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(71) Applicant: **MERCK SHARP & DOHME CORP.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

(72) Inventors; and

(71) Applicants *(for US only)*: **GINDY, Marian, E.** [US/US]; 770 Sumneytown Pike, West Point, Pennsylvania 19486 (US). **CASIMIRO, Danilo, R.** [US/US]; 626 Westbourne Road, Harleysville, Pennsylvania 19438 (US). **KAUFMAN, David, R.** [US/US]; 770 Sumneytown Pike, West Point, Pennsylvania 19486 (US). **PHAN, Uyen Truong** [US/US]; 901 S. California Avenue, Palo Alto, California 94304 (US).

(74) Common Representative: **MERCK SHARP & DOHME CORP.**; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

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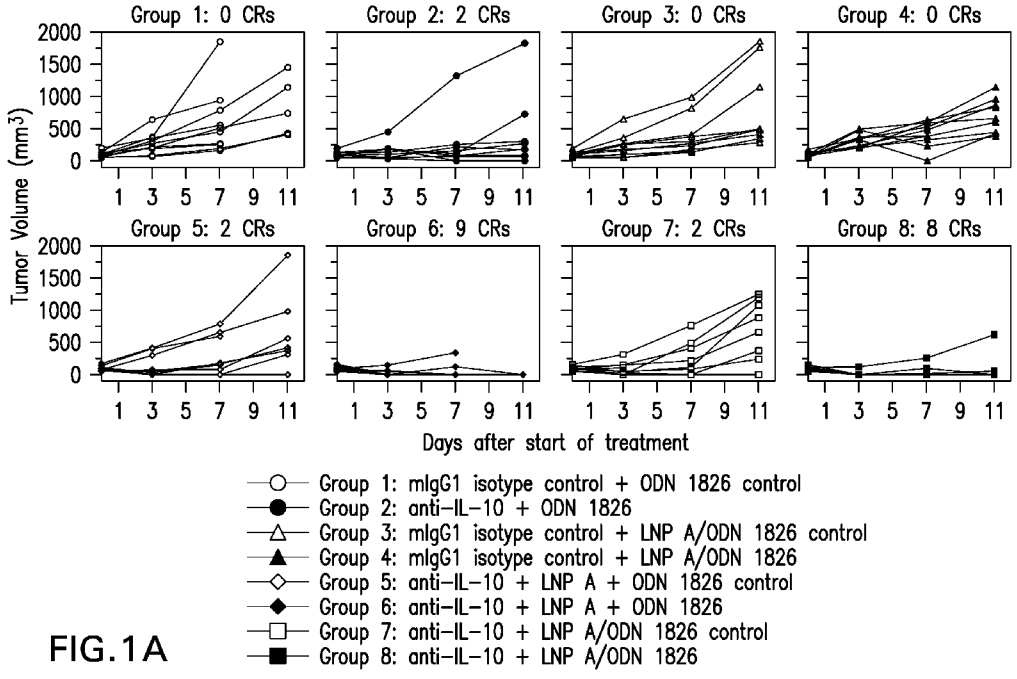

FIG.1A

(57) Abstract: The present invention is directed to a composition comprising: a) a lipid nanoparticle (LNP) comprising one or more cationic lipids and a poly(ethyleneglycol)-lipid (PEG-lipid); b) a Toll-like receptor 9 (TLR9) agonist CpG oligonucleotide; and c) an anti-IL-10 antibody or antigen binding fragment thereof. The present invention is also directed to methods of treating cancer in the subject by co-administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) an anti-IL-10 antibody or antigen binding fragment thereof.

[Continued on next page]

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COMBINATION THERAPY OF ANTI-IL-10 ANTIBODY AND COMPOSITIONS COMPRISING LIPID NANOPARTICLES AND TLR9 AGONIST CpG OLIGONUCLEOTIDES

FIELD OF THE INVENTION

The present invention relates to combinations of an anti-IL-10 antibody and compositions comprising a TLR9 agonist CpG oligonucleotide and lipid nanoparticles. The lipid nanoparticles are composed of a combination of cationic lipids with other lipid components such as PEG-lipids and optionally non-cationic lipids.

