~~WHAT IS CLAIMED IS:~~

CLAIMS

1. ~~A method of treating a bleeding disorder in a subject in need thereof comprising administering to the subject at least one dose of $5x10^{10}$ or less transducing units/kg (TU/kg) of a~~ A lentiviral vector comprising ~~an isolated~~:

a lipid coat which comprises a CD47 polypeptide and does not comprise a MHC-I polypeptide; and

a nucleic acid ~~molecule~~ comprising a nucleotide sequence encoding a polypeptide with FVIII activity, wherein the nucleotide sequence has:

(i) at least 91%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 1;

(ii) at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 2;

(iii) at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 70;

(iv) at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 71;

(v) at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 3;

(vi) at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 4;

(vii) at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 5; or

(viii) at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 58-2277 and 2320-4374 of SEQ ID NO: 6; or.

(ix) or any combination of (i) to (viii).

2. A method of treating a bleeding disorder in a subject in need thereof comprising administering to the subject at least one dose of $5x10^{10}$ or less transducing units/kg (TU/kg) of a A lentiviral vector comprising an isolated:

a lipid coat which comprises a CD47 polypeptide and does not comprise a MHC-I polypeptide; and

a nucleic acid molecule comprising a nucleotide sequence which comprises a first nucleic acid sequence encoding an N-terminal portion of a Factor VIII (FVIII) polypeptide and a second nucleic acid sequence encoding a C-terminal portion of a FVIII polypeptide;

(a) wherein the first nucleic acid sequence hascomprises:

(i) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotidesnucleotide[[s]] 58-2277 and 2320-1791 of SEQ ID NO: 3;

(ii) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotidesnucleotide[[s]] 58-2277 and 2320-1791 of SEQ ID NO: 4;

(iii) at least 60%, at least 70%, at least 8085%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotidesnucleotide[[s]] 58-17912277 of SEQ ID NO: 5; or

(iv) at least 60%, at least 70%, at least 8085%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotidesnucleotide[[s]] 58-17912277 of SEQ ID NO: 6; and

(b) wherein the second nucleotidenucleic acid sequence hascomprises:

(i) at least 60%, at least 70%, at least 8085%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 1792-2277 andnucleotide 2320-4374 of SEQ ID NO: 3;

(ii) at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to nucleotides 1792-2277 andnucleotide 2320-4374 of SEQ ID NO: 4;

(iii) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to ~~nucleotides 1792-2277 and~~nucleotide 2320-4374 of SEQ ID NO: 5; or

(iv) at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to ~~nucleotides 1792-2277 and~~nucleotide 2320-4374 of SEQ ID NO: 6; ~~or~~

~~(c) any combination of (a) and (b); and~~

wherein the N-terminal portion and the C-terminal portion together have a FVIII polypeptide activity.

~~3. The method of claim 1 or 2, wherein~~

3. The lentiviral vector of claim 1 or claim 2, wherein the lentiviral vector is for use in a method of treating hemophilia A in a subject in need thereof that comprises administering to the subject in need thereof at least one dose ranging from about $1x10^8$ to about $5x10^{10}$ transducing units/kg (TU/kg) of the lentiviral vector.

