 2	115	Var. 36	40-140	Var. 69	70-110	Var. 102
25	Var. 3	120	Var. 37	40-130	Var. 70	70-100	Var. 103
27.5	Var. 4	125	Var. 38	40-120	Var. 71	70-90	Var. 104
30	Var. 5	130	Var. 39	40-110	Var. 72	70-80	Var. 105
32.5	Var. 6	135	Var. 40	40-100	Var. 73	80-150	Var. 106
35	Var. 7	140	Var. 41	40-90	Var. 74	80-140	Var. 107
37.5	Var. 8	145	Var. 42	40-80	Var. 75	80-130	Var. 108
40	Var. 9	150	Var. 43	40-70	Var. 76	80-120	Var. 109
42.5	Var. 10	20-150	Var. 44	40-60	Var. 77	80-110	Var. 110
45	Var. 11	20-140	Var. 45	40-50	Var. 78	80-100	Var. 111
47.5	Var. 12	20-130	Var. 46	50-150	Var. 79	80-90	Var. 112
50	Var. 13	20-120	Var. 47	50-140	Var. 80	90-150	Var. 113
52.5	Var. 14	20-110	Var. 48	50-130	Var. 81	90-140	Var. 114
55	Var. 15	20-100	Var. 49	50-120	Var. 82	90-130	Var. 115
57.5	Var. 16	20-90	Var. 50	50-110	Var. 83	90-120	Var. 116
60	Var. 17	20-80	Var. 51	50-100	Var. 84	90-110	Var. 117
62.5	Var. 18	20-70	Var. 52	50-90	Var. 85	90-100	Var. 118
65	Var. 19	20-60	Var. 53	50-80	Var. 86	100-150	Var. 119
67.5	Var. 20	20-50	Var. 54	50-70	Var. 87	100-140	Var. 120
70	Var. 21	20-40	Var. 55	50-60	Var. 88	100-130	Var. 121
72.5	Var. 22	30-150	Var. 56	60-150	Var. 89	100-120	Var. 122
75	Var. 23	30-140	Var. 57	60-140	Var. 90	100-110	Var. 123
77.5	Var. 24	30-130	Var. 58	60-130	Var. 91	110-150	Var. 124
80	Var. 25	30-120	Var. 59	60-120	Var. 92	110-140	Var. 125
82.5	Var. 26	30-110	Var. 60	60-110	Var. 93	110-130	Var. 126
85	Var. 27	30-100	Var. 61	60-100	Var. 94	110-120	Var. 127
87.5	Var. 28	30-90	Var. 62	60-90	Var. 95	120-150	Var. 128
90	Var. 29	30-80	Var. 63	60-80	Var. 96	120-140	Var. 129

(mU/ μ g)		(mU/ μ g)		(mU/ μ g)		(mU/ μ g)	
92.5	Var. 30	30-70	Var. 64	60-70	Var. 97	120-130	Var. 130
95	Var. 31	30-60	Var. 65	70-150	Var. 98	130-150	Var. 131
97.5	Var. 32	30-50	Var. 66	70-140	Var. 99	130-140	Var. 132
100	Var. 33	30-40	Var. 67	70-130	Var. 100	140-150	Var. 133
105	Var. 34						
Var. = Variation							

[0098] The rVWF for the use of the present invention is highly multimeric comprising about 10 to about 40 subunits. In further embodiments, the multimeric rVWF produced using methods of the present specification comprise about 10-30, 12-28, 14-26, 16-24, 18-22, 20-21 subunits. In some embodiments, the rVWF is present in multimers varying in size from dimers to multimers of over 40 subunits (> 10 million Daltons). The largest multimers provide multiple binding sites that can interact with both platelet receptors and subendothelial matrix sites of injury, and are the most hemostatically active form of VWF. In some embodiments, the rVWF for the use of the present invention comprises ultralarge multimers (ULMs). Generally, high and ultralarge multimers are considered to be hemostatically most effective (see, for example, Turecek, P., *Hämostaseologie*, (Vol. 37): Supplement 1, pages S15-S25 (2017)). In some embodiments, the rVWF is between 500 kDa and 20,000 kDa. In some embodiments, any desired multimer pattern can be obtained using the methods described. In some embodiments, when anion exchange and/or cation exchanger methods are employed, the pH, conductivity, and/or counterion concentration of the buffers in the one or more wash step(s) or the gradient buffers can be manipulated to obtain the desired multimer pattern. In some embodiments, then size exclusion chromatography methods are employed, the collection criteria can be employed to obtain the desired multimer pattern. In some embodiments, the described multimer pattern comprises ultralarge multimers. In some embodiments, the ultralarge multimers are at least 10,000 kDa, at least 11,000 kDa, at least 12,000 kDa, at least 13,000 kDa, at least 14,000 kDa, at least 15,000 kDa, at least 16,000 kDa, at least 17,000 kDa, at least 18,000 kDa, at least 19,000 kDa, at least 20,000 kDa. In some embodiments, the ultralarge multimers are between about 10,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 11,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 12,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 13,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 14,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 15,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 16,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 17,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 18,000 kDa and 20,000 kDa. In some embodiments, the ultralarge multimers are between about 19,000 kDa and 20,000 kDa. In some embodiments, the rVWF obtained using the present methods includes any multimer pattern present in the loading sample of the rVWF. In some embodiments, the rVWF obtained using the present methods includes physiological occurring multimer patterns as well as ultra large VWF-multimer patterns.

[0099] In some embodiments, the rVWF composition prepared by the purification method described herein has a distribution of rVWF oligomers characterized in that 95% of the oligomers have between 6 subunits and 20 subunits. In some embodiments, the rVWF composition has a distribution of rVWF oligomers characterized in that 95% of the oligomers have a range of subunits selected from variations 458 to 641 found in 4.

Table 4. Exemplary embodiments for the distribution of rVWF oligomers found in the compositions and used in the methods provided herein.

Subunit s		Subunit s		Subunit s		Subunit s	
2-40	Var. 458	6-16	Var. 504	12-20	Var. 550	20-28	Var. 596
2-38	Var. 459	6-14	Var. 505	12-18	Var. 551	20-26	Var. 597
2-36	Var. 460	6-12	Var. 506	12-16	Var. 552	20-24	Var. 598
2-34	Var. 461	6-10	Var. 507	12-14	Var. 553	20-22	Var. 599
2-32	Var. 462	6-8	Var. 508	14-40	Var. 554	22-40	Var. 600
2-30	Var. 463	8-40	Var. 509	14-38	Var. 555	22-38	Var. 601
2-28	Var. 464	8-38	Var. 510	14-36	Var. 556	22-36	Var. 602
2-26	Var. 465	8-36	Var. 511	14-34	Var. 557	22-34	Var. 603
2-24	Var. 466	8-34	Var. 512	14-32	Var. 558	22-32	Var. 604
2-22	Var. 467	8-32	Var. 513	14-30	Var. 559	22-30	Var. 605
2-20	Var. 468	8-30	Var. 514	14-28	Var. 560	22-28	Var. 606
2-18	Var. 469	8-28	Var. 515	14-26	Var. 561	22-26	Var. 607
2-16	Var. 470	8-26	Var. 516	14-24	Var. 562	22-24	Var. 608
2-14	Var. 471	8-24	Var. 517	14-22	Var. 563	24-40	Var. 609
2-12	Var. 472	8-22	Var. 518	14-20	Var. 564	24-38	Var. 610
2-10	Var. 473	8-20	Var. 519	14-18	Var. 565	24-36	Var. 611
2-8	Var. 474	8-18	Var. 520	14-16	Var. 566	24-34	Var. 612
4-40	Var. 475	8-16	Var. 521	16-40	Var. 567	24-32	Var. 613
4-38	Var. 476	8-14	Var. 522	16-38	Var. 568	24-30	Var. 614
4-36	Var. 477	8-12	Var. 523	16-36	Var. 569	24-28	Var. 615
4-34	Var. 478	8-10	Var. 524	16-34	Var. 570	24-26	Var. 616
4-32	Var. 479	10-40	Var. 525	16-32	Var. 571	26-40	Var. 617
4-30	Var. 480	10-38	Var. 526	16-30	Var. 572	26-38	Var. 618
4-28	Var. 481	10-36	Var. 527	16-28	Var. 573	26-36	Var. 619
4-26	Var. 482	10-34	Var. 528	16-26	Var. 574	26-34	Var. 620
4-24	Var. 483	10-32	Var. 529	16-24	Var. 575	26-32	Var. 621
4-22	Var. 484	10-30	Var. 530	16-22	Var. 576	26-30	Var. 622
4-20	Var. 485	10-28	Var. 531	16-20	Var. 577	26-28	Var. 623

Subunit s		Subunit s		Subunit s		Subunit s	
4-18	Var. 486	10-26	Var. 532	16-18	Var. 578	28-40	Var. 624
4-16	Var. 487	10-24	Var. 533	18-40	Var. 579	28-38	Var. 625
4-14	Var. 488	10-22	Var. 534	18-38	Var. 580	28-36	Var. 626
4-12	Var. 489	10-20	Var. 535	18-36	Var. 581	28-34	Var. 627
4-10	Var. 490	10-18	Var. 536	18-34	Var. 582	28-32	Var. 628
4-8	Var. 491	10-16	Var. 537	18-32	Var. 583	28-30	Var. 629
6-40	Var. 492	10-14	Var. 538	18-30	Var. 584	30-40	Var. 630
6-38	Var. 493	10-12	Var. 539	18-28	Var. 585	30-38	Var. 631
6-36	Var. 494	12-40	Var. 540	18-26	Var. 586	30-36	Var. 632
6-34	Var. 495	12-38	Var. 541	18-24	Var. 587	30-34	Var. 633
6-32	Var. 496	12-36	Var. 542	18-22	Var. 588	30-32	Var. 634
6-30	Var. 497	12-34	Var. 543	18-20	Var. 589	32-40	Var. 635
6-28	Var. 498	12-32	Var. 544	20-40	Var. 590	32-38	Var. 636
6-26	Var. 499	12-30	Var. 545	20-38	Var. 591	32-36	Var. 637
6-24	Var. 500	12-28	Var. 546	20-36	Var. 592	32-34	Var. 638
6-22	Var. 501	12-26	Var. 547	20-34	Var. 593	34-40	Var. 639
6-20	Var. 502	12-24	Var. 548	20-32	Var. 594	36-38	Var. 640
6-18	Var. 503	12-22	Var. 549	20-30	Var. 595	38-40	Var. 641
Var. = Variation							

[0100] In some embodiments, the rVWF composition prepared by the methods provided herein can be characterized according to the percentage of rVWF molecules that are present in a particular higher order rVWF multimer or larger multimer. For example, in one embodiment, at least 20% of rVWF molecules in a rVWF composition used in the methods described herein are present in an oligomeric complex of at least 10 subunits. In another embodiment, at least 20% of rVWF molecules in a rVWF composition used in the methods described herein are present in an oligomeric complex of at least 12 subunits. In yet other embodiments, a rVWF composition used in the methods provided herein has a minimal percentage (e.g., has at least X%) of rVWF molecules present in a particular higher-order rVWF multimer or larger multimer (e.g., a multimer of at least Y subunits) according to any one of variations 134 to 457 found in Table 5 to Table 7.

Table 5. Exemplary embodiments for the percentage of rVWF molecules that are present in a particular higher order rVWF multimer or larger multimer found in the compositions and used in the methods provided herein.

		Minimal Number of Subunits in rVWF Multimer					
Minimal Percentage of		6	8	10	12	14	16
	10%	Var. 134	Var. 152	Var. 170	Var. 188	Var. 206	Var. 224
	15%	Var. 135	Var. 153	Var. 171	Var. 189	Var. 207	Var. 225
	20%	Var. 136	Var. 154	Var. 172	Var. 190	Var. 208	Var. 226
	25%	Var. 137	Var. 155	Var. 173	Var. 191	Var. 209	Var. 227
	30%	Var. 138	Var. 156	Var. 174	Var. 192	Var. 210	Var. 228
	35%	Var. 139	Var. 157	Var. 175	Var. 193	Var. 211	Var. 229
	40%	Var. 140	Var. 158	Var. 176	Var. 194	Var. 212	Var. 230
	45%	Var. 141	Var. 159	Var. 177	Var. 195	Var. 213	Var. 231
	50%	Var. 142	Var. 160	Var. 178	Var. 196	Var. 214	Var. 232
	55%	Var. 143	Var. 161	Var. 179	Var. 197	Var. 215	Var. 233
	60%	Var. 144	Var. 162	Var. 180	Var. 198	Var. 216	Var. 234
	65%	Var. 145	Var. 163	Var. 181	Var. 199	Var. 217	Var. 235
	70%	Var. 146	Var. 164	Var. 182	Var. 200	Var. 218	Var. 236
	75%	Var. 147	Var. 165	Var. 183	Var. 201	Var. 219	Var. 237
	80%	Var. 148	Var. 166	Var. 184	Var. 202	Var. 220	Var. 238
	85%	Var. 149	Var. 167	Var. 185	Var. 203	Var. 221	Var. 239
	90%	Var. 150	Var. 168	Var. 186	Var. 204	Var. 222	Var. 240
	95%	Var. 151	Var. 169	Var. 187	Var. 205	Var. 223	Var. 241

Var. = Variation

Table 6. Exemplary embodiments for the percentage of rVWF molecules that are present in a particular higher order rVWF multimer or larger multimer found in the compositions and used in the methods provided herein.

		Minimal Number of Subunits in rVWF Multimer					
Minimal Percentage of rVWF Molecules		18	20	22	24	26	28
	10%	Var. 242	Var. 260	Var. 278	Var. 296	Var. 314	Var. 332
	15%	Var. 243	Var. 261	Var. 279	Var. 297	Var. 315	Var. 333
	20%	Var. 244	Var. 262	Var. 280	Var. 298	Var. 316	Var. 334
	25%	Var. 245	Var. 263	Var. 281	Var. 299	Var. 317	Var. 335
	30%	Var. 246	Var. 264	Var. 282	Var. 300	Var. 318	Var. 336
	35%	Var. 247	Var. 265	Var. 283	Var. 301	Var. 319	Var. 337
	40%	Var. 248	Var. 266	Var. 284	Var. 302	Var. 320	Var. 338
	45%	Var. 249	Var. 267	Var. 285	Var. 303	Var. 321	Var. 339
	50%	Var. 250	Var. 268	Var. 286	Var. 304	Var. 322	Var. 340
	55%	Var. 251	Var. 269	Var. 287	Var. 305	Var. 323	Var. 341
	60%	Var. 252	Var. 270	Var. 288	Var. 306	Var. 324	Var. 342
	65%	Var. 253	Var. 271	Var. 289	Var. 307	Var. 325	Var. 343

	10%	Var. 254	Var. 272	Var. 290	Var. 308	Var. 320	Var. 344
Minimal	75%	Var. 255	Var. 273	Var. 291	Var. 309	Var. 327	Var. 345
	80%	Var. 256	Var. 274	Var. 292	Var. 310	Var. 328	Var. 346
	85%	Var. 257	Var. 275	Var. 293	Var. 311	Var. 329	Var. 347
	90%	Var. 258	Var. 276	Var. 294	Var. 312	Var. 330	Var. 348
	95%	Var. 259	Var. 277	Var. 295	Var. 313	Var. 331	Var. 349

Var. = Variation

Table 7. Exemplary embodiments for the percentage of rVWF molecules that are present in a particular higher order rVWF multimer or larger multimer found in the compositions and used in the methods provided herein.

Minimal Number of Subunits in rVWF Multimer							
		30	32	34	36	38	40
Minimal	10%	Var. 350	Var. 368	Var. 386	Var. 404	Var. 422	Var. 440
	15%	Var. 351	Var. 369	Var. 387	Var. 405	Var. 423	Var. 441
	20%	Var. 352	Var. 370	Var. 388	Var. 406	Var. 424	Var. 442
	25%	Var. 353	Var. 371	Var. 389	Var. 407	Var. 425	Var. 443

	30%	Var. 354	Var. 372	Var. 390	Var. 408	Var. 426	Var. 444
	35%	Var. 355	Var. 373	Var. 391	Var. 409	Var. 427	Var. 445
	40%	Var. 356	Var. 374	Var. 392	Var. 410	Var. 428	Var. 446
	45%	Var. 357	Var. 375	Var. 393	Var. 411	Var. 429	Var. 447
	50%	Var. 358	Var. 376	Var. 394	Var. 412	Var. 430	Var. 448
	55%	Var. 359	Var. 377	Var. 395	Var. 413	Var. 431	Var. 449
	60%	Var. 360	Var. 378	Var. 396	Var. 414	Var. 432	Var. 450
	65%	Var. 361	Var. 379	Var. 397	Var. 415	Var. 433	Var. 451
	70%	Var. 362	Var. 380	Var. 398	Var. 416	Var. 434	Var. 452
	75%	Var. 363	Var. 381	Var. 399	Var. 417	Var. 435	Var. 453
	80%	Var. 364	Var. 382	Var. 400	Var. 418	Var. 436	Var. 454
	85%	Var. 365	Var. 383	Var. 401	Var. 419	Var. 437	Var. 455
	90%	Var. 366	Var. 384	Var. 402	Var. 420	Var. 438	Var. 456
	95%	Var. 367	Var. 385	Var. 403	Var. 421	Var. 439	Var. 457

Var. = Variation

[0101] In accordance with the above, the rVWF comprises a significant percentage of high molecular weight (HMW) rVWF multimers. In further embodiments, the HMW rVWF multimer composition comprises at least 10% - 80% rVWF decamers or higher order multimers. In further embodiments, the composition comprises about 10-95%, 20-90%, 30-85%, 40-80%, 50-75%, 60-70% decamers or higher order multimers. In further embodiments, the HMW rVWF multimer composition comprises at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% decamers or higher order multimers.

[0102] Assessment of the number and percentage of rVWF multimers can be conducted using methods known in the art, including without limitation methods using electrophoresis and size exclusion chromatography methods to separate rVWF multimers by size, for example as discussed by Cumming et al. (J Clin Pathol. 1993 May; 46(5): 470-473. Such techniques may further include immunoblotting techniques (such as Western Blot), in which the gel is immunoblotted with a radiolabeled antibody against VWF followed by chemiluminescent detection (see for example Wen et al., (1993), J. Clin. Lab. Anal., 7: 317-323. Further assays for VWF include VWF:Antigen (VWF:Ag), VWF:Ristocetin Cofactor (VWF:RCof), and VWF:Collagen Binding Activity assay (VWF:CBA), which are often used for diagnosis and classification of Von Willebrand Disease. (see for example Favalaro et al., Pathology, 1997, 29(4): 341-456.

[0103] In some embodiments, the ratio of rFVIII procoagulant activity (IU rFVIII:C) to rVWF Ristocetin cofactor activity (IU rVWF:RCo) for the rVWF prepared according to the methods of the present specification is between 3:1 and 1:5. In further embodiments, the ratio is between 2:1 and 1:4. In still further embodiments, the ratio is between 5:2 and 1:4. In further embodiments, the ratio is between 3:2 and 1:3. In still further embodiments, the ratio is about 1:1, 1:2, 1:3, 1:4, 1:5, 2:1, 2:3, 2:4, 2:5, 3:1, 3:2, 3:4, or 3:5. In further embodiments, the ratio is between 1:1 and 1:2. In yet further embodiments, the ratio is 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, or 2:1. In certain embodiments, the ratio of rFVIII procoagulant activity (IU rFVIII:C) to rVWF Ristocetin cofactor activity (IU rVWF:RCo) in a composition useful for a method described herein is selected from variations 1988 to 2140 found in Table 8.

Table 8. Exemplary embodiments for the ratio of rFVIII procoagulant activity (IU rFVIII:C) to rVWF Ristocetin cofactor activity (IU rVWF:RCo) in compositions and used in methods provided herein.

(IU rFVIII:C) to (IU rVWF:RCo)		(IU rFVIII:C) to (IU rVWF:RCo)		(IU rFVIII:C) to (IU rVWF:RCo)		(IU rFVIII:C) to (IU rVWF:RCo)	
4:1	Var. 198 8	3:1-3:5	Var. 202 7	4:3-1:4	Var. 206 5	4:5-2:3	Var. 210 3
3:1	Var. 198 9	3:1-2:3	Var. 202 8	4:3-1:3	Var. 206 6	4:5-3:4	Var. 210 4
2:1	Var. 199 0	3:1-3:4	Var. 202 9	4:3-2:5	Var. 206 7	3:4-1:6	Var. 210 5
3:2	Var. 199 1	3:1-4:5	Var. 203 0	4:3-1:2	Var. 206 8	3:4-1:5	Var. 210 6
4:3	Var. 199 2	3:1-5:6	Var. 203 1	4:3-3:5	Var. 206 9	3:4-1:4	Var. 210 7
1:1	Var. 199 3	3:1-1:1	Var. 203 2	4:3-2:3	Var. 207 0	3:4-1:3	Var. 210 8
5:6	Var. 199 4	3:1-4:3	Var. 203 3	4:3-3:4	Var. 207 1	3:4-2:5	Var. 210 9
4:5	Var. 199 5	3:1-3:2	Var. 203 4	4:3-4:5	Var. 207 2	3:4-1:2	Var. 211 0
3:4	Var. 199 6	3:1-2:1	Var. 203 5	4:3-5:6	Var. 207 3	3:4-3:5	Var. 211 1
2:3	Var. 199 7	2:1-1:6	Var. 203 6	4:3-1:1	Var. 207 4	3:4-2:3	Var. 211 2
3:5	Var. 199 8	2:1-1:5	Var. 203 7	1:1-1:6	Var. 207 5	2:3-1:6	Var. 211 3
1:2	Var. 199 9	2:1-1:4	Var. 203 8	1:1-1:5	Var. 207 6	2:3-1:5	Var. 211 4
2:5	Var. 200 0	2:1-1:3	Var. 203 9	1:1-1:4	Var. 207 7	2:3-1:4	Var. 211 5
1:3	Var. 200 1	2:1-2:5	Var. 204 0	1:1-1:3	Var. 207 8	2:3-1:3	Var. 211 6
1:4	Var. 200 2	2:1-1:2	Var. 204 1	1:1-2:5	Var. 207 9	2:3-2:5	Var. 211 7

(IU rFVIII:C) to (IU rVWF:RCo)		(IU rFVIII:C) to (IU rVWF:RCo)		(IU rFVIII:C) to (IU rVWF:RCo)		(IU rFVIII:C) to (IU rVWF:RCo)	
1:5	Var. 200 3	2:1-3:5	Var. 204 2	1:1-1:2	Var. 208 0	2:3-1:2	Var. 211 8
1:6	Var. 200 4	2:1-2:3	Var. 204 3	1:1-3:5	Var. 208 1	2:3-3:5	Var. 211 9
4:1-1:6	Var. 200 5	2:1-3:4	Var. 204 4	1:1-2:3	Var. 208 2	3:5-1:6	Var. 212 0
4:1-1:5	Var. 200 6	2:1-4:5	Var. 204 5	1:1-3:4	Var. 208 3	3:5-1:5	Var. 212 1
4:1-1:4	Var. 200 7	2:1-5:6	Var. 204 6	1:1-4:5	Var. 208 4	3:5-1:4	Var. 212 2
4:1-1:3	Var. 200 8	2:1-1:1	Var. 204 7	1:1-5:6	Var. 208 5	3:5-1:3	Var. 212 3
4:1-2:5	Var. 200 9	2:1-4:3	Var. 204 8	5:6-1:6	Var. 208 6	3:5-2:5	Var. 212 4
4:1-1:2	Var. 201 0	2:1-3:2	Var. 204 9	5:6-1:5	Var. 208 7	3:5-1:2	Var. 212 5
4:1-3:5	Var. 201 1	3:2-1:6	Var. 205 0	5:6-1:4	Var. 208 8	1:2-1:6	Var. 212 6
4:1-2:3	Var. 201 2	3:2-1:5	Var. 205 1	5:6-1:3	Var. 208 9	1:2-1:5	Var. 212 7
4:1-3:4	Var. 201 3	3:2-1:4	Var. 205 2	5:6-2:5	Var. 209 0	1:2-1:4	Var. 212 8
4:1-4:5	Var. 201 4	3:2-1:3	Var. 205 3	5:6-1:2	Var. 209 1	1:2-1:3	Var. 212 9
4:1-5:6	Var. 201 5	3:2-2:5	Var. 205 4	5:6-3:5	Var. 209 2	1:2-2:5	Var. 213 0
4:1-1:1	Var. 201 6	3:2-1:2	Var. 205 5	5:6-2:3	Var. 209 3	2:5-1:6	Var. 213 1
4:1-4:3	Var. 201 7	3:2-3:5	Var. 205 6	5:6-3:4	Var. 209 4	2:5-1:5	Var. 213 2
4:1-3:2	Var. 201 8	3:2-2:3	Var. 205 7	5:6-4:5	Var. 209 5	2:5-1:4	Var. 213 3
4:1-2:1	Var. 201 9	3:2-3:4	Var. 205 8	4:5-1:6	Var. 209 6	2:5-1:3	Var. 213 4
4:1-3:1	Var. 202 0	3:2-4:5	Var. 205 9	4:5-1:5	Var. 209 7	1:3-1:6	Var. 213 5
3:1-1:6	Var. 202 1	3:2-5:6	Var. 206 0	4:5-1:4	Var. 209 8	1:3-1:5	Var. 213 6
3:1-1:5	Var. 202 2	3:2-1:1	Var. 206 1	4:5-1:3	Var. 209 9	1:3-1:4	Var. 213 7
3:1-1:4	Var. 202 3	3:2-4:3	Var. 206 2	4:5-2:5	Var. 210 0	1:4-1:6	Var. 213 8
3:1-1:3	Var. 202 4	4:3-1:6	Var. 206 3	4:5-1:2	Var. 210 1	1:4-1:5	Var. 213 9
3:1-2:5	Var. 202 5	4:3-1:5	Var. 206 4	4:5-3:5	Var. 210 2	1:5-1:6	Var. 214 0
3:1-1:2	Var. 202 6						
Var. = Variation							

[0104] In further embodiments, higher order rVWF multimers for the use of the invention are stable for about 1 to about 90 hours post-administration. In still further embodiments, the higher order rVWF multimers are stable for about 5-80, 10-70, 15-60, 20-50, 25-40, 30-35 hours post-administration. In yet further embodiments, the higher order rVWF multimers are stable for at least 3, 6, 12, 18, 24, 36, 48, 72 hours post-administration. In certain embodiments the stability of the rVWF multimers is assessed *in vitro*.

