with FX are based on the therapeutic use of non-activated zymogen because it is considered to be a safer approach. There is a need for improved or alternative FX therapeutics.

SUMMARY

Provided herein are modified or variant Factor X (FX) polypeptides that exhibit altered properties or activities compared to an unmodified FX polypeptide such as increased cofactor (FVa) dependence, increased half-life, increased resistance to inhibitors (e.g. antithrombin III) and/or altered glycosylation. The modified FX polypeptides provided herein can exhibit some or all of the above properties or activities. The modified FX polypeptides can be mature, zymogen, active or activated or catalytically active forms thereof.

In one aspect, there is provided an isolated modified Factor X (FX) polypeptide, comprising:

an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134;, and

comprising a further amino acid replacement(s) in the light chain or in the protease domain of the heavy chain, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide whose set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the further amino acid replacement(s) confers altered glycosylation, increased resistance to an inhibitor, increased co-factor dependence, increased half-life and/or increased catalytic activity; and

the further amino acid replacement(s) is/are at a position corresponding to a position selected from among 211, 214, 216, 218, 219, 273, 276, 306, 326, 332, 338, 420 and 424, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134;

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 95%, 96%, 97%, 98%, 99% or more

sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement: and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

In one aspect, there is provided an isolated modified Factor X (FX) polypeptide, comprising an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide whose set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement: and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

In one aspect, there is provided a modified active Factor X (FXa) polypeptide, comprising an amino acid replacement at a position 196 and at position 332 in an

unmodified FXa polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

the replacement at position 196 is serine (S), and the replacement at 332 alanine (A), aspartic acid (D), glutamic acid (E), serine (S) or glycine (G);

the modified FXa polypeptide contains only up to seven amino acid replacements;

the unmodified FXa comprises a light chain consisting of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain consisting of the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134;

the modified FXa exhibits at least 50-fold increased FVa cofactor dependence compared to the unmodified FXa polypeptide not containing the amino acid replacements; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

In one aspect, there is provided a nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FX polypeptide as described herein.

In one aspect, there is provided a vector, comprising the nucleic acid molecule as described herein.

In one aspect, there is provided an isolated cell or cell culture, comprising the vector as described herein.

In one aspect, there is provided a method of producing a modified FX polypeptide as described herein, comprising culturing a cell of a cell as described herein, under conditions whereby the modified FX polypeptide is produced.

In one aspect, there is provided a pharmaceutical composition, comprising a modified FX polypeptide as described herein, a nucleic acid molecule as described herein in a pharmaceutically acceptable vehicle.

In one aspect, there is provided method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a modified FX polypeptide as described herein, wherein the modified FX polypeptide is a single chain zymogen;

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide;

or, comprising:

a) providing a composition comprising a modified FX zymogen selected from among a zymogen comprising:

a light chain having the sequence of amino acids 1-139 set forth in SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 1-139 of any of SEQ ID NOS:134 and that contains the amino acid replacement(s) selected from S and T at position 196; and

a heavy chain having the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265 and that contains amino acid replacement(s) compared to an unmodified FX polypeptide; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

In another aspect, there is provided a composition, comprising a modified FX polypeptide as described herein for use for treating a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

In another aspect, there is provided a of a modified FX polypeptide as described herein in the preparation of a medicament for treatment of a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

For example, provided herein are modified FX polypeptide that contain an amino acid replacement in an unmodified FX polypeptide such that the active FX (FXa) form of the modified FX polypeptide exhibits at least 2-fold, 10-fold, 20-fold, 30-fold, 40-fold, and generally at least 50-fold increased FVa cofactor dependence compared to the FXa polypeptide that is the same as the modified FX polypeptide, but not containing the amino acid replacement(s). The unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 75% sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof. In one example, the unmodified FX polypeptide can be a zymogen FX polypeptide having a light chain containing the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain containing the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the zymogen FX polypeptide having a light chain containing the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain containing the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134. In another example, the unmodified FX polypeptide is an active FX (FXa) polypeptide having a light chain containing the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain containing the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at

CLAIMS:

1. An isolated modified Factor X (FX) polypeptide, comprising an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide whose set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement: and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

2. The isolated modified FX polypeptide of claim 1 that is a FXa polypeptide, the unmodified FXa comprises a sequence of amino acids that exhibits at least 85% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a catalytically active form thereof.

