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(54) Benevnelse **COMBINATION OF FACTOR VII AND A ANTI-FACTOR IX AND FACTOR X BISPECIFIC ANTIBODY**

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COMBINATION OF FACTOR VII AND AN ANTI-FACTOR IX AND ANTI-FACTOR X BISPECIFIC ANTIBODY

The invention relates to pharmaceutical compositions useful for the treatment of a coagulation disorder, like haemophilia A, especially in a patient with type A haemophilia with the development of factor VIII inhibitor antibodies.

Technological background

Coagulation involves two routes, an intrinsic one and an extrinsic one, finishing in a common final route. The combination of both mechanisms ensures the formation of a solid and flexible blood clot which resists blood pressure. By the action of thrombin, fibrinogen undergoes chemical modifications which lead to the formation of fibrin. Fibrin is necessary to the formation of a clot.

The intrinsic route includes the factors present in the bloodstream and the coagulation process starts within the blood vessel itself. In turn, the extrinsic route involves tissue factors which are not normally present in the bloodstream and which are released upon a vascular lesion.

The factor VII is a glycoprotein which intervenes in the extrinsic coagulation route. In order to initiate the coagulation cascade, FVII must be activated into FVIIa. Once activated, FVIIa complexes with the tissue factor (TF), a protein associated with two phospholipids, which is released upon a vascular lesion. FVIIa alone (not complexed with the tissue factor) features a low proteolytic activity. Afterwards, the FVIIa-FT complex transforms the factor X into factor Xa in the presence of calcium ions. This complex also acts on the activation of the factor FIX into FIXa, thereby catalysing the intrinsic route. In return, the factors IXa and Xa activate the activated factor VII.

The factor IX and the factor X intervene in the coagulation intrinsic route. The activated factor IX enables the activation of the factor X into factor Xa.

The factor Xa complexed with the activated factor FV and prothrombinase transforms prothrombin into thrombin. Thrombin then acts on fibrinogen to transform it into fibrin and also enables the activation of FVIII and FV into FVIIIa and FVa, respectively. In turn, thrombin allows activating the factor XIII into FXIIIa, responsible for the consolidation of the fibrin clot, in the presence of calcium naturally present in the plasma.

Nevertheless, when a coagulation factor is absent, the coagulation cascade is interrupted or deficient and then we talk about abnormal coagulation.

The activated factor VII acts locally in the presence of the tissue factor released after a lesion of tissues generating bleeding, even in the absence of the factor VIII or IX. This is why the factor VII, preferably in the activated form, is used for the treatment of some blood coagulation disorders reflected by bleeding.

Thus, the factor VII is used for treating patients with haemophilia, with a factor VIII deficiency (type A haemophilia) or a factor IX deficiency (type B haemophilia), as well as patients with other coagulation factor deficiencies, for example, a hereditary FVII deficiency. FVII is also recommended in cerebrovascular accident treatment.

Some haemophilia patients develop antibodies that inhibit the factor VIII administered, generally in the concentrated form, as haemophilia treatment. Nowadays, this is the most common complication of haemophilia treatment.

Bispecific antibodies targeting FIX or FIXa and FX or FXa, such as emicizumab, are used to treat patients with haemophilia A with anti-factor VIII antibodies. These antibodies functionally replace FVIII by promoting the activation of FX by FIXa by bringing these two molecules together. These antibodies have a long-lasting effect.

The combination of recombinant FVIIa derived from cell culture (such as Novoseven®, produced in BHK cells) with Emicizumab (such as ACE910 or Hemlibra®) has been tested *(R. HARTMANN, et al. OR 36 | Synergistic Effects of a Procoagulant Bispecific Antibody and FEIBA or Factor VIIA on Thrombin Generation (Haemophilia (2017), 23 (Suppl. 2), 11-27))*. This combination demonstrated just an additive effect on the treatment of coagulation disorders.

Consequently, there is a need for a pharmaceutical combination enabling a better management of haemophilia A patients, and more particularly patients with anti-factor VIII.

Summary of the invention

The invention suggests combining the transgenic factor VII with a multispecific antibody directed against the factors IX and X.

According to the invention, the combination of the invention of factor VII obtained by transgenesis and antibodies directed against the factor IX and factor X induces a synergistic effect in the treatment of coagulation disorders, and particularly for the

treatment of patients with haemophilia A with anti-FVIII inhibitors and patients with a FVII deficiency.

Hence, one aspect of the invention is a pharmaceutical composition comprising:

a. transgenic factor VII, and

b. a multispecific, preferably bispecific, antibody directed against the factor IX and the factor X, such as, for example emicizumab.

Preferably, the factor VII is in the form of activated factor VII (FVIIa).

In a particular embodiment, the factor IX is in the form of activated factor IX (FIXa) and/or the factor X is in the form of activated factor X (FXa).

Preferably, said transgenic factor VII is a human factor VII derived from a production by the epithelial cells of the mammary glands of a transgenic non-human mammal, for example a transgenic rabbit for the human factor VII.

The invention also provides a combination product comprising:

a. transgenic factor VII, and

b. a multispecific antibody directed against the factor IX and the factor X,

for use thereof in the prevention or treatment of a coagulation disorder, such as haemophilia A, more particularly haemophilia A with factor VIII inhibitors (FVIII).

Preferably, the combination product is in the form of a pharmaceutical composition which comprises both the transgenic factor VII and said antibody.

Alternatively, the transgenic factor VII and the antibody are in the form of separate compositions, suited for simultaneous or separate (for example sequential) administration to the patient.

Another object of the invention also relates to a kit comprising:

- A container containing a transgenic factor FVII; and
- Another container containing an antibody directed against the factor IX and the factor X.

List of the figures:

Figure 1: Assessment of the synergic thrombogenic effect of the Sevenfact™ + Hemlibra® combination on Batch 1 of Haemophiliac A plasma after induction with TF/PL. (A) Assessment of the synergic thrombogenic effect on the ETP, (B) Assessment of the synergic thrombogenic effect on the thrombin generation peak, (C) Assessment of the synergic thrombogenic effect on the velocity.

Figure 2: Assessment of the synergic thrombogenic effect of the Sevenfact™ + Hemlibra® combination on Batch 2 of Haemophiliac A plasma after induction with TF/PL. (A) Assessment of the synergic thrombogenic effect on the ETP, (B) Assessment of the synergic thrombogenic effect on the thrombin generation peak, (C) Assessment of the synergic thrombogenic effect on the velocity.

Detailed description of the invention

General definitions

The coagulation phenomenon consists of a cascade of enzymatic reactions involving coagulation factors present in the form of proenzymes which, in the presence of some cofactors, are converted by proteolytic cleavage into their "**activated**" form. The activated form of each factor present in the form of inactive precursor is designated by the letter a. Thus, FVIIa results, in vivo, from the cleavage of zymogen by different proteases (FIXa, FXa, FVIIa) into two chains joined by a disulphide bridge.

The term "**treatment**" or "**treat**" generally refers to an improvement, prophylaxis, or reversal of a disease or disorder, or at least a symptom, for example, the slow-down of the progress of a disease or the stabilisation of a symptom. The delay of the apparition of a disease or disorder, or of at least one symptom, is also included.

The term "**prevention**" or "**prevent**" refers to a reduction in the risk of developing or acquiring a specific disease or disorder.

In the context of the present invention, by "**patient**" or "**subject**", it should be understood any mammal, and more particularly human beings, males or females, of any age, including children.

The term "**pharmaceutical composition**" refers to preparations enabling the biological activity of active ingredients and containing no additional component toxic for the subjects to whom the composition is administered.

Transgenic factor VII

The term "**Factor VII**" or "**FVII**" includes polypeptides comprising the sequence 1-406 of the wildtype human factor VII (as described in the U.S. patent No. 4,784,950) or FVII derived from another species (for example, bovine, porcine, canine, murine). It further comprises natural allelic variations of the factor VII that might exist, in any form or degree of glycosylation or other post-translational modification. The term "factor VII"

also includes the FVII variants that have the same or superior biological activity compared to the activity of the wildtype, these variants including in particular polypeptides different from the wildtype FVII by insertion, suppression or substitution of one or more amino acid(s).

Unless indicated otherwise, in the present description, the term “factor VII” will refer indifferently to the uncleaved FVII (zymogen) or to the activated factor VII (FVIIa).

Hence, FVIIa is composed of a light chain of 152 amino acids with a molecular weight of about 20 kDa and a heavy chain of 254 amino acids with a molecular weight of about 30 kDa bonded together by a single disulphide bridge (Cys135-Cys262).

By “**Recombinant factor VII**”, it should be understood any factor VII derived from genetic engineering and resulting from the expression of the corresponding gene in any microorganism, plant or transgenic plant. By microorganism, it should be understood any bacterial, fungal, viral or cellular system. The recombinant factor VII may also be produced from eukaryote cells in culture, such as plant or mammal cells, for example, animal or human cells.