[0105] In one embodiment, higher order rVWF multimers used in the compositions and methods provided herein have a half-life of at least 12 hour post administration. In another embodiment, the higher order rVWF multimers have a half-life of at least 24 hour post administration. In yet other embodiments, the higher order rVWF multimers have a half-life selected from variations 642 to 1045 found in Table 9.

Table 9. Exemplary embodiments for the half-life of higher order rVWF multimers found in the compositions prepared by the methods provided herein.

Hours		Hours		Hours		Hours	
at least 1	Var. 642	4-22	Var. 743	14-78	Var. 844	24-30	Var. 945
at least 2	Var. 643	4-20	Var. 744	14-72	Var. 845	24-27	Var. 946
at least 3	Var. 644	4-18	Var. 745	14-66	Var. 846	27-90	Var. 947
at least 4	Var. 645	4-16	Var. 746	14-60	Var. 847	27-84	Var. 948
at least 5	Var. 646	4-14	Var. 747	14-54	Var. 848	27-78	Var. 949
at least 6	Var. 647	4-12	Var. 748	14-48	Var. 849	27-72	Var. 950
at least 7	Var. 648	4-10	Var. 749	14-45	Var. 850	27-66	Var. 951
at least 8	Var. 649	4-8	Var. 750	14-42	Var. 851	27-60	Var. 952
at least 9	Var. 650	4-6	Var. 751	14-39	Var. 852	27-54	Var. 953
at least 10	Var. 651	6-90	Var. 752	14-36	Var. 853	27-48	Var. 954
at least 11	Var. 652	6-84	Var. 753	14-33	Var. 854	30-90	Var. 955
at least 12	Var. 653	6-78	Var. 754	14-30	Var. 855	30-84	Var. 956
at least 14	Var. 654	6-72	Var. 755	14-27	Var. 856	30-78	Var. 957
at least 16	Var. 655	6-66	Var. 756	14-24	Var. 857	30-72	Var. 958
at least 18	Var. 656	6-60	Var. 757	14-22	Var. 858	30-66	Var. 959
at least 20	Var. 657	6-54	Var. 758	14-20	Var. 859	30-60	Var. 960
at least 22	Var. 658	6-48	Var. 759	14-18	Var. 860	30-54	Var. 961
at least 24	Var. 659	6-45	Var. 760	14-16	Var. 861	30-48	Var. 962
at least 27	Var. 660	6-42	Var. 761	16-90	Var. 862	30-45	Var. 963
at least 30	Var. 661	6-39	Var. 762	16-84	Var. 863	30-42	Var. 964
at least 33	Var. 662	6-36	Var. 763	16-78	Var. 864	30-39	Var. 965
at least 36	Var. 663	6-33	Var. 764	16-72	Var. 865	30-36	Var. 966

Hours		Hours		Hours		Hours	
at least 39	Var. 664	6-30	Var. 765	16-66	Var. 866	30-33	Var. 967
at least 42	Var. 665	6-27	Var. 766	16-60	Var. 867	33-90	Var. 968
at least 45	Var. 666	6-24	Var. 767	16-54	Var. 868	33-84	Var. 969
at least 48	Var. 667	6-22	Var. 768	16-48	Var. 869	33-78	Var. 970
at least 54	Var. 668	6-20	Var. 769	16-45	Var. 870	33-72	Var. 971
at least 60	Var. 669	6-18	Var. 770	16-42	Var. 871	33-66	Var. 972
at least 66	Var. 670	6-16	Var. 771	16-39	Var. 872	33-60	Var. 973
at least 72	Var. 671	6-14	Var. 772	16-36	Var. 873	33-54	Var. 974
at least 78	Var. 672	6-12	Var. 773	16-33	Var. 874	33-48	Var. 975
at least 84	Var. 673	6-10	Var. 774	16-30	Var. 875	33-45	Var. 976
at least 90	Var. 674	6-8	Var. 775	16-27	Var. 876	33-42	Var. 977
2-90	Var. 675	8-90	Var. 776	16-24	Var. 877	33-29	Var. 978
2-84	Var. 676	8-84	Var. 777	16-22	Var. 878	33-36	Var. 979
2-78	Var. 677	8-78	Var. 778	16-20	Var. 879	36-90	Var. 980
2-72	Var. 678	8-72	Var. 779	16-18	Var. 880	36-84	Var. 981
2-66	Var. 679	8-66	Var. 780	18-90	Var. 881	36-78	Var. 982
2-60	Var. 680	8-60	Var. 781	18-84	Var. 882	36-72	Var. 983
2-54	Var. 681	8-54	Var. 782	18-78	Var. 883	36-66	Var. 984
2-48	Var. 682	8-48	Var. 783	18-72	Var. 884	36-60	Var. 985
2-45	Var. 683	8-45	Var. 784	18-66	Var. 885	36-54	Var. 986
2-42	Var. 684	8-42	Var. 785	18-60	Var. 886	36-48	Var. 987
2-39	Var. 685	8-39	Var. 786	18-54	Var. 887	36-45	Var. 988
2-36	Var. 686	8-36	Var. 787	18-48	Var. 888	36-42	Var. 989
2-33	Var. 687	8-33	Var. 788	18-45	Var. 889	36-39	Var. 990
2-30	Var. 688	8-30	Var. 789	18-42	Var. 890	39-90	Var. 991
2-27	Var. 689	8-27	Var. 790	18-39	Var. 891	39-84	Var. 992
2-24	Var. 690	8-24	Var. 791	18-36	Var. 892	39-78	Var. 993
2-22	Var. 691	8-22	Var. 792	18-33	Var. 893	39-72	Var. 994
2-20	Var. 692	8-20	Var. 793	18-30	Var. 894	39-66	Var. 995
2-18	Var. 693	8-18	Var. 794	18-27	Var. 895	39-60	Var. 996
2-16	Var. 694	8-16	Var. 795	18-24	Var. 896	39-54	Var. 997
2-14	Var. 695	8-14	Var. 796	18-22	Var. 897	39-48	Var. 998
2-12	Var. 696	8-12	Var. 797	18-20	Var. 898	39-45	Var. 999
2-10	Var. 697	8-10	Var. 798	20-90	Var. 899	39-42	Var. 1000
2-8	Var. 698	10-90	Var. 799	20-84	Var. 900	42-90	Var. 1001
2-6	Var. 699	10-84	Var. 800	20-78	Var. 901	42-84	Var. 1002
2-4	Var. 700	10-78	Var. 801	20-72	Var. 902	42-78	Var. 1003
3-90	Var. 701	10-72	Var. 802	20-66	Var. 903	42-72	Var. 1004
3-84	Var. 702	10-66	Var. 803	20-60	Var. 904	42-66	Var. 1005
3-78	Var. 703	10-60	Var. 804	20-54	Var. 905	42-60	Var. 1006
3-72	Var. 704	10-54	Var. 805	20-48	Var. 906	42-54	Var. 1007
3-66	Var. 705	10-48	Var. 806	20-45	Var. 907	42-48	Var. 1008
3-60	Var. 706	10-45	Var. 807	20-42	Var. 908	42-45	Var. 1009
3-54	Var. 707	10-42	Var. 808	20-39	Var. 909	45-90	Var. 1010
3-48	Var. 708	10-39	Var. 809	20-36	Var. 910	45-84	Var. 1011
3-45	Var. 709	10-36	Var. 810	20-33	Var. 911	45-78	Var. 1012
3-42	Var. 710	10-33	Var. 811	20-30	Var. 912	45-72	Var. 1013
3-39	Var. 711	10-30	Var. 812	20-27	Var. 913	45-66	Var. 1014
3-36	Var. 712	10-27	Var. 813	20-24	Var. 914	45-60	Var. 1015
3-33	Var. 713	10-24	Var. 814	20-22	Var. 915	45-54	Var. 1016
3-30	Var. 714	10-22	Var. 815	22-90	Var. 916	45-48	Var. 1017
3-27	Var. 715	10-20	Var. 816	22-84	Var. 917	48-90	Var. 1018
3-24	Var. 716	10-18	Var. 817	22-78	Var. 918	48-84	Var. 1019
3-22	Var. 717	10-16	Var. 818	22-72	Var. 919	48-78	Var. 1020
3-20	Var. 718	10-14	Var. 819	22-66	Var. 920	48-72	Var. 1021
3-18	Var. 719	10-12	Var. 820	22-60	Var. 921	48-66	Var. 1022
3-16	Var. 720	12-90	Var. 821	22-54	Var. 922	48-60	Var. 1023
3-14	Var. 721	12-84	Var. 822	22-48	Var. 923	48-54	Var. 1024

Hours		Hours		Hours		Hours	
3-12	Var. 722	12-78	Var. 823	22-45	Var. 924	54-90	Var. 1025
3-10	Var. 723	12-72	Var. 824	22-42	Var. 925	54-84	Var. 1026
3-8	Var. 724	12-66	Var. 825	22-39	Var. 926	54-78	Var. 1027
3-6	Var. 725	12-60	Var. 826	22-36	Var. 927	54-72	Var. 1028
3-4	Var. 726	12-54	Var. 827	22-33	Var. 928	54-66	Var. 1029
4-90	Var. 727	12-48	Var. 828	22-30	Var. 929	54-60	Var. 1030
4-84	Var. 728	12-45	Var. 829	22-27	Var. 930	60-90	Var. 1031
4-78	Var. 729	12-42	Var. 830	22-24	Var. 931	60-84	Var. 1032
4-72	Var. 730	12-39	Var. 831	24-90	Var. 932	60-78	Var. 1033
4-66	Var. 731	12-36	Var. 832	24-84	Var. 933	60-72	Var. 1034
4-60	Var. 732	12-33	Var. 833	24-78	Var. 934	60-66	Var. 1035
4-54	Var. 733	12-30	Var. 834	24-72	Var. 935	66-90	Var. 1036
4-48	Var. 734	12-27	Var. 835	24-66	Var. 936	66-84	Var. 1037
4-45	Var. 735	12-24	Var. 836	24-60	Var. 937	66-78	Var. 1038
4-42	Var. 736	12-22	Var. 837	24-54	Var. 938	66-72	Var. 1039
4-39	Var. 737	12-20	Var. 838	24-48	Var. 939	72-90	Var. 1040
4-36	Var. 738	12-18	Var. 839	24-45	Var. 940	72-84	Var. 1041
4-33	Var. 739	12-16	Var. 840	24-42	Var. 941	72-78	Var. 1042
4-30	Var. 740	12-14	Var. 841	24-39	Var. 942	78-90	Var. 1043
4-27	Var. 741	14-90	Var. 842	24-36	Var. 943	78-84	Var. 1044
4-24	Var. 742	14-84	Var. 843	24-33	Var. 944	84-90	Var. 1045
Var. = Variation							

[0106] In some embodiments, the pro-VWF and/or purified rVWF purified in accordance with the present specification is not modified with any conjugation, post-translation or covalent modifications. In particular embodiments, the pro-VWF and/or purified rVWF for the use of the present invention is not modified with a water soluble polymer, including without limitation, a polyethylene glycol (PEG), a polypropylene glycol, a polyoxyalkylene, a polysialic acid, hydroxyl ethyl starch, a poly-carbohydrate moiety, and the like.

[0107] In some embodiments, the pro-VWF and/or purified rVWF purified in accordance with the present specification is modified through conjugation, post-translation modification, or covalent modification, including modifications of the N- or C- terminal residues as well as modifications of selected side chains, for example, at free sulfhydryl-groups, primary amines, and hydroxyl-groups. In one embodiment, a water soluble polymer is linked to the protein (directly or via a linker) by a lysine group or other primary amine. In some embodiments, the pro-VWF and/or purified rVWF for the use of the present invention may be modified by conjugation of a water soluble polymer, including without limitation, a polyethylene glycol (PEG), a polypropylene glycol, a polyoxyalkylene, a polysialic acid, hydroxyl ethyl starch, a poly-carbohydrate moiety, and the like.

[0108] Water soluble polymers that may be used to modify the pro-VWF and/or purified rVWF include linear and branched structures. The conjugated polymers may be attached directly to the coagulation proteins for the use of the invention, or alternatively may be attached through a linking moiety. Non-limiting examples of protein conjugation with water soluble polymers can be found in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192, and 4,179,337, as well as in Abuchowski and Davis "Enzymes as Drugs," Holcenberg and Roberts, Eds., pp. 367-383, John Wiley and Sons, New York (1981), and Hermanson G., Bioconjugate Techniques 2nd Ed., Academic Press, Inc. 2008.

[0109] Protein conjugation may be performed by a number of well-known techniques in the art, for example, see Hermanson G., Bioconjugate Techniques 2nd Ed., Academic Press, Inc. 2008. Examples include linkage through the peptide bond between a carboxyl group on one of either the coagulation protein or water-soluble polymer moiety and an amine group of the other, or an ester linkage between a carboxyl group of one and a hydroxyl group of the other. Another linkage by which a coagulation protein for the use of the invention could be conjugated to a water-soluble polymer compound is via a Schiff base, between a free amino group on the polymer moiety being reacted with an aldehyde group formed at the non-reducing end of the polymer by periodate oxidation (Jennings and Lugowski, J. Immunol. 1981; 127:1011-8; Fernandes and Gregoradis, Biochim Biophys Acta. 1997; 1341; 26-34). The generated Schiff Base can be stabilized by specific reduction with NaCNBH₃ to form a secondary amine. An alternative approach is the generation of terminal free amino groups on the polymer by reductive amination with NH₄Cl after prior oxidation. Bifunctional reagents can be used for linking two amino or two hydroxyl groups. For example, a polymer containing an amino group can be coupled to an amino group of the coagulation protein with reagents like BS3 (Bis(sulfosuccinimidyl)suberate/Pierce, Rockford, Ill.). In addition, heterobifunctional cross linking reagents like Sulfo-EMCS (N-ε-Maleimidocaproyloxy) sulfosuccinimide ester/Pierce) can be used for instance to link amine and thiol groups. In other embodiments, an aldehyde reactive group, such as PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, and PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, succinimidyl active esters, activated dithiocarbonate P.E.G., 2,4,5-trichlorophenylchloroformate and P-nitrophenylchloroformate activated P.E.G., may be used in the conjugation of a coagulation protein.

[0110] Another method for measuring the biological activity of VWF is the collagen binding assay, which is based on ELISA technology (Brown and Bosak, Thromb. Res., 1986, 43:303-311; Favaloro, Thromb. Haemost., 2000, 83 127-135). A microtiter plate is coated with type I or III collagen. Then the VWF is bound to the collagen surface and subsequently detected with an enzyme-labeled polyclonal antibody. The last step is a substrate reaction, which can be photometrically monitored with an ELISA reader.

[0111] Immunological assays of von Willebrand factors (VWF:Ag) are immunoassays that measure the concentration of the VWF protein in plasma. They give no indication as to VWF function. A number of methods exist for measuring VWF:Ag and these include both enzyme-linked immunosorbent assay (ELISA) or automated latex immunoassays (LIA.) Many laboratories now use a fully automated latex immunoassay. Historically laboratories used a variety of

techniques including Laurell electroimmunoassay 'Laurell Rockets' but these are rarely used in most labs today.

III. Kits

[0112] As an additional aspect which is not part of the invention, the specification includes kits which comprise one or more lyophilized compositions packaged in a manner which facilitates their use for administration to subjects. In one embodiment, such a kit includes pharmaceutical formulation described herein (e.g., a composition comprising a therapeutic protein or peptide), packaged in a container such as a sealed bottle or vessel, with a label affixed to the container or included in the package that describes use of the compound or composition in practicing the method. In one embodiment, the pharmaceutical formulation is packaged in the container such that the amount of headspace in the container (e.g., the amount of air between the liquid formulation and the top of the container) is very small. Preferably, the amount of headspace is negligible (e.g., almost none). In one embodiment, the kit contains a first container having a therapeutic protein or peptide composition and a second container having a physiologically acceptable reconstitution solution for the composition. In one aspect, the pharmaceutical formulation is packaged in a unit dosage form. The kit may further include a device suitable for administering the pharmaceutical formulation according to a specific route of administration. Preferably, the kit contains a label that describes use of the pharmaceutical formulations.

IV. rVWF for Methods of Pretreating Subjects with VWD Undergoing Surgery

[0113] One of the advantages of administering rVWF to subjects with severe VWD to pretreat for surgery is that the higher specific activity of rVWF as compared to pdVWF allows flexibility in the amount of rVWF administered and the number of times the subject is re-dosed. As will be appreciated and as is discussed in further detail herein, the co-administered FVIII may be recombinant or plasma derived

[0114] Single or multiple administrations of rVWF are carried out with the dose levels and pattern being selected by the treating physician. For the prevention or treatment of disease, the appropriate dosage depends on the type of disease to be treated (e.g., von Willebrand disease), the severity and course of the disease, whether drug is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the drug, and the discretion of the attending physician.

[0115] In some aspects, rVWF is administered prior to a surgical procedure to a subject at a range from 20-60 IU/kg, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 20-60, 35-70, 20-40, 35-60, 45-60, 45-55, 45-50, 50-60, 55-60, or 50-55 IU/kg. In some embodiments, rVWF is administered between 12 hours and 24 hours, e.g., 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 12 hours and 24 hours, 14 hours and 24 hours, 16 and 24 hours, 18 hours and 24 hours, or 20 hours and 24 hours prior to the surgical procedure. In some aspects, Factor VIII (FVIII) is not administered with the rVWF prior to the surgical procedure.

[0116] In some embodiments, rVWF is administered to the subject at a range of 5-90 IU/kg, e.g., 5-90, 5-50, 10-90, 15-90, 20-90, 30-90, 40-90, 50-90, 60-90, 70-90, 80-90, 5-80, 10-70, 20-60, 30-50, 35-60, 5-50, 5-40, 5-30, 5-20, 10-90, 10-50, or 20-40 IU/kg 1 hour prior to surgery. In other embodiments, rVWF is administered at a dose of 70-200 IU/kg, e.g., 70-200, 80-200, 90-200, 100-200, 110-200, 120-200, 130-200, 130-200, 140-200, 150-200, 160-200, 170-200, 180-200, 190-200, 70-170, 80-180, 60-160, 50-150, 40-140, 30, 130, 20-120, 10-110, 70-100, or 70-90 IU/kg after the surgery. In some cases, the surgical procedure is selected from a group consisting of major surgery, minor surgery, and oral surgery.

[0117] In some embodiments, the subject is administered 35-60 IU/kg rVWF between 12 hours and 24 hours prior to major surgery. In other embodiments, the subject is administered 15-90 IU/kg rVWF 1 hour prior to major surgery. In another embodiment, the subject is administered 150-220 IU/kg rVWF after major surgery. In some instances, the subject undergoing major surgery is administered a total dosage of 220-320 IU/kg.

[0118] In some embodiments, the subject is administered 50-60 IU/kg rVWF between 12 hours and 24 hours prior to minor surgery. In other embodiments, the subject is administered 5-50 IU/kg rVWF 1 hour prior to minor surgery. In another embodiment, the subject is administered 70-150 IU/kg rVWF after minor surgery. In some instances, the subject undergoing minor surgery is administered a total dosage of 100-220 IU/kg.

[0119] In some embodiments, the subject is administered 20-40 IU/kg rVWF between 12 hours and 24 hours prior to oral surgery. In other embodiments, the subject is administered 20-50 IU/kg rVWF 1 hour prior to oral surgery. In another embodiment, the subject is administered 10-50 IU/kg rVWF during oral surgery. In another embodiment, the subject is administered 20-50 IU/kg rVWF after oral surgery. In some instances, the subject undergoing oral surgery is administered a total dosage of 70-190 IU/kg.

[0120] Compositions of rVWF can be contained in pharmaceutical formulations, as described herein. Such formulations can be administered orally, topically, transdermally, parenterally, by inhalation spray, vaginally, rectally, or by intracranial injection. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. Administration by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary injection and or surgical implantation at a particular site is contemplated as well. Generally, compositions are essentially free of pyrogens, as well as other impurities that could be harmful to the recipient.

[0121] In one aspect, formulations for the use of the invention are administered by an initial bolus followed by a continuous infusion to maintain therapeutic circulating levels of drug product. As another example, the inventive compound is administered as a one-time dose. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient. The route of administration can be, but is not limited to, by intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The frequency of dosing depends on the pharmacokinetic parameters of the agents and the route of administration. The optimal pharmaceutical formulation is determined by one skilled in the art depending upon the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed., 1990, Mack Publishing Co., Easton, Pa. 18042 pages 1435-1712. Such formulations influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the administered agents. Depending on the route of administration, a suitable dose is calculated according to body weight, body surface area or organ size. Appropriate dosages may be ascertained through use of established assays for determining blood level dosages in conjunction with appropriate dose-response data. The final dosage regimen is determined by the attending physician, considering various factors which modify the action of drugs, e.g. the drug's specific activity, the severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. By way of example, a typical dose of a recombinant VWF for the use of

the present invention is approximately 50 IU/kg, equal to 500 µg/kg. As studies are conducted, further information will emerge regarding the appropriate dosage levels and duration of treatment for various diseases and conditions.

[0122] The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV), *Using Antibodies: A Laboratory Manual*, *Cells: A Laboratory Manual*, *PCR Primer: A Laboratory Manual*, and *Molecular Cloning: A Laboratory Manual* (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) *Biochemistry* (4th Ed.) Freeman, Highly stabilized York, Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press, London, Nelson and Cox (2000), Lehninger, *Principles of Biochemistry* 3rd Ed., W. H. Freeman Pub., Highly stabilized York, N.Y. and Berg et al. (2002) *Biochemistry*, 5th Ed., W. H. Freeman Pub., Highly stabilized York, N.Y.

[0123] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polymerase" refers to one agent or mixtures of such agents, and reference to "the method" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[0124] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polymerase" refers to one agent or mixtures of such agents, and reference to "the method" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[0125] 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 invention belongs.

[0126] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[0127] In the above description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

[0128] Although the present invention is described primarily with reference to specific embodiments, it is also envisioned that other embodiments will become apparent to those skilled in the art upon reading the present disclosure, and it is intended that such embodiments be contained within the present inventive method.

a. Lyophilized VWF Formulations

[0129] The present method also provides formulations of rVWF for use in the treatment methods provided herein. In some embodiments, the rVWF composition is used for the production of a pharmaceutical composition. In some embodiments, the rVWF can be formulated into a lyophilized formulation.

[0130] In some embodiments, the formulations comprising a VWF polypeptide for the use of the invention are lyophilized after purification and prior to administration to a subject. Lyophilization is carried out using techniques common in the art and should be optimized for the composition being developed (Tang et al., *Pharm Res.* 21:191-200, (2004) and Chang et al., *Pharm Res.* 13:243-9 (1996)).

[0131] A lyophilization cycle is, in one aspect, composed of three steps: freezing, primary drying, and secondary drying (A. P. Mackenzie, *Phil Trans R Soc London, Ser B, Biol* 278:167 (1977)). In the freezing step, the solution is cooled to initiate ice formation. Furthermore, this step induces the crystallization of the bulking agent. The ice sublimates in the primary drying stage, which is conducted by reducing chamber pressure below the vapor pressure of the ice, using a vacuum and introducing heat to promote sublimation. Finally, adsorbed or bound water is removed at the secondary drying stage under reduced chamber pressure and at an elevated shelf temperature. The process produces a material known as a lyophilized cake. Thereafter the cake can be reconstituted with either sterile water or suitable diluent for injection.

[0132] The lyophilization cycle not only determines the final physical state of excipients but also affects other parameters such as reconstitution time, appearance, stability and final moisture content. The composition structure in the frozen state proceeds through several transitions (e.g., glass transitions, wettings, and crystallizations) that occur at specific temperatures and the structure may be used to understand and optimize the lyophilization process. The glass transition temperature (T_g and/or T_g') can provide information about the physical state of a solute and can be determined by differential scanning calorimetry (DSC). T_g and T_g' are an important parameter that must be taken into account when designing the lyophilization cycle. For example, T_g' is important for primary drying. Furthermore, in the dried state, the glass transition temperature provides information on the storage temperature of the final product.

b. Pharmaceutical Formulations and Excipients in General

[0133] Excipients are additives that either impart or enhance the stability and delivery of a drug product (e.g., protein). Regardless of the reason for their inclusion, excipients are an integral component of a formulation and therefore need to be safe and well tolerated by patients. For protein drugs, the choice of excipients is particularly important because they can affect both efficacy and immunogenicity of the drug. Hence, protein formulations need to be developed with appropriate selection of excipients that afford suitable stability, safety, and marketability.

[0134] A lyophilized formulation is, in one aspect, at least comprised of one or more of a buffer, a bulking agent, and a stabilizer. In this aspect, the utility of

a surfactant is evaluated and selected in cases where aggregation during the lyophilization step or during reconstitution becomes an issue. An appropriate buffering agent is included to maintain the formulation within stable zones of pH during lyophilization. A comparison of the excipient components contemplated for liquid and lyophilized protein formulations is provided in Table 10.

Table 1: Excipient components of lyophilized protein formulations

Excipient Component	Function in Formulation
Buffer	Maintain pH of formulation during lyophilization and upon reconstitution
Tonicity agent/stabilizer	- Stabilizers include amino acids, lyoprotectants - Excipients include Polyols, sugars and polymers - Cryoprotectants protect proteins from freezing stresses - Lyoprotectants stabilize proteins in the reconstituted state
Bulking agent	- Used to enhance product stability and to prevent osmotic - Provides structural strength to the lyo cake - Excipients include mannitol and glycine
Surfactant	- Employed if aggregation during the lyophilization process is an issue - May serve to reduce reconstitution times - Excipients include polysorbates 20 and 80
Anti-oxidant	Usually not employed, molecular structures in the lyo cake are generally resistant
Metal chelating agent	- May be included if a specific metal ion is indicated only as a co-factor or where the metal is required for protease activity - Chelating agents are generally not needed in lyo formulations
Preservative	- For multi-dose formulations only - Provides protection against microbial growth in formulation - Is usually included in the reconstitution solvent (e.g., BWW)

[0135] The principal challenge in developing formulations for proteins is stabilizing the product against the stresses of manufacturing, shipping and storage. The role of formulation excipients is to provide stabilization against these stresses. Excipients are also employed to reduce viscosity of high concentration protein formulations in order to enable their delivery and enhance patient convenience. In general, excipients can be classified on the basis of the mechanisms by which they stabilize proteins against various chemical and physical stresses. Some excipients are used to alleviate the effects of a specific stress or to regulate a particular susceptibility of a specific protein. Other excipients have more general effects on the physical and covalent stabilities of proteins. The excipients described herein are organized either by their chemical type or their functional role in formulations. Brief descriptions of the modes of stabilization are provided when discussing each excipient type.

