[0015] 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

[0016] The present disclosure provides methods and pharmaceutical compositions for inhibiting (*i.e.*, reducing or suppressing) an 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) and inhibitors for inhibiting an immune response (*e.g.*, an innate immune response). In one aspect, the present disclosure refers to the use of a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector) in the manufacture of a medicament for inhibiting an immune response when a transgene is expressed in a cell, wherein the ceDNA vector comprises at least one heterologous nucleotide sequence operably positioned between two flanking inverted terminal repeat sequences (ITRs); and at least one inhibitor of the immune response. In another aspect, the present disclosure refers to a host cell comprising a ceDNA expression construct that encodes the ceDNA vector produced from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA expression construct in the presence of at least one Rep protein, wherein the ceDNA expression construct encodes the ceDNA vector, under conditions effective and for a time sufficient to induce production of the ceDNA vector within the insect cells; and (b) isolating the ceDNA vector from the insect cells. In another aspect, the present disclosure refers to a method of producing a ceDNA vector, comprising: (a) incubating the host cell as disclosed herein under conditions effective and for time sufficient to induce production of the ceDNA vector; and (b) isolating the ceDNA from the host cells. In another aspect, the present disclosure refers to a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises at least one heterologous nucleotide sequence operably positioned between two flanking inverted terminal repeat sequences (ITRs), wherein the ceDNA vector further encodes the at least one inhibitor of the immune response. In another aspect, the present disclosure refers to the use of the composition as disclosed herein in the manufacture of a medicament for expressing an inhibitor of the immune response in a cell. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the composition as disclosed herein. In another aspect, the present disclosure refers to a cell comprising the composition as disclosed herein. In yet another aspect, the present disclosure refers to a kit comprising the composition as disclosed herein, the pharmaceutical composition as disclosed herein, or the cell as disclosed herein. According to some examples, the pharmaceutical compositions and formulations may include one or more inhibitors of the immune response (*e.g.*, the innate immune response), such

as rapamycin and rapamycin analogs thereof, TLR antagonists (*e.g.*, TLR9 antagonists), cGAS antagonists and inflammasome antagonists (*e.g.*, any one or more of: an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof).

[0017] According to some examples, the disclosure provides compositions and methods for inhibiting (*i.e.*, reducing or suppressing) an immune response (*e.g.*, an innate immune response) using non-viral, capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) for expressing an inhibitor of the innate immune response 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 nucleic acid sequence or codon optimized versions thereof of an inhibitor of the immune response (*e.g.*, the innate immune response).

[0018] According to some examples, the disclosure provides compositions and methods for inhibiting (*i.e.*, reducing or suppressing) an immune response (*e.g.*, an innate immune response) using non-viral, capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) for expressing rapamycin and rapamycin analogs thereof, 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 nucleic acid sequence or codon optimized versions thereof of rapamycin and rapamycin analogs thereof. Accordingly, these ceDNA vectors can be used to produce rapamycin and rapamycin analogs thereof, for inhibiting the immune system (*e.g.*, the innate immune system).

[0019] According to some examples, the disclosure provides compositions and methods for inhibiting (*i.e.*, reducing or suppressing) an immune response (*e.g.*, an innate immune response) using non-viral, capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) for expressing a TLR antagonist, 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 nucleic acid sequence or codon optimized versions thereof of a TLR antagonist. Accordingly, these ceDNA vectors can be used to produce a TLR antagonist, for inhibiting the immune system (*e.g.*, the innate immune system).

[0020] According to some examples, the disclosure provides compositions and methods for inhibiting (*i.e.*, reducing or suppressing) an immune response (*e.g.*, an innate immune response) using non-viral, capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) for expressing a cGAS antagonist, 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 nucleic acid sequence or codon optimized versions thereof of a cGAS antagonist. Accordingly, these ceDNA vectors can be used to produce a cGAS antagonist, for inhibiting the immune system (*e.g.*,the innate immune system).

[0021] According to some examples, the disclosure provides compositions and methods for inhibiting (*i.e.*, reducing or suppressing) an immune response (*e.g.*, an innate immune response) using non-viral, capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) for expressing an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof, 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 nucleic acid sequence or codon optimized versions thereof of an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof. Accordingly, these ceDNA vectors can be used to produce an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof, for inhibiting the immune system (*e.g.*, innate immune system).

