FVIIa also has been approved for the treatment of patients with congenital FVII deficiency, and is increasingly being utilized in off-label uses, such as the treatment of bleeding associated with other congenital or acquired bleeding disorders, trauma, and surgery in non-hemophilic patients.

The use of recombinant FVIIa to promote clot formation underlines its growing importance as a therapeutic agent. FVIIa therapy leaves significant unmet medical need. For example, based on clinical trial data, an average of 3 doses of FVIIa over a 6 hour or more time period are required to manage acute bleeding episodes in hemophilia patients. More efficacious variants of FVIIa are needed to reduce these requirements. Therefore, among the objects herein, it is an object to provide modified FVII polypeptides that are designed to have improved therapeutic properties.

SUMMARY

Provided herein is a modified factor VII (FVII) polypeptide, wherein:

the modified FVII polypeptide has up to 7 amino acid modifications compared to the unmodified FVII polypeptide;

two of the modifications are replacements at positions corresponding to position 286 and position 298 in a FVII polypeptide having the sequence of amino acids set forth in SEQ ID NO:3;

corresponding positions are identified by alignment with the polypeptide of SEQ ID NO:3;

the replacement at the position corresponding to position 286 is an amino acid replacement with an arginine (Arg, R);

the replacement at the position corresponding to position 298 is an amino acid replacement with a glutamine (Gln, Q);

the unmodified FVII polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 1-3 or a portion thereof that has coagulant activity and contains residues at positions 286 and 298; and

the modified FVII polypeptide exhibits increased coagulant activity compared to an unmodified FVII polypeptide that, when in an active form, does not have the modification at position 286.

Provided herein is a nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FVII polypeptide as described herein.

Provided herein is a vector, comprising the nucleic acid molecule as described herein.

Provided herein is a cell, comprising the vector as described herein.

Provided herein is a method of producing a modified FVII polypeptide, comprising culturing a cell as described herein to express the encoded modified FVII polypeptide.

Provided herein is a pharmaceutical composition, comprising a therapeutically effective concentration or amount of a modified FVII polypeptide as described herein, or a nucleic acid molecule as described herein or a vector as described herein or a cell as described herein, in a pharmaceutically acceptable vehicle.

Provided herein is a modified FVII polypeptide as described herein, for use in treating a disease or condition that is treated by administration of FVII or a pro-coagulant.

Provided herein is the use of a pharmaceutical composition as described herein in the preparation of a medicament for treatment of a disease or condition that is treated by administration of FVII or a pro-coagulant.

Provided herein are modified Factor VII (FVII) polypeptides. In particular, provided herein are modified FVII polypeptides that exhibit procoagulant activities. The FVII polypeptides are modified in primary sequence compared to an unmodified FVII polypeptide, and can include amino acid insertions, deletions and replacements. Modified FVII polypeptides provided herein include FVII polypeptides that exhibit those that have increased resistance to inhibitors such as antithrombin III (AT-III) and tissue factor pathway inhibitor (TFPI), those that have increased resistance to the inhibitory effects of Zn^{2+}, those that have increased catalytic activity in the presence and/or absence of TF, those that have improved pharmacokinetic properties, such as increased half-life, those that have increased binding and/or affinity for the platelet surface, those that have increased binding and/or affinity for serum albumin, and those that have increased binding and/or affinity for platelet integrin $\alpha_{IIb}\beta_3$. The modified FVII polypeptides can contain any combination of modifications provided herein, whereby one or more activities or properties of the polypeptide are altered compared to an unmodified FVII polypeptide. Typically the modified FVII polypeptide retains procoagulant activity. Also provided herein are nucleic acid molecules, vectors and cells that encode/express modified FVII polypeptides. Pharmaceutical compositions, articles of manufacture, kits and methods of treatment

CLAIMS:

1. A modified factor VII (FVII) polypeptide, wherein:

the modified FVII polypeptide has up to 7 amino acid modifications compared to the unmodified FVII polypeptide;

two of the modifications are replacements at positions corresponding to position 286 and position 298 in a FVII polypeptide having the sequence of amino acids set forth in SEQ ID NO:3;

corresponding positions are identified by alignment with the polypeptide of SEQ ID NO:3;

the replacement at the position corresponding to position 286 is an amino acid replacement with an arginine (Arg, R);

the replacement at the position corresponding to position 298 is an amino acid replacement with a glutamine (Gln, Q);

the unmodified FVII polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 1-3 or a portion thereof that has coagulant activity and contains residues at positions 286 and 298; and

the modified FVII polypeptide exhibits increased coagulant activity compared to an unmodified FVII polypeptide that, when in an active form, does not have the modification at position 286.

2. The modified FVII polypeptide of claim 1, wherein the unmodified FVII polypeptide comprises the sequence of amino acids set forth in SEQ ID NO:3.