3. The isolated FX polypeptide of claim 1 or claim 2, wherein the modified FX polypeptide comprises an amino acid replacement with S at a position corresponding to position 196 with reference to amino acid position set forth in SEQ ID NO:134, or the same replacement at a corresponding amino acid residue in the unmodified FX polypeptide that does not comprise S at the position corresponding to 196.

4. The isolated modified FX polypeptide of any of claims 1-3, comprising a further amino acid replacement(s) in the light chain or in the protease domain of the heavy chain, wherein:

the further amino acid replacement(s) confers altered glycosylation, increased resistance to an inhibitor, increased co-factor dependence, increased half-life and/or increased catalytic activity; and

is at a position corresponding to a position selected from among 211, 214, 216, 218, 219, 273, 276, 306, 326, 332, 338, 420 and 424, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134.

5. The isolated FX polypeptide of claim 4 , wherein:

the replacement confers increased resistance to an inhibitor; and

the inhibitor is a tissue factor pathway inhibitor (TFPI) or is anti-thrombin III (ATIII).

6. The isolated modified FX polypeptide of claim 5, wherein the inhibitor is anti-thrombin III (ATIII).

7. The isolated modified FX polypeptide of any of claims 1-5, comprising only one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acid replacement(s) compared to the unmodified FXa polypeptide of the same form.

8. The isolated modified FX polypeptide of any of claims 1-6, comprising a further amino acid replacement selected from among amino acid replacement with:

S at a position corresponding to position 211; D at a position corresponding to position 214; A at a position corresponding to position 214; S at a position corresponding to position 214; R at a position corresponding to position 216; K at a position corresponding to position 216; A at a position corresponding to position 216; S at a position corresponding to position 216; R at a position corresponding to position 218; K at a position corresponding to position 218; A at a position corresponding to position 218; H at a position corresponding to position 219; A at a position corresponding to position 273; E at a position corresponding to position 273; A at a position corresponding to position 276; E at a position corresponding to position 276; E at a position corresponding to position 306; S at a position corresponding to position 326; T at a position corresponding to position 326; V at a position corresponding to position 326; Q at a position corresponding to position 326; N at a position corresponding to position 326; M at a position corresponding to position 326; K at a position corresponding to position 326; Y at a position corresponding to position 326; E at a position corresponding to position 326; D at a position corresponding to position 326; A at a position corresponding to position 332; D at a position corresponding to position 332; E at a position corresponding to position 332; S at a position corresponding to position 332; G at a position corresponding to position 332; A at a position

corresponding to position 338; S at a position corresponding to position 338; N at a position corresponding to position 338; R at a position corresponding to position 338; V at a position corresponding to position 338; Y at a position corresponding to position 338; M at a position corresponding to position 338; A at a position corresponding to position 420; E at a position corresponding to position 420; A at a position corresponding to position 424; and E at a position corresponding to position 424, with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

9. The isolated modified FX polypeptide of any of claims 1-8, comprising an amino acid replacement with S or T at a position corresponding to position 196, and an amino acid replacement selected from among amino acid replacement with A, D, E, S or G at a position corresponding to 332 in the unmodified FX polypeptide.

10. The isolated modified FX polypeptide of any of claims 1-9, comprising amino acid replacements with S at a position corresponding to position 196 and A at a position corresponding to position 332, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134 or the same amino acid replacement at a corresponding amino acid residue in the unmodified FXa polypeptide.