By “**Transgenic factor VII**”, it should be understood any recombinant factor VII obtained from a transgenic animal for the factor VII.

By “**Transgenic animal**”, it should be understood any non-human animal with a modification in its genome intended to enable the expression of a protein of interest (herein the factor VII). The modification of the genome may result from an alteration, a modification or an insertion of a gene. This modification may be due to the action of conventional altering or mutagenic agents or performed by directed mutagenesis. The modification of the genome may also result from an insertion of a gene or genes or a or substitution of a gene or genes in their wildtype or mutated form. The transgenic animal may be selected, without limitation, from among rabbit, goat, cow, camel, hamster, mouse, rat, horse, sow, dromedary, sheep or llama. In a particular embodiment, it is possible to select an animal that does not express α1,3-galactosyltransferase.

By the expression “**biological activity of the factor VIIa**”, it should be understood the ability of FVIIa to generate thrombin, for example, at the surface of the activated platelets. The activity of the factor VII may be assessed in different manners. The biological activity of FVIIa may be quantified, for example, by measuring the ability of an FVII composition to promote blood clotting by using a FVII and thromboplastin deficient plasma, as described, for example, in the US patent No. 5,997,864. In this test, the

biological activity is assessed with respect to a control sample and is converted into “FVII units” by comparison with a pooled standard human serum containing 1 unit/mL of factor VII activity. Alternatively, the biological activity of the factor VII may be quantified by (i) measuring the ability of the factor VIIa to produce the factor Xa in a system comprising a tissue factor (TF) embedded in a lipid membrane and the factor X (Persson et al. J. Biol. Chem. 272: 19919-19924, 1997); (ii) measuring the hydrolysis of the factor X in an aqueous system; (iii) measuring the physical bond of FVIIa to TF by surface plasmon resonance (Persson, FEBS letts, 413:359-363, 1997), (iv) measuring the hydrolysis of a synthetic substrate or (v) measuring the generation of thrombin in an in-vitro system independent of the TF.

In a preferred embodiment, the FVII described herein is a polypeptide whose peptide sequence may be that of natural human FVII, i.e., the sequence present in humans who do not suffer from FVII-related disorders. Such a technique is described in the document EP 0 200 421.

Advantageously, the FVII sequence used in the invention is the sequence SEQ ID NO: 1.

By “**Synergy**” or “**synergistic effect**”, it should be preferably understood an effect of the combination of two products that is greater than 2 times the sum of the effects of each of the products considered separately. According to the present invention, a synergistic effect is obtained when the use of a transgenic FVIIa in combination with a multispecific antibody directed against the factor IX and the factor X allows obtaining an effect greater than 2 times the sum of the effect obtained with a transgenic FVIIa alone and the effect obtained with a multispecific antibody directed against the factor IX and the factor X alone, on at least one thrombin generation parameter. The thrombin generation parameter is selected amongst the peak height, the velocity or the endogenous thrombin potential (ETP).

In a particular embodiment, FVIIa is administered at a concentration lower than or equal to 105 nM, preferably lower than 100 nM.

In a particular embodiment, the multispecific antibody directed against the factor IX and the factor X is administered at a concentration lower than 600 nM, preferably lower than 550 nM, preferably lower than 500 nM, preferably lower than 450 nM, preferably lower than 400 nM, preferably lower than 350 nM, preferably lower than 325 nM.

In a particular embodiment, the factor VII is obtained from the milk of a transgenic animal.

A method for producing a recombinant protein in the milk of a transgenic animal may include the following steps: a synthetic DNA molecule comprising a gene coding for a protein of interest (herein, for example, the human FVII), this gene being under the control of a promoter of a protein naturally secreted in the milk, is integrated into an embryo of a non-human mammal. Afterwards, the embryo is implanted in a female mammal of the same species. Once the mammal derived from the embryo is developed enough, the lactation of the mammal is induced, and the milk is then collected. The milk then contains the FVII of interest secreted by the transgenic animal.

An example of protein preparation in the milk of a female mammal other than a human being is given in patent application EP0527063, the teaching of which could be replicated for the production of the factor VII of the invention.

Secretion of the factor VII by the mammary glands, enabling secretion thereof into the milk of the transgenic mammal, involves the control of the expression of the factor VII in a tissue-dependent manner. Such control methods are well known to a person skilled in the art. Control of the expression is performed thanks to sequences enabling the expression of the protein towards a particular tissue. In particular, these consist of WAP, beta-casein and beta-lactoglobulin promoter sequences and signal peptide sequences; the list not being exhaustive.

In a preferred embodiment, the factor VII according to the invention is produced in the milk of transgenic rabbits.

In a particularly advantageous manner, the expression in the mammary glands of the rabbit is performed under the control of the beta-casein promoter well known to a person skilled in the art. In particular, a plasmid containing the beta-casein promotor is made by introduction of a sequence containing the beta-casein gene promoter, this plasmid being made so as to be able to receive a foreign gene placed under the control of this promoter. The gene coding for the human FVII is integrated, and placed under the control of the beta-casein promoter. The plasmid containing the promoter and the sequence coding for the protein of interest is digested by restriction enzymes to release the DNA fragment containing the beta-casein promoter and the human FVII sequence. After purification, the fragments are introduced, by microinjection, into the male pronucleus of wildtype rabbit embryos. Afterwards, the embryos are cultured before

transfer into the hormonally-prepared oviduct of wildtype females. When these females give birth, the descendants are assessed by PCR to determine the transgenic animals. The number of copies of the transgene and its integrity are revealed by the Southern technique from the DNA extracted from the obtained young transgenic rabbits. The concentration of human FVII expressed in the milk of the female transgenic descendants is assessed through immune-enzymatic tests.

In a particular embodiment, the factor VII useful in the invention is obtained by a method comprising the following steps:

(a) inserting a DNA sequence comprising a gene coding for the factor VII into a non-human mammal embryo, said gene being under the transcriptional control of the beta-casein promoter,

(b) transferring the embryos obtained in step a) into the oviduct of non-human mammal females so that it develops into an adult non-human mammal,

(c) inducting lactation in the female type adult non-human mammal obtained in step b) or in a female descendent of this non-human mammal in which the gene and the promoter are present in its genome,

(d) collecting milk from said non-human mammal, and

(e) purifying the FVII present in the collected milk.

The useful transgenic FVII herein has a substantially homogenous isoelectric point.

By "**isoelectric point**" or "**pI**", it should be understood the pH for which the net elementary charge of the factor VII or factor VIIa molecule is zero, i.e., the pH at which the molecule is electrically neutral (zwitterionic form). The isoelectric point of the factor VII according to the invention may be measured by implementing a technique well known to a person skilled in the art such as iso-electric focusing ("IEF"). This electrophoretic technique enables the separation of the proteins on the basis of their isoelectric point. It consists of a migration, induced by a uniform electrical current, of the proteins in a pH gradient until they reach a pH equivalent to their specific isoelectric point, at which time they stop migrating since their net charge is zero. The IEF gels are used to determine the isoelectric point of a given protein.

By "**Substantially homogenous**", it should be understood that at least 90%, preferably at least 95% of the factor VII molecules of the composition have an isoelectric point comprised within a pH unit difference smaller than or equal to 1.2. In another embodiment of the invention, at least 50%, preferably at least 55%, preferably 60% of the

transgenic factor VII molecules of the composition have an isoelectric point comprised within a pH unit difference smaller than 1, preferably smaller than 0.5. In another preferred embodiment, at least 50%, preferably at least 55%, preferably 60% of the factor VII molecules of the composition have an isoelectric point comprised within a pH unit difference of 0.4.

By "**N-glycan forms**", it should be understood all of the N-glycan forms present at the two N-glycosylation sites of the factor VII of the invention. The N-glycan forms are so-called monocharged, if their total charge is equal to 1. In the context of the present invention, by "charge", it should be understood a phosphate group, a sulphate group, or a sialic acid molecule. Thus, the N-glycan forms are called monocharged if they contain only one phosphate group or one sulphate group or one sialic acid molecule. In contrast with the term "monocharged", the term "bicharged" means that the total charge carried by the N-glycan forms is equal to 2, i.e., they have two charges selected from among a phosphate group, a sulphate group and/or a sialic acid molecule. In other words, the bicharged N-glycan forms have one sialic acid molecule and one phosphate group, or one sialic acid molecule and one sulphate group, or two sialic acid molecules, or two phosphate groups, or two sulphate groups, or one phosphate group and one sulphate group. The term "tricharged" means that the total charge carried by the N-glycan forms is equal to 3, i.e. they have three charges selected from among a phosphate group, a sulphate group and/or a sialic acid molecule. In other words, the tricharged N-glycan forms have one sialic acid molecule, one phosphate group and one sulphate group, or two sialic acid molecules and one phosphate group, or two sialic acid molecules and one sulphate group, or one sialic acid molecule and two phosphate groups, or one sialic acid group and two sulphate groups, or one phosphate group and two sulphate groups, or one sulphate group and two phosphate groups or three sialic acid molecules, or three phosphate groups, or three sulphate groups. In turn, the term "neutral" means that the N-glycan forms do not contain any charge.