[0022] According to some examples, the pharmaceutical compositions and formulations may include one or more inhibitors of the immune response (*e.g.*, innate immune response), as described herein, in, in conjunction with various types of therapeutic nucleic acids (TNA) and carriers (*e.g.*, lipid nanoparticle). According to some examples, the composition further comprises an excipient or carrier. According to some examples, the pharmaceutical composition comprises a lipid nanoparticle (LNP). In one example, the LNP comprises a cationic lipid. According to some examples, the LNP comprises polyethylene glyclol (PEG). According to some examples, the LNP comprises a cholesterol.

[0023] The methods described herein generally include use of one or more inhibitors of the immune response (*e.g.*, innate immune response) (*e.g.*, rapamycin and analogs thereof, TLR antagonists, cGAS antagonists) for preventing, reducing, attenuating or even eliminating immune responses associated with administration of a transgene (*e.g.*, a therapeutic nucleic acid (TNA)). Methods comprising administering the same are described herein.

[0024] In one example, the therapeutic nucleic acid is an RNA molecule, or a derivative thereof. In one example, the RNA molecule is an antisense oligonucleotide. In one example, the antisense oligonucleotide is an antisense RNA. In one example, the RNA is RNA interference (RNAi).

[0025] In one example, the therapeutic nucleic acid is an mRNA molecule.

[0026] In one example, the therapeutic nucleic acid is a DNA molecule, or a derivative thereof.

[0027] In one example, the therapeutic nucleic acid is a DNA antisense oligonucleotide. In one example, the DNA antisense oligonucleotide is morpholino based nucleic acid. In one example, the morpholino based nucleic acid is a phosphorodiamidate morpholino oligomer (PMO).

[0028] In one example, the therapeutic nucleic acid is a closed-ended DNA (ceDNA). In one example, the ceDNA comprises an expression cassette comprising a promoter sequence and a

transgene. In one example, the ceDNA comprises expression cassette comprising a polyadenylation sequence. In one example, the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of the expression cassette. In one example, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. In one example, the expression cassette is connected to an ITR at 3' end (3' ITR). In one example, the expression cassette is connected to an ITR at 5' end (5' ITR). In one example, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.

[0029] In one example, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. In one example, the spacer sequence is at least 5 base pair long in length. In one example, the spacer sequence is 5 to 200 base pairs long in length. In one example, the spacer sequence is 5 to 500 base pairs long in length.

[0030] In one example, the ITR is an ITR derived from an AAV serotype. In one example, the AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12. In one example, the ITR is derived from an ITR of goose virus. In one example, the ITR is derived from a B19 virus ITR. In one example, the ITR is a wild-type ITR from a parvovirus. In one example, the ITR is a mutant ITR. In one example, the ceDNA comprises two mutant ITRs in both 5' and 3' ends of the expression cassette.

[0031] In one example, the ceDNA has a nick or a gap.

[0032] In one example, the ceDNA is synthetically produced in a cell-free environment.

[0033] In one example, the ceDNA is produced in a cell. In one example, the ceDNA is produced in insect cells. In one example, the insect cell is Sf9. In one example, the ceDNA is produced in a mammalian cell. In one example, the mammalian cell is human cell line.

[0034] In one example, the therapeutic nucleic acid is a closed-ended DNA comprising at least one protelomerase target sequence in its 5' and 3' ends of the expression cassette.

[0035] In one example, the therapeutic nucleic acid is a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in 5' and 3' ends of an expression cassette.

[0036] In one example, the therapeutic nucleic acid is a DNA-based minicircle or a MIDGE.

[0037] In one example, the therapeutic nucleic acid is a linear covalently closed-ended DNA vector. In one example, the linear covalently closed-ended DNA vector is a ministring DNA.

[0038] In one example, the therapeutic nucleic acid is a doggybone (dbDNA™) DNA.

[0039] In one example, the therapeutic nucleic acid is a minigene.

[0040] In one example, the therapeutic nucleic acid is a plasmid.