[0136] Given the teachings and guidance provided herein, those skilled in the art will know what amount or range of excipient can be included in any particular formulation to achieve a biopharmaceutical formulation for the use of the invention that promotes retention in stability of the biopharmaceutical (e.g., a protein). For example, the amount and type of a salt to be included in a biopharmaceutical formulation for the use of the invention is selected based on the desired osmolality (e.g., isotonic, hypotonic or hypertonic) of the final solution as well as the amounts and osmolality of other components to be included in the formulation.

[0137] By way of example, inclusion of about 5% sorbitol can achieve isotonicity while about 9% of a sucrose excipient is needed to achieve isotonicity. Selection of the amount or range of concentrations of one or more excipients that can be included within a biopharmaceutical formulation for the use of the invention has been exemplified above by reference to salts, polyols and sugars. However, those skilled in the art will understand that the considerations described herein and further exemplified by reference to specific excipients are equally applicable to all types and combinations of excipients including, for example, salts, amino acids, other tonicity agents, surfactants, stabilizers, bulking agents, cryoprotectants, lyoprotectants, anti-oxidants, metal ions, chelating agents and/or preservatives.

[0138] Further, where a particular excipient is reported in molar concentration, those skilled in the art will recognize that the equivalent percent (%) w/v (e.g., (grams of substance in a solution sample/mL of solution) X 100%) of solution is also contemplated.

[0139] Of course, a person having ordinary skill in the art would recognize that the concentrations of the excipients described herein share an interdependency within a particular formulation. By way of example, the concentration of a bulking agent may be lowered where, e.g., there is a high protein concentration or where, e.g., there is a high stabilizing agent concentration. In addition, a person having ordinary skill in the art would recognize that, in order to maintain the isotonicity of a particular formulation in which there is no bulking agent, the concentration of a stabilizing agent would be adjusted accordingly (e.g., a "tonicifying" amount of stabilizer would be used). Common excipients are known in the art and can be found in Powell et al., Compendium of Excipients for Parenteral Formulations (1998), PDA J. Pharm. Sci. Technology, 52:238-311.

c. Pharmaceutical Buffers and Buffering Agents

[0140] The stability of a pharmacologically active protein formulation is usually observed to be maximal in a narrow pH range. This pH range of optimal stability needs to be identified early during pre-formulation studies. Several approaches, such as accelerated stability studies and calorimetric screening studies, are useful in this endeavor (Remmele R.L. Jr., et al., Biochemistry, 38(16): 5241-7 (1999)). Once a formulation is finalized, the protein must be manufactured and maintained throughout its shelf-life. Hence, buffering agents are almost always employed to control pH in the formulation.

[0141] The buffer capacity of the buffering species is maximal at a pH equal to the pKa and decreases as pH increases or decreases away from this value. Ninety percent of the buffering capacity exists within one pH unit of its pKa. Buffer capacity also increases proportionally with increasing buffer concentration.

[0142] Several factors need to be considered when choosing a buffer. First and foremost, the buffer species and its concentration need to be defined based on its pKa and the desired formulation pH. Equally important is to ensure that the buffer is compatible with the protein and other formulation excipients, and does not catalyze any degradation reactions. A third important aspect to be considered is the sensation of stinging and irritation the buffer may induce upon administration. For example, citrate is known to cause stinging upon injection (Laursen T, et al., *Basic Clin Pharmacol Toxicol.*, 98(2): 218-21 (2006)). The potential for stinging and irritation is greater for drugs that are administered via the subcutaneous (SC) or intramuscular (IM) routes, where the drug solution remains at the site for a relatively longer period of time than when administered by the IV route where the formulation gets diluted rapidly into the blood upon administration. For formulations that are administered by direct IV infusion, the total amount of buffer (and any other formulation component) needs to be monitored. One has to be particularly careful about potassium ions administered in the form of the potassium phosphate buffer, which can induce cardiovascular effects in a patient (Hollander-Rodriguez JC, et al., *Am. Fam. Physician.*, 73(2): 283-90 (2006)).

[0143] Buffers for lyophilized formulations need additional consideration. Some buffers like sodium phosphate can crystallize out of the protein amorphous phase during freezing resulting in shifts in pH. Other common buffers such as acetate and imidazole may sublime or evaporate during the lyophilization process, thereby shifting the pH of formulation during lyophilization or after reconstitution.

[0144] The buffer system present in the compositions is selected to be physiologically compatible and to maintain a desired pH of the pharmaceutical formulation. In one embodiment, the pH of the solution is between pH 2.0 and pH 12.0. For example, the pH of the solution may be 2.0, 2.3, 2.5, 2.7, 3.0, 3.3, 3.5, 3.7, 4.0, 4.3, 4.5, 4.7, 5.0, 5.3, 5.5, 5.7, 6.0, 6.3, 6.5, 6.7, 7.0, 7.3, 7.5, 7.7, 8.0, 8.3, 8.5, 8.7, 9.0, 9.3, 9.5, 9.7, 10.0, 10.3, 10.5, 10.7, 11.0, 11.3, 11.5, 11.7, or 12.0.

[0145] The pH buffering compound may be present in any amount suitable to maintain the pH of the formulation at a predetermined level. In one embodiment, the pH buffering concentration is between 0.1 mM and 500 mM (1 M). For example, it is contemplated that the pH buffering agent is at least 0.1, 0.5, 0.7, 0.8 0.9, 1.0, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, or 500 mM.

[0146] Exemplary pH buffering agents used to buffer the formulation as set out herein include, but are not limited to organic acids, glycine, histidine, glutamate, succinate, phosphate, acetate, citrate, Tris, HEPES, and amino acids or mixtures of amino acids, including, but not limited to aspartate, histidine, and glycine. In one embodiment of the use of the present invention, the buffering agent is citrate.

d. Pharmaceutical Stabilizers and Bulking Agents

[0147] In one aspect of the present pharmaceutical formulations, a stabilizer (or a combination of stabilizers) is added to prevent or reduce storage-induced aggregation and chemical degradation. A hazy or turbid solution upon reconstitution indicates that the protein has precipitated or at least aggregated. The term "stabilizer" means an excipient capable of preventing aggregation or physical degradation, including chemical degradation (for example, autolysis, deamidation, oxidation, etc.) in an aqueous state. Stabilizers contemplated include, but are not limited to, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, mannitol, sorbitol, glycine, arginine HCL, poly-hydroxy compounds, including polysaccharides such as dextran, starch, hydroxyethyl starch, cyclodextrins, N-methyl pyrrolidene, cellulose and hyaluronic acid, sodium chloride, (Carpenter et al., *Develop. Biol. Standard* 74:225, (1991)). In the present formulations, the stabilizer is incorporated in a concentration of about 0.1, 0.5, 0.7, 0.8 0.9, 1.0, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 700, 900, or 1000 mM. In one embodiment of the use of the present invention, mannitol and trehalose are used as stabilizing agents.

[0148] If desired, the formulations also include appropriate amounts of bulking and osmolality regulating agents. Bulking agents include, for example and without limitation, mannitol, glycine, sucrose, polymers such as dextran, polyvinylpyrrolidone, carboxymethylcellulose, lactose, sorbitol, trehalose, or xylitol. In one embodiment, the bulking agent is mannitol. The bulking agent is incorporated in a concentration of about 0.1, 0.5, 0.7, 0.8 0.9, 1.0, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 700, 900, or 1000 mM.

e. Pharmaceutical Surfactants

[0149] Proteins have a high propensity to interact with surfaces making them susceptible to adsorption and denaturation at air-liquid, vial-liquid, and liquid-liquid (silicone oil) interfaces. This degradation pathway has been observed to be inversely dependent on protein concentration and results in either the formation of soluble and insoluble protein aggregates or the loss of protein from solution via adsorption to surfaces. In addition to container surface adsorption, surface-induced degradation is exacerbated with physical agitation, as would be experienced during shipping and handling of the product.

[0150] Surfactants are commonly used in protein formulations to prevent surface-induced degradation. Surfactants are amphipathic molecules with the capability of outcompeting proteins for interfacial positions. Hydrophobic portions of the surfactant molecules occupy interfacial positions (*e.g.*, air/liquid), while hydrophilic portions of the molecules remain oriented towards the bulk solvent. At sufficient concentrations (typically around the detergent's critical micellar concentration), a surface layer of surfactant molecules serves to prevent protein molecules from adsorbing at the interface. Thereby, surface-induced degradation is minimized. Surfactants contemplated herein include, without limitation, fatty acid esters of sorbitan polyethoxylates, *e.g.*, polysorbate 20 and polysorbate 80. The two differ only in the length of the aliphatic chain that imparts hydrophobic character to the molecules, C-12 and C-18, respectively. Accordingly, polysorbate-80 is more surface-active and has a lower critical micellar concentration than polysorbate-20.

[0151] Detergents can also affect the thermodynamic conformational stability of proteins. Here again, the effects of a given detergent excipient will be protein specific. For example, polysorbates have been shown to reduce the stability of some proteins and increase the stability of others. Detergent destabilization of proteins can be rationalized in terms of the hydrophobic tails of the detergent molecules that can engage in specific binding with partially or wholly unfolded protein states. These types of interactions could cause a shift in the conformational equilibrium towards the more expanded protein states (*e.g.* increasing the exposure of hydrophobic portions of the protein molecule in complement to binding polysorbate). Alternatively, if the protein native state exhibits some hydrophobic surfaces, detergent binding to the native state may stabilize that conformation.

[0152] Another aspect of polysorbates is that they are inherently susceptible to oxidative degradation. Often, as raw materials, they contain sufficient quantities of peroxides to cause oxidation of protein residue side-chains, especially methionine. The potential for oxidative damage arising from the addition of stabilizer emphasizes the point that the lowest effective concentrations of excipients should be used in formulations. For surfactants, the effective concentration for a given protein will depend on the mechanism of stabilization.

[0153] Surfactants are also added in appropriate amounts to prevent surface related aggregation phenomenon during freezing and drying (Chang, B, J. Pharm. Sci. 85:1325, (1996)). Thus, exemplary surfactants include, without limitation, anionic, cationic, nonionic, zwitterionic, and amphoteric surfactants including surfactants derived from naturally-occurring amino acids. Anionic surfactants include, but are not limited to, sodium lauryl sulfate, dioctyl sodium sulfo succinate and dioctyl sodium sulfonate, chenodeoxycholic acid, N-lauroylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate, sodium deoxycholate, and glycodeoxycholic acid sodium salt. Cationic surfactants include, but are not limited to, benzalkonium chloride or benzethonium chloride, cetylpyridinium chloride monohydrate, and hexadecyltrimethylammonium bromide. Zwitterionic surfactants include, but are not limited to, CHAPS, CHAPSO, SB3-10, and SB3-12. Non-ionic surfactants include, but are not limited to, digitonin, Triton X-100, Triton X-114, TWEEN-20, and TWEEN-80. Surfactants also include, but are not limited to laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 40, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, soy lecithin and other phospholipids such as dioleoyl phosphatidyl choline (DOPC), dimyristoylphosphatidyl glycerol (DMPG), dimyristoylphosphatidyl choline (DMPC), and (dioleoyl phosphatidyl glycerol) DOPG; sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Compositions comprising these surfactants, either individually or as a mixture in different ratios, are therefore further provided. In one embodiment of the use of the present invention, the surfactant is TWEEN-80. In the present formulations, the surfactant is incorporated in a concentration of about 0.01 to about 0.5 g/L. In formulations provided, the surfactant concentration is 0.005, 0.01, 0.02, 0.03, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0 g/L.

f. Pharmaceutical Salts

[0154] Salts are often added to increase the ionic strength of the formulation, which can be important for protein solubility, physical stability, and isotonicity. Salts can affect the physical stability of proteins in a variety of ways. Ions can stabilize the native state of proteins by binding to charged residues on the protein's surface. Alternatively, salts can stabilize the denatured state by binding to peptide groups along the protein backbone (-CONH-). Salts can also stabilize the protein native conformation by shielding repulsive electrostatic interactions between residues within a protein molecule. Salts in protein formulations can also shield attractive electrostatic interactions between protein molecules that can lead to protein aggregation and insolubility. In formulations provided, the salt concentration is between 0.1, 1, 10, 20, 30, 40, 50, 80, 100, 120, 150, 200, 300, and 500 mM.

g. Other Common Excipient Components: Pharmaceutical Amino Acids

[0155] Amino acids have found versatile use in protein formulations as buffers, bulking agents, stabilizers and antioxidants. Thus, in one aspect histidine and glutamic acid are employed to buffer protein formulations in the pH range of 5.5 - 6.5 and 4.0 - 5.5 respectively. The imidazole group of histidine has a pKa = 6.0 and the carboxyl group of glutamic acid side chain has a pKa of 4.3 which makes these amino acids suitable for buffering in their respective pH ranges. Glutamic acid is particularly useful in such cases. Histidine is commonly found in marketed protein formulations, and this amino acid provides an alternative to citrate, a buffer known to sting upon injection. Interestingly, histidine has also been reported to have a stabilizing effect, with respect to aggregation when used at high concentrations in both liquid and lyophilized presentations (Chen B, et al., Pharm Res., 20(12): 1952-60 (2003)). Histidine was also observed by others to reduce the viscosity of a high protein concentration formulation. However, in the same study, the authors observed increased aggregation and discoloration in histidine containing formulations during freeze-thaw studies of the antibody in stainless steel containers. Another note of caution with histidine is that it undergoes photo-oxidation in the presence of metal ions (Tomita M, et al., Biochemistry, 8(12): 5149-60 (1969)). The use of methionine as an antioxidant in formulations appears promising; it has been observed to be effective against a number of oxidative stresses (Lam XM, et al., J Pharm Sci., 86(11): 1250-5 (1997)).

[0156] In various aspects, formulations are provided which include one or more of the amino acids glycine, proline, serine, arginine and alanine have been shown to stabilize proteins by the mechanism of preferential exclusion. Glycine is also a commonly used bulking agent in lyophilized formulations. Arginine has been shown to be an effective agent in inhibiting aggregation and has been used in both liquid and lyophilized formulations.

[0157] In formulations provided, the amino acid concentration is between 0.1, 1, 10, 20, 30, 40, 50, 80, 100, 120, 150, 200, 300, and 500 mM. In one embodiment of the use of the present invention, the amino acid is glycine.

h. Other Common Excipient Components: Pharmaceutical Antioxidants

[0158] Oxidation of protein residues arises from a number of different sources. Beyond the addition of specific antioxidants, the prevention of oxidative protein damage involves the careful control of a number of factors throughout the manufacturing process and storage of the product such as atmospheric oxygen, temperature, light exposure, and chemical contamination. The invention therefore contemplates the use of the pharmaceutical antioxidants including, without limitation, reducing agents, oxygen/free-radical scavengers, or chelating agents. Antioxidants in therapeutic protein formulations are, in one aspect, water-soluble and remain active throughout the product shelf -life. Reducing agents and oxygen/free-radical scavengers work by ablating active oxygen species in solution. Chelating agents such as EDTA are effective by binding trace metal contaminants that promote free-radical formation. For example, EDTA was utilized in the liquid formulation of acidic fibroblast growth factor to inhibit the metal ion catalyzed oxidation of cysteine residues.

[0159] In addition to the effectiveness of various excipients to prevent protein oxidation, the potential for the antioxidants themselves to induce other covalent or physical changes to the protein is of concern. For example, reducing agents can cause disruption of intramolecular disulfide linkages, which can lead to disulfide shuffling. In the presence of transition metal ions, ascorbic acid and EDTA have been shown to promote methionine oxidation in a number of proteins and peptides (Akers MJ, and Defelippis MR. Peptides and Proteins as Parenteral Solutions. In: Pharmaceutical Formulation Development of Peptides and Proteins. Sven Frokjaer, Lars Hovgaard, editors. Pharmaceutical Science. Taylor and Francis, UK (1999)); Fransson J.R., J. Pharm. Sci. 86(9): 4046-1050 (1997); Yin J, et al., Pharm Res., 21(12): 2377-83 (2004)). Sodium thiosulfate has been reported to reduce the levels of light and temperature induced methionine-oxidation in rhuMab HER2; however, the formation of a thiosulfate-protein adduct was also reported in this study (Lam XM, Yang JY, et al., J Pharm Sci. 86(11): 1250-5 (1997)). Selection of an appropriate antioxidant is made according to the specific stresses and sensitivities of the protein.

Antioxidants contemplated in certain aspects include, without limitation, reducing agents and oxygen/free-radical scavengers, EDTA, and sodium thiosulfate.

i. Other Common Excipient Components: Pharmaceutical Metal Ions

[0160] In general, transition metal ions are undesired in protein formulations because they can catalyze physical and chemical degradation reactions in proteins. However, specific metal ions are included in formulations when they are co-factors to proteins and in suspension formulations of proteins where they form coordination complexes (e.g., zinc suspension of insulin). Recently, the use of magnesium ions (10 - 120 mM) has been proposed to inhibit the isomerization of aspartic acid to isoaspartic acid (WO 2004039337).

[0161] Two examples where metal ions confer stability or increased activity in proteins are human deoxyribonuclease (rhDNase, Pulmozyme®), and Factor VIII. In the case of rhDNase, Ca⁺² ions (up to 100 mM) increased the stability of the enzyme through a specific binding site (Chen B, et al., *J. Pharm. Sci.*, 88(4): 477-82 (1999)). In fact, removal of calcium ions from the solution with EGTA caused an increase in deamidation and aggregation. However, this effect was observed only with Ca⁺² ions; other divalent cations Mg⁺², Mn⁺² and Zn⁺² were observed to destabilize rhDNase. Similar effects were observed in Factor VIII. Ca⁺² and Sr⁺² ions stabilized the protein while others like Mg⁺², Mn⁺² and Zn⁺², Cu⁺² and Fe⁺² destabilized the enzyme (Fatouros, A., et al., *Int. J. Pharm.*, 155, 121-131 (1997)). In a separate study with Factor VIII, a significant increase in aggregation rate was observed in the presence of Al⁺³ ions (Derrick TS, et al., *J. Pharm. Sci.*, 93(10): 2549-57 (2004)). The authors note that other excipients like buffer salts are often contaminated with Al⁺³ ions and illustrate the need to use excipients of appropriate quality in formulated products.

j. Other Common Excipient Components: Pharmaceutical Preservatives

[0162] Preservatives are necessary when developing multi-use parenteral formulations that involve more than one extraction from the same container. Their primary function is to inhibit microbial growth and ensure product sterility throughout the shelf life or term of use of the drug product. Commonly used preservatives include, without limitation, benzyl alcohol, phenol and m-cresol. Although preservatives have a long history of use, the development of protein formulations that includes preservatives can be challenging. Preservatives almost always have a destabilizing effect (aggregation) on proteins, and this has become a major factor in limiting their use in multi-dose protein formulations (Roy S, et al., *J. Pharm. Sci.*, 94(2): 382-96 (2005)).

[0163] To date, most protein drugs have been formulated for single-use only. However, when multi-dose formulations are possible, they have the added advantage of enabling patient convenience, and increased marketability. A good example is that of human growth hormone (hGH) where the development of preserved formulations has led to commercialization of more convenient, multi-use injection pen presentations. At least four such pen devices containing preserved formulations of hGH are currently available on the market. Norditropin® (liquid, Novo Nordisk), Nutropin AQ® (liquid, Genentech) & Genotropin (lyophilized - dual chamber cartridge, Pharmacia & Upjohn) contain phenol while Somatrop® (Eli Lilly) is formulated with m-cresol.

[0164] Several aspects need to be considered during the formulation development of preserved dosage forms. The effective preservative concentration in the drug product must be optimized. This requires testing a given preservative in the dosage form with concentration ranges that confer anti-microbial effectiveness without compromising protein stability. For example, three preservatives were successfully screened in the development of a liquid formulation for interleukin-1 receptor (Type I), using differential scanning calorimetry (DSC). The preservatives were rank ordered based on their impact on stability at concentrations commonly used in marketed products (Remmele RL Jr., et al., *Pharm. Res.*, 15(2): 200-8 (1998)).

[0165] Development of liquid formulations containing preservatives are more challenging than lyophilized formulations. Freeze-dried products can be lyophilized without the preservative and reconstituted with a preservative containing diluent at the time of use. This shortens the time for which a preservative is in contact with the protein significantly minimizing the associated stability risks. With liquid formulations, preservative effectiveness and stability have to be maintained over the entire product shelf-life (-18 -24 months). An important point to note is that preservative effectiveness has to be demonstrated in the final formulation containing the active drug and all excipient components.

[0166] Some preservatives can cause injection site reactions, which is another factor that needs consideration when choosing a preservative. In clinical trials that focused on the evaluation of preservatives and buffers in Norditropin, pain perception was observed to be lower in formulations containing phenol and benzyl alcohol as compared to a formulation containing m-cresol (Kappelgaard A.M., *Horm. Res. Suppl.* 3:98-103 (2004)). Interestingly, among the commonly used preservative, benzyl alcohol possesses anesthetic properties (Minogue SC, and Sun DA., *Anesth. Analg.*, 100(3): 683-6 (2005)). In various aspects the use of preservatives provide a benefit that outweighs any side effects.

k. Methods of Preparation of Pharmaceutical Formulations

[0167] The present specification further contemplates methods for the preparation of pharmaceutical formulations whereby these are not part of the invention.

[0168] The present methods further comprise one or more of the following steps: adding a stabilizing agent as described herein to said mixture prior to lyophilizing, adding at least one agent selected from a bulking agent, an osmolality regulating agent, and a surfactant, each of which as described herein, to said mixture prior to lyophilization.

[0169] The standard reconstitution practice for lyophilized material is to add back a volume of pure water or sterile water for injection (WFI) (typically equivalent to the volume removed during lyophilization), although dilute solutions of antibacterial agents are sometimes used in the production of pharmaceuticals for parenteral administration (Chen, *Drug Development and Industrial Pharmacy*, 18:1311-1354 (1992)). Accordingly, methods are provided for preparation of reconstituted rVWF compositions comprising the step of adding a diluent to a lyophilized rVWF composition for the use of the invention.

[0170] The lyophilized material may be reconstituted as an aqueous solution. A variety of aqueous carriers, e.g., sterile water for injection, water with preservatives for multi dose use, or water with appropriate amounts of surfactants (for example, an aqueous suspension that contains the active compound

in admixture with excipients suitable for the manufacture of aqueous suspensions). In various aspects, such excipients are suspending agents, for example and without limitation, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents are a naturally-occurring phosphatide, for example and without limitation, lecithin, or condensation products of an alkylene oxide with fatty acids, for example and without limitation, polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example and without limitation, heptadecaethyl-eneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example and without limitation, polyethylene sorbitan monooleate. In various aspects, the aqueous suspensions also contain one or more preservatives, for example and without limitation, ethyl, or n-propyl, p-hydroxybenzoate.

I. Exemplary rVWF Formulation for Administration

[0171] In some embodiments, the present methods provide for an enhanced formulation that allows a final product with high potency (high rVWF concentration and enhanced long term stability) in order to reduce the volume for the treatment (100 IU/ml to 10000 IU/ml). In some embodiments, the rVWF concentration in the formulation for administration is about 100 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 500 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 1000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 2000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 3000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 4000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 5000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 6000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 7000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 8000 IU/ml to 10000 IU/ml. In some embodiments, the rVWF concentration in the formulation for administration is about 9000 IU/ml to 10000 IU/ml.

[0172] In some embodiments, the formulation for administration comprises one or more zwitterionic compounds, including for example, amino acids like Histidine, Glycine, Arginine. In some embodiments, the formulation for administration comprises a component with amphipathic characteristic having a minimum of one hydrophobic and one hydrophilic group, including for example polysorbate 80, octylpyranosid, dipeptides, and/or amphipathic peptides. In some embodiments, the formulation for administration comprises a non reducing sugar or sugar alcohol or disaccharides, including for example, sorbitol, mannitol, sucrose, or trehalose. In some embodiments, the formulation for administration comprises a nontoxic water soluble salt, including for example, sodium chloride, that results in a physiological osmolality. In some embodiments, the formulation for administration comprises a pH in a range from 6.0 to 8.0. In some embodiments, the formulation for administration comprises a pH of about 6.0, about 6.5, about 7, about 7.5 or about 8.0. In some embodiments, the formulation for administration comprises one or more bivalent cations that stabilize rVWF, including for example, Ca²⁺, Mg²⁺, Zn²⁺, Mn²⁺ and/or combinations thereof. In some embodiments, the formulation for administration comprises about 1 mM to about 50 mM Glycine, about 1 mM to about 50 mM Histidine, about zero to about 300 mM sodium chloride (e.g., less than 300 mM sodium), about 0.01 % to about 0.05% polysorbate 20 (or polysorbate 80), and about 0.5 % to about 20% (w/w) sucrose with a pH of about 7.0 and having a physiological osmolality at the time point of administration.

[0173] In some embodiments, the formulation for administration can be freeze dried. In some embodiments, the formulation for administration is stable and can be stored in liquid state at about 2°C to about 8°C, as well as at about 18°C to about 25°C. In some embodiments, the formulation for administration is stable and can be stored in liquid state at about 2°C to about 8°C. In some embodiments, the formulation for administration is stable and can be stored in liquid state at about 18°C to about 25°C.

m Administration/Dosing

[0174] To administer compositions to human or test animals, in one aspect, the compositions comprises one or more pharmaceutically acceptable carriers. The phrases "pharmaceutically" or "pharmacologically" acceptable refer to molecular entities and compositions that are stable, inhibit protein degradation such as aggregation and cleavage products, and in addition do not produce allergic, or other adverse reactions when administered using routes well-known in the art, as described below. "Pharmaceutically acceptable carriers" include any and all clinically useful solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like, including those agents disclosed above.