The charge of the N-glycan forms of the factor VII according to the invention may be measured by implementing a technique well known to a person skilled in the art, in particular by ultra-high-performance liquid-phase chromatography with an anion exchange resin coupled to a detection by fluorescence (AEX-UPLC/FD). This method allows separating the different N-glycan forms according to their apparent charge (cf., in particular, Hermentin et al, Glycobiology, vol. 6, No. 2, 1996). In the context of anion

exchange chromatography, a positively-charged resin is used as a stationary phase. In general, these positively-charged resins consist of a crosslinked polymer or gel, onto which positively-charged groups are grafted. In an advantageous embodiment of the invention, an aminopropyl-type weak anion exchange column is used.

In the case of the factor VII composition according to the invention, it turns out that among all the N-glycan forms of the factor VII of the composition, at least 50% of the N-glycan forms, at least 60% of the N-glycan forms, preferably at least 65%, preferably at least 70%, preferably at least 75%, preferably at least 80%, preferably at least 85%, preferably at least 90%, preferably at least 95%, are monocharged. In a preferred embodiment, the factor VII molecules having monocharged N-glycan forms represent between 50% and 95% of the factor VII molecules of the composition, preferably between 50% and 90% of the factor VII molecules of the composition, preferably between 50% and 80% of the factor VII molecules of the composition, preferably between 50% and 75% of the factor VII molecules of the composition, preferably between 50% and 70% of the factor VII molecules of the composition, preferably between 50 and 65% of the factor VII molecules of the composition, preferably between 50% and 60% of the factor VII molecules of the composition.

The substantially homogenous isoelectric point of the composition of the factor VII of the combination according to the invention results from the combination of the glycosylation and γ-carboxylation properties of the FVII molecules that compose it.

The transgenic factor VII useful herein features post-translational modification characteristics. In particular, these consist of glycosylation modifications, such as two N-glycosylation sites with a zero or very low level of Galα1,3Gal in the FVII composition, or still low enough so as not to be immunogenic. In contrast, the FVII described herein is not a plasma FVII, i.e., it is not a purified product from human or animal plasma. More particularly, the transgenic FVII useful herein features post-translational modifications, as well as two O-glycosylation sites with defined glycan units, a γ-carboxylation, and specific disulphide bridges.

The FVIIa useful herein may include several post-translational modifications: the first nine or ten N-terminal glutamic acids are γ-carboxylated, Asp_{63} is partially hydroxylated, Ser_{52} and Ser_{60} are O-glycosylated and respectively carry $Glucose(Xylose)_{0-2}$ and Fucose units, Asn_{145} and Asn_{322} are N-glycosylated predominantly by mono-sialylated biantennary complex structures.

Advantageously, at least 80% of the transgenic factor VII molecules useful herein have a γ-carboxylation on nine glutamic acid residues. In another embodiment, at least 85% of said molecules have a γ-carboxylation on nine glutamic acid residues. In another embodiment, between 85% and 100%, preferably between 90% and 100%, preferably between 95% and 100% of said molecules have a γ-carboxylation on nine glutamic acid residues. Advantageously, the degree of γ-carboxylation on the glutamic acid residue 35 (Glu 35) of the factor VII molecules of the composition is lower than 20%. In another embodiment, the degree of γ-carboxylation of the residue Glu35 is lower than 15%, preferably lower than 10%, preferably lower than 5%.

The Galα1,3Gal unit is a structure composed of two α1,3 bonded galactoses. It is located at the end of the oligosaccharide antennas of the N-bonded structures. This unit is known for its immunogenicity. Thus, it is preferred to produce a FVII or a FVIIa whose level of Galα1,3Gal structures is zero or so low that it cannot be distinguished from the background noise obtained by the measurements implemented by currently available analysis devices. This expression equivalently refers to any transgenic FVII whose Galα1,3Gal level is close to that of the plasma FVII. Advantageously, the level of Galα1,3Gal of the FVII composition described herein is not immunogenic for humans. In addition, the FVII useful herein preferably includes, like human FVII, two N-glycosylation sites, at the position 145 and 322, and 2 O-glycosylation sites, at the position 52 and 60. In an N-glycosylation site, the oligosaccharide chains are bonded to an asparagine (N-bonded). In an O-glycosylation site, the oligosaccharide chains are bonded to a serine. The units bonded to these amino acids will be different for each protein of the composition. Nonetheless, for the entire composition, it is possible to quantify the level of each glycan unit, or of each sugar.

The percentages of the different glycans given in the present application do not take the O-glycosylation into account.

Preferably, the FVII composition is characterised in that, among all of the glycan units of the FVII of the composition, at least 40% are mono-sialylated biantennary glycan forms. In another embodiment, the mono-sialylated biantennary forms are present in at least 50%. In another embodiment, the mono-sialylated biantennary forms are present in at least 60%, preferably at least 65%, preferably at least 70%.

Advantageously, the mono-sialylated biantennary glycan forms of FVII are predominant. The FVII composition is characterised in that at least some of the sialic acids of the factor VII involve α2-6 bonds.

Advantageously, at least 65% of the sialic acids of FVII involve α2,6 bonds. Quite advantageously, at least 70%, and possibly 80% and, in particular, at least 90% of the sialic acids of the FVII involve α2,6 bonds.

In a particularly preferred manner, all sialic acids involve α2,6 bonds, i.e., all sialic acids are bound to galactose by an α2,6 bond. The FVII composition described herein may further comprise sialic acids with α2-3 bonds.

According to some embodiments of the invention, 65% to 100% of the sialic acids of the FVII involve α2,6 bonds. More preferably, 70% or 80% to 100% of the sialic acids of the FVII involve α2,6 bonds.

Advantageously, among the mono-sialylated biantennary glycan forms of the FVII, the predominant glycan forms are non-fucosylated.

Preferably, these non-fucosylated mono-sialylated biantennary glycan forms are present in the FVII of the composition at a level higher than 20%. Advantageously, this level is higher than 25%, or higher than 40%. In a particularly advantageous manner, the degree of fucosylation of the FVII composition is comprised between 20% and 50%. In one embodiment, this level may be lower than 20%.

In a particular embodiment, at least 10%, preferably at least 15%, preferably at least 20%, preferably at least 25% of the N-glycan forms of the factors FVII of the composition are high mannose/hybrid.

Preferably, the glycosylation profile described herein convers an improved biological activity and stability on the FVII. The factor VII compositions with a substantially homogenous isoelectric point facilitate the formulation step at an optimum pH, preferably at an optimum pH of 6.0 ± 0.2, of the pharmaceutical composition while avoiding FVII precipitation. Indeed, it is known that at the isoelectric point of a molecule, these will have a tendency to aggregate and precipitate. The factor VII molecules used in the composition of the invention have an isoelectric point comprised between 6.6 and 7.0. This results in a better stability of the factor VII composition, in particular when the latter is formulated at a pH lower than the isoelectric point, and in particular at a pH of 6.0. The improvement in the stability of the factor VII composition allows avoiding the electrostatic interactions responsible for the soluble and insoluble type precipitation and

aggregation phenomena, as well as avoiding the loss of raw materials and therefore a drop in yield leading to a loss of the amount of active principle and therefore potentially in a loss of activity.

In a preferred embodiment, the transgenic FVII is produced by the rabbit in its milk, allowing obtaining a composition where each factor VII molecule of the composition has two N-glycosylation sites. Preferably, all of the FVII molecules of the composition have a level of Galα1,3Gal glycan units lower than 4%, and even zero. Thus, advantageously, the transgenic FVII produced by the rabbit does not have a Galα1,3Gal unit.

The FVII may be purified from the milk by techniques known to a person skilled in the art. For example, a method for purifying a protein of interest from milk, as described in US patent 6,268,487, may comprise the following steps consisting in: a) subjecting the milk to a tangential filtration on a membrane with a sufficient porosity to form a retentate and a permeate, the permeate containing the exogenous protein, b) subjecting the permeate to a chromatographic capturing apparatus so as to move the exogenous protein and obtain an effluent, c) combining the effluent and the retentate, d) repeating steps a) to c) until the FVII is separated from the lipids, casein micelles, and the FVII is recovered.

Advantageously, the FVII of the invention is in the activated form. In one embodiment, the FVII may be activated in vitro by the factors Xa, VIIa, IIa, IXa or XIIa. The FVII may also be typically activated throughout its purification process, in particular by passage on positively-charged chromatography columns.