4. The lentiviral vector of claim 3, wherein:

the dose is about $5x10^{10}$ TU/kg, about $4.5x10^{10}$ TU/kg, about $4x10^{10}$ TU/kg, about $3.5x10^{10}$ TU/kg, about $3x10^{10}$ TU/kg, about $2.5x10^{10}$ TU/kg, about $2x10^{10}$ TU/kg, about $1.5x10^{10}$ TU/kg, about $1x10^{10}$ TU/kg, about $9.5x10^9$ TU/kg, about $9x10^9$ TU/kg, about $8.5x10^9$ TU/kg, about $8x10^9$ TU/kg, about $7.5x10^9$ TU/kg, about $7x10^9$ TU/kg, about $6.5x10^9$ TU/kg, about $6x10^9$ TU/kg, about $5.5x10^9$ TU/kg, about $5x10^9$ TU/kg, about $4.5x10^9$ TU/kg, about $4x10^9$ TU/kg, about $3.5x10^9$ TU/kg, about $3x10^9$ TU/kg, about $2.5x10^9$ TU/kg, about $2x10^9$ TU/kg, about $1.5x10^9$ TU/kg, about $1x10^9$ TU/kg, about $9.5x10^8$ TU/kg, about $9x10^8$ TU/kg, about $8.5x10^8$ TU/kg, about $8x10^8$ TU/kg, about $7.5x10^8$ TU/kg, about $7x10^8$ TU/kg, about $6.5x10^8$ TU/kg, about $6x10^8$ TU/kg, about $5.5x10^8$ TU/kg, about $5x10^8$ TU/kg, about $4.5x10^8$ TU/kg, about $4x10^8$ TU/kg, about $3.5x10^8$ TU/kg, about $3x10^8$ TU/kg, about $2.5x10^8$ TU/kg, about $2x10^8$ TU/kg, about $1.5x10^8$ TU/kg, or about $1x10^8$ TU/kg~~.~~;

~~4. The method of claim 1 or 2, wherein the dose is less than about $5x10^{10}$ TU/kg, less than about $4.5x10^{10}$ TU/kg, less than about $4x10^{10}$ TU/kg, less than about $3.5x10^{10}$ TU/kg, less than about $3x10^{10}$ TU/kg, less than about $2.5x10^{10}$ TU/kg, less than about $2x10^{10}$ TU/kg, less than about $1.5x10^{10}$ TU/kg, less than about $1x10^{10}$ TU/kg, less than about $9.5x10^9$ TU/kg, less than about $9x10^9$ TU/kg, less than about $8.5x10^9$ TU/kg, less than about $8x10^9$ TU/kg, less than~~

about $7.5x10^9$ TU/kg, less than about $7x10^9$ TU/kg, less than about $6.5x10^9$ TU/kg, less than about $6x10^9$ TU/kg, less than about $5.5x10^9$ TU/kg, less than about $5x10^9$ TU/kg, less than about $4.5x10^9$ TU/kg, less than about $4x10^9$ TU/kg, less than about $3.5x10^9$ TU/kg, less than about $3x10^9$ TU/kg, less than about $2.5x10^9$ TU/kg, less than about $2x10^9$ TU/kg, less than about $1.5x10^9$ TU/kg, less than about $1x10^9$ TU/kg, less than about $9.5x10^8$ TU/kg, less than about $9x10^8$ TU/kg, less than about $8.5x10^8$ TU/kg, less than about $8x10^8$ TU/kg, less than about $7.5x10^8$ TU/kg, less than about $7x10^8$ TU/kg, less than about $6.5x10^8$ TU/kg, less than about $6x10^8$ TU/kg, less than about $5.5x10^8$ TU/kg, less than about $5x10^8$ TU/kg, less than about $4.5x10^8$ TU/kg, less than about $4x10^8$ TU/kg, less than about $3.5x10^8$ TU/kg, less than about $3x10^8$ TU/kg, less than about $2.5x10^8$ TU/kg, less than about $2x10^8$ TU/kg, less than about $1.5x10^8$ TU/kg, or less than about $1x10^8$ TU/kg.

5. The method of claim 1 or 2, wherein the dose is between $1x10^8$ and $5x10^{10}$ TU/kg,the dose is between $1x10^8$ and $5x10^9$ TU/kg, between $1x10^8$ and $1x10^9$ TU/kg, between $1x10^8$ and $1x10^{10}$ TU/kg, between $1x10^9$ and $5x10^{10}$ TU/kg, between $2x10^9$ and $5x10^{10}$ TU/kg, between $3x10^9$ and $5x10^{10}$ TU/kg, between $4x10^9$ and $5x10^{10}$ TU/kg, between $5x10^9$ and $5x10^{10}$ TU/kg, between $6x10^9$ and $5x10^{10}$ TU/kg, between $7x10^9$ and $5x10^{10}$ TU/kg, $8x10^9$ and $5x10^{10}$ TU/kg, between $9x10^9$ and $5x10^{10}$ TU/kg, between 10^{10} and $5x10^{10}$ TU/kg, between $1.5x10^{10}$ and $5x10^{10}$ TU/kg, between $2x10^{10}$ and $5x10^{10}$ TU/kg, between $2.5x10^{10}$ and $5x10^{10}$ TU/kg, between $3x10^{10}$ and $5x10^{10}$ TU/kg, between $3.5x10^{10}$ and $5x10^{10}$ TU/kg, between $4x10^{10}$ and $5x10^{10}$ TU/kg, or between $4.5x10^{10}$ and $5x10^{10}$ TU/kg;