[0175] The pharmaceutical formulations are administered orally, topically, transdermally, parenterally, by inhalation spray, vaginally, rectally, or by intracranial injection. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. Administration by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, retrobulbar, and/or intrapulmonary injection at a particular site is contemplated as well. Generally, compositions are essentially free of pyrogens, as well as other impurities that could be harmful to the recipient.

[0176] According to the use of the present invention, rVWF is administered in the absence of Factor VIII (FVIII). In some embodiments, FVIII is not administered.

[0177] In some embodiments, the rVWF is administered prior to the surgical procedure, as discussed herein. In some embodiments, the rVWF is administered at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, or at least 24 hours, prior to the surgical procedure. In some embodiments, the surgical procedure is a minor surgical procedure. In some embodiments, the surgical procedure is a major surgical procedure. In some embodiments, FVIII is not administered.

[0178] In some embodiments, 35-60 IU/kg rVWF is administered at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, or at least 24 hours, prior to the surgical procedure. In some embodiments, 50-60 IU/kg rVWF is administered at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, or at least 24 hours, prior to the surgical procedure. In some embodiments, 20-40 IU/kg rVWF is administered at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, or at least 24 hours, prior to the surgical procedure.

embodiments, a total dosage of about 220 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 230 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 240 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 250 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 260 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 270 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 280 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 290 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 300 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 310 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, a total dosage of about 320 IU/kg rVWF is administered when the surgical procedure is a major surgical procedure. In some embodiments, FVIII is not administered.

[0192] In some embodiments, a total dosage of about 70-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 80-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 90-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 100-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 110-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 120-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 130-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 140-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 150-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 160-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 170-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 180-190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 70 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 80 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 90 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 100 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 110 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 120 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 130 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 140 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 150 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 160 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 170 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 180 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, a total dosage of about 190 IU/kg rVWF is administered when the surgical procedure is an oral surgical procedure. In some embodiments, FVIII is not administered.

[0193] In some embodiments, when the surgical procedure is a major surgical procedure, the pre-treatment comprises administering at least two approximately equal doses of rVWF prior to the surgical procedure. In some embodiments, the dosage is a dosage as listed above. In some embodiments, FVIII is not administered. In some embodiments, when the surgical procedure is a minor surgical procedure, the pre-treatment comprises administering at least two doses of rVWF prior to the surgical procedure. In some embodiments, when the surgical procedure is a minor surgical procedure, the pre-treatment comprises administering at least two doses of rVWF prior to the surgical procedure, wherein the first dose is larger than the second dose. In some embodiments, the dosage is a dosage as listed above. In some embodiments, FVIII is not administered. In some embodiments, the surgical procedure is an oral surgical procedure and the pre-treatment comprises administering at least two approximately equal doses of rVWF prior to the surgical procedure. In some embodiments, the dosage is a dosage as listed above. In some embodiments, FVIII is not administered.

[0194] Generally, Type 1 VWD is indicated by <30 IU/dL VWF:RCo, <30 IU/dL VWF:Ag, low or normal FVIII, and $>0.5-0.7$ IU/dLVWF:RCo/VWF:Ag Ratio. Type 2A VWD is indicated by <30 IU/dL VWF:RCo, $<30-200$ IU/dL VWF:Ag, low or normal FVIII, and $<0.5-0.7$ IU/dLVWF:RCo/VWF:Ag Ratio. Type 2B VWD is indicated by $<30-200$ IU/dL VWF:RCo, <30 IU/dL VWF:Ag, low or normal FVIII, and usually $<0.5-0.7$ IU/dLVWF:RCo/VWF:Ag Ratio. Type 2M VWD is indicated by <30 IU/dL VWF:RCo, $<30-200$ IU/dL VWF:Ag, low or normal FVIII, and $<0.5-0.7$ IU/dLVWF:RCo/VWF:Ag Ratio. Type 2N VWD is indicated by $30-2000$ IU/dL VWF:RCo, $30-200$ IU/dL VWF:Ag, very low FVIII, and $>0.5-0.7$ IU/dLVWF:RCo/VWF:Ag Ratio. Type 3 VWD is indicated by <3 IU/dL VWF:RCo, <3 IU/dL VWF:Ag, extremely low (<10 IU/dL) FVIII, and the VWF:RCo/VWF:Ag Ratio is not applicable. Normal is indicated by $50-200$ IU/dL VWF:RCo, $50-200$ IU/dL VWF:Ag, normal FVIII, and $>0.5-0.7$ IU/dLVWF:RCo/VWF:Ag Ratio. In some embodiments, the subject has Type 3 VWD. In some embodiments, the subject has severe type 1 VWD. In some embodiments, the subject has severe type 2 VWD.

V. Surgical Procedures

[0195] The surgical procedure according to the use of the present invention can be a major surgical procedure or a minor surgical procedure.

[0196] Generally, major surgery includes any invasive operative procedure in which a more extensive resection is performed, e.g., a body cavity is entered, organs are removed, or normal anatomy is altered. Generally, if a mesenchymal barrier is opened (for example, pleural cavity, peritoneum, meninges), the surgery is considered major. In some embodiments, a major surgery is one in which there is an expected blood loss of greater than 500 mL, significant fluid shifts and typically, at least one night in hospital. Exemplary major surgical procedures include but are not limited to bariatric surgeries/gastric bypass, septal myotomy, pancreatectomy, thoracic aortic dissection repair, esophagectomy, bladder cystectomy, coronary revascularization, spinal osteomyelitis surgery, surgical ventricular restoration, craniectomy, laparoscopic surgery (except cholecystectomy and tubal ligation), open resection of organs, large joint replacements, mastectomy with reconstruction, and/or spine, thoracic, vascular, and/or intracranial surgery. Further examples of major surgeries include but are not limited to maxillary or mandibular osteotomy, laryngectomy, resection of large benign or malignant mass and/or lymph node dissection requiring overnight stay in hospital (with or without reconstructive surgery), mastectomy with immediate tissue reconstruction (with or without lymph node biopsy or axillary dissection), laparoscopic or open repair or resection (of, for example, stomach, small bowel, colon, liver, pancreas, spleen, adrenals or liver), open cholecystectomy, large incisional, epigastric or ventral hernia repairs, hysteroscopic resection or ablation, hysterectomy and/or adnexal surgery, laparoscopy

for extensive endometriosis, abdominal or transvaginal pelvic floor surgery, intracranial surgery, spinal laminectomy and/or fusion, knee replacement, hip replacement, shoulder replacement, elbow joint replacement, hardware removal or revision for infection or failure, amputation, spinal laminectomy and/or fusion, free flap reconstruction (plastic surgery), panniculectomy, mediastinoscopy, lung resection, esophagus resection, mediastinal mass resection (thoroscopic or open), hiatal hernia repair (thoroscopic or open), bladder tumor resection (transurethral or open), prostate tumor (transurethral or open), resection of kidney resection (laparoscopic or open), ureteral resection (laparoscopic or open), resection of testis (transscrotal or abdominal), amputation, peripheral arterial bypass surgery, aortic aneurysm repair (endovascular or open), and/or carotid endarterectomy.

[0197] Minor surgery is any invasive operative procedure in which only skin or mucus membranes and connective tissue is resected e.g. vascular cutdown for catheter placement, implanting pumps in subcutaneous tissue. A minor surgical procedure typically includes any procedure that can be safely performed in an outpatient setting, without the use of general anesthesia or the need for respiratory assistance. In some embodiments, a minor surgery is one in which there is an expected blood loss of less than 500 mL, minimal fluid shifts and is typically done on an ambulatory basis (day surgery/same day discharge). Such outpatient surgical procedures can include but are not limited to cataract surgery, breast surgery without reconstruction, laparoscopic cholecystectomy and tubal ligation, and most cutaneous, superficial, endoscopic and arthroscopic procedures. Further examples of minor surgeries include but are not limited to tooth extraction, tonsillectomy, adenoidectomy, septoplasty, turbinectomy, rhinoplasty, pharyngeal biopsy, laryngeal biopsy, minor excision by laser or other means, middle ear surgery, mastoidectomy, cochlear implantation, endoscopic sinus surgery, small resections of benign and malignant masses (done on an ambulatory basis; *i.e.*, mandibular tori, brachial cleft cyst, small tongue cancer), thyroidectomy, breast lumpectomy (with or without lymph node biopsy or axillary dissection), mastectomy (with or without lymph node biopsy or axillary dissection), inguinal hernia repair (laparoscopic or open approach), umbilical hernia repair (laparoscopic or open approach), laparoscopic cholecystectomy, hemorrhoidectomy, dilation, curettage, diagnostic hysteroscopy, laparoscopy, endometrial ablation by thermal balloon, tubal ligation, laparoscopy - limited endometriosis, transvaginal tape insertion, discectomy, cataract extraction, most ophthalmological procedures, arthroscopic surgery (including ACL repair), routine hardware removal (not for infection), tendon surgery, bunionectomy, discectomy, carpal tunnel release, Dupuytren's contracture release, major tendone surgery, minor tendon surgery, small rotational flaps and skin grafts, basal cell carcinoma resection, lipoma excision, reduction mammoplasty and other surgery for benign breast disease, cosmetic breast surgery, bronchoscopy, cystoscopy, ureteroscopy, renoscopy for stricture, renoscopy for biopsy, hydrocele excision, varicocele excision, vasectomy, circumcision, and/or varicose vein excision.

[0198] Oral surgical procedures include, but are not limited to, various dental procedures and oral surgeries, including for example tooth extractions.

[0199] In some embodiments, the surgical procedure is a major surgical procedure. In some embodiments, the surgical procedure is a minor surgical procedure. In some embodiments, the surgical procedure is an oral surgical procedure.

EXAMPLES

EXAMPLE 1: HEMOSTATIC EFFICACY AND SAFETY OF RWVF

[0200] This study evaluated the hemostatic efficacy and safety of rVWF with or without ADVATE (antihemophilic factor [recombinant]), Baxalta US Inc., Westlake Village, CA (rFVIII) in patients with severe VWD undergoing elective surgery.

METHODS

[0201] Phase 3, open-label, uncontrolled, nonrandomized study at 14 sites in 10 countries (NCT02283268) in patients ≥ 18 y of age who had severe VWD and were scheduled to undergo elective surgery. Patients were monitored for 14 d after surgery.

TREATMENT

[0202] 12-24 h before surgery, rVWF 40-60 IU/kg rVWF:RCo was given intravenously to allow endogenous FVIII:C levels to increase to ≥ 30 IU/dL (minor/oral surgery) or ≥ 60 IU/dL (major surgery). FVIII:C levels were assessed within 3 h of initiation of surgery. If target FVIII:C levels were achieved, rVWF alone was administered 1 h before surgery to achieve the peak levels described in Table 2. If target FVIII:C levels were achieved, rVWF alone was administered 1 h before surgery to achieve the peak levels described in Table 2. Intraoperative and postoperative dosing were individualized to maintain target trough levels according to pharmacokinetic (PK) and pharmacodynamic (PD) results, as well as the intensity and duration of the hemostatic challenge.

Table 2. VWF:RCo and FVIII:C Target Levels: Recommendations for the Prevention of Excessive Bleeding During and After Surgery

Type of Surgery	VWF:RCo Target Peak Plasma Level (IU/dL)	FVIII:C Target Peak Plasma Level* (IU/dL)	Calculation of rVWF Dose (IU VWF:RCo Required)†
Minor/Oral	50-60	40-50	$\Delta\text{VWF:RCo} \times \text{BW (kg)}/\text{IR}‡$
Major	100	80-100	$\Delta\text{VWF:RCo} \times \text{BW (kg)}/\text{IR}‡$

BW=body weight; FVIII:C=factor VIII activity; IR=incremental recovery;
 rVWF=recombinant von Willebrand factor; VWF:RCo= von Willebrand factor ristocetin cofactor activity.
 *Additional rFVIII may be required to attain the recommended FVIII:C target peak plasma levels.
 †Administered within 1 h before surgery.
 ‡If the IR was not available, assume an IR of 2.0 IU/dL per IU/kg.
 $\Delta\text{VWF:RCo} = \text{target peak plasma VWF:RCo} - \text{baseline plasma VWF:RCo}$.

ASSESSMENT

[0203] Overall hemostatic efficacy (primary outcome) was assessed by the investigator at 24 h after the last perioperative infusion or at study completion,

whichever occurred earlier (Table 3). Intraoperative hemostatic efficacy was assessed by the surgeon (Table 3), along with intraoperative actual versus predicted blood loss. Safety evaluations included adverse events (AEs) and antibodies to rVWF, rFVIII, Chinese hamster ovary (CHO) proteins, murine immunoglobulin G (IgG), and rFurin.

Table 3. Overall* and Intraoperative† Hemostatic Efficacy Rating Scale

Rating	Assessment
Excellent	Hemostasis achieved with rVWF with or without rFVIII was as good or better than that expected for the type of surgical procedure performed in a hemostatically normal subject
Good	Hemostasis achieved with rVWF with or without rFVIII was probably as good as that expected for the type of surgical procedure performed in a hemostatically normal subject
Moderate	Hemostasis with rVWF with or without rFVIII was clearly less than optimal for the type of procedure performed but was maintained without the need to change the rVWF concentrate
None	Patient experienced uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of rVWF concentrate
rFVIII=recombinant factor VIII; rVWF=recombinant von Willebrand factor. *As assessed by the investigator. †As assessed by the surgeon.	

STATISTICS

[0204] Descriptive analyses included point estimates and 90% CIs for the number of patients with hemostatic efficacy rated "excellent/good" using a Clopper Pearson test. PK/PD and safety were summarized using descriptive statistics.

RESULTS

PATIENTS

[0205]

Table 4. Baseline Demographics and Clinical Characteristics

Parameter	N=15
Sex, n (%)	
Male	7 (46.7)
Female	8 (53.3)
Median age (range), y	40 (20-70)
Median weight (range), kg	73.5 (52.0-127.2)
Median BMI (range), kg/m²	25.6 (17.1-38.0)
VWD type, n (%)	
1	3 (20.0)
2A	2 (13.3)
2B	1 (6.7)
2M	1 (6.7)
3	8 (53.3)
Surgery classification, n (%)	
Major	10 (66.7)
Minor	4 (26.7)
Oral	1 (6.7)
Mean (SD) FVIII:C, IU/dL	
All VWD types (n=11)	20.6 (23.7)
Type 3 VWD (n=5)	1.8 (1.1)
Mean (SD) VWF:RCo, IU/dL	
All VWD types (n=11)	9.7 (11.0)
Type 3 VWD (n=5)	<8 (0.0)
BMI=body mass index; FVIII:C=factor VIII activity; VWD=von Willebrand disease; VWF:RCo=von Willebrand factor ristocetin cofactor activity.	

[0206] Overall hemostatic efficacy was rated as "excellent" or "good" for all 15 patients (90% CI: 81.9-100.0) (Figure 1).

EFFICACY

[0207] Overall hemostatic efficacy was rated as "excellent" or "good" for all 15 patients (90% CI: 81.9-100.0) (Figure 1).

[0208] Intraoperative hemostatic efficacy was rated "excellent" or "good" for all 15 patients (90% CI: 81.9-100.0) (Figure 2). Among the 8 patients with type 3 VWD, overall and intraoperative hemostatic efficacy were both rated "excellent" for 7 patients and "good" for 1 patient. Mean $\pm$ SD intraoperative actual blood loss relative to predicted blood loss was 70% $\pm$ 45% and was rated "excellent" for 13 patients and "good" for 2 patients.

EXPOSURE

[0209] Patients received a total of 104 infusions of rVWF to prevent or treat surgical bleeding; the median overall surgical dose of rVWF was 220.4 IU/kg (range, 63.8-648.4 IU/kg) (Table 4). 93 (89.4%) infusions of rVWF alone: 15 (12-24 h before surgery), 12 (1 h before surgery), and 66 (postoperatively). 11 (10.6%) infusions of rVWF with rFVIII: 3 (1 h before surgery), 1 (intraoperatively), and 7 (postoperatively). 5 patients received the 11 infusions of rVWF with rFVIII, and 6 of the 7 postoperative infusions of rVWF with rFVIII were in 1 patient.

[0210] Of the 10 patients undergoing major surgery, 7 (70%) did not require coadministration of rFVIII.

Table 5. Median rVWF Exposure Overall and by Surgery Classification

	Surgery Classification			
	Minor (n=4)	Major (n=10)	Oral (n=1)	Overall (N=15)
Median total number of infusions* (range)	3 (2-4)	7.5 (4-15)	5	6 (2-15)
Median exposure (range), d	3 (2-4)	6.5 (4-15)	4	6 (2-15)
Median dose 12-24 h before surgery (range), IU/kg	57.2 (55.0-59.9)	49.3 (37.4-57.6)	36.1 55.0	(36.1-59.9)
Median dose 1 h before surgery (range), IU/kg	39.3 (8.0-46.4)	37.6 (15.7-82.7)	18.1	35.8 (8.0-82.7)
Median intraoperative dose (range), IU/kg	0	0	18.1	18.1
Median postoperative dose (range), IU/kg	79.3 (42.8-115.9)	214.8 (47.7-533.3)	36.1	189.8 (36.1-533.3)
Median total surgical dose (range), IU/kg	119.9 (63.8-217.3)	307.6 (125.2-648.4)	108.4	220.4 (63.8-648.4)
rVWF=recombinant von Willebrand factor. *Total number of preoperative priming infusions, preoperative initial loading doses, preoperative supplemental loading doses, intraoperative doses, and postoperative doses.				

SAFETY

[0211] 6 patients reported 12 treatment-emergent AEs; none considered related to treatment. patients had serious AEs (diverticulitis and deep vein thrombosis [DVT]; each occurred in 1 patient); neither event was considered related to factor replacement treatment.

[0212] The serious DVT occurred on postoperative day 8 (initially reported as a nonserious DVT on postoperative day 4). The event was asymptomatic and observed after routine duplex scan. The event was assessed as unlikely related to rVWF and not related to rFVIII or the study procedures; causally associated with the patient's major surgery (total hip replacement) and ongoing history of obesity. Postoperative levels of FVIII:C never exceeded 150 IU/dL. The event resulted in placement of caval filter and subsequently resolved without sequelae. No severe allergic reactions; neutralizing antibodies to rFVIII, CHO proteins, murine IgG, or rFurin.

[0213] One patient with VWD type 3 who had an intraoperative transfusion of packed red blood cells during major total knee replacement surgery tested positive for binding antibodies to VWF on postoperative day 7.

[0214] The PK parameters for VWF:RCo for the patients who underwent PK analysis (n=11) are shown in FIG. 4. Mean concentrations of VWF:RCo, VWF:Ag, and VWF collagen binding activity (VWF:CB) reached peak levels by 30 min and gradually declined over a period of 72 h post-infusion (FIG. 4).

[0215] Administration of rVWF alone resulted in substantial, rapid stabilization of endogenous FVIII:C levels 6-12 h after infusion, with peak FVIII:C levels reached by 24 h among all patients assessed (n=11; FIG. 5A), as well as in the subset of patients with type 3 VWD (n=5; FIG. 5B). Overall, patients achieved mean FVIII:C >60 IU/dL by 6 h postinfusion (FIG. 5A), and patients with higher baseline FVIII:C (e.g., with type 1 or type 2 VWD) were able to achieve target levels more rapidly. Despite having mean FVIII:C levels <2 IU/dL at baseline, administration of rVWF alone allowed patients with type 3 VWD to achieve target VWF:RCo and FVIII:C levels quickly, with FVIII:C >60 IU/dL achieved by 12 h post-infusion (FIG. 5B).

CONCLUSIONS

[0216] In this surgery study, overall and intraoperative hemostatic efficacies were rated as "excellent" or "good" for all 15 patients. For major surgeries, overall hemostatic efficacy was "excellent" in 7 patients and "good" in 3 patients, and intraoperative efficacy was "excellent" in 8 patients and "good" in 2 patients. Nearly 90% of infusions to achieve intraoperative and postoperative hemostasis were with rVWF alone; 70% of major surgeries were managed with rVWF alone. rVWF targets the primary dysfunction of VWD and allows physicians to focus on achieving optimal efficacy without concern for FVIII accumulation. These data support the safe and effective use of rVWF in major and minor surgeries.

EXAMPLE 2: RECOMBINANT VON WILLEBRAND FACTOR IN SUBJECTS WITH SEVERE VON WILLEBRAND DISEASE UNDERGOING SURGERY

[0217] This example provides the study results from a study examining treatment of subjects with severe von Willebrand Disease (VWD) undergoing

surgery.

OUTCOME MEASURES:

Primary Outcome Measures:

[0218] Overall Hemostatic Efficacy as Assessed by the Investigator (Hemophilia Physician) [Time Frame: 24 hours after last peri-operative infusion or at completion of Day 14 ($\pm$ 2 days) visit, whichever occurs earlier]. Hemostatic efficacy was rated on a scale of excellent - good - moderate - none.

- Excellent: Intra-, and postoperative hemostasis achieved with rVWF with or without ADVATE was as good or better than that expected for the type of surgical procedure performed in a hemostatically normal subject.
- Good: Intra-, and postoperative hemostasis achieved with rVWF with or without ADVATE was probably as good as that expected for the type of surgical procedure performed in a hemostatically normal subject.
- Moderate: Intra-, and postoperative hemostasis with rVWF with or without ADVATE was clearly less than optimal for the type of procedure performed but was maintained without the need to change the rVWF concentrate.
- None: Participant experienced uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of rVWF concentrate.

Secondary Outcome Measures:

[0219]

#1: Intraoperative Actual Versus Predicted Blood Loss as Assessed by the Operating Surgeon [Time Frame: Day 0 (at completion of surgery)]. The predicted blood loss was estimated preoperatively by the operating surgeon based on a hemostatically normal individual of the same sex, age, stature and co-morbidities as the participant. The actual blood loss was assessed consisting of the estimated blood loss, including into swabs, towels and suction during the procedure, per the anesthesiologist's record.

#2: Intraoperative Actual Blood Loss Relative to Predicted Blood Loss [Time Frame: Day 0 (at completion of surgery)]. Actual blood loss relative to predicted blood loss was calculated as [Actual Blood loss (mL)] divided by [Predicted Blood Loss (mL) multiplied by 100].

#3: Intraoperative Actual Versus Predicted Blood Loss Score as Assessed by the Operating Surgeon [Time Frame: Day 0 (at completion of surgery)]

- Hemostatic efficacy was rated on a scale of excellent - good - moderate - none.
- Excellent: Intraoperative blood loss was less than or equal to the maximum blood loss expected for the type of procedure performed in a hemostatically normal subject ($\leq$ 100%).
- Good: Intraoperative blood loss was up to 50% more than the maximum expected blood loss for the type of procedure performed in a hemostatically normal subject (101-150%) Moderate: Intraoperative blood loss was more than 50% of the maximum expected blood loss for the type of procedure performed in a hemostatically normal subject (>150%).
- None: Uncontrolled hemorrhage that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of clotting factor replacement regimen.

#4: Intraoperative Hemostatic Efficacy Score as Assessed by the Operating Surgeon [Time Frame: Day 0 (at completion of surgery)]

- Hemostatic efficacy was rated on a scale of excellent - good - moderate - none.
- Excellent: Intraoperative hemostasis achieved with rVWF with or without ADVATE was as good or better than that expected for the type of surgical procedure performed in a hemostatically normal subject.
- Good: Intraoperative hemostasis achieved with rVWF with or without ADVATE was probably as good as that expected for the type of surgical procedure performed in a hemostatically normal subject.
- Moderate: Intraoperative hemostasis with rVWF with or without ADVATE was clearly less than optimal for the type of procedure performed but was maintained without the need to change the rVWF concentrate.
- None: Participant experienced uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of rVWF concentrate.

#5: Daily Intra- and Postoperative Weight-adjusted Dose of rVWF With or Without ADVATE [Time Frame: Daily, from day of surgery through postoperative Day 14 ($\pm$ 2 days)]

#6: Occurrence of Adverse Events [Time Frame: From first infusion of investigational product through study completion (i.e., 14 ($\pm$ 2) days post-surgery)]. Treatment emergent adverse events (TEAEs) and treatment emergent serious adverse events (TESAEs) were evaluated.

#7: Occurrence of Thrombotic Events [Time Frame: From first infusion of investigational product through study completion (i.e., 14 ($\pm$ 2) days post-surgery)]. Treatment emergent adverse events (TEAEs) and treatment emergent serious adverse events (TESAEs) were evaluated for thrombotic events.

#8: Occurrence of Severe Allergic Reactions (e.g., Anaphylaxis) [Time Frame: From first infusion of investigational product through study completion (i.e., 14 ($\pm$ 2) days post-surgery)]. Treatment emergent adverse events (TEAEs) and treatment emergent serious adverse events (TESAEs) were evaluated for severe allergic reactions.

#9: Number of Participants Who Developed Inhibitory and Total Binding Antibodies to Von Willebrand Factor (VWF) and Inhibitory Antibodies to Factor VIII (FVIII) [Time Frame: Testing occurred throughout the study at screening, prior PK infusion, pre-surgery, post-surgery in case of excessive bleeding or unexplained bleeding, at postoperative day 7 and at study completion visit (ie. 14 ($\pm$ 2) days post-surgery)]. Participants were treated with recombinant van Willebrand Factor (rVWF) with or without ADVATE.

#10: Number of Participants Who Developed Antibodies to Chinese Hamster Ovary (CHO) Proteins, Mouse Immunoglobulin G (IgG) or Recombinant Furin (rFurin) [Time Frame: Testing occurred throughout the study at screening, prior PK infusion, pre-surgery, post-surgery in case of excessive bleeding or unexplained bleeding, at postoperative day 7 and at study completion visit (ie. 14 (± 2) days post-surgery).] Participants were treated with recombinant von Willebrand Factor (rVWF) with or without ADVATE.