Multispecific antibody

By "**multispecific antibody**", it should be understood any antibody having at least two bonding sites specific to at least two different antigens, or different epitopes of the same antigen. The term "specific" means that the antibody has the ability to recognise and bond an antigen, substantially with no cross reaction with another antigen. Advantageously, the antibody has, with respect to each antigen, an affinity constant Kd of at least 10^{-6} M, preferably at least 10^{-7} M, more preferably at least 10^{-8} M, 10^{-9} M, or 10^{-10} M.

Thus, the antibodies useful in the invention have the ability of bonding specifically to both the coagulation factor IX and the coagulation factor X in the activated form, or not.

Preferably, the antibody used in the invention, which has the ability of bonding specifically to both the coagulation factor IX and the coagulation factor X has the ability to act as a substitute for the factor VIII (FVIII), which means that this antibody promotes the activation of FX by FIXa.

Such multispecific, preferably bispecific, antibodies may be obtained by various methods known to a person skilled in the art, for example by chemical conjugation, or by using quadromas, which result from the fusion between two hybridomas producing two different monoclonal antibodies; or by genetic recombination.

Thus, the polynucleotides coding for such antibodies may be inserted into expression vectors and expressed in host cells or organisms adapted by techniques well known to a person skilled in the art.

The antibodies useful herein may have a very simple format, built from single-chain Fv fragments (scFv) of two or more antibodies, associated by a suitable peptide linker.

By “Fv”, it should be understood the smallest antibody fragment preserving the antigen recognition and bonding properties. An “Fv” fragment is a dimer (V_H + V_L dimer) consisting of a variable region (V_H) borne by a heavy chain (H) and an adjacent variable region (V_L) borne by a light chain (L).

Alternatively, it may consist of a full-length antibody, preferably containing an Fc region. Several formats are possible. For example, in a first format, scFv fragments of an antibody A are fused at the ends (in general N-terminus) of the heavy chains of an antibody B. The resulting antibody has one single type of heavy chain, which contains the VH, CH1, CH2 and CH3 domains of the antibody B and the VH and VL domains of the antibody A, and one single type of light chain which contains the VL and CL domains of the antibody B (Qu et al. Blood, 111, 2211-2219, 2008). In a second format, the heavy chain and the light chain of an antibody A are associated with the heavy chain and the light chain of an antibody B. Where appropriate, mutations, for example of the “knobs into holes” type (Ridgway et al, Protein Eng, 9, 617-21, 1996; US patent No. 7,695,936) may be introduced to avoid mismatches.

Unless indicated otherwise, in the present description, the term “factor IX” indifferently refers to the inactivated factor IX or to the activated factor IX (FIXa).

Unless indicated otherwise, in the present description, the term “factor X” indifferently refers to the inactivated factor X or to the activated factor X (FXa).

An antibody recognising (i) the FIX and/or the FIXa, and (ii) the FX and/or the FXa may be obtained, in particular according to the methods described in patent applications WO2005/035756, WO2006/109592 or WO2012/067176.

In a preferred embodiment, said antibody is emicizumab. For example, the production of this antibody is described in the patent application WO2018047813 or the patent application EP1688488.

Pharmaceutical compositions and posologies

The factor VII and the antibodies may be formulated in the form of separate pharmaceutical compositions, or combined within the same pharmaceutical composition. In the case of separate administration, the FVII and the antibodies may be formulated in a manner suited to administration according to different or identical routes.

Thus, for example, FVII may be administered intravenously, subcutaneously or intramuscularly.

The antibody may also be administered, for example, intravenously, subcutaneously or intramuscularly.

For example, a Factor VII composition may be like that one described in the patent application WO2010/149907.

Thus, in one embodiment, the composition comprises:

- factor VII, preferably in the factor VIIa form;
- arginine, possibly in the hydrochloride form;
- isoleucine;
- lysine;
- glycine;
- trisodium citrate or calcium chloride;
- and, where appropriate, polysorbate 80 or polysorbate 20.

More particularly, the composition may comprise:

- factor VII, preferably in the factor VIIa form;
- from 10 to 40 g/L of arginine, possibly in the hydrochloride form;
- from 4.2 to 6.6 g/L of isoleucine;
- from 0.6 to 1.8 g/L of lysine;
- from 0.6 to 1.8 g/L of glycine;
- from 0 to 0.2 g/L of trisodium citrate or from 1 to 2 g/L of calcium chloride;

- and, where appropriate, from 0 to 0.5 g/L of polysorbate 80.

The FVII composition, which possibly also comprises at least one multispecific antibody as described herein, may be stored in the liquid form or in the solid form, typically obtained by desiccation. The compositions disclosed hereinabove are determined with respect to the compositions in the liquid form, before desiccation, or after reconstitution in the form of an injectable preparation.

Desiccation is a method for high-stage water elimination. It consists of a dehydration intended to eliminate as much water as possible. This phenomenon may be natural or forced. This desiccation may be carried out by means of lyophilisation, atomisation and cryoatomisation techniques.

The preferred method for obtaining the solid form of the composition for pharmaceutical usage described herein is lyophilisation.

Lyophilisation methods are well known to a person skilled in the art, cf., for example [Wang et al, Lyophilisation and development of solid protein pharmaceuticals, International Journal of Pharmaceutics, Vol 203, p 1-60, 2000].

Other suitable processes for reducing the degree of humidity or the water content of the composition may be considered. Preferably, the degree of humidity is lower than or equal to 3% by weight, preferably lower than or equal to 2.5%, preferably lower than or equal to 2%, preferably lower than or equal to 1.5%.

The solid composition, preferably in the Lyophilised form, may be dissolved in water for injectable preparations (or “water for injection or WFI”), to obtain a formulation for therapeutic usage.

The injectable formulation may be administered parenterally (intravenously, subcutaneously, intramuscularly), in an amount determined by the practitioner. The administration of the liquid form (before desiccation) or of the solid form, by any suitable route and means, is not excluded.

The FVII dosage useful in the invention may be determined in an appropriate manner according to the formulation type, administration method, age and weight of the patient, symptoms of the patient, severity of the disease, etc.

Advantageously, the FVII dose to be administered according to the invention may be selected between 270 µg/kg and 2.70 µg/kg. Preferably, the FVII dose to be administered is lower than 270 µg/kg of bodyweight, preferably it is lower than 225 µg/kg of bodyweight, preferably it is lower than 180 µg/kg of bodyweight, preferably it is lower

than 135 µg/kg of bodyweight, preferably it is lower than 90 µg/kg of bodyweight, preferably it is lower than 45 µg/kg of bodyweight, preferably it is lower than 9 µg/kg, preferably it is lower than 5.4 µg/kg, preferably it is lower than 2.7 µg/kg.

A multispecific antibody composition, like the emicizumab antibody, is, for example, like those described in the patent applications WO2017/188356 and WO2018/047813.

Thus, in one embodiment, the composition is a liquid composition.

In one embodiment, the composition comprises:

- an antibody bispecific for the factor IX and the factor X,
- a surfactant such as poloxamer 188 or polysorbate 20,
- a histidine-aspartic acid buffer,
- arginine

More particularly, the composition may comprise:

- from 20 mg/mL to 180 mg/mL of antibodies bispecific for the factor IX and the factor X,
- from 0.2 mg/mL to 1 mg/mL of poloxamer 188,
- from 10 mM to 40 mM of a histidine-aspartic acid buffer
- from 100 mM to 300 mM of arginine,

at a pH comprised between 4.5 and 6.5

The dosage of the multispecific antibody composition, such as the emicizumab antibody, useful in the invention, may be appropriately determined according to the formulation type, the method administration, the age and weight of the patient, the symptoms of the patient, the severity of the disease, etc. For example, the antibody dose may be from 0.3 to 5 mg/kg, preferably at most 3 mg/kg a week during an initiation period, which could last 4 weeks, for example, followed by a maintenance dose, which is preferably lower, for example, 1.5 mg/kg a week. Preferably, the administered antibody dose is lower than 5 mg/kg of bodyweight, preferably it is lower than 3 mg/kg of bodyweight, preferably it is lower than 1.5 mg/kg of bodyweight, preferably it is lower than 1 mg/kg of bodyweight, preferably it is lower than 0.5 mg/kg of bodyweight, preferably it is lower than 0.1 mg/kg of bodyweight, preferably it is lower than 0.05 mg/kg of bodyweight.

The antibody composition useful in the invention may be administered to a patient by any appropriate route, for example intravenously, intramuscularly, intraperitoneally,

intra-cerebrospinally, transdermally, subcutaneously, intra-articularly, sublingually, intrasynovially, orally or by inhalation. Preferably, the intravenous route or the subcutaneous route is preferred.

According to a particular embodiment, the factor VII and the antibody are administered to the patient simultaneously.

According to another particular embodiment, the factor VII and the antibody are administered to the patient separately, preferably sequentially.

Therapeutic indications

The combination described herein allows preventing or treating coagulation disorders, in particular haemophilia with a factor VIII deficit (type A haemophilia, preferably acquired type A haemophilia).