6. The method of claim 1 or 2, wherein the dose is between $1x10^9$ and $5x10^{10}$ TU/kg, between $1x10^9$ and $4.5x10^{10}$ TU/kg, between $1x10^9$ and $4x10^{10}$ TU/kg, between $1x10^9$ and $3.5x10^{10}$ TU/kg, between $1x10^9$ and $3x10^{10}$ TU/kg, between $1x10^9$ and $2.5x10^{10}$ TU/kg, between $1x10^9$ and $2x10^{10}$ TU/kg, between $1x10^9$ and $1.5x10^{10}$ TU/kg, between $1x10^9$ and $1x10^{10}$ TU/kg, between $1x10^9$ and $9x10^9$ TU/kg, between $1x10^9$ and $8x10^9$ TU/kg, between $1x10^9$ and $7x10^9$ TU/kg, between $1x10^9$ and $6x10^9$ TU/kg, between $1x10^9$ and $5x10^9$ TU/kg, between $1x10^9$ and $4x10^9$ TU/kg, between $1x10^9$ and $3x10^9$ TU/kg, and between $1x10^9$ and $2x10^9$ TU/kg.;

7. The method of claim 1 or 2, wherein the dose is between $1x10^{10}$ and $2x10^{10}$ TU/kg, between $1.1x10^{10}$ and $1.9x10^{10}$ TU/kg, between $1.2x10^{10}$ and $1.8x10^{10}$ TU/kg, between $1.3x10^{10}$ and $1.7x10^{10}$ TU/kg, or between $1.4x10^{10}$ and $1.6x10^{10}$ TU/kg.;

8. The method of claim 1 or 2, wherein the dose is about $1.5x10^{10}$ TU/kg.

9. The method of claim 1 or 2, wherein the dose is about $1.0x10^{9}$ TU/kg

10. The method of claim 1 or 2, wherein the dose is about $3.0x10^{9}$ TU/kg.

11. The method of claim 1 or 2, wherein the dose is about $6.0x10^{9}$ TU/kg.

12. The method of claim 1 or 2, wherein the dose is about $1x10^{8}$ TU/kg, about $8.3x10^{8}$ TU/kg, about $1.5x10^{9}$ TU/kg, about $4.5x10^{9}$ TU/kg, or about $1.3x10^{10}$ TU/kg.

13. The method of claim 1 or 2, wherein the dose is between $2.5x10^{9}$ TU/kg and $3.5x10^{9}$ TU/kg, between 2.6 $x10^{9}$ TU/kg and $3.4x10^{9}$ TU/kg, between $2.7x10^{9}$ TU/kg and $3.3x10^{9}$ TU/kg, between $2.8x10^{9}$ TU/kg and $3.2x10^{9}$ TU/kg, or between $2.9x10^{9}$ TU/kg and $3.1x10^{9}$ TU/kg.; or

14. The method of claim 1 or 2, wherein the dose is between $5.5x10^{9}$ TU/kg and $6.5x10^{9}$ TU/kg, between 5.6 $x10^{9}$ TU/kg and $6.4x10^{9}$ TU/kg, between $5.7x10^{9}$ TU/kg and $6.3x10^{9}$ TU/kg, between $5.8x10^{9}$ TU/kg and $6.2x10^{9}$ TU/kg, or between $5.9x10^{9}$ TU/kg and $6.1x10^{9}$ TU/kg.

