

CLAIMS

1. A method of treating a subject with hemophilia A and low bone mineral density (BMD), the method comprising:
- 5 (i) selecting a subject having hemophilia A and low BMD, and
(ii) administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIIIIFc);
- wherein administration of the chimeric protein inhibits reduction of BMD in the subject.
2. The method of claim 1, wherein the chimeric protein comprises an amino acid sequence at least 95% identical to an amino acid sequence according to SEQ ID NO: 1.
- 10 3. The method of claim 1, wherein the chimeric protein comprises an amino acid sequence at least 95% identical to an amino acid sequence according to SEQ ID NO: 2.
4. The method of claim 1, wherein the chimeric protein comprises an amino acid sequence according to SEQ ID NO: 1.
- 15 5. The method of claim 1, wherein the chimeric protein comprises an amino acid sequence at least 95% identical to an amino acid sequence according to SEQ ID NO: 5.
6. The method of claim 1, wherein the chimeric protein comprises an amino acid sequence according to SEQ ID NO: 5.
7. The method of claim 1, wherein the chimeric protein comprises a first polypeptide chain comprising an amino acid sequence at least 95% identical to the amino acid sequence according to SEQ ID NO: 5 and a second polypeptide chain comprising an amino acid sequence at least 95% identical to the amino acid sequence according to SEQ ID NO: 4.
- 20 8. The method of claim 1, wherein the chimeric protein comprises a first polypeptide chain comprising an amino acid sequence according to SEQ ID NO: 5 and a second polypeptide chain comprising an amino acid sequence according to SEQ ID NO: 4.
- 25 9. The method of claim 8, wherein the first polypeptide chain is covalently bound to the second polypeptide chain via a disulfide bond.

10. The method of claim 9, wherein the first polypeptide chain is covalently bound to the second polypeptide chain via two disulfide bonds in a hinge region of the Fc domain.
11. The method of claim 1 or 10, wherein the chimeric protein is efmoroctocog alfa.
12. The method of any one of claims 1 to 11, wherein the chimeric protein has been
5 produced by human cells.
13. The method of claim 12, wherein the human cells are human embryonic kidney 293 (HEK293) cells.
14. The method of any one of claims 1 to 13, wherein the chimeric protein is administered at a dose of 25-65 IU/kg every 3-5 days.
- 10 15. The method of any one of claims 1 to 14, wherein the Fc domain is the Fc domain of human immunoglobulin G1 (IgG1).
16. The method of any one of claims 1 to 15, wherein BMD in the subject is measured by Dual X-Ray Absorptiometry (DXA).
17. The method of any one of claims 1 to 16, wherein the subject is 50 years of age or
15 older.
18. The method of any one of claims 1 to 17, wherein BMD in the subject is determined by T-score.
19. The method of claim 18, wherein the subject is determined to have low BMD if the subject has a T-score of less than -1.0.
- 20 20. The method of claim 18, wherein the subject is determined to have low BMD and osteopenia if the subject has T-score between -1.0 and -2.4.
21. The method of claim 18, wherein the subject is determined to have low BMD and osteoporosis if the subject has a T-score of less than -2.5.
22. The method of any one of claims 1 to 16, wherein the subject is younger than 50
25 years of age.
23. The method of claim 22, wherein BMD in the subject is determined by Z-score.

24. The method of claim 23, wherein the subject is determined to have low BMD if the subject has a Z-score of less than -2.0.
25. The method of any one of claims 1 to 24, wherein the subject is predicted to have low BMD based on the levels of one or more biomarkers of bone formation, bone resorption,
5 and/or bone loss.
26. The method of claim 25, wherein the biomarker is assessed from the peripheral blood or urine of the subject.
27. The method of claim 26, wherein the one or more biomarkers of bone formation comprise bone-specific alkaline phosphatase, procollagen type 1 N-terminal propeptide
10 (P1NP), procollagen type 1 C-terminal propeptide (P1CP), and/or osteocalcin.
28. The method of claim 26, wherein the one or more biomarkers of bone resorption comprise total alkaline phosphatase in serum, the receptor activator of nuclear factor kappa B (RANKL), osteoprotegerin (OPG), tartrate-resistant acid phosphatase (TRAP),
15 hydroxylysine, hydroxyproline, deoxypyridinoline (DPD), pyridinoline (PYD), bone sialoprotein, cathepsin K, tartrate-resistant acid phosphatase 5b (TRAP5b), matrix metalloproteinase 9 (MMP9), and/or C- and/or N-terminal cross-linked telopeptide for type 1 collagen (CTX-1 and NTX-1).
29. The method of any one of claims 1-28, wherein the subject does not have a vitamin D deficiency.
30. The method of any one of claims 1-29, wherein the subject has been previously
20 treated with a Factor VIII without an Fc portion.
31. A method of treating a subject with hemophilia A and an increased risk of fracture, the method comprising:
- 25 (i) selecting a subject having hemophilia A and an increased risk of fracture,
and
(ii) administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII-Fc);
wherein administration of the chimeric protein reduces the risk of fracture in the subject.

