[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 in the manufacture of a medicament for inhibiting an immune response when at least one transgene is expressed in a cell, wherein the composition comprises ~~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~~ the at least one transgene ~~operably~~ positioned between two flanking inverted terminal repeats ~~sequences~~ (ITRs) , and wherein the at least one transgene is used to treat a genetic disease; and at least one inhibitor of the immune response, wherein the inhibitor of the immune response is an inhibitor of a toll like receptor (TLR), an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, a caspase-1 inhibitor, or an inhibitor of cyclic GMP-AMP Synthase (cGAS). In another aspect, the present disclosure refers to the use of a composition for inhibiting an immune response when at least one transgene is expressed in a cell in vitro, wherein the composition comprises a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises the at least one transgene positioned between two flanking inverted terminal repeat sequences (ITRs); and at least one inhibitor of the immune response, wherein the inhibitor of the immune response is an inhibitor of a toll like receptor (TLR), an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, a caspase-1 inhibitor, or an inhibitor of cyclic GMP-AMP Synthase (cGAS). ~~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~~transgene positioned between two flanking inverted terminal repeats ~~sequences~~ (ITRs), wherein the ceDNA vector further encodes ~~the~~ at least one inhibitor of the immune response, wherein the inhibitor of the immune response is an inhibitor of a toll like receptor (TLR), an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, a caspase-1 inhibitor, or an inhibitor of cyclic GMP-AMP Synthase (cGAS). 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, wherein the at least one transgene treats a genetic disease. In another aspect, the present disclosure refers to the use of the composition as disclosed herein for expressing an inhibitor of the immune response in a cell *in vitro*. 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

CLAIMS

1. Use of a composition in the manufacture of a medicament for inhibiting an immune response when at least one transgene is expressed in a cell, wherein the composition comprises ~~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~~ the at least one transgene ~~operably~~ positioned between two flanking inverted terminal repeats ~~sequences~~ (ITRs) , and wherein the at least one transgene is used to treat a genetic disease; and

at least one inhibitor of the immune response, wherein the inhibitor of the immune response is an inhibitor of a toll like receptor (TLR), an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, a caspase-1 inhibitor, or an inhibitor of cyclic GMP-AMP Synthase (cGAS).

2. Use of a composition for inhibiting an immune response when at least one transgene is expressed in a cell *in vitro*, wherein the composition comprises

a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises the at least one transgene positioned between two flanking inverted terminal repeat sequences (ITRs); and

at least one inhibitor of the immune response, wherein the inhibitor of the immune response is an inhibitor of a toll like receptor (TLR), an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, a caspase-1 inhibitor, or an inhibitor of cyclic GMP-AMP Synthase (cGAS).

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

~~3.~~4. The use of any one of claims ~~1 or claim 2~~1-3, wherein the inhibitor of the immune response is an inhibitor of the innate immune response.

~~4.~~5. The use of any one of claims 1-~~3~~4, wherein the ceDNA vector further encodes the at least one inhibitor of the immune response.

~~5.~~6. The use of any one of claims 1-~~4~~5, wherein the inhibitor of the immune response is to be administered separately from the ceDNA vector.

~~6.~~7. The use of any one of claims 1-~~5~~6, 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~~ is rapamycin or a rapamycin analog.

~~7.~~8. The use of claim ~~6~~1, 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.

9. The use of any one of claims 1-~~7~~8, wherein:

the at least one transgene is ~~heterologous nucleotide sequence is operably~~ positioned between two flanking wild-type inverted terminal repeats~~ sequences~~ (WT-ITRs);

the at least one transgene is ~~heterologous nucleotide sequence is operably~~ positioned between two flanking mutant inverted terminal repeats~~ sequences~~ (mutant ITRs); or

~~8.~~ the at least one transgene is ~~heterologous nucleotide sequence is operably~~ positioned between two flanking inverted terminal repeats~~ sequences~~, wherein one ITR is a WT-ITR and one ITR is a mutant ITR; wherein the flanking ITRs are symmetric ITRs; or the flanking ITRs are asymmetric ITRs.~~.~~

~~9.~~10. The use of any one of claims 1-~~8~~9, wherein one or both of the flanking ITRs are 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; the flanking ITRs are symmetric~~al~~ or substantially symmetric~~al~~; the flanking ITRs are asymmetric; one ~~or both~~ of the flanking ITRs ~~are~~ is a wild~~-~~-type ITR, or wherein both of the flanking ITRs are wild-type ITRs; the flanking ITRs are from different viral serotypes; 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 synthetic ITRs; or one ~~or both~~ of the flanking ITRs is not a wild~~-~~-type ITR, or wherein both of the flanking ITRs are not wild-type ITRs.

~~10.~~11. The use of any one of claims 1-~~9~~10, 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; 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 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; 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 C and C' 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 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 flanking ITRs comprise a single stem-loop structure in the region that, in a wild-type ITR, would comprise ~~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 flanking ITRs comprise a single stem and two loops in the region that, in a wild-type ITR, would comprise ~~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 flanking ITRs comprise a single stem and a single loop in the region that, in a wild-type ITR, would comprise ~~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.~~12. The use of any one of claims 1-~~10~~11, wherein the at least one ~~heterologous nucleotide sequence~~transgene is under the control of at least one regulatory switch.

~~12.~~13. The use of any one of claims 1-~~11~~12, wherein the ceDNA vector and/or the inhibitor of the immune response is in a nanocarrier, wherein the nanocarrier ~~that~~ comprises a lipid nanoparticle (LNP).

~~13.~~14. The use of any one of the previous claims, wherein the at least one ~~heterologous nucleotide sequence~~transgene, 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.~~15. The use of claim ~~13~~14, wherein the subject is a human patient in need of treatment.

~~15.~~16. The use of any one of the previous claims, wherein the at least one transgene~~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 transgene~~heterologous nucleotide sequence~~ encodes a protein; wherein the at least one transgene~~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 transgene~~heterologous nucleotide sequence~~ encodes an antibody.

~~16.~~17. The use of any one of the previous claims, ~~further comprising co-administration of~~ wherein the use further comprises that an additional inhibitor of the immune response is to be administered.

~~17.~~18. 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.~~19. 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~~transgene positioned between two flanking inverted terminal repeat~~s sequences~~ (ITRs), wherein the ceDNA vector further encodes ~~the~~ at least one inhibitor of the immune response, wherein the inhibitor of the immune response is an inhibitor of a toll like receptor (TLR), an inhibitor of the NLRP3 inflammasome, an inhibitor of the AIM2 inflammasome, a caspase-1 inhibitor, or an inhibitor of cyclic GMP-AMP Synthase (cGAS).

~~21.~~20. The composition of claim ~~20~~19, wherein the immune response is an innate immune response.

~~22.~~21. Use of the composition of claim 19~~20~~ or ~~21~~ 20 in the manufacture of a medicament for expressing an inhibitor of the immune response in a cell, wherein the at least one transgene treats a genetic disease.

22. Use of the composition of claim 19 or 20 for expressing an inhibitor of the immune response in a cell *in vitro*.

23. A pharmaceutical composition comprising the composition of claim 19 or 20~~20 or 21~~.

24. A cell comprising the composition of claim 19 or 20~~20 or 21~~.

25. A kit comprising the composition of claim 19 or 20~~20 or 21~~, the pharmaceutical composition of claim 23, or the cell of claim 24.