Preferably, the patients are patients who have type A haemophilia, with anti-factor VIII.

The combination described herein allows preventing or treating coagulation disorders, in particular factor VII deficiencies.

The combination described herein combines the rapid effect of FVII activating the extrinsic route of the coagulation cascade and the prolonged effect of the multispecific antibodies described herein which activate the intrinsic route of the coagulation cascade. The combination allows for better patient management.

Examples:

Example 1: Purification and extraction of transgenic FVII

The factor VII purification and extraction method implemented in this example is that one described in the application EP12305882. The steps of this method are described hereinafter. The transgenic rabbit milk is obtained from the transgenic rabbit line. The frozen milk from transgenic rabbits is thawed and concentrated in the form of a pool of transgenic rabbit milk.

Afterwards, the pool of transgenic rabbit milk thus obtained is subjected to a clarification step using a depth filter with a porosity of 0.2 µm, in order to remove lipids and insoluble compounds. Afterwards, the milk thus clarified is subjected to a viral inactivation step by treatment with a detergent-type solvent, for example, polysorbate 80 or Tri-n-Butyl phosphate at 25°C ± 2°C for at least two hours. In particular, such a

treatment allows effectively inactivating viruses, and, in particular, non-enveloped viruses. Afterwards, the clarified and virally-inactivated milk is subjected to an affinity chromatography step using an affinity ligand specific for the factor VII/factor VIIa. Afterwards, the factor VII eluate obtained upon completion of this chromatography step is subjected to an ultrafiltration and formulation step, thereby allowing obtaining an intermediate factor VII concentrate having a purity of 95%.

Afterwards, the intermediate factor VII concentrate is subjected to a filtration step using a filter having a porosity of 0.1 µm to 0.2 µm followed by a nanofiltration step on filters having a porosity of 20 nm then 15 nm. Afterwards, the product thus obtained and containing the factor VII is subjected to Q Sepharose XL gel type chromatography then to a CHT-I type chromatography step followed by a SEC Superdex 200 type chromatography. Afterwards, the factor VII concentrate thus obtained is subjected to a stabilisation step then to filtration on a filter having a porosity of 0.2 µm.

The method thus described allows obtaining a factor VII concentrate having a purity of about 99.9995%.

Example 2: Comparison of the thrombogenic potential of Novoseven®, Sevenfact® and Hemlibra®

A person skilled in the art can measure the thrombogenic potential of Novoseven®, Sevenfact® and Hemlibra® (also called emicizumab) by carrying out the following protocol.

Reagents:

- thrombin calibrator (Stago)
- 5 pM PPP reagent (Stago)
- PPP low reagent (Stago)
- CK-Prest (Stago)
- Fluo-buffer (Stago)
- Fluo-substrate (Stago)
- FVIII-deficient plasma (Siemens)
- Sevenfact®/transgenic factor VII produced in rabbits 1 mg/ml (LFB)
- PNP (Cryopep)
- Novoseven® (NovoNordisk)

- Hemlibra®/Emicizumab (Roche/Genentech/Chugai)

Method:

The thrombin generation test consists in activating coagulation ex vivo either with a mixture of tissue factor and phospholipids (TF/PL), or by using cephalin and then by measuring the concentration of generated thrombin over time.

- Measurement of the thrombogenic potential of Novoseven® after induction of coagulation with TF/P:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of PPP reagent (Stago) containing 0.5 pM of Tissue Factor (TF) and 4 µM of phospholipids (PL). The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The therapeutic dose of FVIIa is 270 µg/kg, which corresponds to 6 µg/mL of FVIIa in the plasma, considering a 100% recovery. The thrombin generation test is carried out at Novoseven® doses of 0 µg/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, and 6 µg/mL, in the presence of 0.5 pM TF / 2 µM PL (coagulation inducer).

- Measurement of the thrombogenic potential of Novoseven® after induction of coagulation with cephalin:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of cephalin (CK-Prest reconstituted with 5 mL of distilled H_20).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$ which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out at Novoseven® doses of 0 µg/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, and 6 µg/mL, in the presence of 20 µL of cephalin (coagulation inducer).

- Measurement of the thrombogenic potential of Sevenfact® after induction of coagulation with TF/PL:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of PPP reagent (Stago) containing 0.5 pM of Tissue Factor (TF) and 4 µM of phospholipids (PL). The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out at Sevenfact® doses of 0 µg/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, and 6 µg/mL, in the presence of 0.5 pM TF/2 µM PL (coagulation inducer).

- Measurement of the thrombogenic potential of Sevenfact® after induction of coagulation with cephalin:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of cephalin (CK-Prest reconstituted with 5 mL of distilled H_20).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out at Sevenfact® doses of 0 µg/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, and 6 µg/mL, in the presence of 20 µL of cephalin.

- Measurement of the thrombogenic potential of Hemlibra® after induction of coagulation with TF/PL:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of PPP reagent (Stago) containing 0.5 pM of Tissue Factor (TF) and 4 µM of phospholipids (PL).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

Hemlibra® (Roche / Genentech / Chugai, USA), a bispecific antibody mimicking the function of FVIII, is used at the maximum concentration of 50 µg/mL, which is the concentration detected in patients on treatment (Oldenburg et al. NEJM, 2017). The thrombin generation test is carried out at Hemlibra® doses of 0 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL, in the presence of 0.5 pM TF / 4 µM PL (coagulation inducer).

- Measurement of the thrombogenic potential of Hemlibra® after induction of coagulation with cephalin:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of cephalin (CK-Prest reconstituted with 5 mL of distilled H_20).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out at Helibra® doses of 0 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL, in the presence of 20 µL of cephalin.

For all of these tests, the apparition of fluorescence is measured on a Fluoroskan Ascent fluorometer (ThermoLabsystems) at an excitation wavelength of 390 nm and an emission wavelength of 460 nm. Afterwards, the thrombinograms (curves showing the intensity of fluorescence over time) are analysed by using the Thrombinoscope™ software, which converts the fluorescence value into nM of thrombin by a comparative calculation.

Thrombin is generated and the key variables for assessing the potency of the different drugs are recorded and compared: endogenous thrombin potential (ETP), peak height, latency time and velocity.

Example 3: Assessment of the synergistic thrombogenic potentials of Novoseven® and Hemlibra® or SevenFact® and Hemlibra®

A person skilled in the art can measure the thrombogenic potential of the Novoseven®/Hemlibra® and Sevenfact®/Hemlibra® combinations by carrying out the following protocol.

Reagents:

The reagents, the apparatus and the experimental protocol in FVIII-deficient plasma are identical to those described in Example 2.

Method:

- Measurement of the thrombogenic potential of the Novoseven® + Hemlibra® combination after induction of coagulation with TF/PL:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of PPP reagent (Stago) containing 0.5 pM of Tissue Factor (TF) and 4 µM of phospholipids (PL).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out in the presence of 0.5 pM TF / 4 µM PL (coagulation inducer) on several Novoseven®/Hemlibra® combinations. The composition

containing the largest amount of product consists of 6 µg/mL of Novoseven® and 50 µg/mL of Hemlibra® at their maximum.

The thrombogenic potential obtained in the presence of the combination of products is compared to the potential of the single products. To consider a synergistic effect of the product combination, lower doses are assessed to be sure not to saturate the thrombin detection.

The tested compositions contain:

Combination	Hemlibra® (µg/mL)	NovoSeven® (µg/mL)
Combination 1	50	6
Combination 2	50	5
Combination 3	40	4
Combination 4	30	3
Combination 5	20	2
Combination 6	10	1

- Measurement of the thrombogenic potential of the Novoseven® + Hemlibra® combination after induction of coagulation with cephalin

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of cephalin (CK-Prest reconstituted with 5 mL of distilled H_20).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out in the presence of 20 µL of cephalin on several Novoseven®/Hemlibra® combinations. The composition containing the largest amount of product consists of 6 µg/mL of Novoseven® and 50 µg/mL of Hemlibra® at their maximum.

The thrombogenic potential obtained in the presence of the combination of products is compared to the potential of the single products. To consider a synergistic effect of the product combination, lower doses are assessed to be sure not to saturate the thrombin detection.

The tested compositions contain:

Combination	Hemlibra® (µg/mL)	NovoSeven® (µg/mL)
Combination 1	50	6
Combination 2	50	5

Combination 3	40	4
Combination 4	30	3
Combination 5	20	2
Combination 6	10	1

- Measurement of the thrombogenic potential of the Sevenfact® + Hemlibra® combination after induction of coagulation with TF/PL:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of PPP reagent (Stago) containing 0.5 pM of Tissue Factor (TF) and 4 µM of phospholipids (PL).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out in the presence of 0.5 pM TF / 4 µM PL (coagulation inducer) on several Sevenfact®/Hemlibra® combinations. The composition containing the largest amount of product consists of 6 µg/mL of Sevenfact® and 50 µg/mL of Hemlibra® at their maximum.