BACKGROUND OF THE INVENTION

Initially known as cytokine synthesis inhibitor factor or CSIF, interleukin-10 (IL-10) is a potent immunomodulator of hematopoietic cells, particularly immune cells. Cells such as activated Th2 cells, B cells, keratinocytes, monocytes and macrophages produce IL-10. *See, e.g.*, Moore et al., *Annu. Rev. Immunol.* 11:165 (1993). IL-10 inhibits activation and effector functions of a number of cells that include T cells, monocytes and macrophages. In particular, IL-10 inhibits cytokine synthesis, including that of IL-1, IFN-γ, and TNF, by cells such as Th1 cells, natural killer cells, monocytes, and macrophages. *See, e.g.*, Fiorentino et al., *J. Exp. Med.*, 170:2081-2095 (1989); Fiorentino et al., *J. Immunol.* 146:3444 (1991); Hsu et al., *Int. Immunol.* 4:563 (1992); Hsu et al., *Int. Immunol.* 4:563 (1992); D'Andrea et al., *J. Exp. Med.* 178:1041 (1993); de Waal Malefyt et al., *J. Exp. Med.* 174:915 (1991); Fiorentino et al., *J. Immunol.* 147:3815 (1991).

The production of IL-10 in the tumor microenvironment by tumor infiltrating macrophages, dendritic cells, and $CD4^+$ and $CD8^+$ T cells has been shown to inhibit tumor eradication by the immune system (see, e.g., Jarnicki, et al. (2006) *J. Immunol.* 896-904). Targeting IL-10 with an antagonist of IL-10 could provide potent immunostimulatory activity and tumor eradication.

Administration of certain DNA sequences, generally known as immunostimulatory sequences, induces an immune response with a Th1-type bias as indicated by secretion of Th1-associated cytokines. Administration of an immunostimulatory polynucleotide with an antigen results in a Th1-type immune response to the administered

antigen. Roman et al. (1997) Nature Med. 3:849-854. For example, mice injected intradermally with Escherichia coli (E. coli) β-galactosidase (β-Gal) in saline or in the adjuvant alum responded by producing specific IgG1 and IgE antibodies, and $CD4^+$ cells that secreted IL-4 and IL-5, but not IFN-γ, demonstrating that the T cells were predominantly of the Th2 subset. However, mice injected intradermally (or with a tyne skin scratch applicator) with plasmid DNA (in saline) encoding β-Gal and containing an immunostimulatory sequence responded by producing IgG2a antibodies and $CD4^+$ cells that secreted IFN-γ, but not IL-4 and IL-5, demonstrating that the T cells were predominantly of the Th1 subset. Moreover, specific IgE production by the plasmid DNA-injected mice was reduced 66-75%. Raz et al. (1996) Proc. Natl. Acad. Sci. USA 93:5141-5145. In general, the response to naked DNA immunization is characterized by production of IL-2, TNFα and IFN-γ by antigen-stimulated $CD4^+$ T cells, which is indicative of a Th1-type response. This is particularly important in treatment of allergy and asthma as shown by the decreased IgE production. The ability of immunostimulatory polynucleotides to stimulate a Th1-type immune response has been demonstrated with bacterial antigens, viral antigens and with allergens (see, for example, WO 98/55495).

Lipid nanoparticles (LNPs) constitute an alternative to other particulate systems, such as emulsions, liposomes, micelles, microparticles and/or polymeric nanoparticles, for the delivery of active ingredients, such as oligonucleotides, peptides, monoclonal antibodies and small molecule pharmaceuticals. LNPs and their use for the delivery of oligonucleotides have been previously disclosed. See U.S. Pat. No. 7,691,405, U.S. Patent Application Publication Nos: US 2006/0083780, US 2006/0240554, US 2008/0020058, US 2009/0263407 and US 2009/0285881; and International Patent Application Publication Nos.: WO 2009/086558, WO2009/127060, WO2009/132131, WO2010/042877, WO2010/054384, WO2010/054401, WO2010/054405 and WO2010/054406. See also Semple *et al.*, 2010, *Nat. Biotechnol. 28*:172-176. Lipid-based nanoparticles as pharmaceutical drug carriers have also been disclosed. See Puri *et al.*, 2009, *Crit. Rev. Ther. Drug Carrier Syst.* 26:523-580.

Other cationic lipids are disclosed in U.S. Patent Application Publication Nos. US 2009/0263407, US 2009/0285881, US 2010/0055168, US 2010/0055169, US 2010/0063135, US 2010/0076055, US 2010/0099738 and US 2010/0104629. Other formulations for delivery of active agents having charged lipids are described in U.S. Pat. No. 6,890,557. Lipid nanoparticle capsules are described in U.S. Patent Application Publication No. 2013/0017239.

SUMMARY OF THE INVENTION

The present invention is directed to a composition comprising: a) a lipid nanoparticle (LNP) comprising one or more cationic lipids and a poly(ethyleneglycol)-lipid (PEG-lipid); b) a Toll-like receptor 9 (TLR9) agonist CpG oligonucleotide; and c) an anti-IL-10 antibody or an antigen-binding fragment thereof.

