

COMPOSITIONS AND METHODS FOR TREATMENT OF BLEEDING DISORDERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Nos.: 63/117,545, filed on November 24, 2020; 63/155,012, filed on March 01, 2021; and 63/216,601, filed on June 30, 2021; the contents of each of which are incorporated herein by reference in their entirety.

REFERENCE TO THE SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 20591009PCTSEQLST.txt, created on November 24, 2021, which is 40,029 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure relates to agents, compositions and methods for treatment of bleeding disorders such as hereditary bleeding disorders (e.g., hemophilia A and von Willebrand disease (VWD)). The methods use agents binding to VWF and pharmaceutical compositions and formulations thereof.

BACKGROUND OF THE DISCLOSURE

[0004] Bleeding disorders are a heterogeneous group of conditions in which the blood cannot properly coagulate. As a result, patients with bleeding disorders will experience extensive bleeding after injury, trauma or surgery, etc. Some patients could develop severe, and spontaneous bleeding. Bleeding disorders can be inherited or acquired. Inherited bleeding disorders are often caused by deficiencies in factors involved on blood coagulation (i.e., coagulation factors; also known as clotting factors), and abnormalities of blood vessels and platelets. Hereditary bleeding disorders caused by deficient coagulation proteins include hemophilia A and B, von Willebrand Disease (VWD) Type 1, Type 2 (including subtypes 2a, 2b, 2m and 2n), and Type 3 and other rare bleeding disorders. Platelet-caused bleeding disorders include the inherited thrombocytopenia with less platelet counts Bernard-Soulier syndrome and Glanzmann's thrombasthenia.

[0005] Blood coagulation is a complex process including the sequential interaction of a series of components, in particular of fibrinogen, Factor II (FII), Factor V(FV), Factor VII (FVII), Factor VIII (FVIII), Factor IX (FIX), Factor X (FX), Factor XI (FXI), Factor XII (FXII) and von Willebrand Factor (VWF). The clotting components interact with platelet in the blood to maintain normal hemostasis.

[0006] Deficiencies in any of the clotting components and/or disruptions of the regulation between clotting components and platelet could result in bleeding disorders. Genetic defects in clotting factors, e.g., mutations in the genes encoding these factors cause rare hereditary bleeding, e.g., hemophilia. Hemophilia is an X-linked inherited bleeding disorder with a frequency of about one in 10,000 births. Hemophilia is caused by a deficiency of coagulation factor VIII (FVIII) (hemophilia A) or factor IX (FIX) (hemophilia B) (e.g., reviewed by Samuelson et al., *Blood Rev.*, 2019, 35:43-50). Mutations in the gene coding FVIII can result in hemophilia A. The clinical presentation for hemophilia A is usually characterized by episodes of spontaneous and prolonged bleedings, ranging from mild, moderate to severe.

[0007] Von Willebrand factor (VWF), a large plasma glycoprotein, circulates in plasma and plays essential roles in normal hemostasis. VWF mediates platelet adhesion to exposed sub-endothelial collagen at sites of vascular injury to facilitate platelet mediated plug formation. VWF also performs its hemostatic functions through binding to Factor VIII (FVIII) and to platelets surface glycoproteins, and to localize FVIII to sites of platelet plug and subsequent clot formation. VWF complexed with FVIII also stabilizes the FVIII protein in plasma and protects it from proteolytic degradation by activated protein C in the circulation. The FVIII-VWF complex therefore extends the circulatory life of FVIII.

[0008] Deficiencies and/or defects of VWF can result in von Willebrand disease (VWD). The most common symptoms of VWD include mucocutaneous bleeding, hematomas, and bleeding after trauma or surgery, similar to hemophilia A due to the rapid degradation FVIII caused by lacking VWF cofactor. VWD is the most common inherited bleeding disorder with an estimated prevalence of ~1%. Clinically relevant bleeding symptoms are present in approximately 1:10,000 individuals. VWD can be caused by a quantitative and/or qualitative defect in VWF. Quantitative deficiencies of VWF are often associated with severe VWD Type 1 and Type 3. VWD Type 2 is usually caused by qualitative defects in VWF (i.e., functional defects in VWF). For example, VWD Type 2b is characterised by an increased binding affinity of VWF to platelet glycoprotein Ib, which leads to consumption of VWF and thrombocytopenia in some VWD patients with a resulting severe bleeding phenotype.

[0009] Currently hemophilia A is treated with protein replacement therapy using either plasma derived or recombinant FVIII. Although FVIII replacement/substitute could markedly improve the life of patients suffering from hemophilia, hemophilia patients are still at risk for severe bleeding episodes and chronic joint damage, since prophylactic treatment is restricted by the short half-life, the limited availability, and the high cost of purified FVIII proteins. Treatment and prevention of bleedings in VWD patients focus on increasing plasma VWF and FVIII levels to adequate hemostatic levels. Current treatments include stimulation of the release of endogenous VWF by administration of desmopressin (DDAVP) and infusion of VWF-containing factor concentrates (e.g., VWF/FVIII concentrates) or recombinant VWF preparations. Choice of treatment is dependent on the type of disease and the severity of the bleeding.

[0010] The present disclosure provides VWF targeting agents to treat bleeding disorders, in particular hemophilia A, including mild, moderate and severe hemophilia, and von Willebrand disease (VWD) Type 1, Type 2 (e.g., Type 2a, Type 2b, Type 2m and Type 2n) and Type 3. The VWF targeting agents include VWF binding aptamers and variants thereof.

SUMMARY OF THE DISCLOSURE

[0011] In one aspect of the present disclosure, methods for treating a bleeding disorder in a patient are provided. The methods comprise administering to the patient a pharmaceutically efficient amount of a composition comprising a VWF targeting agent.

[0012] In accordance with the present disclosure, the patient is diagnosed with a bleeding disorder. The bleeding disorder is a hereditary bleeding disorder, including hemophilia A (e.g., mild, moderate or severe hemophilia), and VWD (e.g., VWD Type 1, VWD Type 2a, Type 2b, Type 2m and Type 2n, and VWD Type 3), inherited thrombocytopenia and a rare bleeding disorder. The bleeding disorder may also be acquired, e.g., inhibitor induced thrombocytopenia. In one embodiment, the patient is diagnosed with hemophilia A. The patient may have mild hemophilia, moderate hemophilia, or severe hemophilia. In another embodiment, the patient is diagnosed with VWD Type 1, Type 2 such as Type 2a, Type 2b and Type 2n, or Type 3.

[0013] In some embodiments, the VWF targeting agent is a VWF binding agent that binds to VWF. The VWF targeting agent is an antibody, a nanobody, a peptide, an oligonucleotide, an RNA (e.g., siRNA, microRNA), a synthetic polynucleotide (e.g., an aptamer), or a small molecule.

[0014] In some embodiments, the VWF binding agent is a synthetic polynucleotide selected from SEQ ID No.: 3, BT99 (SEQ ID No.: 4), BT100 (SEQ ID No.: 5), BT200 (SEQ ID No.: 6), ARC15105 (SEQ ID No.: 7), ARC1779 (SEQ ID No.: 8) or a variant thereof.

[0015] In some embodiments, the patient having a bleeding disorder may receive a single dose, or multiple doses of the VWF targeting agent. In one embodiment, the patient receives multiple doses of the VWF binding agent.

[0016] In one preferred embodiment, the VWF targeting agent is BT200, a PEGylated aptamer that specifically binds to the A1 domain of human VWF. In one embodiment, BT200 is administered at a dose ranging from 1.0mg to 10.0 mg, or from 1.0mg to 6.0mg.

[0017] In another aspect, the present disclosure provides methods for increasing the circulatory level of VWF in a blood system of a subject. The method comprises administering to the subject a composition comprising a VWF targeting agent. The VWF targeting agent binds to VWF and increase the VWF levels in the circulation. In some embodiments, the VWF targeting agent is a VWF binding agent that binds to VWF. As non-limiting examples, the VWF binding agent is a synthetic polynucleotide selected from SEQ ID No.: 3, BT99 (SEQ ID No.: 4), BT100 (SEQ ID No.: 5), BT200 (SEQ ID No.: 6), ARC15105 (SEQ ID No.: 7), ARC1779 (SEQ ID No.: 8) or a variant thereof. In one preferred embodiment, the VWF target agent is BT200, a PEGylated aptamer that specifically binds to the A1 domain of human VWF. BT200 is administered at a dose ranging from 1.0mg to 10.0 mg, or from 1.0mg to 6.0mg. In some embodiments, the subject is diagnosed with VWD, e.g., VWD Type 1, VWD Type 2 including Type 2a, Type 2b and Type 2n, and VWD Type 3. In other embodiments, the subject is diagnosed with VWD including Type 1, Type 2 and Type 3 and receives a factor replacement treatment.

[0018] In yet another aspect, the present disclosure provides methods for extending the circulatory life of FVIII in a blood system of a subject. The method comprises administering to the subject a composition comprising a VWF targeting agent. The VWF targeting agent binds to VWF and increases the FVIII levels in the circulation. In some embodiments, the VWF targeting agent is a VWF binding agent that binds to VWF. As non-limiting examples, the VWF binding agent is a synthetic polynucleotide selected from SEQ ID No.: 3, BT99 (SEQ ID No.: 4), BT100 (SEQ ID No.: 5), BT200 (SEQ ID No.: 6), ARC15105 (SEQ ID No.: 7), ARC1779 (SEQ ID No.: 8) or a variant thereof. In one preferred embodiment, the VWF target agent is BT200, a PEGylated aptamer that specifically binds to the A1 domain of human VWF. BT200 is administered at a dose ranging from 1.0mg to 10.0 mg, or from

1.0mg to 6.0mg. In some embodiments, the subject is diagnosed with hemophilia A, e.g., mild, moderate or severe hemophilia. In some examples, the subject is diagnosed with hemophilia A and receives a factor replacement treatment. In other embodiments, the subject is diagnosed with VWD Type 1, or VWD Type 2 (including Type 2a, Type 2b and Type 2n), or VWD Type 3. In some examples, the subject is diagnosed with VWD and receives a VWF replacement treatment.

BRIEF DESCRIPTION OF DRAWINGS

[0019] Figure 1 displays additive effects on the levels of VWF and FVIII by coadministration of BT200 and desmopressin in healthy volunteers.

[0020] Figure 2 is a histogram demonstrating platelet counts in patients with VWD Type 2b, after subcutaneous injection of BT200.

[0021] Figure 3 displays increased FVIII levels and activity (aPTT FS) in patients with VWD Type2b after subcutaneous injection of BT200.

[0022] Figure 4A shows increased VWF levels after subcutaneous injection of BT200; Figure 4B shows increased VWF activity after subcutaneous injection of BT200 in patients with VWD Type 2b.

[0023] Figure 5 shows increased Factor VIII activity (%) after BT200 prophylactic treatment in patients with mild hemophilia A.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0024] The details of one or more embodiments of the disclosure are set forth in the accompanying description below. Although any materials and methods similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred materials and methods are now described. Other features, objects and advantages of the disclosure will be apparent from the description. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the case of conflict, the present description will control.

[0025] The present disclosure relates to methods, agents, and pharmaceutical compositions and formulations thereof, for treating a bleeding disorder such as an inherited bleeding disorder (e.g., hemophilia A and VWD). A method for treating a bleeding disorder

in a patient comprises administering to the patient a therapeutically effective amount of a pharmaceutical composition comprising an agent targeting to von Willebrand factor (VWF).

[0026] Particularly the agents herein relate to any molecules that can target to VWF in the blood. The VWF targeting agents can bind to VWF to mediate VWF hemostatic function. A VWF targeting agent may be an antibody, a nanobody, a peptide, an oligonucleotide, an RNA (e.g., siRNA, microRNA), an aptamer and a variant thereof, or a small molecule. In accordance with the present disclosure, the VWF targeting agent is a synthetic polynucleotide derived from an aptamer that specifically binds to VWF. The targeting agent may be further modified with one or more chemical modifications and conjugates. The agent and VWF complexes protect circulating VWF from clearance, thereby increasing the levels of VWF and FVIII in the blood to increase clotting.

Definitions

[0027] To more clearly and concisely describe the subject matter of the claimed disclosure, the following definitions are provided for specific terms, which are used in the following description and the appended claims. Throughout the specification, exemplification of specific terms should be considered as non-limiting examples.

[0028] *Pharmaceutical composition*: As used herein, the term “pharmaceutical composition” is sometimes used interchangeably with the term “pharmaceutical formulation”. It refers to the combination of an active compound with a pharmaceutically acceptable carrier or excipient, inert or active, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*. In the context of the present disclosure, the active compound may be one or more compound that can be used to treat a bleeding disorder.

[0029] *Pharmaceutically acceptable excipient*: As used herein, the term “pharmaceutically acceptable excipient” means a carrier or excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes a carrier or excipient that is acceptable for veterinary use as well as human pharmaceutical use. A “pharmaceutically acceptable carrier or excipient” as used in the specification and claims includes both one and more than one such carrier or excipient. As used herein, the term “pharmaceutically acceptable carrier” encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. In some examples, the compositions and formulations also can include

stabilizers and preservatives. The term “pharmaceutically acceptable” refers to molecular entities and compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a human. Preferably, as used herein, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans, or generally recognized as safe for use in parenteral products.

[0030] *Therapeutically effective amount:* As used herein, the term “therapeutically effective amount” refers to the amount of the compound that is sufficient to result in a therapeutic response. In connection with the present disclosure, the term “therapeutically effective amount” may refer to the amount of an anti-VWF aptamer that is sufficient to result in a therapeutic response. A therapeutic response may be any response that a user (e.g., a clinician) will recognize as an effective response to the therapy. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the particular therapeutic agent, its mode and/or route of administration, and the like. It will be understood, however, that the total daily usage of the compounds and compositions of the present disclosure can be decided by an attending physician within the scope of sound medical judgment.

[0031] *Preventing, prevention or prevent:* As used herein, the terms “prevent,” “preventing,” and “prevention” and grammatical variations thereof are used interchangeably. These terms refer to a method of partially or completely delaying or precluding the onset or recurrence of a disorder or conditions and/or one or more of its attendant symptoms or barring a subject from acquiring or reacquiring a disorder or condition or reducing a subject's risk of acquiring or reacquiring a disorder or condition or one or more of its attendant symptoms.

[0032] *Treating, treatment or treat:* As used herein, the terms “treating,” “treatment” and “to treat” and grammatical variations thereof, refer to administering to a subject an effective amount of a pharmaceutical composition, such that at least one symptom of a disease is reversed, cured, alleviated, improved, reduced, decreased, or reaches beneficial or desired clinical results such as diminishment of extent of disease, stabilized (e.g., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (partial or total; and detectable or undetectable).

[0033] *Subject*: As used herein, the term “subject” is used interchangeably with the terms “individual” and “patient”, and refers to a vertebrate, preferably a mammal, more preferably a human.

[0034] *Injection*: As used herein, the terms “injection” or “injectable formulation” refer to a composition that can be drawn into a syringe and injected subcutaneously, intraperitoneally, or intramuscularly into a subject (e.g., human).

[0035] *Parenteral administration*: As used herein, the term “parenteral administration” of a pharmaceutical formulation means administration to a subject by a route other than topical or oral (i.e., a non-topical and non-oral route). Examples of parenteral routes include subcutaneous, intramuscular, intravascular (including intraarterial or intravenous), intraperitoneal, intraorbital, retrobulbar, peribulbar, intranasal, intrapulmonary, intrathecal, intraventricular, intraspinal, intracisternal, intracapsular, intrasternal or intralesional administration. Parenteral administration may be, e.g., by bolus injection or continuous infusion, either constant or intermittent and/or pulsatile, and may be via a needle or via a catheter or other tubing.

[0036] *Subcutaneous administration*: As used herein, the term “subcutaneous administration” refers to a common route of administration of a pharmaceutical composition, including but not limited to subcutaneous injection and infusion. The infusion may be continuous or in spurts using infusion pumps or any other commercially available devices.

Hemostasis and Clotting Components

[0037] Blood coagulation is a complex and dynamic biological process involving the sequential interactions of clotting components including coagulation factors: Factor II (FII), Factor V(FV), Factor VII (FVII), Factor VIII (FVIII), Factor IX (FIX), Factor X (FX), Factor XI (FXI), Factor XII (FXII) and cofactor, von Willebrand Factor (VWF).

