

WHAT IS CLAIMED IS:

1. A Factor VIII (FVIII) encoding nucleic acid variant, wherein the FVIII encoded by said nucleic acid variant exhibits greater expression when compared to wild type FVIII expression.
2. A Factor VIII (FVIII) encoding nucleic acid variant, wherein the FVIII encoded by said
5 nucleic acid variant comprises a B domain deletion and exhibits greater expression when compared to wild type FVIII comprising a B domain deletion expression.
3. The variant FVIII as claimed in claim 1 or 2, wherein said nucleic acid variant is more efficiently packaged into an adenovirus-associated virus (AAV) vector.
4. The variant FVIII as claimed in any of claims 1-3, wherein said FVIII encoded by said
10 nucleic acid variant exhibits greater biological activity when compared to wild type FVIII or when compared to wild type FVIII comprising a B domain deletion.
5. The variant FVIII as claimed in any of claims 1-3, wherein said biological activity is determined by a clotting assay or reduced bleeding in a FVIII assay or FVIII deficiency model.
6. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has
15 one or more leucine codons changed to CTG compared to TTA, TTG, CTT, CTC or CTA in wild type FVIII.
7. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has 2-5, 5-10, 10-20, 20-50, 50-100, 100-250, 250-500, 500-750 or 750-850 CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.
- 20 8. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has greater than 85% CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild type FVIII.
9. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has all CTG leucine codons modified from TTA, TTG, CTT, CTC or CTA leucine codons in wild
25 type FVIII.
10. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant has one or more AAG lysine codons compared to AAA lysine codons in wild type FVIII.
11. The variant FVIII as claimed in claim 1, wherein said nucleic acid variant has between about 50-59%, or 50-56%, or 50-53% GC content.

12. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant is at least 75% identical to wild type human FVIII nucleic acid or wild type human FVIII nucleic acid comprising a B domain deletion.
13. The variant FVIII as claimed in any of claims 1-4, wherein said nucleic acid variant is human FVIII, and/or wherein said wild type FVIII or wild type FVIII comprising a B domain deletion is human FVIII.
14. The variant FVIII as claimed in claim 1, wherein said nucleic acid variant comprises any of SEQ ID NOs:1-7 and 9.
15. The variant FVIII as claimed in any of claims 1-14, wherein 1, 2, 3 or all 4 of the codons encoding the PACE/furin cleavage site is/are deleted.
16. The variant FVIII as claimed in any of claims 1-14, wherein 1, 2, 3 or all 4 of the codons encoding the PACE/furin cleavage site set forth as HHQR or RHQR from positions 1645-1648 is/are deleted.
17. An expression vector comprising the variant FVIII of any of claims 1-16.
18. The expression vector of claim 17, selected from the group consisting of an adenovirus-associated virus (AAV) vector, a retroviral vector, an adenoviral vector, a plasmid, or a lentiviral vector.
19. The expression vector of claim 18, wherein said AAV vector comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 AAV serotype.
20. The expression vector of claim 17, further comprising an intron, an expression control element, one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs) and/or a filler polynucleotide sequence.
21. The expression vector of claim 20, wherein the intron is within or flanks the variant FVIII, or wherein the expression control element is operably linked to the variant FVIII, or wherein the AAV ITR(s) flanks the 5' or 3' terminus of the variant FVIII, or wherein the filler polynucleotide sequence flanks the 5' or 3' terminus of the variant FVIII.
22. The expression vector of claim 20, wherein the expression control element comprises a constitutive or regulatable control element, or a tissue-specific expression control element or promoter.

23. The expression vector of claim 20, wherein the expression control element comprises an element that confers expression in liver.
24. The expression vector of claim 20, wherein the expression control element comprises a TTR promoter or mutant TTR promoter.
- 5 25. The expression vector of claim 24, wherein the mutant TTR promoter comprises SEQ ID NO:8.
26. The expression vector of claim 20, wherein the ITR comprises one or more ITRs of any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 AAV serotypes, or a combination thereof.
- 10 27. A host cell expressing the FVIII encoded by said nucleic acid variant of any of claims 1-16.
28. A host cell comprising the nucleic acid variant of any of claims 1-16 or the expression vector of any of claims 17-26.
29. A virus vector comprising the nucleic acid variant of any of claims 1-16 or the expression
15 vector of any of claims 17-26.
30. An AAV vector comprising the nucleic acid variant of any of claims 1-16 or the expression vector of any of claims 17-26.
31. The AAV vector of claim 30, wherein the AAV vector comprises a VP1, VP2 and/or VP3 capsid sequence having 75% or more sequence identity to AAV1, AAV2, AAV3, AAV4, AAV5,
20 AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 or AAV-2i8 VP1, VP2 and/or VP3 sequences.
32. The AAV vector of claim 30, wherein the AAV vector comprises a VP1, VP2 and/or VP3 capsid sequence selected from any of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74 and AAV-2i8 AAV serotypes.
- 25 33. A pharmaceutical composition comprising the nucleic acid variant of any of claims 1-16, the expression vector of any of claims 17-26, or the virus or AAV vector of any of claims 30-32 in a biologically compatible carrier or excipient, optionally including empty capsid AAV.