3. The modified FVII polypeptide of claim 1, wherein the unmodified FVII polypeptide consists of the sequence of amino acids set forth in any of SEQ ID NOS:1-3.

4. The modified FVII polypeptide of claim 1, wherein the unmodified FVII polypeptide consists of the sequence of amino acids set forth in SEQ ID NO:3.

5. The modified FVII polypeptide of any of claims 1-4, wherein an amino acid modification in addition to replacements corresponding to the replacements 286R and 298Q is at a position corresponding to a position selected from among 51, 52, 54, 60, 66, 68, 109, 119, 122, 124, 130, 132, 158, 161, 175, 196, 197, 199, 202, 216, 222, 237, 239, 257, 287, 290, 292, 294, 296, 305, 314, 318, 321, 337, 341, 366, 373, 374, 394, 395 and 396.

6. The modified FVII polypeptide of any of claims 1-5, comprising modifications selected from among Q286R/M298Q/K341Q, Q286R/M298Q/K199E, S222A/H257A/Q286R/M298Q, Q286R/M298Q/Q366N, Q286R/M298Q/H373F, T128N/P129A/Q286R/M298Q, T128N/P129A/S222A/H257A/Q286R/M298Q, T128N/P129A/Q286R/M298Q/H373F, S52A/S60A/Q286R/M298Q, S52A/S60A/S222A/H257A/Q286R/M298Q, S52A/S60A/Q286R/M298Q/H373F, T239V/Q286R/M298Q, S222A/T239V/H257A/Q286R/M298Q, T239V/Q286R/M298Q/H373F, T239I/Q286R/M298Q, S222A/T239I/H257A/Q286R/M298Q, T239I/Q286R/M298Q/H373F, V158D/Q286R/E296V/M298Q/H373F, H257A/Q286R/M298Q, H257S/Q286R/M298Q, S222A/H257S/Q286R/M298Q, H257S/Q286R/M298Q/H373F, S222A/Q286R/M298Q/H373F, S222A/Q286R/M298Q, T128N/P129A/A175S/Q286R/M298Q, A122N/G124S/A175S/Q286R/M298Q, T128N/P129A/A175S/S222A/H257A/Q286R/M298Q, A122N/G124S/A175S/S222A/H257A/Q286R/M298Q, T128N/P129A/A175S/Q286R/M298Q/H373F, A122N/G124S/A175S/Q286R/M298Q/H373F, T128N/P129A/Q286R/M298Q/Q366N, V158D/Q286R/E296V/M298Q, T128N/P129A/Q286R/M298Q/Q366N/H373F, T239V/Q286R/M298Q/Q366N, T239I/Q286R/M298Q/Q366N, T128N/P129A/T239V/Q286R/M298Q, T128N/P129A/S222A/T239V/H257A/Q286R/M298Q, T128N/P129A/T239V/Q286R/M298Q/H373F, T128N/P129A/T239I/Q286R/M298Q and T128N/P129A/T239I/Q286R/M298Q/H373F.

7. The modified FVII polypeptide of any of claims 1-5, comprising modifications selected from among T128N/P129A/Q286R/M298Q, T239I/Q286R/M298Q/Q366N, T239V/Q286R/M298Q/Q366N, T128N/P129A/Q286R/M298Q/Q366N/H373F, V158D/Q286R/E296V/M298Q/H373F, A122N/G124S/A175S/Q286R/M298Q/H373F, T128N/P129A/A175S/Q286R/M298Q/H373F, A122N/G124S/A175S/S222A/H257A/Q286R/M298Q, T128N/P129A/A175S/S222A/H257A/Q286R/M298Q, A122N/G124S/A175S/Q286R/M298Q, T128N/P129A/A175S/Q286R/M298Q, H257A/Q286R/M298Q, ~~Gla~~ S222A/Q286R/M298Q, S222A/Q286R/M298Q/H373F, S222A/H257S/Q286R/M298Q, T239I/Q286R/M298Q/H373F, S222A/T239I/H257A/Q286R/M298Q, T128N/P129A/T239I/Q286R/M298Q, T239I/Q286R/M298Q, T128N/P129A/S222A/T239V/H257A/Q286R/M298Q, S222A/T239V/H257A/Q286R/M298Q, T128N/P129A/T239V/Q286R/M298Q, V158D/Q286R/E296V/M298Q, T128N/P129A/Q286R/M298Q/H373F, Q286R/M298Q/H373F, S52A/S60A/S222A/H257A/Q286R/M298Q, T128N/P129A/S222A/H257A/Q286R/M298Q, S222A/H257A/Q286R/M298Q.

8. The modified FVII polypeptide of any of claims 1-7 that comprises the modifications Q286R/M298Q or T128N/P129A/A175S/Q286R/M298Q or T128N/P129A/A175S/Q286R/M298Q.