[0041] Accordingly, provided herein, in some examples are methods for inhibiting or suppressing immune responses when expressing a transgene in a cell, comprising: co-administering to a cell (1) a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector) and (2) an inhibitor of an immune response (*e.g.*, an innate immune response), as

described herein. The ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two different AAV inverted terminal repeat sequences (ITRs), one of the ITRS comprising a functional AAV terminal resolution site and a Rep binding site, one of the ITRs comprising a deletion, insertion, or substitution relative to the other ITR, and such that 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. As shown herein, in some examples, the inhibitor of the immune response (*e.g.*, the innate immune response) is co-administered using a synthetic nanocarrier as described in WO 2016/073799, the contents of which are incorporated herein by reference in their entirety. In some examples, the ceDNA vector is also present in the nanocarrier. According to some examples, one or more inhibitors of the immune response (*e.g.*, the innate immune response), are selected from rapamycin and rapamycin analogs thereof, TLR antagonists (*e.g.*, TLR9 antagonists), cGAS antagonists and inflammasome antagonists (*e.g.*, any one or more of: an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof). According to some examples, the TLR9 inhibitory oligonucleotide is present on at least one of the ITRs. According to some examples, the inhibitor of cGAS is encoded by the ceDNA and operably linked to a promoter, such as an inducible promoter. In other examples, the inhibitor of cGAS is not encoded by the ceDNA.

[0042] Further, provided herein, in one example is a composition comprising (i) a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding the transgene operably positioned between two different AAV inverted terminal repeat sequences (ITRs), one of the ITRsS comprising a functional AAV terminal resolution site and a Rep binding site, one of the ITRs comprising a deletion, insertion, or substitution relative to the other ITR, 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, and (ii) an inhibitor of the immune response (*e.g.*, the innate immue response). As shown herein, in some examples, the components of the composition are formulated in separate synthetic nanocarriers. In one example, the components of the composition are formulated in the same synthetic nanocarrier. According to some examples, one or more inhibitors of the immune response (*e.g.*, the innate immune response), are selected from rapamycin and rapamycin analogs thereof, TLR antagonists (*e.g.*, TLR9 antagonists), cGAS antagonists and inflammasome antagonists (*e.g.*, any one or more of: an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof).

[0043] The non-viral capsid free DNA vectors described herein can be produced in permissive host cells from an expression construct (*e.g.*, a plasmid, a Bacmid, a baculovirus, or an integrated cell-line) *e.g.*, see the Examples disclosed in International Patent Application PCT/US18/49996 filed on September 7, 2018, or using synthetic production, *e.g.*, see the Examples disclosed in International Patent Application PCT/US19/14122, filed December 6, 2018, each of which are incorporated herein in their entirety by reference. In some examples, the ceDNA vectors useful in the methods and compositions as disclosed herein comprise a heterologous nucleic acid, *e.g.* a transgene positioned between two inverted terminal repeat (ITR) sequences. In some examples, at least one of the ITRs is modified by deletion, insertion, and/or substitution as compared to a wild-type ITR sequence (*e.g.* AAV ITR); and at least one of the ITRs comprises a functional terminal resolution site (TRS) and a Rep binding site.

[0044] According to another example, the disclosure features a method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] **FIG. 1** is a schematic illustrating one embodiment of an upstream process for making baculo-infected insect cells (BIICs) that are useful in the production of ceDNA vector in the process described in the schematic in **FIG. 2.** i) Two populations of Naïve insect cells are transfected with either Rep protein plasmid or DNA vector producing plasmid; ii) viral supernatant is harvested and used to infect tow new naïve populations of insect cells to generate BIICS-1 of DNA vector construct

CLAIMS

1. Use of a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector) in the manufacture of a medicament for inhibiting an immune response when a transgene is expressed in a cell, wherein the ceDNA vector comprises at least one heterologous nucleotide sequence operably positioned between two flanking inverted terminal repeat sequences (ITRs); and

   at least one inhibitor of the immune response.

2. The use of claim 1, wherein the immune response is an innate immune response.

3. The use of claim 1 or claim 2, wherein the inhibitor of the immune response is an inhibitor of the innate immune response.

4. The use of any one of claims 1-3, wherein the ceDNA vector further encodes the at least one inhibitor of the immune response.

5. The use of any one of claims 1-4, wherein the inhibitor of the immune response is to be administered separately from the ceDNA vector.

6. The use of any one of claims 1-5, wherein the inhibitor of the immune response is an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, or a caspase-1 inhibitor, an inhibitor of cyclic GMP-AMP Synthase (cGAS), an inhibitor of a toll like receptor (TLR), or rapamycin or a rapamycin analog.