[0038] Factor VIII (FVIII) (also known as anti-hemophilic factor A), a large, plasma glycoprotein, is a key component of the fluid phase blood coagulation cascade. FVIII is primarily produced by hepatocytes and vascular endothelial cells. Human FVIII is one of the largest coagulation factors including three A- domains, a unique B-domain, and two C- domains and activated via proteolytic cleavage by FXa and thrombin to generate the activated FVIII heterotrimer (FVIIIa). FVIIIa acts as a non-enzymatic cofactor for the prothrombinase and tenase complex in the coagulation cascade that accelerates FX activation in the presence of FIXa, phospholipids and calcium ions (Fay et al., *Blood Reviews*, 2004, 18:

1-15). The half-life of FVIII is about 12 hours. To avoid excessive coagulation, FVIIIa must be inactivated soon after activation, by Protein C (APC) mediated cleavage. The inactivation of FVIIIa is a rapid process, which explains the short half-life of FVIIIa in the blood. As used herein, the terms "Factor VIII(a)" and "FVIII(a)" include both FVIII and FVIIIa. Similarly, the term "Factor VIII" and "FVIII" may include both FVIII and FVIIIa.

[0039] FVIII present in plasma is in association with von Willebrand factor (VWF), forming a noncovalent FVIII-VWF complex. Von Willebrand factor (VWF) is a large, multimeric glycoprotein and plays a key role in hemostasis and thrombosis. Human VWF preproprotein (GeneBank Ref. No. NP_000543.2; SEQ ID No.: 1) (encoded by cDNA: GeneBank Ref. No. NM_000552.3; SEQ ID No.: 2) is processed to be a mature polypeptide comprising multiple subdomains with different functions (Hassan and Saxena, *Blood Coagul. Fibrinolysis*, 2012, 23(1):11-22). VWF is the carrier protein for FVIII, binding FVIII to platelets surface glycoproteins and localizing FVIII to sites of platelet plug and subsequent clot formation. VWF also acts as a stabilizer of FVIII in the circulation by the formation of the non-covalently bound VWF-FVIII complex that protects FVIII from degradation by activated protein C (APC), thereby preventing FVIII from premature proteolysis (Koppelman et al., *Blood*, 1996; 87:2292-2300). In addition, VWF blocks the interaction of FVIII with lipoprotein-related receptors and thereby increases the half-life of FVIII in the circulation.

[0040] Furthermore, the interaction between VWF and FVIII plays a crucial role in FVIII function, immunogenicity, and clearance, with VWF essentially serving as a chaperone for FVIII. VWF has an important protective role for FVIII both under normal physiological conditions and in patients with hemophilia. In hemophilia patients who have developed FVIII inhibitors (e.g., antibodies to FVIII substitute) after treatment with FVIII replacement therapy, VWF may protect exogenous FVIII from the binding of inhibitory antibodies (Gensana et al., *Hemophilia*, 2001; 7:369-374).

[0041] Plasma levels of VWF can affect risk of bleeding (lower level of VWF) or thrombosis (higher level of VWF). Quantitative deficiencies (low levels) of plasma VWF (e.g., <50%) are associated with an increased risk for bleeding, while high plasma levels of VWF (e.g., >150%) increase the risk for thrombosis (e.g., higher risk for venous thromboembolic disease, ischemic stroke, coronary artery disease, myocardial infarction and peripheral vascular disease).

Bleeding and Bleeding Disorders

[0042] A deficiency in the coagulation process may increase the risk of bleeding. For example, coagulation factor deficiencies and defects in platelet number or function cause bleeding disorders. Bleeding can occur inside the body (internal bleeding) or underneath the skin or from the surface of the skin (external bleeding).

[0043] As used herein, the term “bleeding disorders” refer to a heterogeneous group of conditions that result when the blood cannot clot properly. In normal clotting, platelets stick together and form a plug at the site of an injured blood vessel. Coagulation factors in the blood interact to form a fibrin clot, essentially a gel plug, which holds the platelets in place and allows healing to occur at the site of the injury while preventing blood from escaping the blood vessel. The inability to form clots can be very dangerous, resulting in excessive bleeding. Bleeding can result from either too few or abnormal platelets, abnormal or low amounts of coagulation factors, dysfunctional coagulation factors (e.g., mutations in the genes encoding coagulation factors), or abnormal blood vessels. The bleeding severity can be assessed according to guidelines such as the assessment tool reviewed by Rodeghiero et al., ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. *J Thromb Haemost.* Sep 2010;8(9):2063-5. doi:10.1111/j.1538-7836.2010.03975.x). A person with a bleeding disorder can have both internal and external bleeds. Common symptoms of a bleeding disorder include but not limited to, extended bleeding after injury, surgery, trauma, or menstruation; excessive bruising; bleeding into joints, muscles and soft tissues; gastrointestinal bleeding; spontaneous bleeding without a known or identifiable cause.

[0044] Bleeding disorders can be hereditary or acquired. As used herein, the term “hereditary bleeding disorders” (also called “congenital bleeding disorders”) refers to a group of rare disorders caused by genetic deficiency of clotting components. The most common bleeding disorders include hemophilia (e.g., hemophilia A and B) and VWD (e.g., Types 1, 2 and 3). Hemophilia A or Hemophilia B is a rare, hereditary bleeding disorder that can range from mild, moderate to severe, depending on how much the residual factor activity is present in a patient’s plasma. The incidence of hemophilia A is ~1 in 5000 live male births and that of hemophilia B is 1 in 25 000 live male births. Collectively, they are among the most common inherited bleeding disorders in the world. Von Willebrand disease (VWD) is the most common inherited bleeding disorder in America affecting up to 1% of the US population.

[0045] Bleeding may also result from a low platelet (thrombocyte) count in the blood, a condition called thrombocytopenia. A normal platelet count in adults ranges from 150,000 to 450,000 platelets per microliter of blood. A platelet count of less than 150,000 platelets per microliter is lower than normal (thrombocytopenia). The risk for serious bleeding does not occur until the count becomes very low—less than 10,000 or 20,000 platelets per microliter. When the count is less than 50,000 platelets per microliter, mild bleeding sometimes occurs. If the count is very low, less than 10,000 or 20,000 platelets per microliter, the risk for serious bleeding may occur. In rare cases, the number of platelets can be so low that dangerous internal bleeding occurs. Thrombocytopenia may occur in various conditions, such as the inherited bleeding disorders and acquired conditions (e.g., a side effect from medications). A low platelet count (thrombocytopenia) occurs may be due to not enough platelets made by bone marrow, deficiency in maintaining enough platelets in the blood, and/or abnormality in spleen which holds on too many platelets.

[0046] Conditions that cause a low platelet count (thrombocytopenia) include, for example, thrombotic thrombocytopenic purpura (TTP); disseminated intravascular coagulation (DIC); immune thrombocytopenia (ITP); an autoimmune disease; an enlarged spleen condition caused by cancer, severe liver disease, and a bone marrow deficiency; a viral or bacterial infection; and a side reaction to medicine.

Hemophilia A

[0047] Classic hemophilia or hemophilia A (also known as Factor FVIII deficiency) is an inherited bleeding disorder caused by a chromosome X-linked deficiency of FVIII and affects almost exclusively males. A mutation in the gene coding FVIII (i.e., the F8 gene) results in hemophilia A. The genetic mutations could cause absence or decreased synthesis of FVIII or abnormal protein synthesis (Hong et al., *Thrombosis Res.*, 2007, 119:1-13). The X-chromosome defect is transmitted by female carriers who are not themselves hemophiliacs. Due to random chromosome activation, some women carriers may range from asymptomatic to symptomatic depending on how much of their FVIII is inactivated.

[0048] Because blood does not clot properly without enough FVIII, the clinical manifestation of hemophilia A is an increased bleeding tendency, e.g., a small cut or injury carrying the risk of excessive bleeding. In addition, people with hemophilia may suffer from internal bleeding that can damage joints (including the knee, ankle, elbow and hip joints), muscles, organs, and tissues (e.g., soft tissue under the skin) over time. Hemophilia bleeding

intensity depends on the level of FVIII deficiency. There are three forms of hemophilia A: mild, moderate or severe in individual patients (Table 1), which are determined based on deficient plasma FVIII (IU) (Bolton-Maggs and Pasi, *Lancet*, 2003, 361: 1801-1809).

Table 1: Three forms of hemophilia

Form of hemophilia	FVIII activity (IU/ml)	Main symptoms
mild	>0.05 but <0.50 (>5-50% of normal range)	Excessive bleeding after injuries, trauma, accidents, tooth extractions, surgeries
moderate	0.01-0.05 (1-5% of normal range)	Joint and muscle bleedings following mild trauma; excessive bleeding after injuries, trauma, accidents, tooth extractions, surgeries
severe	<0.01 (<1% of normal range)	Spontaneous joint and muscle bleedings; excessive bleeding after injuries, trauma, accidents, tooth extractions, surgeries

*IU: international unit

[0049] With regard to mild inherited bleeding disorders, bleeding symptoms also occur in otherwise healthy individuals. Although patients with mild bleeding disorders may not often suffer from bleeding in daily life, problems may occur after a hemostatic challenge, including but not limited to trauma, dental extractions and surgery.

[0050] The treatment of hemophilia A mainly focuses on increasing blood activity of deficient coagulation factor VIII with factor substitute, in order to inhibit and/or prevent active bleedings in patients. Current available therapeutics include human plasma derived lyophilized FVIII concentrates and recombinant coagulation factors produced by genetically engineered cells. FVIII concentrates have a short half-life in plasma, with an average of about 12 hours in adults, ranging in individual patients with hemophilia A between 6 hours and 29 hours, and even shorter in younger children. FVIII substitute treatment for hemophilia often requires frequent intravenous injections of therapeutics.

[0051] For patients with severe hemophilia A, an increased amount and dosing intervals are required for prophylactic treatment.

[0052] Several technologies are developing to extend the half-life of FVIII in the blood. As the vast majority of plasma FVIII circulates in a high-affinity complex with VWF, FVIII is mostly cleared while not coupled to VWF. It has been supported by studies showing that FVIII in hemophilia patients is significantly influenced by plasma VWF levels. For example, Valentino has shown that the FVIII half-life is significantly longer in patients with elevated VWF levels (Valentino et al., *Haemophilia*, 2014, 20: 607-615). Targeting VWF chaperon to

increase plasma FVIII-VWF complex levels may provide alternative strategies to extend half-life of FVIII in plasma.

[0053] In accordance with the present disclosure, VWF targeting agents can bind to VWF and extend the half-life of VWF, thereby extending the half-life of FVIII.

von Willebrand's disease (VWD)

[0054] Although deficiencies of Factor VIII (hemophilia A) and Factor IX (hemophilia B) are well recognized, von Willebrand disease (VWD) is much more common. Deficiencies in VWF (e.g., quantitatively reduced, functional defect, or completely missing) lead to different types of VWD. VWD can affect both males and females. Conversely, abnormally elevated VWF concentrations or function can also cause severe medical disorders like venous thromboembolic disease (VTE). VWD is classified into three different types (Types 1, 2, and 3), based on the levels of VWF and FVIII activity in the blood.

[0055] VWD Type 3 is the most severe and least common form with complete deficiency of VWF. Patients with Type 3 VWD have little or no VWF in their blood. The amount of FVIII in the blood also drops to low levels without VWF to act as a carrier. Type 3 VWD patients have trouble making both a platelet plug and a fibrin clot. VWD Type 3 patients have spontaneous bleedings into their joints and muscles, frequent bleeding from their noses and mouths. Women with Type 3 VWD may have long menstrual periods with very heavy bleeding.

[0056] VWD Type 2 relates to qualitative defects of VWF and can be as severe as VWD Type 3 in some patients, which include four subtypes: Types 2a, 2b, 2m and 2n. Type 2a is caused by defects in VWF multimerization due to the wrong size of the VWF protein. The abnormal VWF multimers stop the platelets from making a good platelet plug. In Type 2b, the VWF protein is abnormally active with variants of spontaneous platelet binding. The attachment of VWF causes the body to quickly get rid of the platelets, which causes a shortage of both platelets and VWF in the blood. Type 2m is caused by VWF defects in ligand binding with intact multimers. In Type 2n, VWF has defects in FVIII binding, failing to act as the carrier and protector of FVIII. The level of FVIII in the blood is low because of lacking enough VWF to keep it from being degradation. A Type 2n VWD patient can appear to have mild hemophilia with some of the same symptoms. VWD Type 2b in particular results from a mutation in the A1 domain of VWF causing constitutive activation of A1

domain binding to the GPIb receptor on platelets, leading to a consumptive deficiency of VWF, FVIII, and platelets.

[0057] VWD Type 1 is the mildest and most common form accounting for up to 85% of VWD (reviewed by Robertson et al., *Pediatr Clin North Am.*, 2008, 55(2): 377-392), which relates to a quantitative loss of VWF but with qualitatively normal VWF. Because there is not enough VWF in the blood, a lower level of FVIII may be seen in Type 1. A small number of patients with Type 1 VWD can have severe bleedings. VWD Type 1 is a very heterogeneous family of genetic defects representing 85 unique SNPs at last count and has only partial deficiency of VWF. For example, type Vicenza variant of VWD Type 1 is characterized by a low plasma VWF level and supranormal VWF multimers, caused by increased rate of VWF clearance (Alessandra et al., *Blood* 2002, 99(1): 180-184).

Other rare bleeding disorders

[0058] Deficiencies in other coagulation factors could cause rare bleeding disorders, e.g., rare factor deficiencies including Factor I, II, V, VII, X, XI, XII and XIII deficiency. In addition to hereditary disorders, other bleeding disorders may be acquired, e.g., platelet disorders which are the most common cause of acquired bleeding disorder.

[0059] Currently, the standard treatment of Hemophilia A and VWD involves frequent intravenous infusions of FVIII and VWF preparations or concentrates comprising a complex of FVIII and VWF derived from the plasmas of human donors, recombinant FVIII preparations, or recombinant VWF preparations. In severe hemophilia A patients, FVIII injection is used for prophylactic treatment. Because of the short plasma half-life of FVIII, patients have to be administered intravenously about 3 times per week. VWD can be treated by replacement therapy with concentrates containing VWF of plasmatic or recombinant origin. One approach to increase functional half-life of VWF is by PEGylation of VWF, which pegylated VWF by having an increased half-life would indirectly also enhance the half-life of FVIII present in plasma (PCT Application Publication No.: WO2006/071801; the contents of which are incorporated by reference in their entirety.) PEGylation of VWF antigen at specific sites can protect VWF from macrophage-mediated clearance in plasma (Fazavaza et al., *J. Thromb. Haemost.*, 2020; 18:1278-1290; the contents of which are incorporated herein by reference in their entirety).

[0060] It is highly desirable to create new treatments that can increase functional half-life of FVIII and VWF in patients with bleeding disorders (e.g., hemophilia and VWD), while

drugs can be administered less frequently or by other less cumbersome and less painful means of administration.

[0061] The inventors of the present disclosure surprisingly found that a VWF targeting aptamer that is modified with PEG conjugation can increase the VWF and FVIII levels in the blood after a single dose treatment. The VWF targeting nucleic acid does not interfere the functionality of FVIII in plasma at any concentration, and at relatively low concentrations does not interfere with the function of VWF in plasma.

[0062] In accordance with the present disclosure, a VWF targeting agent is provided to bind to VWF in plasma and block its clearance, thereby increasing half-life of the VWF-FVIII complex in the plasma. The VWF targeting agent can be used to treat a hereditary bleeding disease, in particular, hemophilia A and VWD (e.g., Type 1, Vicenza subtype, Type 2 including subtypes 2a, 2b, 2m and 2n, and Type 3).

VWF Targeting Agents

[0063] As used herein, the term “targeting agent” refers to an agent that specifically binds to and interacts with a molecule of interest. A VWF targeting agent is an agent that binds to VWF, directly or indirectly through another agent, to regulate VWF biologic activities (also referred to as “VWF binding agent), e.g., interaction of VWF with FVIII and half-life of VWF and FVIII-VWF complex. In general, the targeting agent may be an antibody, a nanobody, a peptide, an oligonucleotide, an RNA (e.g., siRNA, microRNA), a synthetic polynucleotide (e.g., an aptamer), or a small molecule that can bind to VWF. The VWF targeting agent also includes an agent that binds to VWF, thereby blocking the clearance mechanism. In some embodiments, the VWF targeting agent can be used for treatment of bleeding disorders, particularly hemophilia A and VWD. The VWF targeting agent may also be used for blocking VWF clearance in the blood.

[0064] In some embodiments, the VWF targeting agents may be nucleic acid based, particularly aptamers that bind to VWF, and variants thereof. In some examples, a VWF binding agent is an aptamer or salt thereof. Aptamers are short (i.e., typically 12-80 nucleotides in length) single-stranded nucleic acid polymers that bind with high affinity and specificity to VWF. The aptamer is a synthetic polynucleotide that can be isolated using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process.

