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(54) **Titre : VACCIN CONTRE UN NOROVIRUS ET METHODES D'UTILISATION**
(54) **Title: NOROVIRUS VACCINE AND METHODS OF USE**

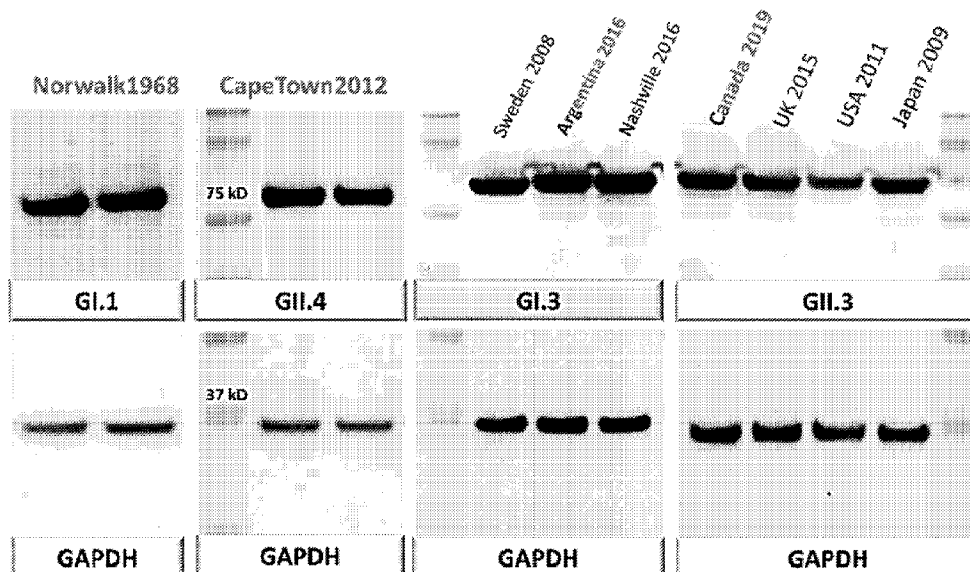


Fig. 1

(57) **Abrégé/Abstract:**

Provided is a Norovirus vaccine comprising mRNA molecules encoding Norovirus VP1 antigens and methods of use thereof to treat or prevent a disease or disorder associated with Norovirus infection.

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Abstract:

Provided is a Norovirus vaccine comprising mRNA molecules encoding Norovirus VP1 antigens and methods of use thereof to treat or prevent a disease or disorder associated with Norovirus infection.

TITLE OF THE INVENTION

Norovirus Vaccine and Methods of Use

5 CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/272,439, filed October 27, 2021 which is hereby incorporated by reference herein in its entirety.

10 BACKGROUND OF THE INVENTION

Human noroviruses (HuNoV) are the most common viral cause of acute gastroenteritis worldwide, causing an estimated 200 million cases and ~200,000 deaths in young children annually. Noroviruses are classified into 10 genogroups of which 5 infect humans, >90% of human infections are caused by strains of either genogroup I or II with genogroup II, genotype 4 causing the majority of HuNoV infections and cyclical pandemic waves of disease every few years.

Noroviruses are non-enveloped, single-stranded positive-sense RNA viruses in the family Caliciviridae. Its genome is approximately 7.5 kb to 7.7 kb in size and contains three open reading frames (ORFs); ORF2 encodes the major capsid protein (VP1) that determines the antigenicity of the virus and consists of a shell domain located at the base of the capsid.

Noroviruses (NoV) classified into 10 genogroups (GI to GX) based on the complete major capsid protein VP1 sequences. However, only GI, GII and GIV infect humans.

25 Globally GII.4 is the most predominant genotype accounting for ~70-80% of all NoV outbreaks in most countries over the past 20 years.

There are no approved vaccines or specific therapeutics to treat the norovirus disease. Thus, there is a need in the art for improved norovirus vaccines. The present invention addresses this need.

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SUMMARY OF THE INVENTION

In one embodiment, the invention relates to a composition for inducing an immune response against Norovirus (NoV) comprising at least one mRNA molecule encoding at least one NoV antigen. In one embodiment, the NoV antigen is p48,
5 nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, a fragment thereof, or any combination thereof.

In one embodiment, the NoV antigen from a GI, GII, GIV, GVIII or GIX genogroup.

In one embodiment, the composition comprises a combination of mRNA
10 molecules encoding at least two NoV VP1 antigens. In one embodiment, the composition comprises a combination of mRNA molecules encoding a GI VP1 antigen and a GII VP1 antigen.

In one embodiment, the composition comprises a combination of mRNA molecules encoding at least two of a GI.1 antigen, a GI.3 VP1 antigen, a GI.5 VP1
15 antigen, a GII.3 VP1 antigen and a GII.4 VP1 antigen.

In one embodiment, the composition comprises a combination of an mRNA molecule encoding a NoV VP1 GI.1 antigen and an mRNA molecule encoding a NoV VP1 GII.4 antigen.

In one embodiment, the composition comprises a combination of mRNA
20 molecules encoding each of a NoV VP1 GI.1 antigen, a NoV VP1 GII.4 antigen, a NoV VP1 GI.3 antigen and a NoV VP1 GII.3 antigen.

In one embodiment, the composition comprises at least one mRNA molecule encoding an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ
25 ID NO:9 or SEQ ID NO:10.

In one embodiment, the composition further comprises an adjuvant.

In one embodiment, the mRNA molecule is encapsulated within a lipid nanoparticle (LNP).

In one embodiment, the composition comprises a combination of two or
30 more LNP encapsulating two or more mRNA molecules encoding NoV VP1 antigens.

In one embodiment, the mRNA molecule is a nucleoside modified mRNA

molecule comprising at least one pseudouridine, 1-methyl pseudouridine, or 5-methyl-uridine modified nucleoside.

In one embodiment, the invention relates to a method of inducing an immune response against at least one strain of Norovirus (NoV) in a subject comprising administering to the subject an effective amount of a composition comprising a mRNA molecule encoding at least one NoV antigen or a fragment thereof. In one embodiment, the NoV antigen is p48, nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, a fragment thereof, or any combination thereof. In one embodiment, the NoV antigen from a GI, GII, GIV, GVIII or GIX genogroup.

In one embodiment, the method comprises administering a combination of mRNA molecules encoding at least two NoV VP1 antigens. In one embodiment, the composition comprises a combination of mRNA molecules encoding a GI VP1 antigen and a GII VP1 antigen.

In one embodiment, the method comprises administering a combination of mRNA molecules encoding at least two of a GI.1 antigen, a GI.3 VP1 antigen, a GI.5 VP1 antigen, a GII.3 VP1 antigen and a GII.4 VP1 antigen.

In one embodiment, the method comprises administering a combination of an mRNA molecule encoding a NoV VP1 GI.1 antigen and an mRNA molecule encoding a NoV VP1 GII.4 antigen.

In one embodiment, the method comprises administering a combination of mRNA molecules encoding each of a NoV VP1 GI.1 antigen, a NoV VP1 GII.4 antigen, a NoV VP1 GI.3 antigen and a NoV VP1 GII.3 antigen.

In one embodiment, the method comprises administering at least one mRNA molecule encoding an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

In one embodiment, the method further comprises administering an adjuvant.

In one embodiment, the method comprises administering at least one mRNA molecule encapsulated within a lipid nanoparticle (LNP).

In one embodiment, the method comprises administering a combination of two or more LNP encapsulating two or more mRNA molecules encoding NoV VP1 antigens.

5 In one embodiment, the method comprises administering at least one nucleoside modified mRNA molecule comprising at least one pseudouridine, 1-methyl pseudouridine, or 5-methyl-uridine modified nucleoside.

In one embodiment, the composition is administered by an intradermal, subcutaneous, inhalation, intranasal, or intramuscular delivery route.

10 In one embodiment, the method comprises a single administration of the composition. In one embodiment, the method comprises multiple administrations of the composition.

In one embodiment, the composition treats or prevents a disease or disorder associated with NoV infection. In one embodiment, the disease or disorder associated with NoV infection is gastroenteritis, food poisoning, vomiting or diarrhea.
15

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of embodiments of the invention will be better understood when read in conjunction with the appended drawings. It should be understood that the invention is not limited to the precise arrangements and
20 instrumentalities of the embodiments shown in the drawings.

Figure 1 depicts exemplary experimental data demonstrating that nucleoside-modified mRNAs encoding capsid protein VP1 sequences of GI.1/Norwalk1968, GII.4/CapeTown2012, GI.3/Sweden2008, GI.3/Argentina2016, GI.3/Nashville2019, GII.3/Canada2019, GII.2/UK2015, GII.3/USA2011 and
25 GII.3/Japan2009 viruses express the correct protein. Reducing conditions were used. Loading per well - 10 ug; GI.1- Anti-Norovirus (VP1 GI.1 capsid protein), Rabbit Polyclonal Rb G1; GII.4 - Anti-Norovirus (VP1 GII.4 capsid protein), Rabbit Polyclonal Rb 428, GI.3 - Anti-Norovirus (VP1 GI.3 capsid protein), Rabbit Polyclonal Rb 402, GII.3 - Anti-Norovirus (VP1 GII.3 capsid protein), Rabbit Polyclonal Rb 397, GAPDH
30 (14C10) – Rabbit mAb, (Cell Signaling, # 2118S); o/n +4°C incubation, dilution 1:2,500; 2nd GAR- HRP, 1 h RT, Dilution 1:10,000; ECL+.

Figure 2 depicts an exemplary immunization scheme with bivalent mRNA vaccine (GI.1 and GII.4).

Figure 3 depicts exemplary experimental data demonstrating the titers of blockade antibody persisting at high levels up to day 180.

5 Figure 4 depicts exemplary experimental data demonstrating that the vaccine induces a good, but genotype specific blockade antibody response.

Figure 5 depicts an exemplary immunization scheme for the T cell stimulation assay.

10 Figure 6 depicts the results of the T cell stimulation assay with VLPs (GI.1, GI.3, GII.3, GII.4).

Figure 7 depicts exemplary experimental data demonstrating the human Norovirus bivalent mRNA vaccine dose response.

Figure 8 depicts exemplary experimental data demonstrating that the low dose mRNA vaccine induces strong Th1 response.

15 Figure 9 depicts an exemplary immunization scheme for a tetravalent Norovirus mRNA vaccine.

Figure 10 depicts exemplary experimental data demonstrating the human Norovirus tetravalent vaccine dose response.

20 Figure 11 depicts exemplary experimental data demonstrating infection of stem cell-derived human 3D enteroids with live HNoV.

Figure 12 depicts exemplary images demonstrating infection of stem cell-derived human 3D enteroids with live HNoV.

25 Figure 13 depicts exemplary experimental data demonstrating protection of human intestinal enteroids against GII.4 infections by serum from mRNA-LNP vaccinated mice vaccinated with 10 µg per vaccine/mouse, by intradermal (i.d.) injection.

Figure 14 depicts exemplary experimental data demonstrating protection of human intestinal enteroids against GII.4 infections by serum from mRNA-LNP vaccinated mice vaccinated with 0.25 µg per vaccine/mouse by intramuscular (i.m.) injection.

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DETAILED DESCRIPTION

The present invention relates to compositions and methods for inducing an immune response against Norovirus (NoV) in a subject. In some embodiments, the invention provides a composition comprising at least one lipid nanoparticle (LNP) comprising at least one nucleoside-modified RNA molecule encoding at least one

5 Norovirus major capsid protein (VP1) from one or more Norovirus genogroup. In some embodiments, the invention provides a composition comprising at least one lipid nanoparticle (LNP) comprising at least one nucleoside-modified RNA molecule encoding at least one VP1 from a GI, GII, GIII, GIV, GV, GVI, GVII, GVIII, GIX or GX genogroup. In some embodiments, the invention provides a composition comprising a

10 combination of lipid nanoparticles (LNPs) comprising nucleoside-modified RNA molecules encoding at least two NoV major capsid proteins (VP1s). In some embodiments, the composition comprises mRNA molecules encoding at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than twenty NoV VP1 antigens.

15 In one embodiment, at least two VP1s are from the same NoV genogroup. In one embodiment, at least two VP1s are from two or more different NoV genogroups.

In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from GII and at least one LNP comprising an mRNA molecule encoding a VP1 from GI.

20 In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from GII, at least one LNP comprising an mRNA molecule encoding a VP1 from GI, and at least one LNP comprising an mRNA molecule encoding a VP1 from GIV.

In one embodiment, the composition comprises a combination of at least

25 one LNP comprising an mRNA molecule encoding a VP1 from GII.4 and at least one LNP comprising an mRNA molecule encoding a VP1 from GI.1.

In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from GII.4, at least one LNP comprising an mRNA molecule encoding a VP1 from GI.1, at least one LNP comprising

30 an mRNA molecule encoding a VP1 from GI.3, and at least one LNP comprising an mRNA molecule encoding a VP1 from GII.3.

The Invention also relates to method of treating Norovirus infection or treating or preventing a disease or disorder associated with Norovirus infection using the compositions of the invention, such as vomiting and diarrhea,

5 Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, each of the following terms has the meaning associated
10 with it in this section.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an
15 amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

The term “antibody,” as used herein, refers to an immunoglobulin molecule, which specifically binds with an antigen. Antibodies can be intact
20 immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab)₂, as well as single chain antibodies and humanized antibodies (Harlow
25 et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

The term “antibody fragment” refers to a portion of an intact antibody and
30 refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments,

linear antibodies, scFv antibodies, and multispecific antibodies formed from antibody fragments.

5 An “antibody heavy chain,” as used herein, refers to the larger of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations.

An “antibody light chain,” as used herein, refers to the smaller of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations. κ and λ light chains refer to the two major antibody light chain isotypes.

10 By the term “synthetic antibody” as used herein, is meant an antibody, which is generated using recombinant DNA technology. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology
15 which is available and well known in the art. The term should also be construed to mean an antibody, which has been generated by the synthesis of an RNA molecule encoding the antibody. The RNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the RNA has been obtained by transcribing DNA (synthetic or cloned), synthesizing the RNA, or other technology, which is
20 available and well known in the art.

By the term “specifically binds,” as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from
25 one or more other species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific. In some instances, the terms “specific binding” or “specifically binding,” can be used in reference
30 to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g.,

an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope “A”, the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled “A” and the antibody, will reduce the amount of labeled A bound to the antibody.

The term “immunogen” as used herein, is intended to denote a substance of matter, which is capable of inducing an adaptive immune response in an individual, where said adaptive immune response is capable of inducing an immune response, which significantly engages pathogenic agents, which share immunological features with the immunogen. “Immunogen” refers to any substance introduced into the body in order to generate an immune response. That substance can be a physical molecule, such as a protein, or can be encoded by a vector, such as DNA, mRNA, or a virus.

The term “antigen” or “Ag” as used herein is defined as a molecule that provokes an adaptive immune response. This immune response may involve either antibody production, or the activation of specific immunogenically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA or RNA. A skilled artisan will understand that any DNA or RNA, which comprises a nucleotide sequence or a partial nucleotide sequence encoding a protein that elicits an adaptive immune response therefore encodes an “antigen” as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full-length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

“Immune response,” as the term is used herein, means a process involving the activation and/or induction of an effector function in, by way of non-limiting

examples, a T cell, B cell, natural killer (NK) cell, and/or an antigen-presenting cell (APC). Thus, an immune response, as would be understood by the skilled artisan, includes, but is not limited to, any detectable antigen-specific activation and/or induction of a helper T cell or cytotoxic T cell activity or response, production of antibodies, antigen presenting cell activity or infiltration, macrophage activity or infiltration, neutrophil activity or infiltration, and the like.

As used herein, an “immunogenic composition” may comprise an antigen (e.g., a peptide or polypeptide), a nucleic acid encoding an antigen, a cell expressing or presenting an antigen or cellular component, a virus expressing or presenting an antigen or cellular component, or a combination thereof. In particular embodiments, the composition comprises or encodes all or part of any peptide antigen described herein, or an immunogenically functional equivalent thereof. In other embodiments, the composition is in a mixture that comprises an additional immunostimulatory agent or nucleic acids encoding such an agent. Immunostimulatory agents include but are not limited to an additional antigen, an immunomodulator, an antigen presenting cell, lipid nanoparticle, or an adjuvant. In other embodiments, one or more of the additional agent(s) is covalently bonded to the antigen or an immunostimulatory agent, in any combination.

As used herein, the term “vaccine” refers to a composition that induces an immune response upon inoculation into a subject. In some embodiments, the induced immune response provides protective immunity.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or

cDNA.

A “vector” is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear
5 polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are
10 not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, and the like.

“Expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting
15 elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) RNA, and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

20 “Homologous” refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology
25 between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared X 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two
30 sequences are aligned to give maximum homology.

As used herein, a nucleotide sequence is “substantially homologous” to

any of the nucleotide sequences described herein when its nucleotide sequence has a degree of identity with respect to the original nucleotide sequence at least 60%, of at least 65%, of at least 70%, of at least 75%, of at least 80%, of at least 85%, of at least 90%, of at least 91%, of at least 92%, of at least 93%, of at least 94%, of at least 95%, of at least 96%, of at least 97%, of at least 98%, of at least 99%, or of at least 99.5%.

As used herein, an amino acid sequence is “substantially homologous” to any of the amino acid sequences described herein when its amino acid sequence has a degree of identity with respect to the original amino acid sequence of at least 60%, of at least 65%, of at least 70%, of at least 75%, of at least 80%, of at least 85%, of at least 90%, of at least 91%, of at least 92%, of at least 93%, of at least 94%, of at least 95%, of at least 96%, of at least 97%, of at least 98%, of at least 99%, or of at least 99.5%. The identity between two amino acid sequences can be determined by using the BLASTN algorithm (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894, Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990)).

The term “variant” as used herein with respect to a nucleic acid refers (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto. A variant may be a nucleic acid sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The nucleic acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof.

The term “variant” as used with respect to a peptide or polypeptide refers to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also refer to a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and

distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., 1982, J. Mol. Biol. 157:105-132). The hydropathic index of an amino acid is based on a consideration of its

5 hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ± 2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide

10 permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be

15 performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino

20 acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. A variant may be an amino acid sequence that is substantially identical over the full length of the amino acid sequence or fragment thereof. The amino acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino

25 acid sequence or a fragment thereof.

As used herein, the terms “fragment” or “functional fragment” refer to a fragment of an antigen or a nucleic acid sequence encoding an antigen that, when administered to a subject, provides an increased immune response. Fragments are generally 10 or more amino acids or nucleic acids in length. “Fragment” may mean a

30 polypeptide fragment of an antigen that is capable of eliciting an immune response in a subject. A fragment of an antigen may be 100% identical to the full length except missing

at least one amino acid from the N and/or C terminal, in each case with or without signal peptides and/or a methionine at position 1. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length antigen, excluding any heterologous signal peptide added. The fragment may comprise a fragment of a polypeptide that is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more identical to the antigen and additionally comprise an N terminal methionine or heterologous signal peptide which is not included when calculating percent identity.

A fragment of a nucleic acid sequence that encodes an antigen may be 100% identical to the full length except missing at least one nucleotide from the 5' and/or 3' end, in each case with or without sequences encoding signal peptides and/or a methionine at position 1. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length coding sequence, excluding any heterologous signal peptide added. The fragment may comprise a fragment that encode a polypeptide that is 95% or more, 96% or more, 97% or more, 98% or more or 99% or more identical to the antigen and additionally optionally comprise sequence encoding an N terminal methionine or heterologous signal peptide which is not included when calculating percent identity.

“Isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living subject is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

In the context of the present invention, the following abbreviations for the

commonly occurring nucleosides (nucleobase bound to ribose or deoxyribose sugar via N-glycosidic linkage) are used. “A” refers to adenosine, “C” refers to cytidine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s). In addition, the nucleotide sequence may contain modified nucleosides that are capable of being translated by translational machinery in a cell. Exemplary modified nucleosides are described elsewhere herein. For example, an mRNA where some or all of the uridines have been replaced with pseudouridine, 1-methyl pseudouridine, 5-methyl-uridine or another modified nucleoside, such as those described elsewhere herein. In some embodiments, the nucleotide sequence may contain a sequence where some or all cytosines are replaced with methylated cytidine, or another modified nucleoside, such as those described elsewhere herein.

The term “operably linked” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA or RNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

The term “polynucleotide” as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric “nucleotides.” The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic

acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR™, and the like, and by synthetic means.

5 In some instances, the polynucleotide or nucleic acid of the invention is a “nucleoside-modified nucleic acid,” which refers to a nucleic acid comprising at least one modified nucleoside. A “modified nucleoside” refers to a nucleoside with a modification. For example, over one hundred different nucleoside modifications have been identified in RNA (Rozenski, et al., 1999, The RNA Modification Database: 1999 update. Nucl Acids
10 Res 27: 196-197).

 As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a
15 protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include,
20 for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

25 The term “promoter” as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence. By way of one non-limiting example, a promoter that is recognized by bacteriophage RNA polymerase and is used to generate the mRNA by in vitro transcription.

30 The term “adjuvant” as used herein is defined as any molecule to enhance an antigen-specific adaptive immune response.

In some embodiments, “pseudouridine” refers to $m^1acp^3\Psi$ (1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine). In another embodiment, the term refers to $m^1\Psi$ (1-methylpseudouridine). In another embodiment, the term refers to Ψm (2'-O-methylpseudouridine). In another embodiment, the term refers to m^5D (5-methyldihydrouridine). In another embodiment, the term refers to $m^3\Psi$ (3-methylpseudouridine). In another embodiment, the term refers to a pseudouridine moiety that is not further modified. In another embodiment, the term refers to a monophosphate, diphosphate, or triphosphate of any of the above pseudouridines. In another embodiment, the term refers to any other pseudouridine known in the art. Each possibility represents a separate embodiment of the present invention.

The term “lipid nanoparticle” refers to a particle having at least one dimension on the order of nanometers (e.g., 1-1,000 nm), which includes one or more lipids.

The term “lipid” refers to a group of organic compounds that are derivatives of fatty acids (e.g., esters) and are generally characterized by being insoluble in water but soluble in many organic solvents. Lipids are usually divided in at least three classes: (1) “simple lipids” which include fats and oils as well as waxes; (2) “compound lipids” which include phospholipids and glycolipids; and (3) “derived lipids” such as steroids.

As used herein, the term “cationic lipid” refers to a lipid that is cationic or becomes cationic (protonated) as the pH is lowered below the pK of the ionizable group of the lipid, but is progressively more neutral at higher pH values. At pH values below the pK, the lipid is then able to associate with negatively charged nucleic acids. In some embodiments, the cationic lipid comprises a zwitterionic lipid that assumes a positive charge on pH decrease.

The term “neutral lipid” refers to any one of a number of lipid species that exist in either an uncharged or neutral zwitterionic form at physiological pH. Representative neutral lipids include diacylphosphatidylcholines, diacylphosphatidylethanolamines, ceramides, sphingomyelins, dihydro sphingomyelins, cephalins, and cerebroside.

The term “anionic lipid” refers to any lipid that is negatively charged at

physiological pH.

The term “polymer conjugated lipid” refers to a molecule comprising both a lipid portion and a polymer portion. An example of a polymer conjugated lipid is a pegylated lipid.

5 The term “pegylated lipid” refers to a molecule comprising both a lipid portion and a polyethylene glycol portion. Pegylated lipids are known in the art and include 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-s- DMG) and the like.

10 “Liposome” is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components
15 undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated
20 are lipofectamine-nucleic acid complexes.

 The terms “subject,” “patient,” “individual,” and the like are used interchangeably herein, and refer to any animal, or cells thereof whether in vitro or in situ, amenable to the methods described herein. In some non-limiting embodiments, the patient, subject or individual is a mammal, bird, poultry, cattle, pig, horse, sheep, ferret,
25 primate, dog, cat, guinea pig, rabbit, bat, or human.

 A “disease” is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein if the disease is not ameliorated then the subject’s health continues to deteriorate.

 In contrast, a “disorder” in a subject is a state of health in which the
30 subject is able to maintain homeostasis, but in which the subject’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does

not necessarily cause a further decrease in the subject's state of health.

By the term "modulating," as used herein, is meant mediating a detectable increase or decrease in the level of a response in a subject compared with the level of a response in the subject in the absence of a treatment or compound, and/or compared with
5 the level of a response in an otherwise identical but untreated subject. The term encompasses perturbing and/or affecting a native signal or response thereby mediating a beneficial therapeutic response in a subject, such as a human.

To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced
10 by a subject.

An "effective amount" as used herein, means an amount which provides a therapeutic or prophylactic benefit.

The term "therapeutic" as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, diminution, remission,
15 prevention, or eradication of at least one sign or symptom of a disease or disorder.

The term "therapeutically effective amount" refers to the amount of the subject compound that will elicit the biological or medical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term "therapeutically effective amount" includes that amount of a
20 compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease being treated. The therapeutically effective amount will vary depending on the compound, the disease and its severity and the age, weight, etc., of the subject to be treated.

The term "transfected" or "transformed" or "transduced" as used herein
25 refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The phrase "under transcriptional control" or "operatively linked" as used
30 herein means that the promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and

expression of the polynucleotide.

As used herein, “additional ingredients” include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; 5 sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable 10 polymeric or hydrophobic materials. Other “additional ingredients” which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Remington’s Pharmaceutical Sciences (1985, Genaro, ed., Mack Publishing Co., Easton, PA), which is incorporated herein by reference.

Ranges: throughout this disclosure, various aspects of the invention can be 15 presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 20 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

25 Description

The present invention relates to compositions and methods for inducing an immune response against one or more strains of Norovirus in a subject. In some 30 embodiments, the invention provides a composition comprising at least one RNA molecule encoding at least one VP1 from a GI, GII, GIII, GIV, GV, GVI, GVII, GVIII, GIX or GX genogroup. In some embodiments, the mRNA molecules are nucleoside modified mRNA molecules.

In some embodiments, the invention provides a composition comprising a combination of mRNA molecules (e.g., nucleoside-modified mRNA molecules) encoding at least two NoV major capsid proteins (VP1s). In some embodiments, the composition comprises mRNA molecules (e.g., nucleoside-modified mRNA molecules) encoding at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than twenty NoV VP1 antigens.

In one embodiment, at least two VP1s are from different strains within the same NoV genogroup. In one embodiment, at least two VP1s are from two or more different NoV genogroups.

10 In one embodiment, the composition comprises a combination of at least one mRNA molecule encoding a VP1 from at least one GII strain and at least one mRNA molecule encoding a VP1 from at least one GI strain.

In one embodiment, the composition comprises a combination of at least one mRNA molecule encoding a VP1 from GII, at least one LNP comprising an mRNA molecule encoding VP1 from GI, and at least one mRNA molecule encoding VP1 from GIV.

15 In one embodiment, the composition comprises a combination of at least one mRNA molecule encoding a VP1 from GII.4 and at least one mRNA molecule encoding a VP1 from GI.1.

20 In one embodiment, the composition comprises a combination of at least one mRNA molecule encoding a VP1 from GII.4, at least one mRNA molecule encoding a VP1 from GI.1, at least one mRNA molecule encoding a VP1 from GI.3, and at least one mRNA molecule encoding a VP1 from GII.3.

In some embodiments, the composition further comprises mRNA molecules encoding at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than twenty additional viral antigens. In some embodiments, one or more additional viral antigens may be from human immunodeficiency virus (HIV), Chikungunya virus (CHIKV), dengue fever virus, papilloma viruses, for example, human papillomoma virus (HPV), polio virus, hepatitis viruses, for example, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV), smallpox virus (Variola major and minor), vaccinia virus,

rhinoviruses, equine encephalitis viruses, rubella virus, yellow fever virus, Norwalk virus, hepatitis A virus, human T-cell leukemia virus (HTLV-I), hairy cell leukemia virus (HTLV-II), California encephalitis virus, Hanta virus (hemorrhagic fever), rabies virus, Ebola fever virus, Marburg virus, measles virus, mumps virus, respiratory syncytial virus (RSV), herpes simplex 1 (oral herpes), herpes simplex 2 (genital herpes), herpes zoster (varicella-zoster, a.k.a., chickenpox), cytomegalovirus (CMV), for example human CMV, Epstein-Barr virus (EBV), flavivirus, foot and mouth disease virus, lassa virus, arenavirus, Merkel cell polyoma virus (MCV), influenza virus, a coronavirus including but not limited to severe acute respiratory syndrome-coronavirus 2, a cancer causing virus, or any combination thereof. In one embodiment, one or more additional viral antigen are NoV antigens. Exemplary additional NoV antigens include, but are not limited to, p48, nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, fragments thereof, or any combination thereof.

In some embodiments, the nucleoside-modified mRNA molecules (e.g., nucleoside-modified mRNA molecules) are encapsulated in one or more LNP. In some embodiments, the composition comprises at least one LNP comprising at least one nucleoside-modified RNA molecule encoding at least one VP1 antigen from a GI, GII, GIII, GIV, GV, GVI, GVII, GVIII, GIX or GX genogroup. In some embodiments, the composition comprises a combination of LNPs comprising nucleoside-modified RNA molecules encoding a combination of at least two VP1 antigens from a GI, GII, GIII, GIV, GV, GVI, GVII, GVIII, GIX or GX genogroup.

In some embodiments, the composition comprises a combination of LNPs comprising mRNA molecules encoding at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than twenty NoV VP1 antigens.

In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from at least one GII strain and at least one LNP comprising an mRNA molecule encoding a VP1 from at least one GI strain.

In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from GII, at least one LNP

comprising an mRNA molecule encoding VP1 from GI, and at least one LNP comprising an mRNA molecule encoding VP1 from GIV.

In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from GII.4 and at least one
5 LNP comprising an mRNA molecule encoding a VP1 from GI.1.

In one embodiment, the composition comprises a combination of at least one LNP comprising an mRNA molecule encoding a VP1 from GII.4, at least one LNP comprising an mRNA molecule encoding a VP1 from GI.1, at least one LNP comprising an mRNA molecule encoding a VP1 from GI.3, and at least one LNP comprising an
10 mRNA molecule encoding a VP1 from GII.3.

Vaccine

In one embodiment, the present invention provides an immunogenic composition for inducing an immune response against Norovirus (NoV) in a subject. For
15 example, in one embodiment, the immunogenic composition is a vaccine. For a composition to be useful as a vaccine, the composition must induce an immune response against the Norovirus antigen in a cell, tissue or subject. In some embodiments, the composition induces a broad immune response against multiple strains of NoV in a cell, tissue or subject. In some instances, the vaccine induces a protective immune response in
20 the subject.

A vaccine of the present invention may vary in its composition of nucleic acid and/or cellular components. In one embodiment, the vaccine comprises a nucleic acid molecule encoding a NoV VP1 antigen. In one embodiment, the vaccine comprises a combination of nucleic acid molecules encoding NoV VP1 antigens from two or more
25 NoV genogroups. In one embodiment, the vaccine comprises a combination of nucleic acid molecules encoding NoV VP1 antigens from one or more strain of NoV GII and one or more strain of NoV GI.

In a non-limiting example, one or more nucleic acid molecule encoding an NoV VP1 antigen might also be formulated with an adjuvant. In another non-limiting
30 example, the vaccine may comprise one or more adjuvants. A vaccine of the present invention, and its various components, may be prepared and/or administered by any

method disclosed herein or as would be known to one of ordinary skill in the art, in light of the present disclosure.

In various embodiments, the induction of immunity by the expression of the NoV VP1 antigens can be detected by observing in vivo or in vitro the response of all or any part of the immune system in the host against one or more NoV VP1 antigen.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). Some T cells that respond to the antigen presented by APC in an antigen specific manner differentiate into cytotoxic T cells (also referred to as cytotoxic T lymphocytes or CTLs) due to stimulation by the antigen. These antigen-stimulated cells then proliferate. This process is referred to herein as “activation” of T cells. Therefore, CTL induction by an epitope of a polypeptide or peptide or combinations thereof can be evaluated by presenting an epitope of a polypeptide or peptide or combinations thereof to a T cell by APC, and detecting the induction of CTL. Furthermore, APCs have the effect of activating B cells, CD4+ T cells, CD8+ T cells, macrophages, eosinophils and NK cells.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having a robust CTL inducing action among APCs. In the methods of the invention, the epitope of a polypeptide or peptide or combinations thereof is initially expressed by the DC and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the epitope of a polypeptide or peptide or combinations thereof has an activity of inducing the cytotoxic T cells. Furthermore, the induced immune response can also be examined by measuring IFN-gamma produced and released by CTL in the presence of antigen-presenting cells that carry immobilized peptide or a combination of peptides by visualizing using anti-IFN-gamma antibodies, such as an ELISPOT assay.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported to be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

The antigens confirmed to possess CTL-inducing activity by these methods are antigens having DC activation effect and subsequent CTL-inducing activity. Furthermore, CTLs that have acquired cytotoxicity due to presentation of the antigen by APC can be also used as vaccines against antigen-associated disorders.

5 The induction of immunity by expression of the NoV antigens can be further confirmed by observing the induction of antibody production against the NoV antigens. For example, when antibodies against an antigen are induced in a laboratory subject immunized with the composition encoding the antigens, and when antigen-associated pathology is suppressed by those antibodies, the composition is determined to
10 induce immunity.

The specificity of the antibody response induced in a subject can include binding to many regions of the delivered antigen, as well as, the induction of neutralization capable antibodies that that prevent infection or reduce disease severity.

 The induction of immunity by expression of the NoV antigens can be
15 further confirmed by observing the induction of T cells, such as CD4⁺ T cells, CD8⁺ T cells, or a combination thereof. For example, CD4⁺ T cells can also lyse target cells, but mainly supply help in the induction of other types of immune responses, including CTL and antibody generation. The type of CD4⁺ T cell help can be characterized, as Th1, Th2, Th9, Th17, Tregulatory (Treg), or T follicular helper (Tfh) cells. Each subtype of CD4⁺
20 T cell supplies help to certain types of immune responses. In one embodiment, the composition selectively induces T follicular helper cells, which drive potent antibody responses.

 The therapeutic compounds or compositions of the invention may be administered prophylactically (i.e., to prevent a disease or disorder) or therapeutically
25 (i.e., to treat a disease or disorder) to subjects suffering from, or at risk of (or susceptible to) developing a disease or disorder. Such subjects may be identified using standard clinical methods. In the context of the present invention, prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or alternatively delayed in its progression. In the context
30 of the field of medicine, the term “prevent” encompasses any activity, which reduces the burden of mortality or morbidity from disease. Prevention can occur at primary,

secondary and tertiary prevention levels. While primary prevention avoids the development of a disease, secondary and tertiary levels of prevention encompass activities aimed at preventing the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already established disease by
5 restoring function and reducing disease-related complications.

Antigen

The present invention provides a composition that induces an immune response in a subject. In one embodiment, the composition comprises at least one mRNA
10 molecule encoding at least one Norovirus (NoV) antigen. Norovirus antigens that can be included in the composition of the invention include, but are not limited to p48, nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, fragments thereof, or any combination thereof.

In one embodiment, the antigen is a VP1 antigen. In one embodiment, the
15 VP1 antigen is from a GI, GII, GIII, GIV, GV, GVI, GVII, GVIII, GIX or GX genogroup. In one embodiment, the VP1 antigen is from a GI, GII, GIV, GVIII or GIX genogroup. In one embodiment, the VP1 antigen is from GII.3, GII.4, GI.1, GI.3, GI.5 or any combination thereof.

In some embodiments, the vaccine further comprises one or more
20 additional nucleic acid molecules encoding at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than twenty additional viral antigens. In some embodiments, one or more additional viral antigens may be from human immunodeficiency virus (HIV), Chikungunya virus (CHIKV), dengue fever virus, papilloma viruses, for example, human papillomoma virus (HPV), polio virus, hepatitis
25 viruses, for example, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV), smallpox virus (Variola major and minor), vaccinia virus, rhinoviruses, equine encephalitis viruses, rubella virus, yellow fever virus, Norwalk virus, hepatitis A virus, human T-cell leukemia virus (HTLV-I), hairy cell leukemia virus (HTLV-II), California encephalitis virus, Hanta virus
30 (hemorrhagic fever), rabies virus, Ebola fever virus, Marburg virus, measles virus, mumps virus, respiratory syncytial virus (RSV), herpes simplex 1 (oral herpes), herpes

simplex 2 (genital herpes), herpes zoster (varicella-zoster, a.k.a., chickenpox), cytomegalovirus (CMV), for example human CMV, Epstein-Barr virus (EBV), flavivirus, foot and mouth disease virus, lassa virus, arenavirus, Merkel cell polyoma virus (MCV), influenza virus, a coronavirus including but not limited to severe acute
5 respiratory syndrome-coronavirus 2, a cancer causing virus, or any combination thereof. In one embodiment, one or more additional viral antigen are NoV antigens.

