examples, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

[0017] According to another example, the disclosure provides a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs), and (ii) a modified dexamethasone compound. According to ~~another~~ one aspect, the disclosure provides a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a ~~heterologous~~ nucleic acid sequence encoding a transgene ~~operably~~ positioned between two flanking AAV inverted terminal repeat~~s sequences~~ (ITRs), and (ii) a ~~modified~~ dexamethasone compound modified with a fatty acid. According to some examples, the ceDNA vector when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some examples, the at least one of the ITRs comprises a functional AAV terminal resolution site (TRS) and a Rep binding site. According to some examples, both ITRs are naturally occurring AAV ITRs from the same AAV strain. According to some examples, one ITR comprises a deletion, insertion, or substitution relative to the other ITR. According to some examples, one ITR comprises a deletion, insertion, or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR. According to some examples, the modified dexamethasone compound comprises at least one fatty acid. According to some examples, the modified dexamethasone compound is dexamethasone palmitate. According to some examples, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some examples, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some examples, the modified dexamethasone compound is co-administered with the ceDNA vector being administered to the cell but is not co-encapsulated with the ceDNA vector.

[0018] According to any of the foregoing examples and aspects, the composition further comprises at least one additional innate immune pathway inhibitor. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of the cGAS/STING pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

[0019] According to some examples, the disclosure provides a method for inhibiting immune responses when expressing a transgene in a cell, comprising administering to a cell a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein

the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs) and (ii) a modified dexamethasone compound. In one aspect, the disclosure provides a use of a composition in the manufacture of a medicament for inhibiting an immune response when expressing a transgene in a cell, the composition comprising: (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a ~~heterologous~~ nucleic acid sequence encoding a transgene ~~operably~~ positioned between two flanking AAV inverted terminal repeats ~~sequences~~ (ITRs), and (ii) a ~~modified~~ dexamethasone compound modified with a fatty acid. According to some examples, one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site. According to some examples, one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR. According to some examples, the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some examples, the modified dexamethasone compound comprises at least one fatty acid. According to some examples, the modified dexamethasone compound is dexamethasone palmitate. According to some examples, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some examples, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some examples, both ITRs are naturally occurring AAV ITRs from the same AAV strain. In another example, one ITR comprises a deletion, insertion, or substitution relative to the other ITR. In another example, one ITR comprises a deletion, insertion or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR. In another example, the two ITRs are a pair of ITRs selected from the group consisting of (a) SEQ ID NO: 1 (3' WT-ITR) and SEQ ID NO: 4 (5' mod ITR); and (b) SEQ ID NO: 3 (3' mod ITR) and SEQ ID NO: 2 (5' WT-ITR). According to some examples, the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier. According to some examples, increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell. In another example, the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.

[0020] According to any of the foregoing examples and aspects, at least one additional innate immune pathway inhibitor is administered. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of the cGAS/STING pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some examples, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

[0021] In another example, the ceDNA vector is obtained from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA vector polynucleotide, which is devoid of viral capsid coding sequences in the presence of Rep protein under conditions effective and for time sufficient to induce production of the closed-ended linear, capsid-free, DNA vector within the insect cells, wherein the insect cells do not comprise production of closed-ended linear, capsid-free, DNA within the insect cells; and (b) harvesting and isolating the closed-ended linear capsid-free, DNA from the insect cells; wherein the presence of the linear, capsid-free, DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA vector and analyzing the digested DNA material on a non-denaturing gel to confirm the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA. According to some examples, the ceDNA vector is obtained by cell-free synthesis. According to some examples, the ceDNA vector is encapsulated. According to some examples, the encapsulation is with a liposome. According to some examples, the encapsulation is by a lipid nanoparticle.

[0022] According to some examples, the disclosure provides a method for treating a disease in a subject, comprising: administering to a subject in need thereof a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs) and (ii) a modified dexamethasone compound. In one aspect, the disclosure provides a use of a composition in the manufacture of a medicament for treating a disease in a subject, the composition comprising: (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a ~~heterologous~~ nucleic acid sequence encoding a transgene ~~operably~~ positioned between two flanking AAV inverted terminal repeat<u>s</u> ~~sequences~~ (ITRs), and (ii) a ~~modified~~ dexamethasone compound <u>modified with a fatty acid</u>. According to some examples, one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site. According to some examples, one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR. According to some examples, the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some examples, the modified dexamethasone compound comprises at least one fatty acid. According to some examples, the modified dexamethasone compound is dexamethasone palmitate. According to some examples, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some examples, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some examples, both ITRs are naturally occurring AAV ITRs from the same AAV strain. In another example, one ITR comprises a deletion, insertion, or substitution relative to the other ITR. In another example, one ITR comprises a deletion, insertion or substitution relative to the other ITR and neither ITR is a naturally

CLAIMS

What is claimed is:

1. A composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a ~~heterologous~~ nucleic acid sequence encoding a transgene ~~operably~~ positioned between two flanking AAV inverted terminal repeats ~~sequences~~ (ITRs), and (ii) a ~~modified~~ dexamethasone compound modified with a fatty acid.
2. The composition of claim 1, wherein the ceDNA vector, when digested with a restriction enzyme having a single recognition site on the ceDNA vector, has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel.
3. The composition of claim 1, wherein:
   at least one of the flanking ITRs comprises a functional AAV terminal resolution site (TRS) and a Rep binding site: both of the flanking ITRs are naturally occurring AAV ITRs from the same AAV strain;
   one of the flanking ITR comprises a deletion, an insertion, and/or a substitution relative to the other flanking ITR; and/or
   one of the flanking ITR comprises a deletion, an insertion, and/or a substitution relative to the other flanking ITR and neither flanking ITR is a naturally occurring AAV ITR.
4. The composition of any of claims 1-3, wherein the ~~modified~~ dexamethasone compound modified with a fatty acid is dexamethasone palmitate.
5. The composition of any of claims 1-3, wherein the ~~modified~~ dexamethasone compound modified with a fatty acid is co-encapsulated with the ceDNA vector.
6. The composition of any of claims 1-3, wherein the ~~modified~~ dexamethasone compound modified with a fatty acid is not co-encapsulated with the ceDNA vector.
7. The composition of any of claims 1-6, wherein the composition further comprises at least one additional innate immune pathway inhibitor.
8. The composition of claim 7, wherein the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway.
9. Use of a composition in the manufacture of a medicament for inhibiting an immune response when expressing a transgene in a cell, the composition comprising: (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a ~~heterologous~~ nucleic acid sequence encoding a transgene ~~operably~~ positioned

between two flanking AAV inverted terminal repeats ~~sequences~~ (ITRs), and (ii) a ~~modified~~ dexamethasone compound modified with a fatty acid.

10. Use of a composition in the manufacture of a medicament for treating a disease in a subject, the composition comprising: (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a ~~heterologous~~ nucleic acid sequence encoding a transgene ~~operably~~ positioned between two flanking AAV inverted terminal repeats ~~sequences~~ (ITRs), and (ii) a ~~modified~~ dexamethasone compound modified with a fatty acid.

11. The use of claim 9 or 10, wherein one of the flanking ITRs comprises a functional AAV terminal resolution site and a Rep binding site, and one of the flanking ITRs comprises a deletion, an insertion, and/or a substitution relative to the other ITR.

12. The use of claim 9 or 10, wherein the ~~modified~~ dexamethasone compound modified with a fatty acid is to be co-encapsulated with the ceDNA vector being administered to the cell.

13. The use of claim 9 or 10, wherein the ~~modified~~ dexamethasone compound modified with a fatty acid is to be co-administered with the ceDNA vector being administered to the cell but is not co-encapsulated with the ceDNA vector.

14. The use of claim 9 or 10, wherein the ~~modified~~ dexamethasone compound modified with a fatty acid is to be administered prior to, at the same time as, or after the administration of the ceDNA vector.

15. The use of any of claims 9-14, wherein the ~~modified~~ dexamethasone compound is dexamethasone palmitate modified with a fatty acid.

16. The use of claim 9 or 10 wherein:

both of the flanking ITRs are naturally occurring AAV ITRs from the same AAV strain:

one of the flanking ITRs comprises a deletion, an insertion, and/or a substitution relative to the other flanking ITR;

one of the flanking ITRs comprises a deletion, an insertion and/or a substitution relative to the other flanking ITR and neither flanking ITR is a naturally occurring AAV ITR; and/or

the two flanking ITRs are a pair of ITRs selected from the group consisting of:

a. SEQ ID NO: 1 and SEQ ID NO: 4; and

b. SEQ ID NO: 3 and SEQ ID NO: 2.

17. The use of any one of claims 9-16, wherein the ceDNA vector is to be administered in combination with a pharmaceutically acceptable carrier.

18. The use of claim 9 or 10, wherein increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell.

19. The use of claim 9 or 10, wherein the ~~heterologous~~ nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.

20. The use of claim 9 or 10, wherein at least one additional innate immune inhibitor is to be co-administered with the ceDNA vector and the ~~modified~~ dexamethasone compound modified with a fatty acid.

21. The use of claim 20, wherein the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway.

22. The use of any one of claims 9-21, wherein the ceDNA vector is obtained from a process comprising the steps of:

    a. incubating a population of insect cells harboring a ceDNA vector polynucleotide, which is devoid of viral capsid coding sequences in the presence of Rep protein under conditions effective and for time sufficient to induce production of the closed-ended, linear, capsid-free DNA vector within the insect cells, wherein the insect cells do not comprise production of closed-ended linear, capsid-free DNA within the insect cells; and

    b. harvesting and isolating the closed-ended linear, capsid-free DNA from the insect cells.

23. The use of claim 9 or 10, wherein the ceDNA, when digested with a restriction enzyme having a single recognition site on the DNA vector, has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA when analyzed on a non-denaturing gel.

24. The use of any of claims 9-21, wherein the ceDNA vector is obtained by cell-free synthesis.

25. The use of any of claims 9-24, wherein the ceDNA vector is encapsulated.