11. The modified FX of any of claims 1-10, comprising the amino acid replacements corresponding to 196S and 332A, wherein the modified FX is a factor FXa.

12. The isolated modified FX polypeptide of any of claims 1-11, comprising a further amino acid replacement(s) that introduce a non-native glycosylation site.

13. The isolated modified FX polypeptide of any of claims 1-11, wherein the unmodified FX polypeptide is selected from among:

a) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; or

b) a catalytically active form of a).

14. The isolated modified FX polypeptide of any of claims 1-12, wherein the unmodified polypeptide contains a heavy and light chain and consists of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134.

15. The isolated modified FX polypeptide of any of claims 1-12, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265); and

b) a catalytically active form of a) that includes the amino acid replacement(s) and exhibits catalytic activity.

16. A modified active Factor X (FXa) polypeptide, comprising an amino acid replacement at a position 196 and at position 332 in an unmodified FXa polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

the replacement at position 196 is serine (S), and the replacement at 332 alanine (A), aspartic acid (D), glutamic acid (E), serine (S) or glycine (G);

the modified FXa polypeptide contains only up to seven amino acid replacements;

the unmodified FXa comprises a light chain consisting of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain consisting of the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134;

the modified FXa exhibits at least 50-fold increased FVa cofactor dependence compared to the unmodified FXa polypeptide not containing the amino acid replacements; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

17. The modified FXa polypeptide of claim 16, wherein the replacement at position 332 is A.

18. The modified FX polypeptide of any of claims 1-15, wherein the unmodified FX polypeptide is selected from among:

a) a zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134;

b) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the

sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; and

c) a catalytically active form of b).

19. The modified FX polypeptide of any of claims 1-16 that comprises the sequence of amino acids of the polypeptide of any of SEQ ID NOs: 92-96 and 224-228.

20. The modified FX polypeptide of any of claims 1-16, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

b) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

c) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s); and

d) a catalytically active form of c) that includes the modification(s) and exhibits catalytic activity.

21. The modified FX polypeptide of any of claims 1-20 comprising an amino acid replacement corresponding to 195L with reference to amino acid positions set forth in SEQ ID

NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

22. The modified FX polypeptide of any of claims 1-21, wherein the unmodified polypeptide is a human polypeptide.

23. The modified FX polypeptide of any of claims 1-22 that is selected from among a single chain zymogen polypeptide, a two-chain polypeptide and a single-chain polypeptide.

24. The modified FX polypeptide of any of claims 1-23 that is a FXa activated polypeptide.

25. The modified FX polypeptide of any of claims 1-24, wherein only the primary sequence is modified.

26. The modified FX polypeptide of any of claims 1-25 , wherein the modified FX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated or conjugated to a polyethylene glycol (PEG) moiety.

27. The modified FX polypeptide of any claims 1-26 that is a chimeric or fusion protein.

28. The modified FX polypeptide of claim 27, comprising sequence of acid residues set forth in any of SEQ ID NOs.:505-509.

29. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FX polypeptide of any of claims 1-20.

30. A vector, comprising the nucleic acid molecule of claim 26.

31. The vector of claim 30, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

32. The vector of claim 30 that is a viral vector s selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus vector.

33. An isolated cell or cell culture, comprising the vector of any of claims 30-32.

34. The cell or cell culture of claim 33, that is a eukaryotic cell or comprises eukaryotic cells..

35. The cell or cell culture of claim 34, wherein the eukaryotic cell is a mammalian cell.

36. The cell or cell culture of claim 35, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

37. A method of producing a modified FX polypeptide of any of claims 1-25, comprising culturing a cell of a cell of any of claims 30-33, under conditions whereby the modified FX

polypeptide is produced.

38. The method of claim 37, further comprising isolating the modified FX polypeptide.

39. A pharmaceutical composition, comprising a modified FX polypeptide of any of claims 1-28, a nucleic acid molecule of claim 29 or a vector of any of claims 30-32 in a pharmaceutically acceptable vehicle.