For example, in some embodiments, the composition comprises a nucleoside-modified RNA encoding a combination of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more than 20 NoV VP1 antigens from NoV GI, GII
10 and GIV genogroups, or fragments or variants thereof.

In some embodiments, the NoV VP1 antigen comprises a full length VP1 antigen, or a fragment or variant thereof.

In one embodiment, the mRNA molecule encoding the NoV VP1 antigen encodes SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5,
15 SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

In one embodiment, the nucleic acid molecule encoding the NoV VP1 antigen comprises a sequence encoding a tag or signal peptide (SP). Other signal peptides that may be used include, but are not limited to, signal sequences derived from IL-2, tPA, mouse and human IgG, and synthetic optimized signal sequences. In some instances, the
20 nucleic acid sequence comprises include additional sequences that encode linker or tag sequences that are linked to the antigen by a peptide bond.

In some embodiments, the NoV VP1 antigen comprises an amino acid sequence that is substantially homologous to the amino acid sequence of a NoV VP1 antigen described herein and retains the immunogenic function of the original amino acid
25 sequence. For example, in some embodiments, the nucleotide sequence of the nucleic acid molecule encoding the NoV VP1 antigen has a degree of identity with respect to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10 of at least 60%, of at least 65%, of at least 70%, of at least 75%, of at least 80%,
30 of at least 85%, of at least 90%, of at least 91%, of at least 92%, of at least 93%, of at least 94%, of at least 95%, of at least 96%, of at least 97%, of at least 98%, of at least

99%, or of at least 99.5%.

Adjuvant

In one embodiment, the composition comprises an adjuvant. In one
 5 embodiment, the composition comprises a nucleic acid molecule encoding an adjuvant.
 In one embodiment, the adjuvant-encoding nucleic acid molecule is IVT RNA. In one
 embodiment, the adjuvant-encoding nucleic acid molecule is nucleoside-modified RNA.
 In one embodiment, the adjuvant-encoding nucleic acid molecule is nucleoside-modified
 mRNA.

10 Exemplary adjuvants include, but are not limited to, alpha-interferon,
 gamma-interferon, platelet derived growth factor (PDGF), $\text{TNF}\alpha$, $\text{TNF}\beta$, GM-CSF,
 epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK),
 epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial
 chemokine (MEC), IL-12, IL-15, MHC, CD80, CD86. Other genes which may be useful
 15 adjuvants include those encoding: MCP-I, MIP-Ia, MIP-1p, IL-8, RANTES, L-selectin, P-
 selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-I, VLA-I, Mac-1, p150.95,
 PECAM, ICAM-I, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms
 of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve
 growth factor, vascular endothelial growth factor, Fas, TNF receptor, Fit, Apo-1, p55,
 20 WSL-I, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2,
 TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-I, Ap-I, Ap-2, p38, p65Rel, MyD88, IRAK,
 TRAF6, Ikb, Inactive NIK, SAP K, SAP-I, JNK, interferon response genes, NFkB, Bax,
 TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND,
 O $\times$ 40, O $\times$ 40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E,
 25 NKG2F, TAP 1, TAP2, anti-CTLA4-sc, anti-LAG3-Ig, anti-TIM3-Ig, and functional
 fragments thereof.

In some embodiments, the composition comprises an LNP, where the LNP
 acts as an adjuvant.

30 Nucleic Acids

In one embodiment, the invention includes at least one mRNA molecule

(e.g., a nucleoside modified mRNA molecule) encoding at least one NoV antigen.

Norovirus antigens that can be encoded by the mRNA molecule of the invention include, but are not limited to p48, nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, fragments thereof, or any
5 combination thereof.

In one embodiment, the mRNA molecule encodes a VP1 antigen. In one embodiment, the VP1 antigen is from a GI, GII, GIII, GIV, GV, GVI, GVII, GVIII, GIX or GX genogroup. In one embodiment, the VP1 antigen is from a GI, GII, GIV, GVIII or GIX genogroup. In one embodiment, the VP1 antigen is from GII.3, GII.4, GI.1, GI.3 or
10 GI.5 or any combination thereof.

The nucleic acid molecule can be made using any methodology in the art, including, but not limited to, in vitro transcription, chemical synthesis, or the like.

The nucleotide sequences encoding at least one NoV antigen as described herein, can alternatively comprise sequence variations with respect to the original
15 nucleotide sequences, for example, substitutions, insertions and/or deletions of one or more nucleotides, with the condition that the resulting polynucleotide encodes a polypeptide according to the invention. Therefore, the scope of the present invention includes nucleotide sequences that are substantially homologous or substantially identical to the nucleotide sequences recited herein and encode the NoV antigens of the invention.

A nucleotide sequence that is substantially homologous to a nucleotide sequence encoding an antigen can typically be isolated from a producer organism of the antigen based on the information contained in the nucleotide sequence by means of introducing conservative or non-conservative substitutions, for example. Other examples
20 of possible modifications include the insertion of one or more nucleotides in the sequence, the addition of one or more nucleotides in any of the ends of the sequence, or the deletion of one or more nucleotides in any end or inside the sequence. The degree of identity between two polynucleotides is determined using computer algorithms and methods that are widely known for the persons skilled in the art.

Further, the scope of the invention includes nucleotide sequences that
30 encode amino acid sequences that are substantially homologous to the amino acid sequences recited herein and preserve the immunogenic function of the original amino

acid sequence.

In one embodiment, the invention relates to a construct, comprising a nucleotide sequence encoding a NoV VP1 antigen. In one embodiment, the invention comprises a plurality of nucleotide sequences encoding a plurality of NoV antigens. For example, in some embodiments, the invention comprises a plurality of nucleotide sequences encoding 1 or more, 2 or more, 3 or more, or 4 or more NoV VP1 antigens. In one embodiment, the invention relates to a construct comprising a nucleotide sequence encoding an adjuvant. In one embodiment, the invention comprises a combination of a plurality of nucleotide sequences encoding 1 or more, 2 or more, 3 or more, or 4 or more NoV VP1 antigens and an adjuvant.

In one embodiment, the composition comprises a plurality of constructs, each construct encoding a NoV VP1 antigen. In one embodiment, the plurality of NoV VP1 antigens are from NoV genogroups GI, GII, GIV, GVIII or GIX or any combination thereof. In one embodiment, the composition comprises a first construct, comprising a nucleotide sequence encoding a NoV GII.4 VP1 antigen; and a second construct, comprising a nucleotide sequence encoding a NoV GI.1 VP1 antigen. In one embodiment, the composition comprises a first construct, comprising a nucleotide sequence encoding a NoV GII.4 VP1 antigen; a second construct, comprising a nucleotide sequence encoding a NoV GI.1 VP1 antigen, a third construct, comprising a nucleotide sequence encoding a NoV GII.3 VP1 antigen, and a fourth construct, comprising a nucleotide sequence encoding a NoV GI.3 VP1 antigen. In one embodiment, the mRNA molecule encoding the NoV GI.1 antigen encodes an amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2. In one embodiment, the mRNA molecule encoding the NoV GII.4 antigen encodes an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:4. In one embodiment, the mRNA molecule encoding the NoV GI.3 antigen encodes an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. In one embodiment, the mRNA molecule encoding the NoV GII.3 antigen encodes an amino acid sequence of SEQ ID NO:7 or SEQ ID NO:8. In one embodiment, the mRNA molecule encoding the NoV GI antigen encodes an amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10.

In another particular embodiment, the construct is operatively bound to a translational control element. The construct can incorporate an operatively bound

regulatory sequence for the expression of the nucleotide sequence of the invention, thus forming an expression cassette.

Vectors

5 The nucleic acid sequence(s) coding for the NoV antigen(s) of the invention can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be
10 produced synthetically.

 The nucleic acid molecules can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, a PCR-generated linear DNA sequence, and a cosmid. Vectors of particular interest include expression
15 vectors, replication vectors, probe generation vectors, sequencing vectors and vectors optimized for in vitro transcription.

 Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles,
20 mixed micelles, carbohydrates, peptides, cationic polymers, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle).

 In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the
25 introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome,
30 complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with

a micelle, or otherwise associated with a lipid. Lipid, lipid/RNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a “collapsed” structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine (“DMPC”) can be obtained from Sigma, St. Louis, MO; dicetyl phosphate (“DCP”) can be obtained from K & K Laboratories (Plainview, NY); cholesterol (“Choi”) can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol (“DMPG”) and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as it is more readily evaporated than methanol.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to a composition of the present invention, in order to confirm the presence of the mRNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Northern blotting and RT-PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, e.g., by immunogenic means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

In vitro transcribed RNA

In one embodiment, the composition of the invention comprises a combination of in vitro transcribed (IVT) RNA molecules encoding the NoV antigens of the invention. In one embodiment, an IVT RNA can be introduced to a cell as a form of transient transfection. The RNA is produced by in vitro transcription using a plasmid

DNA template generated synthetically. DNA of interest from any source can be directly converted by PCR into a template for in vitro mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any other appropriate source of DNA. In one embodiment, the desired template for in vitro transcription is a NoV antigen capable of inducing an adaptive immune response. In one embodiment, the desired template for in vitro transcription is an adjuvant capable of enhancing an adaptive immune response.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one embodiment, the DNA is a full-length gene or a portion of a gene. The gene can include some or all of the 5' and/or 3' untranslated regions (UTRs). The gene can include exons and introns. In one embodiment, the DNA to be used for PCR is a human gene. In another embodiment, the DNA to be used for PCR is a human gene including the 5' and 3' UTRs. In another embodiment, the DNA to be used for PCR is a gene from a pathogenic or commensal organism, including bacteria, viruses, parasites, and fungi. In another embodiment, the DNA to be used for PCR is from a pathogenic or commensal organism, including bacteria, viruses, parasites, and fungi, including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

Genes that can be used as sources of DNA for PCR include genes that encode polypeptides that induce or enhance an adaptive immune response in an organism. In some instances, the genes are useful for a short term treatment. In some instances, the genes have limited safety concerns regarding dosage of the expressed gene.

In various embodiments, a plasmid is used to generate a template for in vitro transcription of mRNA, which is used for transfection.

Chemical structures with the ability to promote stability and/or translation efficiency may also be used. In some embodiments, the RNA has 5' and 3' UTRs. In one

embodiment, the 5' UTR is between zero and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the gene of interest. Alternatively, UTR sequences that are not endogenous to the gene of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the gene of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous gene. Alternatively, when a 5' UTR that is not endogenous to the gene of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments the 5' UTR can be derived from an RNA virus whose RNA genome is stable in cells. In other embodiments various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

To enable synthesis of RNA from a DNA template, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added to the 5' end of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product upstream of the open reading frame that is to be transcribed. In one embodiment, the promoter is a T7 RNA polymerase promoter, as

described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

5 In one embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability of mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatameric product, which is not suitable for expression in eukaryotic cells. The transcription of plasmid DNA linearized at the end of the 3' UTR results in normal sized mRNA, which is effective in eukaryotic transfection when it is
10 polyadenylated after transcription.

On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270:1485-65 (2003)).

15 The conventional method of integration of polyA/T stretches into a DNA template is molecular cloning. However, polyA/T sequence integrated into plasmid DNA can cause plasmid instability, which can be ameliorated through the use of recombination incompetent bacterial cells for plasmid propagation.

Poly(A) tails of RNAs can be further extended following in vitro
20 transcription with the use of a poly(A) polymerase, such as E. coli polyA polymerase (E-PAP) or yeast polyA polymerase. In one embodiment, increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the attachment of different chemical groups to the 3' end can increase mRNA stability. Such attachment
25 can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP analogs can be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogs can further increase the stability of the RNA.

5' caps also provide stability to mRNA molecules. In one embodiment, RNAs produced by the methods to include a 5' cap1 structure. Such cap1 structure can be
30 generated using Vaccinia capping enzyme and 2'-O-methyltransferase enzymes (CellScript, Madison, WI). Alternatively, 5' cap is provided using techniques known in

the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7:1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendorf, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as “gene guns” (see, for example, Nishikawa, et al. Hum Gene Ther., 12(8):861-70 (2001)). In some embodiments RNA of the invention is introduced to a cell with a method comprising the use of TransIT[®]-mRNA transfection Kit (Mirus, Madison WI), which, in some instances, provides high efficiency, low toxicity, transfection.

15

Nucleoside-modified RNA

In one embodiment, the composition of the present invention comprises a nucleoside-modified nucleic acid encoding a NoV antigen as described herein. In one embodiment, the composition of the present invention comprises a plurality of nucleoside-modified nucleic acid molecules encoding a plurality of NoV antigens as described herein. In one embodiment, the composition of the present invention comprises a nucleoside-modified nucleic acid encoding an adjuvant as described herein. In one embodiment, the composition of the present invention comprises a nucleoside-modified nucleic acid encoding one or more NoV antigen and one or more adjuvants.

25

For example, in one embodiment, the composition comprises a nucleoside-modified RNA. In one embodiment, the composition comprises a nucleoside-modified mRNA. Nucleoside-modified mRNA have particular advantages over non-modified mRNA, including for example, increased stability, low or absent innate immunogenicity, and enhanced translation. Nucleoside-modified mRNA useful in the present invention is further described in U.S. Patent Nos. 8,278,036, 8,691,966, and 8,835,108, each of which is incorporated by reference herein in its entirety.

30

In some embodiments, nucleoside-modified mRNA does not activate any pathophysiologic pathways, translates very efficiently and almost immediately following delivery, and serve as templates for continuous protein production in vivo lasting for several days to weeks (Karikó et al., 2008, Mol Ther 16:1833-1840; Karikó et al., 2012, Mol Ther 20:948-953). The amount of mRNA required to exert a physiological effect is small, making it applicable for human therapy. For example, as described herein, nucleoside-modified mRNA encoding a NoV antigen has demonstrated the ability to induce antigen-specific antibody production. For example, in some instances, antigen encoded by nucleoside-modified mRNA induces greater production of antigen-specific antibody production as compared to antigen encoded by non-modified mRNA.

In some instances, expressing a protein by delivering the encoding mRNA has many benefits over methods that use protein, plasmid DNA or viral vectors. During mRNA transfection, the coding sequence of the desired protein is the only substance delivered to cells, thus avoiding all the side effects associated with plasmid backbones, viral genes, and viral proteins. More importantly, unlike DNA- and viral-based vectors, the mRNA does not carry the risk of being incorporated into the genome and protein production starts immediately after mRNA delivery. For example, high levels of circulating proteins have been measured within 15 to 30 minutes of in vivo injection of the encoding mRNA. In some embodiments, using mRNA rather than the protein also has many advantages. Half-lives of proteins in the circulation or in tissues are often short, thus protein treatment would need frequent dosing, while mRNA provides a template for continuous protein production for several days to weeks. Purification of proteins is problematic and they can contain aggregates and other impurities that cause adverse effects (Kromminga and Schellekens, 2005, Ann NY Acad Sci 1050:257-265).

In some embodiments, the nucleoside-modified RNA comprises the naturally occurring modified-nucleoside pseudouridine. In some embodiments, inclusion of pseudouridine makes the mRNA more stable, non-immunogenic, and highly translatable (Karikó et al., 2008, Mol Ther 16:1833-1840; Anderson et al., 2010, Nucleic Acids Res 38:5884-5892; Anderson et al., 2011, Nucleic Acids Research 39:9329-9338; Karikó et al., 2011, Nucleic Acids Research 39:e142; Karikó et al., 2012, Mol Ther 20:948-953; Karikó et al., 2005, Immunity 23:165-175).

It has been demonstrated that the presence of modified nucleosides, including pseudouridines in RNA suppress their innate immunogenicity (Karikó et al., 2005, *Immunity* 23:165-175). Further, protein-encoding, in vitro-transcribed RNA containing pseudouridine can be translated more efficiently than RNA containing no or
5 other modified nucleosides (Karikó et al., 2008, *Mol Ther* 16:1833-1840). Subsequently, it is shown that the presence of pseudouridine improves the stability of RNA (Anderson et al., 2011, *Nucleic Acids Research* 39:9329-9338) and abates both activation of PKR and inhibition of translation (Anderson et al., 2010, *Nucleic Acids Res* 38:5884-5892).

Similar effects as described for pseudouridine have also been observed for
10 RNA containing 1-methyl-pseudouridine.

In some embodiments, the nucleoside-modified nucleic acid molecule is a purified nucleoside-modified nucleic acid molecule. For example, in some embodiments, the composition is purified to remove double-stranded contaminants. In some instances, a preparative high-performance liquid chromatography (HPLC) purification procedure is
15 used to obtain pseudouridine-containing RNA that has superior translational potential and no innate immunogenicity (Karikó et al., 2011, *Nucleic Acids Research* 39:e142). Administering HPLC-purified, pseudouridine-containing RNA coding for erythropoietin into mice and macaques resulted in a significant increase of serum EPO levels (Karikó et al., 2012, *Mol Ther* 20:948-953), thus confirming that pseudouridine-containing mRNA
20 is suitable for in vivo protein therapy. In some embodiments, the nucleoside-modified nucleic acid molecule is purified using non-HPLC methods. In some instances, the nucleoside-modified nucleic acid molecule is purified using chromatography methods, including but not limited to HPLC and fast protein liquid chromatography (FPLC). An exemplary FPLC-based purification procedure is described in Weissman et al., 2013,
25 *Methods Mol Biol*, 969: 43-54. Exemplary purification procedures are also described in U.S. Patent Application Publication No. US2016/0032316, which is hereby incorporated by reference in its entirety.

The present invention encompasses RNA, oligoribonucleotide, and polyribonucleotide molecules comprising pseudouridine or a modified nucleoside. In
30 some embodiments, the composition comprises an isolated nucleic acid encoding an antigen, wherein the nucleic acid comprises a pseudouridine or a modified nucleoside. In

some embodiments, the composition comprises a vector, comprising an isolated nucleic acid encoding an antigen, adjuvant, or combination thereof, wherein the nucleic acid comprises a pseudouridine or a modified nucleoside.

In one embodiment, the nucleoside-modified RNA of the invention is IVT RNA, as described elsewhere herein. For example, in some embodiments, the nucleoside-modified RNA is synthesized by T7 phage RNA polymerase. In another embodiment, the nucleoside-modified mRNA is synthesized by SP6 phage RNA polymerase. In another embodiment, the nucleoside-modified RNA is synthesized by T3 phage RNA polymerase.

In one embodiment, the modified nucleoside is $m^1acp^3\Psi$ (1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine). In another embodiment, the modified nucleoside is $m^1\Psi$ (1-methylpseudouridine). In another embodiment, the modified nucleoside is Ψm (2'-O-methylpseudouridine). In another embodiment, the modified nucleoside is m^5D (5-methyldihydrouridine). In another embodiment, the modified nucleoside is $m^3\Psi$ (3-methylpseudouridine). In another embodiment, the modified nucleoside is a pseudouridine moiety that is not further modified. In another embodiment, the modified nucleoside is a monophosphate, diphosphate, or triphosphate of any of the above pseudouridines. In another embodiment, the modified nucleoside is any other pseudouridine-like nucleoside known in the art.

In another embodiment, the nucleoside that is modified in the nucleoside-modified RNA the present invention is uridine (U). In another embodiment, the modified nucleoside is cytidine (C). In another embodiment, the modified nucleoside is adenosine (A). In another embodiment, the modified nucleoside is guanosine (G).

In another embodiment, the modified nucleoside of the present invention is m^5C (5-methylcytidine). In another embodiment, the modified nucleoside is m^5U (5-methyluridine). In another embodiment, the modified nucleoside is m^6A (N^6 -methyladenosine). In another embodiment, the modified nucleoside is s^2U (2-thiouridine). In another embodiment, the modified nucleoside is Ψ (pseudouridine). In another embodiment, the modified nucleoside is Um (2'-O-methyluridine).

In other embodiments, the modified nucleoside is m^1A (1-methyladenosine); m^2A (2-methyladenosine); Am (2'-O-methyladenosine); ms^2m^6A (2-

- methylthio-N⁶-methyadenosine); i⁶A (N⁶-isopentenyladenosine); ms²i⁶A (2-methylthio-N⁶-isopentenyladenosine); io⁶A (N⁶-(cis-hydroxyisopentenyl)adenosine); ms²io⁶A (2-methylthio-N⁶-(cis-hydroxyisopentenyl) adenosine); g⁶A (N⁶-glycinyln carbamoyladenosine); t⁶A (N⁶-threonyln carbamoyladenosine); ms²t⁶A (2-methylthio-N⁶-threonyln carbamoyladenosine); m⁶t⁶A (N⁶-methyl-N⁶-threonyln carbamoyladenosine); hn⁶A (N⁶-hydroxynorvalyln carbamoyladenosine); ms²hn⁶A (2-methylthio-N⁶-hydroxynorvalyln carbamoyladenosine); Ar(p) (2'-O-ribosyladenosine (phosphate)); I (inosine); m¹I (1-methylinosine); m¹Im (1,2'-O-dimethylinosine); m³C (3-methylcytidine); Cm (2'-O-methylcytidine); s²C (2-thiocytidine); ac⁴C (N⁴-acetylcytidine); f⁵C (5-formylcytidine); m⁵Cm (5,2'-O-dimethylcytidine); ac⁴Cm (N⁴-acetyl-2'-O-methylcytidine); k²C (lysidine); m¹G (1-methylguanosine); m²G (N²-methylguanosine); m⁷G (7-methylguanosine); Gm (2'-O-methylguanosine); m²₂G (N²,N²-dimethylguanosine); m²Gm (N²,2'-O-dimethylguanosine); m²₂Gm (N²,N²,2'-O-trimethylguanosine); Gr(p) (2'-O-ribosylguanosine (phosphate)); yW (wybutosine); o₂yW (peroxywybutosine); OHyW (hydroxywybutosine); OHyW* (undermodified hydroxywybutosine); imG (wyosine); mimG (methylwyosine); Q (queuosine); oQ (epoxyqueuosine); galQ (galactosyl-queuosine); manQ (mannosyl-queuosine); preQ₀ (7-cyano-7-deazaguanosine); preQ₁ (7-aminomethyl-7-deazaguanosine); G⁺ (archaeosine); D (dihydrouridine); m⁵Um (5,2'-O-dimethyluridine); s⁴U (4-thiouridine); m⁵s²U (5-methyl-2-thiouridine); s²Um (2-thio-2'-O-methyluridine); acp³U (3-(3-amino-3-carboxypropyl)uridine); ho⁵U (5-hydroxyuridine); mo⁵U (5-methoxyuridine); cmo⁵U (uridine 5-oxyacetic acid); mcmo⁵U (uridine 5-oxyacetic acid methyl ester); chm⁵U (5-(carboxyhydroxymethyl)uridine); mchm⁵U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm⁵U (5-methoxycarbonylmethyluridine); mcm⁵Um (5-methoxycarbonylmethyl-2'-O-methyluridine); mcm⁵s²U (5-methoxycarbonylmethyl-2-thiouridine); nm⁵s²U (5-aminomethyl-2-thiouridine); mnm⁵U (5-methylaminomethyluridine); mnm⁵s²U (5-methylaminomethyl-2-thiouridine); mnm⁵se²U (5-methylaminomethyl-2-selenouridine); ncm⁵U (5-carbamoylmethyluridine); ncm⁵Um (5-carbamoylmethyl-2'-O-methyluridine); cmnm⁵U (5-carboxymethylaminomethyluridine); cmnm⁵Um (5-carboxymethylaminomethyl-2'-O-methyluridine); cmnm⁵s²U (5-carboxymethylaminomethyl-2-thiouridine); m⁶₂A (N⁶,N⁶-

dimethyladenosine); Im (2'-O-methylinosine); m⁴C (N⁴-methylcytidine); m⁴Cm (N⁴,2'-O-dimethylcytidine); hm⁵C (5-hydroxymethylcytidine); m³U (3-methyluridine); cm⁵U (5-carboxymethyluridine); m⁶Am (N⁶,2'-O-dimethyladenosine); m⁶₂Am (N⁶,N⁶,O-2'-trimethyladenosine); m^{2,7}G (N²,7-dimethylguanosine); m^{2,2,7}G (N²,N²,7-trimethylguanosine); m³Um (3,2'-O-dimethyluridine); m⁵D (5-methyldihydrouridine); f⁵Cm (5-formyl-2'-O-methylcytidine); m¹Gm (1,2'-O-dimethylguanosine); m¹Am (1,2'-O-dimethyladenosine); τ m⁵U (5-taurinomethyluridine); τ m^{5s}U (5-taurinomethyl-2-thiouridine)); imG-14 (4-demethylwyosine); imG2 (isowyosine); or ac⁶A (N⁶-acetyladenosine).

10 In another embodiment, a nucleoside-modified RNA of the present invention comprises a combination of 2 or more of the above modifications. In another embodiment, the nucleoside-modified RNA comprises a combination of 3 or more of the above modifications. In another embodiment, the nucleoside-modified RNA comprises a combination of more than 3 of the above modifications.

15 In various embodiments, between 0.1% and 100% of the residues in the nucleoside-modified RNA of the present invention are modified (e.g., either by the presence of pseudouridine, 1-methyl-pseudouridine, 5-methyl-uridine or another modified nucleoside base). In one embodiment, the fraction of modified residues is 0.1%. In another embodiment, the fraction of modified residues is 0.2%. In another
20 embodiment, the fraction is 0.3%. In another embodiment, the fraction is 0.4%. In another embodiment, the fraction is 0.5%. In another embodiment, the fraction is 0.6%. In another embodiment, the fraction is 0.7%. In another embodiment, the fraction is 0.8%. In another embodiment, the fraction is 0.9%. In another embodiment, the fraction is 1%. In another embodiment, the fraction is 1.5%. In another embodiment, the fraction
25 is 2%. In another embodiment, the fraction is 2.5%. In another embodiment, the fraction is 3%. In another embodiment, the fraction is 4%. In another embodiment, the fraction is 5%. In another embodiment, the fraction is 6%. In another embodiment, the fraction is 7%. In another embodiment, the fraction is 8%. In another embodiment, the fraction is 9%. In another embodiment, the fraction is 10%. In another embodiment, the fraction is
30 12%. In another embodiment, the fraction is 14%. In another embodiment, the fraction is 16%. In another embodiment, the fraction is 18%. In another embodiment, the fraction is

20%. In another embodiment, the fraction is 25%. In another embodiment, the fraction is 30%. In another embodiment, the fraction is 35%. In another embodiment, the fraction is 40%. In another embodiment, the fraction is 45%. In another embodiment, the fraction is 50%. In another embodiment, the fraction is 55%. In another embodiment, the fraction is 60%. In another embodiment, the fraction is 65%. In another embodiment, the fraction is 70%. In another embodiment, the fraction is 75%. In another embodiment, the fraction is 80%. In another embodiment, the fraction is 85%. In another embodiment, the fraction is 90%. In another embodiment, the fraction is 91%. In another embodiment, the fraction is 92%. In another embodiment, the fraction is 93%. In another embodiment, the fraction is 94%. In another embodiment, the fraction is 95%. In another embodiment, the fraction is 96%. In another embodiment, the fraction is 97%. In another embodiment, the fraction is 98%. In another embodiment, the fraction is 99%. In another embodiment, the fraction is 100%.

In another embodiment, the fraction is less than 5%. In another embodiment, the fraction is less than 3%. In another embodiment, the fraction is less than 1%. In another embodiment, the fraction is less than 2%. In another embodiment, the fraction is less than 4%. In another embodiment, the fraction is less than 6%. In another embodiment, the fraction is less than 8%. In another embodiment, the fraction is less than 10%. In another embodiment, the fraction is less than 12%. In another embodiment, the fraction is less than 15%. In another embodiment, the fraction is less than 20%. In another embodiment, the fraction is less than 30%. In another embodiment, the fraction is less than 40%. In another embodiment, the fraction is less than 50%. In another embodiment, the fraction is less than 60%. In another embodiment, the fraction is less than 70%.

In another embodiment, 0.1% of the residues of a given nucleoside (i.e., uridine, cytidine, guanosine, or adenosine) are modified. In another embodiment, the fraction of modified residues is 0.2%. In another embodiment, the fraction is 0.3%. In another embodiment, the fraction is 0.4%. In another embodiment, the fraction is 0.5%. In another embodiment, the fraction is 0.6%. In another embodiment, the fraction is 0.7%. In another embodiment, the fraction is 0.8%. In another embodiment, the fraction is 0.9%. In another embodiment, the fraction is 1%. In another embodiment, the fraction

is 1.5%. In another embodiment, the fraction is 2%. In another embodiment, the fraction is 2.5%. In another embodiment, the fraction is 3%. In another embodiment, the fraction is 4%. In another embodiment, the fraction is 5%. In another embodiment, the fraction is 6%. In another embodiment, the fraction is 7%. In another embodiment, the fraction is 8%. In another embodiment, the fraction is 9%. In another embodiment, the fraction is 10%. In another embodiment, the fraction is 12%. In another embodiment, the fraction is 14%. In another embodiment, the fraction is 16%. In another embodiment, the fraction is 18%. In another embodiment, the fraction is 20%. In another embodiment, the fraction is 25%. In another embodiment, the fraction is 30%. In another embodiment, the fraction is 35%. In another embodiment, the fraction is 40%. In another embodiment, the fraction is 45%. In another embodiment, the fraction is 50%. In another embodiment, the fraction is 55%. In another embodiment, the fraction is 60%. In another embodiment, the fraction is 65%. In another embodiment, the fraction is 70%. In another embodiment, the fraction is 75%. In another embodiment, the fraction is 80%. In another embodiment, the fraction is 85%. In another embodiment, the fraction is 90%. In another embodiment, the fraction is 91%. In another embodiment, the fraction is 92%. In another embodiment, the fraction is 93%. In another embodiment, the fraction is 94%. In another embodiment, the fraction is 95%. In another embodiment, the fraction is 96%. In another embodiment, the fraction is 97%. In another embodiment, the fraction is 98%. In another embodiment, the fraction is 99%. In another embodiment, the fraction is 100%. In another embodiment, the fraction of the given nucleotide that is modified is less than 8%. In another embodiment, the fraction is less than 10%. In another embodiment, the fraction is less than 5%. In another embodiment, the fraction is less than 3%. In another embodiment, the fraction is less than 1%. In another embodiment, the fraction is less than 2%. In another embodiment, the fraction is less than 4%. In another embodiment, the fraction is less than 6%. In another embodiment, the fraction is less than 12%. In another embodiment, the fraction is less than 15%. In another embodiment, the fraction is less than 20%. In another embodiment, the fraction is less than 30%. In another embodiment, the fraction is less than 40%. In another embodiment, the fraction is less than 50%. In another embodiment, the fraction is less than 60%. In another embodiment, the fraction is less than 70%.

In some embodiments, the composition comprises a purified preparation

of single-stranded nucleoside modified RNA. For example, in some embodiments, the purified preparation of single-stranded nucleoside modified RNA is substantially free of double stranded RNA (dsRNA). In some embodiments, the purified preparation is at least 90%, or at least 91%, or at least 92%, or at least 93 % or at least 94%, or at least 95%, or
5 at least 96%, or at least 97%, or at least 98%, or at least 99%, or at least 99.5%, or at least 99.9% single stranded nucleoside modified RNA, relative to all other nucleic acid molecules (DNA, dsRNA, etc.).

In another embodiment, a nucleoside-modified RNA of the present invention is translated in the cell more efficiently than an unmodified RNA molecule
10 with the same sequence. In another embodiment, the nucleoside-modified RNA exhibits enhanced ability to be translated by a target cell. In another embodiment, translation is enhanced by a factor of 2-fold relative to its unmodified counterpart. In another embodiment, translation is enhanced by a 3-fold factor. In another embodiment, translation is enhanced by a 4-fold factor. In another embodiment, translation is enhanced
15 by a 5-fold factor. In another embodiment, translation is enhanced by a 6-fold factor. In another embodiment, translation is enhanced by a 7-fold factor. In another embodiment, translation is enhanced by an 8-fold factor. In another embodiment, translation is enhanced by a 9-fold factor. In another embodiment, translation is enhanced by a 10-fold factor. In another embodiment, translation is enhanced by a 15-fold factor. In another
20 embodiment, translation is enhanced by a 20-fold factor. In another embodiment, translation is enhanced by a 50-fold factor. In another embodiment, translation is enhanced by a 100-fold factor. In another embodiment, translation is enhanced by a 200-fold factor. In another embodiment, translation is enhanced by a 500-fold factor. In another embodiment, translation is enhanced by a 1000-fold factor. In another
25 embodiment, translation is enhanced by a 2000-fold factor. In another embodiment, the factor is 10-1000-fold. In another embodiment, the factor is 10-100-fold. In another embodiment, the factor is 10-200-fold. In another embodiment, the factor is 10-300-fold. In another embodiment, the factor is 10-500-fold. In another embodiment, the factor is 20-1000-fold. In another embodiment, the factor is 30-1000-fold. In another embodiment,
30 the factor is 50-1000-fold. In another embodiment, the factor is 100-1000-fold. In another embodiment, the factor is 200-1000-fold. In another embodiment, translation is enhanced

by any other significant amount or range of amounts.

In another embodiment, the nucleoside-modified antigen-encoding RNA of the present invention induces a significantly more robust adaptive immune response as compared with an unmodified in vitro-synthesized RNA molecule of the same sequence.

5 In another embodiment, the modified RNA molecule induces an adaptive immune response that is 2-fold greater than its unmodified counterpart. In another embodiment, the adaptive immune response is increased by a 3-fold factor. In another embodiment, the adaptive immune response is increased by a 4-fold factor. In another embodiment, the adaptive immune response is increased by a 5-fold factor. In another embodiment, the adaptive immune response is increased by a 6-fold factor. In another embodiment, the adaptive immune response is increased by a 7-fold factor. In another embodiment, the adaptive immune response is increased by an 8-fold factor. In another embodiment, the adaptive immune response is increased by a 9-fold factor. In another embodiment, the adaptive immune response is increased by a 10-fold factor. In another embodiment, the adaptive immune response is increased by a 15-fold factor. In another embodiment, the adaptive immune response is increased by a 20-fold factor. In another embodiment, the adaptive immune response is increased by a 50-fold factor. In another embodiment, the adaptive immune response is increased by a 100-fold factor. In another embodiment, the adaptive immune response is increased by a 200-fold factor. In another embodiment, the adaptive immune response is increased by a 500-fold factor. In another embodiment, the adaptive immune response is increased by a 1000-fold factor. In another embodiment, the adaptive immune response is increased by a 2000-fold factor. In another embodiment, the adaptive immune response is increased by another fold difference.

In another embodiment, “induces significantly more robust adaptive
25 immune response” refers to a detectable increase in an adaptive immune response. In another embodiment, the term refers to a fold increase in the adaptive immune response (e.g., 1 of the fold increases enumerated above). In another embodiment, the term refers to an increase such that the nucleoside-modified RNA can be administered at a lower dose or frequency than an unmodified RNA molecule while still inducing a similarly
30 effective adaptive immune response. In another embodiment, the increase is such that the nucleoside-modified RNA can be administered using a single dose to induce an effective

adaptive immune response.

In another embodiment, the nucleoside-modified RNA of the present invention exhibits significantly less innate immunogenicity than an unmodified in vitro-synthesized RNA molecule of the same sequence. In another embodiment, the modified RNA molecule exhibits an innate immune response that is 2-fold less than its unmodified counterpart. In another embodiment, innate immunogenicity is reduced by a 3-fold factor. In another embodiment, innate immunogenicity is reduced by a 4-fold factor. In another embodiment, innate immunogenicity is reduced by a 5-fold factor. In another embodiment, innate immunogenicity is reduced by a 6-fold factor. In another embodiment, innate immunogenicity is reduced by a 7-fold factor. In another embodiment, innate immunogenicity is reduced by a 8-fold factor. In another embodiment, innate immunogenicity is reduced by a 9-fold factor. In another embodiment, innate immunogenicity is reduced by a 10-fold factor. In another embodiment, innate immunogenicity is reduced by a 15-fold factor. In another embodiment, innate immunogenicity is reduced by a 20-fold factor. In another embodiment, innate immunogenicity is reduced by a 50-fold factor. In another embodiment, innate immunogenicity is reduced by a 100-fold factor. In another embodiment, innate immunogenicity is reduced by a 200-fold factor. In another embodiment, innate immunogenicity is reduced by a 500-fold factor. In another embodiment, innate immunogenicity is reduced by a 1000-fold factor. In another embodiment, innate immunogenicity is reduced by a 2000-fold factor. In another embodiment, innate immunogenicity is reduced by another fold difference.

