[0009] Coagulation Factor IX (FIX) gene therapy has been used effectively to treat individuals with hemophilia B, a related blood coagulation condition characterized by diminished Factor IX activity (Manno C.S., et al., Nat Med., 12(3):342-47 (2006)). However, Factor VIII gene therapy presents several unique challenges. For example, the full-length, wild-type Factor VIII polypeptide (2351 amino acids; UniProt accession number P00451) is five times larger than the full-length, wild-type Factor IX polypeptide (461 amino acids; UniProt accession number P00740). As such, the coding sequence of wild-type Factor VIII is 7053 base pairs, which is too large to be packaged in conventional AAV gene therapy vectors. Further, reported recombinant expression of B-domain deleted variants of Factor VIII (BDD-FVIII) has been poor. As such, several groups have attempted to alter the codon usage of BDD-FVIII constructs, with limited success.

SUMMARY

[0010] Accordingly, there is a need for Factor VIII variants whose coding sequences are more efficiently packaged into, and delivered via, gene therapy vectors. There is also a need for synthetic, codon-altered nucleic acids which express Factor VIII more efficiently. There is also a need for codon-altered nucleic acids encoding Factor VIII polypeptides with improved folding properties, improved secretion from expressing cells, increased activity, and/or improved circulating half-life in vivo, as compared to wild-type Factor VIII or wild-type B-domain deleted Factor VIII. Such Factor VIII variants and codon-altered nucleic acids allow for improved treatment of Factor VIII deficiencies (e.g., hemophilia A). The above deficiencies and other problems associated with the treatment of Factor VIII deficiencies (e.g., hemophilia A) are reduced or eliminated by the disclosed codon-altered Factor VIII variants.

[0011] In one aspect, the present disclosure refers to a nucleic acid composition comprising a Factor VIII polynucleotide encoding a Factor VIII protein, said Factor VIII polynucleotide having the nucleic acid sequence of CS12-FL-NA (SEQ ID NO:1). For example, nucleic acid compositions (e.g., codon-altered polynucleotides) encoding Factor VIII variants are described. In some examples, the nucleic acid compositions include polynucleotides with high sequence identity to the CS04 (SEQ ID NO:37) or CS12 (SEQ ID NO:1) sequences encoding Factor VIII variants, as described herein. In some examples, the nucleic acid compositions described herein provide increased Factor VIII expression and/or increased Factor VIII activity in the blood of an animal relative to wild-type Factor VIII coding sequences and/or other codon-optimized Factor VIII coding sequences. In some examples, the nucleic acid compositions also allow for increased production of AAV-based gene therapy virions. In some examples, the nucleic

acid compositions described herein have decreased GC content and or include fewer CpG dinucleotides, as compared to wild-type sequences encoding Factor VIII. In some examples, the Factor VIII variant encoded by the nucleic acid compositions is secreted into the blood more effectively, in vivo, and/or has an increased circulating half-life in the blood, in vivo, relative to wild-type Factor VIII and/or other Factor VIII variants.

[0012] In some examples, a nucleic acid composition includes a polynucleotide encoding a Factor VIII polypeptide having an amino acid sequence of CS12-FL-AA (SEQ ID NO:2), where the polynucleotide has a sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity) to a CS12-FL-NA (SEQ ID NO:1).

[0013] In some examples, the nucleic acid composition further includes a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter polynucleotide has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to hTTR (SEQ ID NO:6). In some examples, the promoter is directly attached to the Factor VIII polynucleotide, e.g., as depicted in Figure 11.

[0014] In some examples, the nucleic acid composition further includes a liver-specific element operatively linked to the Factor VIII polynucleotide. In some examples, the liver-specific element is an enhancer element with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100%) to CRM8 (SEQ ID NO:5). In some examples, the nucleic acid composition includes two such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the nucleic acid composition includes three such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the one or more liver-specific elements and the promoter are directly attached, e.g., as depicted in Figure 11.

[0015] In some examples, the nucleic acid composition has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vr (SEQ ID NO:3).

[0016] In some examples, the acid composition has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vrp (SEQ ID NO:10).