The thrombogenic potential obtained in the presence of the combination of products is compared to the potential of the single products. To consider a synergistic effect of the product combination, lower doses are assessed to be sure not to saturate the thrombin detection.

The tested compositions contain:

Combination	Hemlibra® (µg/mL)	Sevenfact® (µg/mL)
Combination 1	50	6
Combination 2	50	5
Combination 3	40	4
Combination 4	30	3
Combination 4	20	2
Combination 6	10	1

- Measurement of the thrombogenic potential of the Sevenfact® + Hemlibra® combination after induction of coagulation with cephalin:

The thrombin generation test is carried out in 80 µL of an FVIII-deficient plasma pool that mimics a haemophilia A plasma in the presence of 20 µL of cephalin (CK-Prest reconstituted with 5 mL of distilled H_20).

The reaction is initiated by adding 20 µL of Fluca-kit (substrate + $CaCl_2$) which is the starting point of the measurement of thrombin generation.

The thrombin generation test is carried out in the presence of 20 µL of cephalin on several Sevenfact®/Hemlibra® combinations. The composition containing the largest amount of product consists of 6 µg/mL of Sevenfact® and 50 µg/mL of Hemlibra® at their maximum.

The thrombogenic potential obtained in the presence of the combination of products is compared to the potential of the single products. To consider a synergistic effect of the product combination, lower doses are assessed to be sure not to saturate the thrombin detection.

The tested compositions contain:

Combination	Hemlibra® (µg/mL)	Sevenfact® (µg/mL)
Combination 1	50	6
Combination 2	50	5
Combination 3	40	4
Combination 4	30	3
Combination 5	20	2
Combination 6	10	1

For all of these tests, the apparition of fluorescence is measured on a Fluoroskan Ascent fluorometer (ThermoLabsystems) at an excitation wavelength of 390 nm and an emission wavelength of 460 nm. Afterwards, the thrombinograms (curves showing the intensity of fluorescence over time) are analysed by using the Thrombinoscope™ software, which converts the fluorescence value into nM of thrombin by a comparative calculation.

A synergistic effect is considered, for example, when at least one of the parameters calculated from the thrombin generation test for a given combination is greater than the sum of each of these parameters obtained with the components alone, deducted from the background noise of the experiment.

Example 4: Comparison of the potential of Sevenfact™, Hemlibra® and of the combination of both in Haemophilia A plasma

Reagents:

- thrombin calibrator (Stago)

- 1 pM TF PRP reagent (Stago)
- 4 µM PL MP reagent (Stago)
- Fluo-buffer (Stago)
- Fluo-substrate (Stago)
- Sevenfact™: Transgenic factor VII produced in rabbits 1 mg/ml (LFB)
- Hemlibra®: Emicizumab (Roche / Genentech / Chugai)
- Haemophilia A plasma (Cryopep)
- Owren Koller (Stago)

Method:

The thrombin generation test consists in activating coagulation ex vivo, for example with a mixture of tissue factor and phospholipids (TF / PL), then measuring the concentration of generated thrombin over time. The thrombin generation tests are carried out with 80 µL of haemophilia A plasma (Cryopep), in the presence of 20 µL of a mixture of PRP and MP reagents (Stago) containing 0.5 pM of tissue factor and 4 µM of phospholipids.

The reaction is initiated by the addition of 20 µL of Fluca-kit (Fluo substrate + $CaCl_2$), which corresponds to the starting point of the measurement of thrombin generation (TG).

Fluorescence is measured by fluorimetry using the Fluoroskan Ascent (ThermoLabsystems) apparatus at an excitation wavelength of 390 nm and an emission wavelength of 460 nm. The thrombinograms are analysed using the Thrombinoscope™ software which converts the fluorescence intensity into a molar concentration of thrombin (nM) by comparative calculation.

To measure the thrombogenic potential of the two molecules, several haemophilia A plasmas are studied. The highest therapeutic dose of FVIIa is 270 µg/kg, which corresponds to 6 µg/mL of FVIIa (or 120 nM) in the plasma. The use of this dose may be considered as a maximum potential for thrombin generation. On the basis of the concentrations of product in circulation in the bloodstream obtained in the patients, Sevenfact™ concentrations comprised between 20 and 100 nM are also studied. Hemlibra® (Roche / Genentech / Chugai, USA), a bispecific antibody imitating the function of FVIII, is used at the maximum concentration of 120 µg/mL. The concentration currently detected in patients on treatment is 50 µg/mL (or 300 nM) (Oldenburg et al. NEJM, 2017).

Thus, Hemlibra® is herein used at about 300 nM (50 µg/mL). The variables studied to measure the thrombogenic potential of Hemlibra® and Sevenfact™ are:

- the endogenous thrombin potential (ETP): area under the curve representing the total amount of generated thrombin,
- the peak height: measured maximum concentration of thrombin, and
- the thrombin generation velocity: the thrombin formation rate.

2 - Results

2.1 - Effect of Sevenfact™ or Hemlibra® on Haemophilia A plasmas

2.1.1 - Assessment in Batch 1 of Haemophilia A plasma

In this matrix, very low thrombin generation signals originating from the two compounds are obtained, regardless of the used concentrations. Indeed, the observed thrombin generation is almost zero for Hemlibra® and Sevenfact™ at concentrations of 20 and 40 nM. With 100 nM of Sevenfact™, a very low thrombin generation peak is observed (Table 1).

	Sevenfact™ 20nM	Sevenfact™ 40nM	Sevenfact™ 100nM	Hemlibra® 300nM
ETP (nM/min)	125	154.25	128.75	128.25
Peak (nM)	7.105	9.46	16.55	6.41
Velocity (nM/min)	0.59	0.84	1.64	0.42

Table 1: Thrombin generation parameters from Batch 1 of Haemophilia A plasma treated with Sevenfact™ or Hemlibra®

Thus, each molecule used individually induces just a very small thrombin generation.

2.1.2 - Assessment in Batch 2 of Haemophilia A plasma

A second batch of Haemophilia A plasma has been tested. Herein again, a very small thrombin generation is observed with the use of Hemlibra® and Sevenfact™, with a maximum thrombin generation peak at a concentration of 100 nM of Sevenfact™ (Table 2).

	Sevenfact™ 20 nM	Sevenfact™ 40 nM	Sevenfact™ 100 nM	Hemlibra® 300 nM
ETP (nM/min)	221.25	280.75	414.25	196
Peak (nM)	9.93	12.84	21.04	8.895
Velocity (nM/min)	0.7	0.96	1.75	0.51

Table 2: Thrombin generation parameters from Batch 2 of Haemophilia A plasma treated with Sevenfact™ or Hemlibra®

In this matrix, Sevenfact™ and Hemlibra® used separately have a low thrombogenic potential.

Example 5: Assessment of the synergistic combination of Sevenfact™ + Hemlibra®

1 - Protocol

The reagents, the apparatus and the experimental protocol in the haemophilia A plasma are identical to those described in Example 2.

2 - Results

As seen in Example 2, Sevenfact™ and Hemlibra® used individually induce a small thrombin generation in Haemophilia A plasmas. The synergistic effect of the Sevenfact™ and Hemlibra® combination is studied here. Three concentrations of Sevenfact™ are studied (20 nM, 40 nM and 100 nM) in the presence of a Hemlibra® concentration of 300 nM. A synergistic effect is taken into account if the effect of the Sevenfact™+Hemlibra® combination is at least 2 times greater than the sum of the effects of Sevenfact™ and Hemlibra® considered separately for at least one of the parameters of the thrombin generation test (ETP, peak thrombin generation and velocity).

2.1 - Effect of Sevenfact™ and Hemlibra® on Haemophilia A plasmas after coagulation induction with TF/PL

2.1.1 - Assessment in Batch 1 of Haemophilia A plasma

The results are reported in Table 3 and Figure 1. At a very low Sevenfact™ concentration of 20 nM, the ratios for the ETP (Figure 1A), for the thrombin peak (Figure 1B) and for the velocity (Figure 1C) of the Sevenfact™+Hemlibra® combination are,

respectively, 2.14, 2.95 and 4.19. Thus, even at the tested lowest concentration, a synergistic thrombogenic effect is observed.

At a concentration of 40 nM, for all of the tested parameters, the ratio is greater than 2. The ratio obtained for the ETP is 2.75 (Figure 1A), the ratio obtained for the thrombin peak is 3.96 (Figure 1B) and that obtained for the velocity reaches a value of 6.21 (Figure 1C). In other words, the thrombin formation rate is multiplied by 6 when Sevenfact™ and Hemlibra® are used in combination.