In another embodiment, “exhibits significantly less innate immunogenicity” refers to a detectable decrease in innate immunogenicity. In another embodiment, the term refers to a fold decrease in innate immunogenicity (e.g., 1 of the fold decreases enumerated above). In another embodiment, the term refers to a decrease such that an effective amount of the nucleoside-modified RNA can be administered without triggering a detectable innate immune response. In another embodiment, the term refers to a decrease such that the nucleoside-modified RNA can be repeatedly administered without eliciting an innate immune response sufficient to detectably reduce production of the protein encoded by the modified RNA. In another embodiment, the

decrease is such that the nucleoside-modified RNA can be repeatedly administered without eliciting an innate immune response sufficient to eliminate detectable production of the protein encoded by the modified RNA.

5 Lipid Nanoparticle

 In one embodiment, delivery of nucleoside-modified RNA comprises any suitable delivery method, including exemplary RNA transfection methods described elsewhere herein. In some embodiments, delivery of a nucleoside-modified RNA to a subject comprises mixing the nucleoside-modified RNA with a transfection reagent prior
10 to the step of contacting. In another embodiment, a method of present invention further comprises administering nucleoside-modified RNA together with the transfection reagent. In another embodiment, the transfection reagent is a cationic lipid reagent. In another embodiment, the transfection reagent is a cationic polymer reagent.

 In another embodiment, the transfection reagent is a lipid-based
15 transfection reagent. In another embodiment, the transfection reagent is a protein-based transfection reagent. In another embodiment, the transfection reagent is a carbohydrate-based transfection reagent. In another embodiment, the transfection reagent is a cationic lipid-based transfection reagent. In another embodiment, the transfection reagent is a cationic polymer-based transfection reagent. In another embodiment, the transfection
20 reagent is a polyethyleneimine based transfection reagent. In another embodiment, the transfection reagent is calcium phosphate. In another embodiment, the transfection reagent is Lipofectin[®], Lipofectamine[®], or TransIT[®]. In another embodiment, the transfection reagent is any other transfection reagent known in the art.

 In another embodiment, the transfection reagent forms a liposome.
25 Liposomes, in another embodiment, increase intracellular stability, increase uptake efficiency and improve biological activity. In another embodiment, liposomes are hollow spherical vesicles composed of lipids arranged in a similar fashion as those lipids, which make up the cell membrane. They have, in another embodiment, an internal aqueous space for entrapping water-soluble compounds and range in size from 0.05 to several
30 microns in diameter. In another embodiment, liposomes can deliver RNA to cells in a biologically active form.

In one embodiment, the composition comprises a lipid nanoparticle (LNP) and one or more nucleic acid molecules described herein. For example, in one embodiment, the composition comprises an LNP and one or more nucleoside-modified RNA molecules encoding one or more antigens, adjuvants, or a combination thereof.

5 In some embodiments, the lipid nanoparticle is a particle having at least one dimension on the order of nanometers (e.g., 1-1,000 nm). In some embodiments, the lipid nanoparticle comprises one or more lipids. For example, in some embodiments, the lipid comprises a lipid of Formula (I), (II) or (III).

10 In some embodiments, lipid nanoparticles are included in a formulation comprising a nucleoside-modified RNA as described herein. In some embodiments, such lipid nanoparticles comprise a cationic lipid (e.g., a lipid of Formula (I), (II) or (III)) and one or more excipient selected from neutral lipids, charged lipids, steroids and polymer conjugated lipids (e.g., a pegylated lipid such as a pegylated lipid of structure (IV). In some embodiments, the nucleoside-modified RNA is encapsulated in the lipid portion of
15 the lipid nanoparticle or an aqueous space enveloped by some or all of the lipid portion of the lipid nanoparticle, thereby protecting it from enzymatic degradation or other undesirable effects induced by the mechanisms of the host organism or cells, e.g., an adverse immune response.

20 In various embodiments, the lipid nanoparticles have a mean diameter of from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm, from about 70 nm to about 80 nm, or about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm,
25 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm, and are substantially non-toxic. In some embodiments, the nucleoside-modified RNA, when present in the lipid nanoparticles, is resistant in aqueous solution to degradation with a nuclease.

30 The LNP may comprise any lipid capable of forming a particle to which the one or more nucleic acid molecules are attached, or in which the one or more nucleic

acid molecules are encapsulated.

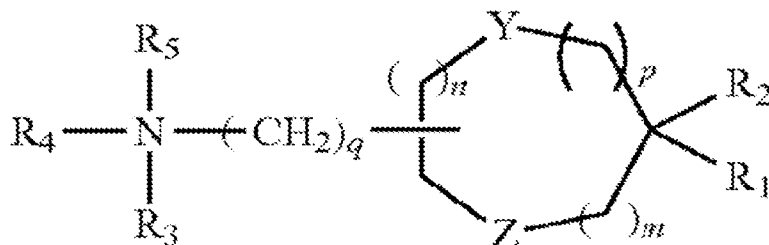
In one embodiment, the LNP comprises one or more cationic lipids, and one or more stabilizing lipids. Stabilizing lipids include neutral lipids and pegylated lipids.

5 In one embodiment, the LNP comprises a cationic lipid. In some embodiments, the cationic lipid comprises any of a number of lipid species which carry a net positive charge at a selective pH, such as physiological pH. Such lipids include, but are not limited to, N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC); N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); N,N-distearyl-N,N-dimethylammonium bromide (DDAB); N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP); 3-(N—(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol), N-(1-(2,3-dioleoyloxy)propyl)-N-2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA), dioctadecylamidoglycyl carboxyspermine (DOGS), 1,2-dioleoyl-3-dimethylammonium propane (DODAP), N,N-dimethyl-2,3-dioleoyloxy)propylamine (DODMA), and N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE). Additionally, a number of commercial preparations of cationic lipids are available which can be used in the present invention. These include, for example, LIPOFECTIN[®] (commercially available cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine (DOPE), from GIBCO/BRL, Grand Island, N. Y.); LIPOFECTAMINE[®] (commercially available cationic liposomes comprising N-(1-(2,3-dioleoyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA) and (DOPE), from GIBCO/BRL); and TRANSFECTAM[®] (commercially available cationic lipids comprising dioctadecylamidoglycyl carboxyspermine (DOGS) in ethanol from Promega Corp., Madison, Wis.). The following lipids are cationic and have a positive charge at below physiological pH: DODAP, DODMA, DMDMA, 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA).

30 In one embodiment, the cationic lipid is an amino lipid. Suitable amino lipids useful in the invention include those described in WO 2012/016184, incorporated herein by reference in its entirety. Representative amino lipids include, but are not limited

to, 1,2-dilinoleyloxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-dilinoleyloxy-3-morpholinopropane (DLin-MA), 1,2-dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), 3-(N,N-dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-dioleylamino)-1,2-propanediol (DOAP), 1,2-dilinoleyloxy-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), and 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA).

Suitable amino lipids include those having the formula:



wherein R₁ and R₂ are either the same or different and independently optionally substituted C₁₀-C₂₄ alkyl, optionally substituted C₁₀-C₂₄ alkenyl, optionally substituted C₁₀-C₂₄ alkynyl, or optionally substituted C₁₀-C₂₄ acyl;

R₃ and R₄ are either the same or different and independently optionally substituted C₁-C₆ alkyl, optionally substituted C₂-C₆ alkenyl, or optionally substituted C₂-C₆ alkynyl or R₃ and R₄ may join to form an optionally substituted heterocyclic ring of 4 to 6 carbon atoms and 1 or 2 heteroatoms chosen from nitrogen and oxygen;

R₅ is either absent or present and when present is hydrogen or C₁-C₆ alkyl;

m, n, and p are either the same or different and independently either 0 or 1 with the proviso that m, n, and p are not simultaneously 0;

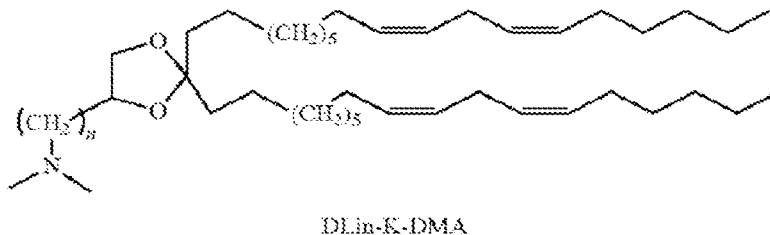
q is 0, 1, 2, 3, or 4; and

Y and Z are either the same or different and independently O, S, or NH.

In one embodiment, R₁ and R₂ are each linoleyl, and the amino lipid is a

dilinoleyl amino lipid. In one embodiment, the amino lipid is a dilinoleyl amino lipid.

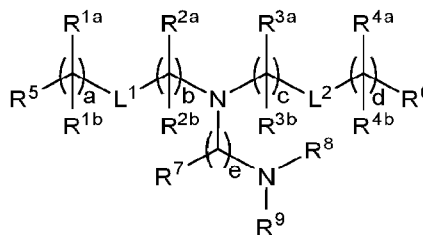
A representative useful dilinoleyl amino lipid has the formula:



wherein n is 0, 1, 2, 3, or 4.

5 In one embodiment, the cationic lipid is a DLin-K-DMA. In one embodiment, the cationic lipid is DLin-KC2-DMA (DLin-K-DMA above, wherein n is 2).

In one embodiment, the cationic lipid component of the LNPs has the structure of Formula (I):



(I)

or a pharmaceutically acceptable salt, tautomer, prodrug or stereoisomer thereof, wherein:

15 L^1 and L^2 are each independently $-O(C=O)-$, $-(C=O)O-$ or a carbon-carbon double bond;

R^{1a} and R^{1b} are, at each occurrence, independently either (a) H or C_1-C_{12} alkyl, or (b) R^{1a} is H or C_1-C_{12} alkyl, and R^{1b} together with the carbon atom to which it is bound is taken together with an adjacent R^{1b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

20 R^{2a} and R^{2b} are, at each occurrence, independently either (a) H or C_1-C_{12} alkyl, or (b) R^{2a} is H or C_1-C_{12} alkyl, and R^{2b} together with the carbon atom to which it is bound is taken together with an adjacent R^{2b} and the carbon atom to which it is bound to

form a carbon-carbon double bond;

R^{3a} and R^{3b} are, at each occurrence, independently either (a) H or C₁-C₁₂ alkyl, or (b) R^{3a} is H or C₁-C₁₂ alkyl, and R^{3b} together with the carbon atom to which it is bound is taken together with an adjacent R^{3b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

R^{4a} and R^{4b} are, at each occurrence, independently either (a) H or C₁-C₁₂ alkyl, or (b) R^{4a} is H or C₁-C₁₂ alkyl, and R^{4b} together with the carbon atom to which it is bound is taken together with an adjacent R^{4b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

R^5 and R^6 are each independently methyl or cycloalkyl;

R^7 is, at each occurrence, independently H or C₁-C₁₂ alkyl;

R^8 and R^9 are each independently C₁-C₁₂ alkyl; or R^8 and R^9 , together with the nitrogen atom to which they are attached, form a 5, 6 or 7-membered heterocyclic ring comprising one nitrogen atom;

a and d are each independently an integer from 0 to 24;

b and c are each independently an integer from 1 to 24; and

e is 1 or 2.

In some embodiments of Formula (I), at least one of R^{1a} , R^{2a} , R^{3a} or R^{4a} is C₁-C₁₂ alkyl, or at least one of L^1 or L^2 is $-O(C=O)-$ or $-(C=O)O-$. In other embodiments, R^{1a} and R^{1b} are not isopropyl when a is 6 or n-butyl when a is 8.

In still further embodiments of Formula (I), at least one of R^{1a} , R^{2a} , R^{3a} or R^{4a} is C₁-C₁₂ alkyl, or at least one of L^1 or L^2 is $-O(C=O)-$ or $-(C=O)O-$; and

R^{1a} and R^{1b} are not isopropyl when a is 6 or n-butyl when a is 8.

In other embodiments of Formula (I), R^8 and R^9 are each independently unsubstituted C₁-C₁₂ alkyl; or R^8 and R^9 , together with the nitrogen atom to which they are attached, form a 5, 6 or 7-membered heterocyclic ring comprising one nitrogen atom;

In some embodiments of Formula (I), any one of L^1 or L^2 may be $-O(C=O)-$ or a carbon-carbon double bond. L^1 and L^2 may each be $-O(C=O)-$ or may each be a carbon-carbon double bond.

In some embodiments of Formula (I), one of L^1 or L^2 is $-O(C=O)-$. In

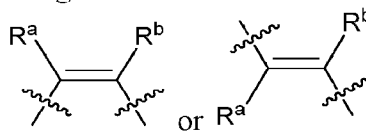
other embodiments, both L^1 and L^2 are $-\text{O}(\text{C}=\text{O})-$.

In some embodiments of Formula (I), one of L^1 or L^2 is $-(\text{C}=\text{O})\text{O}-$. In other embodiments, both L^1 and L^2 are $-(\text{C}=\text{O})\text{O}-$.

In some other embodiments of Formula (I), one of L^1 or L^2 is a carbon-carbon double bond. In other embodiments, both L^1 and L^2 are a carbon-carbon double bond.

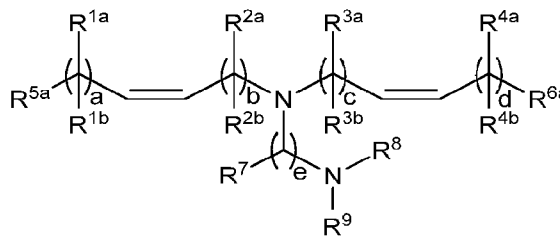
In still other embodiments of Formula (I), one of L^1 or L^2 is $-\text{O}(\text{C}=\text{O})-$ and the other of L^1 or L^2 is $-(\text{C}=\text{O})\text{O}-$. In more embodiments, one of L^1 or L^2 is $-\text{O}(\text{C}=\text{O})-$ and the other of L^1 or L^2 is a carbon-carbon double bond. In yet more
10 other embodiments, one of L^1 or L^2 is $-(\text{C}=\text{O})\text{O}-$ and the other of L^1 or L^2 is a carbon-carbon double bond.

It is understood that “carbon-carbon” double bond, as used throughout the specification, refers to one of the following structures:



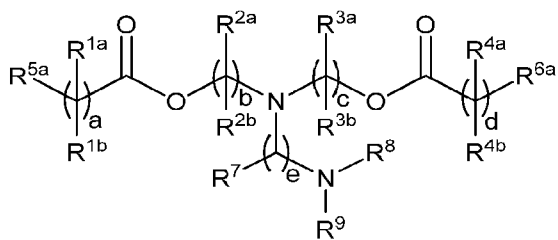
15 wherein R^a and R^b are, at each occurrence, independently H or a substituent. For example, in some embodiments R^a and R^b are, at each occurrence, independently H, C_1 - C_{12} alkyl or cycloalkyl, for example H or C_1 - C_{12} alkyl.

In other embodiments, the lipid compounds of Formula (I) have the following structure (Ia):



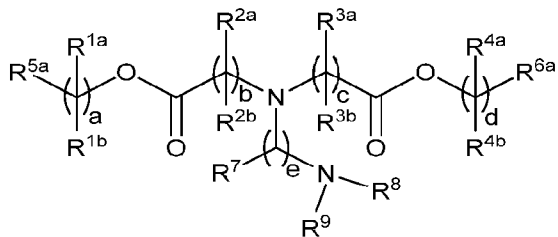
(Ia)

In other embodiments, the lipid compounds of Formula (I) have the following structure (Ib):



(Ib)

In yet other embodiments, the lipid compounds of Formula (I) have the following structure (Ic):



(Ic)

In some embodiments of the lipid compound of Formula (I), a, b, c and d are each independently an integer from 2 to 12 or an integer from 4 to 12. In other embodiments, a, b, c and d are each independently an integer from 8 to 12 or 5 to 9. In some embodiments, a is 0. In some embodiments, a is 1. In other embodiments, a is 2. In more embodiments, a is 3. In yet other embodiments, a is 4. In some embodiments, a is 5. In other embodiments, a is 6. In more embodiments, a is 7. In yet other embodiments, a is 8. In some embodiments, a is 9. In other embodiments, a is 10. In more embodiments, a is 11. In yet other embodiments, a is 12. In some embodiments, a is 13. In other embodiments, a is 14. In more embodiments, a is 15. In yet other embodiments, a is 16.

In some other embodiments of Formula (I), b is 1. In other embodiments, b is 2. In more embodiments, b is 3. In yet other embodiments, b is 4. In some embodiments, b is 5. In other embodiments, b is 6. In more embodiments, b is 7. In yet other embodiments, b is 8. In some embodiments, b is 9. In other embodiments, b is 10. In more embodiments, b is 11. In yet other embodiments, b is 12. In some embodiments, b is 13. In other embodiments, b is 14. In more embodiments, b is 15. In yet other embodiments, b is 16.

In some more embodiments of Formula (I), c is 1. In other embodiments, c

is 2. In more embodiments, c is 3. In yet other embodiments, c is 4. In some
embodiments, c is 5. In other embodiments, c is 6. In more embodiments, c is 7. In yet
other embodiments, c is 8. In some embodiments, c is 9. In other embodiments, c is 10. In
more embodiments, c is 11. In yet other embodiments, c is 12. In some embodiments, c is
5 13. In other embodiments, c is 14. In more embodiments, c is 15. In yet other
embodiments, c is 16.

In some other embodiments of Formula (I), d is 0. In some embodiments,
d is 1. In other embodiments, d is 2. In more embodiments, d is 3. In yet other
embodiments, d is 4. In some embodiments, d is 5. In other embodiments, d is 6. In more
10 embodiments, d is 7. In yet other embodiments, d is 8. In some embodiments, d is 9. In
other embodiments, d is 10. In more embodiments, d is 11. In yet other embodiments, d is
12. In some embodiments, d is 13. In other embodiments, d is 14. In more embodiments,
d is 15. In yet other embodiments, d is 16.

In some other various embodiments of Formula (I), a and d are the same.
15 In some other embodiments, b and c are the same. In some other specific embodiments, a
and d are the same and b and c are the same.

The sum of a and b and the sum of c and d in Formula (I) are factors
which may be varied to obtain a lipid of Formula (I) having the desired properties. In one
embodiment, a and b are chosen such that their sum is an integer ranging from 14 to 24.
20 In other embodiments, c and d are chosen such that their sum is an integer ranging from
14 to 24. In further embodiment, the sum of a and b and the sum of c and d are the same.
For example, in some embodiments the sum of a and b and the sum of c and d are both
the same integer which may range from 14 to 24. In still more embodiments, a, b, c and d
are selected such the sum of a and b and the sum of c and d is 12 or greater.

25 In some embodiments of Formula (I), e is 1. In other embodiments, e is 2.

The substituents at R^{1a} , R^{2a} , R^{3a} and R^{4a} of Formula (I) are not particularly
limited. In some embodiments R^{1a} , R^{2a} , R^{3a} and R^{4a} are H at each occurrence. In some
other embodiments at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is C_1 - C_{12} alkyl. In some other
embodiments at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is C_1 - C_8 alkyl. In some other
30 embodiments at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is C_1 - C_6 alkyl. In some of the foregoing

embodiments, the C₁-C₈ alkyl is methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-hexyl or n-octyl.

In some embodiments of Formula (I), R^{1a}, R^{1b}, R^{4a} and R^{4b} are C₁-C₁₂ alkyl at each occurrence.

5 In further embodiments of Formula (I), at least one of R^{1b}, R^{2b}, R^{3b} and R^{4b} is H or R^{1b}, R^{2b}, R^{3b} and R^{4b} are H at each occurrence.

In some embodiments of Formula (I), R^{1b} together with the carbon atom to which it is bound is taken together with an adjacent R^{1b} and the carbon atom to which it is bound to form a carbon-carbon double bond. In other embodiments of the foregoing R^{4b} together with the carbon atom to which it is bound is taken together with an adjacent R^{4b} and the carbon atom to which it is bound to form a carbon-carbon double bond.

The substituents at R⁵ and R⁶ of Formula (I) are not particularly limited in the foregoing embodiments. In some embodiments one or both of R⁵ or R⁶ is methyl. In some other embodiments one or both of R⁵ or R⁶ is cycloalkyl for example cyclohexyl. In these embodiments the cycloalkyl may be substituted or not substituted. In some other embodiments the cycloalkyl is substituted with C₁-C₁₂ alkyl, for example tert-butyl.

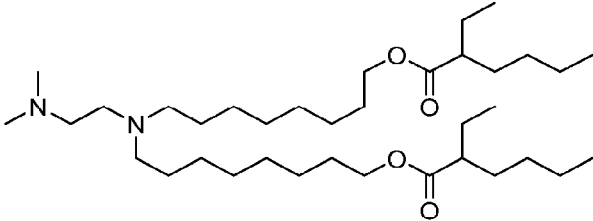
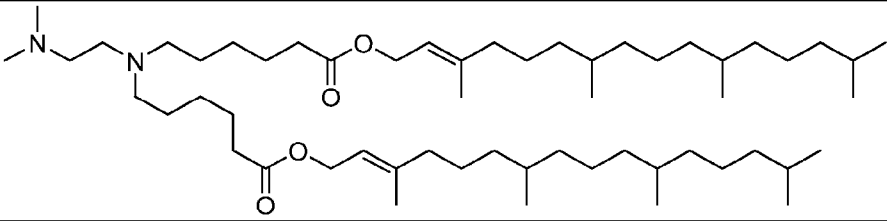
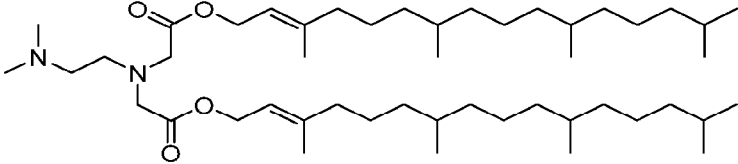
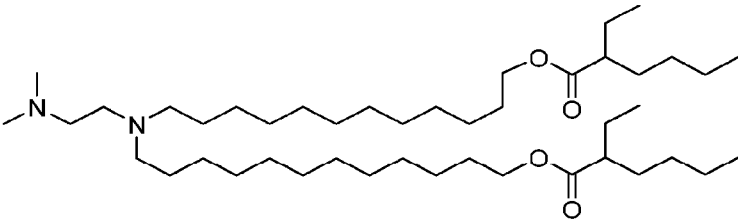
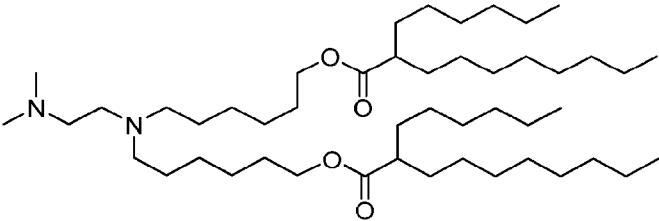
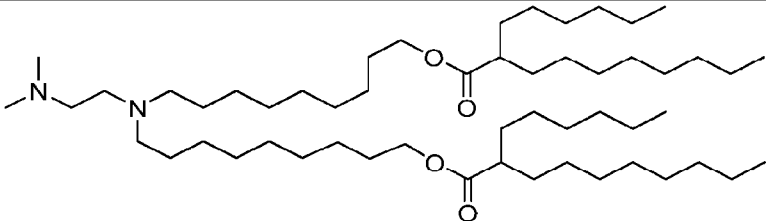
The substituents at R⁷ are not particularly limited in the foregoing embodiments of Formula (I). In some embodiments at least one R⁷ is H. In some other embodiments, R⁷ is H at each occurrence. In some other embodiments R⁷ is C₁-C₁₂ alkyl.

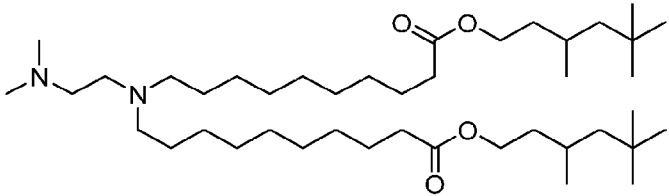
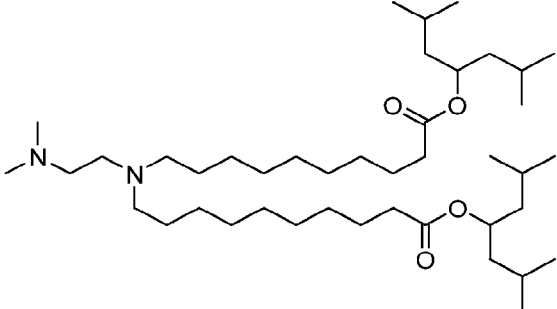
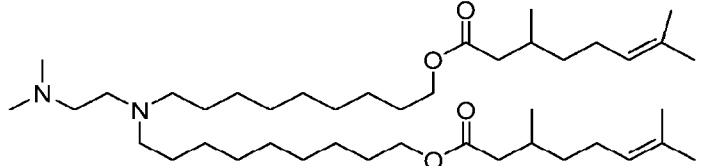
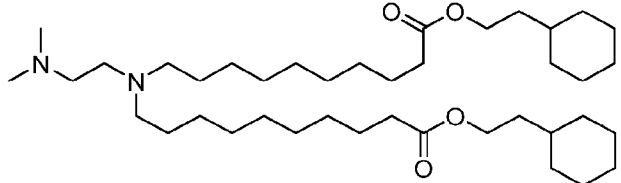
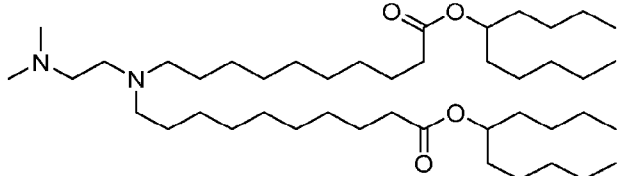
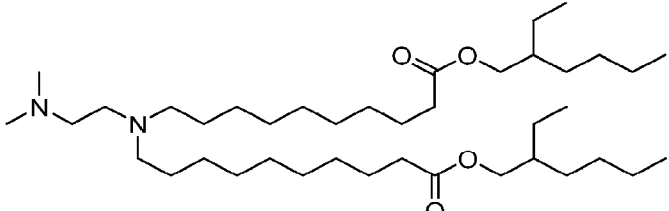
20 In some other of the foregoing embodiments of Formula (I), one of R⁸ or R⁹ is methyl. In other embodiments, both R⁸ and R⁹ are methyl.

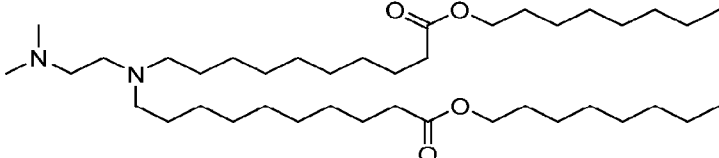
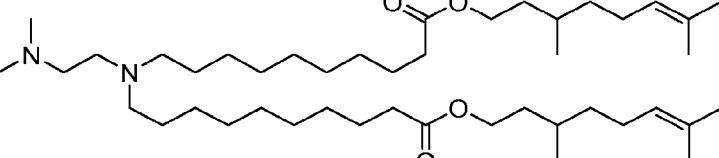
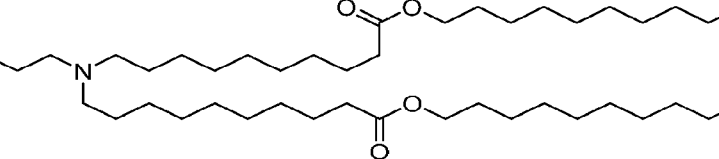
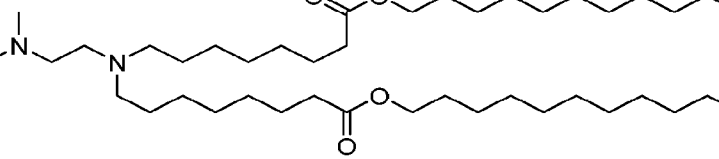
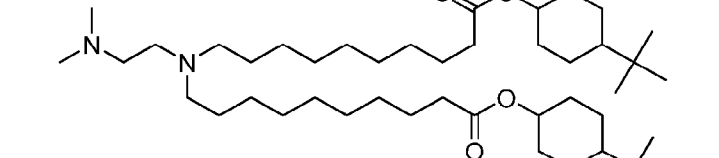
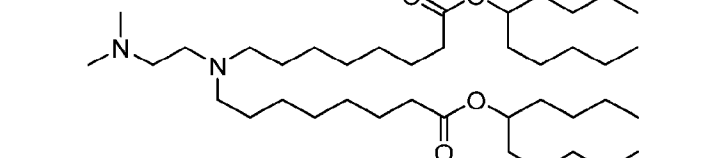
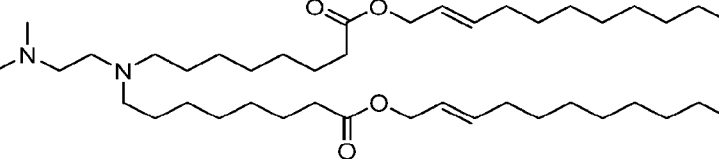
In some different embodiments of Formula (I), R⁸ and R⁹, together with the nitrogen atom to which they are attached, form a 5, 6 or 7-membered heterocyclic ring. In some embodiments of the foregoing, R⁸ and R⁹, together with the nitrogen atom to which they are attached, form a 5-membered heterocyclic ring, for example a pyrrolidinyl ring.

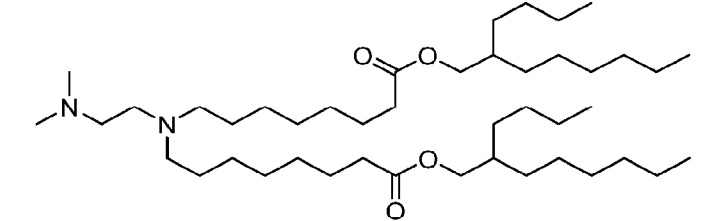
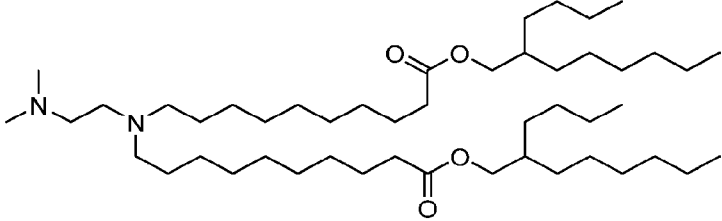
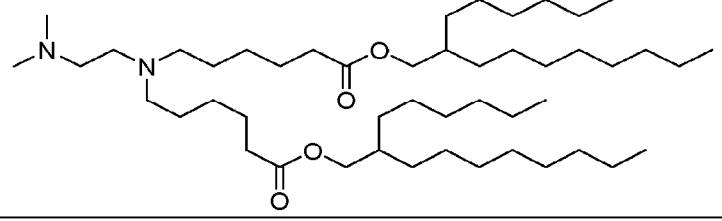
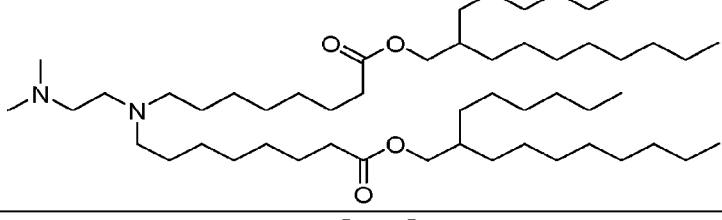
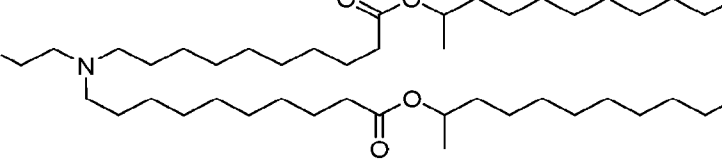
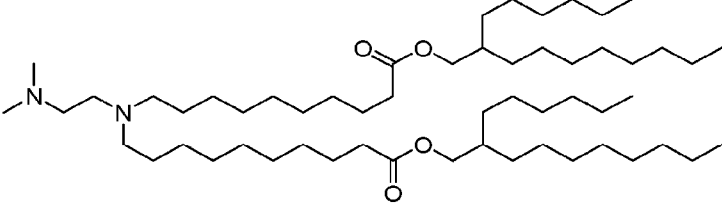
In various different embodiments, the lipid of Formula (I) has one of the structures set forth in Table 1 below.

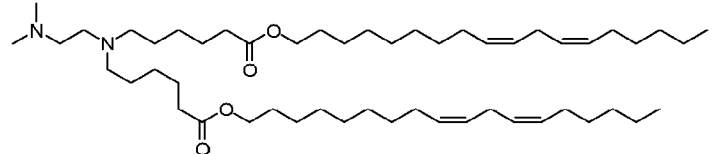
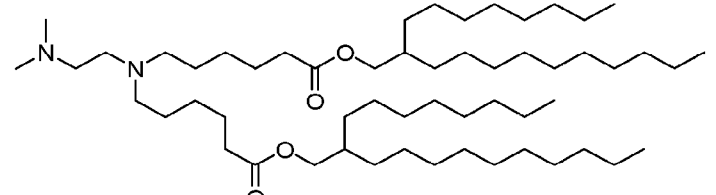
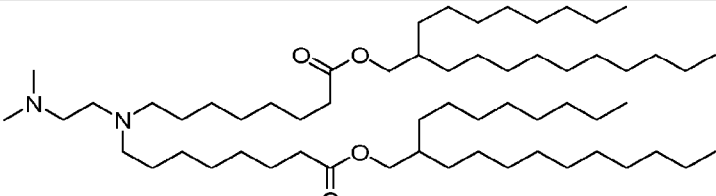
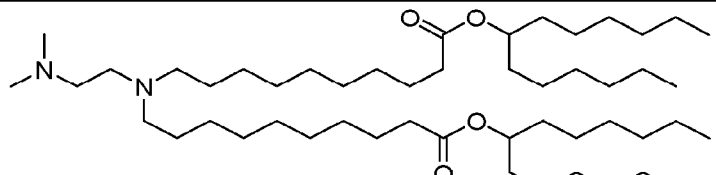
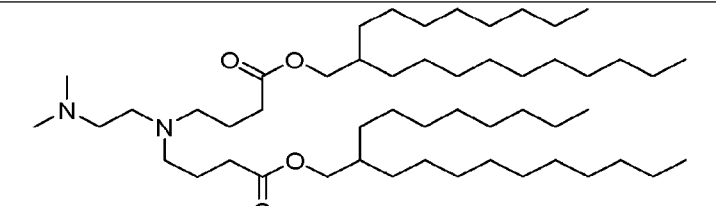
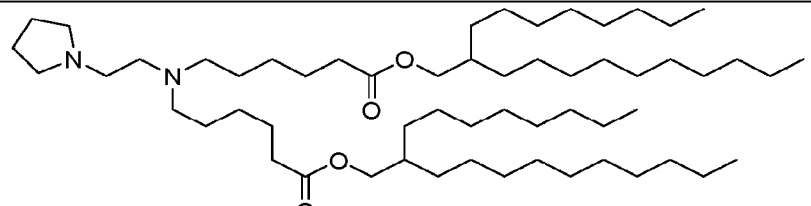
30 Table 1: Representative Lipids of Formula (I).