In one embodiment, the cationic lipid is an ionizable cationic lipid, which may be selected from DLinDMA; DlinKC2DMA; DLin-MC3-DMA; CLinDMA; S-Octyl CLinDMA; (2 S)-1-{7-[(3 β)-cholest-5-en-3-yloxy]heptyloxy}-3-[(4 Z)-dec-4-en-1-yloxy]-N, N -dimethylpropan-2-amine; (2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-3-[(4 Z)-dec-4-en-1-yloxy]- N, N -dimethylpropan-2-amine; 1-[(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-3-(octyloxy)propan-2-yl]guanidine; 1-[(2 R)-1-{7-[(3 β)-cholest-5-en-3-yloxy]heptyloxy}-N,N-dimethyl-3-[(9 Z, 12 Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine; 1-[(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-N,N-dimethyl-3-[(9 Z, 12 Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine; (2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine; (3β)-3-[6-{ [(2S)-3-[(9Z)-octadec-9-en-1-yloxyl]-2-(pyrrolidin-1-yl)propyl]oxy}hexyl)oxy] cholest-5-ene; (2R)-1-{4-[(3β)-cholest-5-en-3-yloxy]butoxy}-3-(octyloxy)propan-2-amine; (2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-(pentyloxy)propan-2-amine; (2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-3-(heptyloxy)-N,N-dimethylpropan-2-amine; (2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(2Z)-pent-2-en-1-yloxy]propan-2-amine; (2S)-1-butoxy-3-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethylpropan-2-amine; (2S-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-3-[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononyl)oxy]-N,N-dimethylpropan-2-amine; 2-amino-2-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}propane-1,3-diol; 2-amino-3-((9-(((3S,10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)nonyl)oxy)-2-((((9Z,12Z)-octadeca-9,12-dien-1-yl)oxy)methyl)propan-1-ol; 2-amino-3-((6-(((3S,10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)hexyl)oxy)-2-((((Z)-octadec-9-en-1-yl)oxy)methyl)propan-1-ol; (20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine; (17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-9-amine; (16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-8-amine; (13Z,16Z)-N,N-dimethyldocosa-13,16-dien-5-amine; (12Z,15Z)-N,N-dimethylhenicosa-12,15-dien-4-amine; (14Z,17Z)-N,N-dimethyltricosa-14,17-dien-6-amine;

(15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-7-amine; (18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-10-amine; (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-5-amine; (14Z,17Z)-N,N-dimethyltricosa-14,17-dien-4-amine; (19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-9-amine; (18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-8-amine; (17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-7-amine; (16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-6-amine; (22Z,25Z)-N,N-dimethylhentriaconta-22,25-dien-10-amine; (21Z,24Z)-N,N-dimethyltriaconta-21,24-dien-9-amine; (18Z)-N,N-dimethylheptacos-18-en-10-amine; (17Z)-N,N-dimethylhexacos-17-en-9-amine; (19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-7-amine; N,N-dimethylheptacosan-10-amine; (20Z,23Z)-N-ethyl-N-methylnonacosa-20,23-dien-10-amine; 1-[(11Z,14Z)-1-nonylicosa-11,14-dien-1-yl]pyrrolidine; (20Z)-N,N-dimethylheptacos-20-en-10-amine; (15Z)-N,N-dimethylheptacos-15-en-10-amine; (14Z)-N,N-dimethylnonacos-14-en-10-amine; (17Z)-N,N-dimethylnonacos-17-en-10-amine; (24Z)-N,N-dimethyltritriacont-24-en-10-amine; (20Z)-N,N-dimethylnonacos-20-en-10-amine; (22Z)-N,N-dimethylhentriacont-22-en-10-amine; (16Z)-N,N-dimethylpentacos-16-en-8-amine; (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine; (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine; N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine; 1-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine; N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]nonadecan-10-amine; N,N-dimethyl-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine; N,N-dimethyl-1-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]nonadecan-10-amine; N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine; N,N-dimethyl-1-[(1R,2S)-2-undecylcyclopropyl]tetradecan-5-amine; N,N-dimethyl-3-{7-[(1S,2R)-2-octylcyclopropyl]heptyl}dodecan-1-amine; 1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine; 1-[(1S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine; N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine; and (11E,20Z,23Z)-N,N-dimethylnonacosa-11,20,23-trien-10-amine; or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the recited compounds or salts, or any combination thereof. In one aspect of this embodiment, the ionizable cationic lipid is selected from (2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine; (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine; and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine; or a pharmaceutically acceptable salt thereof, or a stereoisomer of any of the foregoing, or any combination of the foregoing.