9. The modified Factor VII polypeptide of claim 1, comprising amino acid substitutions at positions corresponding to positions 128, 129, 286 and 298 in SEQ ID NO:3, wherein position 128 is substituted with asparagine (N), position 129 is substituted with alanine (A), position 286 is substituted with arginine (R) and position 298 is substituted with glutamine (Q).

10. The modified FVII polypeptide of any of claims 1-9, wherein the polypeptide is a FVIIa polypeptide.

11. The modified Factor VII polypeptide of claim 9 that is a two-chain activated Factor VII (FVIIa) polypeptide.

12. The modified factor VII (FVII) polypeptide of claim 1, wherein the primary amino acid sequence consists of the sequence of amino acids set forth in SEQ ID NO:280.

13. The modified Factor VII polypeptide of claim 12 that is a two-chain activated Factor VII (FVIIa) polypeptide, consisting of the amino acid sequence of SEQ ID NO:280 cleaved between the arginine at position 152 and the isoleucine at position 153.

14. The modified FVII polypeptide of any of claims 1-13 that comprises post-translational modifications.

15. The modified FVII polypeptide claim 14, wherein the post-translational modifications comprise glycosylation.

16. The modified FVII polypeptide of any of claims 1-11 that comprises modifications that introduce an additional glycosylation site.

17. The modified FVII polypepeptide of claim 16, wherein the modifications are amino acid substitutions selected from among T128N/P129A, A122N/G124S, S52A/S60A, T128N/P129A/A175S and A122N/G124S/A175S.

18. The modified FVII polypeptide of claim 16, wherein the additional glycosylation site is introduced by the modification A122N/G124S or T128N/P129A.

19. The modified FVII polypeptide of any of claims 1-18, comprising amino acid replacements T128N/P129A/Q286R/M298Q.

20. The modified FVII polypeptide of any of claims 1-19, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 138-141, 150, 155, 157, 280, 282, 287, 288, 293, 295, 297, 302, 303, 306, 311, 312, 315, 320, 321, 322, 324-326, 328, 337-342, 355-358 and 364-371.

21. The modified FVII polypeptide of any of claims 1-20 that is a mature polypeptide.

22. The modified FVII polypeptide of any of claims 1-21 that is a single-chain polypeptide or a two-chain polypeptide.

23. The modified FVII polypeptide of any of claims 1-20 that is an activated two-chain polypeptide.

24. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FVII polypeptide of any of claims 1-23.

25. A vector, comprising the nucleic acid molecule of claim 24.

26. The vector of claim 25, wherein the vector is a prokaryotic vector, a viral vector, or an eukaryotic vector.

27. A cell, comprising the vector of claim 25 or claim 26.

28. The cell of claim 27, that is a eukaryotic cell.

29. The cell of claim 28, wherein the eukaryotic cell is a mammalian cell.

30. The cell of claim 29, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

31. A method of producing a modified FVII polypeptide, comprising culturing a cell of any of claims 27-30 to express the encoded modified FVII polypeptide.

32. The method of claim 31, further comprising isolating the modified FVII polypeptide.

33. A pharmaceutical composition, comprising a therapeutically effective concentration or amount of a modified FVII polypeptide of any of claims 1-23, or a nucleic acid molecule of claim 24 or a vector of claim 25 or claim 26 or a cell of any of claims 27-30, in a pharmaceutically acceptable vehicle.

34. The pharmaceutical composition of claim 33 that is formulated for single-dosage administration.

35. A modified FVII polypeptide of any of claims 1-23, for use in treating a disease or condition that is treated by administration of FVII or a pro-coagulant.

36. Use of a pharmaceutical composition of claim 33 or claim 34 in the preparation of a medicament for treatment of a disease or condition that is treated by administration of FVII or a pro-coagulant.

37. The modified FVII polypeptide of claim 35 or the use of claim 36, wherein the disease or condition is treated by administration of a zymogen or active form of FVII.

38. The modified FVII polypeptide of claim 35 or claim 37, or the use of claim 36 or claim 37, wherein treatment with the pharmaceutical composition ameliorates or alleviates the symptoms associated with the disease or condition.

39. The modified FVII polypeptide of any of claims 35, 37 and 38, or the use of any of claims 36-38, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias, factor VII deficiency, bleeding disorders, surgical bleeding, or bleeding resulting from trauma.

40. The modified FVII polypeptide or use of claim 39 wherein the disease or condition is hemophilia and the hemophilia is hemophilia A or hemophilia B or hemophilia C.

41. The modified FVII polypeptide or use of claim 39, wherein the disease or condition is hemophilia and the hemophilia is congenital or is acquired.

42. The modified FVII polypeptide or use of claim 39, wherein the disease or condition is due to a bleeding complication due to surgery or trauma.