40. A method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a modified FX polypeptide of claim 23, wherein the modified FX polypeptide is a single chain zymogen;

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide;

or, comprising:

a) providing a composition comprising a modified FX zymogen selected from among a zymogen comprising:

a light chain having the sequence of amino acids 1-139 set forth in SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 1-139 of any of SEQ ID NOS:134 and that contains the amino acid replacement(s) selected from S and T at position 196; and

a heavy chain having the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265 and that contains amino acid replacement(s) compared to an unmodified FX polypeptide; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

41. The method of claim 40, wherein activation cleavage is effected *in vivo* or *in vitro*.

42. The method of claim 40 or claim 41, further comprising removing the activation peptide from the composition.

43. The method of any of claims 40-42, wherein the FX polypeptide comprises the an amino acid replacement corresponding to 196S.

44. The method of any of claims 40-43, wherein the FX zymogen is produced by expression of a nucleic acid encoding a polypeptide set forth in any of SEQ ID NOS: 4-133 or 136-265 and 417-546, or a nucleic acid encoding a polypeptide having a sequence of amino acids that has

at least 95% sequence identity to any of SEQ ID NOS: 4-133 or 136-265 and 417-546 and that contains an amino acid replacement(s) compared to an unmodified FX polypeptide.

45. A composition, comprising a modified FX polypeptide of any of claims 1-28 for use for treating a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

46. Use of a modified FX polypeptide of any of claims 1-28 in the preparation of a medicament for treatment of a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

47. The modified FX polypeptide of claim 45, wherein the bleeding disorder is a hemophilia.

48. The use of claim 46, wherein the bleeding disorder is a hemophilia.

CLAIMS:

1. An isolated modified Factor X (FX) polypeptide, comprising:

an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134:, and

comprising a further amino acid replacement(s) in the light chain or in the protease domain of the heavy chain, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide whose set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the further amino acid replacement(s) confers altered glycosylation, increased resistance to an inhibitor, increased co-factor dependence, increased half-life and/or increased catalytic activity; and

the further amino acid replacement(s) is/are at a position corresponding to a position selected from among 211, 214, 216, 218, 219, 273, 276, 306, 326, 332, 338, 420 and 424, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134;

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement: and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

2. The isolated modified FX polypeptide of claim 1 that is a FXa polypeptide, the unmodified FXa comprises a sequence of amino acids that exhibits at least 85% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID N-0:134, or a catalytically active form thereof.

3. The isolated FX polypeptide of claim 1 or claim 2, wherein the modified FX polypeptide comprises an amino acid replacement with S at a position corresponding to position 196 with reference to amino acid position set forth in SEQ ID NO:134, or the same replacement at a corresponding amino acid residue in the unmodified FX polypeptide that does not comprise S at the position corresponding to 196.

4. The isolated modified FX polypeptide of any of claims 1-3, wherein the further amino acid replacement is at position 332 based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134.

5. The isolated FX polypeptide of any of claims 1-3, wherein:

the replacement confers increased resistance to an inhibitor; and

the inhibitor is a tissue factor pathway inhibitor (TFPI) or is anti-thrombin III (ATIII).

6. The isolated modified EX polypeptide of claim 5, wherein the inhibitor is anti-thrombin III (ATM).

7. The isolated modified FX polypeptide of any of claims 1-5, comprising only one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acid replacement(s) compared to the unmodified FXa polypeptide of the same form.

