to a desired expression level or desired expression level range based on the concentration of ceDNA vector in the re-dose administration, allowing for a controlled and specific increases in the expression of the transgene in the cell or subject.

[0012a] In one aspect, the present disclosure refers to the use of a capsid-free close-ended DNA (ceDNA) vector in the manufacture of a medicament for treating a disease associated with reduced expression, lack of expression or dysfunction of a gene in a subject, wherein: a. a first dose of a capsid-free closed-ended DNA (ceDNA) vector comprising a nucleic acid cassette containing at least one transgene linked to a promoter between flanking inverted terminal repeats (ITRs) is to be administered, wherein the first dose comprises an amount of the ceDNA vector that is sufficient to express a measurable level of the transgene, wherein the transgene encodes a desired protein; and b. at least a second dose of the ceDNA vector comprising the at least one transgene between the flanking ITRs is to be administered, wherein the second dose is formulated for administration to: (i) continue expression of the desired protein at a predetermined level of expression for a predetermined time; (ii) increase expression of the desired protein to a predetermined level, and wherein the second dose, or a dose subsequent to the second dose, of the ceDNA vector is selected from the group consisting of: an amount that is between 2-fold and 10-fold the first dose of the ceDNA vector; an amount that is the same as the first or preceding dose of the ceDNA vector; and an amount that increases the expression of the transgene by 2-20 fold as compared the expression of the transgene after administration of the first or preceding dose of the ceDNA vector, and wherein the second dose does not generate an immune reaction sufficient to prevent obtaining the predetermined level of expression of the desired protein.

[0013] Accordingly, in some examples a ceDNA vector as disclosed herein can be re-administered (also referred to herein as a "redose" or "booster" administration) to continue transgene expression level at a predetermined level for a predetermined time, or to increase the expression level of the transgene above a prior expression level which was achieved on a first, or prior ceDNA vector administration, where the second administration or booster administration does not generate an immune reaction that prevents expression of the transgene by not generating an immune response to the vector itself that impacts expression of the transgene, or where the immune reaction is less as compared to a re-administration of a viral vector comprising viral proteins, including but not limited to a viral vector comprising a capsid, such as a parvovirus or a lentivirus.

[0014] Without wishing to be limited to theory, research shows that long-term transgene expression using conventional AAV viral vectors wanes over time. One way to ensure long-term transgene expression using conventional AAV vectors has traditionally been to increase the titre and dose of the delivered AAV vector at the initial administration. However, it is known that too high a titre or dose of the AAV vector can result in a variety of side-effects. Furthermore, as discussed above, re-administration of an AAV viral vector is traditionally not possible due to immune responses. Ways to circumvent causing an immune response to re-administration of AAV vectors typically require re-administration of an AAV vector with a different capsid configuration as compared to the AAV vector administered on any previous administrations. Such strategies however, can still pose significant risk to the subject by inducing an immune response to the AAV vector and potentially deleterious effects.

[0015] Herein, the disclosure provides a method for controlled expression of a transgene, including long term expression using a close-ended DNA (ceDNA) vector. It is demonstrated herein that a ceDNA vector can titrated to increase the transgene expression levels by, e.g., repeat or re-administration of the ceDNA vector. Additionally, the level of transgene expression can be maintained over a long term, and if any drop in expression levels is observed, a redose administration of the ceDNA vector can be used to maintain the desired level or even to increase the level of transgene expression if desirable for the subject and/or the disease or disorder to be treated.

[0016] Accordingly, provided herein are methods of administering ceDNA vectors for sustaining and/or increasing the level of expression of a transgene from a ceDNA vector in a cell or a subject, and where the expression level can be sustained or increased with one or more subsequent administrations (e.g., a re-dose or a booster administration). In the event that ceDNA-delivered transgene expression decreases for any reason, re-dosing of the ceDNA vector can re-establish or maintain the desired

CLAIMS

1. Use of a capsid-free close-ended DNA (ceDNA) vector in the manufacture of a medicament for treating a disease associated with reduced expression, lack of expression or dysfunction of a gene in a subject, wherein:

a. a first dose of a capsid-free closed-ended DNA (ceDNA) vector comprising a nucleic acid cassette containing at least one transgene linked to a promoter between flanking inverted terminal repeats (ITRs) is to be administered, wherein the first dose comprises an amount of the ceDNA vector that is sufficient to express a measurable level of the transgene, wherein the transgene encodes a desired protein; and

b. at least a second dose of the ceDNA vector comprising the at least one transgene between the flanking ITRs is to be administered, wherein the second dose is formulated for administration to: (i) continue expression of the desired protein at a predetermined level of expression for a predetermined time; (ii) increase expression of the desired protein to a predetermined level, and wherein the second dose, or a dose subsequent to the second dose, of the ceDNA vector is selected from the group consisting of:

an amount that is between 2-fold and 10-fold the first dose of the ceDNA vector;

an amount that is the same as the first or preceding dose of the ceDNA vector; and

an amount that increases the expression of the transgene by 2-20 fold as compared the expression of the transgene after administration of the first or preceding dose of the ceDNA vector, and wherein the second dose does not generate an immune reaction sufficient to prevent obtaining the predetermined level of expression of the desired protein.