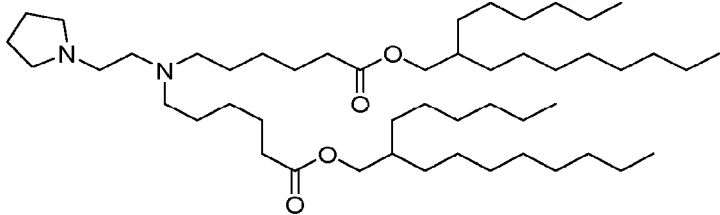
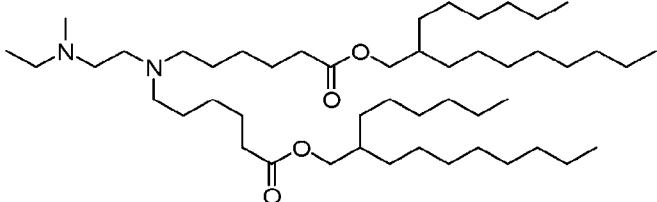
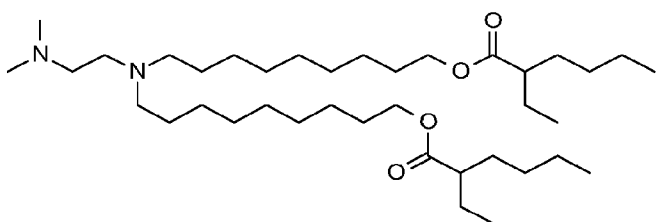
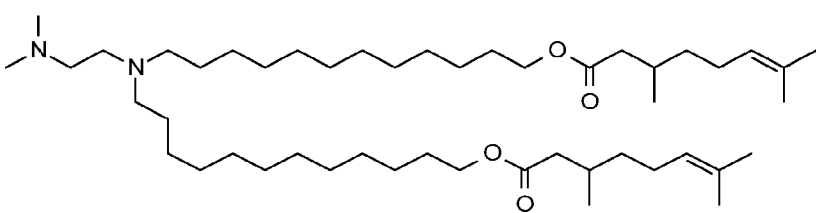
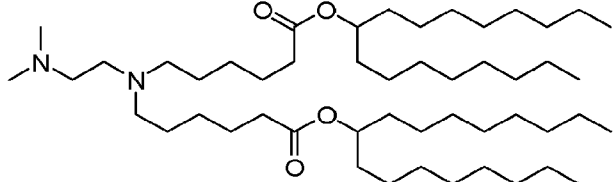
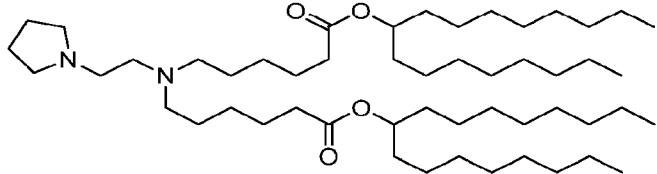
No.	Structure	Prep. Method
I-1		B
I-2		A
I-3		A
I-4		B
I-5		B
I-6		B

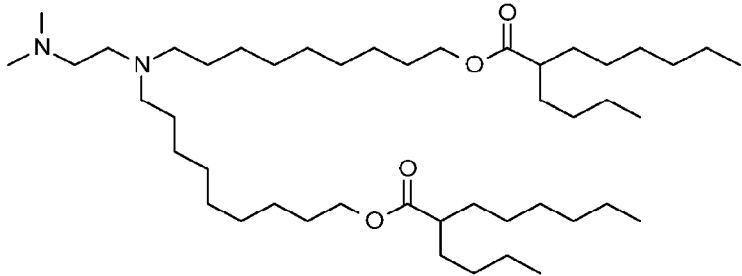
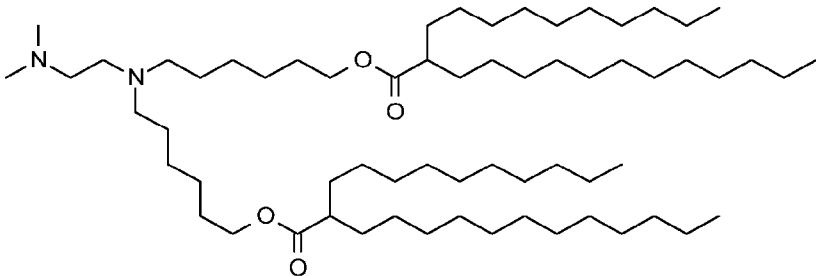
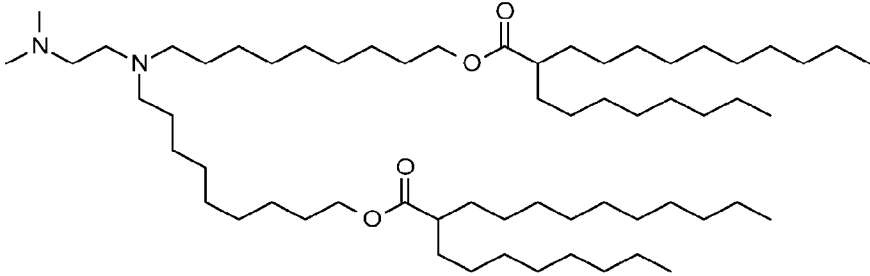
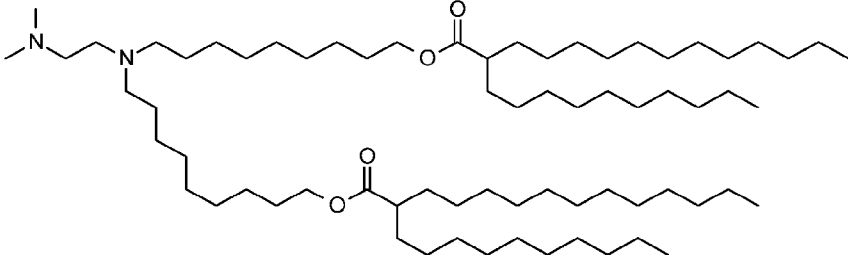
No.	Structure	Prep. Method
I-7		A
I-8		A
I-9		B
I-10		A
I-11		A
I-12		A

No.	Structure	Prep. Method
I-13		A
I-14		A
I-15		A
I-16		A
I-17		A
I-18		A
I-19		A

No.	Structure	Prep. Method
I-20		A
I-21		A
I-22		A
I-23		A
I-24		A
I-25		A

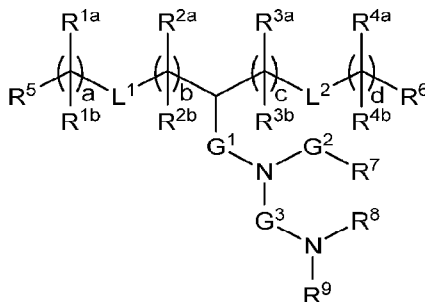
No.	Structure	Prep. Method
I-26		A
I-27		A
I-28		A
I-29		A
I-30		A
I-31		C

No.	Structure	Prep. Method
I-32		C
I-33		C
I-34		B
I-35		B
I-36		C
I-37		C

No.	Structure	Prep. Method
I-38		B
I-39		B
I-40		B
I-41		B

In some embodiments, the LNPs comprise a lipid of Formula (I), a nucleoside-modified RNA and one or more excipients selected from neutral lipids, steroids and pegylated lipids. In some embodiments the lipid of Formula (I) is compound I-5. In some embodiments the lipid of Formula (I) is compound I-6.

In some other embodiments, the cationic lipid component of the LNPs has the structure of Formula (II):



(II)

5 or a pharmaceutically acceptable salt, tautomer, prodrug or stereoisomer thereof, wherein:

L^1 and L^2 are each independently $-O(C=O)-$, $-(C=O)O-$, $-C(=O)-$, $-O-$, $-S(O)_x-$, $-S-S-$, $-C(=O)S-$, $-SC(=O)-$, $-NR^aC(=O)-$, $-C(=O)NR^a-$, $-NR^aC(=O)NR^a$,

10 $-OC(=O)NR^a-$, $-NR^aC(=O)O-$, or a direct bond;

G^1 is C_1-C_2 alkylene, $-(C=O)-$, $-O(C=O)-$, $-SC(=O)-$, $-NR^aC(=O)-$ or a direct bond;

G^2 is $-C(=O)-$, $-(C=O)O-$, $-C(=O)S-$, $-C(=O)NR^a$ or a direct bond;

G^3 is C_1-C_6 alkylene;

15 R^a is H or C_1-C_{12} alkyl;

R^{1a} and R^{1b} are, at each occurrence, independently either: (a) H or C_1-C_{12} alkyl; or (b) R^{1a} is H or C_1-C_{12} alkyl, and R^{1b} together with the carbon atom to which it is bound is taken together with an adjacent R^{1b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

20 R^{2a} and R^{2b} are, at each occurrence, independently either: (a) H or C_1-C_{12} alkyl; or (b) R^{2a} is H or C_1-C_{12} alkyl, and R^{2b} together with the carbon atom to which it is bound is taken together with an adjacent R^{2b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

25 R^{3a} and R^{3b} are, at each occurrence, independently either: (a) H or C_1-C_{12} alkyl; or (b) R^{3a} is H or C_1-C_{12} alkyl, and R^{3b} together with the carbon atom to which it is

bound is taken together with an adjacent R^{3b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

R^{4a} and R^{4b} are, at each occurrence, independently either: (a) H or C₁-C₁₂ alkyl; or (b) R^{4a} is H or C₁-C₁₂ alkyl, and R^{4b} together with the carbon atom to which it is bound is taken together with an adjacent R^{4b} and the carbon atom to which it is bound to form a carbon-carbon double bond;

R⁵ and R⁶ are each independently H or methyl;

R⁷ is C₄-C₂₀ alkyl;

R⁸ and R⁹ are each independently C₁-C₁₂ alkyl; or R⁸ and R⁹, together with the nitrogen atom to which they are attached, form a 5, 6 or 7-membered heterocyclic ring;

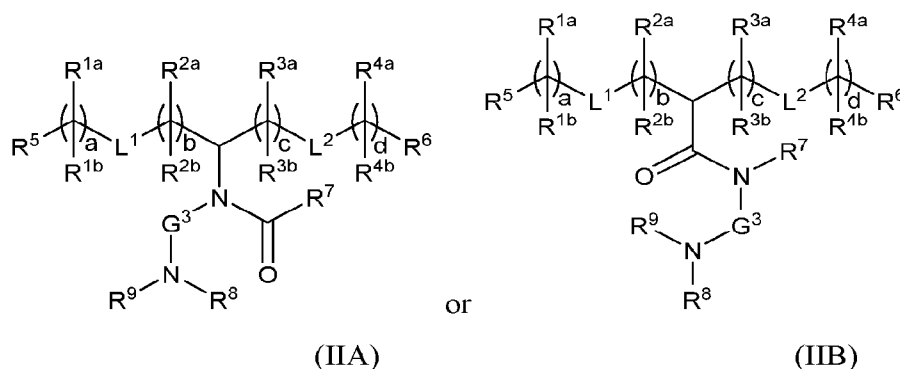
a, b, c and d are each independently an integer from 1 to 24; and
x is 0, 1 or 2.

In some embodiments of Formula (II), L¹ and L² are each independently -O(C=O)-, -(C=O)O- or a direct bond. In other embodiments, G¹ and G² are each independently -(C=O)- or a direct bond. In some different embodiments, L¹ and L² are each independently -O(C=O)-, -(C=O)O- or a direct bond; and G¹ and G² are each independently -(C=O)- or a direct bond.

In some different embodiments of Formula (II), L¹ and L² are each independently -C(=O)-, -O-, -S(O)_x-, -S-S-, -C(=O)S-, -SC(=O)-, -NR^a-, -NR^aC(=O)-, -C(=O)NR^a-, -NR^aC(=O)NR^a-, -OC(=O)NR^a-, -NR^aC(=O)O-, -NR^aS(O)_xNR^a-,

-NR^aS(O)_x- or -S(O)_xNR^a-.

In other of the foregoing embodiments of Formula (II), the lipid compound has one of the following structures (IIA) or (IIB):



In some embodiments of Formula (II), the lipid compound has structure (IIA). In other embodiments, the lipid compound has structure (IIB).

5 In any of the foregoing embodiments of Formula (II), one of L¹ or L² is -O(C=O)-. For example, in some embodiments each of L¹ and L² are -O(C=O)-.

In some different embodiments of Formula (II), one of L¹ or L² is -(C=O)O-. For example, in some embodiments each of L¹ and L² is -(C=O)O-.

In different embodiments of Formula (II), one of L¹ or L² is a direct bond.

10 As used herein, a “direct bond” means the group (e.g., L¹ or L²) is absent. For example, in some embodiments each of L¹ and L² is a direct bond.

In other different embodiments of Formula (II), for at least one occurrence of R^{1a} and R^{1b}, R^{1a} is H or C₁-C₁₂ alkyl, and R^{1b} together with the carbon atom to which it is bound is taken together with an adjacent R^{1b} and the carbon atom to which it is bound to form a carbon-carbon double bond.

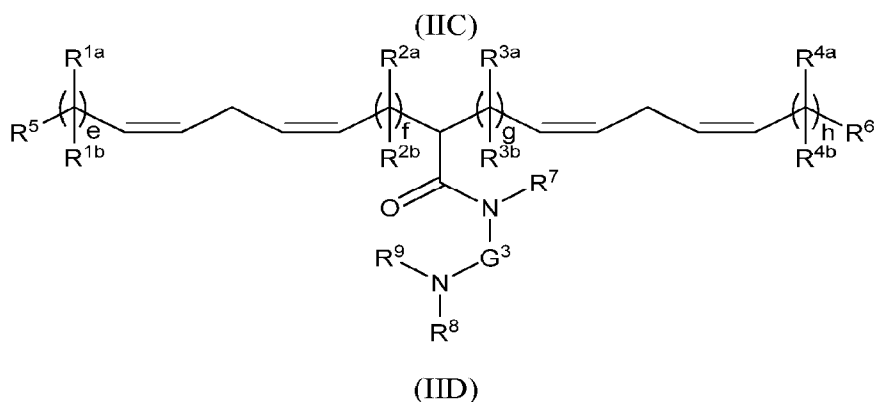
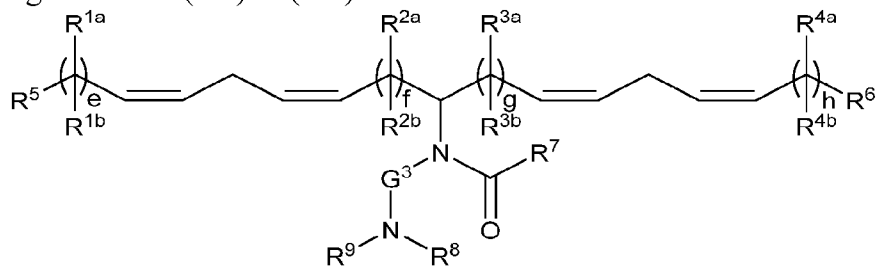
In still other different embodiments of Formula (II), for at least one occurrence of R^{4a} and R^{4b} , R^{4a} is H or C₁-C₁₂ alkyl, and R^{4b} together with the carbon atom to which it is bound is taken together with an adjacent R^{4b} and the carbon atom to which it is bound to form a carbon-carbon double bond.

20 In more embodiments of Formula (II), for at least one occurrence of R^{2a} and R^{2b} , R^{2a} is H or C₁-C₁₂ alkyl, and R^{2b} together with the carbon atom to which it is bound is taken together with an adjacent R^{2b} and the carbon atom to which it is bound to form a carbon-carbon double bond.

In other different embodiments of Formula (II), for at least one occurrence
25 of R^{3a} and R^{3b}, R^{3a} is H or C₁-C₁₂ alkyl, and R^{3b} together with the carbon atom to which it

is bound is taken together with an adjacent R^{3b} and the carbon atom to which it is bound to form a carbon-carbon double bond.

In various other embodiments of Formula (II), the lipid compound has one of the following structures (IIC) or (IID):



wherein e, f, g and h are each independently an integer from 1 to 12.

10 In some embodiments of Formula (II), the lipid compound has structure (IIC). In other embodiments, the lipid compound has structure (IID).

In various embodiments of structures (IIC) or (IID), e, f, g and h are each independently an integer from 4 to 10.

15 In some embodiments of Formula (II), a, b, c and d are each independently an integer from 2 to 12 or an integer from 4 to 12. In other embodiments, a, b, c and d are each independently an integer from 8 to 12 or 5 to 9. In some embodiments, a is 0. In some embodiments, a is 1. In other embodiments, a is 2. In more embodiments, a is 3. In yet other embodiments, a is 4. In some embodiments, a is 5. In other embodiments, a is 6. In more embodiments, a is 7. In yet other embodiments, a is 8. In some embodiments, a is 9. In other embodiments, a is 10. In more embodiments, a is 11. In yet other
20 embodiments, a is 12. In some embodiments, a is 13. In other embodiments, a is 14. In

more embodiments, a is 15. In yet other embodiments, a is 16.

In some embodiments of Formula (II), b is 1. In other embodiments, b is 2. In more embodiments, b is 3. In yet other embodiments, b is 4. In some embodiments, b is 5. In other embodiments, b is 6. In more embodiments, b is 7. In yet other
5 embodiments, b is 8. In some embodiments, b is 9. In other embodiments, b is 10. In more embodiments, b is 11. In yet other embodiments, b is 12. In some embodiments, b is 13. In other embodiments, b is 14. In more embodiments, b is 15. In yet other embodiments, b is 16.

In some embodiments of Formula (II), c is 1. In other embodiments, c is 2.
10 In more embodiments, c is 3. In yet other embodiments, c is 4. In some embodiments, c is 5. In other embodiments, c is 6. In more embodiments, c is 7. In yet other embodiments, c is 8. In some embodiments, c is 9. In other embodiments, c is 10. In more embodiments, c is 11. In yet other embodiments, c is 12. In some embodiments, c is 13. In other embodiments, c is 14. In more embodiments, c is 15. In yet other embodiments, c is 16.

15 In some embodiments of Formula (II), d is 0. In some embodiments, d is 1. In other embodiments, d is 2. In more embodiments, d is 3. In yet other embodiments, d is 4. In some embodiments, d is 5. In other embodiments, d is 6. In more embodiments, d is 7. In yet other embodiments, d is 8. In some embodiments, d is 9. In other embodiments, d is 10. In more embodiments, d is 11. In yet other embodiments, d is 12.
20 In some embodiments, d is 13. In other embodiments, d is 14. In more embodiments, d is 15. In yet other embodiments, d is 16.

In some embodiments of Formula (II), e is 1. In other embodiments, e is 2. In more embodiments, e is 3. In yet other embodiments, e is 4. In some embodiments, e is 5. In other embodiments, e is 6. In more embodiments, e is 7. In yet other embodiments, e
25 is 8. In some embodiments, e is 9. In other embodiments, e is 10. In more embodiments, e is 11. In yet other embodiments, e is 12.

In some embodiments of Formula (II), f is 1. In other embodiments, f is 2. In more embodiments, f is 3. In yet other embodiments, f is 4. In some embodiments, f is 5. In other embodiments, f is 6. In more embodiments, f is 7. In yet other embodiments, f
30 is 8. In some embodiments, f is 9. In other embodiments, f is 10. In more embodiments, f

is 11. In yet other embodiments, f is 12.

In some embodiments of Formula (II), g is 1. In other embodiments, g is 2. In more embodiments, g is 3. In yet other embodiments, g is 4. In some embodiments, g is 5. In other embodiments, g is 6. In more embodiments, g is 7. In yet other
5 embodiments, g is 8. In some embodiments, g is 9. In other embodiments, g is 10. In more embodiments, g is 11. In yet other embodiments, g is 12.

In some embodiments of Formula (II), h is 1. In other embodiments, e is 2. In more embodiments, h is 3. In yet other embodiments, h is 4. In some embodiments, e is 5. In other embodiments, h is 6. In more embodiments, h is 7. In yet other
10 embodiments, h is 8. In some embodiments, h is 9. In other embodiments, h is 10. In more embodiments, h is 11. In yet other embodiments, h is 12.

In some other various embodiments of Formula (II), a and d are the same. In some other embodiments, b and c are the same. In some other specific embodiments and a and d are the same and b and c are the same.

15 The sum of a and b and the sum of c and d of Formula (II) are factors which may be varied to obtain a lipid having the desired properties. In one embodiment, a and b are chosen such that their sum is an integer ranging from 14 to 24. In other embodiments, c and d are chosen such that their sum is an integer ranging from 14 to 24. In further embodiment, the sum of a and b and the sum of c and d are the same. For
20 example, in some embodiments the sum of a and b and the sum of c and d are both the same integer which may range from 14 to 24. In still more embodiments, a, b, c and d are selected such that the sum of a and b and the sum of c and d is 12 or greater.

The substituents at R^{1a} , R^{2a} , R^{3a} and R^{4a} of Formula (II) are not particularly limited. In some embodiments, at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is H. In
25 some embodiments R^{1a} , R^{2a} , R^{3a} and R^{4a} are H at each occurrence. In some other embodiments at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is C₁-C₁₂ alkyl. In some other embodiments at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is C₁-C₈ alkyl. In some other embodiments at least one of R^{1a} , R^{2a} , R^{3a} and R^{4a} is C₁-C₆ alkyl. In some of the foregoing
30 embodiments, the C₁-C₈ alkyl is methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-hexyl or n-octyl.

In some embodiments of Formula (II), R^{1a} , R^{1b} , R^{4a} and R^{4b} are C_1 - C_{12} alkyl at each occurrence.

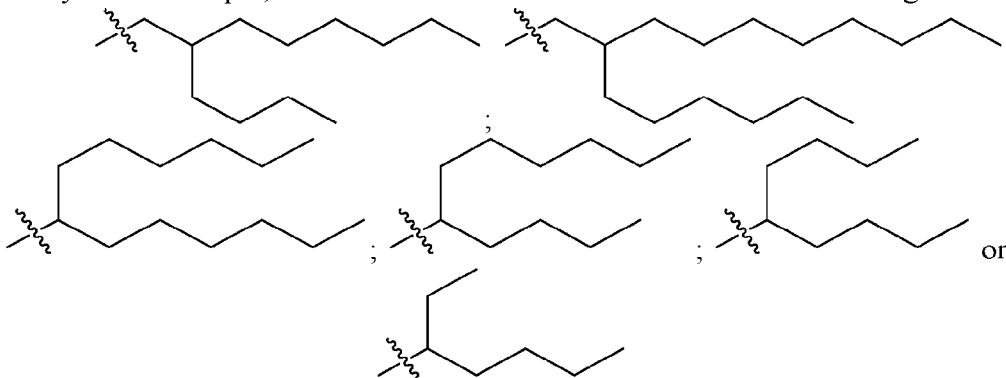
In further embodiments of Formula (II), at least one of R^{1b} , R^{2b} , R^{3b} and R^{4b} is H or R^{1b} , R^{2b} , R^{3b} and R^{4b} are H at each occurrence.

5 In some embodiments of Formula (II), R^{1b} together with the carbon atom to which it is bound is taken together with an adjacent R^{1b} and the carbon atom to which it is bound to form a carbon-carbon double bond. In other embodiments of the foregoing R^{4b} together with the carbon atom to which it is bound is taken together with an adjacent R^{4b} and the carbon atom to which it is bound to form a carbon-carbon double bond.

10 The substituents at R^5 and R^6 of Formula (II) are not particularly limited in the foregoing embodiments. In some embodiments one of R^5 or R^6 is methyl. In other embodiments each of R^5 or R^6 is methyl.

The substituents at R^7 of Formula (II) are not particularly limited in the foregoing embodiments. In some embodiments R^7 is C_6 - C_{16} alkyl. In some other
 15 embodiments, R^7 is C_6 - C_9 alkyl. In some of these embodiments, R^7 is substituted with $-(C=O)OR^b$, $-O(C=O)R^b$, $-C(=O)R^b$, $-OR^b$, $-S(O)_xR^b$, $-S-SR^b$, $-C(=O)SR^b$, $-SC(=O)R^b$, $-NR^aR^b$, $-NR^aC(=O)R^b$, $-C(=O)NR^aR^b$, $-NR^aC(=O)NR^aR^b$, $-OC(=O)NR^aR^b$, $-NR^aC(=O)OR^b$, $-NR^aS(O)_xNR^aR^b$, $-NR^aS(O)_xR^b$ or $-S(O)_xNR^aR^b$, wherein: R^a is H or C_1 - C_{12} alkyl; R^b is C_1 - C_{15} alkyl; and x is 0, 1 or 2.
 20 For example, in some embodiments R^7 is substituted with $-(C=O)OR^b$ or $-O(C=O)R^b$.

In various of the foregoing embodiments of Formula (II), R^b is branched C_1 - C_{15} alkyl. For example, in some embodiments R^b has one of the following structures:



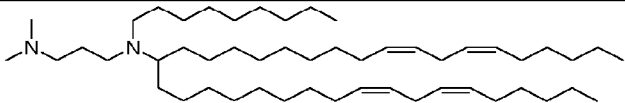
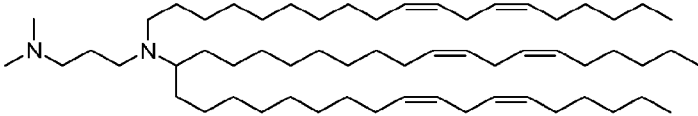
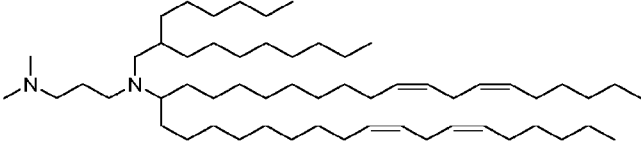
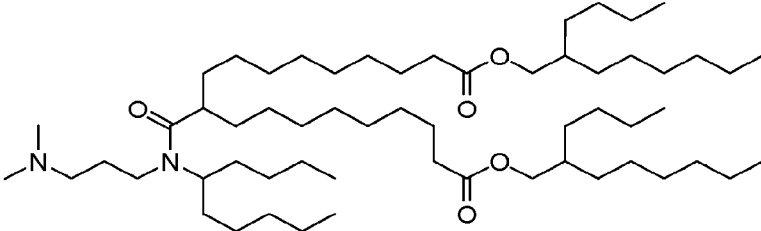
In some other of the foregoing embodiments of Formula (II), one of R⁸ or R⁹ is methyl. In other embodiments, both R⁸ and R⁹ are methyl.

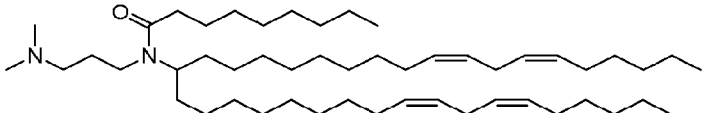
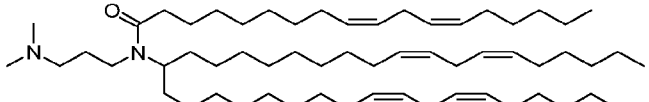
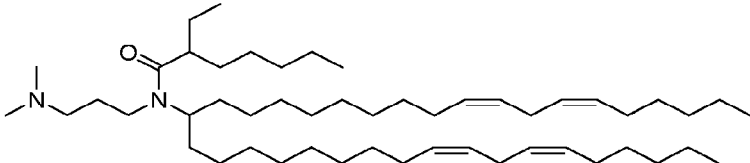
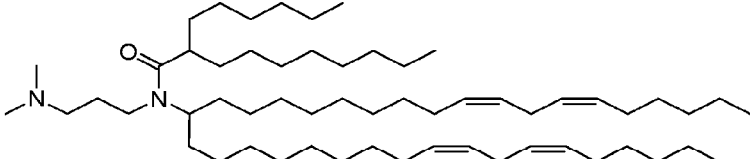
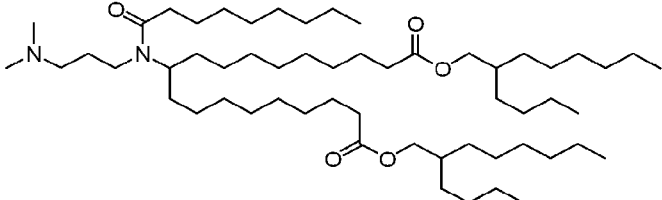
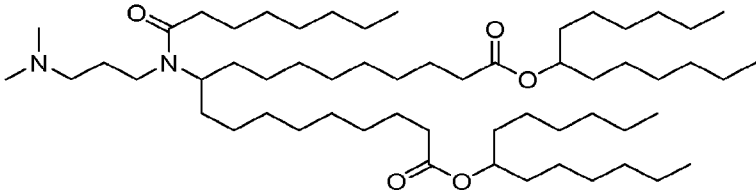
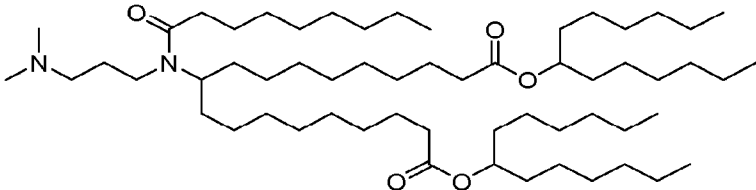
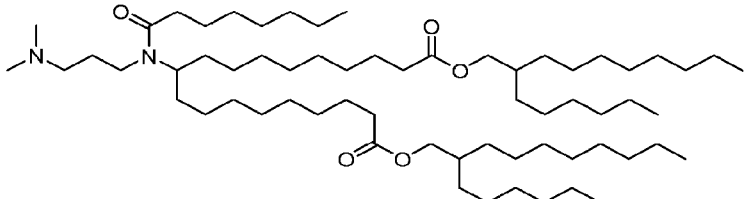
In some different embodiments of Formula (II), R⁸ and R⁹, together with the nitrogen atom to which they are attached, form a 5, 6 or 7-membered heterocyclic ring. In some embodiments of the foregoing, R⁸ and R⁹, together with the nitrogen atom to which they are attached, form a 5-membered heterocyclic ring, for example a pyrrolidinyl ring. In some different embodiments of the foregoing, R⁸ and R⁹, together with the nitrogen atom to which they are attached, form a 6-membered heterocyclic ring, for example a piperazinyl ring.

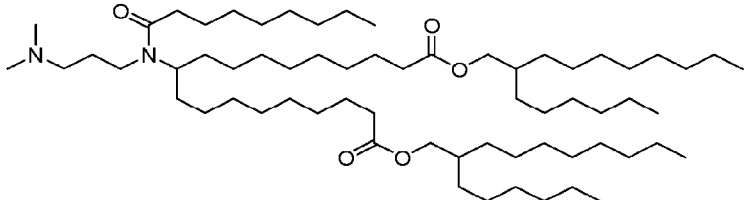
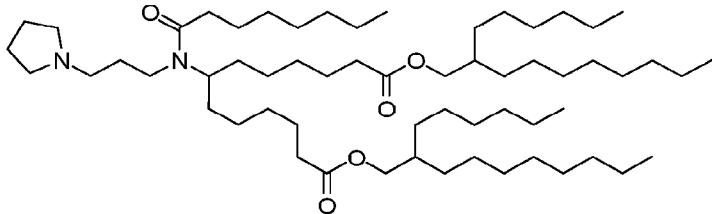
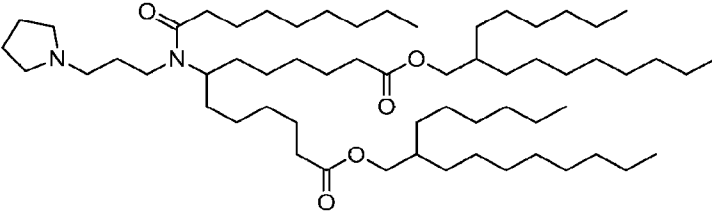
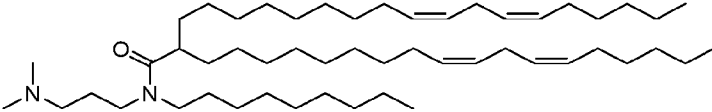
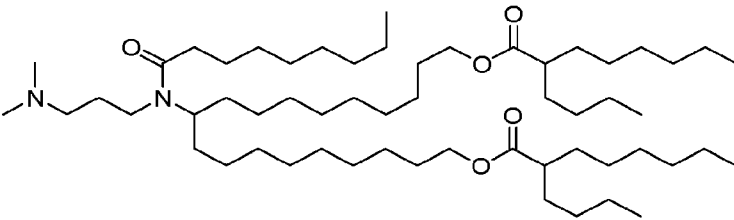
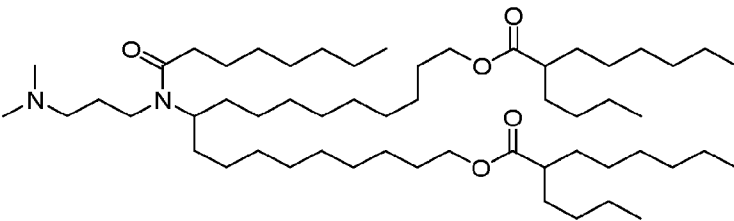
In still other embodiments of the foregoing lipids of Formula (II), G³ is C₂-C₄ alkylene, for example C₃ alkylene.

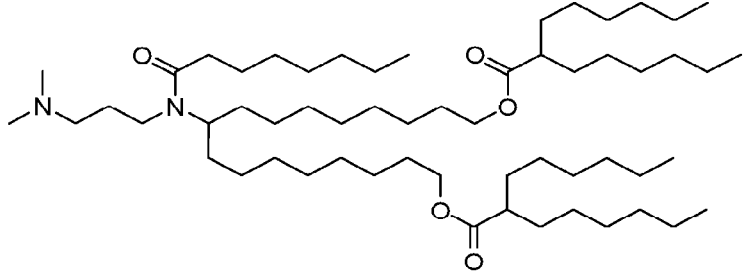
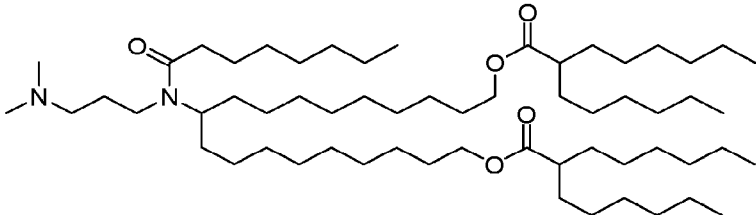
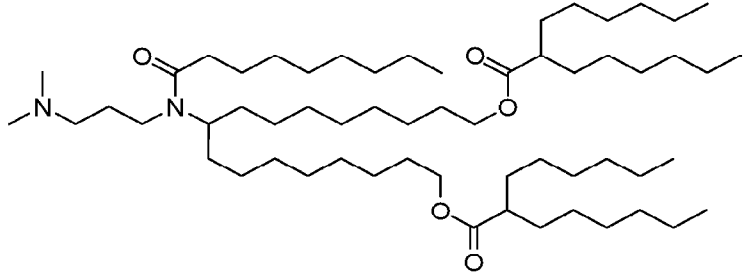
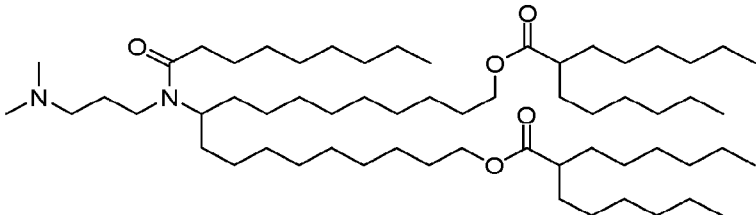
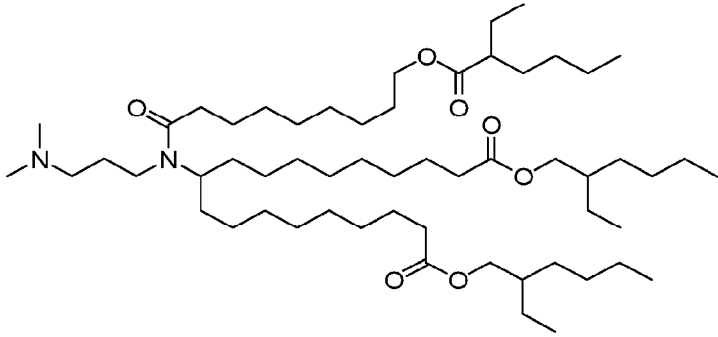
In various different embodiments, the lipid compound has one of the structures set forth in Table 2 below.

