and/or destruction of such DNA. Macrophage lineage cells in particular have multiple DNA sensor pathways, including the TLR9 pathway, the cGAS/STING pathway, and inflammasome pathways.

[0010] Although conceptually elegant, the prospect of using nucleic-acid molecules for gene therapy for treating human diseases remains uncertain. The main cause of this uncertainty is the apparent adverse events relating to host's innate immune response to nucleic acid therapeutics and, thus, the way in which these materials modulate expression of their intended targets in the context of the immune response. The current state of the art surrounding the creation, function, behavior and optimization of nucleic acid molecules that may be adopted for clinical applications has a particular focus on: (1) antisense oligonucleotides and duplex RNAs that directly regulate translation and gene expression; (2) transcriptional gene silencing RNAs that result in long-term epigenetic modifications; (3) antisense oligonucleotides that interact with and alter gene splicing patterns; (4) creation of synthetic or viral vectors that mimic physiological functionalities of naturally occurring AAV or lentiviral genome; and (5) the *in vivo* delivery of therapeutic oligonucleotides. However, despite the advances made in the development of nucleic acid therapeutics that are evident in recent clinical achievements, the field of gene therapy is still severely limited by unwanted adverse events in recipients triggered by the therapeutic nucleic acids, themselves.

[0011] Administration of exogenous DNA for gene therapy may contend with clearance and/or destruction prior to reaching the desired intracellular location or expression of the encoded gene(s) of interest. Modulating such host immune defense against exogenous DNA would be potentially of benefit in facilitating gene therapy efficacy.

[0012] Accordingly, there is a need in the field for a new technology that inhibits (*e.g.*, reduces, ameliorates, mitigates, prevents) the immune response on administration of vectors or nucleic acid to a subject that permits expression of a therapeutic protein in a cell, tissue or subject for the treatment of a wide variety of diseases.

SUMMARY

[0013] The present disclosure provides methods and pharmaceutical compositions for minimizing or reducing an innate immune response in a subject suffering from a genetic disorder and receiving gene or nucleic acid therapy ("nucleic acid therapeutics" or "therapeutic nucleic acid" (TNA)) Provided herein are non-viral capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) in compositions comprising inhibitors for minimizing and reducing innate immune responses, and methods comprising the same.

[0014] According to some examples, the disclosure provides methods for inhibiting immune responses when expressing a transgene in a cell, comprising co-administering to a cell a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector) and a modified dexamethasone, such that the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two different AAV inverted terminal repeat

sequences (ITRs). 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 is dexamethasone palmitate.

[0015] Also provided herein, in some examples, are methods for treating disease in a subject comprising administering to a subject in need thereof, a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), such that the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two different AAV inverted terminal repeat sequences (ITRs). 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 method comprises separately administering a modified dexamethasone. 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 administration is prior to the administration of the ceDNA vector. According to some examples, the administration is simultaneous with the administration of the ceDNA vector. According to some examples, the administration is subsequent to the administration of the ceDNA vector. 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. According to some examples, increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell. According to some examples, the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.

[0016] 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.

[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 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 sequences (ITRs), and (ii) a modified dexamethasone compound. 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 medicamentfor 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 repeat sequences (ITRs), and (ii) a modified dexamethasone compound. 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 sequences (ITRs), and (ii) a modified dexamethasone compound. 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).

[0023] In another example, the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier. In another example, 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.

[0024] In any of the foregoing examples and aspects, at least one additional innate immune pathway inhibitor is co-administered with the ceDNA vector and the modified dexamethasone compound. 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. 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. According to some examples, 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 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIG. 1A** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein, comprising asymmetric ITRs. In this embodiment, the exemplary ceDNA vector comprises an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene, can be inserted into the cloning site (R3/R4) between the CAG

promoter and WPRE. The expression cassette is flanked by two inverted terminal repeats (ITRs) – the wild-type AAV2 ITR on the upstream (5'-end) and the modified ITR on the downstream (3'-end) of the expression cassette, therefore the two ITRs flanking the expression cassette are asymmetric with respect to each other.

[0026] **FIG. 1B** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein comprising asymmetric ITRs with an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene, can be inserted into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two inverted terminal repeats (ITRs) – a modified ITR on the upstream (5'-end) and a wild-type ITR on the downstream (3'-end) of the expression cassette.

[0027] **FIG. 1C** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein comprising asymmetric ITRs, with an expression cassette containing an

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 repeat sequences (ITRs), and (ii) a modified dexamethasone compound.
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, insertion, or substitution relative to the other ITR; and/or
   one of the flanking ITR comprises a deletion, insertion, or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR.
4. The composition of any of claims 1-3, wherein the modified dexamethasone compound is dexamethasone palmitate.
5. The composition of any of claims 1-3, wherein the modified dexamethasone compound is co-encapsulated with the ceDNA vector.
6. The composition of any of claims 1-3, wherein the modified dexamethasone compound 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 medicamentfor 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 repeat sequences (ITRs), and (ii) a modified dexamethasone compound.

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 repeat sequences (ITRs), and (ii) a modified dexamethasone compound.
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, insertion, or substitution relative to the other ITR.
12. The use of claim 9 or 10, wherein the modified dexamethasone compound 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 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 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.
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, or a substitution relative to the other ITR;
    one of the flanking ITRs comprises a deletion, an insertion or a substitution relative to the other ITR and neither 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.

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.