32. The method of claim 31, wherein the risk of fracture in the subject is determined by the fracture risk assessment tool (FRAX).
33. The method of claim 32, wherein the risk of fracture in the subject is determined by assessment of low BMD risk factors.
- 5 34. The method of claim 33, wherein the low BMD risk factors comprise arthropathy, reduced physical activity, infection with HIV or HCV, vitamin D deficiency, low body mass index (BMI), and/or hypogonadism.
35. A method of reducing the rate of bone mineral density (BMD) loss in a subject, the method comprising:
- 10 (i) selecting a subject with low BMD; and
- (ii) administering to the subject a therapeutically effective amount of a chimeric protein comprising a coagulation factor and a Fc domain (rFVIII_{IFc}), such that administration of the chimeric protein reduces the rate of BMD loss in the subject.
- 15 36. The method of any one of claims 1 to 35, wherein the subject has mild hemophilia A.
37. The method of any one of claims 1 to 35, wherein the subject has moderate hemophilia A.
38. The method of any one of claims 1 to 35, wherein the subject has severe hemophilia A.
- 20 39. A method of increasing bone mineral density (BMD) and prophylactically treating bleeding episodes in a subject who has hemophilia A, the method comprising:
- (i) identifying a subject who is receiving treatment for hemophilia A with a FVIII protein without an Fc portion, wherein the subject has had adequate blood clotting during the treatment, and wherein the subject has low BMD; and
- 25 (ii) discontinuing treatment with the FVIII protein without an Fc portion and administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII_{IFc}), wherein administration of the chimeric protein increases BMD and prophylactically treats bleeding episodes in the subject.

40. A method of increasing bone mineral density (BMD) and prophylactically treating bleeding episodes in a subject who has hemophilia A, the method comprising:
- 5 (i) identifying a subject who is receiving treatment for hemophilia A with a non-factor replacement protein, wherein the subject has had adequate blood clotting during the treatment, and wherein the subject has low BMD; and
- (ii) discontinuing treatment with the non-factor replacement protein and administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII-Fc),
- 10 wherein administration of the chimeric protein increases BMD and prophylactically treats bleeding episodes in the subject.
41. A method of increasing bone mineral density (BMD) and prophylactically treating bleeding episodes in a subject, the method comprising administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII-Fc), wherein the subject has been identified as having hemophilia A
- 15 and low BMD, and wherein administration of the chimeric protein increases BMD and prophylactically treats bleeding episodes in the subject.
42. A method of reducing risk of fracture and prophylactically treating bleeding episodes in a subject, the method comprising administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain
- 20 (rFVIII-Fc), wherein the subject has been identified as having hemophilia A and an increased risk of fracture, and wherein administration of the chimeric protein reduces the risk of fracture and prophylactically treats bleeding episodes in the subject.
43. A method of reducing rate of bone mineral density (BMD) loss and prophylactically treating bleeding episodes in a subject, the method comprising administering to the subject
- 25 a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII-Fc), wherein the subject has been identified as having hemophilia A and BMD loss, and wherein administration of the chimeric protein reduces the rate of BMD loss and prophylactically treats bleeding episodes in the subject.
44. A method of increasing bone mineral density (BMD) and prophylactically treating bleeding episodes in a subject who has hemophilia A and is being treated with a FVIII
- 30 protein without an Fc portion, the method comprising discontinuing treatment with the FVIII

protein without an Fc portion and administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII-Fc), wherein the subject has been identified as having low BMD and adequate blood clotting during treatment with the FVIII protein without an Fc portion, and wherein
5 administration of the chimeric protein increases BMD and prophylactically treats bleeding episodes in the subject.

45. A method of increasing bone mineral density (BMD) and prophylactically treating bleeding episodes in a subject who has hemophilia A and is being treated with a non-factor replacement protein, the method comprising discontinuing treatment with the non-factor
10 replacement protein and administering to the subject a therapeutically effective amount of a chimeric protein comprising a recombinant FVIII protein and a Fc domain (rFVIII-Fc), wherein the subject has been identified as having low BMD and adequate blood clotting during treatment with the non-factor replacement protein, and wherein administration of the chimeric protein increases BMD and prophylactically treats bleeding episodes in the subject.

15 46. The method of any one of claims 1-45, wherein the subject has been previously treated to reduce bleeding associated with hemophilia A using a Factor VIII protein without an Fc portion.

47. The method of any one of claims 39, 44, or 46, wherein the Factor VIII protein without an Fc portion is PEGylated FVIII that is not fused to a Fc domain.

20 48. The method of any one of claims 39, 44, or 46, wherein the Factor VIII protein without an Fc portion is single-chain FVIII that is not fused to a Fc domain.

49. The method of any one of claims 39, 44, or 46, wherein the Factor VIII protein without an Fc portion is recombinant FVIII that does not comprise a moiety that extends half-life thereof in humans.

25 50. The method of any one of claims 39, 44, or 46, wherein the Factor VIII protein without an Fc portion is blood-derived FVIII or plasma-derived FVIII.

51. The method of any one of claims 39, 44, or 46, wherein the Factor VIII protein without an Fc portion is damoctocog alfa pegol, turoctocog alfa pegol, turoctocog alfa, lonoctocog alfa, simoctocog alfa, ruriocog alfa pegol, or octocog alfa.

52. The method of any one of claims 1-51, wherein the subject has been previously treated to reduce bleeding associated with hemophilia A using a non-factor replacement protein.
53. The method of claim 52, wherein the non-factor replacement protein is emicizumab.
- 5 54. The method of claim 53, wherein the emicizumab is emicizumab-kxwh.
55. The method of any one of claims 30 or 36-54, wherein the subject had adequate blood clotting during treatment with the Factor VIII protein without an Fc portion or the non-factor replacement protein.
- 10 56. The method of any one of claims 1-55, wherein the subject has low BMD at a bone site and/or joint where bleeding has not been detected.