2. The use of claim 1, wherein a pharmaceutically acceptable carrier is to be administered in combination with the ceDNA vector of the first dose, and/or the second or the dose subsequent to the second dose.

3. The use of claim 1 or 2, wherein the second dose is formulated for administration at a time when the level of the expression of the transgene decreases from a predetermined level.

4. The use of any of claims 1-3, wherein the second dose is formulated for administration at least 90 days after the first administration; or wherein the administrations are on a periodic schedule.

5. The use of any of claims 1-4, wherein the second dose is formulated for administration to prolong the expression of the desired protein at a predetermined level of expression.

6. The use of any of claims 1-5, wherein the desired protein is an inhibitor protein; or the desired protein replaces a defective protein expressed in the subject or a protein that is not being expressed.

7. The use of claim 1, wherein the promoter is an inducible promoter or a repressible promoter.

8. The use of any of claims 1-7, wherein the ceDNA vector further comprises a regulatory switch.

9. The use of any of claims 1-8, wherein, the two flanking ITRsare AAV ITRs.

10. The use of any of claims 1-9, wherein at least one of the flanking ITRs comprises a functional terminal resolution site and a Replication protein (Rep) binding site.

11. The use of any of claims 1-10, wherein:
the flanking ITRs are symmetric or asymmetric;
the flanking ITRs are symmetric or substantially symmetric;
the flanking ITRs are asymmetric; and/or
the flanking ITRs are derived from the same viral serotype or are derived from different viral serotypes.

12. The use of any of claims 1-11, wherein:
one of the flanking ITRs is not a wild-type ITR;
both of the flanking ITRs are not wild-type ITRs;
one of the flanking ITRs is a wild-type ITR; or
both of the flanking ITRs are wild-type ITRs.

13. The use of any of claims 1-11, wherein one or both of the flanking ITRs are synthetic.

14. The use of any of claims 1-13, wherein:
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;
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; and/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 and/or all or part of a stem-loop structure formed by the C and C' regions.

15. The use of any of claims 1-14, 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 first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions, or wherein one or both of the ITRs comprise a single stem and two loops 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.

16. The use of any of claims 1-15, wherein the subject has a disease or disorder selected from the group consisting of: a genetic disease, a metabolic disease, an ocular disease, a cancer, an autoimmune disease, a neurodegenerative disorder, hypercholesterolemia, an acute organ rejection, multiple sclerosis, post-menopausal osteoporosis, a skin condition, asthma, and hemophilia.

17. The use of claim 16, wherein:
the cancer is selected from the group consisting of: a solid tumor, soft tissue sarcoma, lymphoma, and leukemia;
the metabolic disease is selected from the group consisting of: Gaucher disease and phenylketonuria; the hemophilia is selected from the group consisting of: hemophilia A and hemophilia B;
the ocular disease is selected from the group consisting of: vascular or wet macular degeneration, Stargardt disease, Leber Congenital Amaurosis (LCA), and Usher syndrome;
the autoimmune disease is selected from the group consisting of: rheumatoid arthritis and Crohn's disease;
the skin condition is selected from the group consisting of: psoriasis and atopic dermatitis; and
the neurodegenerative disorder is Alzheimer's disease.

18. The use of any of claims 1-16, wherein the second dose, or the dose subsequent to the second dose, of the ceDNA vector is formulated to increase the expression level of the transgene as compared to the level of expression of transgene achieved after the second dose, or to increase the expression level of the transgene to achieve a desired expression level.

19. The use of any of claims 1-18, wherein the second dose, or the dose subsequent to the second dose, of the ceDNA vector is determined using a dose-dependent relationship between the preceding dose and the level of transgene expression from the ceDNA vector to achieve the desired level of expression of the transgene in the subject.

20. The use of any one of claims 1-19, wherein the second dose, or the dose subsequent to the second dose, of the ceDNA vector is selected from: an amount that increases the expression of the transgene by at least 3-fold, or at least 5-fold, or least 10-fold, as compared to the expression of the transgene after administration of the preceding dose of the ceDNA vector.