Aptamers

[0065] An aptamer is a biomolecule that binds to a specific target molecule and modulates the target's activity, structure, or function. Aptamers often are referred to as "chemical antibodies," having similar characteristics as antibodies. An aptamer can be nucleic acid or amino acid based, i.e., either a nucleic acid aptamer or peptide aptamer. Nucleic acid aptamers have specific binding affinity to target molecules through interactions other than classic Watson-Crick base pairing. Nucleic acid aptamers are capable of specifically binding to selected targets and, through binding, block their targets' ability to function. Aptamers of the present disclosure are synthetic oligonucleotides. A typical nucleic acid aptamer is approximately 10-15 kDa in size, binds its target with sub-nanomolar affinity, and discriminates against closely related targets. A target of a nucleic acid aptamer may be but is not limited to, a protein, a nucleic acid molecule, a peptide, a small molecule and a whole cell.

[0066] Nucleic acid aptamers may be ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or mixed ribonucleic acid and deoxyribonucleic acid (DNA/RNA hybrid). Aptamers may be single stranded. A suitable nucleotide length for an aptamer ranges from about 15 to about 150 nucleotide (nt), and in various other preferred embodiments, 15-30 nt, 20-25 nt, 20-45 nt, 30-100 nt, 30-60 nt, 25-70 nt, 25-60 nt, 40-60 nt, 25-40 nt, 30-40 nt, any of 15, 16, 17, 18, 19, 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 or 50 nt, or 30-50 nt, 40-70 nt, or 50-100 nt in length. However, the sequence can be designed with sufficient flexibility such that it can accommodate interactions of aptamers with targets.

[0067] As used herein, the terms "nucleic acid," "polynucleotide," "oligonucleotide" are used interchangeably. A nucleic acid molecule is a polymer of nucleotides consisting of at least two nucleotides covalently linked together. A nucleic acid molecule is a DNA (deoxyribonucleotide), an RNA (ribonucleotide), as well as a recombinant RNA and DNA molecule or an analogue of DNA or RNA generated using nucleotide analogues. The nucleic acids may be single stranded or double stranded, linear or circular. The term also comprises fragments of nucleic acids, such as naturally occurring RNA or DNA which may be recovered using the extraction methods disclosed, or artificial DNA or RNA molecules that are artificially synthesized *in vitro* (i.e., synthetic polynucleotides). Molecular weights of nucleic acids are also not limited, may be optional in a range from several base pairs (bp) to several hundred base pairs, for example from about 2 nucleotides to about 1,0000

nucleotides, or from about 10 nucleotides to 5,000 nucleotides, or from about 10 nucleotides to about 1,000 nucleotides.

[0068] The term “nucleotide (nt)” refers to the monomer of nucleic acids, a chemical compound comprised of a heterocyclic base, a sugar and one or more phosphate groups. The base is a derivative of purine and pyrimidine and the sugar is a pentose, either deoxyribose or ribose.

[0069] As used herein, the term “modification” refers to the technique of chemically reacting a nucleic acid, e.g., an oligonucleotide, with chemical reagents. A nucleic acid may be modified in the base moiety, sugar moiety or phosphate backbone. The modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine, modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil, backbone modifications, phosphorothioate or alkyl phosphate modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping. The nucleic acid molecule may also be modified by conjugation to a moiety having desired biological properties. Such moiety may include, but is not limited to, compounds, peptides and proteins, carbohydrates, antibodies, enzymes, polymers, drugs and fluorophores. In some examples, the polynucleotide is conjugated to a lipophilic compound such as cholesterol, dialkyl glycerol, diacyl glycerol, or a non-immunogenic, high molecular weight compound or polymer such as PEG (polyethylene glycol) or other water soluble pharmaceutically acceptable polymers including, but not limited to, polyaminoamines (PAMAM) and polysaccharides such as dextran, or polyoxazolines (POZ). The modifications may be intended, for example, to increase the in vivo stability of nucleic acid molecules or to enhance or to mediate delivery of the molecules.

[0070] Aptamers may be either monovalent or multivalent. Aptamers may be monomeric, dimeric, trimeric, tetrameric or other higher multimeric. Individual aptamer monomers may be linked to form multimeric aptamer fusion molecules. As a non-limiting example, a linking oligonucleotide (i.e., linker) may be designed to contain sequences complementary to both 5'-arm and 3'-arm regions of random aptamers to form dimeric aptamers. For trimeric or tetrameric aptamers, a small trimeric or tetrameric (i.e., a Holliday junction-like) DNA nanostructure will be engineered to include sequences complementary to the 3'-arm regions of the random aptamers, therefore creating multimeric aptamer fusion through hybridization.

In addition, 3 to 5 or 5 to 10 dT rich nucleotides can be engineered into the linker polynucleotides as a single stranded region between the aptamer-binding motifs, which offers flexibility and freedom of multiple aptamers to coordinate and synergize multivalent interactions with cellular ligands or receptors. Alternatively, multimeric aptamers can also be formed by mixing biotinylated aptamers with streptavidin.

[0071] As used herein, the term “multimeric aptamer” or “multivalent aptamer” refers to an aptamer that comprises multiple monomeric units, wherein each of the monomeric units can be an aptamer on its own. Multivalent aptamers have multivalent binding characteristics. A multimeric aptamer can be a homomultimer or a heteromultimer. The term “homomultimer” refers to a multimeric aptamer that comprises multiple binding units of the same kind, i.e., each unit binds to the same binding site of the same target molecule. The term “heteromultimer” refers to a multimeric aptamer that comprises multiple binding units of different kinds, i.e., each binding unit binds to a different binding site of the same target molecule, or each binding unit binds to a binding site on different target molecule. Thus, a heteromultimer can refer to a multimeric aptamer that binds to one target molecule at different binding sites or a multimeric aptamer that binds to different target molecules. A heteromultimer that binds to different target molecules can also be referred to as a multi-specific multimer.

[0072] Aptamers can be generated against a target molecule (e.g., VWF) using a process called either *in vitro* selection (*Ellington and Szostak*, Nature, 1990; 346: 818-822) or SELEX (*Tuerk and Gold*, Science, 1990, 249: 505-510). This method allows the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules. The SELEX method is described in, for example, U.S. Pat. No. 7,087,735, U.S. Pat. No. 5,475,096 and U.S. Pat. No. 5,270,163; the contents of each of which are incorporated herein by reference in their entirety. Nucleic acid aptamers can be synthesized using methods well-known in the art. For example, the disclosed aptamers may be synthesized using standard oligonucleotide synthesis technology known in the art.

VWF Aptamers

[0073] In accordance with the present disclosure, VWF targeting agents are polynucleotides, salts thereof, or derivatives thereof, that target VWF to, e.g., regulate the interaction between VWF and FVIII in plasma. In some embodiments, VWF targeting agents

are aptamers specifically binding to VWF. Accordingly, anti-VWF aptamers are also referred to as “VWF binding agents”.

[0074] The synthetic polynucleotide, when binding to VWF, can block the clearance mechanism in the blood, thereby increasing the levels of VWF antigen in the blood. The blockage could increase the level of Factor VIII in the blood as the FVIII-VWF complex is protected from protein degradation.

[0075] In some embodiments, the synthetic polynucleotide binding to VWF may be 15 to 50 nucleotides in length, or 20 to 30 nucleotides in length (e.g., 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, and 30 nucleotides in length). Said synthetic polynucleotide may further comprise a double stranded region. The double stranded region may be from about 6 to about 9 nucleotides. The double stranded region is formed by 6 nucleotides, or 7 nucleotides, or 8 nucleotides, or 9 nucleotides. Furthermore, the double stranded region is formed by 6 or more of the 3' and 5' nucleotides at or near the termini of the sequence.

[0076] In some embodiments, the synthetic polynucleotides may be chemically modified. Each nucleotide may contain at least one chemical modification. Such chemical modification may be at the sugar, nucleobase or inter-nucleoside linker of the synthetic polynucleotide. Modifications to the sugar may comprise but are not limited to 2'-amino, 2'-O-alkyl, 2'-fluoro, and 2'-O-methyl modification. As a non-limiting example, the synthetic polynucleotide targeting to VWF comprises 2'-O-methyl modification. In some embodiments, a terminal cap structure may be incorporated to the 3' and/or 5' termini of the synthetic polynucleotide. The cap structure includes 5'-5' inverted nucleotide cap at the 5' terminus of the synthetic polynucleotide and a 3'-3' inverted nucleotide cap at the 3' terminus of the synthetic polynucleotide. The cap structure may be an inverted deoxythymidine or amino group (NH₂).

[0077] The synthetic polynucleotide may be further modified to comprise one or more conjugates such as a PEG (polyethylene glycol) moiety at the 5' or 3' termini of the synthetic polynucleotides. The PEG moiety may be of any size or branch configuration. PEGs can range in size from about 5kD to about 200 kD. PEGs may be linear chain PEGs or branched PEGs with multiple PEG chains attached together. The 3'-and 5'-short PEG conjugates do not interfere with the binding specificity and they do not influence the target affinity of the synthetic polynucleotides.

[0078] In one embodiment, the VWF binding agent is a synthetic polynucleotide comprising at least 21 contiguous nucleotides of SEQ ID No.: 3 (5'GCCAGGGACCUAAGACACAUGUCCCCUGGC-3'); the sequence is derived from an aptamer that binds specifically to VWF. In one embodiment, the VWF binding agent may be a synthetic polynucleotide comprising at least 21 contiguous nucleotides of SEQ ID No.: 3. Additionally, the synthetic polynucleotide may exhibit a double stranded region having at least 6 nucleotides, or at least 7 nucleotides, or at least 8 nucleotides, or at least 9 nucleotides. In one embodiment, the double stranded region of 6 or more nucleotides is at or near (e.g., within 1-10 nucleotides) the termini of the synthetic polynucleotide.

[0079] In some embodiments, the synthetic polynucleotide comprises an inverted deoxythymidine at the 3' terminus of the sequence. As a non-limiting example, the VWF targeting agent is a synthetic polynucleotide comprising the structure: mGmCmCmAmGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmCmUmGmGmGmC-idT (SEQ ID No.: 4) (BT99), where "NH" is a 5'-hexylamine linker phosphoramidite, "idT" is an inverted deoxythymidine, "mN" is a 2'-O-Methyl containing residue.

[0080] In some embodiments, the synthetic polynucleotide comprises an inverted deoxythymidine at the 3' terminus of the sequence and an amino group (NH₂) at the 5' terminus of the sequence. As a non-limiting example, the VWF targeting agent is a synthetic polynucleotide comprising the structure: NH₂-mGmCmCmAmGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmCmUmGmGmGmC-idT (SEQ ID No.: 5) (BT100), where "NH" is a 5'-hexylamine linker phosphoramidite, "idT" is an inverted deoxythymidine, "mN" is a 2'-O-Methyl containing residue.

[0081] In some embodiments, the synthetic polynucleotide binding to VWF further comprises a PEG moiety conjugated at the 5' terminus of the sequence. As a non-limiting example, the VWF targeting agent is a synthetic polynucleotide comprising the structure: PEG40K-NH-mGmCmCmAmGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmCmUmGmGmGmC-idT (SEQ ID No.: 6) (BT200), where "NH" is a 5'-hexylamine linker phosphoramidite, "idT" is an inverted deoxythymidine, "mN" is a 2'-O-Methyl containing residue and "PEG" is a polyethylene glycol and PEG40K is a PEGylation moiety having a molecular weight of approximately 40KDa.

[0082] The PEGylated BT200 specifically binds to the A1 domain of VWF in plasma, interfering its regulation and functionality (Zhu et al., *J Thromb Haemost.* 2020 May;18(5):1113-1123; the contents of which are incorporated herein by reference in their entirety). One mechanism is that the binding of BT200 to VWF may protect the nucleic acid-protein complex from clearance by inhibiting the interaction of VWF with the macrophage low density lipoprotein receptor related protein-1 (LRP1) (Fazavana et al., *J Thromb Haemost.*, 2020, 1278-1290). In this regard, BT200 can be used as an anti-bleeding agent. The inventors of the present disclosure have found that at low doses, BT200 blocks the clearance of VWF Antigen from the circulation and leads to a sustained increase in concentrations of both VWF antigen (VWF Ag) and FVIII but has a negligible effect on the activity of either. At higher doses, BT200 blocks clearance of VWF and inhibits the activity of VWF but does not inhibit FVIII activity. Therefore, BT200 may be used to correct deficiency of VWF and/or FVIII in patients with hereditary bleeding disorders (e.g., VWD Type 1, Type 2b and Type 3).

[0083] BT200 by subcutaneous injections demonstrates an extended half-life, lasting 7-12 days in the circulation that provides good subcutaneous bioavailability (Zhu et al., *J Thromb Haemost.* 2020, 1113-1123).

[0084] In other embodiments, the VWF binding agents are aptamers comprising the sequence: PEG40K-NH-mGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmC-idT (ARC15105) (SEQ ID No.: 7), or the sequence: PEG20K-NH-mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmGsdTmGdCdGdGdTmGmCdCmUdCdCmGmUdCmAmCmGmCidT (ARC1779) (SEQ ID No.: 8), where "NH" is a 5'-hexylamine linker phosphoramidite, "idT" is an inverted deoxythymidine, "mN" is a 2'-O-Methyl containing residue, "dN" is a deoxynucleotide residue, "sdT" is a phosphorothioate deoxythymidine residue and "PEG" is a polyethylene glycol and PEG20K is a PEGylation moiety having a molecular weight of approximately 20Kda.

[0085] In some embodiments, the VWF binding agent may include a synthetic polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID No.: 3.

Table 2: VWF aptamers and variants

Agent	Sequence (5'-3') and modifications	SEQ ID NO.:
VWF	GCCAGGGACC UAAGACACAUGUCCUGGC	3

aptamer		
BT99	mGmCmCmAmGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmCmUmGmGmC-idT	4
BT100	NH ₂ -mGmCmCmAmGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmCmUmGmGmC-idT	5
BT200	PEG40K-NH-mGmCmCmAmGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmCmUmGmGmC-idT	6
ARC15105	PEG40K-NH-mGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUmGmUmCmCmC-idT	7
ARC1779	PEG20K-NH-mGmCmGmUdGdCdAmGmUmGmCmCmUmUmCmGmGmCdCmGsdTmGdCdGdGdTmGmCdCmUdCdCmGmUdCmAmCmGmCidT	8

Pharmaceutical Compositions

[0086] In another aspect of the present disclosure, pharmaceutical compositions and formulations including any one of anti-VWF aptamers of the present disclosure are provided. The VWF binding agents are formulated in formulations suitable for administration to a subject in need. Formulations which are suitable for the pharmaceutical compositions of the present disclosure, particularly the nucleic acid-based compositions, are taught in International Patent Publication No. WO2013/090648 (Application PCT/US2012/069610), the contents of which are incorporated herein by reference in their entirety. Methods of making formulations are well known in the art, for example, in Remington: The Science and Practice of Pharmacy (20th ed., ed. A.R. Gennaro AR.), *Lippincott Williams & Wilkins*, 2000; the contents of which are incorporated herein by reference in their entirety.

[0087] Pharmaceutical compositions of the present disclosure may comprise at least one VWF targeting agent as active ingredient, e.g., BT200.

[0088] The compositions further include at least one pharmaceutically acceptable carrier, diluent or excipient, e.g., saline or distilled water. Optionally, the compositions may include excipients that stabilize the aptamer agent, thereby maintaining therapeutic activity of the agent. In some embodiments, the compositions may comprise excipients such as salts, sugars and alcohols that facilitate diffusion of the aptamer agent.

[0089] As non-limiting examples, excipients may include saccharides such as sucrose, trehalose, fructose, galactose, mannitol, dextran and glucose; poly-alcohols such as glycerol and sorbitol; proteins such as albumin; hydrophobic molecules such as oils; hydrophilic polymers such as polyethylene glycol; isomers such as diastereomers and enantiomers, mixtures of isomers, including racemic mixtures, salts, solvates, and polymorphs thereof.

[0090] In some embodiments, the composition may be in the form of liquid solutions or suspensions. In some cases, the composition is sterile for injection.

[0091] In some embodiments, the VWF targeting agents of the present disclosure are formulated for oral administration. Formulations may be in the form of tablets or capsules. In some embodiments, the VWF agents of the present disclosure are formulated for intranasal administration. Intranasal formulations may be in the form of powders, nasal drops, or aerosols. In some embodiments, the VWF agents of the present disclosure are formulated for parenteral administration. The formulations include only pharmaceutically acceptable excipients, diluents, carriers and adjuvants that are safe for parenteral administration to humans at the concentrations used, under the same or similar standards as for excipients, diluents, carriers and adjuvants deemed safe by the Federal Drug Administration or other foreign national authorities. The pharmaceutical formulation may be in a ready-to-use solution form, concentrated form, or a lyophilized preparation that may be reconstituted with a directed amount of diluent suitable for parenteral injection such as water, salt solution, or buffer solution. In some examples, the formulation is a stable aqueous pharmaceutical formulation that remains within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life. In some embodiments, the VWF targeting agents of the present disclosure are formulated for inhalation.