5. The method lentiviral vector of claim 3, wherein the dose is about $1.5x10^{10}$ TU/kg, about $1.0x10^{9}$ TU/kg, about $3.0x10^{9}$ TU/kg, about $6.0x10^{9}$ TU/kg, about $1x10^{8}$ TU/kg, about $8.3x10^{8}$ TU/kg, about $1.5x10^{9}$ TU/kg, about $4.5x10^{9}$ TU/kg, or about $1.3x10^{10}$ TU/kg.

15. 6. The lentiviral vector of any one of claims 1 to 143-5, wherein when the lentiviral vector is administered to a subject, plasma FVIII activity in the subject is increased at 24 hours to 48 hours post administration of the lentiviral vector–is increased relative to a subject

administered a reference vector comprising a nucleic acid molecule comprising SEQ ID NO: 16.; and

16. The method of claim 15,optionally wherein the plasma FVIII activity is increased by at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 11-fold, at least about 12-fold, at least about 13-fold, at least about 14-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, at least about 100-fold, at least about 110-fold, at least about 120-fold, at least about 130-fold, at least about 140-fold, at least about 150-fold, at least about 160-fold, at least about 170-fold, at least about 180-fold, at least about 190-fold, or at least about 200-fold.

17. 7. The method lentiviral vector of any one of claims 13 to 166, wherein the lentiviral vector is formulated to be administered to the subject as a single dose or multiple doses.

18. 8. The method lentiviral vector of any one of claims 13 to 177, wherein the lentiviral vector is formulated to be administered to the subject via intravenous injection.

19. 9. The methodlentiviral vector of any one of claims 1 to 18, wherein the subject is a pediatric subject.

20. The method of any one of claims 1 to 183 to 8, wherein the subject is an adult subject.

21. 10. The methodlentiviral vector of any one of claims 1 to 209, wherein the lentiviral vector comprises a tissue specific promoter.

22. The method of claim 21,, optionally wherein the tissue specific promoter selectively enhances expression of the polypeptide with FVIII activity in a target liver cell.

23. The method of claim 22,; and/or wherein the tissue specific promoter that selectively enhances expression of the polypeptide with FVIII activity in a target liver cell comprises an mTTR promoter.

24. 11. The methodlentiviral vector of claims 22 or 23claim 10, wherein the target liver cell is a hepatocyte.

25. The method of claim 24,, and optionally wherein the isolated nucleic acid molecule is stably integrated into the genome of the hepatocyte.

12. The lentiviral vector of any one of claims 1 to 11, wherein nucleotide sequence encoding a polypeptide with FVIII activitycomprises LV-coFVIII-6 (SEQ ID NO:71).

26. 13. The methodlentiviral vector of any one of claims 1 to 2511, wherein the bleeding disorder is hemophilia A.

27. The method of any one of claims 1 to 26, wherein the isolated nucleic acid molecule comprises LV-coFVIII-6 (SEQ ID NO:71).

28. The method of any one of claims 1 to 26, wherein the isolated nucleic acid moleculenucleotide sequence encoding a polypeptide with FVIII activity comprises LV-coFVIII-6-XTEN (SEQ ID NO:72).

29. 14. The method of any one of claims 13 to 2813, wherein the dose of lentivirus vector is administered to the subject once or divided into at least two sub-doses.

30. 15. The methodlentiviral vector of any one of claims 1 to 28, wherein the dose of lentivirus vector is repeated at least twice.

31. The method of any one of claims 1 to 3014, wherein the nucleotide sequence encoding a polypeptide with FVIII activity further comprises a nucleic acid sequence encoding a signal peptide, wherein the nucleic acid sequence encoding a signal peptide has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to:

(i) nucleotides 1 to 57 of SEQ ID NO: 1;

(ii) nucleotides 1 to 57 of SEQ ID NO: 2;

(iii) nucleotides 1 to 57 of SEQ ID NO: 3;

(iv) nucleotides 1 to 57 of SEQ ID NO: 4;

(v) nucleotides 1 to 57 of SEQ ID NO: 5;

(vi) nucleotides 1 to 57 of SEQ ID NO: 6;

(vii) nucleotides 1 to 57 of SEQ ID NO: 70;

(viii) nucleotides 1 to 57 of SEQ ID NO: 71; or

(ix) nucleotides 1 to 57 of SEQ ID NO: 68.