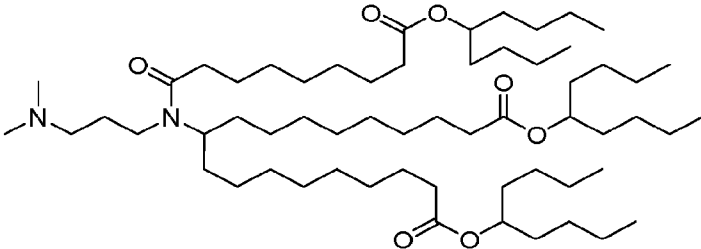
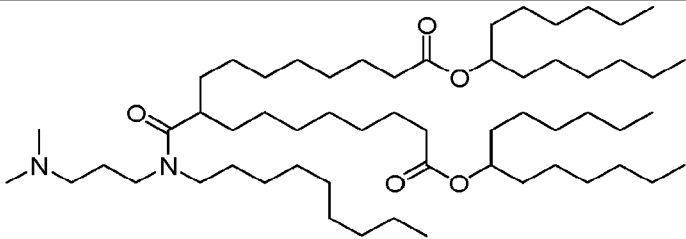
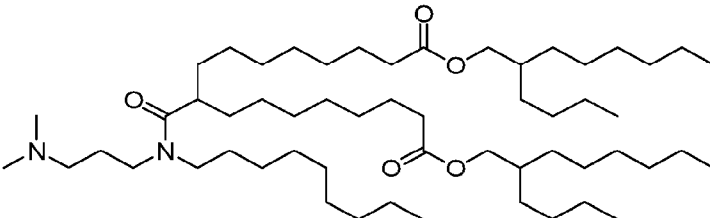
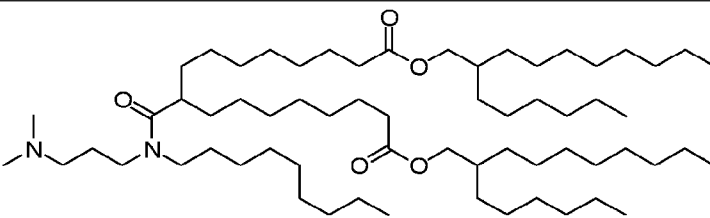
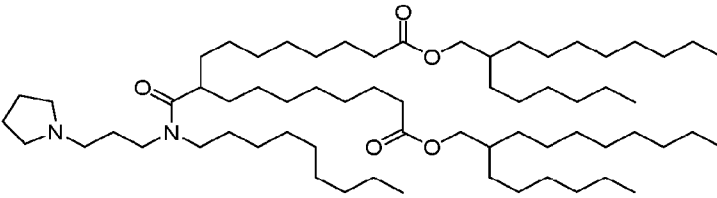
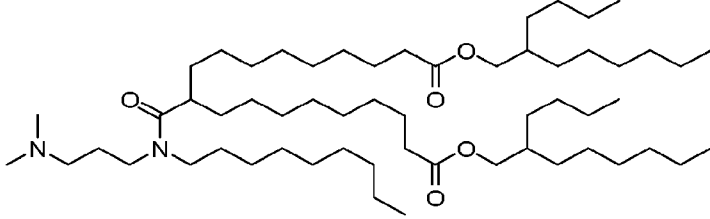
Table 2: Representative Lipids of Formula (II).

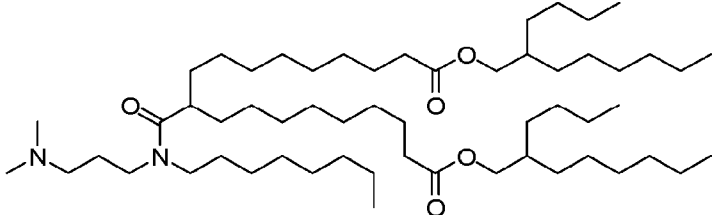
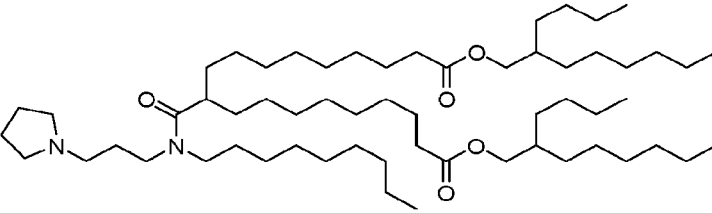
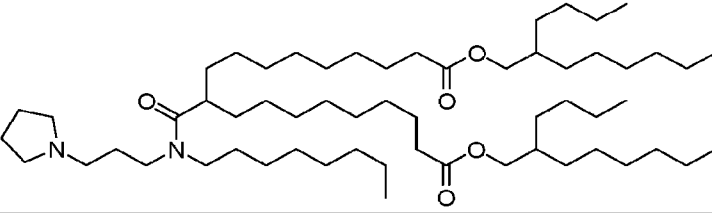
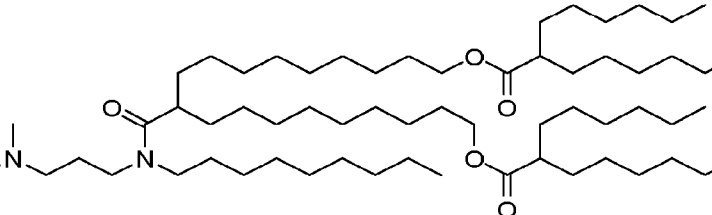
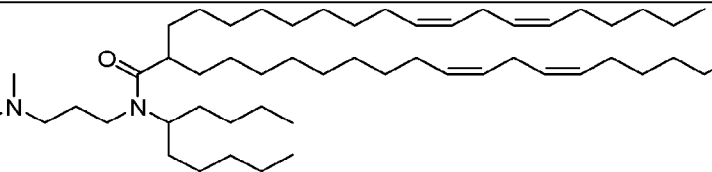
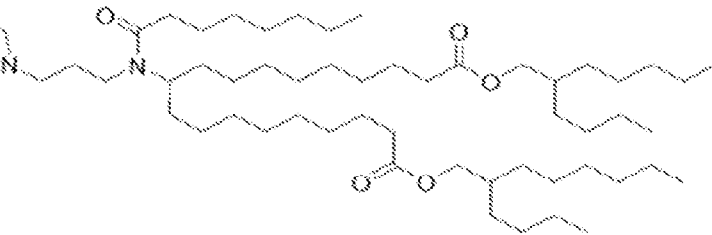
No.	Structure	Prep. Method
II-1		D
II-2		D
II-3		D
II-4		E

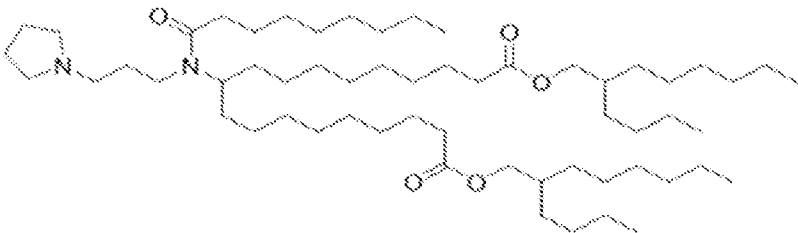
No.	Structure	Prep. Method
II-5		D
II-6		D
II-7		D
II-8		D
II-9		D
II-10		D
II-11		D
II-12		D

No.	Structure	Prep. Method
II-13		D
II-14		D
II-15		D
II-16		E
II-17		D
II-18		D

No.	Structure	Prep. Method
II-19		D
II-20		D
II-21		D
II-22		D
II-23		D

No.	Structure	Prep. Method
II-24		D
II-25		E
II-26		E
II-27		E
II-28		E
II-29		E

No.	Structure	Prep. Method
II-30		E
II-31		E
II-32		E
II-33		E
II-34		E
II-35		D

No.	Structure	Prep. Method
II-36		D

In some embodiments, the LNPs comprise a lipid of Formula (II), a nucleoside-modified RNA and one or more excipient selected from neutral lipids, steroids and pegylated lipids. In some embodiments the lipid of Formula (II) is compound II-9. In some embodiments the lipid of Formula (II) is compound II-10. In some embodiments the lipid of Formula (II) is compound II-11. In some embodiments the lipid of Formula (II) is compound II-12. In some embodiments the lipid of Formula (II) is compound II-32.

In some other embodiments, the cationic lipid component of the LNPs has the structure of Formula (III):

(III)

or a pharmaceutically acceptable salt, tautomer, prodrug or stereoisomer thereof, wherein:

one of L^1 or L^2 is $-O(C=O)-$, $-(C=O)O-$, $-C(=O)-$, $-O-$, $-S(O)_x-$, $-S-S-$, $-C(=O)S-$, $SC(=O)-$, $-NR^aC(=O)-$, $-C(=O)NR^a-$, $NR^aC(=O)NR^a-$, $-OC(=O)NR^a-$ or $-NR^aC(=O)O-$, and the other of L^1 or L^2 is $-O(C=O)-$, $-(C=O)O-$, $-C(=O)-$, $-O-$, $-S(O)_x-$, $-S-S-$, $-C(=O)S-$, $SC(=O)-$, $-NR^aC(=O)-$, $-C(=O)NR^a-$, $NR^aC(=O)NR^a-$, $-OC(=O)NR^a-$ or $-NR^aC(=O)O-$ or a direct bond;
 G^1 and G^2 are each independently unsubstituted C_1 - C_{12} alkylene or C_1 - C_{12}

alkenylene;

G^3 is C₁-C₂₄ alkylene, C₁-C₂₄ alkenylene, C₃-C₈ cycloalkylene, C₃-C₈ cycloalkenylene;

R^a is H or C₁-C₁₂ alkyl;

5 R^1 and R^2 are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl;

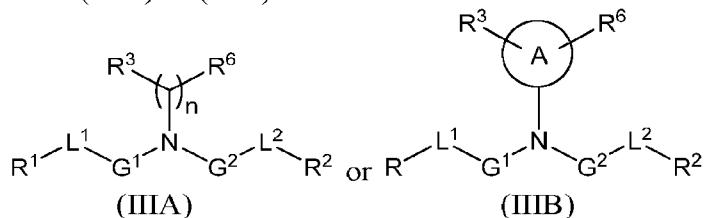
R^3 is H, OR⁵, CN, -C(=O)OR⁴, -OC(=O)R⁴ or -NR⁵C(=O)R⁴;

R^4 is C₁-C₁₂ alkyl;

R^5 is H or C₁-C₆ alkyl; and

x is 0, 1 or 2.

10 In some of the foregoing embodiments of Formula (III), the lipid has one of the following structures (IIIA) or (IIIB):



wherein:

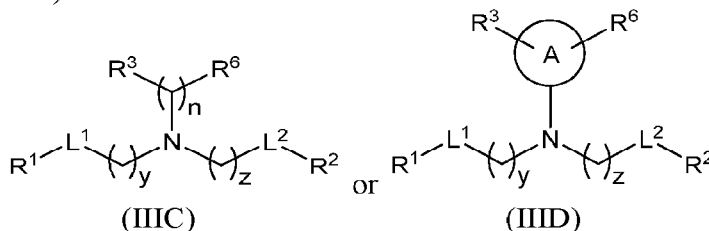
15 A is a 3 to 8-membered cycloalkyl or cycloalkylene ring;

R^6 is, at each occurrence, independently H, OH or C₁-C₂₄ alkyl;

n is an integer ranging from 1 to 15.

In some of the foregoing embodiments of Formula (III), the lipid has structure (IIIA), and in other embodiments, the lipid has structure (IIIB).

20 In other embodiments of Formula (III), the lipid has one of the following structures (IIIC) or (IIID):

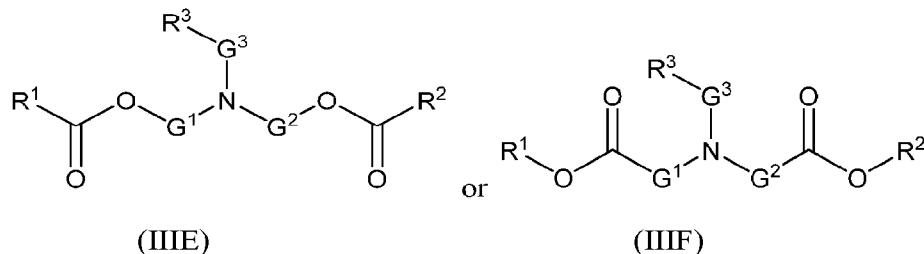


wherein y and z are each independently integers ranging from 1 to 12.

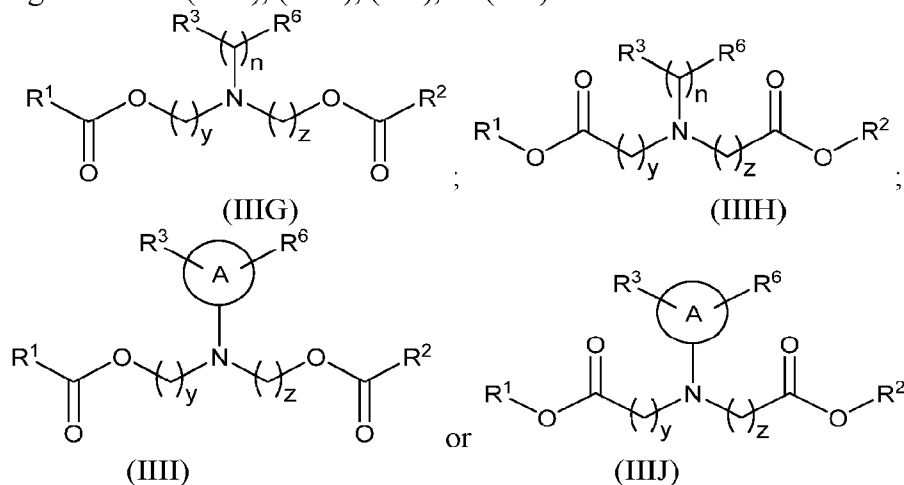
25 In any of the foregoing embodiments of Formula (III), one of L^1 or L^2

is $-\text{O}(\text{C}=\text{O})-$. For example, in some embodiments each of L^1 and L^2 are $-\text{O}(\text{C}=\text{O})-$. In some different embodiments of any of the foregoing, L^1 and L^2 are each independently $-(\text{C}=\text{O})\text{O}-$ or $-\text{O}(\text{C}=\text{O})-$. For example, in some embodiments each of L^1 and L^2 is $-(\text{C}=\text{O})\text{O}-$.

- 5 In some different embodiments of Formula (III), the lipid has one of the following structures (IIIE) or (IIIF):



- 10 In some of the foregoing embodiments of Formula (III), the lipid has one of the following structures (IIIG), (IIIH), (IIII), or (IIIJ):



- 15 In some of the foregoing embodiments of Formula (III), n is an integer ranging from 2 to 12, for example from 2 to 8 or from 2 to 4. For example, in some embodiments, n is 3, 4, 5 or 6. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6.

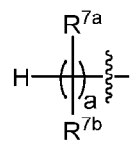
- 20 In some other of the foregoing embodiments of Formula (III), y and z are each independently an integer ranging from 2 to 10. For example, in some embodiments, y and z are each independently an integer ranging from 4 to 9 or from 4 to 6.

In some of the foregoing embodiments of Formula (III), R^6 is H. In other

of the foregoing embodiments, R^6 is C_1 - C_{24} alkyl. In other embodiments, R^6 is OH.

In some embodiments of Formula (III), G^3 is unsubstituted. In other embodiments, G^3 is substituted. In various different embodiments, G^3 is linear C_1 - C_{24} alkylene or linear C_1 - C_{24} alkenylene.

5 In some other foregoing embodiments of Formula (III), R^1 or R^2 , or both, is C_6 - C_{24} alkenyl. For example, in some embodiments, R^1 and R^2 each, independently have the following structure:



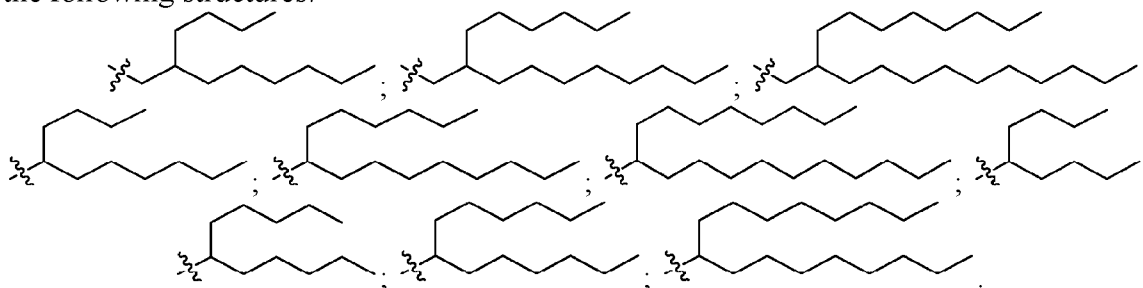
wherein:

10 R^{7a} and R^{7b} are, at each occurrence, independently H or C_1 - C_{12} alkyl; and a is an integer from 2 to 12,

wherein R^{7a} , R^{7b} and a are each selected such that R^1 and R^2 each independently comprise from 6 to 20 carbon atoms. For example, in some embodiments a is an integer ranging from 5 to 9 or from 8 to 12.

15 In some of the foregoing embodiments of Formula (III), at least one occurrence of R^{7a} is H. For example, in some embodiments, R^{7a} is H at each occurrence. In other different embodiments of the foregoing, at least one occurrence of R^{7b} is C_1 - C_8 alkyl. For example, in some embodiments, C_1 - C_8 alkyl is methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-hexyl or n-octyl.

20 In different embodiments of Formula (III), R^1 or R^2 , or both, has one of the following structures:

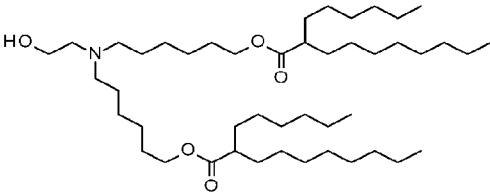
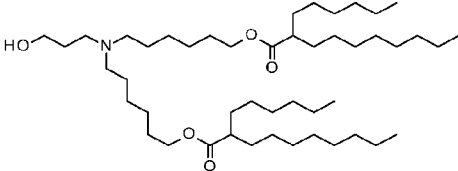
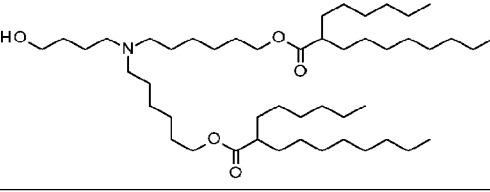
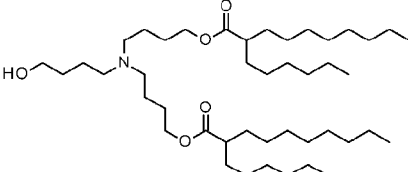
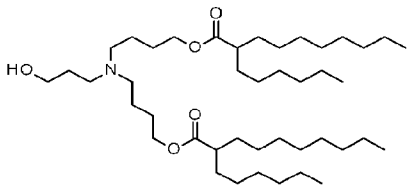
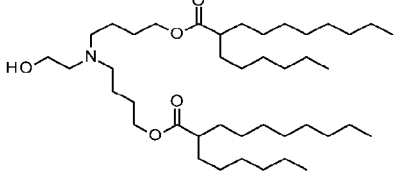


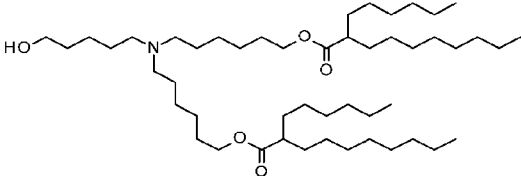
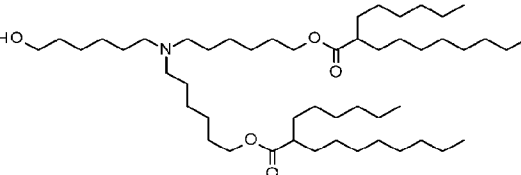
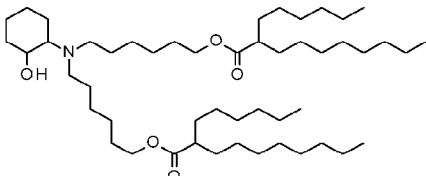
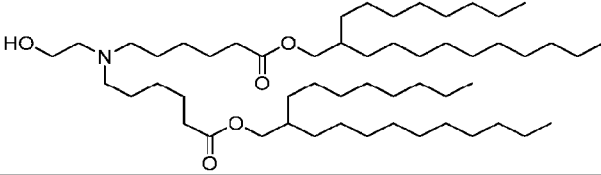
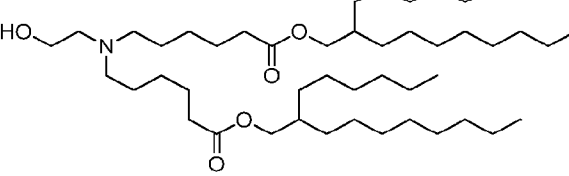
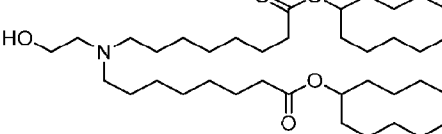
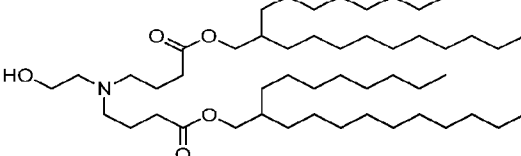
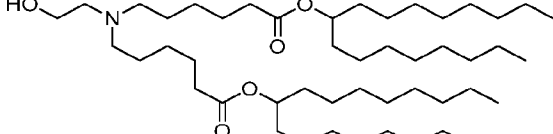
25 In some of the foregoing embodiments of Formula (III), R^3 is OH, CN, $-C(=O)OR^4$, $-OC(=O)R^4$ or $-NHC(=O)R^4$. In some embodiments, R^4 is methyl or

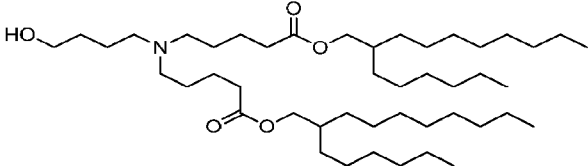
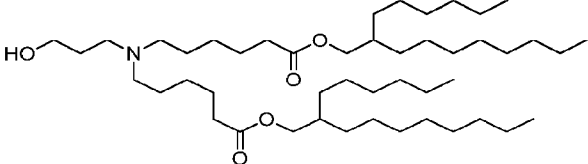
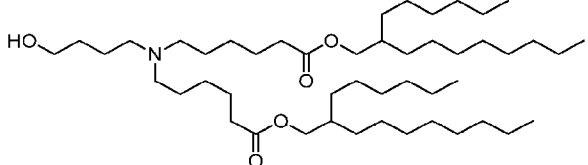
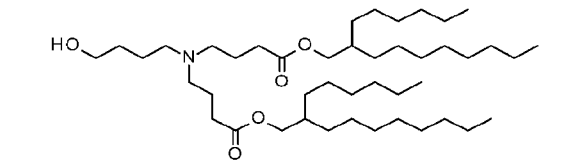
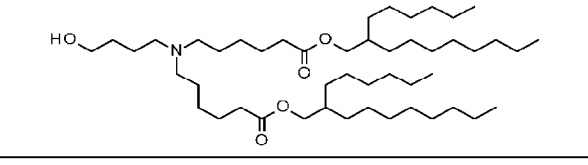
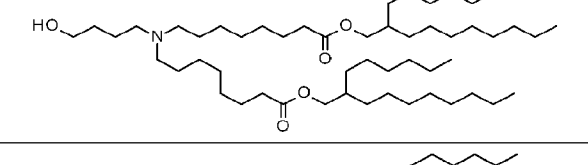
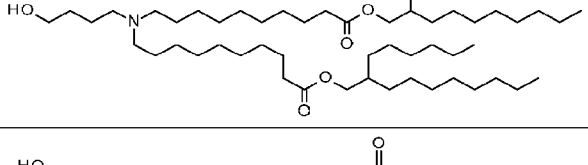
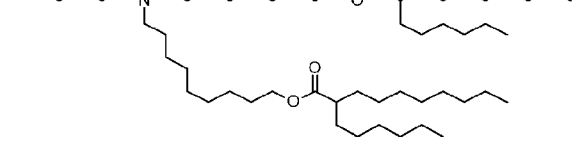
ethyl.

In various different embodiments, the cationic lipid of Formula (III) has one of the structures set forth in Table 3 below.

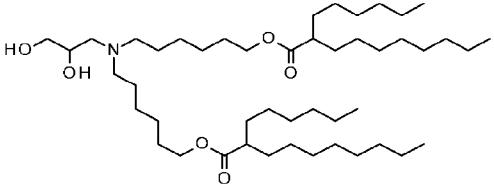
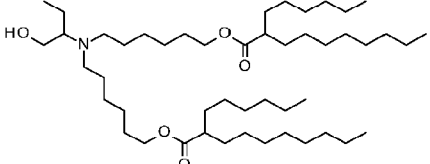
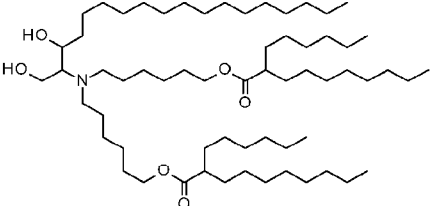
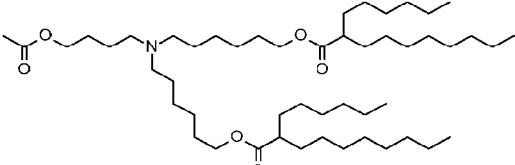
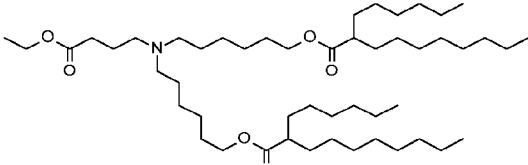
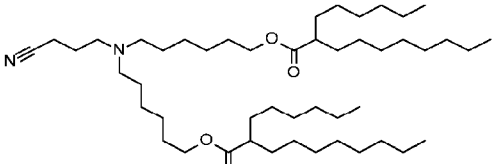
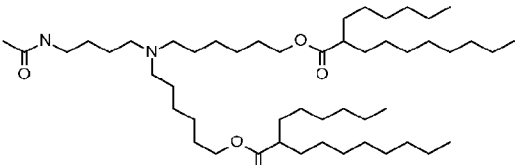
5 Table 3: Representative Compounds of Formula (III).

No.	Structure	Prep. Method
III-1		F
III-2		F
III-3		F
III-4		F
III-5		F
III-6		F

No.	Structure	Prep. Method
III-7		F
III-8		F
III-9		F
III-10		F
III-11		F
III-12		F
III-13		F
III-14		F

No.	Structure	Prep. Method
III-15		F
III-16		F
III-17		F
III-18		F
III-19		F
III-20		F
III-21		F
III-22		F

No.	Structure	Prep. Method
III-23		F
III-24		F
III-25		F
III-26		F
III-27		F
III-28		F
III-29		F

No.	Structure	Prep. Method
III-30		F
III-31		F
III-32		F
III-33		F
III-34		F
III-35		F
III-36		F

In some embodiments, the LNPs comprise a lipid of Formula (III), a nucleoside-modified RNA and one or more excipient selected from neutral lipids, steroids and pegylated lipids. In some embodiments the lipid of Formula (III) is compound III-3. In some embodiments the lipid of Formula (III) is compound III-7.

5 In some embodiments, the cationic lipid is present in the LNP in an amount from about 30 to about 95 mole percent. In one embodiment, the cationic lipid is present in the LNP in an amount from about 30 to about 70 mole percent. In one embodiment, the cationic lipid is present in the LNP in an amount from about 40 to about 60 mole percent. In one embodiment, the cationic lipid is present in the LNP in an amount of about 50 mole percent. In one embodiment, the LNP comprises only cationic lipids.

In some embodiments, the LNP comprises one or more additional lipids which stabilize the formation of particles during their formation.

Suitable stabilizing lipids include neutral lipids and anionic lipids.

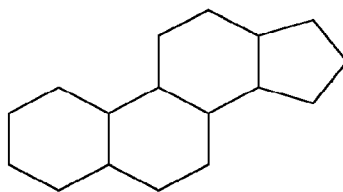
15 Exemplary anionic lipids include, but are not limited to, phosphatidylglycerol, cardiolipin, diacylphosphatidylserine, diacylphosphatidic acid, N-dodecanoylphosphatidylethanolamines, N-succinylphosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol (POPG), and other anionic modifying groups joined to neutral lipids.

Exemplary neutral lipids include, for example, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), 25 palmitoyloleyolphosphatidylcholine (POPC), palmitoyloleyolphosphatidylethanolamine (POPE) and dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-30 phosphatidyethanol amine (SOPE), and 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine (transDOPE). In one embodiment, the neutral lipid is 1,2-distearoyl-sn-glycero-3-

phosphocholine (DSPC).

In some embodiments, the LNPs comprise a neutral lipid selected from DSPC, DPPC, DMPC, DOPC, POPC, DOPE and SM. In various embodiments, the molar ratio of the cationic lipid (e.g., lipid of Formula (I)) to the neutral lipid ranges from about 2:1 to about 8:1.

In various embodiments, the LNPs further comprise a steroid or steroid analogue. A “steroid” is a compound comprising the following carbon skeleton:



In some embodiments, the steroid or steroid analogue is cholesterol. In some of these embodiments, the molar ratio of the cationic lipid (e.g., lipid of Formula (I)) to cholesterol ranges from about 2:1 to 1:1.

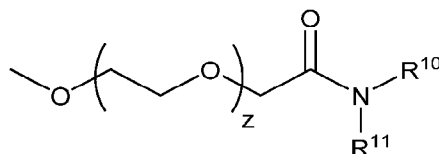
In some embodiments, the LNP comprises glycolipids (e.g., monosialoganglioside GM₁). In some embodiments, the LNP comprises a sterol, such as cholesterol.

In some embodiments, the LNPs comprise a polymer conjugated lipid.

In some embodiments, the LNP comprises an additional, stabilizing -lipid which is a polyethylene glycol-lipid (pegylated lipid). Suitable polyethylene glycol-lipids include PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramides (e.g., PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols. Representative polyethylene glycol-lipids include PEG-c-DOMG, PEG-c-DMA, and PEG-s-DMG. In one embodiment, the polyethylene glycol-lipid is N-[(methoxy poly(ethylene glycol)₂₀₀₀)carbamyl]-1,2-dimyristyloxypropyl-3-amine (PEG-c-DMA). In one embodiment, the polyethylene glycol-lipid is PEG-c-DOMG). In other embodiments, the LNPs comprise a pegylated diacylglycerol (PEG-DAG) such as 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), a pegylated phosphatidylethanolamine (PEG-PE), a PEG succinate diacylglycerol (PEG-S-DAG) such as 4-O-(2',3'-di(tetradecanoyloxy)propyl)-1-O-(ω-

methoxy(polyethoxy)ethyl)butanedioate (PEG-S-DMG), a pegylated ceramide (PEG-cer), or a PEG dialkoxypentylcarbamate such as ω -methoxy(polyethoxy)ethyl-N-(2,3-di(tetradecanoxo)propyl)carbamate or 2,3-di(tetradecanoxo)propyl-N-(ω -methoxy(polyethoxy)ethyl)carbamate. In various embodiments, the molar ratio of the cationic lipid to the pegylated lipid ranges from about 100:1 to about 25:1.

In some embodiments, the LNPs comprise a pegylated lipid having the following structure (IV):



(IV)

or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, wherein:

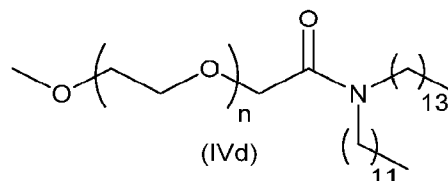
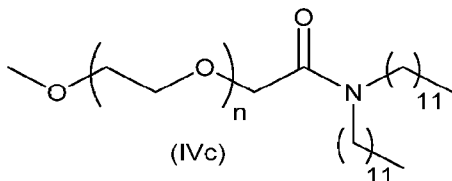
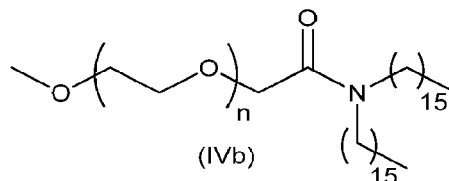
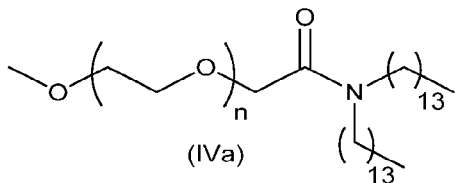
R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and z has mean value ranging from 30 to 60.

In some of the foregoing embodiments of the pegylated lipid (IV), R^{10} and R^{11} are not both *n*-octadecyl when z is 42. In some other embodiments, R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing from 10 to 18 carbon atoms. In some embodiments, R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing from 12 to 16 carbon atoms. In some embodiments, R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing 12 carbon atoms. In some embodiments, R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing 14 carbon atoms. In other embodiments, R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing 16 carbon atoms. In still more embodiments, R^{10} and R^{11} are each independently a straight or branched, saturated or unsaturated alkyl chain containing 18 carbon atoms. In still other embodiments, R^{10} is a straight or branched, saturated or unsaturated alkyl chain containing 12 carbon atoms and R^{11} is a straight or branched, saturated or unsaturated

alkyl chain containing 14 carbon atoms.

In various embodiments, z spans a range that is selected such that the PEG portion of (II) has an average molecular weight of about 400 to about 6000 g/mol. In some embodiments, the average z is about 45.

- 5 In other embodiments, the pegylated lipid has one of the following structures:



wherein n is an integer selected such that the average molecular weight of the pegylated lipid is about 2500 g/mol.

- 10 In some embodiments, the additional lipid is present in the LNP in an amount from about 1 to about 10 mole percent. In one embodiment, the additional lipid is present in the LNP in an amount from about 1 to about 5 mole percent. In one embodiment, the additional lipid is present in the LNP in about 1 mole percent or about 1.5 mole percent.
- 15 In some embodiments, the LNPs comprise a lipid of Formula (I), a nucleoside-modified RNA, a neutral lipid, a steroid and a pegylated lipid. In some embodiments the lipid of Formula (I) is compound I-6. In different embodiments, the neutral lipid is DSPC. In other embodiments, the steroid is cholesterol. In still different embodiments, the pegylated lipid is compound IVa.
- 20 In some embodiments, the LNP comprises one or more targeting moieties, which are capable of targeting the LNP to a cell or cell population. For example, in one embodiment, the targeting moiety is a ligand, which directs the LNP to a receptor found

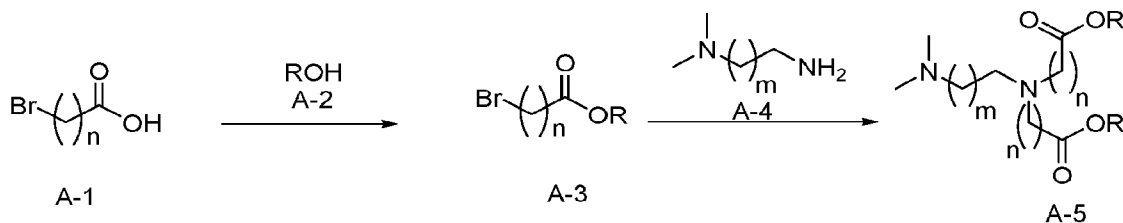
on a cell surface.

In some embodiments, the LNP comprises one or more internalization domains. For example, in one embodiment, the LNP comprises one or more domains, which bind to a cell to induce the internalization of the LNP. For example, in one
 5 embodiment, the one or more internalization domains bind to a receptor found on a cell surface to induce receptor-mediated uptake of the LNP. In some embodiments, the LNP is capable of binding a biomolecule in vivo, where the LNP-bound biomolecule can then be recognized by a cell-surface receptor to induce internalization. For example, in one
 10 embodiment, the LNP binds systemic ApoE, which leads to the uptake of the LNP and associated cargo.

Other exemplary LNPs and their manufacture are described in the art, for example in U.S. Patent Application Publication No. US20120276209, Semple et al., 2010, Nat Biotechnol., 28(2):172-176; Akinc et al., 2010, Mol Ther., 18(7): 1357-1364; Basha et al., 2011, Mol Ther, 19(12): 2186-2200; Leung et al., 2012, J Phys Chem C
 15 Nanomater Interfaces, 116(34): 18440-18450; Lee et al., 2012, Int J Cancer., 131(5): E781-90; Belliveau et al., 2012, Mol Ther nucleic Acids, 1: e37; Jayaraman et al., 2012, Angew Chem Int Ed Engl., 51(34): 8529-8533; Mui et al., 2013, Mol Ther Nucleic Acids, 2, e139; Maier et al., 2013, Mol Ther., 21(8): 1570-1578; and Tam et al., 2013, Nanomedicine, 9(5): 665-74, each of which are incorporated by reference in their
 20 entirety.

The following Reaction Schemes illustrate methods to make lipids of Formula (I), (II) or (III).