#11: Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From 0 to 72 Hours Post-infusion (AUC 0-72 h/Dose) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. The area under the plasma concentration/time curve from 0 to 72 hours post-infusion was computed using the linear trapezoidal rule. For the calculation of AUC(0-72h) the levels at 72 hours was linearly interpolated/extrapolated from the 2 nearest sampling time points. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac), FVIII Coagulation Activity (FVIII :C)

#12: Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From Time 0 to Infinity (AUC 0-∞ /Dose) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. The area under the plasma concentration/time curve from time 0 to infinity and the area under the first moment curve from time 0 to infinity was calculated as the sum of AUC or AUMC from time 0 to the time of last quantifiable concentration plus a tail area correction calculated as C_t/λ_z and $C_t/\lambda_z(t+1/\lambda_z)$, respectively, where C_t was the last quantifiable concentration, t was the time of last quantifiable concentration and λ_z was the terminal or disposition rate constant. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac), FVIII Coagulation Activity (FVIII :C)

#13: Pharmacokinetics: Mean Residence Time (MRT) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Mean residence time was calculated as area under the first moment curve from time 0 to infinity divided by the area under the curve time 0 to infinity minus $T/2$ where T was the duration of the infusion. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac)

#14: Pharmacokinetics: Clearance (CL) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Clearance was calculated as dose (IU/kg) divided by the area under the curve time 0 to infinity. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac)

#15: Pharmacokinetics: Incremental Recovery (IR) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Incremental recovery was calculated as $(C_{max} - C_{preinfusion})$ divided by the dose (IU/kg) where kg refers to the body weight at the time of dosing and C_{max} was the observed maximum concentration before correction for pre-infusion values. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac)

#16: Pharmacokinetics: Elimination Phase Half-life ($T_{1/2}$) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment is only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Terminal or disposition half-life ($T_{1/2}$) was calculated as $\ln 2/\lambda_z$ where λ_z was the terminal elimination rate constant as calculated in WinNonlin NCA using at least three quantifiable concentrations. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac)

#17: Pharmacokinetics: Volume of Distribution at Steady State (V_{ss}) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.] This assessment is only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. V_{ss} was calculated as the clearance multiplied with the mean residence time. PK analysis was performed for the following analytes: VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac)

Eligibility Criteria:

Ages Eligible for Study: 18 Years and older; Sexes Eligible for Study: All

Inclusion Criteria:

[0220] Diagnosis of severe von Willebrand disease (VWD) as listed below and elective surgical procedure planned:

- 1. Type 1 (Von Willebrand factor: Ristocetin cofactor activity (VWF:RCo) <20 IU/dL), or
- 2. Type 2A (as verified by multimer pattern), Type 2B (as diagnosed by genotype), Type 2N (FVIII:C<10% and historically documented genetics), Type 2M, or
- 3. Type 3 (Von Willebrand factor antigen (VWF:Ag) ≤ 3 IU/dL)

[0221] VWD with a history of requiring substitution therapy with von Willebrand factor (VWF) concentrate to control bleeding.

[0222] If type 3 VWD (VWF Antigen/VWF:Ag $\leq$ 3 IU/dL), participant has a medical history of at least 20 exposure days to VWF/FVIII coagulation factor concentrates (including cryoprecipitate or fresh frozen plasma).

[0223] If type 1 or type 2 VWD, participant has a medical history of 5 exposure days or a past major surgery requiring VWF/FVIII coagulation factor concentrates (including cryoprecipitate or fresh frozen plasma).

[0224] Participant was at least 18 years of age.

[0225] If female of childbearing potential, participant presents with a negative pregnancy test.

[0226] If applicable, participant agrees to employ adequate birth control measures for the duration of the study.

[0227] Participant is willing and able to comply with the requirements of the protocol.

Selected Exclusion Criteria:

[0228]

Diagnosis of pseudo VWD or another hereditary or acquired coagulation disorder (e.g., qualitative and quantitative platelet disorders or elevated prothrombin time [PT] / international normalized ratio [INR] $>$ 1.4).

History or presence of a VWF inhibitor at screening.

History or presence of a factor VIII (FVIII) inhibitor with a titer $\geq$ 0.4 BU (Nijmegen-modified Bethesda assay) or $\geq$ 0.6 BU (by Bethesda assay).

Known hypersensitivity to any of the components of the study drugs, such as to mouse or hamster proteins.

Medical history of immunological disorders, excluding seasonal allergic rhinitis/conjunctivitis, mild asthma, food allergies or animal allergies.

Medical history of a thromboembolic event.

HIV positive with an absolute CD4 count $<$ 200/mm³.

Platelet count $<$ 100,000/mL.

Diagnosis of significant liver disease, as evidenced by, but not limited to, any of the following: serum alanine aminotransferase (ALT) 5 times the upper limit of normal; hypoalbuminemia; portal vein hypertension (e.g., presence of otherwise unexplained splenomegaly, history of esophageal varices) or liver cirrhosis classified as Child B or C.

Diagnosis of renal disease, with a serum creatinine level $\geq$ 2.5 mg/dL.

Participant had been treated with an immunomodulatory drug, excluding topical treatment (e.g., ointments, nasal sprays), within 30 days prior to signing the informed consent.

Participant was pregnant or lactating at the time informed content is obtained.

Participant had participated in another clinical study involving an investigational product (IP), other than rVWF with or without ADVATE, or investigational device within 30 days prior to enrollment or was scheduled to participate in another clinical study involving an IP or investigational device during the course of this study. However, eligible patients participating in the rVWF Prophylaxis Study (071301) may be enrolled.

Progressive fatal disease and/or life expectancy of less than 3 months.

RESULTS:

[0229] Enrollment was conducted at 14 study sites in 10 countries (USA, Australia, Taiwan, Germany, Russia, Spain, Ukraine, United Kingdom, Italy, Turkey).

Table 6: Reporting Groups

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 6: Participant: Overall Study

	Recombinant Von Willebrand Factor (rVWF)
STARTED	15
COMPLETED	14
NOT COMPLETED	1
Withdrawal by Subject	1

Table 7: Baseline Measures

		Recombinant Von Willebrand Factor (rVWF)	
Overall Participants Analyzed		15	
[Units: Participants]			
Age		40.0	
[Units: Years]		(20.0 to 70.0)	
Median (Full Range)			
Sex: Female, Male			
[Units: Participants]			
Count of Participants			
	Female	8	53.3%
	Male	7	46.7%

Primary Outcome: Outcome #1**Table 8: Outcome Measures**

[0230] 1. Primary: Overall Hemostatic Efficacy as Assessed by the Investigator (Hemophilia Physician) [Time Frame: 24 hours after last peri-operative infusion or at completion of Day 14 ($\pm$ 2 days) visit, whichever occurred earlier].

Table 9: Primary Outcome #1

Measure Type	Primary
Measure Title	Overall Hemostatic Efficacy as Assessed by the Investigator (Hemophilia Physician)
Measure Description	Hemostatic efficacy was rated on a scale of excellent - good - moderate - none.
	Excellent: Intra-, and postoperative hemostasis achieved with rVWF with or without ADVATE was as good or better than that expected for the type of surgical procedure performed in a hemostatically normal subject.
	Good: Intra-, and postoperative hemostasis achieved with rVWF with or without ADVATE was probably as good as that expected for the type of surgical procedure performed in a hemostatically normal subject.
	Moderate: Intra-, and postoperative hemostasis with rVWF with or without ADVATE was clearly less than optimal for the type of procedure performed but was maintained without the need to change the rVWF concentrate.
	None: Participant experienced uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of rVWF concentrate.
Time Frame	24 hours after last peri-operative infusion or at completion of Day 14 ($\pm$ 2 days) visit, whichever occurs earlier

Population Description Outcome #1

[0231] Number of participants with major, minor and oral surgery and number of participant with Von Willebrand Type 1, 2A, 2B, 2M and 3 do sum up to the overall number of participants analyzed. The full analysis data set, including all participants who received investigational product and have at least 1 hemostatic assessment, was used for analysis.

Table 10: Reporting Groups Outcome #1

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)
Minor Surgery	All participants who underwent minor surgery.
Major Surgery	All participants who underwent major surgery.
Oral Surgery	All participants who underwent oral surgery.
Von Willebrand Disease Type 1	All participants with von Willebrand Disease Type 1.
Von Willebrand Disease Type 2A	All participants with von Willebrand Disease Type 2A.
Von Willebrand Disease Type 2B	All participants with von Willebrand Disease Type 2B.
Von Willebrand Disease Type 2M	All participants with von Willebrand Disease Type 2M.
Von Willebrand Disease Type 3	All participants with von Willebrand Disease Type 3.

Table 11: Measured Values Outcome #1

[illegible]

Secondary Outcome: Outcome #2

[0232] 2. Secondary: Intraoperative Actual Versus Predicted Blood Loss as Assessed by the Operating Surgeon [Time Frame: Day 0 (at completion of surgery)]

Table 12: Primary Outcome #2

Measure Type	Secondary
Measure Title	Intraoperative Actual Versus Predicted Blood Loss as Assessed by the Operating Surgeon
Measure Description	The predicted blood loss was estimated preoperatively by the operating surgeon based on a hemostatically normal individual of the same sex, age, stature and co-morbidities as the participant.
	The actual blood loss was assessed consisting of the estimated blood loss, including into swabs, towels and suction during the procedure, per the anesthesiologist's record.
Time Frame	Day 0 (at completion of surgery)

Population Description Outcome #2

[02333] For predicted blood loss the number of participants analyzed was 14 as for one participant (included in the major surgery reporting group) the predicted blood loss was not collected. The full analysis data set, including all participants who received investigational product and had at least 1 hemostatic assessment, was used for analysis.

Table 13: Reporting Groups Outcome #2

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)
Minor Surgery	All participants who underwent minor surgery.
Major Surgery	All participants who underwent maj or surgery.
Oral Surgery	All participants who underwent oral surgery.
Von Willebrand Disease Type 1	All participants with von Willebrand Disease Type 1.
Von Willebrand Disease Type 2A	All participants with von Willebrand Disease Type 2A.
Von Willebrand Disease Type 2B	All participants with von Willebrand Disease Type 2B.
Von Willebrand Disease Type 2M	All participants with von Willebrand Disease Type 2M.
Von Willebrand Disease Type 3	All participants with von Willebrand Disease Type 3.

Table 26: Measured Values Outcome #2

[illegible]

[illegible]

No statistical analysis provided for Intraoperative Actual Versus Predicted Blood Loss as Assessed by the Operating Surgeon

Secondary Outcome: Outcome #3

3. Secondary: Intraoperative Actual Blood Loss Relative to Predicted Blood Loss [Time Frame: Day 0 (at completion of surgery)]

[0234]

Table 14: Outcome #3

Measure Type	Secondary
Measure Title	Intraoperative Actual Blood Loss Relative to Predicted Blood Loss
Measure Description	Actual blood loss relative to predicted blood loss was calculated as [Actual Blood loss (mL)] divided by [Predicted Blood Loss (mL) multiplied by 100.
Time Frame	Day 0 (at completion of surgery)

Population Description Outcome #3

[0235] Number of participants analyzed was 11, as for 3 participants the actual and the predicted blood loss was zero and for 1 participant the predicted blood loss was not collected. Therefore 'actual blood loss relative to predicted blood loss' could not be calculated. The full analysis data set was used for the analysis of this outcome measure.

Table 15: Reporting Groups Outcome #3

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)
Minor Surgery	All participants who underwent minor surgery.
Major Surgery	All participants who underwent major surgery.
Oral Surgery	All participants who underwent oral surgery.
Von Willebrand Disease Type 1	All participants with von Willebrand Disease Type 1.
Von Willebrand Disease Type 2A	All participants with von Willebrand Disease Type 2A.
Von Willebrand Disease Type 2B	All participants with von Willebrand Disease Type 2B.
Von Willebrand Disease Type 2M	All participants with von Willebrand Disease Type 2M.
Von Willebrand Disease Type 3	All participants with von Willebrand Disease Type 3.

Table 16: Measured Values

[illegible]

[illegible]

No statistical analysis provided for Intraoperative Actual Versus Predicted Blood Loss Score as Assessed by the Operating Surgeon

Secondary Outcome: Outcome #5

5. Secondary: Intraoperative Hemostatic Efficacy Score as Assessed by the Operating Surgeon [Time Frame: Day 0 (at completion of surgery)]

[0237]

Table 21: Outcome #5

Measure Type	Secondary
Measure Title	Intraoperative Hemostatic Efficacy Score as Assessed by the Operating Surgeon
Measure Description	Hemostatic efficacy was rated on a scale of excellent - good - moderate - none.
	Excellent: Intraoperative hemostasis achieved with rVWF with or without ADVATE was as good or better than that expected for the type of surgical procedure performed in a hemostatically normal subject.
	Good: Intraoperative hemostasis achieved with rVWF with or without ADVATE was probably as good as that expected for the type of surgical procedure performed in a hemostatically normal subject.
	Moderate: Intraoperative hemostasis with rVWF with or without ADVATE was clearly less than optimal for the type of procedure performed but was maintained without the need to change the rVWF concentrate.
	None: Participant experienced uncontrolled bleeding that was the result of inadequate therapeutic response despite proper dosing, necessitating a change of rVWF concentrate.
Time Frame	Day 0 (at completion of surgery)

Table 22: Population Description Outcome #5

Number of participants with major, minor and oral surgery and number of participant with Von Willebrand Type 1, 2A, 2B, 2M and 3 do sum up to the overall number of participants analyzed. The full analysis data set, including all participants who received investigational product and have at least 1 hemostatic assessment, was used for analysis.

Table 23: Reporting Groups Outcome #5

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)
Minor Surgery	All participants who underwent minor surgery.
Major Surgery	All participants who underwent major surgery.
Oral Surgery	All participants who underwent oral surgery.
Von Willebrand Disease Type 1	All participants with von Willebrand Disease Type 1.
Von Willebrand Disease Type 2A	All participants with von Willebrand Disease Type 2A.
Von Willebrand Disease Type 2B	All participants with von Willebrand Disease Type 2B.
Von Willebrand Disease Type 2M	All participants with von Willebrand Disease Type 2M.
Von Willebrand Disease Type 3	All participants with von Willebrand Disease Type 3.

Table 24: Measured Values Outcome #5

[illegible]

		Recombinant Von Willebrand Factor (rVWF)		Minor Surgery		Major Surgery		Oral Surgery		Von Willebrand Disease Type 1		Von Willebrand Disease Type 2A		Von Willebrand Disease Type 2B		Von Willebrand Disease Type 2M		Von Willebrand Disease Type 3	
by the Operating Surgeon																			
[Units: Participants]																			
Count of Participants																			
	Excellent	13	86.7%	4	100.0%	8	80.0%	1	100.0%	3	100.0%	1	50.0%	1	100.0%	1	100.0%	7	87.5%
	Good	2	13.3%	0	0.0%	2	20.0%	0	0.0%	0	0.0%	1	50.0%	0	0.0%	0	0.0%	1	12.5%
	Moderate	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	None	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

No statistical analysis provided for Intraoperative Hemostatic Efficacy Score as Assessed by the Operating Surgeon

Secondary Outcome: Outcome #6

[0238] 6. Secondary: Daily Intra- and Postoperative Weight-adjusted Dose of rVWF With or Without ADVATE [Time Frame: Daily, from day of surgery through postoperative Day 14 ($\pm$ 2 days)]

Table 25: Outcome #6

Measure Type	Secondary
Measure Title	Daily Intra- and Postoperative Weight-adjusted Dose of rVWF With or Without ADVATE
Measure Description	No text entered.
Time Frame	Daily, from day of surgery through postoperative Day 14 ($\pm$ 2 days)

Table 25: Population Description Outcome #6

Number of participants analyzed was different for the time points according to individual treatment. The full analysis data set, including all participants who received investigational product and have at least 1 hemostatic assessment, was used for analysis.

Table 40: Reporting Groups Outcome #6

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 26: Measured Values Outcome #6

		Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed		15
Daily Intra- and Postoperative Weight-adjusted Dose of rVWF With or Without ADVATE		
[Units: IU/kg]		
Median (Inter-Quartile Range)		
intraoperative		
	Participants Analyzed	1
	intraoperative	18.1
		(18.1 to 18.1)
postoperative day 1		
	Participants Analyzed	3
	postoperative day 1	23.5 (16.9 to 47.2)
postoperative day 2		
	Participants Analyzed	11
	postoperative day 2	42.3 (23.2 to 50.6)
postoperative day 3		
	Participants Analyzed	12
	postoperative day 3	28.6 (20.6 to 48.9)
postoperative day 4		
	Participants Analyzed	9
	postoperative day 4	33.9 (23.2 to 44.3)
postoperative day 5		
	Participants Analyzed	7
	postoperative day 5	31.5 (18.8 to 47.2)

		Recombinant Von Willebrand Factor (rVWF)
	postoperative day 6	
	Participants Analyzed	5
	postoperative day 6	23.2 (18.8 to 23.6)
	postoperative day 7	
	Participants Analyzed	5
	postoperative day 7	23.8 (23.6 to 50.8)
	postoperative day 8	
	Participants Analyzed	7
	postoperative day 8	33.9 (23.6 to 53.6)
	postoperative day 9	
	Participants Analyzed	3
	postoperative day 9	23.6 (16.3 to 53.6)
	postoperative day 10	
	Participants Analyzed	3
	postoperative day 10	23.6 (16.3 to 34.8)
	postoperative day 11	
	Participants Analyzed	3
	postoperative day 11	23.6 (16.3 to 53.6)
	postoperative day 12	
	Participants Analyzed	4
	postoperative day 12	29.3 (20.1 to 44.2)
	postoperative day 13	
	Participants Analyzed	1
	postoperative day 13	16.3 (16.3 to 16.3)
	postoperative day 14	
	Participants Analyzed	2
	postoperative day 14	25.5 (16.3 to 34.8)
	postoperative day 15	
	Participants Analyzed	1
	postoperative day 15	16.3 (16.3 to 16.3)

No statistical analysis provided for Daily Intra- and Postoperative Weight-adjusted Dose of rVWF With or Without ADVATE

Secondary Outcome: Outcome #7

7. Secondary: Occurrence of Adverse Events [Time Frame: From first infusion of investigational product through study completion (i.e., 14 (± 2) days post-surgery)].

[0239]

Table 27: Outcome #7

Measure Type	Secondary
Measure Title	Occurrence of Adverse Events
Measure Description	Treatment emergent adverse events (TEAEs) and treatment emergent serious adverse events (TESAEs) was evaluated.
Time Frame	From first infusion of investigational product through study completion (i.e., 14 (± 2) days post-surgery)

Table 28: Population Description Outcome #7

The safety analysis data set, including all participants who received any amount of investigational product, was used for analysis of this outcome measure.

Table 29: Reporting Groups Outcome #7

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 30: Measured Values

	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	15
Occurrence of Adverse Events	
[Units: Adverse Events]	

	Recombinant Von Willebrand Factor (rVWF)
Treatment emergent Adverse Events (TEAEs)	12
Severe TEAEs	1
TEAEs related to rVWF	0
TEAEs related to ADVATE	0
TEAEs related to both rVWF and ADVATE	0
Treatment emergent Serious Adverse Events (TESAEs)	2
TESAEs related to rVWF	0
TESAEs related to ADVATE	0
TESAEs related to both rVWF and ADVATE	0
TEAEs leading to discontinuation of rVWF	0
TEAEs leading to discontinuation of ADVATE	0
TEAEs leading to discontinuation of study	0
TEAEs leading to death	0
TEAEs related to study procedure	0
TESAEs related to study procedure	0

No statistical analysis provided for Occurrence of Adverse Events

Secondary Outcome: Outcome #8

[0240] 8. Secondary: Occurrence of Thrombotic Events [Time Frame: From first infusion of investigational product through study completion (*i.e.*, 14 ($\pm$ 2) days post-surgery)]

Table 31: Outcome #8

Measure Type	Secondary
Measure Title	Occurrence of Thrombotic Events
Measure Description	Treatment emergent adverse events (TEAEs) and treatment emergent serious adverse events (TESAEs) were evaluated for thrombotic events.
Time Frame	From first infusion of investigational product through study completion (<i>i.e.</i> , 14 ($\pm$ 2) days post-surgery)

Table 32: Population Description Outcome #8

The safety analysis data set, including all participants who received any amount of investigational product, was used for analysis of this outcome measure.

Table 33: Reporting Groups

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 34: Measured Values Outcome #8

	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	15
Occurrence of Thrombotic Events	
[Units: Adverse Events]	
Thrombotic TEAEs	2
Thrombotic TESAEs	1

No statistical analysis provided for Occurrence of Thrombotic Events

Secondary Outcome: Outcome #9

9. Secondary: Occurrence of Severe Allergic Reactions (*e.g.*, Anaphylaxis) [Time Frame: From first infusion of investigational product through study completion (*i.e.*, 14 ($\pm$ 2) days post-surgery)]

[0241]

Table 35: Outcome #9

Measure Type	Secondary
Measure Title	Occurrence of Severe Allergic Reactions (<i>e.g.</i> , Anaphylaxis)
Measure Description	Treatment emergent adverse events (TEAEs) and treatment emergent serious adverse events

	(TESAEs) were evaluated for severe allergic reactions.
Time Frame	From first infusion of investigational product through study completion (i.e., 14 (± 2) days post-surgery)

Table 36: Population Description Outcome #9

The safety analysis data set, including all participants who received any amount of investigational product, was used for analysis of this outcome measure.

Table 37: Reporting Groups Outcome #9

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 38: Measured Values Outcome #9

		Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed		15
Occurrence of Severe Allergic Reactions (e.g., Anaphylaxis)		
[Units: Adverse Events]		
	Severe allergic reaction TEAEs	0
	Severe allergic reaction TESAEs	0

No statistical analysis provided for Occurrence of Severe Allergic Reactions (e.g., Anaphylaxis)

Secondary Outcome: Outcome #10

[0242] 10. Secondary: Number of Participants Who Developed Inhibitory and Total Binding Antibodies to Von Willebrand Factor (VWF) and Inhibitory Antibodies to Factor VIII (FVIII) [Time Frame: Testing occurred throughout the study at screening, prior PK infusion, pre-surgery, post-surgery in case of excessive bleeding or unexplained bleeding, at postoperative day 7 and at study completion visit (ie. 14 (± 2) days post-surgery).]

Table 39: Population Description Outcome #10

Measure Type	Secondary
Measure Title	Number of Participants Who Developed Inhibitory and Total Binding Antibodies to Von Willebrand Factor (VWF) and Inhibitory Antibodies to Factor VIII (FVIII)
Measure Description	Participants were treated with recombinant van Willebrand Factor (rVWF) with or without ADVATE.
Time Frame	Testing occurred throughout the study at screening, prior PK infusion, pre-surgery, post-surgery in case of excessive bleeding or unexplained bleeding, at postoperative day 7 and at study completion visit (ie. 14 (± 2) days post-surgery).

Table 40: Population Description Outcome #10

The safety analysis data set, including all participants who received any amount of investigational product, was used for analysis of this outcome measure.

Table 41: Reporting Groups Outcome #10

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 55: Measured Values Outcome #10

		Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed		15
Number of Participants Who Developed Inhibitory and Total Binding Antibodies to Von Willebrand Factor (VWF) and Inhibitory Antibodies to Factor VIII (FVIII)		
[Units: Participants]		
Count of Participants		
	Development of inhibitory antibodies to VWF	0
	Development of total binding antibodies to VWF	1
	Development of inhibitory antibodies to FVIII	0

No statistical analysis provided for Number of Participants Who Developed Inhibitory and Total Binding Antibodies to Von Willebrand Factor (VWF) and Inhibitory Antibodies to Factor VIII (FVIII)

Secondary Outcome: Outcome #11

11. Secondary: Number of Participants Who Developed Antibodies to Chinese Hamster Ovary (CHO) Proteins, Mouse Immunoglobulin G (IgG) or Recombinant Furin (rFurin) [Time Frame: Testing occurred throughout the study at screening, prior PK infusion, pre-surgery, post-surgery in case of excessive bleeding or unexplained bleeding, at postoperative day 7 and at study completion visit (ie. 14 (± 2) days post-surgery).]

[0243]

Table 42: Outcome #11

Measure Type	Secondary
Measure Title	Number of Participants Who Developed Antibodies to Chinese Hamster Ovary (CHO) Proteins, Mouse Immunoglobulin G (IgG) or Recombinant Furin (rFurin)
Measure Description	Participants were treated with recombinant von Willebrand Factor (rVWF) with or without ADVATE.
Time Frame	Testing occurred throughout the study at screening, prior PK infusion, pre-surgery, post-surgery in case of excessive bleeding or unexplained bleeding, at postoperative day 7 and at study completion visit (ie. 14 ($\pm$ 2) days post-surgery).

Table 43: Population Description Outcome #11

The safety analysis data set, including all participants who received any amount of investigational product, was used for analysis of this outcome measure.

Table 44: Reporting Groups Outcome #11

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 59: Measured Values Outcome #11

	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	15
Number of Participants Who Developed Antibodies to Chinese Hamster Ovary (CHO) Proteins, Mouse Immunoglobulin G (IgG) or Recombinant Furin (rFurin)	0
[Units: Participants]	
Count of Participants	

No statistical analysis provided for Number of Participants Who Developed Antibodies to Chinese Hamster Ovary (CHO) Proteins, Mouse Immunoglobulin G (IgG) or Recombinant Furin (rFurin)

Secondary Outcome: Outcome #12

[0244] 12. Secondary: Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From 0 to 72 Hours Post-infusion (AUC 0-72 h/Dose) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.]

Table 45: Outcome #12

Measure Type	Secondary
Measure Title	Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From 0 to 72 Hours Post-infusion (AUC 0-72 h/Dose)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50 $\pm$ 5 IU/kg rVWF:RCo within 42 days prior to surgery. The area under the plasma concentration/time curve from 0 to 72 hours post-infusion was computed using the linear trapezoidal rule. For the calculation of AUC(0-72h) the levels at 72 hours was linearly interpolated/extrapolated from the 2 nearest sampling time points.
	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac), FVIII Coagulation Activity (FVIII:C)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.

Table 46: Population Description Outcome #12

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.