In certain embodiments, the LNP comprises 80-99.9 mole % ionizable cationic lipid and 0.1-20 mole % PEG-lipid. In certain embodiments of the invention, the LNP further comprises one or more non-cationic lipids which can be selected from a phospholipid, a phospholipid derivative, a fatty acid, a sterol, or a combination thereof. The sterol may be cholesterol, stigmasterol or stigmastanol. Natural phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI), Phosphatidic acid (phosphatidate) (PA), dipalmitoylphosphatidylcholine, monoacyl-phosphatidylcholine (lyso PC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), N-Acyl-PE, phosphoinositides, and phosphosphingolipids. Phospholipid derivatives include phosphatidic acid (DMPA, DPPA, DSPA), phosphatidylcholine (DDPC, DLPC, DMPC, DPPC, DSPC, DOPC, POPC, DEPC), phosphatidylglycerol (DMPG, DPPG, DSPG, POPG), phosphatidylethanolamine (DMPE, DPPE, DSPE DOPE), phosphatidylserine (DOPS). Fatty acids include C14:0, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4), C20:0, C22:0 and lethicin. In certain embodiments of the invention, the phospholipid may be phosphatidylserine, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), dilauroylphosphatidylcholine (DLPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine, or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC).

In certain embodiments of the invention, the PEG-lipid is 1,2-Dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA). In certain aspects of this embodiment, the PEG-lipid comprises a polyethylene glycol having an average molecular weight ranging from about 500 daltons to about 10,000 daltons.

In one embodiment of the invention, the LNP comprises 20-99.8 mole % ionizable cationic lipid, 0.1-65 mole % non-cationic lipids, and 0.1-20 mole % PEG-lipid. In one aspect of this embodiment, the non-cationic lipids comprise a mixture of cholesterol and DSPC.

In one aspect of this embodiment, the TLR9 agonist is selected from A-class CpG ODN, B-class CpG ODN and C-class CpG ODN. In one embodiment, the B-class CpG ODN is 5'TCCATGACGTTCCTGACGTT 3' (SEQ ID NO: 22). The agonist may be

physically encapsulated in the LNP before or after LNP preparation. The agonist may be adsorbed, covalently coupled, ionically-interacted or formulated onto the surface of the LNP.

In certain embodiments of the invention, the lipid nanoparticle comprises 34-59 mole % ionizable cationic lipid selected from the group consisting of (2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine; (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine; and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 30-48 mole % cholesterol, 10-24% DSPC and 1-2 mole % PEG-DMG.

The compositions of the invention can be in the form of an aerosol, dispersion, solution, or suspension. The compositions can be formulated for intramuscular, oral, sublingual, buccal, parenteral, nasal, subcutaneous, intradermal, or topical administration.

The present invention is also directed to methods of treating cancer in the subject by administering to the subject an effective amount of the compositions of the invention.

The present invention is also directed to methods of treating cancer in the subject by co-administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) an anti-IL-10 antibody or antigen binding fragment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: A: individual volumes of injected tumors in tc-1 bilateral mouse tumor model. B: median volumes (mm^3) with 68% confidence intervals (CI) of injected tumors by day in TC-1 bilateral mouse tumor model. C: p-values for comparison of volumes of injected tumors between treatments, by day in TC-1 bilateral mouse tumor model.

FIGURE 2: A: individual volumes of non-injected tumors in bilateral mouse tumor TC-1 model. B: Median volumes (mm^3) with 68% confidence intervals (CI) of non-injected tumors by day in TC-1 bilateral mouse tumor model. C: p-values for comparison of volumes of non-injected tumors between treatments, by day in TC-1 bilateral mouse tumor model.

FIGURE 3 shows amino acid sequences of anti-IL-10 hum12G8, with light chain sequence of SEQ ID NO: 2 and heavy chain sequence of SEQ ID NO: 1. CDR regions are underlined.

FIGURE 4 shows amino acid sequences of mouse anti-IL-10 TC40.11D8, with light chain variable region sequence of SEQ ID NO: 3 and heavy chain variable region sequence of SEQ ID NO: 4. CDR regions are underlined.