8. The isolated modified FX polypeptide of any of claims 1-6, comprising a further amino acid replacement selected from among amino acid replacement with:

S at a position corresponding to position 211; D at a position corresponding to position 214; A at a position corresponding to position 214; S at a position corresponding to position 214; R at a position corresponding to position 216; K at a position corresponding to position 216; A at a position corresponding to position 216; 5 at a position corresponding to position 216; R at a position corresponding to position 218; K at a position corresponding to position 218; A at a position corresponding to position 218; El at a position corresponding to position 219; A at a position corresponding to position 273; E at a position corresponding to position 273; A at a position corresponding to position 276; E at a position corresponding to position 276; E at a position corresponding to position 306; S at a position corresponding to position 326; T at a position corresponding to position 326; V at a position corresponding to position 326; Q at a position corresponding to position 326; N at a position corresponding to position 326; M at a position corresponding to position 326; K at a position corresponding to position 326; Y at a position corresponding to position 326; E at a position corresponding to position 326; D at a position corresponding to position 326; A at a position corresponding to position 332; D at a position corresponding to position 332; E at a position corresponding to position 332; S at a position corresponding to position 332; G at a position corresponding to position 332; A at a position corresponding to position 338; S at a position corresponding to position 338; N at a position corresponding to position 338; R at a position corresponding to position 338; V at a position corresponding to position 338; Y at a position corresponding to position 338; Mat a position corresponding to position 338; A at a position corresponding to position 420; E at a position corresponding to position 420; A at a position corresponding to position 424; and E at a position corresponding to position 424, with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

9. The isolated modified FX polypeptide of any of claims 1-8, comprising an amino acid replacement with S or T at a position corresponding to position 196, and an

amino acid replacement selected from among amino acid replacement with A, D, E, S or G at a position corresponding to 332 in the unmodified FX polypeptide.

10. The isolated modified FX polypeptide of any of claims 1-9, comprising amino acid replacements with S at a position corresponding to position 196 and A at a position corresponding to position 332, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134 or the same amino acid replacement at a corresponding amino acid residue in the unmodified FXa polypeptide.

11. The modified FX of any of claims 1-10, comprising the amino acid replacements corresponding to 196S and 332A, wherein the modified FX is a factor FXa.

12. The isolated modified FX polypeptide of any of claims 1-11, comprising a further amino acid replacement(s) that introduce a non-native glycosylation site.

13. The isolated modified FX polypeptide of any of claims 1-11, wherein the unmodified FX polypeptide is selected from among:

a) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID -N0:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; or

b) a catalytically active form of a).

14. The isolated modified FX polypeptide of any of claims 1-12., wherein the unmodified polypeptide contains a heavy and light chain and consists of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134.

15. The isolated modified FX polypeptide of any of claims 1-12, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265); and

b) a catalytically active form of a) that includes the amino acid replacement(s) and exhibits catalytic activity.

16. A modified active Factor X (FXa) polypeptide, comprising an amino acid replacement at a position 196 and at position 332 in an unmodified FXa polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

the replacement at position 196 is serine (S), and the replacement at 332 alanine (A), aspartic acid (D), glutamic acid (E), serine (S) or glycine (G);

the modified FXa polypeptide contains only up to seven amino acid replacements;

the unmodified FXa comprises a light chain consisting of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain consisting of the sequence of amino

acids set forth as residues 195-448 of SEQ NO:134;

the modified FXa exhibits at least 50-fold increased FVa cofactor dependence compared to the unmodified FXa polypeptide not containing the amino acid replacements; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

17. The modified FXa polypeptide of claim 16, wherein the replacement at position 332 is A.

18. The modified FX polypeptide of any of claims 1-15, wherein the unmodified FX polypeptide is selected from among:

a) a zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ 1D NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134;

b) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; and

c) a catalytically active form of b).

19. The modified FX polypeptide of any of claims 1-16 that comprises the sequence of amino acids of the polypeptide of any of SEQ ID NOs.: 92-96 and 224-228.

20. The modified. FX polypeptide of any of claims 1-16, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

b) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

c) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s); and

d) a catalytically active form of c) that includes the modification(s) and exhibits catalytic activity.