Table 47: Reporting Groups Outcome #12

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 48: Measured Values Outcome #12

		Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed		11
Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From 0 to 72 Hours Post-infusion (AUC 0-72 h/Dose)		
[Units: hours*IU/dL]		
Geometric Mean (Geometric Coefficient of Variation)		
	VWF:RCo	
	Participants Analyzed	11 31.91
	VWF:RCo	(37.5%)
	VWF:Ag	

		Recombinant Von Willebrand Factor (rVWF)
	Participants Analyzed	11
	VWF:Ag	57.08 (25.6%)
VWF:CB		
	Participants Analyzed	11
	VWF:CB	63.91 (29.4%)
VWF:Ac		
	Participants Analyzed	11
	VWF:Ac	54.61 (28.1%)
F VIII: C		
	Participants Analyzed	5
	FVIII: C	67.49 (31.1%)

No statistical analysis provided for Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From 0 to 72 Hours Post-infusion (AUC 0-72 h/Dose)

Secondary Outcome: Outcome #13

[0245] 13. Secondary: Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From Time 0 to Infinity (AUC 0- ∞ /Dose) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.]

Table 49: Outcome #13

Measure Type	Secondary
Measure Title	Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From Time 0 to Infinity (AUC 0- ∞ /Dose)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50 $\pm$ 5 IU/kg rVWF:RCo within 42 days prior to surgery. The area under the plasma concentration/time curve from time 0 to infinity and the area under the first moment curve from time 0 to infinity was calculated as the sum of AUC or AUMC from time 0 to the time of last quantifiable concentration plus a tail area correction calculated as C_t/λ_z and $C_t/\lambda_z(t+1/\lambda_z)$, respectively, where C_t was the last quantifiable concentration, t was the time of last quantifiable concentration and λ_z was the terminal or disposition rate constant.
	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF:Ac), FVIII Coagulation Activity (FVIII:C)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.

Table 50: Population Description Outcome #13

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.

Table 51: Reporting Groups Outcome #13

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 52: Measured Values

	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	11
Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From Time 0 to Infinity (AUC 0- ∞ /Dose)	
[Units: hours*IU/dL]	
Geometric Mean (Geometric Coefficient of Variation)	
VWF:RCo	
Participants Analyzed	11
VWF:RCo	34.43 (43.3%)
VWF:Ag	
Participants Analyzed	11
VWF:Ag	68.87 (31.5%)
VWF:CB	
Participants Analyzed	11
VWF:CB	71.82 (34.1%)
VWF:Ac	

		Recombinant Von Willebrand Factor (rVWF)
	Participants Analyzed	11
	VWF:Ac	61.90 (32.2%)
	FVIII: C	
	Participants Analyzed	3
	FVIII: C	75.00 (30.9%)

No statistical analysis provided for Pharmacokinetics: Area Under the Plasma Concentration Versus Time Curve From Time 0 to Infinity (AUC 0- ∞ /Dose)

Secondary Outcome: Outcome #14

[0246] 14. Secondary: Pharmacokinetics: Mean Residence Time (MRT) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.]

Table 53: Outcome #14

Measure Type	Secondary
Measure Title	Pharmacokinetics: Mean Residence Time (MRT)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50 $\pm$ 5 IU/kg rVWF:RCo within 42 days prior to surgery. Mean residence time was calculated as area under the first moment curve from time 0 to infinity divided by the area under the curve time 0 to infinity minus T/2 where T was the duration of the infusion.
	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF: Ac)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.

Table 54: Population Description Outcome #14

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.
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Table 55: Reporting Groups Outcome #14

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 56: Measured Values

		Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed		11
Pharmacokinetics: Mean Residence Time (MRT)		
[Units: Hours]		
Geometric Mean (Geometric Coefficient of Variation)		
	VWF:RCo	22.69 (41.3%)
	VWF:Ag	37.92 (28.4%)
	VWF:CB	29.35 (31.1%)
	VWF:Ac	29.75 (28.6%)

No statistical analysis provided for Pharmacokinetics: Mean Residence Time (MRT)

Secondary Outcome: Outcome #15

15. Secondary: Pharmacokinetics: Clearance (CL) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 ($\pm$ 5) minutes, 60 ($\pm$ 5) minutes, 6 ($\pm$ 1) hours, 12 ($\pm$ 1) hours, 24 ($\pm$ 2) hours, 48 ($\pm$ 2) hours and 72 ($\pm$ 2) hours.]

[0247]

Table 57: Outcome #15

Measure Type	Secondary
Measure Title	Pharmacokinetics: Clearance (CL)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50 $\pm$ 5 IU/kg rVWF:RCo within 42 days prior to surgery. Clearance was calculated as dose (IU/kg) divided by the area under the curve time 0 to infinity.

	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF: Ac)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.

Table 58: Population Description Outcome #15

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.

Table 59: Reporting Groups Outcome #15

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 60: Measured Values

	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	11
Pharmacokinetics: Clearance (CL)	
[Units: dL/hour/kg]	
Geometric Mean (Geometric Coefficient of Variation)	
VWF:RCo	0.02904 (43.3%)
VWF:Ag	0.01452 (31.5%)
VWF:CB	0.01392 (34.1%)
VWF:Ac	0.01616 (32.2%)

No statistical analysis provided for Pharmacokinetics: Clearance (CL)

Secondary Outcome: Outcome #16

[0248] 16. Secondary: Pharmacokinetics: Incremental Recovery (IR) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.]

Table 61: Outcome #16

Measure Type	Secondary
Measure Title	Pharmacokinetics: Incremental Recovery (IR)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Incremental recovery was calculated as (Cmax minus Cpreinfusion) divided by the dose (IU/kg) where kg refers to the body weight at the time of dosing and Cmax was the observed maximum concentration before correction for pre-infusion values.
	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF: Ac)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.

Table 77: Population Description Outcome #16

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.

Table 62: Reporting Groups Outcome #16

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)

Table 63: Measured Values Outcome #16

	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	11
Pharmacokinetics: Incremental Recovery (IR)	
[Units: IU/dL]	
Mean (Standard Deviation)	
VWF:RCo	1.961 (0.45445)
VWF:Ag	1.991 (0.38395)
VWF:CB	2.780 (0.56640)
VWF:Ac	2.635 (0.38050)

No statistical analysis provided for Pharmacokinetics: Incremental Recovery (IR)

Secondary Outcome: Outcome #17

[0249] 17. Secondary: Pharmacokinetics: Elimination Phase Half-life (T1/2) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.]

Table 64: Outcome #17

Measure Type	Secondary
Measure Title	Pharmacokinetics: Elimination Phase Half-life (T1/2)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Terminal or disposition half-life (T1/2) was calculated as $\ln 2/\lambda_z$ where λ_z was the terminal elimination rate constant as calculated in WinNonlin NCA using at least three quantifiable concentrations.
	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF: Ac)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.

Table 65: Population Description Outcome #17

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.

Table 66: Reporting Groups Outcome #17

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)
Measured Values	
	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	11
Pharmacokinetics: Elimination Phase Half-life (T1/2)	
[Units: Hours]	
Geometric Mean (Geometric Coefficient of Variation)	
VWF:RCo	16.52 (42.7%)
VWF:Ag	26.88 (26.5%)
VWF:CB	21.07 (33.2%)
VWF:Ac	22.19 (28.5%)

No statistical analysis provided for Pharmacokinetics: Elimination Phase Half-life (T1/2)

Secondary Outcome: Outcome #18

[0250] 18. Secondary: Pharmacokinetics: Volume of Distribution at Steady State (Vss) [Time Frame: PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.]

Table 67: Outcome #18

Measure Type	Secondary
Measure Title	Pharmacokinetics: Volume of Distribution at Steady State (Vss)
Measure Description	This assessment was only required for subjects undergoing major surgery. Subjects received a PK infusion at a dose of 50±5 IU/kg rVWF:RCo within 42 days prior to surgery. Vss was calculated as the clearance multiplied with the mean residence time.
	PK analysis was performed for the following analytes:
	VWF Ristocetin Cofactor Activity (VWF:RCo), VWF Antigen Activity (VWF:Ag), VWF Collagen Binding Activity (VWF:CB), VWF Activity Measured INNOVANCE VWF Ac Assay (VWF: Ac)
Time Frame	PK measurements were done within 30 minutes pre-infusion, and post infusion at 30 (± 5) minutes, 60 (± 5) minutes, 6 (± 1) hours, 12 (± 1) hours, 24 (± 2) hours, 48 (± 2) hours and 72 (± 2) hours.

Table 68: Population Description Outcome #18

The PK analysis data set, including all participants who underwent PK assessment with data collected at the relevant time points, was used for analysis of this outcome measure.

Table 69: Reporting Groups Outcome #18

	Description
Recombinant Von Willebrand Factor (rVWF)	Surgery participants treated with Recombinant von Willebrand Factor (rVWF)
Measured Values Outcome #18	
	Recombinant Von Willebrand Factor (rVWF)
Participants Analyzed	11
Pharmacokinetics: Volume of Distribution at Steady State	

Measured Values Outcome #18		
(Vss)		Recombinant Von Willebrand Factor (rVWF)
[Units: dL/kg]		
Geometric Mean (Geometric Coefficient of Variation)		
	VWF:RCo	0.6591 (28.8%)
	VWF:Ag	0.5506 (18.4%)
	VWF:CB	0.4086 (24.0%)
	VWF:Ac	0.4806 (21.5%)

No statistical analysis provided for Pharmacokinetics: Volume of Distribution at Steady State (Vss)

[0251] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions for the use of the present invention and the further described systems and methods, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains.

[0252] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the scope of the invention described in the claims.

[0253] Many modifications and variations of this application can be made without departing from its scope of the claims, as will be apparent to those skilled in the art.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- [US8254699](#) [0001]
- [US8253002A](#) [0001]
- [EP1593390A](#) [0004]
- [WO198806096A](#) [0018] [0032]
- [US5585066Q](#) [0018] [0032] [0077]
- [WO2012171031A](#) [0037] [0042]
- [US4640635A](#) [0045] [0108]
- [US4486689A](#) [0045] [0108]
- [US4301144A](#) [0045] [0108]
- [US4670417A](#) [0045] [0108]
- [US4791132A](#) [0045] [0108]
- [US4178337A](#) [0045] [0108]
- [US2006009040A](#) [0054] [0071]
- [US20070212770A](#) [0054] [0071]
- [US20060094104A](#) [0071]
- [US8652688B](#) [0074]
- [US9409971B](#) [0074]
- [US8597910B](#) [0075]
- [US20160129090A](#) [0075]
- [WO8828096A](#) [0077]
- [US3941763A](#) [0084]
- [US20060160348A](#) [0091]
- [US7005502B](#) [0092]
- [WO2004038337A](#) [0160]

Non-patent literature cited in the description

- VEYRADIER A et al. *Medicine* (Baltimore), 2016, vol. 95, 11e3038- [f00031](#)
- EIKENBOOM et al. *Haemophilia*, 1995, vol. 1, 77-90 [f00041](#)
- MANNUCCI PMFRANCHINI M. *Haemophilia*, 2017, vol. 23, 2182-187 [f00041](#)
- The Diagnosis, Evaluation, and Management of von Willebrand Disease NIH Publication No. 08-5832, 2007, [f00041](#)
- TURECEK PL et al. *Hamostaseologie*, 2009, vol. 29, 1532-38 [f00041](#)
- MANNUCCI PM et al. *Blood*, 2013, vol. 122, 5648-657 [f00041](#)
- GILL JC et al. *Blood*, 2015, vol. 126, 172038-2046 [f00041](#)
- TURECEK et al. *Semin. Thromb. Hemost.*, 2010, vol. 36, 5510-521 [f00211](#)
- ANDERSSON et al. *Proc. Natl. Acad. Sci. USA*, 1986, vol. 83, 2979-2983 [f00221](#)
- LIEBERMAN *Pharmaceutical Dosage Forms*, 1992, vol. 1-3, [f00271](#)
- LLOYD *The Art, Science and Technology of Pharmaceutical Compounding*, 1999, [f00271](#)
- PICKAR *Dosage Calculations*, 1999, [f00271](#)
- Remington: The Science and Practice of Pharmacy Lippincott, Williams & Wilkins 20030000 [f00271](#)
- TURECEK et al. *Semin. Thromb. Hemost.*, 2002, vol. 28, 149-160 [f00331](#)
- WEISS et al. *J. Clin. Invest.*, 1973, vol. 52, 2708-2716 [f00341](#) [f00961](#)
- MACFARLANE et al. *Thromb. Diath. Haemorrh.*, 1975, vol. 34, 306-308 [f00341](#) [f00961](#)
- BROWNBOSAK *Thromb. Res.*, 1986, vol. 43, 303-311 [f00341](#) [f01101](#)
- FAVALORO *Thromb. Haemost.*, 2000, vol. 83, 127-135 [f00341](#) [f01101](#)
- CUMMING et al. *J Clin Pathol.*, 1993, vol. 46, 5470-473 [f00401](#) [f00941](#) [f01021](#)
- WEN et al. *J. Clin. Lab. Anal.*, 1993, vol. 7, 317-323 [f00401](#) [f00941](#) [f01021](#)
- FAVALORO et al. *Pathology*, 1997, vol. 29, 4341-456 [f00401](#) [f00941](#) [f01021](#)
- ABUCHOWSKIDAVIS *Enzymes as Drugs* John Wiley and Sons 19810000367-383 [f00451](#) [f01091](#)
- HERMANSON G. *Bioconjugate Techniques* Academic Press, Inc. 20080000 [f00451](#) [f00461](#) [f01091](#) [f01091](#)
- JENNINGS LUGOWSKI J. *Immunol.*, 1981, vol. 127, 1011-8 [f00461](#) [f01091](#)
- FEMANDESGREGONRADIS *Biochim Biophys Acta*, 1997, vol. 1341, 26-34 [f00461](#) [f01091](#)
- VAN WEZEL, A. L. *Nature*, 1967, vol. 216, 64-5 [f00521](#) [f00681](#)
- SPIERGRIF FITHS *Animal Cell Biotechnology*, 1988, vol. 3, 283-303 [f00531](#) [f00691](#)
- TANG et al. *Pharm Res.*, 2004, vol. 21, 191-200 [f00571](#) [f01301](#)
- CHANG et al. *Pharm Res.*, 1996, vol. 13, 243-9 [f00571](#) [f01301](#)
- CHEN *Drug Development and Industrial Pharmacy*, 1992, vol. 18, 1311-1354 [f00591](#) [f01691](#)
- LANKHOF et al. *Thromb. Haemost.*, 1997, vol. 77, 1008-1013 [f00761](#)
- PIETU et al. *Biochem. Biophys. Res. Commun.*, 1989, vol. 164, 1339-1347 [f00761](#)
- MERRIFIELD *Chem. Polypeptides* 19730000335-61 [f00841](#)
- MERRIFIELD *J. Am. Chem. Soc.*, 1963, vol. 85, 2149- [f00841](#)
- DAVIS et al. *Biochem. Intl.*, 1985, vol. 10, 394-414 [f00841](#)
- STEWART YOUNG *Solid Phase Peptide Synthesis*, 1969, [f00841](#)
- FINN et al. *The Proteins* 19760000vol. 2, 105-253 [f00841](#)
- ERICKSON et al. *The Proteins* 19760000vol. 2, 257-527 [f00841](#)
- *Biochim. Biophys. Acta*, 1997, vol. 1341, 26-34 [f00911](#)
- SAENKO et al. *Haemophilia*, 2006, vol. 12, 42-51 [f00911](#)
- MIGNEAULT et al. *Biotechniques*, 2004, vol. 37, 790-796 [f00911](#)
- SAMBROOK et al. *Molecular Cloning* Cold Spring Harbor Laboratory Press 19890000 [f00931](#)
- SADLER, JE *Annu Rev Biochem.*, 1998, vol. 67, 395-424 [f00941](#)
- TURECEK et al. *Semin Thromb Hemost.*, 2010, vol. 36, 510-521 [f00941](#) [f00951](#)
- TURECEK, P. *Hamostaseologie*, 2017, vol. 37, 1S15-S25 [f00991](#)
- Remington's *Pharmaceutical Sciences* Mack Publishing Co. 199000001435-1712 [f01211](#)
- *Genome Analysis: A Laboratory Manual Series* vol. I-IV, [f01221](#)
- *Molecular Cloning: A Laboratory Manual* Cold Spring Harbor Laboratory Press [f01221](#)
- STRYER, L. *Biochemistry* Freeman 19950000 [f01221](#)
- GAITO *Oligonucleotide Synthesis: A Practical Approach* IRL Press 19840000 [f01221](#)
- NELSON COX *Lehninger, Principles of Biochemistry* W. H. Freeman Pub. 20000000 [f01221](#)
- BERG et al. *Biochemistry* W. H. Freeman Pub. 20020000 [f01221](#)
- A. P. MACKENZIE *Phil Trans R Soc London, Ser B, Biol.*, 1977, vol. 278, 167- [f01241](#)
- POWELL et al. *Compendium of Excipients for Parenteral Formulations* PDA J. Pharm. Sci. Technology, 1998, vol. 52, 238-311 [f01391](#)
- REMMELE R. L. JR. et al. *Biochemistry*, 1999, vol. 38, 165241-7 [f01401](#)
- LAURSEN T et al. *Basic Clin Pharmacol Toxicol.*, 2006, vol. 98, 2218-21 [f01421](#)
- HOLLANDER-RODRIGUEZ JC et al. *Am. Fam. Physician.*, 2006, vol. 73, 2283-90 [f01421](#)
- CARPENTER et al. *Develop. Biol. Standard*, 1991, vol. 74, 225- [f01471](#)
- CHANG, B. J. *Pharm. Sci.*, 1996, vol. 85, 1325- [f01531](#)
- CHEN B et al. *Pharm Res.*, 2003, vol. 20, 121952-60 [f01551](#)
- TOMITA M et al. *Biochemistry*, 1969, vol. 8, 125149-60 [f01551](#)
- LAM XM et al. *J Pharm Sci.*, 1997, vol. 86, 111250-5 [f01551](#)
- *Peptides and Proteins as Parenteral Solutions* AKERS MJDEFELIPPIS MR *Pharmaceutical Formulation Development of Peptides and Proteins* Taylor and Francis 19990000 [f01581](#)
- FRANSSON J. R. *Pharm. Sci.*, 1997, vol. 86, 94046-1050 [f01591](#)
- YIN J et al. *Pharm Res.*, 2004, vol. 21, 122377-83 [f01591](#)
- LAM XM YANG JY et al. *J Pharm Sci.*, 1997, vol. 86, 111250-5 [f01591](#)
- CHEN B et al. *Pharm Sci.*, 1999, vol. 88, 4477-82 [f01611](#)
- FATOUROS, A. et al. *Int. J. Pharm.*, 1997, vol. 155, 121-131 [f01611](#)
- DERRICK TS et al. *Pharm. Sci.*, 2004, vol. 93, 102549-57 [f01611](#)

- **ROY S et al.**J Pharm Sci., 2005, vol. 94, 2382-96 [\[0162\]](#)
- **REMMELE RL JR. et al.**Pharm Res., 1998, vol. 15, 2200-8 [\[0164\]](#)
- **KAPPELGAARD A.M.**Horm Res., 2004, vol. 62, 398-103 [\[0166\]](#)
- **MINOGUE SCSUN DA**AnesthAnalg., 2005, vol. 100, 3683-6 [\[0168\]](#)

Patentkrav

1. Rekombinant von Willebrand-faktor (rVWF) til anvendelse i en fremgangsmåde til forbehandling af et individ med svær von Willebrand-sygdom (VWD) inden et kirurgisk indgreb, hvor forbehandlingen omfatter administration af 20-60 IU/kg af rVWF'en til individet mellem 12 timer og 24 timer inden det kirurgiske indgreb, og hvor der inden det kirurgiske indgreb ikke administreres faktor VIII (FVIII) med VWF'en.
2. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor forbehandlingen yderligere omfatter administration af 5-90 IU/kg rVWF til individet 1 time inden det kirurgiske indgreb.
3. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor der efter det kirurgiske indgreb ikke administreres FVIII.
4. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor det kirurgiske indgreb er valgt fra gruppen bestående af et større kirurgisk indgreb, et mindre kirurgisk indgreb og kirurgi i munden.
5. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet får administreret 50-60 IU/kg rVWF mellem 12 timer og 24 timer inden det kirurgiske indgreb, og det kirurgiske indgreb er et mindre kirurgisk indgreb, eller hvor individet får administreret 35-60 IU/kg rVWF mellem 12 timer og 24 timer inden det kirurgiske indgreb, og det kirurgiske indgreb er et større kirurgisk indgreb, eller hvor individet får administreret 20-40 IU/kg rVWF mellem 12 timer og 24 timer inden det kirurgiske indgreb, og det kirurgiske indgreb er et kirurgisk indgreb i munden.
6. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor forbehandlingen yderligere omfatter administration af 5-50 IU/kg rVWF til individet 1 time inden det kirurgiske indgreb, og det kirurgiske indgreb er et mindre kirurgisk indgreb.

7. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet yderligere får administreret 15-90 IU/kg rVWF 1 time inden det kirurgiske indgreb, og det kirurgiske indgreb er et større kirurgisk indgreb.

5

8. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet yderligere får administreret 20-50 IU/kg rVWF 1 time inden det kirurgiske indgreb, og det kirurgiske indgreb er et kirurgisk indgreb i munden.

10 9. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet får administreret 10-50 IU/kg rVWF under det kirurgiske indgreb, og det kirurgiske indgreb er et kirurgisk indgreb i munden.

15 10. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet yderligere får administreret 70-220 IU/kg rVWF efter det kirurgiske indgreb.

11. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet yderligere får administreret 70-150 IU/kg rVWF efter det kirurgiske indgreb, og det kirurgiske indgreb er et mindre kirurgisk indgreb, eller
20 hvor individet yderligere får administreret 150-220 IU/kg rVWF efter det kirurgiske indgreb, og det kirurgiske indgreb er et større kirurgisk indgreb, eller
eller
hvor individet yderligere får administreret 20-50 IU/kg rVWF efter det kirurgiske indgreb, og det kirurgiske indgreb er et kirurgisk indgreb i munden.

25

12. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor individet får administreret en samlet dosis på 100-220 IU/kg rVWF, og det kirurgiske indgreb er et mindre kirurgisk indgreb, eller
hvor individet får administreret en samlet dosis på 220-320 IU/kg rVWF, og det
30 kirurgiske indgreb er et større kirurgisk indgreb, eller
hvor individet får administreret en samlet dosis på 70-190 IU/kg rVWF, og det kirurgiske indgreb er et kirurgisk indgreb i munden.

13. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor det kirurgiske indgreb er et større kirurgisk indgreb, og forbehandlingen omfatter administration af mindst to omtrent ens doser rVWF inden det kirurgiske indgreb.

5

14. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor det kirurgiske indgreb er et mindre kirurgisk indgreb, og forbehandlingen omfatter administration af mindst to doser rVWF inden det kirurgiske indgreb, hvor den første dosis er større end den anden dosis.

10

15. Rekombinant von Willebrand-faktor (rVWF) til anvendelse ifølge krav 1, hvor det kirurgiske indgreb er et kirurgisk indgreb i munden, og forbehandlingen omfatter administration af mindst to omtrent ens doser rVWF inden det kirurgiske indgreb.

DRAWINGS

Drawing

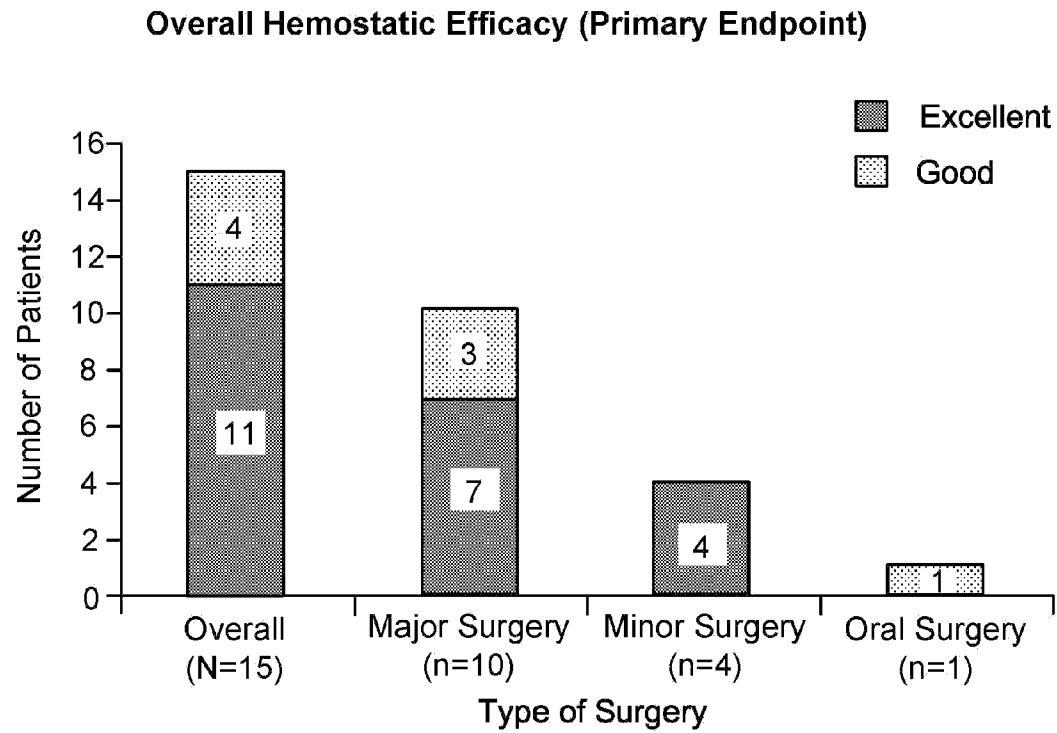


FIG. 1

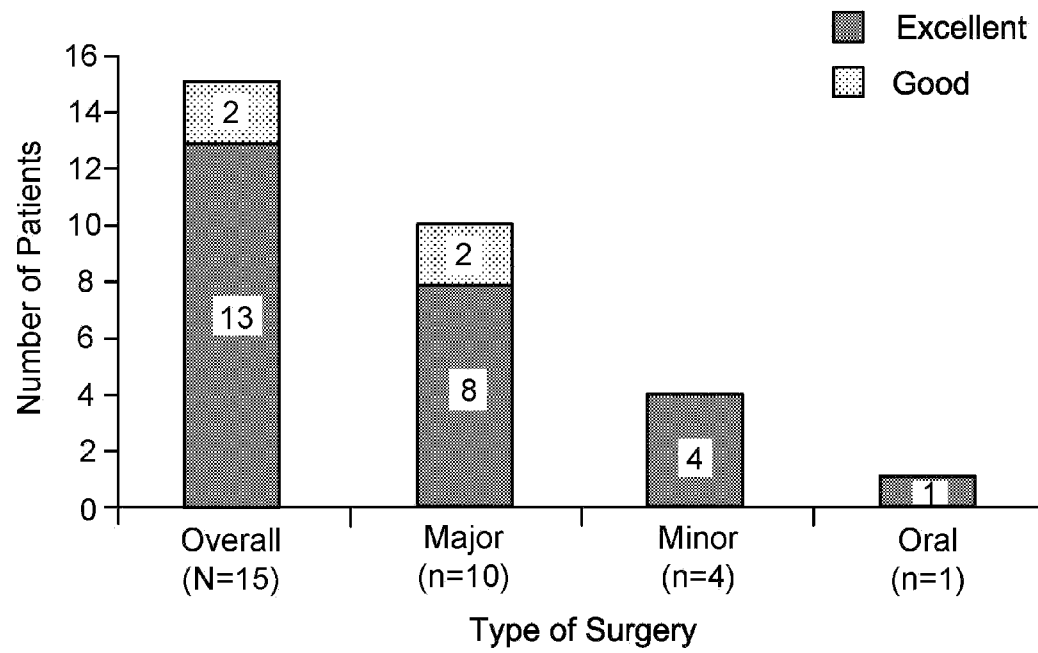
Intraoperative Hemostatic Efficacy (Secondary Endpoint)**FIG. 2**

Table 3. Baseline Demographics and Clinical Characteristics

Parameter	N = 15
Sex, n (%)	
Male	7 (46.7)
Female	8 (53.3)
Median age (range), y	40 (20-70)
Median weight (range), kg	D
Median BMI (range), kg/m ²	D
VWD type, n (%)	
1	3 (20.0)
2A	2 (13.3)
2B	1 (6.7)
2M	1 (6.7)
3	8 (53.3)
Surgery classification, n (%)	
Major	10 (66.7)
Minor	4 (26.7)
Oval	1 (6.7)
Mean (SD) FVIII:C, IU/dL	16.4 (19.9)
Mean (SD) VWF:Rco, IU/dL	10.6 (13.3)

BMI=body mass index; FVIII:C=factor VIII activity; VWD=von Willebrand disease; VWF:RCo=von Willebrand factor ristocetin cofactor activity.