[0092] In some embodiments, the VWF targeting agents of the present disclosure may be formulated for controlled release of the active agent. As non-limiting examples, formulations for controlled release may comprise biocompatible polymers. The choice of the polymer depending on the rate of drug release required in a particular treatment regimen. Biocompatible polymers suitable for sustained release include, but are not limited to, biodegradable polymers such as polyamides such as poly(amino acids) and poly(peptides); polyesters such as poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and poly(caprolactone); poly(anhydrides); polyorthoesters; polycarbonates and chemical derivatives thereof; copolymers and mixtures thereof. Biocompatible polymers may include non-degradable polymers such as polysaccharides; polyethers (e.g., poly(ethylene oxide), poly(ethylene glycol), and poly(tetramethylene oxide)); vinyl polymers (e.g., polyacrylates, acrylic acids, poly(vinyl alcohol), poly(vinyl pyrrolidone), and poly(vinyl acetate)); polyurethanes; cellulose-based polymers (e.g., cellulose, alkyl cellulose, hydroxyalkyl cellulose, cellulose ethers, cellulose esters, nitrocellulose, and cellulose acetates); polysiloxanes and other silicone derivatives. The sustained release of the agents may last for more than two months, for one month, for three weeks, for two weeks, for one week, for six days, for five days, for four days, for three days, for two days, or for one day. The amount of

the agents in the compositions for sustained release may comprise about 0.1% to about 30%, or about 0.1% to about 10%, or about 1% to about 10%, or about 0.5% to about 5% (w/w) of the formulation, e.g., about 0.1% (w/w), about 0.2% (w/w), about 0.5% (w/w), about 1.0 % (w/w), about 2.0% (w/w), about 5.0 % (w/w), about 10% (w/w), about 12% (w/w), about 15% (w/w), about 20% (w/w), about 25% (w/w), or about 30% (w/w) of the formulation.

[0093] In some embodiments, the VWF targeting agents are formulated as injectable thermosensitive gel formulations. As used herein, the term “thermosensitive gel formulation” means a formulation that exists as a mobile viscous liquid at low temperatures but forms a rigid semisolid gel at higher temperatures. Specifically, the formulation is liquid at room temperature or at lower temperature, but gels once injected, thus producing a depot of drug at the injection site. The formulations may contain thermo-sensitive polymers. As used herein, the term “gel” refers to the semi-solid phase that spontaneously occurs as the temperature of the thermo-sensitive polymer solution is raised to or above the gelation temperature of the polymer. Exemplary thermo-sensitive polymers may include PLGA-PEG-PLGA triblock copolymer, poloxamer, Pluronic acid F127, and polyoxyethylene-polyoxypropylene (PEO-PPO) block copolymers. In some embodiments, the gelation temperature of the formulation is from about 30°C to about 40°C.

[0094] In some embodiments, the VWF targeting agents may be encapsulated with liposomal formulations. As used herein, the term “liposome” means a formulation consisting of at least one lipid bilayer membrane enclosing an aqueous internal compartment. Liposomes may be small unilamellar vesicles (SUVs) having a single membrane and typically range between 0.02 and 0.05µm in diameter, or large unilamellar vesicles (LUVs) that are typically larger than 0.05µm, or oliglamellar large vesicles having multiple, usually concentric, membrane layers that are typically larger than 0.1µm. Liposomes are formulated to carry the VWF agents contained within the aqueous interior space or partitioned into the lipid bilayer.

[0095] In some embodiments, the VWF targeting agents may be formulated as implantable solid formulations, or coated to the surface of implants (e.g., stent).

[0096] The concentrations of the VWF targeting agent in the pharmaceutical formulation vary depending on a variety of factors, including the dosage of the drug to be administered, and the route of administration.

Treatment, Administration and Dosage

[0097] In one aspect, the VWF targeting agents of the present disclosure may be used for treating a bleeding disorder, and more specifically for treating a hereditary bleeding disorder, e.g., hemophilia A (mild, moderate and severe hemophilia), and VWD (Type 1, Type 2b and Type 3).

[0098] In some embodiments, the VWF targeting agents of the present disclosure may be used in the therapy and prophylactic treatment of a bleeding disorder. As used herein, the term “prophylactic treatment” is commonly referred to as a preventive treatment to avoid bleeding, by regular infusion of blood coagulation factor concentrates to a patient with a bleeding disorder. Patients with a severe form of the bleeding disorder (e.g., severe hemophilia and VWD Type 3) who receive the prophylactic treatment may experience reduced risk of bleeding and less joint damages.

[0099] In some embodiments, the VWF targeting agents of the present disclosure may be used in combination with coagulation factor replacement therapy (also known as coagulation factor substitute). For example, the VWF targeting agents may be used in combination with FVIII replacement therapy for treating hemophilia A, such as mild hemophilia, moderate hemophilia and severe hemophilia, in a patient. In one preferred embodiment, the patient is diagnosed with severe hemophilia.

[0100] Regular treatment with FVIII prophylaxis can significantly protect against spontaneous bleeding symptoms in patients with hemophilia A, particularly with severe hemophilia. Plasma derived FVIII concentrates and recombinant FVIII preparations are currently available treatments of hemophilia A. As the short circulatory half-life of FVIII in the blood requires regular intravenous FVIII infusions to maintain therapeutic plasma FVIII levels, the dosing schedule clinically has important implications for patient compliance. Recent effort focuses on developing new approaches to extend half-lives of FVIII substitute, e.g., modification of recombinant FVIII. However, clinical studies with modified recombinant FVIII molecules have demonstrated more moderate increases in half-life (≈ 1.5 -fold) than for wild-type recombinant FVIII (Pipe et al., *Blood*, 2016, 128: 2007-2016).

[0101] It is recently reported that VWF peptide can effectively prolong endogenous FVIII survival in VWF-deficient mice and that extending the half-life of plasma VWF carrier fragment can result in a significant prolongation in FVIII half-life in VWF-deficient mice (Yee et al., *Blood*, 2014, 124:445-452). Agents that can elevate the VWF levels may be used for extending the half-life of FVIII in the blood, thereby elevating the FVIII levels in patients

with low FVIII activity (e.g., hemophilia and VWD). The studies performed by the inventors demonstrated that administration of a VWF binding agent, BT200 can increase the VWF and FVIII levels in the blood, in a dose-dependent manner. BT200 binds to VWF and prevents VWF from clearance in the blood. Elevated VWF forms FVIII-VWF complexes, thereby increasing the FVIII levels in the blood.

[0102] In some embodiments, the VWF targeting agents of the present disclosure may be used in combination with a recombinant FVIII preparation, or plasma derived FVIII concentrates, to extend the half-life of FVIII in the blood of a hemophilia patient. The patient may have mild, moderate or severe hemophilia. In particular, the patient has severe hemophilia.

[0103] In some embodiments, the VWF targeting agents of the present disclosure may be used in combination with VWF replacement therapy for treating VWD, particularly VWD Type 3 and VWD Type 2 and VWD Type 1. In some examples, the VWF targeting agents may be used in combination with a VWF replacement preparation. The VWF replacement preparation may be a plasma-derived factor concentrate containing both FVIII and VWF (i.e., a FVIII/VWF concentrate), and a recombinant VWF preparation. As a non-limiting example, the recombinant VWF preparation may be Vonicog alfa, rVWF that is produced in genetically altered CHO cells expressing both VWF and FVIII (Turecek et al., *Hamostaseologie*, 2009, 29(Suppl.):S32-38).

[0104] In other examples, the present VWF targeting agents may be used in combination with DDAVP treatment. The VWF targeting agent may stabilize and increase the endogenous VWF and FVIII levels released by stimulation of DDAVP.

[0105] In some embodiments, the VWF targeting agents of the present disclosure may be used to maintain adequate VWF levels in the blood to protect FVIII from degradation. VWF may be endogenous, released by *in vivo* VWF producing cells, or exogenous, infused with *in vitro* VWF substitute. FVIII may be endogenous or exogenous.

[0106] In some embodiments, the VWF targeting agents may be used to increase platelet counts in a patient with VWD Type 2b, in particular, the VWD Type 2b patient with thrombocytopenia which is formed due to platelet aggregation. The present VWF targeting agent such as BT200 may raise platelet counts in the blood.

[0107] The present VWF targeting agent can increase platelet counts in the blood. Accordingly, a VWF targeting agent, can be used to treat thrombocytopenia, a condition in which a patient has a low platelet count in the blood. Thrombocytopenia may occur in various

conditions, e.g., a bone marrow disorder, a side effect from medications, an enlarged spleen associated with cancer and severe liver disease, an autoimmune disease, a condition by exposing a toxic chemical, and an infection.

[0108] In particular, BT200 can maintain sustained elevation of VWF and FVIII levels in the blood.

[0109] In some embodiments, the present VWF targeting agents, compositions and methods may be used to treat other rare deficiencies in clotting components. Rare inherited bleeding disorders (RBDs) include, for example, deficiencies of coagulation factors fibrinogen, FII, FV, combined FV and FVIII, FVII, FX, FXI, FXIII, and congenital deficiency of vitamin K-dependent factors (VKCFDs).

[0110] In accordance with the present disclosure, the methods comprise administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising at least one VWF targeting agent. In some examples, the targeting agent is a VWF binding agent. The VWF binding agent is an antibody, a nanobody, a peptide, an oligonucleotide, an RNA (e.g., siRNA, microRNA), a synthetic polynucleotide (e.g., an aptamer), or a small molecule. In some embodiments, The VWF binding agent is a synthetic polynucleotide selected from the group consisting of SEQ ID No.: 3, BT99 (SEQ ID No.: 4), BT100 (SEQ ID No.: 5), BT200 (SEQ ID No.: 6), ARC15105 (SEQ ID No.: 7), and ARC1779 (SEQ ID No.: 8) and variants thereof. In one preferred embodiment, the VWF binding agent is BT200 (SEQ ID No.: 6).

[0111] The VWF targeting agent may increase the levels of FVIII and VWF in the blood of the subject being treated with said agent. The VWF targeting agent may raise platelet counts in the subject as well.

[0112] As a non-limiting example, the VWF targeting agent is BT200 (SEQ ID No.: 6).

[0113] In accordance with the present disclosure, the treatment regimen such as doses, dose-response relationship, loading and maintenance doses and dosing schedules (such as intervals and timing), administration routes, formulations, etc. are discussed and determined.

[0114] In some embodiments, the VWF targeting agents may be administered to the subject in need in any administration routes. The agents and compositions may be formulated for administration by parental administration or enteral administration, or other appropriate routes. Parental administration may be performed by injection, or by the insertion of an indwelling catheter, including but not limited to intravenous (IV), intramuscular (IM), subcutaneous (SC), epicutaneous injection, peridural injection, intracerebral (into the

cerebrum) administration, intracerebroventricular (into the cerebral ventricles) administration, extra-amniotic administration, nasal administration, intra-arterial, intracardiac, intraosseous infusion (IO), intraperitoneal infusion or injection, transdermal diffusion, enteral and gastrointestinal routes, topical administration and oral routes.

[0115] As a non-limiting example, the VWF targeting agent is administered by subcutaneous injection.

[0116] The therapeutically effective dosage of a medicine varies from patient to patient and depends upon factors such as the age and condition of the patient and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. Factors that may influence the dosage required to effectively treat a subject, include but are not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a therapeutic polynucleotide disclosed herein can include a single treatment or can include a series of treatments. The effective dosage of a therapeutic polynucleotide disclosed herein used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

[0117] In some embodiments, a therapeutically effective amount or dosage of the VWF targeting agent may range from about 0.001 µg/kg or 0.01 µg/kg to about 250 mg/kg or 500 mg/kg body weight, with other ranges including but not limited to, about 0.01 µg/kg to 100 mg/kg body weight, about 0.01 µg/kg to 50 mg/kg body weight, about 0.1 µg/kg to 20 mg/kg body weight, about 0.1 µg/kg to 10 mg/kg body weight, about 1 µg/kg to 100 mg/kg body weight, about 1 µg/kg to 50 mg/kg body weight, about 10 µg/kg to 100 mg/kg body weight, about 10 µg/kg to 50 mg/kg body weight, about 20 µg/kg to 100 mg/kg body weight, about 20 µg/kg to 50 mg/kg body weight, about 1 mg/kg to 100 mg/kg body weight, about 1 mg/kg to 50 mg/kg body weight, or about 20 mg/kg to 50 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present.

[0118] In some embodiments, the pharmaceutical compositions may be administered to the subject in need in a single dose. A single dose of BT200 may be 0.1mg, 0.2mg, 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg, 3.0mg, 3.5mg, 4.0mg, 4.5mg, 5.0mg, 5.5mg, 6.0mg, 6.5g,

7.0mg, 7.5mg, 8.0mg, 8.5mg, 9.0mg, 10.0mg, 11.0mg, 12.0mg, 13.0mg, 14.0mg, 15.0mg, 16.0mg, 17.0mg, 18.0mg, 19.0mg, 20.0mg, 21.0mg, 22.0mg, 23.0mg, 24.0mg, 25.0mg, 26.0mg, 27.0mg, 28.0mg, 29.0mg, 30.0mg, 31.0mg, 32.0mg, 33.0mg, 34.0mg, 35.0mg, 36.0mg, 37.0mg, 38.0mg, 39.0mg, 40.0mg, 41.0mg, 42.0mg, 43.0mg, 44.0mg, 45.0mg, 46.0mg, 47.0mg, 48.0mg, 49.0mg, 50.0mg, 51.0mg, 52.0mg, 53.0mg, 54.0mg, 55.0mg, 56.0mg, 57.0mg, 58.0mg, 59.0mg, 60.0mg, 61.0mg, 62.0mg, 63.0mg, 64.0mg, 65.0mg, 66.0mg, 67.0mg, 68.0mg, 69.0mg, 70.0mg, 71.0mg, 72.0mg, 73.0mg, 74.0mg, 75.0mg, 76.0mg, 77.0mg, 78.0mg, 79.0mg, 80.0mg, 81.0mg, 82.0mg, 83.0mg, 84.0mg, 85.0mg, 86.0mg, 87.0mg, 88.0mg, 89.0mg, 90.0mg, 91.0mg, 92.0mg, 93.0mg, 94.0mg, 95.0mg, 96.0mg, 97.0mg, 98.0mg, 99.0mg, or 100.0mg.

[0119] In one preferred embodiment, the single dose of BT200 is about 1.0 mg to 10.0 mg, or about 1.0 mg to 6.0 mg.

[0120] In some embodiments, the pharmaceutical compositions may be administered to the subject in need in multiple doses. The dosing schedules provided herein are safe and effective to prevent bleeding. As a non-limiting example, a patient may be treated with the present VWF targeting agent one time per week. It will also be appreciated that the effective dosage of an agent used for treatment may increase or decrease over the course of a particular treatment. The agents of the present disclosure can be administered simultaneously or separately.

[0121] In one preferred embodiment, a multiple dosing regimen is provided, including a loading dose of BT200 and sequential maintenance doses of BT200. The loading dose and maintenance doses may range from, but are not limited to, 0.1mg to 10.0mg of BT200, or 1.0mg to 6.0mg of BT200.

[0122] As non-limiting examples, BT200 may be dosed at 0.1mg, 0.2mg, 0.5mg, 1.0mg, 1.5mg, 2.0mg, 2.5mg, 3.0mg, 3.5mg, 4.0mg, 4.5mg, 5.0mg, 5.5mg, 6.0mg, 6.5mg, 7.0mg, 8.0mg, 9.0mg, 10.0mg, 15mg, or 20mg. In some embodiments, BT200 may be dosed at 3.0mg, 6.0mg, 9.0mg or 10.0mg.

[0123] In some embodiments, the VWF targeting agents or pharmaceutical formulations thereof may be administered to a patient about every day, about every other day, about every 3 days, about every 4 days, about every 5 days, about every 6 days, about every 7 days, about every 8 to 10 days, about every 11 to 14 days, or about every three weeks to the patient in need thereof. In one embodiment, the VWF agent or pharmaceutical formulations thereof can be administered about every 7 days.

[0124] In some examples, the VWF targeting agents or pharmaceutical formulations thereof can be administered as a single time administration. In other examples, administration of the VWF targeting agents or pharmaceutical formulations thereof can continue for about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about two months, about three to four months, about four to six months, or about one year. In some examples, administration of the VWF agents or pharmaceutical formulations thereof is about every 7 days for about 5 weeks. In certain embodiments, administration can be intermittent after, for example, about 4 weeks, or about 5 weeks. As a non-limiting example, a subject in need may be treated once a week for about 5 weeks and then treated about 3 to about 4 times over the following years. In some embodiments, the VWF agents or pharmaceutical formulations thereof may be administered to the subject intermittently to maintain the patency of a vessel, or to keep VWF at certain levels. Dosing schedules and use of methods in combination with other bleeding prevention therapies are included.