32. 16. The methodlentiviral vector of any one of claims 1 to 3115, wherein athe nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide comprises one or more propertyproperties selected from the group consisting of:

(a) the human codon adaptation index the nucleic acid molecule or a portion thereofof the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is increased relative to SEQ ID NO: 16;

(b) the frequency of optimal codons of the nucleotide sequence or a portion thereofencoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is increased relative to SEQ ID NO:16;

(c) the nucleotide sequence or a portion thereof(c) the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide contains a higher percentage of G/C nucleotides compared to the percentage of G/C nucleotides in SEQ ID NO: 16;

(d) the relative synonymous codon usage of the nucleotide sequence or a portion thereofencoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid

sequence encoding the C-terminal portion of the FVIII polypeptide is increased relative to SEQ ID NO: 16;

(e) the effective number of codons of the nucleotide sequence or a portion thereof is reduced relative SEQ ID encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide is reduced relative SEQ ID NO: 16;

(f) the nucleotide sequence(f) the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide contains fewer MARS/ARS sequences (SEQ ID NOs: 21 and 22) relative to SEQ ID NO: 16;

(g) the nucleotide sequence encoding a polypeptide with FVIII activity, the first nucleic acid sequence encoding the N-terminal portion of the Factor VIII (FVIII) polypeptide, and/or the second nucleic acid sequence encoding the C-terminal portion of the FVIII polypeptide contains fewer destabilizing elements (SEQ ID NOs: 23 and 24) relative to SEQ ID NO: 16; and

(h) any combination thereof.

33. 17. The method lentiviral vector of any one of claims 1 to 3216, wherein the nucleotide sequence encoding a polypeptide with FVIII activity further comprises a heterologous nucleotide sequence encoding a heterologous amino acid sequence.

34. The method of claim 33,, optionally wherein the heterologous amino acid sequence is an immunoglobulin constant region or a portion thereof, XTEN, transferrin, albumin, or a PAS sequence.

35. The method of claim 33 or 34,, and more preferably wherein the heterologous amino acid sequence is linked to the N-terminus or the C-terminus of the amino acid sequence encoded by a nucleotide sequence encoding a polypeptide with FVIII activity or inserted between two amino acids in the amino acid sequence encoded by the nucleotide sequence at one or more insertion sitesites selected from TABLE 3.

36. 18. The method lentiviral vector of any one of claims 1 to 3517, wherein the FVIII polypeptide is a full length FVIII or a B domain deleted FVIII.

37. 19. The method lentiviral vector of any one of claims 1 to 36, wherein the lentiviral vector comprises a lipid coat.

38. The method of claim 37, wherein the lipid coat comprises one or more CD47 polypeptide.

39. The method of claim 3818, wherein the CD47 polypeptide is a human CD47 polypeptide.

40. The method of any one of claims 37 to 39, wherein the lipid coat comprises a high concentration of CD47 polypeptides.

41. The method of any one of claims 37 to 40, wherein the lipid coat does not comprise an MHC-I polypeptide.

42. The method of any one of claims 37 to 41, wherein the lipid coat comprises a high concentration of CD47 polypeptides and does not comprise an MHC-I polypeptide.

43. The method20. The lentiviral vector of any one of claims 1 to 4219, wherein the lentiviral vector is produced in a host cell., optionally wherein:

44. The method of claim 43, wherein the host cell expresses CD47.;

45. The method of claim 43 or 44, wherein the host cell does not express MHC-I. ;

46. The method of any one of claims 43 to 45, wherein the host cell is CD47^{high}/MHC-I^{-}.; or

The method of any one of claims 43 to 46, wherein the host cell is a CD47^{high}/MHC-I^{-} HEK 293T cell.

21. The lentiviral vector of claim 20, wherein the lentiviral vector is produced in a CD47high/MHC-I- host cell.

22. The lentiviral vector of claim 21, wherein the host cell is a HEK 293T cell modified to have increased expression of CD47 relative to unmodified HEK 293T cells.

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