[0017] In one aspect, mammalian gene therapy vectors that include a nucleic acid that encodes a Factor VIII variant are described. In some examples, the nucleic acid that encodes the Factor VIII includes a polynucleotide with high sequence identity to the CS04 (SEQ ID NO:37) or CS12 (SEQ ID NO:1) sequences encoding Factor VIII variants, as described herein. In some examples, the mammalian gene

therapy vectors described herein provide increased Factor VIII expression and/or increased Factor VIII activity in the blood of an animal relative to gene therapy vectors that include a natively encoded Factor VIII variant polynucleotide or other codon-optimized Factor VIII variant polynucleotides. In some examples, the mammalian gene therapy vectors described herein encode for a Factor VIII variant protein that is secreted into the blood more effectively, in vivo, and/or has an increased circulating half-life in the blood, in vivo, relative to wild-type Factor VIII and/or other Factor VIII variants.

[0018] In some examples, the mammalian gene therapy vector includes a polynucleotide encoding a Factor VIII polypeptide having an amino acid sequence of CS12-FL-AA (SEQ ID NO:2), where the polynucleotide has a sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity) to a CS12-FL-NA (SEQ ID NO:1).

[0019] In some examples, the mammalian gene therapy vector further includes a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter polynucleotide has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to hTTR (SEQ ID NO:6). In some examples, the promoter is directly attached to the Factor VIII polynucleotide, e.g., as depicted in Figure 11.

[0020] In some examples, the mammalian gene therapy vector further includes a liver-specific element operatively linked to the Factor VIII polynucleotide. In some examples, the liver-specific element is an enhancer element with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100%) to CRM8 (SEQ ID NO:5). In some examples, the nucleic acid composition includes two such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the nucleic acid composition includes three such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the one or more liver-specific elements and the promoter are directly attached, e.g., as depicted in Figure 11.

[0021] In some examples, the mammalian gene therapy vector has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vr (SEQ ID NO:3).

[0022] In some examples, the mammalian gene therapy vector is included in a plasmid having a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vrp (SEQ ID NO:10).

[0023] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) particle comprising capsid proteins encapsulating a nucleic acid composition as disclosed herein. In one example, adeno-associated virus (AAV) particles that include a nucleic acid that encodes a Factor VIII variant are

described. In some examples, the nucleic acid that encodes the Factor VIII includes a polynucleotide with high sequence identity to the CS04 (SEQ ID NO:37) or CS12 (SEQ ID NO:1) sequences encoding Factor VIII variants, as described herein. In some examples, the AAV particles described herein provide increased Factor VIII expression and/or increased Factor VIII activity in the blood of an animal relative to AAV particles that include a natively encoded Factor VIII variant polynucleotide or other codon-optimized Factor VIII variant polynucleotides. In some examples, the AAV particles described herein encode for a Factor VIII variant protein that is secreted into the blood more effectively, in vivo, and/or has an increased circulating half-life in the blood, in vivo, relative to wild-type Factor VIII and/or other Factor VIII variants.

[0024] In some examples, the nucleic acid contained in the AAV particles includes a polynucleotide encoding a Factor VIII polypeptide having an amino acid sequence of CS12-FL-AA (SEQ ID NO:2), where the polynucleotide has a sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity) to a CS12-FL-NA (SEQ ID NO:1).

[0025] In some examples, the nucleic acid contained in the AAV particles further includes a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter polynucleotide has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to hTTR (SEQ ID NO:6). In some examples, the promoter is directly attached to the Factor VIII polynucleotide, e.g., as depicted in Figure 11.

[0026] In some examples, the nucleic acid contained in the AAV particles further includes a liver-specific element operatively linked to the Factor VIII polynucleotide. In some examples, the liver-specific element is an enhancer element with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100%) to CRM8 (SEQ ID NO:5). In some examples, the nucleic acid composition includes two such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the nucleic acid composition includes three such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the one or more liver-specific elements and the promoter are directly attached, e.g., as depicted in Figure 11.

[0027] In some examples, the nucleic acid contained in the AAV particles has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vr (SEQ ID NO:3).

[0028] In some examples, the nucleic acid contained in the AAV particles is produced using a plasmid having a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vrp (SEQ ID NO:10).