[0125] In accordance with the present disclosure, the VWF targeting agent may be administered once every other day, once every three days, once every five days, once a week, or once every other week. In one preferred embodiment, the VWF targeting agent may be administered at least one loading dose and at least one maintenance dose. The loading dose and maintenance dose may be the same or different. As a non-limiting example, the VWF targeting agents or pharmaceutical formulations thereof can be administered with 2 loading doses, and 3 maintenance doses every 7 days.

EQUIVALENTS AND SCOPE

[0126] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the disclosure described herein. The scope of the present disclosure is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[0127] In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure

includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[0128] It is also noted that the term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term “comprising” is used herein, the term “consisting of” is thus also encompassed and disclosed.

[0129] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0130] In addition, it is to be understood that any particular embodiment of the present disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the disclosure (e.g., any antibiotic, therapeutic or active ingredient; any method of production; any method of use; etc.) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0131] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the disclosure in its broader aspects.

[0132] While the present disclosure has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the disclosure.

EXAMPLES**Example 1: Dosing window of BT200 for treating a bleeding disorder**

[0133] The blood levels of Factor VIII and VWF were measured after a single dose of BT200 at a dose ranging from 0.18mg up to 48mg in healthy volunteers. One week after dosing, the blood levels of Factor VIII and VWF antigen were increased in a dose- and time-dependent manner (as shown in Table 3 (Factor VIII) and Table 4 (VWF antigen)). In the description column of Table 3 and Table 4, the description includes the cohort number, the therapeutic given and the patient identifier. For example, “1-BT200-A” means the patient A of cohort 1 which was given BT200 and “1-Control-A” means patient A of the control group.

Table 3. Factor VIII Activity

Description	Visit	Result (%)	Absolute Change	Relative Change
1-BT200-A	Day 0, 1h prior IMP	59	-	-
1-BT200-A	Day 2	138	79	1.34
1-BT200-A	Day 7	101	42	0.71
1-BT200-A	Day 14	93	34	0.58
1-BT200-A	Day 21	117	58	0.98
1-BT200-A	Day 28	69	10	0.17
1-BT200-B	Day 0, 1h prior IMP	92	-	-
1-BT200-B	Day 2	103	11	0.12
1-BT200-B	Day 7	79	-13	-0.14
1-BT200-B	Day 14	97	5	0.05
1-BT200-B	Day 21	130	38	0.41
1-BT200-B	Day 28	147	55	0.6
1-BT200-C	Day 0, 1h prior IMP	82	-	-
1-BT200-C	Day 2	58	-24	-0.29
1-BT200-C	Day 7	71	-11	-0.13
1-BT200-C	Day 14	99	17	0.21
1-BT200-C	Day 21	79	-3	-0.04
1-BT200-C	Day 28	72	-10	-0.12
1-BT200-D	Day 0, 1h prior IMP	89	-	-
1-BT200-D	Day 2	129	40	0.45
1-BT200-D	Day 7	62	-27	-0.3
1-BT200-D	Day 14	89	0	0
1-BT200-D	Day 21	123	34	0.38
1-BT200-D	Day 28	154	65	0.73
1-BT200-E	Day 0, 1h prior IMP	49	-	-
1-BT200-E	Day 2	63	14	0.29
1-BT200-E	Day 7	50	1	0.02
1-BT200-E	Day 14	65	16	0.33
1-BT200-E	Day 21	56	7	0.14

1-BT200-E	Day 28	52	3	0.06
1-BT200-F	Day 0, 1h prior IMP	44	-	-
1-BT200-F	Day 2	68	24	0.55
1-BT200-F	Day 7	43	-1	-0.02
1-BT200-F	Day 14	74	30	0.68
1-BT200-F	Day 21	101	57	1.3
1-BT200-F	Day 28	124	80	1.82
1-Control-A	Day 0, 1h prior IMP	66	-	-
1-Control-A	Day 2	67	1	0.02
1-Control-A	Day 7	47	-19	-0.29
1-Control-A	Day 14	115	49	0.74
1-Control-A	Day 21	132	66	1
1-Control-A	Day 28	87	21	0.32
1-Control-B	Day 0, 1h prior IMP	80	-	-
1-Control-B	Day 2	104	24	0.3
1-Control-B	Day 7	101	21	0.26
1-Control-B	Day 21	161	81	1.01
1-Control-B	Day 28	99	19	0.24
2-BT200-A	Day 0, 1h prior IMP	27	-	-
2-BT200-A	Day 2	49	22	0.81
2-BT200-A	Day 7	49	22	0.81
2-BT200-A	Day 14	64	37	1.37
2-BT200-A	Day 21	56	29	1.07
2-BT200-A	Day 28	40	13	0.48
2-BT200-B	Day 0, 1h prior IMP	68	-	-
2-BT200-B	Day 2	133	65	0.96
2-BT200-B	Day 7	185	117	1.72
2-BT200-B	Day 14	158	90	1.32
2-BT200-B	Day 21	176	108	1.59
2-BT200-B	Day 28	118	50	0.74
2-BT200-C	Day 0, 1h prior IMP	112	-	-
2-BT200-C	Day 2	137	25	0.22
2-BT200-C	Day 7	72	-40	-0.36
2-BT200-C	Day 14	132	20	0.18
2-BT200-C	Day 21	132	20	0.18
2-BT200-C	Day 28	150	38	0.34
2-BT200-D	Day 0, 1h prior IMP	70	-	-
2-BT200-D	Day 2	141	71	1.01
2-BT200-D	Day 7	134	64	0.91
2-BT200-D	Day 14	176	106	1.51
2-BT200-D	Day 21	137	67	0.96
2-BT200-D	Day 28	147	77	1.1
2-BT200-E	Day 0, 1h prior IMP	101	-	-
2-BT200-E	Day 2	111	10	0.1
2-BT200-E	Day 7	88	-13	-0.13

2-BT200-E	Day 14	107	6	0.06
2-BT200-E	Day 21	119	18	0.18
2-BT200-E	Day 28	101	0	0
2-BT200-F	Day 0, 1h prior IMP	74	-	-
2-BT200-F	Day 2	141	67	0.91
2-BT200-F	Day 7	116	42	0.57
2-BT200-F	Day 14	113	39	0.53
2-BT200-F	Day 21	86	12	0.16
2-BT200-F	Day 28	135	61	0.82
2-Control-A	Day 0, 1h prior IMP	31	-	-
2-Control-A	Day 2	28	-3	-0.1
2-Control-A	Day 7	86	55	1.77
2-Control-A	Day 14	42	11	0.35
2-Control-A	Day 21	42	11	0.35
2-Control-A	Day 28	44	13	0.42
2-Control-B	Day 0, 1h prior IMP	112	-	-
2-Control-B	Day 2	144	32	0.29
2-Control-B	Day 7	125	13	0.12
2-Control-B	Day 14	160	48	0.43
2-Control-B	Day 21	148	36	0.32
2-Control-B	Day 28	122	10	0.09
3-BT200-A	Day 0, 1h prior IMP	163	-	-
3-BT200-A	Day 2	144	-19	-0.12
3-BT200-A	Day 7	202	39	0.24
3-BT200-A	Day 14	144	-19	-0.12
3-BT200-A	Day 21	134	-29	-0.18
3-BT200-A	Day 28	117	-46	-0.28
3-BT200-B	Day 0, 1h prior IMP	75	-	-
3-BT200-B	Day 2	126	51	0.68
3-BT200-B	Day 7	130	55	0.73
3-BT200-B	Day 14	91	16	0.21
3-BT200-B	Day 21	94	19	0.25
3-BT200-B	Day 28	113	38	0.51
3-BT200-C	Day 0, 1h prior IMP	126	-	-
3-BT200-C	Day 2	197	71	0.56
3-BT200-C	Day 7	197	71	0.56
3-BT200-C	Day 14	189	63	0.5
3-BT200-C	Day 21	188	62	0.49
3-BT200-C	Day 28	167	41	0.33
3-BT200-D	Day 0, 1h prior IMP	70	-	-
3-BT200-D	Day 2	82	12	0.17
3-BT200-D	Day 7	158	88	1.26
3-BT200-D	Day 14	133	63	0.9
3-BT200-D	Day 21	106	36	0.51
3-BT200-D	Day 28	112	42	0.6

3-BT200-E	Day 0, 1h prior IMP	67	-	-
3-BT200-E	Day 2	95	28	0.42
3-BT200-E	Day 7	107	40	0.6
3-BT200-E	Day 14	100	33	0.49
3-BT200-E	Day 21	87	20	0.3
3-BT200-E	Day 28	81	14	0.21
3-BT200-F	Day 0, 1h prior IMP	68	-	-
3-BT200-F	Day 2	105	37	0.54
3-BT200-F	Day 7	120	52	0.76
3-BT200-F	Day 14	75	7	0.1
3-BT200-F	Day 21	110	42	0.62
3-BT200-F	Day 28	93	25	0.37
3-Control-A	Day 0, 1h prior IMP	100	-	-
3-Control-A	Day 2	98	-2	-0.02
3-Control-A	Day 7	117	17	0.17
3-Control-A	Day 14	98	-2	-0.02
3-Control-A	Day 21	114	14	0.14
3-Control-A	Day 28	101	1	0.01
3-Control-B	Day 0, 1h prior IMP	112	-	-
3-Control-B	Day 2	102	-10	-0.09
3-Control-B	Day 7	146	34	0.3
3-Control-B	Day 14	138	26	0.23
3-Control-B	Day 21	106	-6	-0.05
3-Control-B	Day 28	130	18	0.16
4-BT200-A	Day 0, 1h prior IMP	137	-	-
4-BT200-A	Day 2	151	14	0.1
4-BT200-A	Day 7	187	50	0.36
4-BT200-A	Day 14	211	74	0.54
4-BT200-A	Day 21	96	-41	-0.3
4-BT200-A	Day 28	88	-49	-0.36
4-BT200-B	Day 0, 1h prior IMP	101	-	-
4-BT200-B	Day 2	167	66	0.65
4-BT200-B	Day 7	207	106	1.05
4-BT200-B	Day 14	142	41	0.41
4-BT200-B	Day 21	93	-8	-0.08
4-BT200-B	Day 28	88	-13	-0.13
4-BT200-C	Day 0, 1h prior IMP	74	-	-
4-BT200-C	Day 2	175	101	1.36
4-BT200-C	Day 7	138	64	0.86
4-BT200-C	Day 14	90	16	0.22
4-BT200-C	Day 21	98	24	0.32
4-BT200-C	Day 28	50	-24	-0.32
4-BT200-D	Day 0, 1h prior IMP	97	-	-
4-BT200-D	Day 2	190	93	0.96
4-BT200-D	Day 7	165	68	0.7

4-BT200-D	Day 14	128	31	0.32
4-BT200-D	Day 21	116	19	0.2
4-BT200-D	Day 28	91	-6	-0.06
4-BT200-E	Day 0, 1h prior IMP	238	-	-
4-BT200-E	Day 2	143	-95	-0.4
4-BT200-E	Day 7	257	19	0.08
4-BT200-E	Day 14	271	33	0.14
4-BT200-E	Day 21	279	41	0.17
4-BT200-E	Day 28	238	0	0
4-BT200-F	Day 0, 1h prior IMP	201	-	-
4-BT200-F	Day 2	261	60	0.3
4-BT200-F	Day 7	254	53	0.26
4-BT200-F	Day 14	257	56	0.28
4-BT200-F	Day 21	203	2	0.01
4-BT200-F	Day 28	185	-16	-0.08
4-Control-A	Day 0, 1h prior IMP	59	-	-
4-Control-A	Day 2	83	24	0.41
4-Control-A	Day 7	68	9	0.15
4-Control-A	Day 14	73	14	0.24
4-Control-A	Day 21	76	17	0.29
4-Control-A	Day 28	77	18	0.31
4-Control-B	Day 0, 1h prior IMP	79	-	-
4-Control-B	Day 2	71	-8	-0.1
4-Control-B	Day 7	45	-34	-0.43
4-Control-B	Day 14	83	4	0.05
4-Control-B	Day 21	45	-34	-0.43
4-Control-B	Day 28	89	10	0.13
5-BT200-A	Day 0, 1h prior IMP	85	-	-
5-BT200-A	Day 2	221	136	1.6
5-BT200-A	Day 7	312	227	2.67
5-BT200-A	Day 14	218	133	1.56
5-BT200-A	Day 21	183	98	1.15
5-BT200-A	Day 28	132	47	0.55
5-BT200-B	Day 0, 1h prior IMP	80	-	-
5-BT200-B	Day 2	226	146	1.83
5-BT200-B	Day 7	396	316	3.95
5-BT200-B	Day 14	167	87	1.09
5-BT200-B	Day 28	124	44	0.55
5-BT200-C	Day 0, 1h prior IMP	90	-	-
5-BT200-C	Day 2	210	120	1.33
5-BT200-C	Day 7	306	216	2.4
5-BT200-C	Day 14	192	102	1.13
5-BT200-C	Day 21	169	79	0.88
5-BT200-C	Day 28	130	40	0.44
5-BT200-D	Day 0, 1h prior IMP	83	-	-

5-BT200-D	Day 2	185	102	1.23
5-BT200-D	Day 7	258	175	2.11
5-BT200-D	Day 14	155	72	0.87
5-BT200-D	Day 21	133	50	0.6
5-BT200-D	Day 28	91	8	0.1
5-BT200-E	Day 0, 1h prior IMP	63	-	-
5-BT200-E	Day 2	170	107	1.7
5-BT200-E	Day 7	236	173	2.75
5-BT200-E	Day 14	156	93	1.48
5-BT200-E	Day 21	162	99	1.57
5-BT200-E	Day 28	90	27	0.43
5-BT200-F	Day 0, 1h prior IMP	79	-	-
5-BT200-F	Day 2	220	141	1.78
5-BT200-F	Day 7	278	199	2.52
5-BT200-F	Day 14	170	91	1.15
5-BT200-F	Day 21	114	35	0.44
5-BT200-F	Day 28	76	-3	-0.04
5-Control-A	Day 0, 1h prior IMP	67	-	-
5-Control-A	Day 2	77	10	0.15
5-Control-A	Day 7	65	-2	-0.03
5-Control-A	Day 14	50	-17	-0.25
5-Control-A	Day 21	78	11	0.16
5-Control-A	Day 28	69	2	0.03
5-Control-B	Day 0, 1h prior IMP	95	-	-
5-Control-B	Day 2	132	37	0.39
5-Control-B	Day 7	133	38	0.4
5-Control-B	Day 14	72	-23	-0.24
5-Control-B	Day 21	109	14	0.15
5-Control-B	Day 28	93	-2	-0.02
6-BT200-A	Day 0, 1h prior IMP	120	-	-
6-BT200-A	Day 2	164	44	0.37
6-BT200-A	Day 7	231	111	0.93
6-BT200-A	Day 14	238	118	0.98
6-BT200-A	Day 21	171	51	0.43
6-BT200-A	Day 28	98	-22	-0.18
6-BT200-B	Day 0, 1h prior IMP	90	-	-
6-BT200-B	Day 2	151	61	0.68
6-BT200-B	Day 7	280	190	2.11
6-BT200-B	Day 14	241	151	1.68
6-BT200-B	Day 21	188	98	1.09
6-BT200-B	Day 28	119	29	0.32
6-BT200-C	Day 0, 1h prior IMP	104	-	-
6-BT200-C	Day 2	227	123	1.18
6-BT200-C	Day 7	254	150	1.44
6-BT200-C	Day 14	146	42	0.4

6-BT200-C	Day 21	143	39	0.38
6-BT200-C	Day 28	124	20	0.19
6-BT200-D	Day 0, 1h prior IMP	134	-	-
6-BT200-D	Day 2	171	37	0.28
6-BT200-D	Day 7	315	181	1.35
6-BT200-D	Day 14	297	163	1.22
6-BT200-D	Day 21	261	127	0.95
6-BT200-D	Day 28	178	44	0.33
6-BT200-E	Day 0, 1h prior IMP	57	-	-
6-BT200-E	Day 2	-	-	-
6-BT200-E	Day 7	142	85	1.49
6-BT200-E	Day 14	145	88	1.54
6-BT200-E	Day 21	125	68	1.19
6-BT200-E	Day 28	84	27	0.47
6-BT200-F	Day 0, 1h prior IMP	84	-	-
6-BT200-F	Day 2	166	82	0.98
6-BT200-F	Day 7	335	251	2.99
6-BT200-F	Day 14	202	118	1.4
6-BT200-F	Day 21	125	41	0.49
6-BT200-F	Day 28	71	-13	-0.15
6-Control-A	Day 0, 1h prior IMP	122	-	-
6-Control-A	Day 2	90	-32	-0.26
6-Control-A	Day 7	126	4	0.03
6-Control-A	Day 14	107	-15	-0.12
6-Control-A	Day 21	106	-16	-0.13
6-Control-A	Day 28	106	-16	-0.13
6-Control-B	Day 0, 1h prior IMP	59	-	-
6-Control-B	Day 2	103	44	0.75
6-Control-B	Day 7	94	35	0.59
6-Control-B	Day 14	144	85	1.44
6-Control-B	Day 21	105	46	0.78
6-Control-B	Day 28	54	-5	-0.08
7-BT200-A	Day 0, 1h prior IMP	104	-	-
7-BT200-A	Day 2	247	143	1.38
7-BT200-A	Day 7	295	191	1.84
7-BT200-A	Day 14	255	151	1.45
7-BT200-A	Day 21	160	56	0.54
7-BT200-A	Day 28	100	-4	-0.04
7-BT200-B	Day 0, 1h prior IMP	90	-	-
7-BT200-B	Day 2	240	150	1.67
7-BT200-B	Day 7	281	191	2.12
7-BT200-B	Day 14	205	115	1.28
7-BT200-B	Day 21	114	24	0.27
7-BT200-B	Day 28	95	5	0.06
7-BT200-C	Day 0, 1h prior IMP	74	-	-