The synergistic effect is the greatest at a Sevenfact™ concentration of 100 nM. At a concentration of 100 nM, for all of the tested parameters, the ratio is greater than 2. The ratio obtained for the ETP is 4.00 (Figure 1A) and the ratio for the thrombin peak is 4.81 (Figure 1B), which means that the maximum generated thrombin concentration is almost 5 times higher when Hemlibra® and Sevenfact™ are used in combination. The ratio corresponding to the velocity is 9.58 (Figure 1C), which means that thrombin is generated almost 10 times faster when Sevenfact™ and Hemlibra® are used in combination.

		Combinations			Combination/sum ratios		
ETP 40nM Sevenfact™ + ETP 300nM Hemlibra®	ETP 100nM Sevenfact™ + ETP 300nM Hemlibra®	ETP (20nM Sevenfact™ + 300nM Hemlibra®)	ETP (40nM Sevanfact™ + 300nM Hemlibra®)	ETP (100nM Sevenfact™ + 300nM Hemlibra®)	20nM Sevenfact™	40nM Sevenfact™	100nM Sevenfact™
282.50	257	543	777.50	1029	2.14	2.75	4.00

		Combinations			Combination/sum ratios		
Peak 40nM Sevenfact™ + Peak 300nM Hemlibra®	Peak 100nM Sevenfact™ + Peak 300nM Hemlibra®	Peak (20nM Sevenfact™ + 300nM Hemlibra®)	Peak (40nM Sevenfact™ + 300nM Hemlibra®)	Peak (100nM Sevenfact™ + 300nM Hemlibra®)	20nM Sevenfact™	40nM Sevenfact™	100nM Sevenfact™
15.87	22.965	39.915	62.9	110.565	2.95	3.96	4.81

		Combinations			Combination/sum ratios		
Velocity 40nM Sevenfact™ + Velocity 300nM Hemlibra®	Velocity 100nM Sevenfact™ + Velocity 300nM Hemlibra®)	Velocity (20nM Sevenfact™ + 300nM Hemlibra®)	Velocity (40nM Sevenfact™ + 300nM Hemlibra®)	Velocity (100nM Sevenfact™ + 300nM Hemlibra®)	20nM Sevenfact™	40nM Sevenfact™	100nM Sevenfact™
1.42	2.06	4.24	7.86	19.78	4.19	6.21	9.58

Table 3: Thrombin generation parameters from Batch 1 of Haemophilia A plasma treated with the Sevenfact™ + Hemlibra® combination

19742443.5

NO/EP3806891

ETP 20nM Sevenfact™ + ETP 300nM Hemlibra®	253.25

Peak 20nM Sevenfact™ + Peak 300nM Hemlibra®	13.515

Velocity 20nM Sevenfact™ + Velocity 300nM Hemlibra®	1.01

In conclusion, for all of the tested concentrations of Sevenfact™, Sevenfact™ and Hemlibra® used in combination have a synergistic effect on thrombin generation.

2.1.2 - Assessment in Batch 2 of Haemophilia A plasma

The results are reported in Table 4 and Figure 2. At a very low Sevenfact™ concentration of 20 nM, a 2.21 ratio is obtained for the ETP parameter (Figure 2A), a 2.34 ratio is obtained for the thrombin peak (Figure 2B), and a 2.9 ratio is obtained for the velocity parameter (Figure 2C) of the Sevenfact™+Hemlibra® combination. Thus, even at the lowest tested concentration of Sevenfact™, a synergistic thrombogenic effect is observed.

At a concentration of 40 nM, the ratio corresponding to the ETP is 2.29 (Figure 2A), that corresponding to the thrombin peak is 2.79 (Figure 2B) and the ratio corresponding to the velocity is 3.68 (Figure 2C), which means that the use of Sevenfact™ in combination with Hemlibra® enables the formation of thrombin about 4 times faster.

The synergistic effect is the greatest with a Sevenfact™ concentration of 100 nM. At a concentration of 100 nM, the ratio corresponding to the thrombin generation peak is 3.41 (Figure 2B) and that corresponding to the velocity is 5.63 (Figure 2C), which means that thrombin is generated almost 6 times faster and that the reached thrombin concentration is multiplied almost by 4 when Sevenfact™ is used in combination with Hemlibra®.

		Combinations			Combination/sum ratios		
ETP 40nM Sevenfact™ + ETP 300nM Hemlibra®	ETP 100nM Sevenfact™ + ETP 300nM Hemlibra®	ETP (20nM Sevenfact™ + 300nM Hemlibra®)	ETP (40nM Sevenfact™ + 300nM Hemlibra®)	ETP (100nM Sevenfact™ + 300nM Hemlibra®)	20nM Sevenfact™	40nM Sevenfact™	100nM Sevenfact™
476.75	610.25	920.75	1091.25	1275.50	2.21	2.29	2.09

		Combinations			Combination/sum ratios		
Peak 40nM Sevenfact™ + Peak 300nM Hemlibra®	Peak 100nM Sevenfact™ + Peak 300nM Hemlibra®	Peak (20nM Sevenfact™ + 300nM Hemlibra®)	Peak (40nM Sevenfact™ + 300nM Hemlibra®)	Peak (100nM Sevenfact™ + 300nM Hemlibra®)	20nM Sevenfact™	40nM Sevenfact™	100nM Sevenfact™
21.735	29.935	43.96	60.745	101.975	2.34	2.79	3.41

		Combinations			Combination/sum ratios		
Velocity 40nM Sevenfact™ + Velocity 300nM Hemlibra®	Velocity 100nM Sevenfact™ + ETP 300nM Hemlibra®	Velocity (20nM Sevenfact™ + 300nM Hemlibra®)	Velocity (40nM Sevenfact™ + 300nM Hemlibra®)	Velocity (100nM Sevenfact™ + 300nM Hemlibra®)	20nM Sevenfact™	40nM Sevenfact™	100nM Sevenfact™
1.48	2.27	3.52	5.44	12.77	2.9	3.68	5.63

Table 4: Thrombin generation parameters from Batch 2 of Haemophilia A plasma treated with the Sevenfact™ + Hemlibra® combination

19742443.5

NO/EP3806891

ETP 20nM Sevenfact™ + ETP 300nM Hemlibra®	417.25

Peak 20nM Sevenfact™ + Peak 300nM Hemlibra®	18.825

Velocity 20nM Sevenfact™ + Velocity 300nM Hemlibra®	1.21

In conclusion, for all of the tested concentrations of Sevenfact™, Sevenfact™ and Hemlibra® used in combination have a synergistic effect on thrombin generation.

P A T E N T K R A V

1. Farmasøytisk sammensetning som omfatter:
   a. transgen faktor VII, og
   b. et multispesifikt antistoff som er rettet mot faktor IX og faktor X.

2. Farmasøytisk sammensetning i henhold til krav 1, hvor den transgene faktoren VII er en menneskelig eller human faktor VII som er utledet fra produksjon av epitelceller til melkekjertlene eller brystkjertlene til et transgent ikke-humant pattedyr.

3. Farmasøytisk sammensetning i henhold til krav 2, hvor det transgene pattedyret er en kanin.

4. Farmasøytisk sammensetning i henhold til et hvilket som helst av krav 1 til 3, hvor antistoffet er emicizumab.

5. Kombinasjonsprodukt som omfatter:
   a. transgen faktor VII, og
   b. et multispesifikt antistoff som er rettet mot faktor IX og faktor X, for dets anvendelse for forebygging eller behandling av en koagulasjonsforstyrrelse hos en pasient.

6. Kombinasjonsprodukt for dets anvendelse i henhold til krav 5, i behandlingen av hemofili / blødersykdom A.

7. Kombinasjonsprodukt for dets anvendelse i henhold til et av krav 5 eller 6, i behandlingen av hemofili / blødersykdom A med faktor VIII-inhibitorer.