34. The nucleic acid variant of any of claims 1-16, the expression vector of any of claims 17-26, or the virus or AAV vector of any of claims 30-32 encapsulated in a liposome or mixed with phospholipids or micelles.

35. A method for delivering or transferring a nucleic acid sequence into a mammal or a
5 mammalian cell, comprising administering or contacting the nucleic acid variant of any of claims 1-16, the expression vector of any of claims 17-26, or the virus or AAV vector of any of claims 30-32 to said mammal or mammalian cell, thereby delivering or transferring the nucleic acid sequence into the mammal or mammalian cell.

36. The method of claim 35, wherein said mammalian cell is in culture or is present in a
10 subject.

37. A method of treating a mammal in need of Factor VIII, comprising:
(a) providing the nucleic acid variant of any of claims 1-16, the expression vector of any of
claims 17-26, or the virus or AAV vector of any of claims 30-32; and
(b) administering an amount of the nucleic acid variant of any of claims 1-16, the expression
15 vector of any of claims 17-26, or the virus or AAV vector of any of claims 30-32 to the mammal
wherein said Factor VIII is expressed in the mammal.

38. The method of claims 35 or 37, wherein said Factor VIII is expressed at levels having a beneficial or therapeutic effect on the mammal.

39. The method of claims 35 or 37, wherein said Factor VIII is expressed in a cell, tissue or
20 organ of said mammal.

40. The method of claim 35 or 37, wherein, the cell comprises a secretory cell.

41. The method of claim 35 or 37, wherein the cell comprises an endocrine cell or an endothelial cell.

42. The method of claim 35 or 37, wherein the cell comprises an hepatocyte, a sinusoidal
25 endothelial cell, a megakaryocyte, a platelet or hematopoietic stem cell.

43. The method of claim 39, wherein the tissue or organ of said mammal comprises liver.

44. The method of claims 35 or 37, wherein the mammal produces an insufficient amount of Factor VIII protein, or a defective or aberrant Factor VIII protein.

45. The method of claims 35 or 37, wherein the mammal has hemophilia A.

46. A method for treatment of a hemostasis related disorder in a patient in need thereof comprising administration of a therapeutically effective amount of the nucleic acid variant of any of claims 1-16, the expression vector of any of claims 17-26, or the virus or AAV vector of any of claims 30-32 in a biologically acceptable carrier to the patient.

5 47. The method of claims 35, 37 or 46, wherein said mammal or said patient has a disorder selected from the group consisting of hemophilia A, von Willebrand diseases and bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stroke, coagulopathy, disseminated intravascular coagulation (DIC) and over-anticoagulation treatment disorders.

10 48. The method of any of claims 35-47, wherein the nucleic acid variant of any of claims 1-16, the expression vector of any of claims 17-26, or the virus or AAV vector of any of claims 30-32 is delivered to said mammal or said patient intravenously, intraarterially, intramuscularly, subcutaneously, intra-cavity, or by intubation, or via catheter.

49. The method of any of claims 33-45, wherein said mammal or said patient is human.

15 50. The method of any of claims 35-47, wherein said mammal or said patient is sero-positive or sero-negative for an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV-Rh10 or AAV-Rh74 serotype.

51. The method of any of claims 35-47, further comprising administering empty capsid AAV to said mammal or said patient.

20 52. The method of any of claims 35-47, further comprising administering empty capsid of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV-12, AAV-Rh10 and/or AAV-Rh74 serotype to said mammal or said patient.

53. A method of producing FVIII protein comprising expressing in a cell the variant FVIII as claimed in any of claims 1-16, or the expression vector of claim 17 and recovering said FVIII protein produced by the cells.

25 54. The method of claim 53, further comprising purifying or isolating said FVIII protein produced by the cells.