7-BT200-C	Day 2	212	138	1.86
7-BT200-C	Day 7	312	238	3.22
7-BT200-C	Day 14	210	136	1.84
7-BT200-C	Day 21	165	91	1.23
7-BT200-C	Day 28	106	32	0.43
7-BT200-D	Day 0, 1h prior IMP	68	-	-
7-BT200-D	Day 2	125	57	0.84
7-BT200-D	Day 7	223	155	2.28
7-BT200-D	Day 14	139	71	1.04
7-BT200-D	Day 21	131	63	0.93
7-BT200-D	Day 28	116	48	0.71
7-BT200-E	Day 0, 1h prior IMP	101	-	-
7-BT200-E	Day 2	201	100	0.99
7-BT200-E	Day 7	249	148	1.47
7-BT200-E	Day 14	212	111	1.1
7-BT200-E	Day 21	144	43	0.43
7-BT200-E	Day 28	114	13	0.13
7-BT200-F	Day 0, 1h prior IMP	56	-	-
7-BT200-F	Day 2	246	190	3.39
7-BT200-F	Day 7	399	343	6.13
7-BT200-F	Day 14	238	182	3.25
7-BT200-F	Day 21	132	76	1.36
7-BT200-F	Day 28	91	35	0.63
7-Control-A	Day 0, 1h prior IMP	102	-	-
7-Control-A	Day 2	145	43	0.42
7-Control-A	Day 7	123	21	0.21
7-Control-A	Day 14	91	-11	-0.11
7-Control-A	Day 21	102	0	0
7-Control-A	Day 28	119	17	0.17
7-Control-B	Day 0, 1h prior IMP	162	-	-
7-Control-B	Day 2	176	14	0.09
7-Control-B	Day 7	142	-20	-0.12
7-Control-B	Day 14	166	4	0.02
7-Control-B	Day 21	99	-63	-0.39
7-Control-B	Day 28	-	-	-
8-BT200-A	Day 0, 1h prior IMP	143	-	-
8-BT200-A	Day 2	288	145	1.01
8-BT200-A	Day 7	482	339	2.37
8-BT200-A	Day 14	444	301	2.1
8-BT200-A	Day 21	282	139	0.97
8-BT200-A	Day 28	245	102	0.71
8-BT200-B	Day 0, 1h prior IMP	74	-	-
8-BT200-B	Day 2	248	174	2.35
8-BT200-B	Day 7	406	332	4.49
8-BT200-B	Day 14	297	223	3.01

8-BT200-B	Day 21	216	142	1.92
8-BT200-B	Day 28	186	112	1.51
8-BT200-C	Day 0, 1h prior IMP	99	-	-
8-BT200-C	Day 2	295	196	1.98
8-BT200-C	Day 7	451	352	3.56
8-BT200-C	Day 14	389	290	2.93
8-BT200-C	Day 21	256	157	1.59
8-BT200-C	Day 28	226	127	1.28
8-BT200-D	Day 0, 1h prior IMP	87	-	-
8-BT200-D	Day 2	258	171	1.97
8-BT200-D	Day 7	406	319	3.67
8-BT200-D	Day 14	293	206	2.37
8-BT200-D	Day 21	193	106	1.22
8-BT200-D	Day 28	130	43	0.49
8-BT200-E	Day 0, 1h prior IMP	66	-	-
8-BT200-E	Day 2	335	269	4.08
8-BT200-E	Day 7	310	244	3.7
8-BT200-E	Day 14	323	257	3.89
8-BT200-E	Day 21	146	80	1.21
8-BT200-E	Day 28	249	183	2.77
8-BT200-F	Day 0, 1h prior IMP	154	-	-
8-BT200-F	Day 2	247	93	0.6
8-BT200-F	Day 7	359	205	1.33
8-BT200-F	Day 21	277	123	0.8
8-BT200-F	Day 28	242	88	0.57
8-Control-A	Day 0, 1h prior IMP	156	-	-
8-Control-A	Day 2	157	1	0.01
8-Control-A	Day 7	95	-61	-0.39
8-Control-A	Day 14	127	-29	-0.19
8-Control-A	Day 21	114	-42	-0.27
8-Control-A	Day 28	66	-90	-0.58
8-Control-B	Day 0, 1h prior IMP	176	-	-
8-Control-B	Day 2	149	-27	-0.15
8-Control-B	Day 7	197	21	0.12
8-Control-B	Day 14	192	16	0.09
8-Control-B	Day 21	229	53	0.3
8-Control-B	Day 28	195	19	0.11
9-BT200-A	Day 0, 1h prior IMP	143	-	-
9-BT200-A	Day 2	220	77	0.54
9-BT200-A	Day 7	298	155	1.08
9-BT200-A	Day 21	289	146	1.02
9-BT200-A	Day 28	247	104	0.73
9-BT200-B	Day 0, 1h prior IMP	153	-	-
9-BT200-B	Day 2	336	183	1.2
9-BT200-B	Day 7	463	310	2.03

9-BT200-B	Day 14	372	219	1.43
9-BT200-B	Day 21	287	134	0.88
9-BT200-B	Day 28	271	118	0.77
9-BT200-C	Day 0, 1h prior IMP	117	-	-
9-BT200-C	Day 2	263	146	1.25
9-BT200-C	Day 7	400	283	2.42
9-BT200-C	Day 14	300	183	1.56
9-BT200-C	Day 21	229	112	0.96
9-BT200-C	Day 28	196	79	0.68
9-BT200-D	Day 0, 1h prior IMP	87	-	-
9-BT200-D	Day 2	214	127	1.46
9-BT200-D	Day 7	336	249	2.86
9-BT200-D	Day 14	400	313	3.6
9-BT200-D	Day 21	282	195	2.24
9-BT200-D	Day 28	228	141	1.62
9-BT200-E	Day 0, 1h prior IMP	123	-	-
9-BT200-E	Day 2	295	172	1.4
9-BT200-E	Day 7	390	267	2.17
9-BT200-E	Day 14	325	202	1.64
9-BT200-E	Day 21	254	131	1.07
9-BT200-E	Day 28	204	81	0.66
9-BT200-F	Day 0, 1h prior IMP	113	-	-
9-BT200-F	Day 2	246	133	1.18
9-BT200-F	Day 7	392	279	2.47
9-BT200-F	Day 14	326	213	1.88
9-BT200-F	Day 21	185	72	0.64
9-BT200-F	Day 28	241	128	1.13
9-Control-A	Day 0, 1h prior IMP	104	-	-
9-Control-A	Day 2	117	13	0.13
9-Control-A	Day 7	69	-35	-0.34
9-Control-A	Day 14	89	-15	-0.14
9-Control-A	Day 21	60	-44	-0.42
9-Control-A	Day 28	92	-12	-0.12
9-Control-B	Day 0, 1h prior IMP	130	-	-
9-Control-B	Day 2	240	110	0.85
9-Control-B	Day 7	188	58	0.45
9-Control-B	Day 14	196	66	0.51
9-Control-B	Day 21	139	9	0.07
9-Control-B	Day 28	209	79	0.61
10-BT200-A	Day 0, 1h prior IMP	162	-	-
10-BT200-A	Day 2	337	175	1.08
10-BT200-A	Day 7	434	272	1.68
10-BT200-A	Day 14	290	128	0.79
10-BT200-A	Day 21	394	232	1.43
10-BT200-B	Day 0, 1h prior IMP	108	-	-

10-BT200-B	Day 2	224	116	1.07
10-BT200-B	Day 7	318	210	1.94
10-BT200-B	Day 14	305	197	1.82
10-BT200-B	Day 21	233	125	1.16
10-BT200-B	Day 28	92	-16	-0.15
10-BT200-C	Day 0, 1h prior IMP	69	-	-
10-BT200-C	Day 2	224	155	2.25
10-BT200-C	Day 7	245	176	2.55
10-BT200-C	Day 14	268	199	2.88
10-BT200-C	Day 21	241	172	2.49
10-BT200-C	Day 28	160	91	1.32
10-BT200-D	Day 0, 1h prior IMP	195	-	-
10-BT200-D	Day 2	389	194	0.99
10-BT200-D	Day 7	498	303	1.55
10-BT200-D	Day 14	400	205	1.05
10-BT200-D	Day 21	382	187	0.96
10-BT200-D	Day 28	267	72	0.37
10-BT200-E	Day 0, 1h prior IMP	90	-	-
10-BT200-E	Day 2	220	130	1.44
10-BT200-E	Day 7	303	213	2.37
10-BT200-E	Day 14	297	207	2.3
10-BT200-E	Day 21	238	148	1.64
10-BT200-E	Day 28	180	90	1
10-BT200-F	Day 0, 1h prior IMP	145	-	-
10-BT200-F	Day 2	304	159	1.1
10-BT200-F	Day 7	556	411	2.83
10-BT200-F	Day 14	452	307	2.12
10-BT200-F	Day 21	263	118	0.81
10-BT200-F	Day 28	151	6	0.04
10-Control-A	Day 0, 1h prior IMP	125	-	-
10-Control-A	Day 2	122	-3	-0.02
10-Control-A	Day 7	139	14	0.11
10-Control-A	Day 14	60	-65	-0.52
10-Control-A	Day 21	117	-8	-0.06
10-Control-A	Day 28	124	-1	-0.01
10-Control-B	Day 0, 1h prior IMP	165	-	-
10-Control-B	Day 2	193	28	0.17
10-Control-B	Day 7	131	-34	-0.21
10-Control-B	Day 14	235	70	0.42
10-Control-B	Day 21	228	63	0.38
10-Control-B	Day 28	142	-23	-0.14

Table 4. VWF Antigen

Description	Timing	Result (%)	Absolute Change	Relative Change
1-BT200-A	Day 0, 1h prior IMP	79	-	-
1-BT200-A	Day 2	146	67	0.85
1-BT200-A	Day 7	91	12	0.15
1-BT200-A	Day 14	80	1	0.01
1-BT200-A	Day 21	78	-1	-0.01
1-BT200-A	Day 28	68	-11	-0.14
1-BT200-B	Day 0, 1h prior IMP	64	-	-
1-BT200-B	Day 2	64	0	0
1-BT200-B	Day 7	47	-17	-0.27
1-BT200-B	Day 14	51	-13	-0.2
1-BT200-B	Day 21	69	5	0.08
1-BT200-B	Day 28	70	6	0.09
1-BT200-C	Day 0, 1h prior IMP	58	-	-
1-BT200-C	Day 2	67	9	0.16
1-BT200-C	Day 7	63	5	0.09
1-BT200-C	Day 14	57	-1	-0.02
1-BT200-C	Day 21	54	-4	-0.07
1-BT200-C	Day 28	49	-9	-0.16
1-BT200-D	Day 0, 1h prior IMP	111	-	-
1-BT200-D	Day 2	131	20	0.18
1-BT200-D	Day 7	89	-22	-0.2
1-BT200-D	Day 14	97	-14	-0.13
1-BT200-D	Day 21	105	-6	-0.05
1-BT200-D	Day 28	128	17	0.15
1-BT200-E	Day 0, 1h prior IMP	52	-	-
1-BT200-E	Day 2	75	23	0.44
1-BT200-E	Day 7	42	-10	-0.19
1-BT200-E	Day 14	61	9	0.17
1-BT200-E	Day 21	34	-18	-0.35
1-BT200-E	Day 28	45	-7	-0.13
1-BT200-F	Day 0, 1h prior IMP	76	-	-
1-BT200-F	Day 2	80	4	0.05
1-BT200-F	Day 7	-	-	-
1-BT200-F	Day 14	77	1	0.01
1-BT200-F	Day 21	87	11	0.14
1-BT200-F	Day 28	104	28	0.37
1-Control-A	Day 0, 1h prior IMP	73	-	-
1-Control-A	Day 2	88	15	0.21
1-Control-A	Day 7	56	-17	-0.23
1-Control-A	Day 14	97	24	0.33
1-Control-A	Day 21	112	39	0.53

1-Control-A	Day 28	76	3	0.04
1-Control-B	Day 0, 1h prior IMP	89	-	-
1-Control-B	Day 2	122	33	0.37
1-Control-B	Day 7	105	16	0.18
1-Control-B	Day 21	135	46	0.52
1-Control-B	Day 28	103	14	0.16
2-BT200-A	Day 0, 1h prior IMP	29	-	-
2-BT200-A	Day 2	58	29	1
2-BT200-A	Day 7	57	28	0.97
2-BT200-A	Day 14	55	26	0.9
2-BT200-A	Day 21	47	18	0.62
2-BT200-A	Day 28	55	26	0.9
2-BT200-B	Day 0, 1h prior IMP	79	-	-
2-BT200-B	Day 2	167	88	1.11
2-BT200-B	Day 7	154	75	0.95
2-BT200-B	Day 14	146	67	0.85
2-BT200-B	Day 21	194	115	1.46
2-BT200-B	Day 28	145	66	0.84
2-BT200-C	Day 0, 1h prior IMP	133	-	-
2-BT200-C	Day 2	119	-14	-0.11
2-BT200-C	Day 7	66	-67	-0.5
2-BT200-C	Day 14	101	-32	-0.24
2-BT200-C	Day 21	125	-8	-0.06
2-BT200-C	Day 28	113	-20	-0.15
2-BT200-D	Day 0, 1h prior IMP	87	-	-
2-BT200-D	Day 2	114	27	0.31
2-BT200-D	Day 7	99	12	0.14
2-BT200-D	Day 14	107	20	0.23
2-BT200-D	Day 21	121	34	0.39
2-BT200-D	Day 28	94	7	0.08
2-BT200-E	Day 0, 1h prior IMP	134	-	-
2-BT200-E	Day 2	116	-18	-0.13
2-BT200-E	Day 7	91	-43	-0.32
2-BT200-E	Day 14	96	-38	-0.28
2-BT200-E	Day 21	123	-11	-0.08
2-BT200-E	Day 28	121	-13	-0.1
2-BT200-F	Day 0, 1h prior IMP	81	-	-
2-BT200-F	Day 2	105	24	0.3
2-BT200-F	Day 7	93	12	0.15
2-BT200-F	Day 14	85	4	0.05
2-BT200-F	Day 21	59	-22	-0.27
2-BT200-F	Day 28	85	4	0.05
2-Control-A	Day 0, 1h prior IMP	42	-	-
2-Control-A	Day 2	41	-1	-0.02
2-Control-A	Day 7	74	32	0.76

2-Control-A	Day 14	54	12	0.29
2-Control-A	Day 21	47	5	0.12
2-Control-A	Day 28	53	11	0.26
2-Control-B	Day 0, 1h prior IMP	123	-	-
2-Control-B	Day 2	129	6	0.05
2-Control-B	Day 7	117	-6	-0.05
2-Control-B	Day 14	142	19	0.15
2-Control-B	Day 21	103	-20	-0.16
2-Control-B	Day 28	131	8	0.07
3-BT200-A	Day 0, 1h prior IMP	106	-	-
3-BT200-A	Day 2	135	29	0.27
3-BT200-A	Day 7	147	41	0.39
3-BT200-A	Day 14	114	8	0.08
3-BT200-A	Day 21	96	-10	-0.09
3-BT200-A	Day 28	107	1	0.01
3-BT200-B	Day 0, 1h prior IMP	64	-	-
3-BT200-B	Day 2	92	28	0.44
3-BT200-B	Day 7	95	31	0.48
3-BT200-B	Day 14	93	29	0.45
3-BT200-B	Day 21	74	10	0.16
3-BT200-B	Day 28	80	16	0.25
3-BT200-C	Day 0, 1h prior IMP	141	-	-
3-BT200-C	Day 2	217	76	0.54
3-BT200-C	Day 7	193	52	0.37
3-BT200-C	Day 14	227	86	0.61
3-BT200-C	Day 21	175	34	0.24
3-BT200-C	Day 28	180	39	0.28
3-BT200-D	Day 0, 1h prior IMP	96	-	-
3-BT200-D	Day 2	35	-61	-0.64
3-BT200-D	Day 7	127	31	0.32
3-BT200-D	Day 14	96	0	0
3-BT200-D	Day 21	101	5	0.05
3-BT200-D	Day 28	100	4	0.04
3-BT200-E	Day 0, 1h prior IMP	70	-	-
3-BT200-E	Day 2	91	21	0.3
3-BT200-E	Day 7	98	28	0.4
3-BT200-E	Day 14	94	24	0.34
3-BT200-E	Day 21	109	39	0.56
3-BT200-E	Day 28	80	10	0.14
3-BT200-F	Day 0, 1h prior IMP	49	-	-
3-BT200-F	Day 2	80	31	0.63
3-BT200-F	Day 7	73	24	0.49
3-BT200-F	Day 14	71	22	0.45
3-BT200-F	Day 21	66	17	0.35
3-BT200-F	Day 28	63	14	0.29