[0029] In one example, methods for treating hemophilia A by administering a nucleic acid composition that encodes a Factor VIII variant to a patient with hemophilia A are described. In some examples, the nucleic acid composition includes a polynucleotide with high sequence identity to the CS04 (SEQ ID NO:37) or CS12 (SEQ ID NO:1) sequences encoding Factor VIII variants, as described herein. In some examples, the methods for treating hemophilia A described herein result in increases in Factor VIII expression and/or increases in Factor VIII activity in the blood of the patient that are greater than increases in Factor VIII expression and/or increases in Factor VIII activity in the blood of a patient administered a nucleic acid composition that includes a wild type Factor VIII coding sequence and/or a different codon-optimized Factor VIII coding sequence. In some examples, the Factor VIII variant encoded by the nucleic acid composition is secreted into the blood more effectively, in vivo, and/or has an increased circulating half-life in the blood, in vivo, relative to wild-type Factor VIII and/or other Factor VIII variants.

[0030] In some examples, the administered nucleic acid composition includes a polynucleotide encoding a Factor VIII polypeptide having an amino acid sequence of CS12-FL-AA (SEQ ID NO:2), where the polynucleotide has a sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity) to a CS12-FL-NA (SEQ ID NO:1).

[0031] In some examples, the administered nucleic acid composition further includes a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter polynucleotide has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to hTTR (SEQ ID NO:6). In some examples, the promoter is directly attached to the Factor VIII polynucleotide, e.g., as depicted in Figure 11.

[0032] In some examples, the administered nucleic acid composition further includes a liver-specific element operatively linked to the Factor VIII polynucleotide. In some examples, the liver-specific element is an enhancer element with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100%) to CRM8 (SEQ ID NO:5). In some examples, the nucleic acid composition includes two such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the nucleic acid composition includes three such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the one or more liver-specific elements and the promoter are directly attached, e.g., as depicted in Figure 11.

[0033] In some examples, the administered nucleic acid composition has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vr (SEQ ID NO:3).

[0034] In some examples, the administered nucleic acid composition has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vrp (SEQ ID NO:10).

[0035] In another aspect, the present disclosure refers to a nucleic acid composition as disclosed herein for treating hemophilia A. In another aspect, the present disclosure refers to the use of a nucleic acid composition as disclosed herein for the manufacture of a medicament for the treatment of hemophilia A. In another aspect, the present disclosure refers to a mammalian gene therapy vector as disclosed herein for treating hemophilia A. In another aspect, the present disclosure refers to the use of a mammalian gene therapy vector as disclosed herein for the manufacture of a medicament for the treatment of hemophilia A. In another aspect, the present disclosure refers to adeno-associated virus (AAV) particle as disclosed herein for treating hemophilia A. In another aspect, the present disclosure refers to the use of an adeno-associated virus (AAV) particle as disclosed herein for the manufacture of a medicament for the treatment of hemophilia A. In one example, methods for treating hemophilia A by administering a mammalian gene therapy vector that includes a nucleic acid encoding a Factor VIII variant are described. In some examples, the nucleic acid that encodes the Factor VIII includes a polynucleotide with high sequence identity to the CS04 (SEQ ID NO:37) or CS12 (SEQ ID NO:1) sequences encoding Factor VIII variants, as described herein. In some examples, the methods for treating hemophilia A described herein result in increases in Factor VIII expression and/or increases in Factor VIII activity in the blood of the patient that are greater than increases in Factor VIII expression and/or increases in Factor VIII activity in the blood of a patient administered a mammalian gene therapy vector that includes a wild type Factor VIII coding sequence and/or a different codon-optimized Factor VIII coding sequence. In some examples, the Factor VIII variant encoded by the nucleic acid within the mammalian gene therapy vector is secreted into the blood more effectively, in vivo, and/or has an increased circulating half-life in the blood, in vivo, relative to wild-type Factor VIII and/or other Factor VIII variants.

[0036] In some examples, the administered mammalian gene therapy vector includes a polynucleotide encoding a Factor VIII polypeptide having an amino acid sequence of CS12-FL-AA (SEQ ID NO:2), where the polynucleotide has a sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity) to a CS12-FL-NA (SEQ ID NO:1).

