However, such encapsidated AAV virus vectors were found to inefficiently transduce certain cell and tissue types and the capsids also induce an immune response.

[0009] Accordingly, use of adeno-associated virus (AAV) vectors for gene therapy is limited due to the single administration to patients (owing to the patient immune response), the limited range of transgene genetic material suitable for delivery in AAV vectors due to minimal viral packaging capacity (about 4.5kb), and slow AAV-mediated gene expression.

[0010] There is large unmet need for disease-modifying therapies in hemophilia A. Current therapies are burdensome and require intravenous (IV) administrations. First, these Factor VIII injectables do not provide continuous delivery of factors, with trough levels allowing bleeding episodes. Second, there are no approved gene therapies for hemophilia A, and AAV based therapies cannot be used by 25% to 40% of patients due to pre-existing antibodies. AAV can only be administered once, and the resulting Factor VIII levels might not be high enough to be efficacious, or may be supranormal, dose levels cannot be titrated. Third, many hemophilia A patients cannot utilize these therapies because of the development of neutralizing antibodies to these exogenous, artificial clotting factors.

[0011] Accordingly, there is need in the field for a technology that permits expression of a therapeutic FVIII protein in a cell, tissue or subject for the treatment of hemophilia A.

SUMMARY

[0011a] In one aspect, the present disclosure refers to a capsid-free close-ended DNA (ceDNA) vector comprising: at least one heterologous nucleotide sequence operably positioned between flanking inverted terminal repeats (ITRs), wherein the at least one heterologous nucleotide sequence encodes at least one Factor VIII (FVIII) protein, wherein the least one heterologous nucleotide sequence that encodes at least one FVIII protein is selected from a sequence having at least 85% identity to any sequence in Table 1.

[0011b] In another aspect, the present disclosure refers to the use of the ceDNA vector as disclosed herein in the manufacture of a medicament for expressing a FVIII protein in a cell *in vivo.*

[0011c] In another aspect, the present disclosure refers to an *in vitro* method of expressing an FVIII protein in a cell, the method comprising contacting the cell with the ceDNA vector as disclosed herein.

[0011d] In another aspect, the present disclosure refers to the use of the ceDNA vector as disclosed herein in the manufacture of a medicament for treating a subject with hemophilia A.

[0011e] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the ceDNA vector as disclosed herein.

[0011f] In another aspect, the present disclosure refers to a cell containing a ceDNA vector as disclosed herein.

[0011g] In another aspect, the present disclosure refers to a composition comprising a ceDNA vector as disclosed herein and a lipid.

[0011h] In another aspect, the present disclosure refers to a kit comprising the ceDNA vector as disclosed herein, the pharmaceutical composition as disclosed herein, the cell as disclosed herein, or the composition as disclosed herein.

BRIEF DESCRIPTION

[0012] The technology described herein relates to methods and compositions for treatment of Hemophilia A by expression of Factor VIII (FVIII) protein from a capsid-free (*e.g.*, non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector"), where the ceDNA vector comprises a FVIII nucleic acid sequence or codon optimized versions thereof. These ceDNA vector can be used to produce FVIII proteins for treatment, monitoring, and diagnosis. The application of ceDNA vectors expressing FVIII to the subject for the treatment of hemophilia A is useful to: (i) provide disease modifying levels of FVIII enzyme, (ii) be minimally invasive in delivery, (iii) be repeatable and dosed-to-effect, (iv) have rapid onset of therapeutic effect, (v) result in sustained expression of corrective FVIII enzyme in the liver, (vi) restore urea cycle function, and/or (vii) be titratable to achieve the appropriate pharmacologic levels of the defective enzyme.

[0013] In some embodiments, a ceDNA-vector expressing FVIII is optionally present in a liposome nanoparticle formulation (LNP) to the treatment of hemophilia A. The ceDNA vectors described herein can provide one or more benefits including, but not limited to providing disease modifying levels of Factor VIII, being minimally invasive in delivery, being repeatable and dosed-to-effect, providing a rapid onset of therapeutic effect, within days of therapeutic intervention, sustained expression of corrective Factor VIII levels in the circulation, be titratable to achieve the appropriate

CLAIMS

1. A capsid-free close-ended DNA (ceDNA) vector comprising:

at least one heterologous nucleotide sequence operably positioned between flanking inverted terminal repeats (ITRs), wherein the at least one heterologous nucleotide sequence encodes at least one Factor VIII (FVIII) protein, wherein the least one heterologous nucleotide sequence that encodes at least one FVIII protein is selected from a sequence having at least 85% identity to any sequence in Table 1.