3-Control-A	Day 0, 1h prior IMP	78	-	-
3-Control-A	Day 2	77	-1	-0.01
3-Control-A	Day 7	100	22	0.28
3-Control-A	Day 14	83	5	0.06
3-Control-A	Day 21	88	10	0.13
3-Control-A	Day 28	97	19	0.24
3-Control-B	Day 0, 1h prior IMP	101	-	-
3-Control-B	Day 2	84	-17	-0.17
3-Control-B	Day 7	117	16	0.16
3-Control-B	Day 14	113	12	0.12
3-Control-B	Day 21	112	11	0.11
3-Control-B	Day 28	102	1	0.01
4-BT200-A	Day 0, 1h prior IMP	104	-	-
4-BT200-A	Day 2	184	80	0.77
4-BT200-A	Day 7	243	139	1.34
4-BT200-A	Day 14	195	91	0.88
4-BT200-A	Day 21	106	2	0.02
4-BT200-A	Day 28	105	1	0.01
4-BT200-B	Day 0, 1h prior IMP	95	-	-
4-BT200-B	Day 2	146	51	0.54
4-BT200-B	Day 7	214	119	1.25
4-BT200-B	Day 14	181	86	0.91
4-BT200-B	Day 21	102	7	0.07
4-BT200-B	Day 28	76	-19	-0.2
4-BT200-C	Day 0, 1h prior IMP	68	-	-
4-BT200-C	Day 2	176	108	1.59
4-BT200-C	Day 7	148	80	1.18
4-BT200-C	Day 14	105	37	0.54
4-BT200-C	Day 21	89	21	0.31
4-BT200-C	Day 28	55	-13	-0.19
4-BT200-D	Day 0, 1h prior IMP	103	-	-
4-BT200-D	Day 2	156	53	0.51
4-BT200-D	Day 7	172	69	0.67
4-BT200-D	Day 14	140	37	0.36
4-BT200-D	Day 21	119	16	0.16
4-BT200-D	Day 28	76	-27	-0.26
4-BT200-E	Day 0, 1h prior IMP	198	-	-
4-BT200-E	Day 2	212	14	0.07
4-BT200-E	Day 7	328	130	0.66
4-BT200-E	Day 14	271	73	0.37
4-BT200-E	Day 21	265	67	0.34
4-BT200-E	Day 28	275	77	0.39
4-BT200-F	Day 0, 1h prior IMP	150	-	-
4-BT200-F	Day 2	258	108	0.72
4-BT200-F	Day 7	325	175	1.17

4-BT200-F	Day 14	208	58	0.39
4-BT200-F	Day 21	193	43	0.29
4-BT200-F	Day 28	151	1	0.01
4-Control-A	Day 0, 1h prior IMP	61	-	-
4-Control-A	Day 2	67	6	0.1
4-Control-A	Day 7	69	8	0.13
4-Control-A	Day 14	57	-4	-0.07
4-Control-A	Day 21	72	11	0.18
4-Control-A	Day 28	67	6	0.1
4-Control-B	Day 0, 1h prior IMP	67	-	-
4-Control-B	Day 2	69	2	0.03
4-Control-B	Day 7	22	-45	-0.67
4-Control-B	Day 14	67	0	0
4-Control-B	Day 21	47	-20	-0.3
4-Control-B	Day 28	68	1	0.01
5-BT200-A	Day 0, 1h prior IMP	100	-	-
5-BT200-A	Day 2	198	98	0.98
5-BT200-A	Day 7	311	211	2.11
5-BT200-A	Day 14	303	203	2.03
5-BT200-A	Day 21	165	65	0.65
5-BT200-A	Day 28	125	25	0.25
5-BT200-B	Day 0, 1h prior IMP	99	-	-
5-BT200-B	Day 2	218	119	1.2
5-BT200-B	Day 7	408	309	3.12
5-BT200-B	Day 14	247	148	1.49
5-BT200-B	Day 28	117	18	0.18
5-BT200-C	Day 0, 1h prior IMP	107	-	-
5-BT200-C	Day 2	193	86	0.8
5-BT200-C	Day 7	323	216	2.02
5-BT200-C	Day 14	230	123	1.15
5-BT200-C	Day 21	159	52	0.49
5-BT200-C	Day 28	134	27	0.25
5-BT200-D	Day 0, 1h prior IMP	104	-	-
5-BT200-D	Day 2	203	99	0.95
5-BT200-D	Day 7	399	295	2.84
5-BT200-D	Day 14	215	111	1.07
5-BT200-D	Day 21	108	4	0.04
5-BT200-D	Day 28	110	6	0.06
5-BT200-E	Day 0, 1h prior IMP	96	-	-
5-BT200-E	Day 2	183	87	0.91
5-BT200-E	Day 7	304	208	2.17
5-BT200-E	Day 14	275	179	1.86
5-BT200-E	Day 21	171	75	0.78
5-BT200-E	Day 28	112	16	0.17
5-BT200-F	Day 0, 1h prior IMP	66	-	-

5-BT200-F	Day 2	192	126	1.91
5-BT200-F	Day 7	314	248	3.76
5-BT200-F	Day 14	172	106	1.61
5-BT200-F	Day 21	102	36	0.55
5-BT200-F	Day 28	66	0	0
5-Control-A	Day 0, 1h prior IMP	69	-	-
5-Control-A	Day 2	72	3	0.04
5-Control-A	Day 7	75	6	0.09
5-Control-A	Day 14	77	8	0.12
5-Control-A	Day 21	70	1	0.01
5-Control-A	Day 28	71	2	0.03
5-Control-B	Day 0, 1h prior IMP	107	-	-
5-Control-B	Day 2	109	2	0.02
5-Control-B	Day 7	98	-9	-0.08
5-Control-B	Day 14	88	-19	-0.18
5-Control-B	Day 21	97	-10	-0.09
5-Control-B	Day 28	90	-17	-0.16
6-BT200-A	Day 0, 1h prior IMP	129	-	-
6-BT200-A	Day 2	220	91	0.71
6-BT200-A	Day 7	441	312	2.42
6-BT200-A	Day 14	309	180	1.4
6-BT200-A	Day 21	248	119	0.92
6-BT200-A	Day 28	124	-5	-0.04
6-BT200-B	Day 0, 1h prior IMP	115	-	-
6-BT200-B	Day 2	184	69	0.6
6-BT200-B	Day 7	362	247	2.15
6-BT200-B	Day 14	358	243	2.11
6-BT200-B	Day 21	233	118	1.03
6-BT200-B	Day 28	140	25	0.22
6-BT200-C	Day 0, 1h prior IMP	149	-	-
6-BT200-C	Day 2	248	99	0.66
6-BT200-C	Day 7	288	139	0.93
6-BT200-C	Day 14	201	52	0.35
6-BT200-C	Day 21	152	3	0.02
6-BT200-C	Day 28	118	-31	-0.21
6-BT200-D	Day 0, 1h prior IMP	216	-	-
6-BT200-D	Day 2	202	-14	-0.06
6-BT200-D	Day 7	420	204	0.94
6-BT200-D	Day 14	420	204	0.94
6-BT200-D	Day 21	374	158	0.73
6-BT200-D	Day 28	250	34	0.16
6-BT200-E	Day 0, 1h prior IMP	80	-	-
6-BT200-E	Day 2	-	-	-
6-BT200-E	Day 7	148	68	0.85
6-BT200-E	Day 14	121	41	0.51

6-BT200-E	Day 21	109	29	0.36
6-BT200-E	Day 28	100	20	0.25
6-BT200-F	Day 0, 1h prior IMP	73	-	-
6-BT200-F	Day 2	166	93	1.27
6-BT200-F	Day 7	402	329	4.51
6-BT200-F	Day 14	296	223	3.05
6-BT200-F	Day 21	132	59	0.81
6-BT200-F	Day 28	87	14	0.19
6-Control-A	Day 0, 1h prior IMP	95	-	-
6-Control-A	Day 2	97	2	0.02
6-Control-A	Day 7	101	6	0.06
6-Control-A	Day 14	86	-9	-0.09
6-Control-A	Day 21	95	0	0
6-Control-A	Day 28	87	-8	-0.08
6-Control-B	Day 0, 1h prior IMP	93	-	-
6-Control-B	Day 2	107	14	0.15
6-Control-B	Day 7	110	17	0.18
6-Control-B	Day 14	130	37	0.4
6-Control-B	Day 21	119	26	0.28
6-Control-B	Day 28	80	-13	-0.14
7-BT200-A	Day 0, 1h prior IMP	101	-	-
7-BT200-A	Day 2	192	91	0.9
7-BT200-A	Day 7	420	319	3.16
7-BT200-A	Day 14	299	198	1.96
7-BT200-A	Day 21	182	81	0.8
7-BT200-A	Day 28	97	-4	-0.04
7-BT200-B	Day 0, 1h prior IMP	86	-	-
7-BT200-B	Day 2	188	102	1.19
7-BT200-B	Day 7	357	271	3.15
7-BT200-B	Day 14	284	198	2.3
7-BT200-B	Day 21	141	55	0.64
7-BT200-B	Day 28	104	18	0.21
7-BT200-C	Day 0, 1h prior IMP	83	-	-
7-BT200-C	Day 2	207	124	1.49
7-BT200-C	Day 7	420	337	4.06
7-BT200-C	Day 14	351	268	3.23
7-BT200-C	Day 21	217	134	1.61
7-BT200-C	Day 28	115	32	0.39
7-BT200-D	Day 0, 1h prior IMP	92	-	-
7-BT200-D	Day 2	106	14	0.15
7-BT200-D	Day 7	221	129	1.4
7-BT200-D	Day 14	153	61	0.66
7-BT200-D	Day 21	131	39	0.42
7-BT200-D	Day 28	118	26	0.28
7-BT200-E	Day 0, 1h prior IMP	89	-	-

7-BT200-E	Day 2	165	76	0.85
7-BT200-E	Day 7	213	124	1.39
7-BT200-E	Day 14	222	133	1.49
7-BT200-E	Day 21	136	47	0.53
7-BT200-E	Day 28	112	23	0.26
7-BT200-F	Day 0, 1h prior IMP	66	-	-
7-BT200-F	Day 2	207	141	2.14
7-BT200-F	Day 7	366	300	4.55
7-BT200-F	Day 14	297	231	3.5
7-BT200-F	Day 21	139	73	1.11
7-BT200-F	Day 28	93	27	0.41
7-Control-A	Day 0, 1h prior IMP	124	-	-
7-Control-A	Day 2	123	-1	-0.01
7-Control-A	Day 7	104	-20	-0.16
7-Control-A	Day 14	98	-26	-0.21
7-Control-A	Day 21	112	-12	-0.1
7-Control-A	Day 28	129	5	0.04
7-Control-B	Day 0, 1h prior IMP	151	-	-
7-Control-B	Day 2	142	-9	-0.06
7-Control-B	Day 7	107	-44	-0.29
7-Control-B	Day 14	165	14	0.09
7-Control-B	Day 21	116	-35	-0.23
7-Control-B	Day 28	-	-	-
8-BT200-A	Day 0, 1h prior IMP	120	-	-
8-BT200-A	Day 2	300	180	1.5
8-BT200-A	Day 7	420	300	2.5
8-BT200-A	Day 14	420	300	2.5
8-BT200-A	Day 21	234	114	0.95
8-BT200-A	Day 28	204	84	0.7
8-BT200-B	Day 0, 1h prior IMP	84	-	-
8-BT200-B	Day 2	292	208	2.48
8-BT200-B	Day 7	408	324	3.86
8-BT200-B	Day 14	408	324	3.86
8-BT200-B	Day 21	294	210	2.5
8-BT200-B	Day 28	164	80	0.95
8-BT200-C	Day 0, 1h prior IMP	136	-	-
8-BT200-C	Day 2	327	191	1.4
8-BT200-C	Day 7	563	427	3.14
8-BT200-C	Day 14	420	284	2.09
8-BT200-C	Day 21	367	231	1.7
8-BT200-C	Day 28	264	128	0.94
8-BT200-D	Day 0, 1h prior IMP	91	-	-
8-BT200-D	Day 2	253	162	1.78
8-BT200-D	Day 7	485	394	4.33
8-BT200-D	Day 14	345	254	2.79

8-BT200-D	Day 21	125	34	0.37
8-BT200-D	Day 28	119	28	0.31
8-BT200-E	Day 0, 1h prior IMP	68	-	-
8-BT200-E	Day 2	281	213	3.13
8-BT200-E	Day 7	293	225	3.31
8-BT200-E	Day 14	324	256	3.76
8-BT200-E	Day 21	145	77	1.13
8-BT200-E	Day 28	137	69	1.01
8-BT200-F	Day 0, 1h prior IMP	111	-	-
8-BT200-F	Day 2	202	91	0.82
8-BT200-F	Day 7	307	196	1.77
8-BT200-F	Day 21	240	129	1.16
8-BT200-F	Day 28	177	66	0.59
8-Control-A	Day 0, 1h prior IMP	124	-	-
8-Control-A	Day 2	93	-31	-0.25
8-Control-A	Day 7	82	-42	-0.34
8-Control-A	Day 14	98	-26	-0.21
8-Control-A	Day 21	96	-28	-0.23
8-Control-A	Day 28	44	-80	-0.65
8-Control-B	Day 0, 1h prior IMP	156	-	-
8-Control-B	Day 2	163	7	0.04
8-Control-B	Day 7	175	19	0.12
8-Control-B	Day 14	159	3	0.02
8-Control-B	Day 21	182	26	0.17
8-Control-B	Day 28	194	38	0.24
9-BT200-A	Day 0, 1h prior IMP	148	-	-
9-BT200-A	Day 2	261	113	0.76
9-BT200-A	Day 7	468	320	2.16
9-BT200-A	Day 21	420	272	1.84
9-BT200-A	Day 28	337	189	1.28
9-BT200-B	Day 0, 1h prior IMP	132	-	-
9-BT200-B	Day 2	247	115	0.87
9-BT200-B	Day 7	420	288	2.18
9-BT200-B	Day 14	420	288	2.18
9-BT200-B	Day 21	368	236	1.79
9-BT200-B	Day 28	270	138	1.05
9-BT200-C	Day 0, 1h prior IMP	93	-	-
9-BT200-C	Day 2	183	90	0.97
9-BT200-C	Day 7	420	327	3.52
9-BT200-C	Day 14	326	233	2.51
9-BT200-C	Day 21	230	137	1.47
9-BT200-C	Day 28	142	49	0.53
9-BT200-D	Day 0, 1h prior IMP	101	-	-
9-BT200-D	Day 2	231	130	1.29
9-BT200-D	Day 7	468	367	3.63