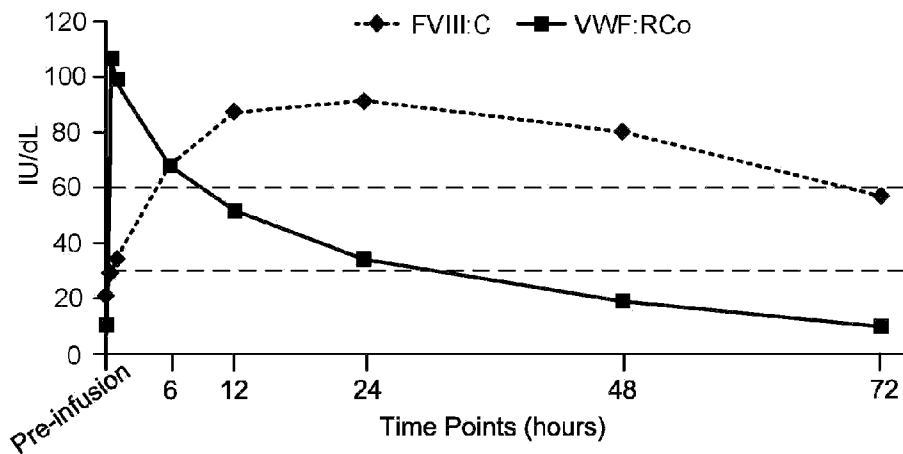
FIG. 3

Table 4. PK Parameters for VWF:RCo (n=11)

Parameter	Mean	SD	Range
AUC _{0-∞} /dose, (h*IU/dL)/(IU/kg)	37.50	18.14	19.2–83.5
AUC _{0-72h} /dose, (h*IU/dL)/(IU/kg)	34.08	14.59	18.3–72.6
C _{max} , IU/dL	96.27	22.01	49.0–118
T _{1/2} , h	17.83	7.34	9.08–31.6
IR at C _{max} , (IU/dL)/(IU/kg)	1.96	0.45	1.06–2.48

AUC=area under the curve; C_{max}=peak concentration; IR=incremental recovery; NC=not calculated; PK=pharmacokinetic; SD=standard deviation; T_{max}=time to peak concentration; T_{1/2}=terminal half-life; VWF:RCo= von Willebrand factor ristocetin cofactor activity.

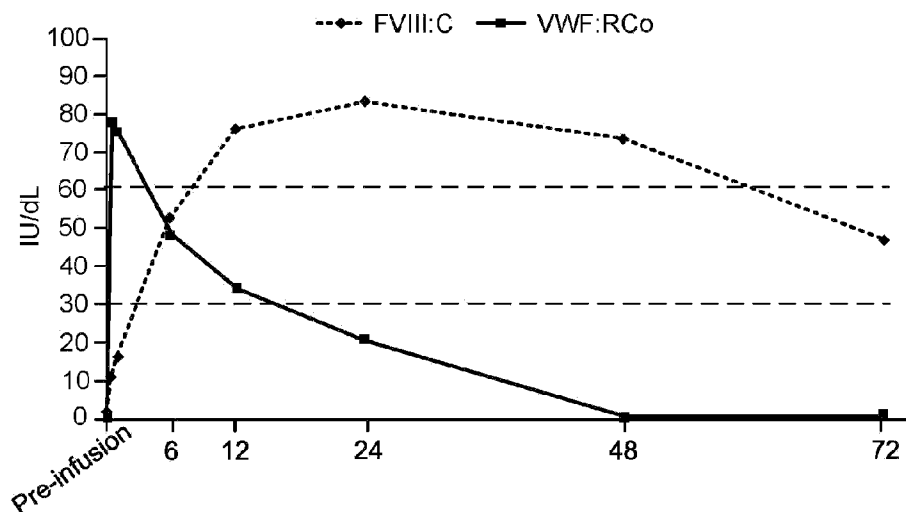
FIG. 4



FVIII:C= factor VIII activity; VWF:RCo= von Willebrand factor ristocetin cofactor activity.

Target endogenous FVIII:C levels (30 IU/dL for minor/oral surgery or 60 IU/dL for major surgery) are indicated by dashed horizontal lines.

FIG. 5A



FVIII:C= factor VIII activity; VWF:RCo= von Willebrand factor ristocetin cofactor activity.

Target endogenous FVIII:C levels (30 IU/dL for minor/oral surgery or 60 IU/dL for major surgery) are indicated by dashed horizontal lines.

FIG. 5B

SEQ ID NO:1

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agcagctgag	agcatggcct	aggtggggcg	gcaccattgt	ccagcagctg	200
agtttcccag	ggaccttgga	gatagccgca	gccctcattt	gcaggggaag	250
atgattccctg	ccagattttgc	cgggggtgctg	cttgcctctgg	ccctcatttt	300
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FIG. 6

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FIG. 6 (Cont.)

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FIG. 6 (Cont.)

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SEQ ID NO:2

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FIG. 6 (Cont.)

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Leu	Ser	Val	Tyr	Leu	Gly	Glu	Phe	Phe	Asp	Ile	His	Leu	Phe	Val	Asn	
			85						90						95	
Gly	Thr	Val	Thr	Gln	Gly	Asp	Gln	Arg	Val	Ser	Met	Pro	Tyr	Ala	Ser	
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Lys	Leu	Glu	Thr	Glu	Ala	Gly	Tyr	Tyr	Lys	Leu	Ser	Gly	Glu	Ala	Tyr	
			115						120						125	
Gly	Phe	Val	Ala	Arg	Ile	Asp	Gly	Ser	Gly	Asn	Phe	Gln	Val	Leu	Leu	
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Ser	Asp	Arg	Tyr	Phe	Asn	Lys	Thr	Cys	Gly	Leu	Cys	Gly	Asn	Phe	Asn	
145						150						155			160	
Ile	Phe	Ala	Glu	Asp	Asp	Phe	Met	Thr	Gln	Glu	Gly	Thr	Leu	Thr	Ser	
			165						170						175	
Asp	Pro	Tyr	Asp	Phe	Ala	Asn	Ser	Trp	Ala	Leu	Ser	Ser	Gly	Glu	Gln	
			180						185						190	
Trp	Cys	Glu	Arg	Pro	Ser	Ser	Ser	Cys	Asn	Ile	Ser	Ser	Gly	Glu	Met	
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FIG. 6 (Cont.)

Ile	Asn	Glu	Met	Cys	Gln	Glu	Arg	Cys	Val	Asp	Gly	Cys	Ser	Cys	Pro	305	310	315	320
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Asn	Glu	Glu	Cys	Pro	Gly	Glu	Cys	Leu	Val	Thr	Gly	Gln	Ser	His	Phe	370	375	380	
Lys	Ser	Phe	Asp	Asn	Arg	Tyr	Phe	Thr	Phe	Ser	Gly	Ile	Cys	Gln	Tyr	385	390	395	400
Leu	Leu	Ala	Arg	Asp	Cys	Gln	Asp	His	Ser	Phe	Ser	Ile	Val	Ile	Glu	405	410	415	
Thr	Val	Gln	Cys	Ala	Asp	Asp	Arg	Asp	Ala	Val	Cys	Thr	Arg	Ser	Val	420	425	430	
Thr	Val	Arg	Leu	Pro	Gly	Leu	His	Asn	Ser	Leu	Val	Lys	Leu	Lys	His	435	440	445	
Gly	Ala	Gly	Val	Ala	Met	Asp	Gly	Gln	Asp	Val	Gln	Leu	Pro	Leu	Leu	450	455	460	
Lys	Gly	Asp	Leu	Arg	Ile	Gln	His	Thr	Val	Thr	Ala	Ser	Val	Arg	Leu	465	470	475	480
Ser	Tyr	Gly	Glu	Asp	Leu	Gln	Met	Asp	Trp	Asp	Gly	Arg	Gly	Arg	Leu	485	490	495	
Leu	Val	Lys	Leu	Ser	Pro	Val	Tyr	Ala	Gly	Lys	Thr	Cys	Gly	Leu	Cys	500	505	510	
Gly	Asn	Tyr	Asn	Gly	Asn	Gln	Gly	Asp	Asp	Phe	Leu	Thr	Pro	Ser	Gly	515	520	525	
Leu	Ala	Glu	Pro	Arg	Val	Glu	Asp	Phe	Gly	Asn	Ala	Trp	Lys	Leu	His	530	535	540	
Gly	Asp	Cys	Gln	Asp	Leu	Gln	Lys	Gln	His	Ser	Asp	Pro	Cys	Ala	Leu	545	550	555	560

FIG. 6 (Cont.)

Asn	Pro	Arg	Met	Thr	Arg	Phe	Ser	Glu	Glu	Ala	Cys	Ala	Val	Leu	Thr	565	570	575	
Ser	Pro	Thr	Phe	Glu	Ala	Cys	His	Arg	Ala	Val	Ser	Pro	Leu	Pro	Tyr	580	585	590	
Leu	Arg	Asn	Cys	Arg	Tyr	Asp	Val	Cys	Ser	Cys	Ser	Asp	Gly	Arg	Glu	595	600	605	
Cys	Leu	Cys	Gly	Ser	Tyr	Ala	Ala	Ala	Cys	Ala	Gly	Arg	Gly	Val	Arg	610	615	620	
Val	Ala	Trp	Arg	Glu	Pro	Gly	Arg	Cys	Glu	Leu	Asn	Cys	Pro	Lys	Gly	625	630	635	640
Gln	Val	Tyr	Leu	Gln	Cys	Gly	Thr	Pro	Cys	Asn	Leu	Thr	Cys	Arg	Ser	645	650	655	
Leu	Ser	Tyr	Pro	Asp	Glu	Glu	Cys	Asn	Glu	Ala	Cys	Leu	Glu	Gly	Cys	660	665	670	
Phe	Cys	Pro	Pro	Met	Asp	Glu	Arg	Gly	Asp	Cys	Val	Pro	Lys	Ala	Gln	675	680	685	
Cys	Pro	Cys	Tyr	Tyr	Asp	Gly	Glu	Ile	Phe	Gln	Pro	Glu	Asp	Ile	Phe	690	695	700	
Ser	Asp	His	His	Thr	Met	Cys	Tyr	Cys	Glu	Asp	Gly	Phe	Met	His	Cys	705	710	715	720
Thr	Met	Ser	Gly	Val	Pro	Gly	Ser	Leu	Leu	Pro	Asp	Ala	Val	Leu	Ser	725	730	735	
Ser	Pro	Leu	Ser	His	Arg	Ser	Lys	Arg	Ser	Leu	Ser	Cys	Arg	Pro	Pro	740	745	750	
Met	Val	Lys	Leu	Val	Cys	Pro	Ala	Asp	Asn	Leu	Arg	Ala	Glu	Gly	Leu	755	760	765	
Glu	Cys	Thr	Lys	Thr	Cys	Gln	Asn	Tyr	Asp	Leu	Glu	Cys	Met	Ser	Met	770	775	780	
Gly	Cys	Val	Ser	Gly	Cys	Leu	Cys	Pro	Pro	Gly	Met	Val	Arg	His	Glu	785	790	795	800
Asn	Arg	Cys	Glu	Arg	Cys	Pro	Cys	Phe	His	Gln	Gly	Lys	Glu	Tyr	Ala	805	810	815	

FIG. 6 (Cont.)

Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr Cys Val Cys Arg Asp		
820	825	830
Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp Ala Thr Cys Ser Thr		
835	840	845
Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe		
850	855	860
Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp Tyr Cys Gly Ser Asn		
865	870	875 880
Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys Gly Cys Ser His Pro		
885	890	895
Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu Val Glu Gly Gly Glu		
900	905	910
Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys Arg Pro Met Lys Asp		
915	920	925
Glu Thr His Phe Glu Val Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu		
930	935	940
Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg His Leu Ser Ile Ser		
945	950	955 960
Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly		
965	970	975
Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln		
980	985	990
Val Glu Glu Asp Pro Val Asp Phe Gly Asn Ser Trp Lys Val Ser Ser		
995	1000	1005
Gln Cys Ala Asp Thr Arg Lys Val Pro Leu Asp Ser Ser Pro Ala		
1010	1015	1020
Thr Cys His Asn Asn Ile Met Lys Gln Thr Met Val Asp Ser Ser		
1025	1030	1035
Cys Arg Ile Leu Thr Ser Asp Val Phe Gln Asp Cys Asn Lys Leu		
1040	1045	1050
Val Asp Pro Glu Pro Tyr Leu Asp Val Cys Ile Tyr Asp Thr Cys		
1055	1060	1065

FIG. 6 (Cont.)

Ser Cys	Glu Ser Ile Gly	Asp	Cys Ala Cys Phe Cys	Asp Thr Ile
1070		1075		1080
Ala Ala	Tyr Ala His Val	Cys	Ala Gln His Gly Lys	Val Val Thr
1085		1090		1095
Trp Arg	Thr Ala Thr Leu	Cys	Pro Gln Ser Cys Glu	Glu Arg Asn
1100		1105		1110
Leu Arg	Glu Asn Gly Tyr	Glu	Cys Glu Trp Arg Tyr	Asn Ser Cys
1115		1120		1125
Ala Pro	Ala Cys Gln Val	Thr	Cys Gln His Pro Glu	Pro Leu Ala
1130		1135		1140
Cys Pro	Val Gln Cys Val	Glu	Gly Cys His Ala His	Cys Pro Pro
1145		1150		1155
Gly Lys	Ile Leu Asp Glu	Leu	Leu Gln Thr Cys Val	Asp Pro Glu
1160		1165		1170
Asp Cys	Pro Val Cys Glu	Val	Ala Gly Arg Arg Phe	Ala Ser Gly
1175		1180		1185
Lys Lys	Val Thr Leu Asn	Pro	Ser Asp Pro Glu His	Cys Gln Ile
1190		1195		1200
Cys His	Cys Asp Val Val	Asn	Leu Thr Cys Glu Ala	Cys Gln Glu
1205		1210		1215
Pro Gly	Gly Leu Val Val	Pro	Pro Thr Asp Ala Pro	Val Ser Pro
1220		1225		1230
Thr Thr	Leu Tyr Val Glu	Asp	Ile Ser Glu Pro Pro	Leu His Asp
1235		1240		1245
Phe Tyr	Cys Ser Arg Leu	Leu	Asp Leu Val Phe Leu	Leu Asp Gly
1250		1255		1260
Ser Ser	Arg Leu Ser Glu	Ala	Glu Phe Glu Val Leu	Lys Ala Phe
1265		1270		1275
Val Val	Asp Met Met Glu	Arg	Leu Arg Ile Ser Gln	Lys Trp Val
1280		1285		1290
Arg Val	Ala Val Val Glu	Tyr	His Asp Gly Ser His	Ala Tyr Ile
1295		1300		1305

FIG. 6 (Cont.)

Gly Leu	Lys Asp Arg Lys Arg	Pro Ser Glu Leu Arg	Arg Ile Ala
1310	1315	1320	
Ser Gln	Val Lys Tyr Ala Gly	Ser Gln Val Ala Ser	Thr Ser Glu
1325	1330	1335	
Val Leu	Lys Tyr Thr Leu Phe	Gln Ile Phe Ser Lys	Ile Asp Arg
1340	1345	1350	
Pro Glu	Ala Ser Arg Ile Thr	Leu Leu Leu Met Ala	Ser Gln Glu
1355	1360	1365	
Pro Gln	Arg Met Ser Arg Asn	Phe Val Arg Tyr Val	Gln Gly Leu
1370	1375	1380	
Lys Lys	Lys Lys Val Ile Val	Ile Pro Val Gly Ile	Gly Pro His
1385	1390	1395	
Ala Asn	Leu Lys Gln Ile Arg	Leu Ile Glu Lys Gln	Ala Pro Glu
1400	1405	1410	
Asn Lys	Ala Phe Val Leu Ser	Ser Val Asp Glu Leu	Glu Gln Gln
1415	1420	1425	
Arg Asp	Glu Ile Val Ser Tyr	Leu Cys Asp Leu Ala	Pro Glu Ala
1430	1435	1440	
Pro Pro	Pro Thr Leu Pro Pro	Asp Met Ala Gln Val	Thr Val Gly
1445	1450	1455	
Pro Gly	Leu Leu Gly Val Ser	Thr Leu Gly Pro Lys	Arg Asn Ser
1460	1465	1470	
Met Val	Leu Asp Val Ala Phe	Val Leu Glu Gly Ser	Asp Lys Ile
1475	1480	1485	
Gly Glu	Ala Asp Phe Asn Arg	Ser Lys Glu Phe Met	Glu Glu Val
1490	1495	1500	
Ile Gln	Arg Met Asp Val Gly	Gln Asp Ser Ile His	Val Thr Val
1505	1510	1515	
Leu Gln	Tyr Ser Tyr Met Val	Thr Val Glu Tyr Pro	Phe Ser Glu
1520	1525	1530	
Ala Gln	Ser Lys Gly Asp Ile	Leu Gln Arg Val Arg	Glu Ile Arg
1535	1540	1545	

FIG. 6 (Cont.)

Tyr	Gln	Gly	Gly	Asn	Arg	Thr	Asn	Thr	Gly	Leu	Ala	Leu	Arg	Tyr
1550						1555					1560			
Leu	Ser	Asp	His	Ser	Phe	Leu	Val	Ser	Gln	Gly	Asp	Arg	Glu	Gln
1565						1570					1575			
Ala	Pro	Asn	Leu	Val	Tyr	Met	Val	Thr	Gly	Asn	Pro	Ala	Ser	Asp
1580						1585					1590			
Glu	Ile	Lys	Arg	Leu	Pro	Gly	Asp	Ile	Gln	Val	Val	Pro	Ile	Gly
1595						1600					1605			
Val	Gly	Pro	Asn	Ala	Asn	Val	Gln	Glu	Leu	Glu	Arg	Ile	Gly	Trp
1610						1615					1620			
Pro	Asn	Ala	Pro	Ile	Leu	Ile	Gln	Asp	Phe	Glu	Thr	Leu	Pro	Arg
1625						1630					1635			
Glu	Ala	Pro	Asp	Leu	Val	Leu	Gln	Arg	Cys	Cys	Ser	Gly	Glu	Gly
1640						1645					1650			
Leu	Gln	Ile	Pro	Thr	Leu	Ser	Pro	Ala	Pro	Asp	Cys	Ser	Gln	Pro
1655						1660					1665			
Leu	Asp	Val	Ile	Leu	Leu	Leu	Asp	Gly	Ser	Ser	Ser	Phe	Pro	Ala
1670						1675					1680			
Ser	Tyr	Phe	Asp	Glu	Met	Lys	Ser	Phe	Ala	Lys	Ala	Phe	Ile	Ser
1685						1690					1695			
Lys	Ala	Asn	Ile	Gly	Pro	Arg	Leu	Thr	Gln	Val	Ser	Val	Leu	Gln
1700						1705					1710			
Tyr	Gly	Ser	Ile	Thr	Thr	Ile	Asp	Val	Pro	Trp	Asn	Val	Val	Pro
1715						1720					1725			
Glu	Lys	Ala	His	Leu	Leu	Ser	Leu	Val	Asp	Val	Met	Gln	Arg	Glu
1730						1735					1740			
Gly	Gly	Pro	Ser	Gln	Ile	Gly	Asp	Ala	Leu	Gly	Phe	Ala	Val	Arg
1745						1750					1755			
Tyr	Leu	Thr	Ser	Glu	Met	His	Gly	Ala	Arg	Pro	Gly	Ala	Ser	Lys
1760						1765					1770			
Ala	Val	Val	Ile	Leu	Val	Thr	Asp	Val	Ser	Val	Asp	Ser	Val	Asp
1775						1780					1785			

FIG. 6 (Cont.)

Ala	Ala	Ala	Asp	Ala	Ala	Arg	Ser	Asn	Arg	Val	Thr	Val	Phe	Pro
1790						1795					1800			
Ile	Gly	Ile	Gly	Asp	Arg	Tyr	Asp	Ala	Ala	Gln	Leu	Arg	Ile	Leu
1805						1810					1815			
Ala	Gly	Pro	Ala	Gly	Asp	Ser	Asn	Val	Val	Lys	Leu	Gln	Arg	Ile
1820						1825					1830			
Glu	Asp	Leu	Pro	Thr	Met	Val	Thr	Leu	Gly	Asn	Ser	Phe	Leu	His
1835						1840					1845			
Lys	Leu	Cys	Ser	Gly	Phe	Val	Arg	Ile	Cys	Met	Asp	Glu	Asp	Gly
1850						1855					1860			
Asn	Glu	Lys	Arg	Pro	Gly	Asp	Val	Trp	Thr	Leu	Pro	Asp	Gln	Cys
1865						1870					1875			
His	Thr	Val	Thr	Cys	Gln	Pro	Asp	Gly	Gln	Thr	Leu	Leu	Lys	Ser
1880						1885					1890			
His	Arg	Val	Asn	Cys	Asp	Arg	Gly	Leu	Arg	Pro	Ser	Cys	Pro	Asn
1895						1900					1905			
Ser	Gln	Ser	Pro	Val	Lys	Val	Glu	Glu	Thr	Cys	Gly	Cys	Arg	Trp
1910						1915					1920			
Thr	Cys	Pro	Cys	Val	Cys	Thr	Gly	Ser	Ser	Thr	Arg	His	Ile	Val
1925						1930					1935			
Thr	Phe	Asp	Gly	Gln	Asn	Phe	Lys	Leu	Thr	Gly	Ser	Cys	Ser	Tyr
1940						1945					1950			
Val	Leu	Phe	Gln	Asn	Lys	Glu	Gln	Asp	Leu	Glu	Val	Ile	Leu	His
1955						1960					1965			
Asn	Gly	Ala	Cys	Ser	Pro	Gly	Ala	Arg	Gln	Gly	Cys	Met	Lys	Ser
1970						1975					1980			
Ile	Glu	Val	Lys	His	Ser	Ala	Leu	Ser	Val	Glu	Leu	His	Ser	Asp
1985						1990					1995			
Met	Glu	Val	Thr	Val	Asn	Gly	Arg	Leu	Val	Ser	Val	Pro	Tyr	Val
2000						2005					2010			
Gly	Gly	Asn	Met	Glu	Val	Asn	Val	Tyr	Gly	Ala	Ile	Met	His	Glu
2015						2020					2025			

FIG. 6 (Cont.)

Val	Arg	Phe	Asn	His	Leu	Gly	His	Ile	Phe	Thr	Phe	Thr	Pro	Gln
2030						2035					2040			
Asn	Asn	Glu	Phe	Gln	Leu	Gln	Leu	Ser	Pro	Lys	Thr	Phe	Ala	Ser
2045						2050					2055			
Lys	Thr	Tyr	Gly	Leu	Cys	Gly	Ile	Cys	Asp	Glu	Asn	Gly	Ala	Asn
2060						2065					2070			
Asp	Phe	Met	Leu	Arg	Asp	Gly	Thr	Val	Thr	Thr	Asp	Trp	Lys	Thr
2075						2080					2085			
Ileu	Val	Gln	Glu	Trp	Thr	Val	Gln	Arg	Pro	Gly	Gln	Thr	Cys	Gln
2090						2095					2100			
Pro	Glu	Gln	Cys	Leu	Val	Pro	Asp	Ser	Ser	His	Cys	Gln	Val	Leu
2105						2110					2115			
Ileu	Leu	Pro	Leu	Phe	Ala	Glu	Cys	His	Lys	Val	Leu	Ala	Pro	Ala
2120						2125					2130			
Thr	Phe	Tyr	Ala	Ile	Cys	Gln	Gln	Asp	Ser	Cys	His	Gln	Glu	Gln
2135						2140					2145			
Val	Cys	Glu	Val	Ile	Ala	Ser	Tyr	Ala	His	Leu	Cys	Arg	Thr	Asn
2150						2155					2160			
Gly	Val	Cys	Val	Asp	Trp	Arg	Thr	Pro	Asp	Phe	Cys	Ala	Met	Ser
2165						2170					2175			
Cys	Pro	Pro	Ser	Leu	Val	Tyr	Asn	His	Cys	Glu	His	Gly	Cys	Pro
2180						2185					2190			
Arg	His	Cys	Asp	Gly	Asn	Val	Ser	Ser	Cys	Gly	Asp	His	Pro	Ser
2195						2200					2205			
Glu	Gly	Cys	Phe	Cys	Pro	Pro	Asp	Lys	Val	Met	Leu	Glu	Gly	Ser
2210						2215					2220			
Cys	Val	Pro	Glu	Glu	Ala	Cys	Thr	Gln	Cys	Ile	Gly	Glu	Asp	Gly
2225						2230					2235			
Val	Gln	His	Gln	Phe	Leu	Glu	Ala	Trp	Val	Pro	Asp	His	Gln	Pro
2240						2245					2250			
Cys	Gln	Ile	Cys	Thr	Cys	Leu	Ser	Gly	Arg	Lys	Val	Asn	Cys	Thr
2255						2260					2265			

FIG. 6 (Cont.)