[0037] In some examples, the administered mammalian gene therapy vector further includes a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter polynucleotide has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to hTTR (SEQ ID NO:6). In some examples, the promoter is directly attached to the Factor VIII polynucleotide, e.g., as depicted in Figure 11.

[0038] In some examples, the administered mammalian gene therapy vector further includes a liver-specific element operatively linked to the Factor VIII polynucleotide. In some examples, the liver-specific element is an enhancer element with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100%) to CRM8 (SEQ ID NO:5). In some examples, the nucleic acid composition includes two such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the nucleic acid composition includes three such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the one or more liver-specific elements and the promoter are directly attached, e.g., as depicted in Figure 11.

[0039] In some examples, the administered mammalian gene therapy vector has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vr (SEQ ID NO:3).

[0040] In some examples, the administered mammalian gene therapy vector is included in a plasmid having a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vrp (SEQ ID NO:10).

[0041] In one example, methods for treating hemophilia A by administering an adeno-associated virus (AAV) particle that includes a nucleic acid encoding a Factor VIII variant are described. In some examples, the nucleic acid that encodes the Factor VIII includes a polynucleotide with high sequence identity to the CS04 (SEQ ID NO:37) or CS12 (SEQ ID NO:1) sequences encoding Factor VIII variants, as described herein. In some examples, the methods for treating hemophilia A described herein result in increases in Factor VIII expression and/or increases in Factor VIII activity in the blood of the patient that are greater than increases in Factor VIII expression and/or increases in Factor VIII activity in the blood of a patient administered an adeno-associated virus (AAV) particle that includes a wild type Factor VIII coding sequence and/or a different codon-optimized Factor VIII coding sequence. In some examples, the Factor VIII variant encoded by the nucleic acid within the adeno-associated virus (AAV) particle is secreted into the blood more effectively, in vivo, and/or has an increased circulating half-life in the blood, in vivo, relative to wild-type Factor VIII and/or other Factor VIII variants.

[0042] In some examples, the nucleic acid contained in the administered AAV particles includes a polynucleotide encoding a Factor VIII polypeptide having an amino acid sequence of CS12-FL-AA (SEQ ID NO:2), where the polynucleotide has a sequence with at least 95% sequence identity (e.g., at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity) to a CS12-FL-NA (SEQ ID NO:1).

[0043] In some examples, the nucleic acid contained in the administered AAV particles further includes a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter

polynucleotide has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to hTTR (SEQ ID NO:6). In some examples, the promoter is directly attached to the Factor VIII polynucleotide, e.g., as depicted in Figure 11.

[0044] In some examples, the nucleic acid contained in the administered AAV particles further includes a liver-specific element operatively linked to the Factor VIII polynucleotide. In some examples, the liver-specific element is an enhancer element with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100%) to CRM8 (SEQ ID NO:5). In some examples, the nucleic acid composition includes two such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the nucleic acid composition includes three such liver-specific elements operatively linked to the Factor VIII polynucleotide. In some examples, the one or more liver-specific elements and the promoter are directly attached, e.g., as depicted in Figure 11.

[0045] In some examples, the nucleic acid contained in the administered AAV particles has a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vr (SEQ ID NO:3).

[0046] In some examples, the nucleic acid contained in the administered AAV particles is produced using a plasmid having a nucleic acid sequence with at least 90% sequence identity (e.g., at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to CS12-CRM8.2-Vrp (SEQ ID NO:10). In yet another aspect, the present disclosure refers to a method for producing an adeno-associated virus (AAV) particle comprising introducing a nucleic acid composition as disclosed herein into a eukaryotic host cell: wherein: the nucleic acid composition comprises a Factor VIII polynucleotide having the nucleic acid sequence of CS12-FL-NA (SEQ ID NO:1) flanked by a 5' inverted terminal repeat sequence (5' ITR) and a 3' inverted terminal repeat sequence (3' ITR), and the mammalian host cell comprises one or more polynucleotides encoding an AAV rep gene, an AAV cap gene, and viral replication helper genes.

BRIEF DESCRIPTION OF DRAWINGS

[0047] Figures 1A and 1B collectively show the CS12 codon-optimized nucleotide sequence (SEQ ID NO:1) encoding a Factor VIII variant in accordance with some embodiments ("CS12-FL-NA" for full-length coding sequence).