2. The ceDNA vector of claim 1, wherein the ceDNA vector comprises a nucleic acid sequence selected from a sequence having at least 85% identity with a sequence in Table 9.

3. The ceDNA vector of claim 1 or claim 2, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 85% identical to SEQ ID NO: 210.

4. The ceDNA vector of claim 1 or claim 2, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 85% identical to SEQ ID NO: 214.

5. The ceDNA vector of any of claims 1-4, wherein the ceDNA vector comprises:

a promoter operatively linked to the at least one heterologous nucleotide sequence that encodes at least one FVIII protein;

an enhancer;

a 5' UTR and/or intron sequence;

a 3' UTR sequence; and/or

at least one poly A sequence.

6. The ceDNA vector of any one of claims 1-5, wherein the at least one heterologous nucleotide sequence is cDNA.

7. The ceDNA vector of any one of claims 1-6, wherein the at least one of the flanking ITRs comprises

a functional terminal resolution site and a Rep binding site;

one or both of the flanking ITRs are from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV);

the flanking ITRs are symmetric or asymmetric;

the flanking ITRs are symmetrical or substantially symmetrical;

the flanking ITRs are asymmetric;

one or both of the flanking ITRs are wild type, or both of the ITRs are wild-type;

the flanking ITRs are from different viral serotypes;

the flanking ITRs are from a pair of viral serotypes shown in Table 2;

one or both of the flanking ITRs comprises a sequence selected from the sequences in Table 3, Table 5A, Table 5B, or Table 6;

at least one of the flanking ITRs is altered from a wild-type AAV ITR sequence by a deletion, addition, or substitution that affects the overall three-dimensional conformation of the ITR;

one or both of the flanking ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12;

one or both of the flanking ITRs are synthetic; and/or

one or both of the flanking ITRs is not a wild type ITR, or both of the ITRs are not wild-type.

8. The ceDNA vector of any one of claims 1-7, wherein:

one or both of the flanking ITRs is modified by a deletion, an insertion, and/or a substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D';

one or both of the flanking ITRs is modified by a deletion, an insertion, and/or a substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D', wherein the deletion, the insertion, and/or the substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B' C, or C' regions;

one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure normally formed by the B and B' regions;

one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure normally formed by the C and C' regions;

one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions;

one or both of the flanking ITRs comprise a single stem-loop structure in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions;

one or both of the flanking ITRs comprise a single stem and two loops in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions;

one or both of the flanking ITRs comprise a single stem and a single loop in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions; and/or

both flanking ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.

9. The ceDNA vector of any one of claims 1-8, wherein at least one heterologous nucleotide

sequence is under the control of at least one regulatory switch.

10. Use of the ceDNA vector of any one of claims 1-9 in the manufacture of a medicament for expressing a FVIII protein in a cell *in vivo*.

11. The use of claim 10, wherein the cell is a liver cell.

12. An *in vitro* method of expressing an FVIII protein in a cell, the method comprising contacting the cell with the ceDNA vector of any one of claims 1-9.

13. The use of any one of claims 10-11 or the method of claim 12, wherein the at least one heterologous nucleotide sequence is codon optimized for expression in the cell.

14. Use of the ceDNA vector of any one of claims 1-9 in the manufacture of a medicament for treating a subject with hemophilia A.

15. The use of claim 14, wherein levels of FVIII in the serum of the subject are increased in subjects administered the ceDNA vector compared to a control.

16. The use of claim 15, wherein the increase in levels of FVIII is greater than about 40% compared to the control.

17. The use of any of claims 14-16, wherein the ceDNA vector is to be administered to a liver cell.

18. The use of any of claims 14-17, wherein the ceDNA vector expresses the FVIII protein in a liver cell.

19. The use of any of claims 14-18, wherein the ceDNA vector is to be administered by any one or more of: subretinal injection, suprachoroidal injection or intravitreal injection.

20. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-9.

21. A cell containing a ceDNA vector of any of claims 1-9.

22. A composition comprising a ceDNA vector of any of claims 1-9 and a lipid.

23. The composition of claim 22, wherein the lipid is a lipid nanoparticle (LNP).

24. A kit comprising the ceDNA vector of any one of claims 1-9, the pharmaceutical composition of claim 20, the cell of claim 21, or the composition of claim 22 or 23.