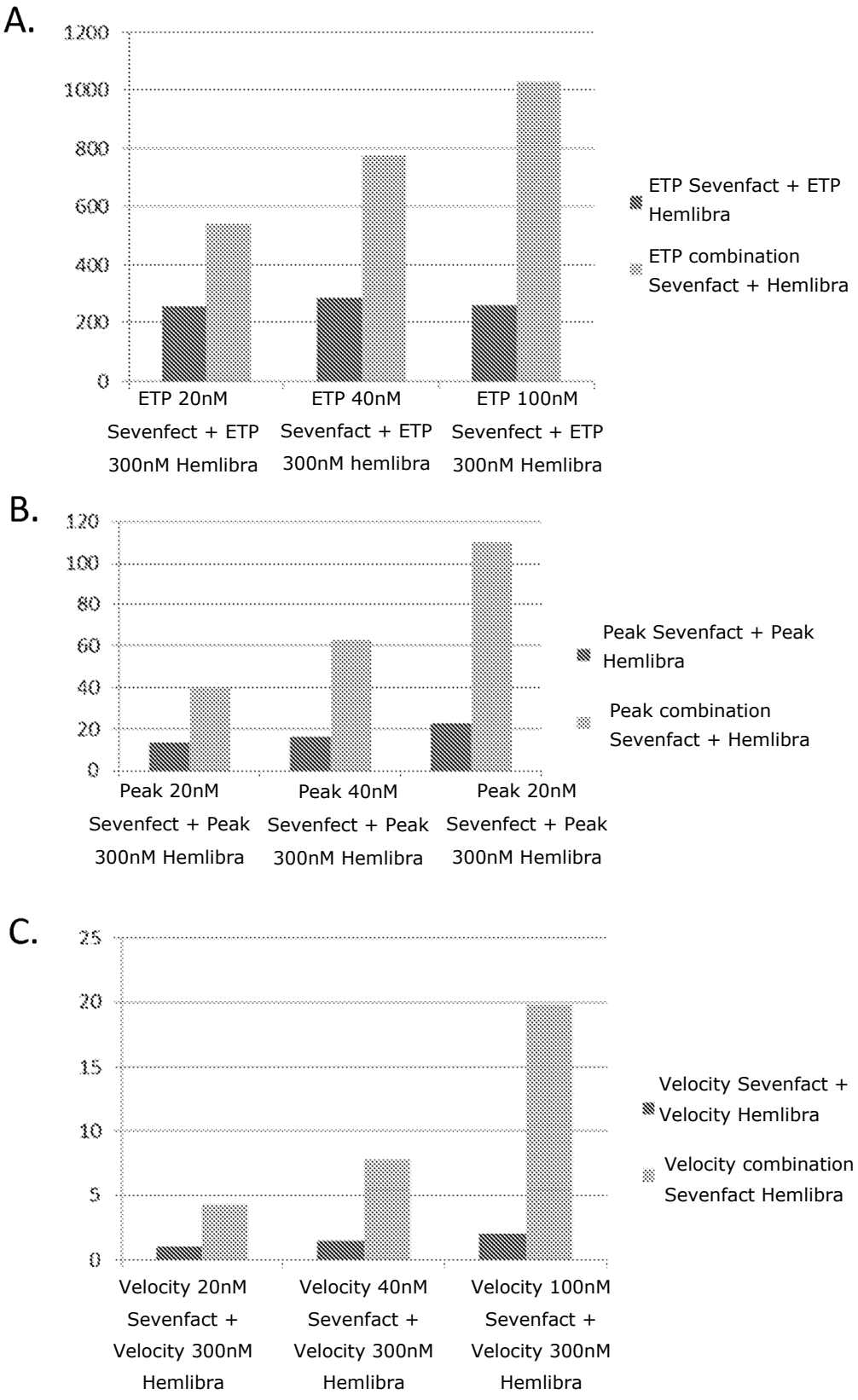
8. Kombinasjonsprodukt for dets anvendelse i henhold til krav 5 til 7, hvor nevnte kombinasjonsprodukt er i form av en farmasøytisk sammensetning slik som definert i et hvilket som helst av krav 1 til 4.

9. Kombinasjonsprodukt for dets anvendelse i henhold til krav 5 til 8, hvor faktor Vila og nevnte antistoff er i en form som er egnet for samtidig administrering til pasienten.

10. Kombinasjonsprodukt for dets anvendelse i henhold til krav 5 til 8, hvor nevnte faktor Vila og nevnte antistoff er i former som er egnet for separat administrering til pasienten.

11. Sett som omfatter:

- en beholder som inneholder transgen faktor FVII; og
- en annen beholder som inneholder et antistoff som er rettet mot faktor IX og faktor X.


A.
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ETP 100nM Sevenfect + ETP 300nM Hemlibra
ETP Sevenfact + ETP Hemlibra
ETP combination Sevenfact + Hemlibra
B.
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Peak 20nM Sevenfect + Peak 300nM Hemlibra
Peak 40nM Sevenfect + Peak 300nM Hemlibra
Peak 20nM Sevenfect + Peak 300nM Hemlibra
Peak Sevenfact + Peak Hemlibra
Peak combination Sevenfact + Hemlibra
C.
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15
10
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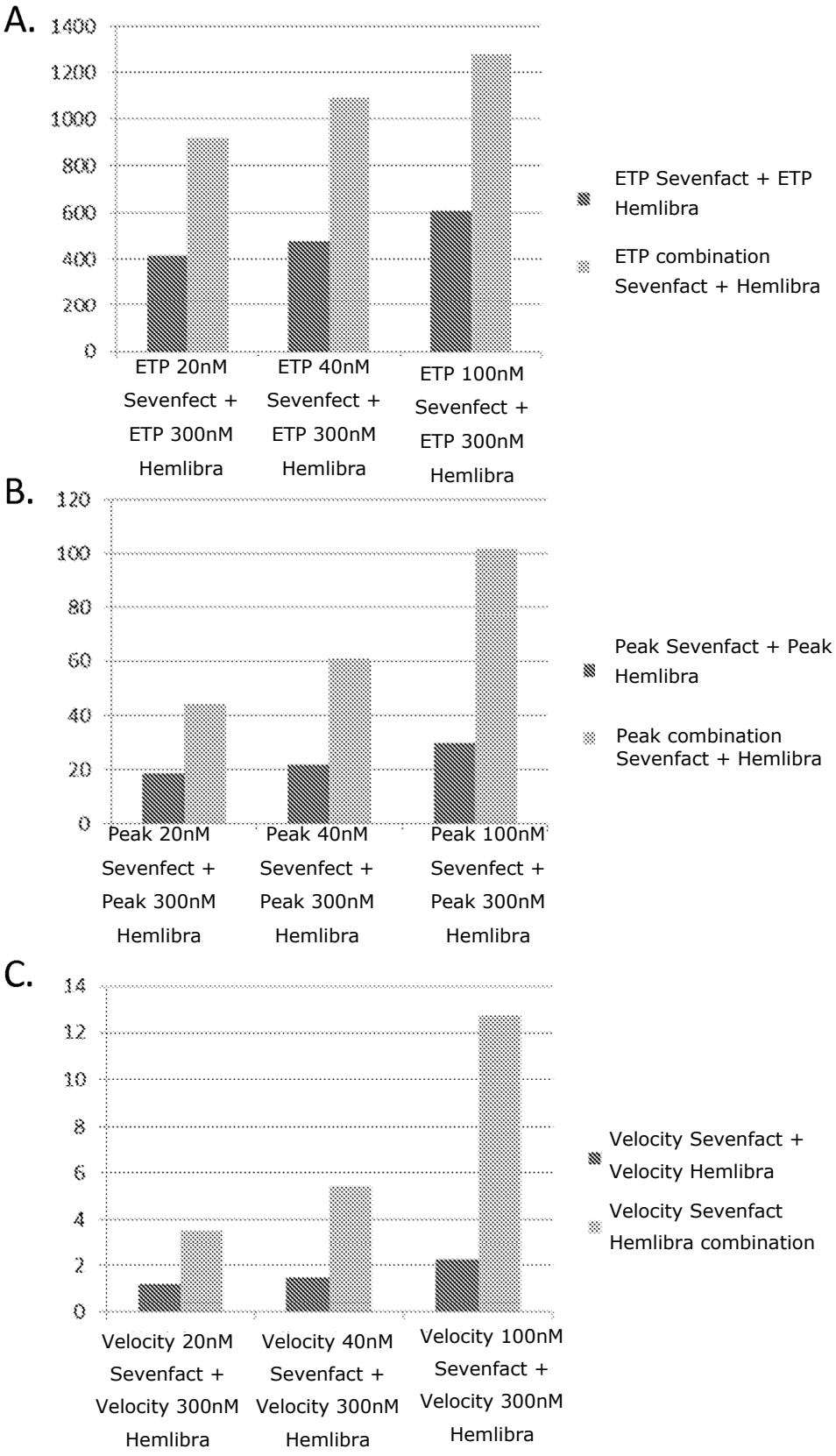
Velocity 20nM Sevenfact + Velocity 300nM Hemlibra
Velocity 40nM Sevenfact + Velocity 300nM Hemlibra
Velocity 100nM Sevenfact + Velocity 300nM Hemlibra
Velocity Sevenfact + Velocity Hemlibra
Velocity combination Sevenfact Hemlibra

FIGURE 1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ETP 100nM Sevenfect + ETP 300nM Hemlibra
ETP Sevenfact + ETP Hemlibra
ETP combination Sevenfact + Hemlibra
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100
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Peak 20nM Sevenfect + Peak 300nM Hemlibra
Peak 40nM Sevenfect + Peak 300nM Hemlibra
Peak 100nM Sevenfect + Peak 300nM Hemlibra
Peak Sevenfact + Peak Hemlibra
Peak combination Sevenfact + Hemlibra
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Velocity 20nM Sevenfact + Velocity 300nM Hemlibra
Velocity 40nM Sevenfact + Velocity 300nM Hemlibra
Velocity 100nM Sevenfact + Velocity 300nM Hemlibra
Velocity Sevenfact + Velocity Hemlibra
Velocity Sevenfact Hemlibra combination

FIGURE 2