[0048] Figure 2 shows the Factor VIII variant amino acid sequence (SEQ ID NO: 2) encoded by the CS12 codon-altered nucleotide sequence in accordance with some embodiments ("CS12-FL-AA" for full-length amino acid sequence).

WHAT IS CLAIMED IS:

1. A nucleic acid composition comprising a Factor VIII polynucleotide encoding a Factor VIII protein, said Factor VIII polynucleotide having the nucleic acid sequence of CS12-FL-NA (SEQ ID NO:1).

2. The nucleic acid composition of claim 1, further comprising a promoter polynucleotide operatively linked to the Factor VIII polynucleotide, wherein the promoter polynucleotide has the nucleic acid sequence of hTTR (SEQ ID NO:6)

3. The nucleic acid composition of claim 2, wherein the promoter is directly attached to the Factor VIII polynucleotide.

4. The nucleic acid composition according to any one of claims 1 to 3, further comprising a liver-specific element operatively linked to the Factor VIII polynucleotide, wherein the liver-specific element has a sequence of CRM8 (SEQ ID NO:5).

5. The nucleic acid composition of claim 4 wherein a second liver-specific element is operatively linked to said Factor VIII polypeptide.

6. The nucleic acid composition of claim 4 or 5, wherein the liver-specific element and the promoter are directly attached.

7. The nucleic acid composition of claim 1, having a nucleic acid sequence comprising CS12-CRM8.2-Vr (SEQ ID NO: 3).

8. A mammalian gene therapy vector comprising a nucleic acid composition according to any one of claims 1 to 7.

9. The mammalian gene therapy vector of claim 8, wherein the mammalian gene therapy vector is an adeno-associated virus (AAV) vector.

10. The mammalian gene therapy vector of claim 9, wherein the AAV vector is a serotype 8 adeno-associated virus (AAV-8) vector.

11. The mammalian gene therapy vector according to any one of claims 8 to 10, wherein the nucleic acid composition is a single-stranded polynucleotide encoding the Factor VIII protein.

12. An adeno-associated virus (AAV) particle comprising capsid proteins encapsulating a nucleic acid composition according to any one of claims 1 to 7.

13. The AAV particles of claim 12, wherein the capsid proteins comprise serotype 8 adeno-associated virus (AAV-8) capsid proteins.

14. The AAV particles of claim 12 or 13, wherein the nucleic acid composition is a single-stranded polynucleotide encoding the Factor VIII protein.

15. A nucleic acid composition according to any one of claims 1 to 7 for treating hemophilia A.

16. Use of a nucleic acid composition according to any one of claims 1 to 7 for the manufacture of a medicament for the treatment of hemophilia A.

17. A mammalian gene therapy vector according to any one of claims 8 to 11 for treating hemophilia A.

18. Use of a mammalian gene therapy vector according to any one of claims 8 to 11 for the manufacture of a medicament for the treatment of hemophilia A.

19. An adeno-associated virus (AAV) particle according to any one of claims 12 to 14 for treating hemophilia A.

20. Use of an adeno-associated virus (AAV) particle according to any one of claims 12 to 14 for the manufacture of a medicament for the treatment of hemophilia A.

21. A method for producing an adeno-associated virus (AAV) particle comprising introducing a nucleic acid composition according to any one of claims 1 to 7 into a eukaryotic host cell: wherein:

the nucleic acid composition comprises a Factor VIII polynucleotide having the nucleic acid sequence of CS12-FL-NA (SEQ ID NO:1) flanked by a 5' inverted terminal repeat sequence (5' ITR) and a 3' inverted terminal repeat sequence (3' ITR), and

the mammalian host cell comprises one or more polynucleotides encoding an AAV *rep* gene, an AAV *cap* gene, and viral replication helper genes.

22. The method of claim 21, wherein the nucleic acid composition is a plasmid having the nucleic acid sequence of CS12-CRM8.2-Vrp (SEQ ID NO:10).

23. The method of claim 21 or 22, wherein the AAV *cap* gene is a serotype 8 adeno-associated virus (AAV-8) *cap* gene.