7. The use of claim 6, wherein the TLR inhibitor is a TLR9 inhibitor, and wherein the TLR9 inhibitor is: a TLR9 inhibitory oligonucleotide; an miRNA specific for TLR9; an siRNA specific for TLR9; or an antibody or antigen-binding fragment that binds TLR9.

8. The use of any one of claims 1-7, wherein the at least one heterologous nucleotide sequence is operably positioned between two flanking wild-type inverted terminal repeat sequences (WT-ITRs); the at least one heterologous nucleotide sequence is operably positioned between two flanking mutant inverted terminal repeat sequences (mutant ITRs); the at least one heterologous nucleotide sequence is operably positioned between two flanking inverted terminal repeat sequences, wherein one ITR is a WT-ITR and one ITR is a mutant ITR; the ITRs are symmetric ITRs; or the ITRs are asymmetric ITRs..

9. The use of any one of claims 1-8, wherein one or both of the 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 ITRs are wild type, or wherein both of the ITRs are wild-type; the flanking ITRs are from different viral serotypes;

at least one of the 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 ITRs are synthetic; or one or both of the ITRs is not a wild type ITR, or wherein both of the ITRs are not wild-type.

10. The use of any one of claims 1-9, wherein one or both of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D'; the deletion, insertion, and/or 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 ITRs are modified by a deletion, insertion, and/or 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 ITRs are modified by a deletion, insertion, and/or 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 ITRs are modified by a deletion, insertion, and/or 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 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 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; or one or both of the 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..

11. The use of any one of claims 1-10, wherein the at least one heterologous nucleotide sequence is under the control of at least one regulatory switch.

12. The use of any one of claims 1-11, wherein the ceDNA vector and/or the inhibitor of the immune response is in a nanocarrier that comprises a lipid nanoparticle (LNP).

13. The use of any one of the previous claims, wherein the at least one heterologous nucleotide sequence, when transcribed or translated, corrects for an abnormal amount of an endogenous protein in a subject or corrects for an abnormal function or activity of an endogenous protein or pathway in a subject.

14. The use of claim 13, wherein the subject is a human patient in need of treatment.

15. The use of any one of the previous claims, wherein the at least one heterologous nucleotide sequence encodes or comprises a nucleotide molecule selected from the group consisting of an RNAi, an siRNA, an miRNA, an lncRNA, and an antisense oligo- or polynucleotide;

wherein the at least one heterologous nucleotide sequence encodes a protein; wherein the at least one heterologous nucleotide sequence encodes an agonist or an antagonist of an endogenous protein or pathway associated with the disease or disorder; or wherein the at least one heterologous nucleotide sequence encodes an antibody.

16. The use of any one of the previous claims, further comprising co-administration of anadditional inhibitor of the immune response.

17. The use of any one of the previous claims, wherein the ceDNA vector is obtained from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA expression construct in the presence of at least one Rep protein, wherein the ceDNA expression construct encodes the ceDNA vector, under conditions effective and for a time sufficient to induce production of the ceDNA vector within the insect cells; and (b) isolating the ceDNA vector from the insect cells.

18. A host cell comprising a ceDNA expression construct that encodes the ceDNA vector produced from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA expression construct in the presence of at least one Rep protein, wherein the ceDNA expression construct encodes the ceDNA vector, under conditions effective and for a time sufficient to induce production of the ceDNA vector within the insect cells; and (b) isolating the ceDNA vector from the insect cells.

19. A method of producing a ceDNA vector, comprising: (a) incubating the host cell of any one of claim 18 under conditions effective and for time sufficient to induce production of the ceDNA vector; and (b) isolating the ceDNA from the host cells.

20. A composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises at least one heterologous nucleotide sequence operably positioned between two flanking inverted terminal repeat sequences (ITRs), wherein the ceDNA vector further encodes the at least one inhibitor of the immune response.

21. The composition of claim 20, wherein the immune response is an innate immune response.

22. Use of the composition of claim 20 or 21 in the manufacture of a medicament for expressing an inhibitor of the immune response in a cell.

23. A pharmaceutical composition comprising the composition of claim 20 or 21.

24. A cell comprising the composition of claim 20 or 21.

25. A kit comprising the composition of claim 20 or 21, the pharmaceutical composition of claim 23, or the cell of claim 24.