21. The modified FX polypeptide of any of claims 1-20 comprising an amino acid replacement corresponding to 195L with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

22. The modified FX polypeptide of any of claims 1-21, wherein the unmodified polypeptide is a human polypeptide.

23. The modified FX polypeptide of any of claims 1-22 that is selected from among a single chain zymogen polypeptide, a two-chain polypeptide and a single-chain polypeptide.

24. The modified FX polypeptide of any of claims 1-23 that is a FXa activated polypeptide.

25. The modified FX polypeptide of any of claims 1-24, wherein only the primary sequence is modified.

26. The modified FX polypeptide of any of claims 1-25 , wherein the modified FX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated or conjugated to a polyethylene glycol (PEG) moiety.

27. The modified FX polypeptide of any claims 1-26 that is a chimeric or fusion protein.

28. The modified FX polypeptide of claim 27, comprising sequence of acid residues set forth in any of SEQ ID NOs.:505-509.

29. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FX polypeptide of any of claims 1-20.

30. A vector, comprising the nucleic acid molecule of claim 26.

31. The vector of claim 30, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

32. The vector of claim 30 that is a viral vectors selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus vector.

33. An isolated cell or cell culture, comprising the vector of any of claims 30-32.

34. The cell or cell culture of claim 33, that is a eukaryotic cell or comprises eukaryotic cells..

35. The cell or cell culture of claim 34, wherein the eukaryotic cell is a mammalian cell.

36. The cell or cell culture of claim 35, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

37. A method of producing a modified FX polypeptide of any of claims 1-25, comprising culturing a cell of a cell of any of claims 30-33, under conditions whereby the modified FX polypeptide is produced.

38. The method of claim 37, further comprising isolating the modified FX polypeptide.

39. A pharmaceutical composition, comprising a modified FX polypeptide of any of claims 1-28, a nucleic acid molecule of claim 29 or a vector of any of claims 30-32 in a pharmaceutically acceptable vehicle.

40. A method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a modified FX polypeptide of claim 23, wherein the modified FX polypeptide is a single chain zymogen;

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide;

or, comprising:

a) providing a composition comprising a modified. FX zymogen selected from among a zymogen comprising:

a light chain having the sequence of amino acids 1-139 set forth in SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 1-139 of any of SEQ ID NOS:134 and that contains the amino acid replacement(s) selected from S and T at position 196; and

a heavy chain having the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265 and that contains amino acid replacement(s) compared to an unmodified FX polypeptide; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

41. The method of claim 40, wherein activation cleavage is effected in vivo or in vitro.

42. The method of claim 40 or claim 41, further comprising removing the activation peptide from the composition.

43. The method of any of claims 40-42, wherein the FX polypeptide comprises the an amino acid replacement corresponding to 196S.

44. The method of any of claims 40-43, wherein the FX zymogen is produced by expression of a nucleic acid encoding a polypeptide set forth in any of SEQ ID NOS:

4-133 or 136–265 and 417-546, or a nucleic acid encoding a polypeptide having a sequence of amino acids that has at least 95% sequence identity to any of SEQ ID NOS: 4-133 or 136-265 and 417-546 and that contains an amino acid replacements) compared to an unmodified FX polypeptide.

45. A composition, comprising a modified FX polypeptide of any of claims 1-28 for use for treating a bleeding disorder, wherein:

the modified EX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase Cl deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia., stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

46. Use of a modified FX polypeptide of any of claims 1-28 in the preparation of a medicament for treatment of a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase Cl deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

47. The modified FX polypeptide of claim 45, wherein the bleeding disorder is a hemophilia.

48. The use of claim 46, wherein the bleed disorder is hemophilia B.

49. Use of a nucleic acid molecule of claim 29 or a vector of any of claims 30-32 in the preparation of a medicament for treatment of a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

50. The composition of claim 49, wherein the bleeding disorder is a hemophilia.

51. The use of claim 50, wherein the bleeding disorder is a hemophilia.