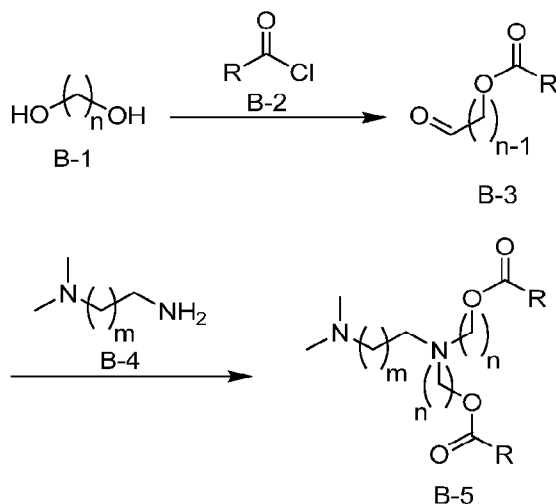
GENERAL REACTION SCHEME 1



25 Embodiments of the lipid of Formula (I) (e.g., compound A-5) can be prepared according to General Reaction Scheme 1 ("Method A"), wherein R is a saturated or unsaturated $\text{C}_1\text{-C}_{24}$ alkyl or saturated or unsaturated cycloalkyl, m is 0 or 1

and n is an integer from 1 to 24. Referring to General Reaction Scheme 1, compounds of structure A-1 can be purchased from commercial sources or prepared according to methods familiar to one of ordinary skill in the art. A mixture of A-1, A-2 and DMAP is treated with DCC to give the bromide A-3. A mixture of the bromide A-3, a base (e.g., N,N-diisopropylethylamine) and the N,N-dimethyldiamine A-4 is heated at a temperature and time sufficient to produce A-5 after any necessarily workup and or purification step.

GENERAL REACTION SCHEME 2

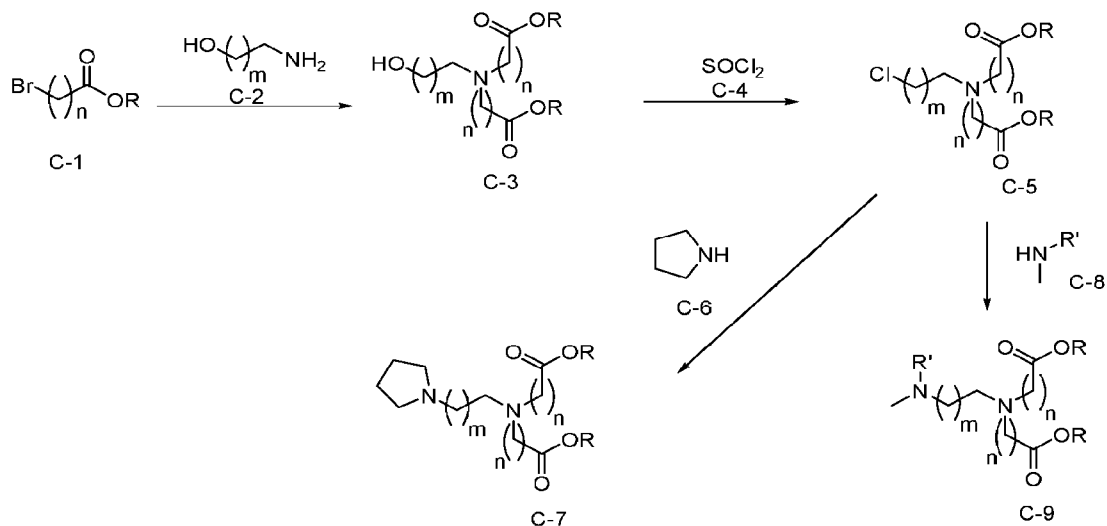


Other embodiments of the compound of Formula (I) (e.g., compound B-5) can be prepared according to General Reaction Scheme 2 ("Method B"), wherein R is a saturated or unsaturated $\text{C}_1\text{-C}_{24}$ alkyl or saturated or unsaturated cycloalkyl, m is 0 or 1 and n is an integer from 1 to 24. As shown in General Reaction Scheme 2, compounds of structure B-1 can be purchased from commercial sources or prepared according to methods familiar to one of ordinary skill in the art. A solution of B-1 (1 equivalent) is treated with acid chloride B-2 (1 equivalent) and a base (e.g., triethylamine). The crude product is treated with an oxidizing agent (e.g., pyridinium chlorochromate) and intermediate product B-3 is recovered. A solution of crude B-3, an acid (e.g., acetic acid), and N,N-dimethylaminoamine B-4 is then treated with a reducing agent (e.g., sodium triacetoxyborohydride) to obtain B-5 after any necessary work up and/or purification.

It should be noted that although starting materials A-1 and B-1 are depicted above as including only saturated methylene carbons, starting materials which include carbon-carbon double bonds may also be employed for preparation of compounds

which include carbon-carbon double bonds.

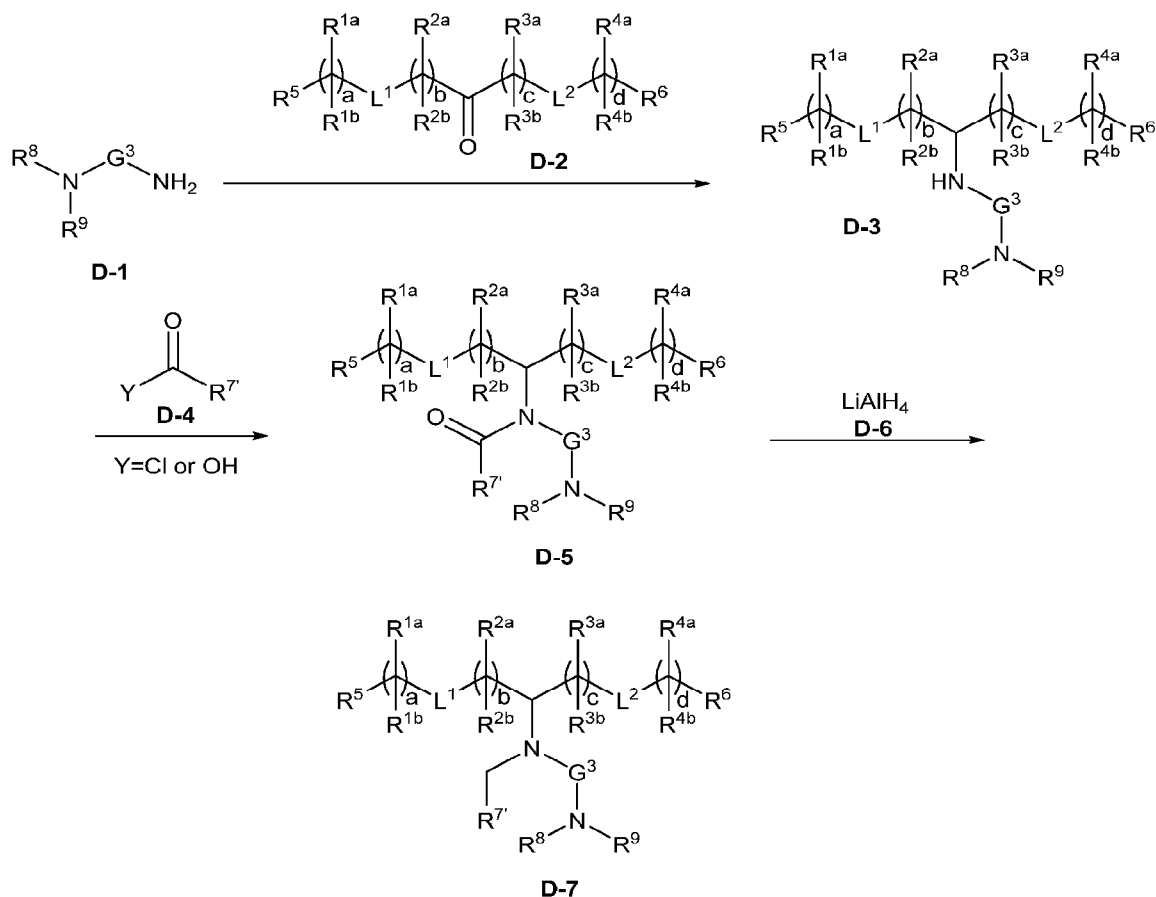
GENERAL REACTION SCHEME 3



- Different embodiments of the lipid of Formula (I) (e.g., compound C-7 or
- 5 C9) can be prepared according to General Reaction Scheme 3 (“Method C”), wherein R is a saturated or unsaturated C₁-C₂₄ alkyl or saturated or unsaturated cycloalkyl, m is 0 or 1 and n is an integer from 1 to 24. Referring to General Reaction Scheme 3, compounds of structure C-1 can be purchased from commercial sources or prepared according to methods familiar to one of ordinary skill in the art.

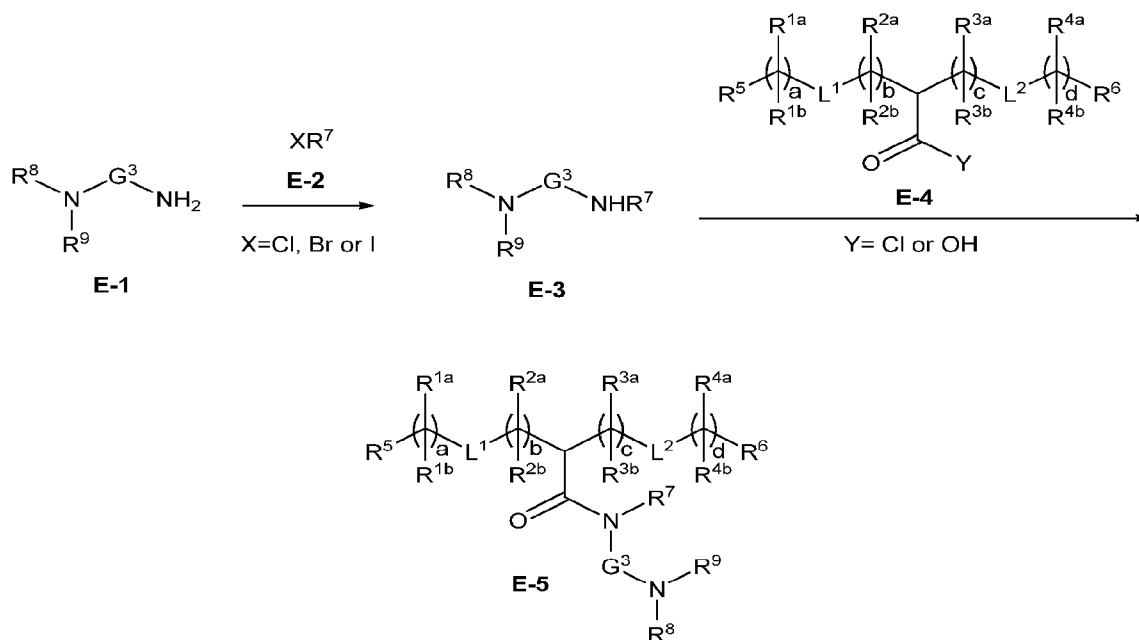
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GENERAL REACTION SCHEME 4



Embodiments of the compound of Formula (II) (e.g., compounds D-5 and D-7) can be prepared according to General Reaction Scheme 4 ("Method D"), wherein R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^{4a}, R^{4b}, R⁵, R⁶, R⁸, R⁹, L¹, L², G¹, G², G³, a, b, c and d are as defined herein, and R⁷ represents R⁷ or a C₃-C₁₉ alkyl. Referring to General Reaction Scheme 1, compounds of structure D-1 and D-2 can be purchased from commercial sources or prepared according to methods familiar to one of ordinary skill in the art. A solution of D-1 and D-2 is treated with a reducing agent (e.g., sodium triacetoxyborohydride) to obtain D-3 after any necessary work up. A solution of D-3 and a base (e.g. trimethylamine, DMAP) is treated with acyl chloride D-4 (or carboxylic acid and DCC) to obtain D-5 after any necessary work up and/or purification. D-5 can be reduced with LiAlH₄ D-6 to give D-7 after any necessary work up and/or purification.

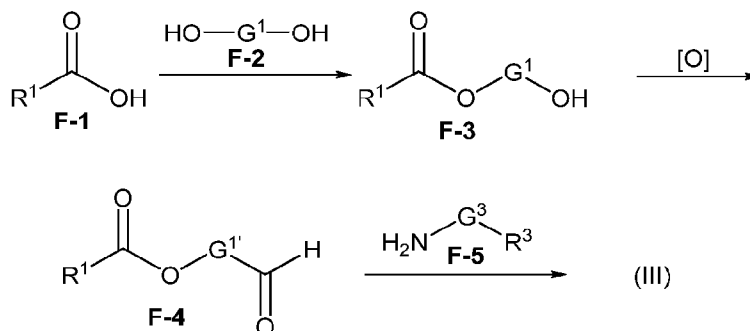
GENERAL REACTION SCHEME 5



Embodiments of the lipid of Formula (II) (e.g., compound E-5) can be prepared according to General Reaction Scheme 5 ("Method E"), wherein R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^{4a}, R^{4b}, R⁵, R⁶, R⁷, R⁸, R⁹, L¹, L², G³, a, b, c and d are as defined herein.

- 5 Referring to General Reaction Scheme 2, compounds of structure E-1 and E-2 can be purchased from commercial sources or prepared according to methods familiar to one of ordinary skill in the art. A mixture of E-1 (in excess), E-2 and a base (e.g., potassium carbonate) is heated to obtain E-3 after any necessary work up. A solution of E-3 and a base (e.g. trimethylamine, DMAP) is treated with acyl chloride E-4 (or carboxylic acid and DCC) to obtain E-5 after any necessary work up and/or purification.
- 10

GENERAL REACTION SCHEME 6



General Reaction Scheme 6 provides an exemplary method (Method F) for

preparation of Lipids of Formula (III). G^1 , G^3 , R^1 and R^3 in General Reaction Scheme 6 are as defined herein for Formula (III), and $G1'$ refers to a one-carbon shorter homologue of $G1$. Compounds of structure F-1 are purchased or prepared according to methods known in the art. Reaction of F-1 with diol F-2 under appropriate condensation conditions (e.g., DCC) yields ester/alcohol F-3, which can then be oxidized (e.g., PCC) to aldehyde F-4. Reaction of F-4 with amine F-5 under reductive amination conditions yields a lipid of Formula (III).

It should be noted that various alternative strategies for preparation of lipids of Formula (III) are available to those of ordinary skill in the art. For example, other lipids of Formula (III) wherein L^1 and L^2 are other than ester can be prepared according to analogous methods using the appropriate starting material. Further, General Reaction Scheme 6 depicts preparation of a lipids of Formula (III), wherein G^1 and G^2 are the same; however, this is not a required aspect of the invention and modifications to the above reaction scheme are possible to yield compounds wherein G^1 and G^2 are different.

It will be appreciated by those skilled in the art that in the process described herein the functional groups of intermediate compounds may need to be protected by suitable protecting groups. Such functional groups include hydroxy, amino, mercapto and carboxylic acid. Suitable protecting groups for hydroxy include trialkylsilyl or diarylalkylsilyl (for example, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl or trimethylsilyl), tetrahydropyranyl, benzyl, and the like. Suitable protecting groups for amino, amidino and guanidino include *t*-butoxycarbonyl, benzyloxycarbonyl, and the like. Suitable protecting groups for mercapto include $-C(O)-R''$ (where R'' is alkyl, aryl or arylalkyl), *p*-methoxybenzyl, trityl and the like. Suitable protecting groups for carboxylic acid include alkyl, aryl or arylalkyl esters. Protecting groups may be added or removed in accordance with standard techniques, which are known to one skilled in the art and as described herein. The use of protecting groups is described in detail in Green, T.W. and P.G.M. Wutz, *Protective Groups in Organic Synthesis* (1999), 3rd Ed., Wiley. As one of skill in the art would appreciate, the protecting group may also be a polymer resin such as a Wang resin, Rink resin or a 2-chlorotrityl-chloride resin.

Pharmaceutical Compositions

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory
5 ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the description of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such
10 compositions are generally suitable for administration to subjects of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various subjects is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which
15 administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as non-human primates, cattle, pigs, horses, sheep, cats, and dogs.

Pharmaceutical compositions that are useful in the methods of the
20 invention may be prepared, packaged, or sold in formulations suitable for ophthalmic, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, intravenous, intracerebroventricular, intradermal, intramuscular, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunogenic-based
25 formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active
30 ingredient is generally equal to the dosage of the active ingredient, which would be administered to a subject or a convenient fraction of such a dosage such as, for example,

one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, intravitreal, subcutaneous, intraperitoneal, intramuscular, intradermal, intrasternal injection, intratumoral, intravenous, intracerebroventricular and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise

one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration
5 of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents,
10 or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are
15 useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

20 A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers. In some embodiments, the formulation may comprise dry particles which comprise the
25 active ingredient and which have a diameter in the range from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient
30 dissolved or suspended in a low-boiling propellant in a sealed container. In some embodiments, such powders comprise particles wherein at least 98% of the particles by

weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. In some embodiments, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. In some embodiments, dry powder compositions include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (in some instances having a particle size of the same order as particles comprising the active ingredient).

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents,

or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations that are useful include those that comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Methods of Treatment or Prevention

The present invention provides methods of inducing an adaptive immune response against Norovirus in a subject comprising administering an effective amount of at least one mRNA molecule (e.g., a nucleoside modified mRNA molecule) encoding an NoV VP1 antigen or a composition (e.g., an LNP) comprising at least one mRNA molecule (e.g., a nucleoside modified mRNA molecule) encoding a NoV VP1 antigen.

In one embodiment, the method provides immunity in the subject to multiple strains of Norovirus, Norovirus infection, or to a disease or disorder associated with Norovirus including, but not limited to, gastroenteritis, food poisoning, vomiting and diarrhea. The present invention thus provides a method of treating or preventing the infection, disease, or disorder associated with Norovirus.

In one embodiment, the composition is administered to a subject having an infection, disease, or disorder associated with Norovirus. In one embodiment, the composition is administered to a subject at risk for developing the infection, disease, or disorder associated with Norovirus. For example, the composition may be administered to a subject who is at risk for being in contact with Norovirus. In one embodiment, the composition is administered to a subject who lives in, traveled to, or is expected to travel to a geographic region in which Norovirus is prevalent. Populations of interest for administration of the vaccine include, but are not limited to, young children (e.g., under 5 years of age), military personnel, cruise ship staff and passengers, institutional long-term

care facility staff and residents (elder care, childcare, school), food handlers, and subject who are traveling. In one embodiment, the composition is administered to a subject who is in contact with or expected to be in contact with another person who lives in, traveled to, or is expected to travel to a geographic region in which Norovirus is prevalent. In one
5 embodiment, the composition is administered to a subject who has knowingly been exposed to Norovirus through their occupation, or other contact.

In one embodiment, the method comprises administering a composition comprising one or more nucleoside-modified nucleic acid molecules encoding one or more NoV antigens. In one embodiment, the method comprises administering a
10 composition comprising a first nucleoside-modified nucleic acid molecule encoding one or more NoV antigens and a second nucleoside-modified nucleic acid molecule encoding one or more NoV antigens. In one embodiment, the method comprises administering a composition comprising a one or more nucleoside-modified nucleic acid molecules encoding a plurality of NoV antigens described herein.

15 In one embodiment, the method comprises administering one or more compositions, each composition comprising one or more nucleoside-modified nucleic acid molecules encoding one or more NoV antigens. In one embodiment, the method comprises administering a first composition comprising one or more nucleoside-modified nucleic acid molecules encoding one or more NoV antigens and administering a second
20 composition comprising one or more nucleoside-modified nucleic acid molecules encoding one or more NoV antigens. In one embodiment, the method comprises administering a plurality of compositions, each composition comprising one or more nucleoside-modified nucleic acid molecules encoding one or more NoV antigens described herein. In some embodiments, the method comprises a staggered administration
25 of the plurality of compositions.

In some embodiments, the method comprises administering to subject a plurality of nucleoside-modified nucleic acid molecules encoding a plurality of NoV antigens, adjuvants, or a combination thereof.

30 In some embodiments, the method of the invention allows for sustained expression of the NoV antigen or adjuvant, described herein, for at least several days following administration. In some embodiments, the method of the invention allows for

sustained expression of the NoV antigen or adjuvant, described herein, for at least 2 weeks following administration. In some embodiments, the method of the invention allows for sustained expression of the NoV antigen or adjuvant, described herein, for at least 1 month following administration. However, the method, in some embodiments, also provides for transient expression, as in some embodiments, the nucleic acid is not integrated into the subject genome.

In some embodiments, the method comprises administering nucleoside-modified RNA, which provides stable expression of the NoV antigen or adjuvant described herein. In some embodiments, administration of nucleoside-modified RNA results in little to no innate immune response, while inducing an effective adaptive immune response.

In some embodiments, the method provides sustained protection against NoV. For example, in some embodiments, the method provides sustained protection against NoV for more than 2 weeks. In some embodiments, the method provides sustained protection against NoV for 1 month or more. In some embodiments, the method provides sustained protection against NoV for 2 months or more. In some embodiments, the method provides sustained protection against NoV for 3 months or more. In some embodiments, the method provides sustained protection against NoV for 4 months or more. In some embodiments, the method provides sustained protection against NoV for 5 months or more. In some embodiments, the method provides sustained protection against NoV for 6 months or more. In some embodiments, the method provides sustained protection against NoV for 1 year or more.

In one embodiment, a single immunization of the composition induces a sustained protection against NoV for 1 month or more, 2 months or more, 3 months or more, 4 months or more, 5 months or more, 6 months or more, or 1 year or more.

Administration of the compositions of the invention in a method of treatment can be achieved in a number of different ways, using methods known in the art. In one embodiment, the method of the invention comprises systemic administration of the subject, including for example enteral or parenteral administration. In some embodiments, the method comprises intradermal delivery of the composition. In another embodiment, the method comprises intravenous delivery of the composition. In some

embodiments, the method comprises intramuscular delivery of the composition. In one embodiment, the method comprises subcutaneous delivery of the composition. In one embodiment, the method comprises inhalation of the composition. In one embodiment, the method comprises intranasal delivery of the composition.

5 It will be appreciated that the composition of the invention may be administered to a subject either alone, or in conjunction with another agent.

 The therapeutic and prophylactic methods of the invention thus encompass the use of pharmaceutical compositions comprising at least one mRNA molecule (e.g. a nucleoside modified mRNA molecule) encoding a NoV antigen, adjuvant, or a
10 combination thereof, described herein to practice the methods of the invention. The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of from 1 ng/kg/day and 100 mg/kg/day. In one embodiment, the invention envisions administration of a dose, which results in a concentration of the compound of the present invention from 10 nM and 10 μ M in a mammal.

15 Typically, dosages which may be administered in a method of the invention to a mammal, such as a human, range in amount from 0.01 μ g to about 50 mg per kilogram of body weight of the mammal, while the precise dosage administered will vary depending upon any number of factors, including but not limited to, the type of mammal and type of disease state being treated, the age of the mammal and the route of
20 administration. In some embodiments, the dosage of the compound will vary from about 0.1 μ g to about 10 mg per kilogram of body weight of the mammal. In some embodiments, the dosage will vary from about 1 μ g to about 1 mg per kilogram of body weight of the mammal.

 The composition may be administered to a mammal as frequently as
25 several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months, several years, or even less frequently, such as every 10-20 years, 15-30 years, or even less frequently, such as every 50-100 years. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors,
30 such as, but not limited to, the type and severity of the disease being treated, the type and age of the mammal, etc.

In some embodiments, administration of an immunogenic composition or vaccine of the present invention may be performed by single administration or boosted by multiple administrations.

5

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be
10 construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the present invention and practice the claimed methods. The following working
15 examples therefore are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: Nucleoside-modified mRNA-LNP vaccine for norovirus

A vaccine platform of nucleoside-modified mRNA complexed with lipid
20 nanoparticles (LNP) was used to develop a vaccine for norovirus. The norovirus vaccine encodes a norovirus capsid protein VP1 from multiple genogroups (GI.1, GI.3, GII.3 and GII.4). The LNP encapsulation allows for an efficient delivery and expression of nucleoside modified mRNA in vivo, and the 1-methylpseudouridine nucleoside modification in the place of uridine is critical for the potency of the antibody response
25 induced by the mRNA platform through its induction of Tfh cells. The nucleoside-modified mRNA-LNP vaccine demonstrated high HBGA blockade antibody titers, protected human enteroids against norovirus infection and elicited strong CD4+ and CD8+ responses, even at low doses (0.25 µg). These data provide proof of concept that a modified-nucleoside mRNA-LNP vaccine based upon norovirus VP1 sequences is
30 immunogenic in vivo and generates neutralizing antibodies that block virus infection in vitro. The mRNA-LNP vaccine is extremely efficacious in low doses and is a non-

replicating vector that is amenable to highly scalable manufacturing; therefore, it is ideally suited to be used in a global public health campaign to immunize people against norovirus.

5 The nucleoside-modified mRNA-LNP norovirus vaccine demonstrated high titer of blockade antibody, good protection of human enteroids against norovirus infection and elicited strong CD4⁺ and CD8⁺ T cells responses. These data provide proof of concept that a modified-nucleoside mRNA-LNP vaccine based upon norovirus VP1 sequences is immunogenic in vivo and generates neutralizing antibodies that block virus infection in vitro.

10 To confirm that the mRNA molecules expressed the correct protein, 293T cells were transfected with mRNA encoded Norwalk1968, CapeTown2012, Sweden2008, Argentina2016, Nashville2016, Canada2019, UK2015, USA2011 or Japan2009. 24 hours later cells were harvested and analyzed by western blot for expression of GI.1, GII.4, GI.3 or GII.3 capsid (Figure 1).

15 Balb/c mice (6-8 wks old) were immunized twice at day 0 and day 28 by i.d. injection with 10 µg of empty-LNP or mRNA bivalent vaccine: Norwalk/2011/GI.1 representing genogroup I strains and CapeTown/2012/GII.4, representing genogroup II strains. Together, genogroup I and II strains account for >90% of human norovirus infection. Figure 2 details the immunization scheme.

20

HuNoV blockade antibody response following mRNA bivalent vaccine

Sera were screened against Norwalk1968/GI.1 and Sydney2012/GII.4 viruses like particles (VLP) for ability to block the interaction of VLP with binding ligand in a surrogate neutralization assay (Figure 3). Sera that did not block at least 50% VLP-ligand binding were assigned a titer of 0.5X LLD (ID₅₀=25) and a marker placed below the limit of detection (dashed line).

25

HuNoV blockade antibody response following mRNA bivalent vaccine

Figure 4 demonstrates that blockade Ab responses to additional VLP demonstrated that vaccine responses are genotype-specific, with no cross reactivity within or between the genogroups tested (GI.3, GI.5, GII.3). Within the GII.4 genotype,

30

blockade Ab titers for GII.4/2009 and GII.4/2002 were about ~20% and ~5%, respectively to the titer of GII.4/2012, in agreement with known antigenic drift within the globally dominant GII.4 pandemic genotype.

5 T cell stimulation assay

Balb/c mice (6-8 wks old) were immunized at day 0 by intramuscular injection with 10 µg per mouse of mRNA bivalent vaccine (GII.4/CapeTown2012, GI.1/Nowalk2012), or with mRNA-Luc as a control. n = 5 per group (Figure 5). T cell stimulation assay was performed with VLPs (GI.1, GI.3, GII.3, GII.4) (Figure 6).

10

Human Noro bivalent mRNA vaccine dose response

To determine the optimal amount of bivalent mRNA vaccine, four doses (0.25, 0.5, 1 or 3 µg of each vaccine per mouse) or mRNA-Luc as a control (12 µg/mouse) were administered by intramuscular (i.m.) injection to mice twice at 28-day intervals (Figure 7). Sera were collected at days 0-, 28-, 56-, 150-days post immunization and were evaluated in a surrogate neutralization assay. n=10 per group, at the indicated time points. The titers of blockade antibody are at a high level with even a small dose of 0.25 µg/mouse.

20 Low dose mRNA vaccine induces strong Th1 response

To evaluate the Th1 response, Balb/c mice (6-8 wks old) were immunized twice at day 0 and day 28 by intramuscular injection with 0.25 µg per mouse of mRNA vaccine (GII.4) or with mRNA-Luc as a control (Figure 8). Two weeks after boost, splenic single cell suspensions were stimulated with GII.4 peptide pools (15 AA length, 25 11 AA overlapping) for 5 hours, 37°C, 5% CO₂, and analyzed for activation of T cells by flow cytometry. n = 10 per each group. Splenocytes isolated from mice vaccinated with GII.4 mRNA-LNP and stimulated with GII.4 peptide pool demonstrated that the mRNA vaccine elicited strong CD4⁺ and CD8 T cell responses.

30 Tetravalent Norovirus mRNA vaccine

A tetravalent norovirus mRNA vaccine was developed which includes

GI.3 and GII.3 genotypes as these are the most prevalent GI and GII genotypes in NoV outbreaks in pediatric NoV acute gastroenteritis. Capsid protein VP1 sequences of GI.3/Argentina2016 and GII.3/Canada2019 were identified on GenBank, the plasmids were designed, and nucleoside-modified mRNA was produced. mRNA encoding GI.1, GII.4, GI.3 and GII.3 was checked for endotoxin level and activation of IFN-alpha before LNP encapsulation.

Balb/c and C57BL/6 mice (6-8 wks old) were immunized twice at day 0 and day 28 by intramuscular injection with 0.125, 0.25, 0.5, 1 µg per mouse of mRNA tetravalent vaccine (GII.4/CapeTown2012, GI.1/Nowalk2012, GI.3/Argentina2016, GII.3/Canada2019), or with empty LNP as a control (n = 5 per group) (Figure 9).

To determine the optimal amount of tetravalent mRNA vaccine, four doses (0.125, 0.25, 0.5, or 1 µg of each vaccine per mouse) or empty LNP as a control (4 µg/mouse) were administered by i.m. injection to mice twice at 28-day intervals (Figure 10). Empty LNP were below level of detection (not shown).

Human intestinal enteroids

The major barrier to development of effective interventions for human noroviruses has been the lack of a robust and reproducible *in vitro* cultivation system. Recently researchers developed human intestinal enteroids (HIE), where three-dimensional *in vitro* cultures are derived from proliferating stem cells in crypts isolated from human gastrointestinal tract biopsies or surgical specimens. HIE cultures possess the multicellular complexity and organization of the intestinal epithelium. HIE are physiologically active, contain all epithelial cell types of the normal GI tract and retain segment-specific properties.

Figure 11 and Figure 12 demonstrate the effects of infection of stem cell-derived human 3D enteroids with live HNoV from a patient stool sample infected with GII.4. HIE can be used to assay HNoV infection as enteroids infected with HNoV display the presence of the VP1 capsid protein and a loss of ZO1 staining, a marker for tight junction integrity (Figure 12).

C57BL/6 mice (6-8 wks old) were immunized twice at day 0 and day 28 by i.m. injection with 0.25 µg per mouse of mRNA vaccine (GII.4) or with mRNA-Luc

as a control. Four weeks after boost, sera were collected, diluted with indicated dilutions, preincubated with HNoV positive stool sample and analyzed to their ability to neutralize HuNoV infection in biopsy-derived human small intestinal organoids. Figure 13 and Figure 14 demonstrates that there is protection of human intestinal enteroids against GII.4 infections by serum from mRNA-LNP vaccinated mice.

These data demonstrate that blockade antibody titers 30 days after prime were higher (GI.1) or comparable (GII.4) with blockade antibody titers from patient infected with HNoV, blockade antibodies are durable at high titer until at least day 240, low dose mRNA vaccine (0.25 µg) elicited strong CD4+ and CD8+ T cell responses, and sera from vaccinated mice neutralized HNoV and protected human intestinal enteroids against GII.4 infection. Therefore, the data support that a modified-nucleoside mRNA-LNP vaccine based upon norovirus VP1 sequences is immunogenic *in vivo* and generates neutralizing antibodies that block virus infection *in vitro*.