9-BT200-D	Day 14	420	319	3.16
9-BT200-D	Day 21	360	259	2.56
9-BT200-D	Day 28	221	120	1.19
9-BT200-E	Day 0, 1h prior IMP	82	-	-
9-BT200-E	Day 2	223	141	1.72
9-BT200-E	Day 7	374	292	3.56
9-BT200-E	Day 14	355	273	3.33
9-BT200-E	Day 21	222	140	1.71
9-BT200-E	Day 28	133	51	0.62
9-BT200-F	Day 0, 1h prior IMP	95	-	-
9-BT200-F	Day 2	231	136	1.43
9-BT200-F	Day 7	418	323	3.4
9-BT200-F	Day 14	416	321	3.38
9-BT200-F	Day 21	268	173	1.82
9-BT200-F	Day 28	263	168	1.77
9-Control-A	Day 0, 1h prior IMP	98	-	-
9-Control-A	Day 2	88	-10	-0.1
9-Control-A	Day 7	88	-10	-0.1
9-Control-A	Day 14	110	12	0.12
9-Control-A	Day 21	89	-9	-0.09
9-Control-A	Day 28	103	5	0.05
9-Control-B	Day 0, 1h prior IMP	137	-	-
9-Control-B	Day 2	242	105	0.77
9-Control-B	Day 7	184	47	0.34
9-Control-B	Day 14	169	32	0.23
9-Control-B	Day 21	136	-1	-0.01
9-Control-B	Day 28	172	35	0.26
10-BT200-A	Day 0, 1h prior IMP	118	-	-
10-BT200-A	Day 2	260	142	1.2
10-BT200-A	Day 7	420	302	2.56
10-BT200-A	Day 14	420	302	2.56
10-BT200-A	Day 21	420	302	2.56
10-BT200-B	Day 0, 1h prior IMP	93	-	-
10-BT200-B	Day 2	218	125	1.34
10-BT200-B	Day 7	395	302	3.25
10-BT200-B	Day 14	387	294	3.16
10-BT200-B	Day 21	234	141	1.52
10-BT200-B	Day 28	73	-20	-0.22
10-BT200-C	Day 0, 1h prior IMP	76	-	-
10-BT200-C	Day 2	171	95	1.25
10-BT200-C	Day 7	329	253	3.33
10-BT200-C	Day 14	417	341	4.49
10-BT200-C	Day 21	274	198	2.61
10-BT200-C	Day 28	156	80	1.05
10-BT200-D	Day 0, 1h prior IMP	127	-	-

10-BT200-D	Day 2	311	184	1.45
10-BT200-D	Day 7	420	293	2.31
10-BT200-D	Day 14	420	293	2.31
10-BT200-D	Day 21	420	293	2.31
10-BT200-D	Day 28	322	195	1.54
10-BT200-E	Day 0, 1h prior IMP	59	-	-
10-BT200-E	Day 2	119	60	1.02
10-BT200-E	Day 7	240	181	3.07
10-BT200-E	Day 14	263	204	3.46
10-BT200-E	Day 21	186	127	2.15
10-BT200-E	Day 28	129	70	1.19
10-BT200-F	Day 0, 1h prior IMP	142	-	-
10-BT200-F	Day 2	255	113	0.8
10-BT200-F	Day 7	420	278	1.96
10-BT200-F	Day 14	420	278	1.96
10-BT200-F	Day 21	269	127	0.89
10-BT200-F	Day 28	104	-38	-0.27
10-Control-A	Day 0, 1h prior IMP	129	-	-
10-Control-A	Day 2	152	23	0.18
10-Control-A	Day 7	146	17	0.13
10-Control-A	Day 14	47	-82	-0.64
10-Control-A	Day 21	96	-33	-0.26
10-Control-A	Day 28	123	-6	-0.05
10-Control-B	Day 0, 1h prior IMP	137	-	-
10-Control-B	Day 2	130	-7	-0.05
10-Control-B	Day 7	138	1	0.01
10-Control-B	Day 14	231	94	0.69
10-Control-B	Day 21	172	35	0.26
10-Control-B	Day 28	104	-33	-0.24

[0134] The functions of VWF were also tested in the treated individuals. In three different activity tests: Multiplate aggregometer, Platelet Function Analyzer and ELISA for free A1 domains of VWF, no effect is observed as compared to non-treated individuals. The function of Factor VIII is also increased in BT200 treated volunteers.

[0135] The results in healthy volunteers show that a lower dose, 0.6 mg BT200, approximately doubles the baseline FVIII activity at 48 hours post-dose and maintains nearly half of that increase for 1 week post-dose. However, in the same time period, no effect on the function of VWF is observed using PFA-100[®] assay, a highly sensitive functional assay which uses undiluted whole blood.

[0136] The results also indicate that a 10-fold higher dose of BT200, 6 mg, maintains the doubling of baseline FVIII for 2 weeks after a single dose, without any evidence of VWF functional inhibition seen with the PFA-100[®] assay.

[0137] These observations indicate that BT200 can dose-dependently enhance the VWF/FVIII levels.

[0138] After single dose administration, the elimination half-life of BT200 in healthy volunteers are approximately 5-12 days, indicating a long half-life and good tolerability of BT200 after subcutaneous injection. No off-target adverse effects were observed upon BT200 administration.

[0139] Co-administration of BT200 and desmopressin demonstrated an additive effect on the levels of VWF and FVIII (as shown in Figure 1) in the blood.

Example 2: Individual dose-titration to dose-response in patients

[0140] A diverse set of up to 25 patients with severe congenital hemophilia A without inhibitors, mild-moderate hemophilia A, heterozygous carriers of hemophilia A with subnormal FVIII levels, VWD Type 1, “Vicenza” type, or VWD Type 2b are enrolled to a study to evaluate dose titration and dose-response.

[0141] Patients are dosed via subcutaneous (SC) injection, with 3mg BT200 on Days 1, Day 4 and Day 7 (± 2 days). BT200 doses were then titrated thereafter between 3 and 9mg on Days 14, 21, and 28 depending on the bleeding disorder patients have. FVIII activity is measured in patients with Hemophilia A or VWD Type 1. Platelet counts and/or FVIII activity are measured in patients with VWD Type 2b.

[0142] Five patients with VWD Type 2b (3 males: 2 females) (Table 5), were dosed with 3mg BT200 on Days 1 and 4 and followed by doses of 6-9mg BT200 every week (Days 7, 14, 21 and 28). The patients have a median age of 61 years (ranging from 24-72 years). Four of five patients presented with thrombocytopenia and two patients were on regular recombinant VWF substitution therapy because of recurrent severe bleedings. Efficacy was measured by VWF parameters, FVIII activity, and platelet counts, and other clinical outcome parameters before, during and after therapy. Statistical analysis was performed by Friedman ANOVA.

Table 5: Patients with VWF Type 2b

Patients	Age: gender	Mutations
#1	54; female	3922 C>T het
#2	61; male	C3916C>T

#3	24; male	C3916C>T
#9	72; male	3946G>A het
#10	61; female	C3939G>C

[0143] The results indicate clinically meaningful responses occurred in patients within 4 days after the first subcutaneous injection of BT200 (first dose 3mg BT200). Platelet counts rose from a median of 60 at baseline to 159 /nL at 28 days ($p=0.012$), i.e., up to 4-fold in all patients with thrombocytopenia, and normalized in $\frac{3}{4}$ of these patients (Figure 2). All thrombocytopenic patients responded with an increase in platelet counts after 96 hours after first dose of BT200. Plasma levels of FVIIIc doubled from 67% (44-91%) to 134% (114-200%) ($p<0.001$), and normalized in the patient with subnormal FVIIIc activity (from 44% at baseline to 135% 1 week after the last dose). This was mirrored by a shortening of the uniformly prolonged activated partial thromboplastin time (aPTT-FS) from 43s to 32s ($p=0.002$) (Figure 3) and increased thrombin generation. BT200 administrations increased circulating VWF antigen levels from 64% (32-106%) to 143% (103-351%, $p<0.001$) (Figure 4A). VWF collagen binding activity (VWF:CBA) increased 2-4 fold in all patients, VWF ristocetin cofactor (VWF:RCO) activity and VWF:GpIbM activity increased 2-3 fold in all thrombocytopenic patients, but only by a small amount in the one patient without thrombocytopenia (all p-values <0.05 for $n=5$) (Figure 4B). The increased ristocetin BT200 was very well tolerated without any relevant adverse effect.

[0144] These clinical observations indicate that BT200 specifically corrects the underlying pathophysiology of VWD Type 2b, rapidly and strongly increasing platelet counts in patients with thrombocytopenia, and elevating VWF and FVIIIc in all patients. It is hypothesized that BT200 may be of potential benefit for VWD Type 2b patients by increasing circulating VWF and FVIII levels in all patients, as well as platelet counts in thrombocytopenic patients.

[0145] Patients with haemophilia A were treated with BT200 with the same dosing schedule demonstrated increased FVIII activity (Figure 5).

[0146] These results indicate that BT200 has a prolong half-life of 7 to 12 days. BT200 can induce sustained increases in VWF levels and activity, as well as increases in FVIII levels and activity measured by increased aPTT shortening and increased thrombin generation.

Example 3: BT200 increases the half-life of FVIII therapy

[0147] Patients were treated with a FVIII therapy (Refacto AF/three patients; Elocta/one patient; Afstylia/ two patients; Kovaltry/one patient; and Advate/one patient). FVIII therapy's

half-life before BT200 treatment was based on historical values known in the art. Patients were dosed with splitting loading doses and weekly doses followed with the loading doses (Table 6). The half-life of each FVIII therapy in each patient post BT200 treatment was measured one month after the BT200 treatment. All the FVIII values were generated through Chromogenic assay (Patients # 014, 017, 020-024), or by one-stage clotting assay (Patient #027). The average increase is 3.1 folder (Median=2.8 folder) (Table 6). These observations suggest that BT200 can increase the half-life of almost all the FVIII product on the market.

Table 6: BT200 increases the half-life of FVIII therapy

Patient ID	FVIII therapy	BT200 dosing	Pre-BT200 FVIII half-life(hr)	Post-BT200 FVIII half-life(hr)	Fold Increase
014	Refacto AF	3mg on Days 1, 4 and 7; 4mg on Day 14; 5mg on day 21; and 6mg on Day 28	19	42.5	2.24
017	Refacto AF	3mg on Days 1, 4 and 7; 5mg on Day 14; 5mg on day 21; and 7mg on Day 28	11	36.5	3.34
020	Elocta	3mg on Days 1, 4 and 7; 5mg on Day 14; 6mg on Day 21; and 7mg on Day 28	24.2	62	2.56
021	Afstyla	3mg on Days 1, 4 and 7; 5mg on Day 14; 6mg on Day 21; and 7mg on Day 28	14.5	29	2.00
022	Kovaltry	3mg on Days 1, 4 and 7; 4mg on Day 14; 5mg on ay 21; and 7mg on Day 28	7.7	26.7	3.47
023	Afstyla	3mg on Days 1, 4 and 7; 6mg on Day 14; 7mg on Day 21; and 9mg on Day 28	7.5	22.8	3.04
024	Advate	3mg on Days 1, 4 and 7; 5mg on Day 14; 6mg on Day 21; and 7mg on Day 28	13.5	34.5	2.56
027	Refacto AF	3mg on Days 1, 4 and 7; 5mg on Day 14, 6mg on day 21; and 8mg on Day 28	10	57	5.70

CLAIMS

1. A method for treating a hereditary bleeding disorder in a subject comprising administering to the subject a therapeutic effective amount of a pharmaceutical composition comprising a VWF targeting agent.
2. The method of claim 1, wherein the hereditary bleeding disorder is an acquired platelet function defect, congenital platelet function defects, disseminated intravascular coagulation (DIC), prothrombin deficiency, fibrinogen deficiency, FV deficiency, FVII deficiency, FX deficiency, FXI deficiency (hemophilia C), FXIII deficiency, combined FV and FVIII deficiency (F5F8D), VKCFD, Glanzmann thrombasthenia, hemophilia A, hemophilia B, immune thrombocytopenic purpura (ITP), von Willebrand disease (VWD) Type 1, von Willebrand disease (VWD) Type 2, and Von Willebrand disease (VWD) Type 3.
3. The method of claim 2, wherein the hereditary bleeding disorder is hemophilia A, VWD Type 1, VWD Type 2, or VWD Type 3.
4. The method of claim 3, wherein the hereditary bleeding disorder is hemophilia A, mild hemophilia, moderate hemophilia, or severe hemophilia.
5. The method of claim 3, wherein the hereditary bleeding disorder is VWD Type 1, VWD Type 1 Vicenza subtype, VWD Type 2a, VWD Type 2b, VWD Type 2b with thrombocytopenia, VWD Type 2m, VWD Type 2n, or VWD Type 3.
6. The method of any one of claims 1-5, wherein the VWF targeting agent is a VWF binding agent selected from the group consisting of an antibody, a nanobody, a peptide, an oligonucleotide, a siRNA, a microRNA, a synthetic polynucleotide, and a small molecule.
7. The method of claim 6, wherein the VWF binding agent is a synthetic polynucleotide selected from the group consisting of SEQ ID No.: 3, BT99 (SEQ ID No.: 4), BT100 (SEQ ID No.: 5), BT200 (SEQ ID No.: 6), ARC15105 (SEQ ID No.: 7), and ARC1779 (SEQ ID No.: 8) and variants thereof.

8. The method of claim 7, wherein the VWF binding agent is BT200 (SEQ ID No.: 6) or a variant thereof.
9. The method of claim 8, wherein BT200 increases the levels and activity of VWF, the levels and activity of FVIII, and/or the platelet counts in the blood.
10. The method of claim 8, wherein BT200 is administered to the subject at a dose ranging from 0.1 mg to 48.0 mg, or from 0.1mg to 20.0 mg, or from 1.0mg to 10mg.
11. The method of claim 10, wherein BT200 is administered to the subject at a dose ranging from 0.6 mg to 6.0 mg.
12. The method of claim 8, wherein the VWF binding agent is administered to the subject via subcutaneous injection.
13. The method of claim 12, wherein the VWF binding agent is administered to the subject with multiple doses, and wherein each dose is administered once every other day, or once every three days, or once every five days, or once a week, or once every other week.
14. The method of any one of claims 1-13, wherein the treatment of the hereditary bleeding disorder is a therapy treatment or a prophylactic treatment.
15. The method of claim 3, wherein the VWF targeting agent is administered in combination with coagulation factor substitute treatment; the coagulation factor substitute is plasma derived FVIII/VWF concentrates, recombinant FVIII preparations, or recombinant VWF preparations.
16. A method for preventing a chronic bleeding condition, and/or spontaneous bleeding in a subject comprising administering to the subject a therapeutic effective amount of a pharmaceutical composition comprising a VWF targeting agent.

17. The method of claim 16, wherein the VWF targeting agent is a VWF binding agent selected from the group consisting of an antibody, a nanobody, a peptide, an oligonucleotide, a siRNA, a microRNA, a synthetic polynucleotide, and a small molecule.
18. The method of claim 17, wherein the VWF binding agent is a synthetic polynucleotide comprising PEGylated nucleic acids.
19. The method of claim 18, wherein the VWF binding agent is BT200 (SEQ ID No.: 6), or a variant thereof.
20. The method of claim 19, wherein BT200 is administered at a dose ranging from 1.0mg to 10.0mg, or alternatively at a dose ranging from 1.0mg to 6.0mg.
21. A method for blocking VWF clearance in the blood comprising providing an effective amount of a VWF targeting agent that is a synthetic polynucleotide comprising a PEGylation moiety.
22. The method of claim 21, wherein the VWF targeting agent is BT200 (SEQ ID No.: 6), or a variant thereof.
23. The method of claim 22, wherein BT200 is provided at an amount ranging from 0.6mg to 6.0mg, or from 1.0mg to 6.0mg, or from 1.0mg to 10.0mg.
24. The method of claim 23, wherein BT 200 increases VWF levels in the blood.
25. A method for elevating the FVIII levels in the blood of a subject comprising administering to the subject an efficient amount of a composition comprising a VWF targeting agent that is a synthetic polynucleotide comprising a PEGylation moiety.
26. The method of claim 25, wherein the subject is diagnosed with hemophilia A, VWD Type 1, VWD Type 2, or VWD Type 3.
27. The method of claim 26, wherein the VWF targeting agent is BT200 (SEQ ID No.: 6).

28. The method of claim 27, wherein BT200 increases FVIII clotting activity.
29. A method for increasing platelet counts in the blood of a patient comprising administering to the patient an efficient amount of a composition comprising BT200 (SEQ ID No.: 6), wherein the patient has a low platelet count (thrombocytopenia).
30. The method of claim 29, wherein the thrombocytopenia is associated with a genetic disorder that causes a low platelet count, an enlarged spleen that holds abnormal platelets, a side reaction to a medicine, an autoimmune disease, or a viral infection.
31. The method of claim 30, wherein the disorder is VWD Type 2a, VWD Type 2b with thrombocytopenia, VWD Type 2b, VWD Type 2m, or VWD Type 2n.
32. A method for treating a hereditary bleeding disorder in a patient with a pharmaceutical composition comprising a VWF binding agent, BT200, comprising:
- a) assessing the hereditary bleeding disorder of said patient and the baseline coagulation function in said patient;
 - b) determining the optimal dose of BT200 with the assessment of step a) for initial treatment; and
 - c) providing a weekly dose of BT200 after one-week treatment with the initial dose of BT200 in said patient.
33. The method of claim 32, wherein the hereditary bleeding disorder may be hemophilia A, severe hemophilia, VWD Type 1, VWD Type 2a, VWD Type 2b, VWD Type 2m, VWD Type 2n, or VWD Type 3.
34. The method of claim 33, wherein the assessment includes FVIII activity, VWF antigen levels and/or platelet counts in the blood.
35. The method of claim 33, wherein the patient is treated with a coagulation factor substitute.