Thr	Gln	Pro	Cys	Pro	Thr	Ala	Lys	Ala	Pro	Thr	Cys	Gly	Leu	Cys
	2270					2275					2280			
Glu	Val	Ala	Arg	Leu	Arg	Gln	Asn	Ala	Asp	Gln	Cys	Cys	Pro	Glu
	2285					2290					2295			
Tyr	Glu	Cys	Val	Cys	Asp	Pro	Val	Ser	Cys	Asp	Leu	Pro	Pro	Val
	2300					2305					2310			
Pro	His	Cys	Glu	Arg	Gly	Leu	Gln	Pro	Thr	Leu	Thr	Asn	Pro	Gly
	2315					2320					2325			
Glu	Cys	Arg	Pro	Asn	Phe	Thr	Cys	Ala	Cys	Arg	Lys	Glu	Glu	Cys
	2330					2335					2340			
Lys	Arg	Val	Ser	Pro	Pro	Ser	Cys	Pro	Pro	His	Arg	Leu	Pro	Thr
	2345					2350					2355			
Leu	Arg	Lys	Thr	Gln	Cys	Cys	Asp	Glu	Tyr	Glu	Cys	Ala	Cys	Asn
	2360					2365					2370			
Cys	Val	Asn	Ser	Thr	Val	Ser	Cys	Pro	Leu	Gly	Tyr	Leu	Ala	Ser
	2375					2380					2385			
Thr	Ala	Thr	Asn	Asp	Cys	Gly	Cys	Thr	Thr	Thr	Thr	Cys	Leu	Pro
	2390					2395					2400			
Asp	Lys	Val	Cys	Val	His	Arg	Ser	Thr	Ile	Tyr	Pro	Val	Gly	Gln
	2405					2410					2415			
Phe	Trp	Glu	Glu	Gly	Cys	Asp	Val	Cys	Thr	Cys	Thr	Asp	Met	Glu
	2420					2425					2430			
Asp	Ala	Val	Met	Gly	Leu	Arg	Val	Ala	Gln	Cys	Ser	Gln	Lys	Pro
	2435					2440					2445			
Cys	Glu	Asp	Ser	Cys	Arg	Ser	Gly	Phe	Thr	Tyr	Val	Leu	His	Glu
	2450					2455					2460			
Gly	Glu	Cys	Cys	Gly	Arg	Cys	Leu	Pro	Ser	Ala	Cys	Glu	Val	Val
	2465					2470					2475			
Thr	Gly	Ser	Pro	Arg	Gly	Asp	Ser	Gln	Ser	Ser	Trp	Lys	Ser	Val
	2480					2485					2490			
Gly	Ser	Gln	Trp	Glu	Asn	Pro	Cys	Leu	Ile	Asn	Glu	Cys	Val	Arg
	2495					2500					2505			

FIG. 6 (Cont.)

Val	Lys	Glu	Glu	Val	Phe	Ile	Gln	Gln	Arg	Asn	Val	Ser	Cys	Pro
2510						2515					2520			
Gln	Leu	Glu	Val	Pro	Val	Cys	Pro	Ser	Gly	Phe	Gln	Leu	Ser	Cys
2525						2530					2535			
Lys	Thr	Ser	Ala	Cys	Cys	Pro	Ser	Cys	Arg	Cys	Glu	Arg	Met	Glu
2540						2545					2550			
Ala	Cys	Met	Leu	Asn	Gly	Thr	Val	Ile	Gly	Pro	Gly	Lys	Thr	Val
2555						2560					2565			
Met	Ile	Asp	Val	Cys	Thr	Thr	Cys	Arg	Cys	Met	Val	Gln	Val	Gly
2570						2575					2580			
Val	Ile	Ser	Gly	Phe	Lys	Leu	Glu	Cys	Arg	Lys	Thr	Thr	Cys	Asn
2585						2590					2595			
Pro	Cys	Pro	Leu	Gly	Tyr	Lys	Glu	Glu	Asn	Asn	Thr	Gly	Glu	Cys
2600						2605					2610			
Cys	Gly	Arg	Cys	Leu	Pro	Thr	Ala	Cys	Thr	Ile	Gln	Leu	Arg	Gly
2615						2620					2625			
Gly	Gln	Ile	Met	Thr	Leu	Lys	Arg	Asp	Glu	Thr	Leu	Gln	Asp	Gly
2630						2635					2640			
Cys	Asp	Thr	His	Phe	Cys	Lys	Val	Asn	Glu	Arg	Gly	Glu	Tyr	Phe
2645						2650					2655			
Trp	Glu	Lys	Arg	Val	Thr	Gly	Cys	Pro	Pro	Phe	Asp	Glu	His	Lys
2660						2665					2670			
Cys	Leu	Ala	Glu	Gly	Gly	Lys	Ile	Met	Lys	Ile	Pro	Gly	Thr	Cys
2675						2680					2685			
Cys	Asp	Thr	Cys	Glu	Glu	Pro	Glu	Cys	Asn	Asp	Ile	Thr	Ala	Arg
2690						2695					2700			
Leu	Gln	Tyr	Val	Lys	Val	Gly	Ser	Cys	Lys	Ser	Glu	Val	Glu	Val
2705						2710					2715			
Asp	Ile	His	Tyr	Cys	Gln	Gly	Lys	Cys	Ala	Ser	Lys	Ala	Met	Tyr
2720						2725					2730			
Ser	Ile	Asp	Ile	Asn	Asp	Val	Gln	Asp	Gln	Cys	Ser	Cys	Cys	Ser
2735						2740					2745			

FIG. 6 (Cont.)

Pro Thr Arg Thr Glu Pro Met Gln His Cys Thr Asn Gly Ser Val
 2750 2755 2760

Val Tyr His Glu Val Leu Asn Ala Met Glu Cys Lys Cys Ser Pro
 2765 2770 2775

Arg Lys Cys Ser Lys
 2780

SEQ ID NO:3

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp
 1 5 10 15

Asn Leu Arg Ala Glu Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr
 20 25 30

Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro
 35 40 45

Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys
 50 55 60

Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys
 65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr
 85 90 95

Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr
 100 105 110

Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr
 115 120 125

Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile
 130 135 140

Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys
 145 150 155 160

Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly
 165 170 175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val
 180 185 190

FIG. 6 (Cont.)

Val	Glu	Ser	Gly	Arg	Tyr	Ile	Ile	Leu	Leu	Leu	Gly	Lys	Ala	Leu	Ser	195	200	205
Val	Val	Trp	Asp	Arg	His	Leu	Ser	Ile	Ser	Val	Val	Leu	Lys	Gln	Thr	210	215	220
Tyr	Gln	Glu	Lys	Val	Cys	Gly	Leu	Cys	Gly	Asn	Phe	Asp	Gly	Ile	Gln	225	230	235
Asn	Asn	Asp	Leu	Thr	Ser	Ser	Asn	Leu	Gln	Val	Glu	Glu	Asp	Pro	Val	245	250	255
Asp	Phe	Gly	Asn	Ser	Trp	Lys	Val	Ser	Ser	Gln	Cys	Ala	Asp	Thr	Arg	260	265	270
Lys	Val	Pro	Leu	Asp	Ser	Ser	Pro	Ala	Thr	Cys	His	Asn	Asn	Ile	Met	275	280	285
Lys	Gln	Thr	Met	Val	Asp	Ser	Ser	Cys	Arg	Ile	Leu	Thr	Ser	Asp	Val	290	295	300
Phe	Gln	Asp	Cys	Asn	Lys	Leu	Val	Asp	Pro	Glu	Pro	Tyr	Leu	Asp	Val	305	310	315
Cys	Ile	Tyr	Asp	Thr	Cys	Ser	Cys	Glu	Ser	Ile	Gly	Asp	Cys	Ala	Cys	325	330	335
Phe	Cys	Asp	Thr	Ile	Ala	Ala	Tyr	Ala	His	Val	Cys	Ala	Gln	His	Gly	340	345	350
Lys	Val	Val	Thr	Trp	Arg	Thr	Ala	Thr	Leu	Cys	Pro	Gln	Ser	Cys	Glu	355	360	365
Glu	Arg	Asn	Leu	Arg	Glu	Asn	Gly	Tyr	Glu	Cys	Glu	Trp	Arg	Tyr	Asn	370	375	380
Ser	Cys	Ala	Pro	Ala	Cys	Gln	Val	Thr	Cys	Gln	His	Pro	Glu	Pro	Leu	385	390	395
Ala	Cys	Pro	Val	Gln	Cys	Val	Glu	Gly	Cys	His	Ala	His	Cys	Pro	Pro	405	410	415
Gly	Lys	Ile	Leu	Asp	Glu	Leu	Leu	Gln	Thr	Cys	Val	Asp	Pro	Glu	Asp	420	425	430
Cys	Pro	Val	Cys	Glu	Val	Ala	Gly	Arg	Arg	Phe	Ala	Ser	Gly	Lys	Lys	435	440	445

FIG. 6 (Cont.)

Val	Thr	Leu	Asn	Pro	Ser	Asp	Pro	Glu	His	Cys	Gln	Ile	Cys	His	Cys	450	455	460	
Asp	Val	Val	Asn	Leu	Thr	Cys	Glu	Ala	Cys	Gln	Glu	Pro	Gly	Gly	Leu	465	470	475	480
Val	Val	Pro	Pro	Thr	Asp	Ala	Pro	Val	Ser	Pro	Thr	Thr	Leu	Tyr	Val	485	490	495	
Glu	Asp	Ile	Ser	Glu	Pro	Pro	Leu	His	Asp	Phe	Tyr	Cys	Ser	Arg	Leu	500	505	510	
Leu	Asp	Leu	Val	Phe	Leu	Leu	Asp	Gly	Ser	Ser	Arg	Leu	Ser	Glu	Ala	515	520	525	
Glu	Phe	Glu	Val	Leu	Lys	Ala	Phe	Val	Val	Asp	Met	Met	Glu	Arg	Leu	530	535	540	
Arg	Ile	Ser	Gln	Lys	Trp	Val	Arg	Val	Ala	Val	Val	Glu	Tyr	His	Asp	545	550	555	560
Gly	Ser	His	Ala	Tyr	Ile	Gly	Leu	Lys	Asp	Arg	Lys	Arg	Pro	Ser	Glu	565	570	575	
Leu	Arg	Arg	Ile	Ala	Ser	Gln	Val	Lys	Tyr	Ala	Gly	Ser	Gln	Val	Ala	580	585	590	
Ser	Thr	Ser	Glu	Val	Leu	Lys	Tyr	Thr	Leu	Phe	Gln	Ile	Phe	Ser	Lys	595	600	605	
Ile	Asp	Arg	Pro	Glu	Ala	Ser	Arg	Ile	Thr	Leu	Leu	Leu	Met	Ala	Ser	610	615	620	
Gln	Glu	Pro	Gln	Arg	Met	Ser	Arg	Asn	Phe	Val	Arg	Tyr	Val	Gln	Gly	625	630	635	640
Leu	Lys	Lys	Lys	Lys	Val	Ile	Val	Ile	Pro	Val	Gly	Ile	Gly	Pro	His	645	650	655	
Ala	Asn	Leu	Lys	Gln	Ile	Arg	Leu	Ile	Glu	Lys	Gln	Ala	Pro	Glu	Asn	660	665	670	
Lys	Ala	Phe	Val	Leu	Ser	Ser	Val	Asp	Glu	Leu	Glu	Gln	Gln	Arg	Asp	675	680	685	
Glu	Ile	Val	Ser	Tyr	Leu	Cys	Asp	Leu	Ala	Pro	Glu	Ala	Pro	Pro	Pro	690	695	700	

FIG. 6 (Cont.)

Thr	Leu	Pro	Pro	Asp	Met	Ala	Gln	Val	Thr	Val	Gly	Pro	Gly	Leu	Leu	705	710	715	720
Gly	Val	Ser	Thr	Leu	Gly	Pro	Lys	Arg	Asn	Ser	Met	Val	Leu	Asp	Val	725	730	735	
Ala	Phe	Val	Leu	Glu	Gly	Ser	Asp	Lys	Ile	Gly	Glu	Ala	Asp	Phe	Asn	740	745	750	
Arg	Ser	Lys	Glu	Phe	Met	Glu	Glu	Val	Ile	Gln	Arg	Met	Asp	Val	Gly	755	760	765	
Gln	Asp	Ser	Ile	His	Val	Thr	Val	Leu	Gln	Tyr	Ser	Tyr	Met	Val	Thr	770	775	780	
Val	Glu	Tyr	Pro	Phe	Ser	Glu	Ala	Gln	Ser	Lys	Gly	Asp	Ile	Leu	Gln	785	790	795	800
Arg	Val	Arg	Glu	Ile	Arg	Tyr	Gln	Gly	Gly	Asn	Arg	Thr	Asn	Thr	Gly	805	810	815	
Leu	Ala	Leu	Arg	Tyr	Leu	Ser	Asp	His	Ser	Phe	Leu	Val	Ser	Gln	Gly	820	825	830	
Asp	Arg	Glu	Gln	Ala	Pro	Asn	Leu	Val	Tyr	Met	Val	Thr	Gly	Asn	Pro	835	840	845	
Ala	Ser	Asp	Glu	Ile	Lys	Arg	Leu	Pro	Gly	Asp	Ile	Gln	Val	Val	Pro	850	855	860	
Ile	Gly	Val	Gly	Pro	Asn	Ala	Asn	Val	Gln	Glu	Leu	Glu	Arg	Ile	Gly	865	870	875	880
Trp	Pro	Asn	Ala	Pro	Ile	Leu	Ile	Gln	Asp	Phe	Glu	Thr	Leu	Pro	Arg	885	890	895	
Glu	Ala	Pro	Asp	Leu	Val	Leu	Gln	Arg	Cys	Cys	Ser	Gly	Glu	Gly	Leu	900	905	910	
Gln	Ile	Pro	Thr	Leu	Ser	Pro	Ala	Pro	Asp	Cys	Ser	Gln	Pro	Leu	Asp	915	920	925	
Val	Ile	Leu	Leu	Leu	Asp	Gly	Ser	Ser	Ser	Phe	Pro	Ala	Ser	Tyr	Phe	930	935	940	
Asp	Glu	Met	Lys	Ser	Phe	Ala	Lys	Ala	Phe	Ile	Ser	Lys	Ala	Asn	Ile	945	950	955	960

FIG. 6 (Cont.)

Gly	Pro	Arg	Leu	Thr	Gln	Val	Ser	Val	Leu	Gln	Tyr	Gly	Ser	Ile	Thr	965	970	975
Thr	Ile	Asp	Val	Pro	Trp	Asn	Val	Val	Pro	Glu	Lys	Ala	His	Leu	Leu	980	985	990
Ser	Leu	Val	Asp	Val	Met	Gln	Arg	Glu	Gly	Gly	Pro	Ser	Gln	Ile	Gly	995	1000	1005
Asp	Ala	Leu	Gly	Phe	Ala	Val	Arg	Tyr	Leu	Thr	Ser	Glu	Met	His		1010	1015	1020
Gly	Ala	Arg	Pro	Gly	Ala	Ser	Lys	Ala	Val	Val	Ile	Leu	Val	Thr		1025	1030	1035
Asp	Val	Ser	Val	Asp	Ser	Val	Asp	Ala	Ala	Ala	Asp	Ala	Ala	Arg		1040	1045	1050
Ser	Asn	Arg	Val	Thr	Val	Phe	Pro	Ile	Gly	Ile	Gly	Asp	Arg	Tyr		1055	1060	1065
Asp	Ala	Ala	Gln	Leu	Arg	Ile	Leu	Ala	Gly	Pro	Ala	Gly	Asp	Ser		1070	1075	1080
Asn	Val	Val	Lys	Leu	Gln	Arg	Ile	Glu	Asp	Leu	Pro	Thr	Met	Val		1085	1090	1095
Thr	Leu	Gly	Asn	Ser	Phe	Leu	His	Lys	Leu	Cys	Ser	Gly	Phe	Val		1100	1105	1110
Arg	Ile	Cys	Met	Asp	Glu	Asp	Gly	Asn	Glu	Lys	Arg	Pro	Gly	Asp		1115	1120	1125
Val	Trp	Thr	Leu	Pro	Asp	Gln	Cys	His	Thr	Val	Thr	Cys	Gln	Pro		1130	1135	1140
Asp	Gly	Gln	Thr	Leu	Leu	Lys	Ser	His	Arg	Val	Asn	Cys	Asp	Arg		1145	1150	1155
Gly	Leu	Arg	Pro	Ser	Cys	Pro	Asn	Ser	Gln	Ser	Pro	Val	Lys	Val		1160	1165	1170
Glu	Glu	Thr	Cys	Gly	Cys	Arg	Trp	Thr	Cys	Pro	Cys	Val	Cys	Thr		1175	1180	1185
Gly	Ser	Ser	Thr	Arg	His	Ile	Val	Thr	Phe	Asp	Gly	Gln	Asn	Phe		1190	1195	1200

FIG. 6 (Cont.)

Lys	Leu	Thr	Gly	Ser	Cys	Ser	Tyr	Val	Leu	Phe	Gln	Asn	Lys	Glu
1205						1210					1215			
Gln	Asp	Leu	Glu	Val	Ile	Leu	His	Asn	Gly	Ala	Cys	Ser	Pro	Gly
1220						1225					1230			
Ala	Arg	Gln	Gly	Cys	Met	Lys	Ser	Ile	Glu	Val	Lys	His	Ser	Ala
1235						1240					1245			
Leu	Ser	Val	Glu	Leu	His	Ser	Asp	Met	Glu	Val	Thr	Val	Asn	Gly
1250						1255					1260			
Arg	Leu	Val	Ser	Val	Pro	Tyr	Val	Gly	Gly	Asn	Met	Glu	Val	Asn
1265						1270					1275			
Val	Tyr	Gly	Ala	Ile	Met	His	Glu	Val	Arg	Phe	Asn	His	Leu	Gly
1280						1285					1290			
His	Ile	Phe	Thr	Phe	Thr	Pro	Gln	Asn	Asn	Glu	Phe	Gln	Leu	Gln
1295						1300					1305			
Leu	Ser	Pro	Lys	Thr	Phe	Ala	Ser	Lys	Thr	Tyr	Gly	Leu	Cys	Gly
1310						1315					1320			
Ile	Cys	Asp	Glu	Asn	Gly	Ala	Asn	Asp	Phe	Met	Leu	Arg	Asp	Gly
1325						1330					1335			
Thr	Val	Thr	Thr	Asp	Trp	Lys	Thr	Leu	Val	Gln	Glu	Trp	Thr	Val
1340						1345					1350			
Gln	Arg	Pro	Gly	Gln	Thr	Cys	Gln	Pro	Ile	Leu	Glu	Glu	Gln	Cys
1355						1360					1365			
Leu	Val	Pro	Asp	Ser	Ser	His	Cys	Gln	Val	Leu	Leu	Leu	Pro	Leu
1370						1375					1380			
Phe	Ala	Glu	Cys	His	Lys	Val	Leu	Ala	Pro	Ala	Thr	Phe	Tyr	Ala
1385						1390					1395			
Ile	Cys	Gln	Gln	Asp	Ser	Cys	His	Gln	Glu	Gln	Val	Cys	Glu	Val
1400						1405					1410			
Ile	Ala	Ser	Tyr	Ala	His	Leu	Cys	Arg	Thr	Asn	Gly	Val	Cys	Val
1415						1420					1425			
Asp	Trp	Arg	Thr	Pro	Asp	Phe	Cys	Ala	Met	Ser	Cys	Pro	Pro	Ser
1430						1435					1440			

FIG. 6 (Cont.)

Leu Val	Tyr Asn His Cys Glu	His Gly Cys Pro Arg	His Cys Asp
1445	1450	1455	
Gly Asn	Val Ser Ser Cys Gly	Asp His Pro Ser Glu	Gly Cys Phe
1460	1465	1470	
Cys Pro	Pro Asp Lys Val Met	Leu Glu Gly Ser Cys	Val Pro Glu
1475	1480	1485	
Glu Ala	Cys Thr Gln Cys Ile	Gly Glu Asp Gly Val	Gln His Gln
1490	1495	1500	
Phe Leu	Glu Ala Trp Val Pro	Asp His Gln Pro Cys	Gln Ile Cys
1505	1510	1515	
Thr Cys	Leu Ser Gly Arg Lys	Val Asn Cys Thr Thr	Gln Pro Cys
1520	1525	1530	
Pro Thr	Ala Lys Ala Pro Thr	Cys Gly Leu Cys Glu	Val Ala Arg
1535	1540	1545	
Leu Arg	Gln Asn Ala Asp Gln	Cys Cys Pro Glu Tyr	Glu Cys Val
1550	1555	1560	
Cys Asp	Pro Val Ser Cys Asp	Leu Pro Pro Val Pro	His Cys Glu
1565	1570	1575	
Arg Gly	Leu Gln Pro Thr Leu	Thr Asn Pro Gly Glu	Cys Arg Pro
1580	1585	1590	
Asn Phe	Thr Cys Ala Cys Arg	Lys Glu Glu Cys Lys	Arg Val Ser
1595	1600	1605	
Pro Pro	Ser Cys Pro Pro His	Arg Leu Pro Thr Leu	Arg Lys Thr
1610	1615	1620	
Gln Cys	Cys Asp Glu Tyr Glu	Cys Ala Cys Asn Cys	Val Asn Ser
1625	1630	1635	
Thr Val	Ser Cys Pro Leu Gly	Tyr Leu Ala Ser Thr	Ala Thr Asn
1640	1645	1650	
Asp Cys	Gly Cys Thr Thr Thr	Thr Cys Leu Pro Asp	Lys Val Cys
1655	1660	1665	
Val His	Arg Ser Thr Ile Tyr	Pro Val Gly Gln Phe	Trp Glu Glu
1670	1675	1680	

FIG. 6 (Cont.)

Gly Cys Asp Val Cys Thr Cys Thr Asp Met Glu Asp Ala Val Met	1685	1690	1695
Gly Leu Arg Val Ala Gln Cys Ser Gln Lys Pro Cys Glu Asp Ser	1700	1705	1710
Cys Arg Ser Gly Phe Thr Tyr Val Leu His Glu Gly Glu Cys Cys	1715	1720	1725
Gly Arg Cys Leu Pro Ser Ala Cys Glu Val Val Thr Gly Ser Pro	1730	1735	1740
Arg Gly Asp Ser Gln Ser Ser Trp Lys Ser Val Gly Ser Gln Trp	1745	1750	1755
Ala Ser Pro Glu Asn Pro Cys Leu Ile Asn Glu Cys Val Arg Val	1760	1765	1770
Lys Glu Glu Val Phe Ile Gln Gln Arg Asn Val Ser Cys Pro Gln	1775	1780	1785
Leu Glu Val Pro Val Cys Pro Ser Gly Phe Gln Leu Ser Cys Lys	1790	1795	1800
Thr Ser Ala Cys Cys Pro Ser Cys Arg Cys Glu Arg Met Glu Ala	1805	1810	1815
Cys Met Leu Asn Gly Thr Val Ile Gly Pro Gly Lys Thr Val Met	1820	1825	1830
Ile Asp Val Cys Thr Thr Cys Arg Cys Met Val Gln Val Gly Val	1835	1840	1845
Ile Ser Gly Phe Lys Leu Glu Cys Arg Lys Thr Thr Cys Asn Pro	1850	1855	1860
Cys Pro Leu Gly Tyr Lys Glu Glu Asn Asn Thr Gly Glu Cys Cys	1865	1870	1875
Gly Arg Cys Leu Pro Thr Ala Cys Thr Ile Gln Leu Arg Gly Gly	1880	1885	1890
Gln Ile Met Thr Leu Lys Arg Asp Glu Thr Leu Gln Asp Gly Cys	1895	1900	1905
Asp Thr His Phe Cys Lys Val Asn Glu Arg Gly Glu Tyr Phe Trp	1910	1915	1920

FIG. 6 (Cont.)

Glu	Lys	Arg	Val	Thr	Gly	Cys	Pro	Pro	Phe	Asp	Glu	His	Lys	Cys
1925						1930					1935			
Leu	Ala	Glu	Gly	Gly	Lys	Ile	Met	Lys	Ile	Pro	Gly	Thr	Cys	Cys
1940						1945					1950			
Asp	Thr	Cys	Glu	Glu	Pro	Glu	Cys	Asn	Asp	Ile	Thr	Ala	Arg	Leu
1955						1960					1965			
Gln	Tyr	Val	Lys	Val	Gly	Ser	Cys	Lys	Ser	Glu	Val	Glu	Val	Asp
1970						1975					1980			
Ile	His	Tyr	Cys	Gln	Gly	Lys	Cys	Ala	Ser	Lys	Ala	Met	Tyr	Ser
1985						1990					1995			
Ile	Asp	Ile	Asn	Asp	Val	Gln	Asp	Gln	Cys	Ser	Cys	Cys	Ser	Pro
2000						2005					2010			
Thr	Arg	Thr	Glu	Pro	Met	Gln	Val	Ala	Leu	His	Cys	Thr	Asn	Gly
2015						2020					2025			
Ser	Val	Val	Tyr	His	Glu	Val	Leu	Asn	Ala	Met	Glu	Cys	Lys	Cys
2030						2035					2040			
Ser	Pro	Arg	Lys	Cys	Ser	Lys								
2045						2050								

FIG. 6 (Cont.)