15 Example 2: Sequences

SEQ ID NO:1

capsid protein VP1 [Norovirus Hu/GI.1/8FIIa/1968/USA]

AFJ38516.1

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPI
 20 DPWIINNFBVQAPQGEFTISPNNTPGDVLFDSLGLPHLNPFLHLSQMYNGWVGN
 MRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPFVIADVRLDPIEVPLE
 DVRNVLFHNNDNRNQQTMRLVCMLYTPLRTGGGTGDSFVAVGRVMTCPSPDFNF
 LFLVPPTVEQKTRPFTLPNLPLSSLSNSRAPLPISSMGISPDNVQSVQFQNGRCTL
 25 GRLVGTTTPVSLSHVAKIRGTSNGTVINLTELDGTPFHPFEGPAPIGFPDLGGCDWH
 INMTQFGHSSQTQYDVDTPDTFVPHLGSIQANGIGSGNYVGVLSWISPPSHPSGS
 QVDLWKIPNYGSSITEATHLAPSVYPPGFGEVLVFFMSKMPGPGAYNLPCLLPQE
 YISHLASEQAPTVEAALLHYVDPDTGRNLGEFKAYPDGFLTCVPNGASSGPQQ
 LPINGVFVFSWVSRYQLKPVGTASSARGRLGLRR

30 SEQ ID NO:2

[Norovirus Hu/GI.1/CHA9A004_20110419/2011/USA]

AGM33223.1

MMMASKDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVATAGQVNPI
 DPWIINNFBVQAPQGEFTISPNNTPGDVLFDLSLGPLNPFLLHLSQMYNGWVGN
 MRVRIMLAGNAFTAGKIIVSCIPPGFGSHNLTIAQATLFPHVIAADVRLDPIEVPLE
 5 DVRNVLFHNNDNRNQQTMRMLVCMLYTPLRTGGGTGDSFVVAGRVMTCSPDFNF
 LFLVPPTVEQKTRPFTLPNLPLSSLSNSRAPLPISSMGISPDNVQSVQFQNGRCTL
 GRLVGTTTPVSLSHVAKIRGTSNGTVINLTELDGTPFHPFEGPAPIGFDPDLGGCDWH
 INMTQFGHSSQTQYDVDTTPTDFVPHLGSIQANGIGSGNYVGVLSWISPPSRPSGS
 QVDLWKIPNYGSSITEATHLAPSVYPPPGFGEVLVFFMSKMPGPGAYNLPCLLPQE
 10 YISHLASEQAPTVEGEAALLHYVDPDTGRNLGEFKAYPDGFLTCVPNGASSGPQQ
 LPINGVFVFSWVSRYFYQLKPVGTASSARGRLGLRR

SEQ ID NO:3**VP1 [Norovirus Hu/GII.4/Sydney/NSW0514/2012/AU]****15 AFV08795.1**

MKMASSDANPSDGSAANLVPEVNNEVMALPVPVGAIAAPVAGQQNVIDPWIR
 NNFVQAPGGGEFTVSPRNAPGEILWSAPLGPDLNPYLHLARMYNGYAGGFEVQV
 ILAGNAFTAGKVIFAAVPPNFPTEGLSPSQVTMFPHIVVDVRQLEPVLIPDPVRN
 NFYHYNQSNPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRSPDFDFIFLVPPT
 20 VESRTKPFSPVLTVEEMTNSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGT
 TQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNDYDPTTEEIPAPLGTPDFVGKI
 QGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRVQFETDTRDFEANQNTKFT
 PVGVIQDGGTTHRNEPQQWVLPSYSGRNTHNVHLAPAVAPTFFGEQLLFFRSTM
 PGCSGYPNMDLDCLLPQEWVQYFYQEAAPAQSDVALLRFVNPDTRVLFECKL
 25 HKSGYVTVAHTGQHDLVIPNGYFRFDSWVNQFYTLAPMGNGTGRRRAV

SEQ ID NO:4**[Norovirus GII/Hu/ZAF/2012/GII.P4_GII.4/CapeTown/9772]****ALX87357.1**

30 MKMASSDANPSDGSAANLVPEVNNEVMALPVPVGAIAAPVAGQQNVIDPWIR
 NNFVQAPGGGEFTVSPRNAPGEILWSAPLGPDLNPYLHLARMYNGYAGGFEVQV

ILAGNAFTAGKVIFAAVPPNFPTEGLSPSQVTMFPHIVVDVRQLEPVLIPDPVRN
 NFYHYNQSNPTIKLIAMLYTPLRANNAGDDVFTVSCRVLTRPSPDFDFIFLVPPT
 VESRTKPFSSVPVLTVEEMTNSRFPIPLEKLFTGPSSAFVVQPQNGRCTTDGVLLGT
 TQLSPVNICTFRGDVTHITGSRNYTMNLASQNWNNYDPTEEIPAPLGTPDFVGEI
 5 QGVLTQTTRTDGSTRGHKATVYTGSADFAPKLGRIQFETDTHDHEANQNTKFT
 PVGVIQDGSTTHRNEPQQWVLPSYSGRNTHNVHLAPAVAPTFPGEQLLFFRSTM
 PGCSGYPNMDLDCLLPQEWVQYFYQEAAAPSQSDVALLRFVNPDTGRVLFECKL
 HKSGYVTVAHTGQHDLVIPNGYFRFDSWVNQFYTLAPMGNGTGRRRVV

10 **SEQ ID NO:5**

capsid protein [Norovirus GI.3] (China 2015)

GenBank: ARI71147.1

MMMASKDAPTNMDGTSGAGQLVPEANTAEPISMEPVAGAATAAATAGQVNMI
 DPWIMNNYVQAPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLAQMYNGWVGN
 15 MKVKVLLAGNAFTAGKIIISCIPPGFAAQNISIAQATMFPHVIADVRLVLEPIEVPLE
 DVARNVLFHNNDNTPTMRLVCMLYTPLRASGSSSGTDPFVIAGRVLTCPSPDFSFL
 FLVPPNVEQKTKPFSSVPNLPLNLTLSNSRVPSLIKSMMSRDLHGQMVQFQNGRVT
 LDGQLQGTTPTSASQLCKIRGSVFHANGGNGYNLTLDGSPYHAFESPAPIGFPD
 LGECDWHMEASPTTQFNTGDVIKQINVKQESAFAPHLGTIQADGLSDVSVNTNM
 20 IAKLGWVSPVSDGHKRDVDPWVIPRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSD
 FPIAHGTNGLSVPCTIPQEFVTHFVNEQAPTRGEAALLHYLDPDTHRNLGEFKLY
 PDGFMTCPVNSSGTGPQTLPIGVFVFSWVSRYQLKPVGTAGPARRLGIRRS

SEQ ID NO:6

25 **VP1 [Norovirus GI.3] (Hu/USA/2016/GI.Pd-GI.3/Nashville-0047)**

GenBank: AZJ17766.1

MMMASKDAPTNMDGTSGAGQLVPEANTAEPISMEPVAGAATAAATAGQVNMI
 DPWIMNNYVQAPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLAQMYNGWVGN
 MKVKVLLAGNAFTAGKIIISCIPPGFVSQNISIAQATMFPHVIADVRLVLEPIEVPLE
 30 DVARNVLFHNNDNTPTMRLVCMLYTPLRASGSSSGTDPFVIAGRVLTCPSPDFSFL
 FLVPPNVEQKTKPFSSVPDLPLNVLSNSRVPSLIKFMMSQDHGQMVQFQNGRVT

LDGQLQGTTPTSASQLCKMRGTVYHASGGQGLNLTEIDGTPYHAFESPAPIGFPD
 IGDSDDWHINASPATAFGSGESVKRLDMEQGSSFAPHLGTVHYTNASYEANADLI
 CSLEWLSPPSGGTPNKVNPWAIIPRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSDFPI
 ANGQDGLRVPCTIPQEFVTHFVNEQAPTRGEAALLHYVDPDTHRNLGEFKLYPE
 5 GFMTCPVNSSSGSGPQTLPIINGVFTFVSWVSRFYQLKPVGTTGPVRRLGIRRS

SEQ ID NO:7**VP1 [Norovirus GII.3] UK 2015****GenBank: AWR17549.1**

10 MKMASNDAAPSNDGAAGLVPEINNEAMALEPVAGAAIAAPLTGQQNIIDPWIMN
 NFVQAPGGGFTVSPRNSPGEILLNLELGPGINPYLAHLARMYNGYAGGFEVQVV
 LAGNAFTAGKIIFAAIPPNFPIDNLSAAQITMCPHVIVDVRQLEPVNLPMPDVRNN
 FFHYNQGSDSRLRLIAMLYTPLRANNSGDDVFTVSCRVLTRPSPDFSFNFLVPPTV
 ESKTKPFSLPILTISEMSNSRFPVPIDSLHTSPTENIVVQCQNGRVTLDGELMGTTQ
 15 LLPSQICAFRGVLTRSTSASDQADTATPRLFNYYWHIQLDNLDGTPYDPAEDIP
 GPLGTPDFRGKVFGVASQRNPDATTRAEAKIDTTSGRFTPKLGSLEISTESSDFD
 QNQPTRFTPVGVGVGDHEADFQQWTLPDYAGQFTHNMNLAPAVAPNFPGEQLLF
 FRSHLPSSGGRSNGILDCLVPQEWVQHFYQESAPSQSQVALIRYVNPDTGRVLFE
 AKLHKLGFMTIAKNGDSPITVPPNGYFRFESWVNPFFYTLAPMGTTGNGRRRIQ

20

SEQ ID NO:8**major capsid protein VP1 [Norovirus GII] (Hu/GII.P16-
 GII.3/RUS/Novosibirsk/NS17-A996/2017)****GenBank: AVO62572.1**

25 MKMASNDATPSNDGAAGLVPEINNEAMALDPVAGAAIAAPLTGQQNIIDPWIMN
 NFVQAPGGGFTVSPRNSPGEVLLNLELGPEINPYLAHLARMYNGYAGGFEVQVV
 LAGNAFTAGKIIFAAIPPNFPTDNLSAAQITMCPHVIVDVRQLEPVNLPMPDVRNN
 FFHYNQGSDSKLRLVAMLYTPLRANNSGDDVFTVSCRVLTRPSPEFSFNFLVPPT
 VESKTKPFTLPILTISEMSNSRFPVPIDSLHTSPTENIVVQCQNGRVTLDGELMGTT
 30 QLLPSQICAFRGVLTRSTSASDQADTATPRLFNYYWHIQLDNLDGTPYDPAEDI
 PGPLGTPDFRGKVFGVASQRNPDSTTRAHEAKVDTTAGRFTPKLGSLEISTESSDD

FDQNQPTRFTPVGIGVDHESDFQQWSLPDYSGQFTHNMNLA PAVAPNFPGEQLL
FFRSQLPSSGGRSNGILDCLVPQEWVQH FYQESAPAQTQVALVRYVNPDTGRVL
FEAKLHKLGFMTIAKNGDSPITVPPNGYFRFESWVNP FYTLAPMGTGNGRRRVQ

5

SEQ ID NO:9**VP1 [Norovirus GI] (Argentina 2016)****GenBank: QPJ58837.1**

MMMASKDAPTNMDGTSGAGQLVPEANTAEPISMEPVAGAATAAATAGQVNMI
10 DPWIMNNYVQAPQGEFTISPNNTPGDILFDLQLGPHLNPFLSHLAQMYNGWVGN
MKVKVLLAGNAFTAGKIIISCIPPGFAAQNISIAQATMFPHVIADVRLVLEPIEVPLE
DVRNVLFHNNDNTPTMRLVCMLYTPLRASGSSSGTDPFVIAGRVLTCPSPDFSFL
FLVPPNVEQKTKPFSSPNLPLNTLSNSRVPSLIKSMMSVRDHGQMVQFQNGRVT
LDGQLQGTTPTSASQLCKIRGSVFHANGGNGYNLTLDGSPYHAFESPAPIGFPD
15 LGECDWHMEASPTTQFNIGDVIKQINVKQESAFAPHLGTVQVDGLSDVSVNTNM
IAKLGWVSPVSDRHKRDVDPWVIPRYGSTLTEAAQLAPPIYPPGFGEAIVFFMSD
FPIAHGTNGLSVPCTIPQEFVTHFVNEQAPTRGEAALLHYLDPDTNRNLGEFKLY
PDGFMTCPVNSSGTGPQTLPIGVFVFSWVSRYQLKPVGTAGPARRLGIRRS

20 **SEQ ID NO:10****VP1 [Norovirus GII] (Canada 2019)****GenBank: QSD58385.1**

MKMASNDATPSNDGAAGLVPEINNEAMALEPVAGAAIAAPLTGQQNIIDPWIMN
NFVQAPGGEFTVSPRNSPGEVLLNLELGPEINPYLAHLARMYNGYAGGFEVQVV
25 LAGNAFTAGKIIFAAIPPFPIDNLSAAQITMCPHVIVDVRQLEPVNLPMPDVRNN
FFHYNQGSDSLRLVAMLYTPLRANNSGDDVFTVSCRVLTRPSPDFSFNFLVPPT
VESKTKPFTLPILTISEMSNSRFPVPIDSLHTSPTENIVVQCQNGRVTLDGELMGTT
QLLPSQICAFRGVLTRSTSRSADQADTATPRLFNYYWHIQLDNLNGTPYDPAEDI
PGPLGTDPDFRGKVFGVASQRNPDSTTRAHEAKVDTTAGRFTPKLGSLEISTESGD
30 FDQNQPTRFTPVGIGVDHEADFQQWSLPDYSGQFTHNMNLA PAVAPNFPGEQLL

FFRSQLPSSGGRSNGILDCLVPQEWVQHIFYQESAPAQTQVALVRYVNPDTGRVL
FEAKLHKLGFM TVAKNGDSPITVPPNGYFRFESWVNPFFYTLAPMGTGNRRRVQ

5 The disclosures of each and every patent, patent application, and
publication cited herein are hereby incorporated herein by reference in their entirety.
While this invention has been disclosed with reference to specific embodiments, it is
apparent that other embodiments and variations of this invention may be devised by
others skilled in the art without departing from the true spirit and scope of the invention.
The appended claims are intended to be construed to include all such embodiments and
10 equivalent variations.

CLAIMS

What is claimed is:

1. A composition for inducing an immune response against Norovirus (NoV) in a subject, the composition comprising at least one mRNA molecule encoding at least one NoV antigen.
2. The composition of claim 1, wherein the NoV antigen is selected from the group consisting of p48, nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, a fragment thereof, and any combination thereof.
3. The composition of claim 1, wherein the NoV antigen is selected from a genogroup consisting of GI, GII, GIV, GVIII and GIX.
4. The composition of claim 1, comprising a combination of mRNA molecules encoding at least two NoV VP1 antigens, wherein the VP1 antigens are selected from a NoV genogroup consisting of GI and GII.
5. The composition of claim 4, comprising a combination of mRNA molecules encoding at least two NoV VP1 antigens, wherein the VP1 antigens are selected the group consisting of GI.1, GI.3, GI.5, GII.3 and GII.4.
6. The composition of claim 4, comprising a combination of an mRNA molecule encoding a NoV VP1 GI.1 antigen and an mRNA molecule encoding a NoV VP1 GII.4 antigen.
7. The composition of claim 4, comprising a combination of mRNA molecules encoding each of a NoV VP1 GI.1 antigen, a NoV VP1 GII.4 antigen, a NoV VP1 GI.3 antigen and a NoV VP1 GII.3 antigen.

8. The composition of claim 1, wherein the composition comprises at least one mRNA molecule encoding an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

9. The composition of claim 1, wherein the composition further comprises an adjuvant.

10. The composition of claim 1, wherein the mRNA molecule is encapsulated within a lipid nanoparticle (LNP).

11. The composition of claim 1, wherein the mRNA molecule is a nucleoside modified mRNA molecule comprising at least one modified nucleoside selected from the group consisting of pseudouridine, 1-methyl pseudouridine, and 5-methyl-uridine.

12. A method of inducing an immune response against at least one strain of Norovirus (NoV) in a subject comprising administering to the subject an effective amount of a composition comprising a mRNA molecule encoding at least one NoV antigen or a fragment thereof.

13. The method of claim 12, wherein the NoV antigen is selected from the group consisting of p48, nucleoside-triphosphatase (NTPase), p22, VPg, protease, and the RNA-dependent RNA polymerase (RdRp), VP1, VP2, a fragment thereof, and any combination thereof.

14. The method of claim 12, wherein the NoV antigen is selected from a genogroup consisting of GI, a GII, GIV, GVIII and GIX.

15. The method of claim 12, comprising a combination of mRNA molecules

encoding at least two NoV VP1 antigens, wherein the VP1 antigens are selected from a NoV genogroup consisting of GI and GII.

16. The method of claim 15, comprising a combination of mRNA molecules encoding at least two NoV VP1 antigens, wherein the VP1 antigens are selected the group consisting of GI.1, GI.3, GI.5, GII.3 and GII.4.

17. The method of claim 15, comprising a combination of an mRNA molecule encoding a NoV VP1 GI.1 antigen and an mRNA molecule encoding a NoV VP1 GII.4 antigen.

18. The method of claim 15, comprising a combination of mRNA molecules encoding each of a NoV VP1 GI.1 antigen, a NoV VP1 GII.4 antigen, a NoV VP1 GI.3 antigen and a NoV VP1 GII.3 antigen.

19. The method of claim 12, wherein the composition comprises at least one mRNA molecule encoding an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

20. The method of claim 12, wherein the composition further comprises an adjuvant.

21. The method of claim 12, wherein the mRNA molecule is encapsulated within a lipid nanoparticle (LNP).

22. The method of claim 12, wherein the mRNA molecule is a nucleoside modified mRNA molecule comprising at least one modified nucleoside selected from the group consisting of pseudouridine, 1-methyl pseudouridine, and 5-methyl-uridine.

23. The method of claim 12, wherein the composition is administered by a

delivery route selected from the group consisting of intradermal, subcutaneous, inhalation, intranasal, and intramuscular.

24. The method of claim 12, wherein the method comprises a single administration of the composition.

25. The method of claim 12, wherein the method comprises multiple administrations of the composition.

26. The method of claim 12, wherein the composition treats or prevents a disease or disorder associated with NoV infection.

27. The method of claim 26, wherein the disease or disorder associated with NoV infection is selected from the group consisting of gastroenteritis, food poisoning, vomiting and diarrhea.

