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 nucleotide sequence positioned between flanking inverted terminal repeats (ITRs), wherein the at least one nucleotide sequence encodes at least one Factor VIII (FVIII) protein and comprises a sequence having at least 90% identity to SEQ ID NO: 395.

[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.

CLAIMS

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

at least one nucleotide sequence positioned between flanking inverted terminal repeats (ITRs), wherein the at least one nucleotide sequence encodes at least one Factor VIII (FVIII) protein and comprises a sequence having at least 90% identity to SEQ ID NO: 395.

2. The ceDNA vector of claim 1, wherein the FVIII protein comprises a sequence having at least 95% identity to SEQ ID NO: 395.

3. The ceDNA vector of claim 1, wherein the FVIII protein comprises SEQ ID NO: 395.

4. The ceDNA vector of any one of claims 1-3, wherein the ceDNA vector comprises a nucleic acid sequence selected from a sequence having at least 90% identity with a sequence in Table 9.

5. The ceDNA vector of any one of claims 1-4, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 85% identical to SEQ ID NO: 214.

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

a promoter operatively linked to the at least one nucleotide sequence that encodes the 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.

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

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

at least one of the flanking ITRs comprises a functional terminal resolution site (TRS) and a Rep binding site;

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

the flanking ITRs are symmetric or asymmetric; optionally wherein

the flanking ITRs are symmetric or substantially symmetric; or

the flanking ITRs are asymmetric;

one of the flanking ITRs is a wild-type ITR, or wherein both of the ITRs are wild-type ITRs;

the flanking ITRs are derived from different viral serotypes;

the flanking ITRs are derived from a pair of viral serotypes set forth in Table 2;

one or both of the flanking ITRs comprise a sequence selected from the group consisting of: the sequences set forth in Table 3, Table 5A, Table 5B, and Table 6;

at least one of the flanking ITRs is altered from a wild-type AAV ITR sequence by a deletion, an addition, and/or a 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 the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12;

and/or

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

9. The ceDNA vector of any one of claims 1-7, wherein one or both of the flaking ITRs are synthetic.

10. The ceDNA vector of any one of claims 1-9, 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 the group consisting of: the A, A', B, B', C, C', D, and D' regions; optionally 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 the group consisting of: the A, A', B, B', C, C', D, and D' regions, wherein the deletion, the insertion, and/or the substitution results in the deletion of all or part of a stem-loop structure formed by the A, A', B, B' C, or C' regions; or

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 formed by the B and B' regions; or

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 formed by the C and C' regions; or

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 and/or part of a stem-loop structure formed by the C and C' regions; further optionally wherein:

one or both of the flanking ITRs comprise a single stem-loop structure in the region that, in a wild-type ITR, would comprise 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;

further optionally wherein:

one or both of the flanking ITRs comprise a single stem and two loops in the region that 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; further optionally wherein:

one or both of the flanking ITRs comprise a single stem and a single loop in the region that, in a wild-type ITR, would comprise 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; further optionally wherein:

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

11. The ceDNA vector of any one of claims 1-10, wherein the ceDNA vector comprises at least one regulatory switch.

12. Use of the ceDNA vector of any one of claims 1-11 in the manufacture of a medicament for expressing a FVIII protein in a cell *in vivo*.

13. The use of claim 12, wherein the cell is a liver cell.

14. 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 1-11.

15. The use of any one of claims 12-13 or the method of claim 14, wherein the at least one nucleotide sequence is codon optimized for expression in the cell.

16. Use of the ceDNA vector of any one of claims 1-11 in the manufacture of a medicament for treating a subject with hemophilia A.

17. The use of claim 16, wherein levels of FVIII in the serum of the subject are increased in subjects administered the ceDNA vector compared to a control.

18. The use of claim 17, wherein the increase in levels of FVIII is greater than about 40% compared to the control.

19. The use of any of claims 16-18, wherein the ceDNA vector is delivered to a liver cell.

20. The use of any of claims 16-19, wherein the ceDNA vector expresses the FVIII protein in a liver cell.

21. The use of any of claims 16-20, wherein the ceDNA vector is to be administered by any one or more routes selected from the group consisting of: intravenous injection, intramuscular injection, and infusion.

22. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-11.

23. A cell containing a ceDNA vector of any of claims 1-11.

24. A composition comprising a ceDNA vector of any of claims 1-11 and a lipid.

25. The composition of claim 24, wherein the lipid is a lipid nanoparticle (LNP).

26. A kit comprising the ceDNA vector of any one of claims 1-11, the pharmaceutical composition of claim 22, the cell of claim 23, or the composition of claim 24 or 25.