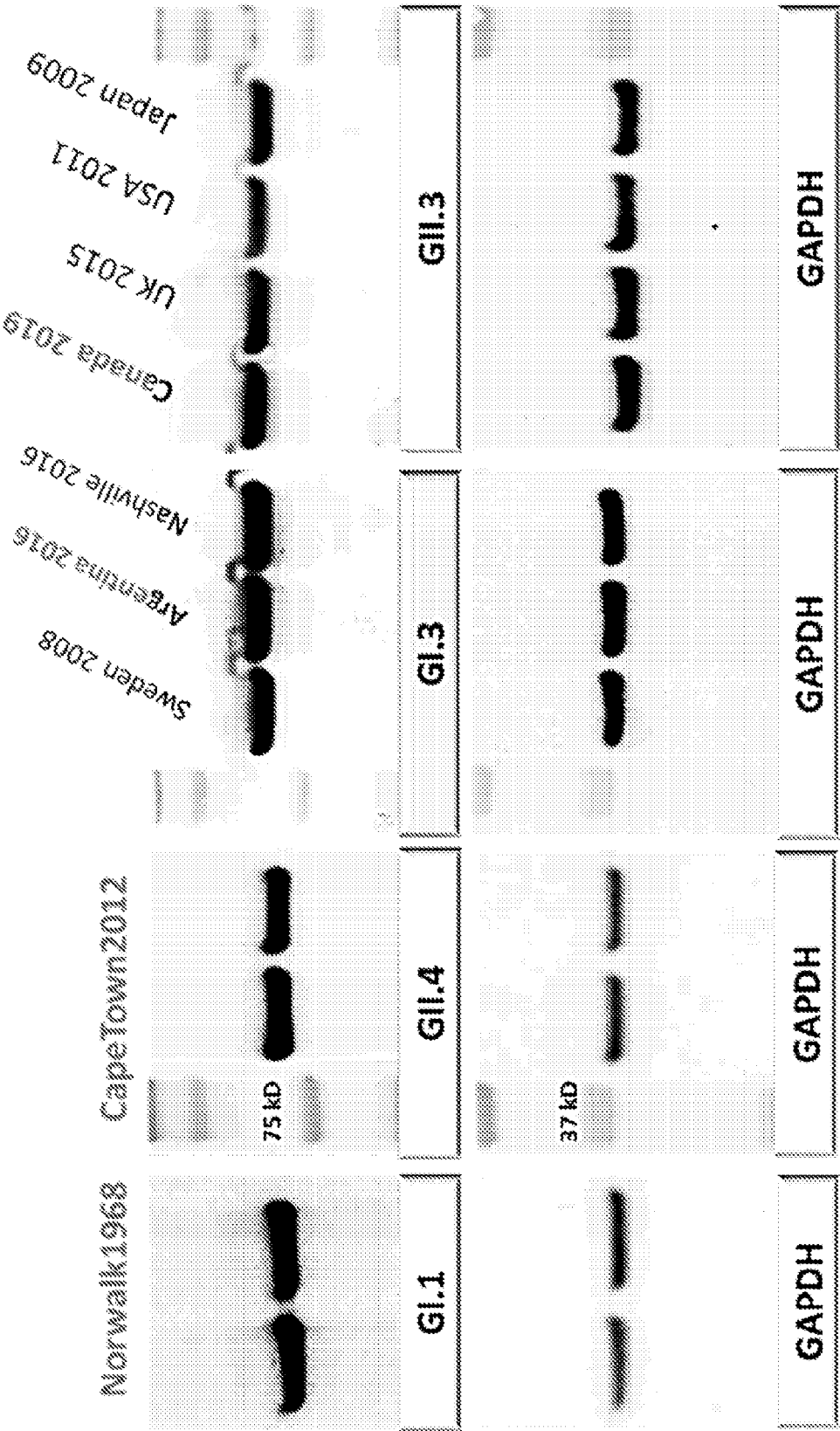


Fig. 1

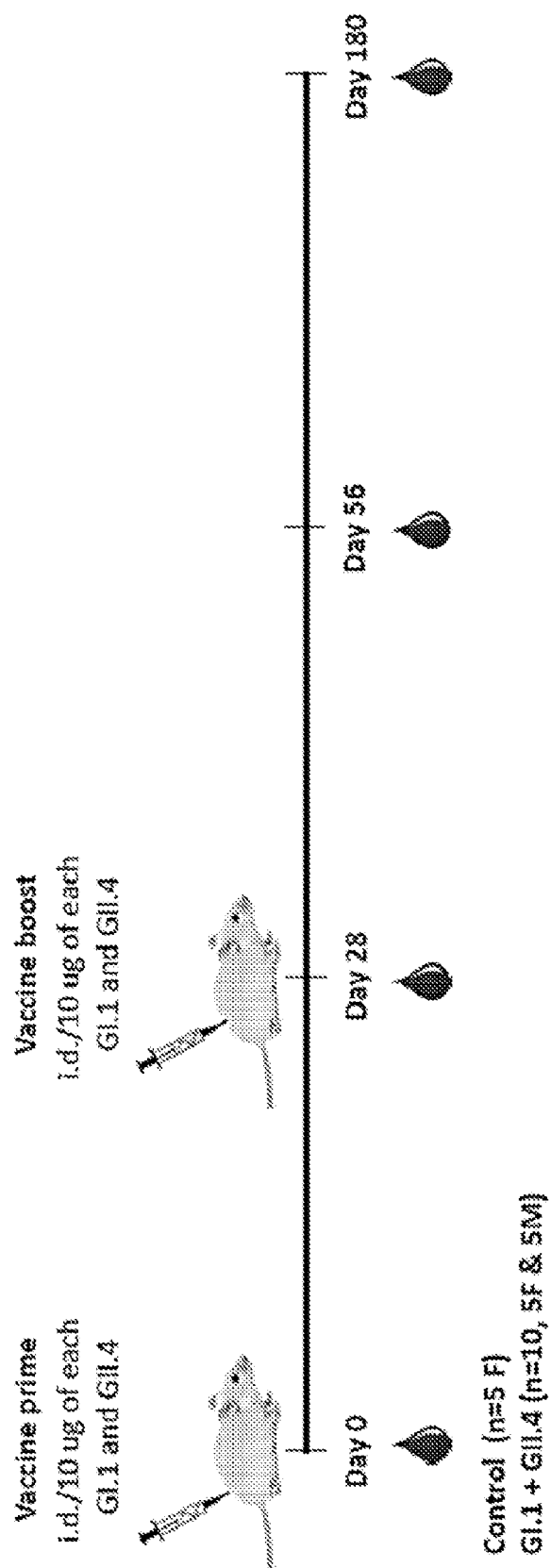


Fig. 2

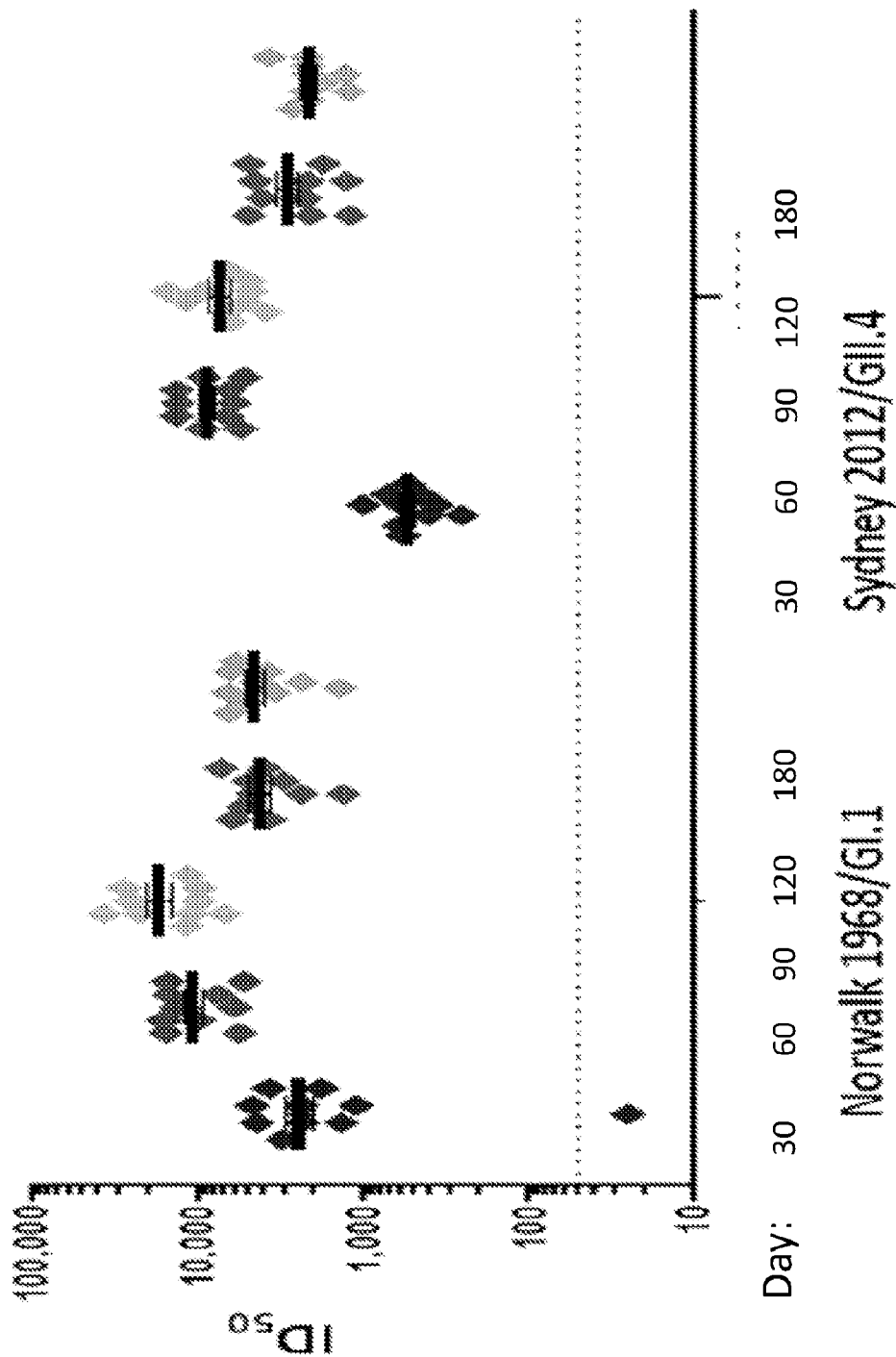


Fig. 3

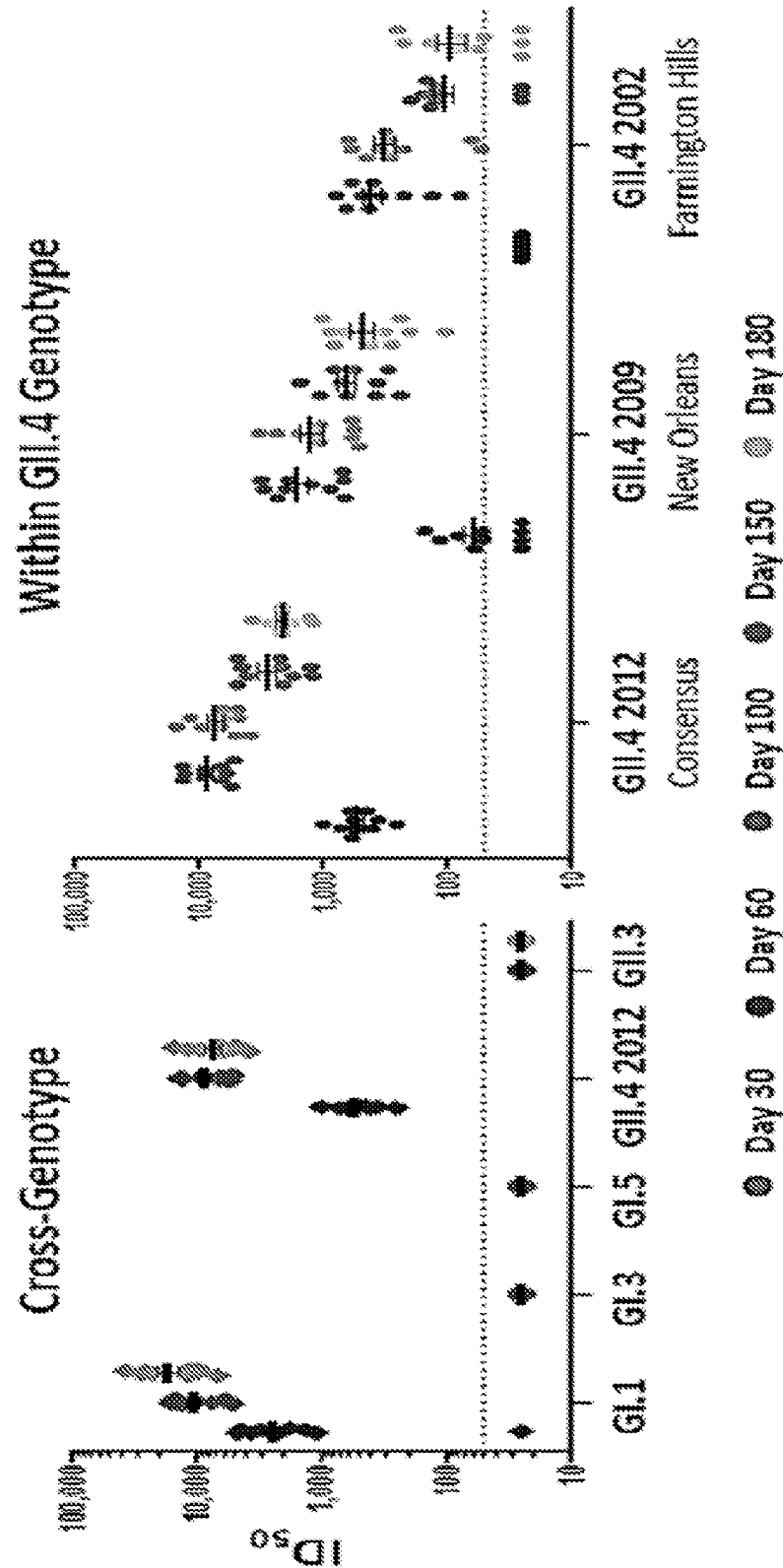


Fig. 4

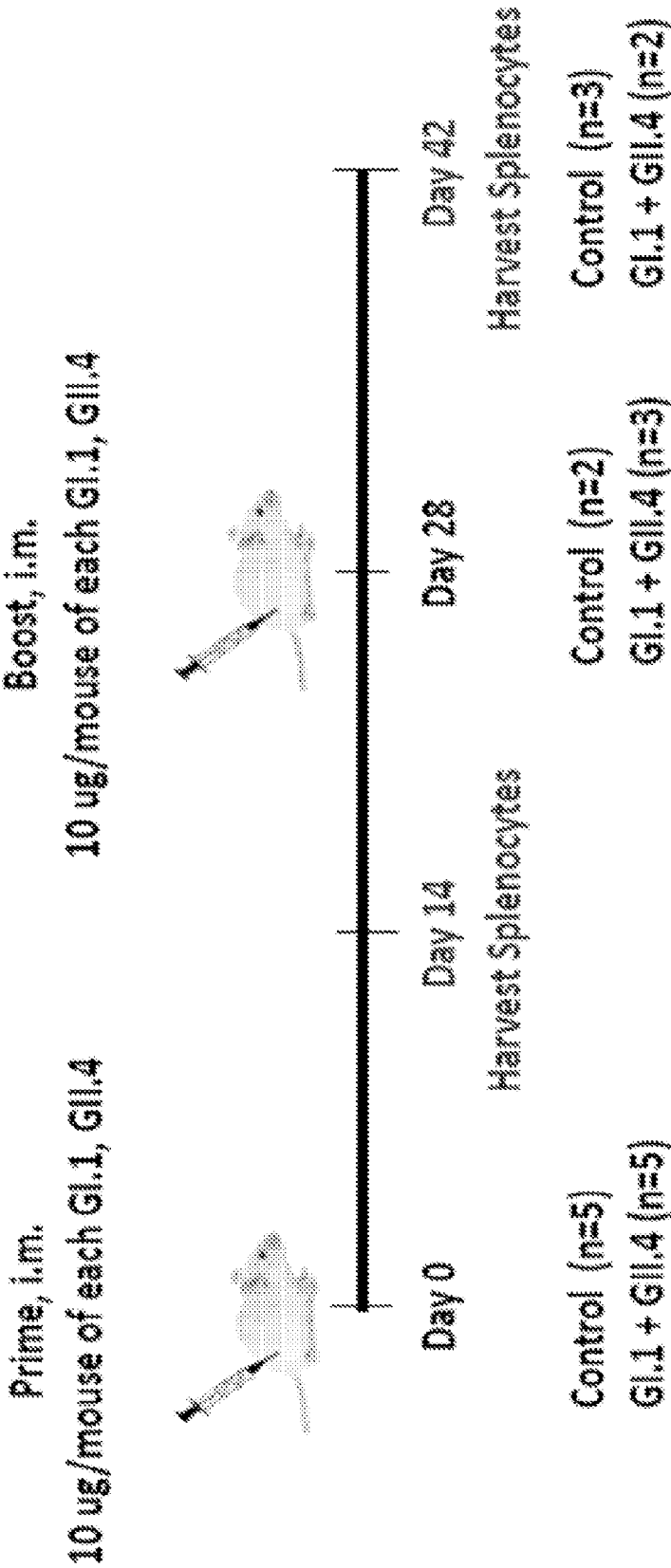


Fig. 5

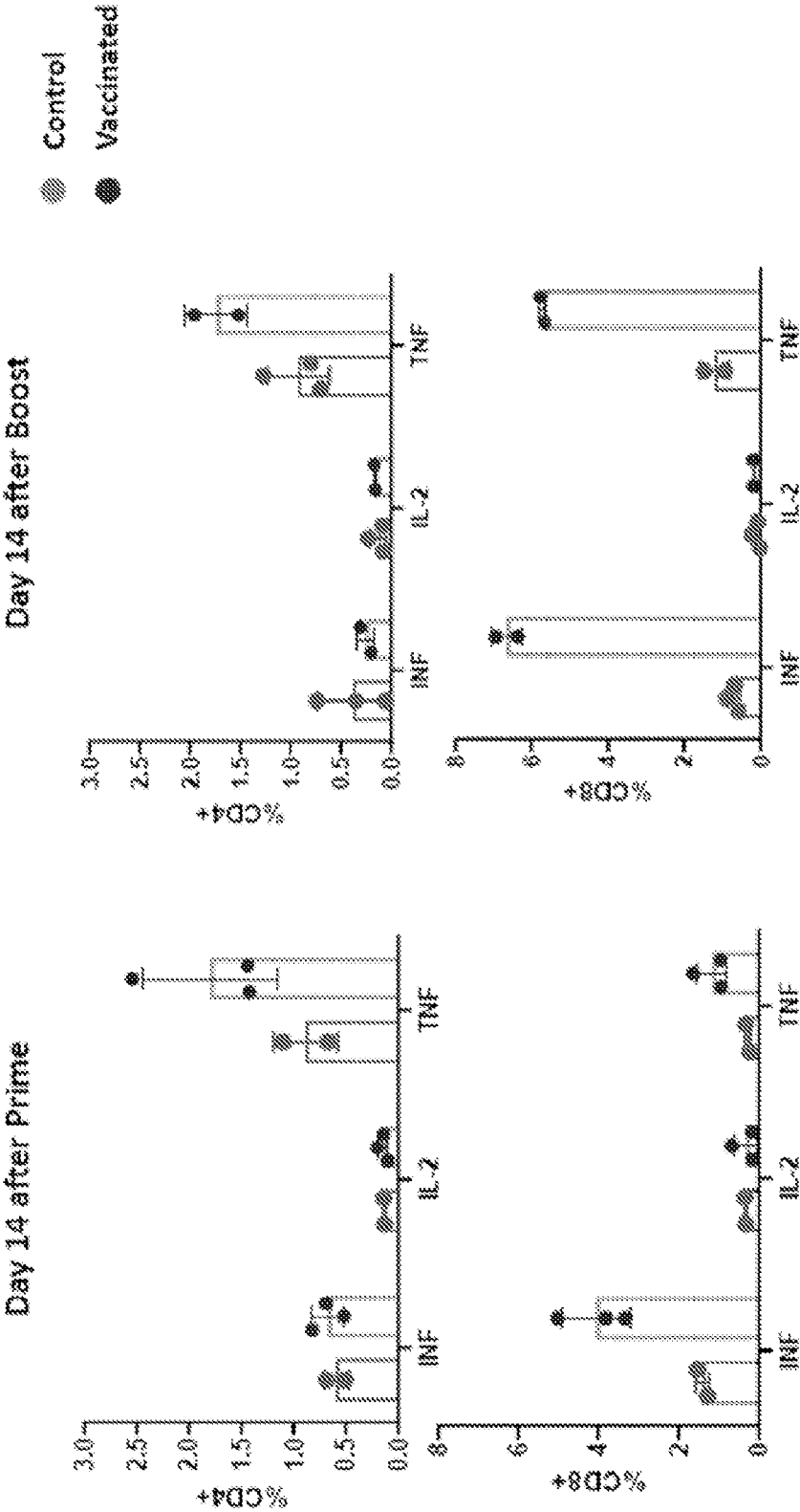


Fig. 6

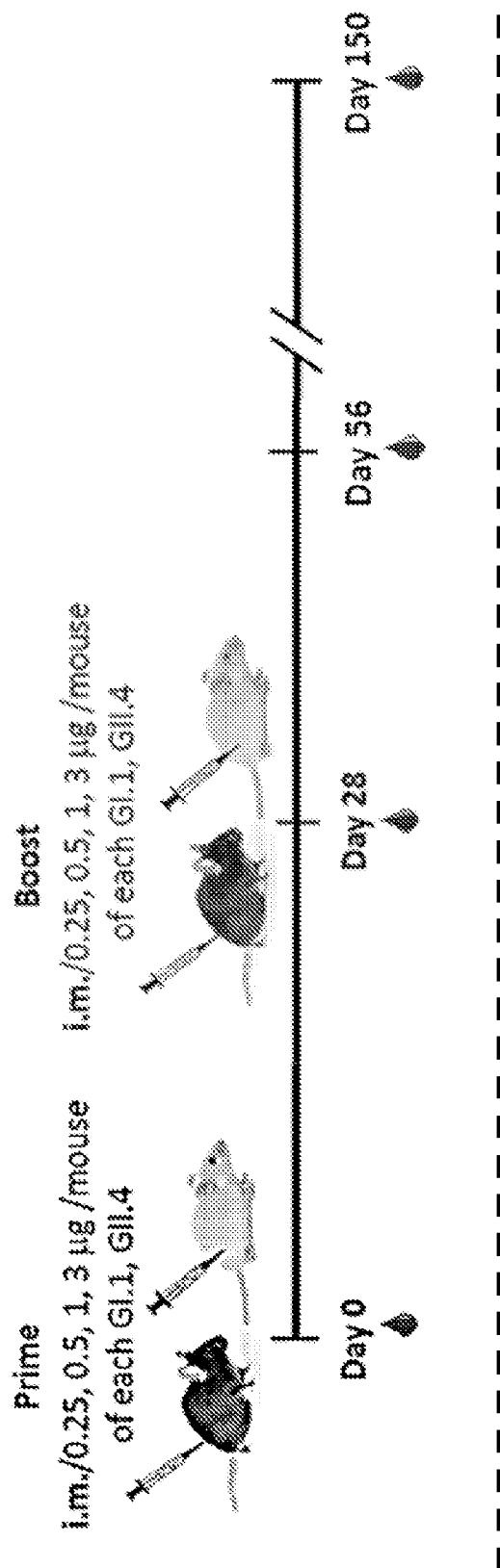


Fig. 7

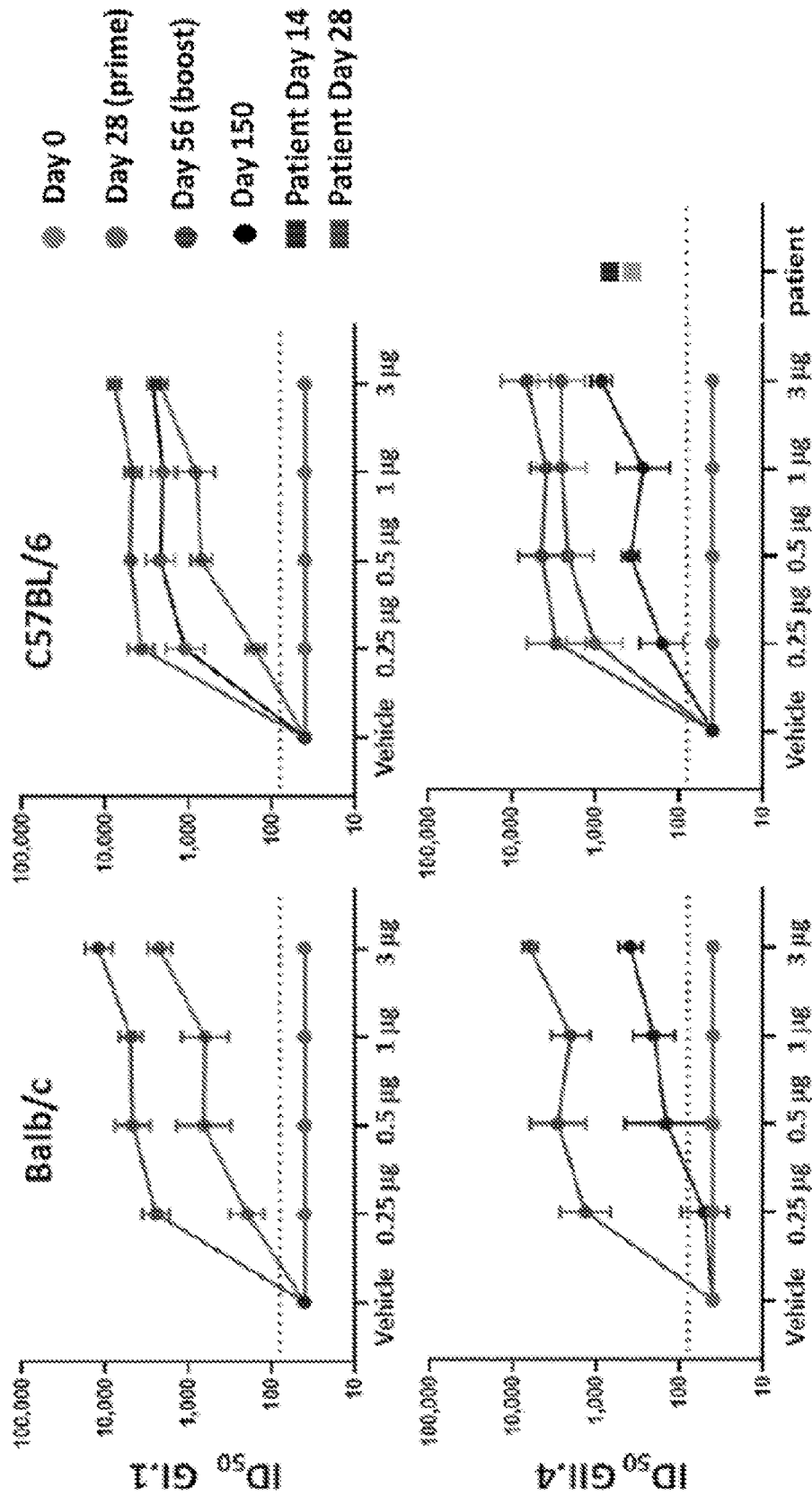
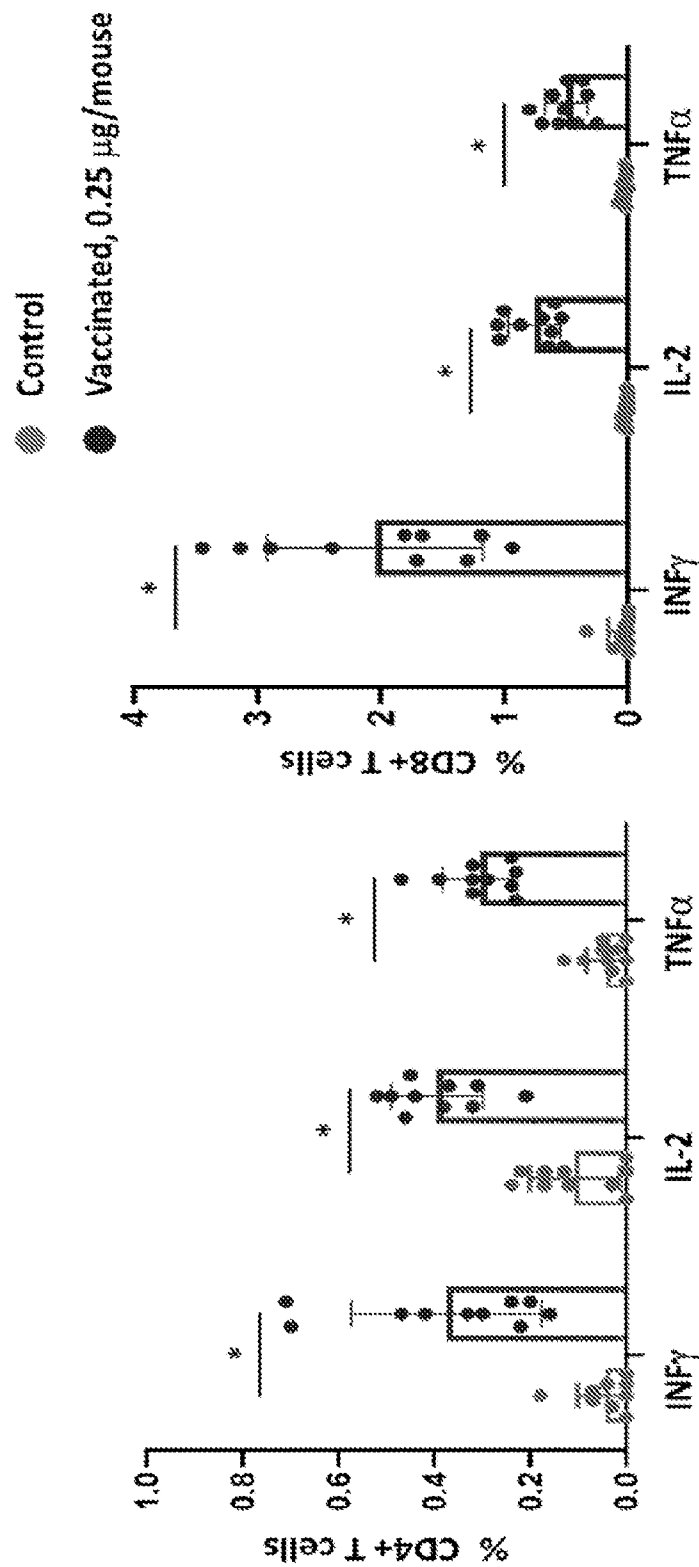


Fig. 7 (Continued)



Mean $\pm$ SEM, n=10, *p<0.05, (ANOVA, Tukey's tests).

Fig. 8

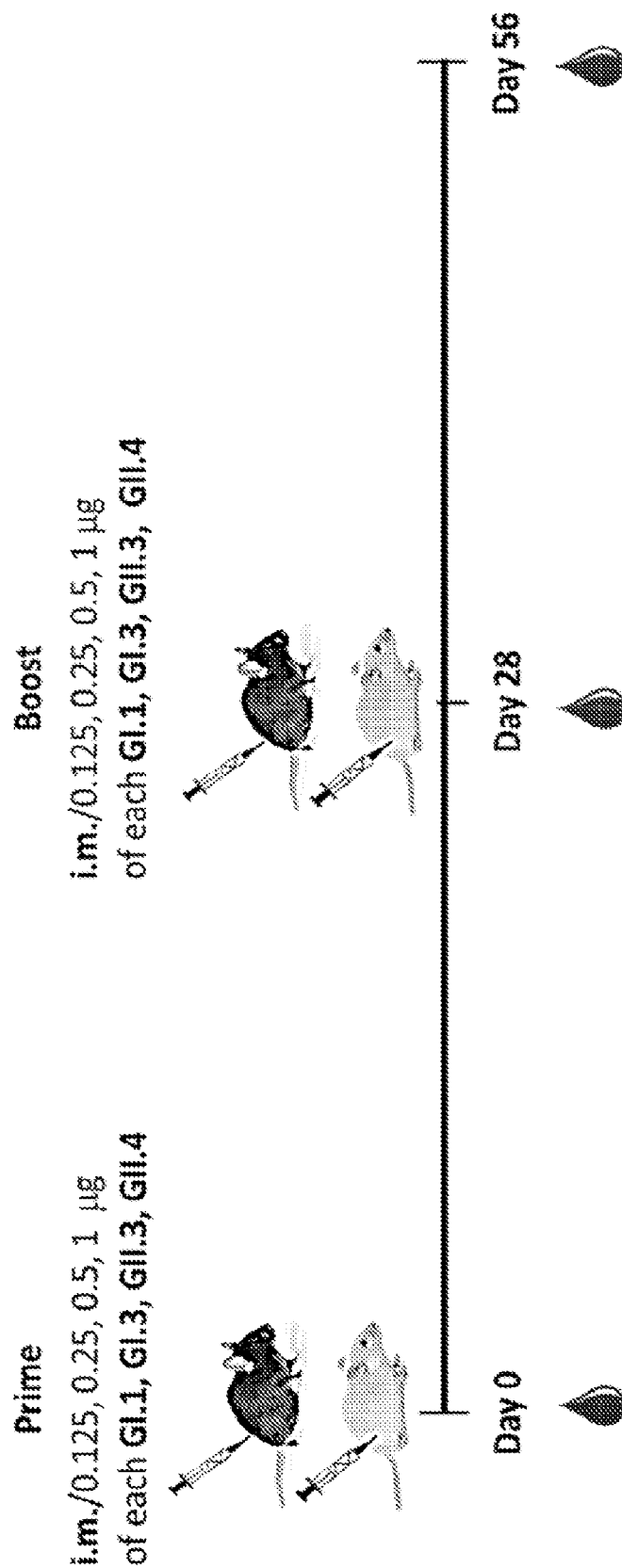


Fig. 9

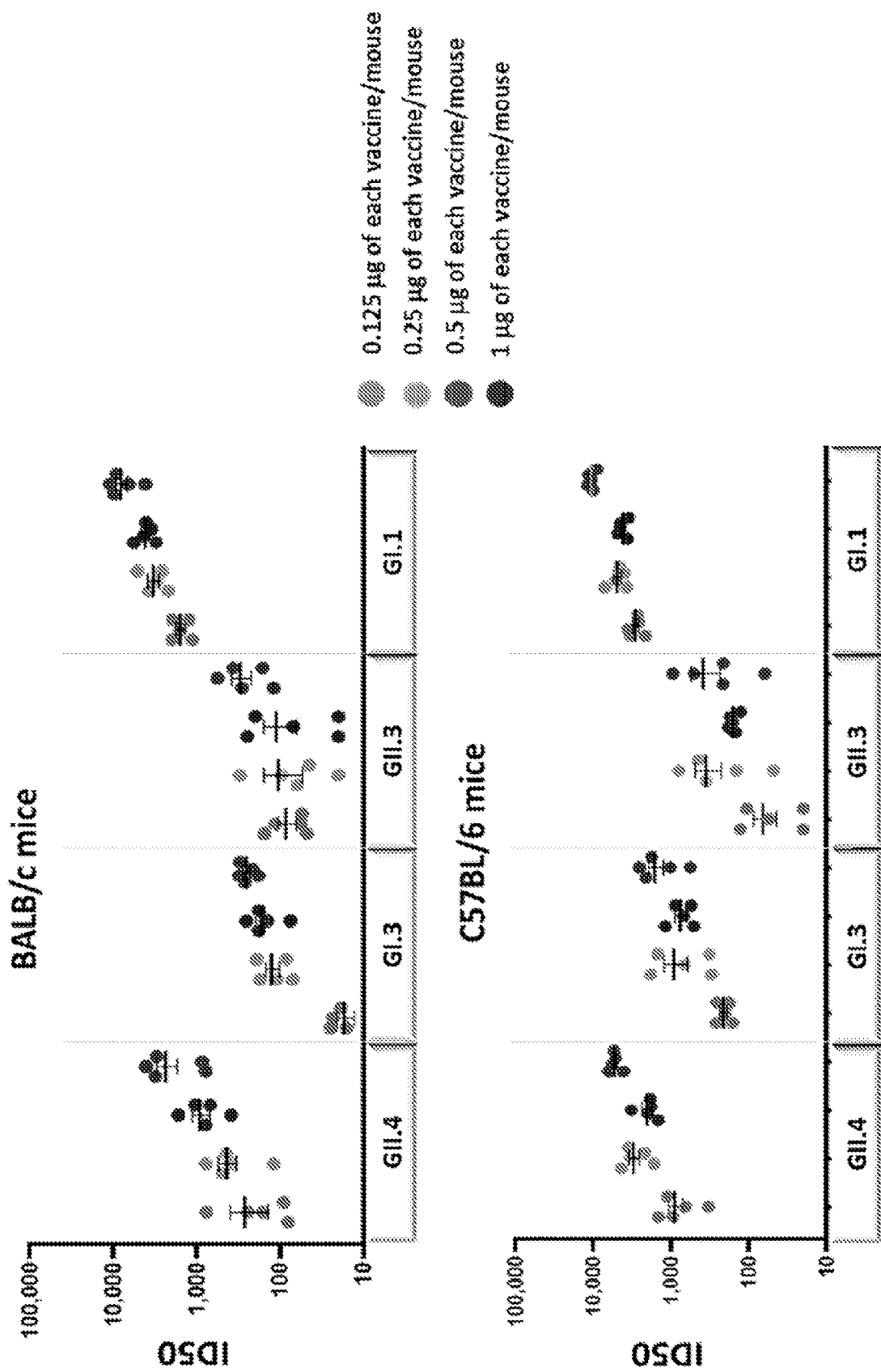
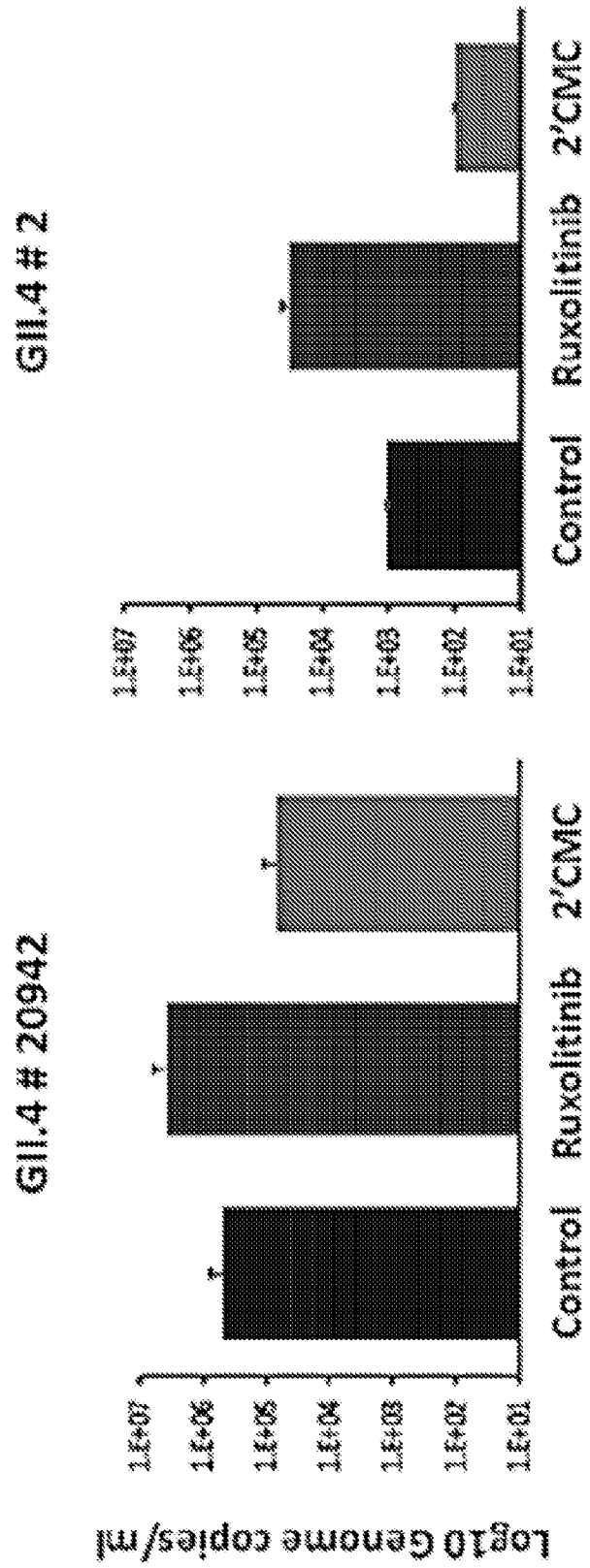


Fig. 10



Ruxolitinib – inhibitor of IFN- γ signaling
2'CMC - RNA polymerase inhibitor

Fig. 11

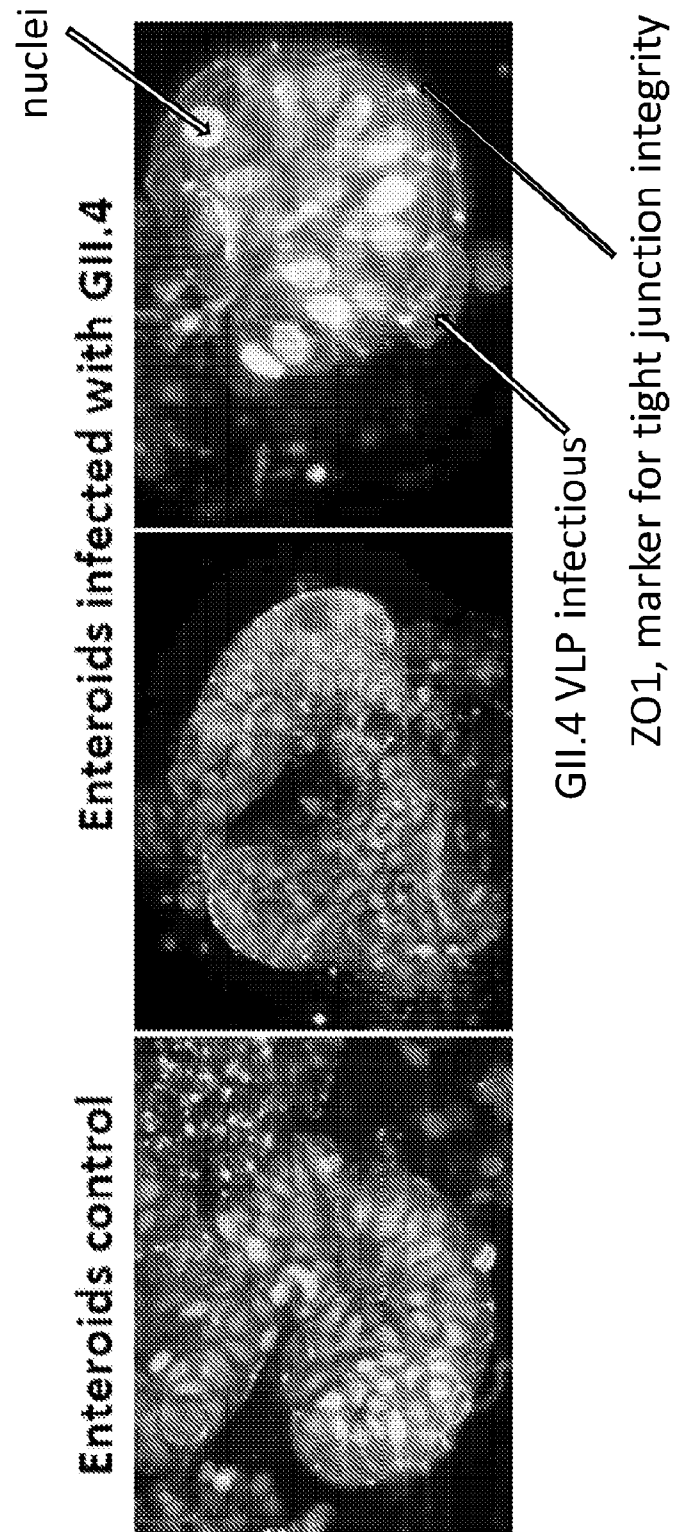


Fig. 12

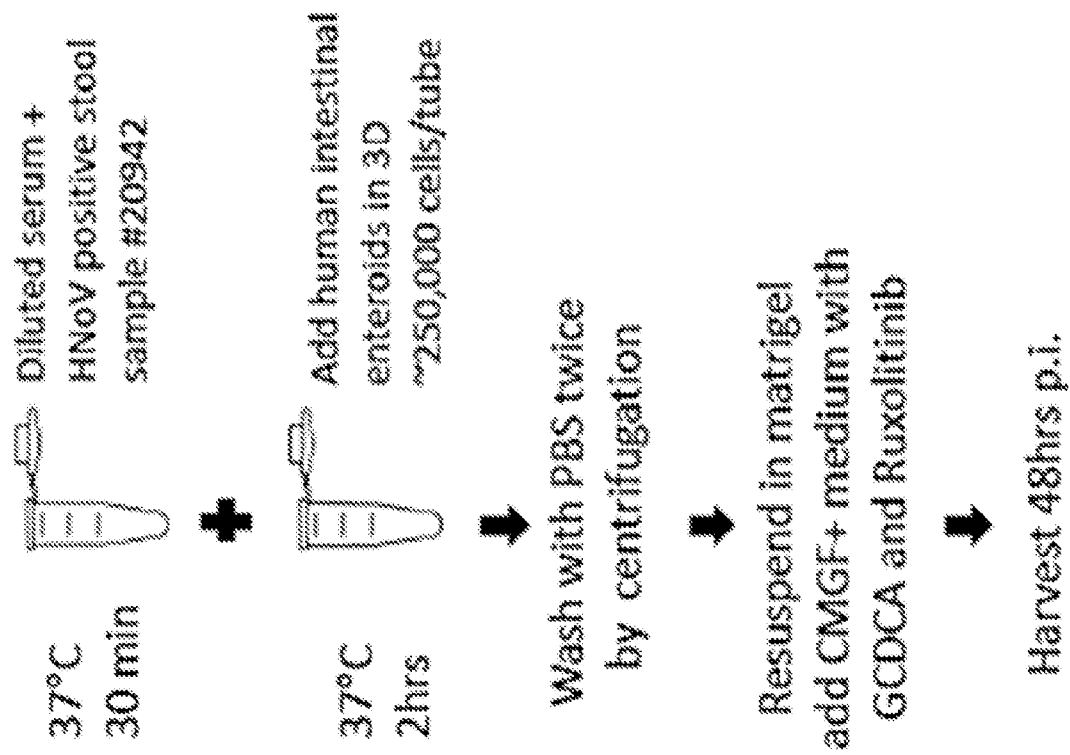


Fig. 13

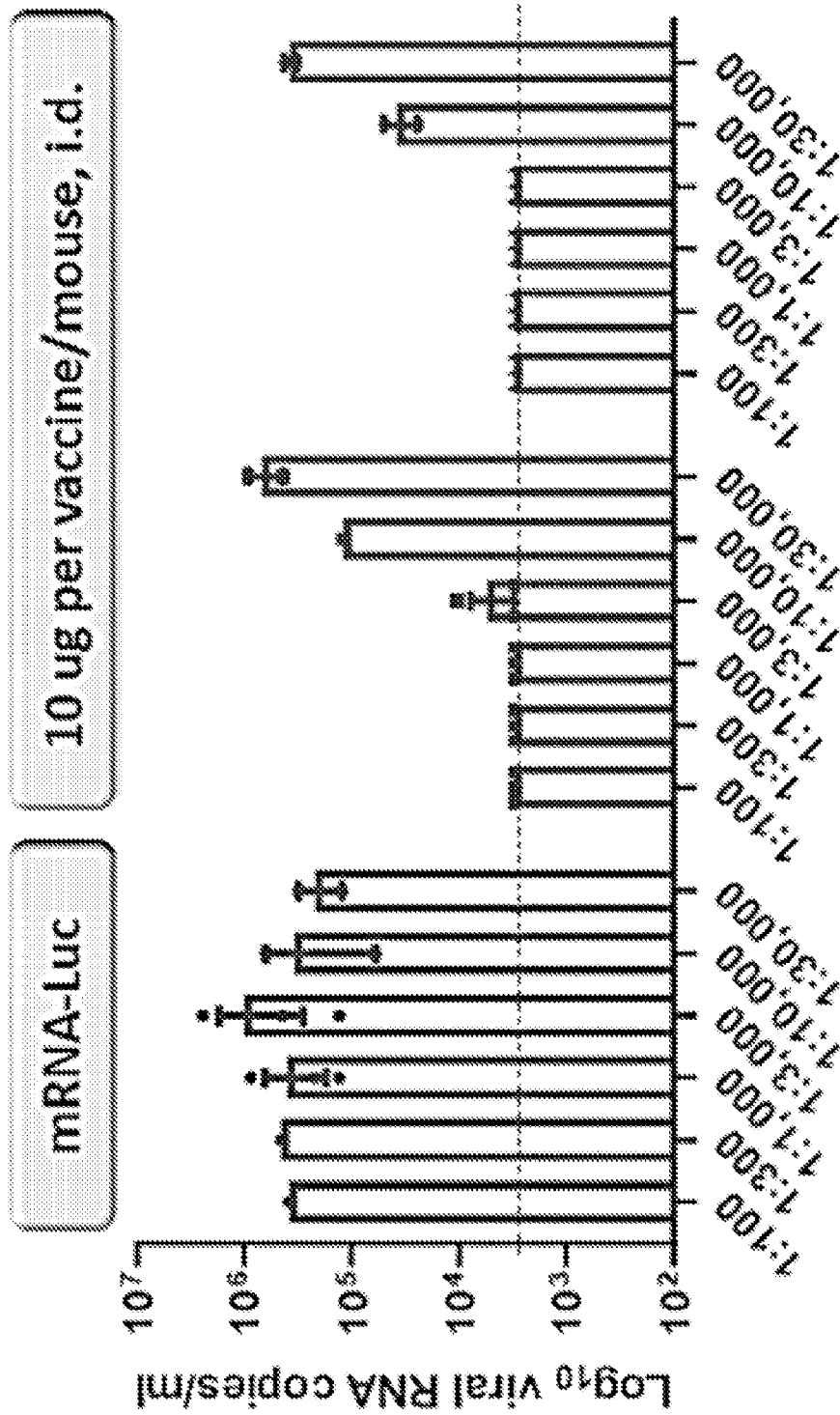


Fig. 13 (Continued)

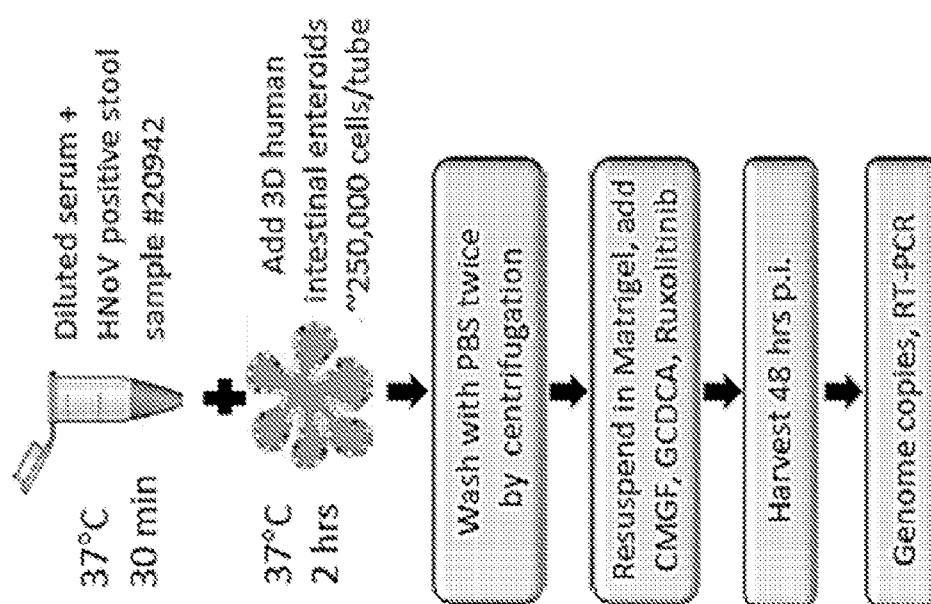


Fig. 14

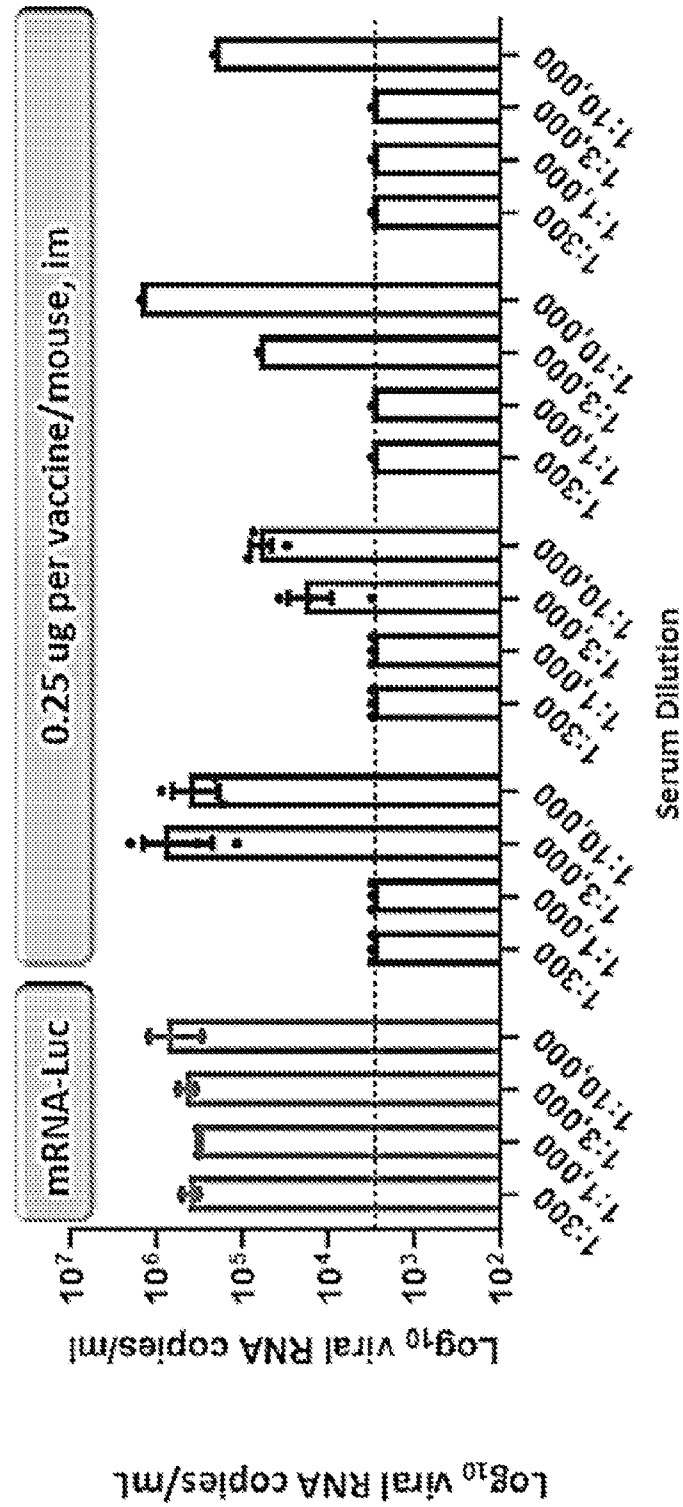


Fig. 14 (Continued)

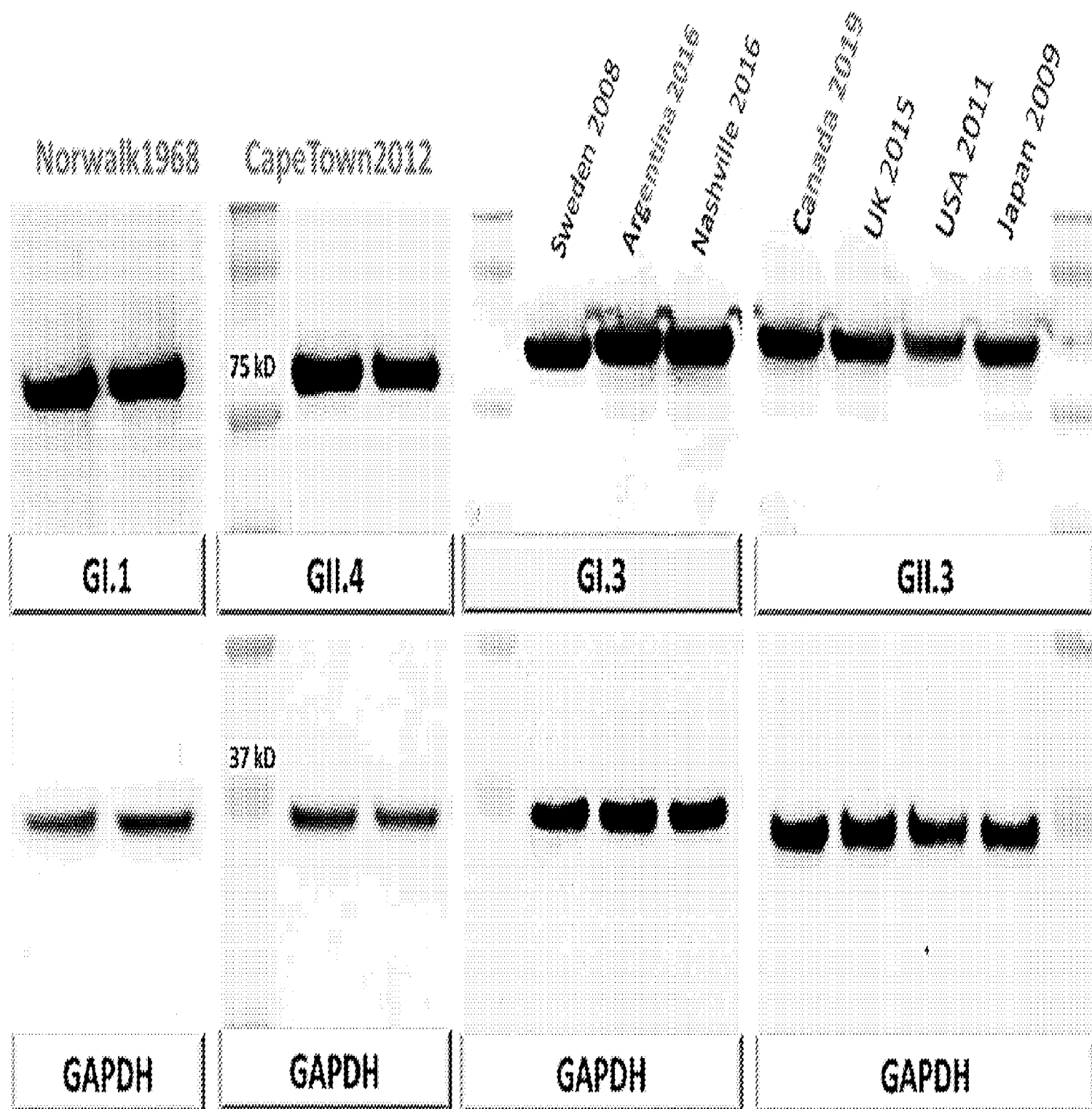


Fig. 1