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DETAILED DESCRIPTION OF THE INVENTION

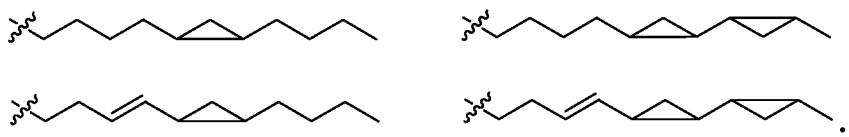
The present invention is directed to a composition comprising: a) a lipid nanoparticle (LNP) comprising one or more cationic lipids and a poly(ethyleneglycol)-lipid (PEG-lipid); b) a Toll-like receptor 9 (TLR9) agonist CpG oligonucleotide; and c) an anti-IL-
10 10 antibody or an antigen-binding fragment thereof.

As used herein, "about" can refer to a variance of 0, 1, 2, 3, 4, or 5 units or ±0, 1, 5, 10, 15, 20 or 25%.

As used herein, "alkyl" means a straight chain, cyclic or branched saturated aliphatic hydrocarbon having the specified number of carbon atoms.

15 As used herein, "alkeny" means a straight chain, cyclic or branched aliphatic hydrocarbon having the specified number of carbon atoms and one or more double bonds including but not limited to diene, triene and tetraene unsaturated aliphatic hydrocarbons.

Examples of a cyclic "alkyl" or "alkenyl include:



20 As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 atoms in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydro-naphthyl, indanyl and biphenyl.

So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this
25 document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

30 "Administration" as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical,

therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any organism, preferably an animal, more preferably a mammal (*e.g.*, rat, mouse, dog, cat, rabbit) and most preferably a human.

As used herein the term "co-administration" or "co-administering" refers to administration of the LNP of the invention and a TLR9 agonist or antibody concurrently, i.e., simultaneously in time, or sequentially, i.e., administration of an LNP of the invention, followed by administration of the agonist or antibody. That is, after administration of the LNP, the agonist or antibody can be administered substantially immediately after the LNP or the agonist or antibody can be administered after an effective time period after the LNP; the effective time period is the amount of time given for realization of maximum benefit from the administration of the LNP. An effective time period can be determined experimentally and can be generally within 1, 2, 3, 5, 10, 15, 20, 25, 30, 45 or 60 minutes.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological or binding activity. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), humanized, fully human antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as humanization of an antibody for use as a human therapeutic.

In general, the basic antibody structural unit comprises a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the heavy chain may define a constant region primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy

chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989).

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), which are located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of Sequences of Proteins of Immunological Interest, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat (1978) Adv. Prot. Chem. 32:1-75; Kabat, *et al.*, (1977) J. Biol. Chem. 252:6609-6616; Chothia, *et al.*, (1987) J Mol. Biol. 196:901-917 or Chothia, *et al.*, (1989) Nature 342:878-883.

As used herein, unless otherwise indicated, "antibody fragment" or "antigen binding fragment" refers to antigen binding fragments of antibodies, i.e. antibody fragments that retain the ability to bind specifically to the antigen bound by the full-length antibody, e.g. fragments that retain one or more CDR regions. Examples of antibody binding fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

An antibody that "specifically binds to" a specified target protein is an antibody that exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g. without producing undesired results such as false positives. Antibodies, or binding fragments thereof, useful in the present invention will bind to the target protein with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with non-target proteins.

"Chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in an antibody

derived from a particular species (e.g., human) or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in an antibody derived from another species (e.g., mouse) or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

"Human antibody" refers to an antibody that comprises human immunoglobulin protein sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" or "rat antibody" refer to an antibody that comprises only mouse or rat immunoglobulin sequences, respectively.

"Humanized antibody" refers to forms of antibodies that contain sequences from non-human (*e.g.*, murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The prefix "hum", "hu" or "h" is added to antibody clone designations when necessary to distinguish humanized antibodies from parental rodent antibodies. The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

"Anti-tumor response" when referring to a cancer patient treated with a therapeutic regimen, such as a combination therapy described herein, means at least one positive therapeutic effect, such as for example, reduced number of cancer cells, reduced tumor size, reduced rate of cancer cell infiltration into peripheral organs, reduced rate of tumor metastasis or tumor growth, or progression free survival. Positive therapeutic effects in cancer can be measured in a number of ways (See, W. A. Weber, J. Null. Med. 50:1S-10S (2009); Eisenhauer et al., *supra*). In some embodiments, an anti-tumor response to a combination therapy described herein is assessed using RECIST 1.1 criteria, bidimentional irRC or unidimensional irRC. In some embodiments, an anti-tumor response is any of SD, PR, CR, PFS, or DFS.

"Biotherapeutic agent" means a biological molecule, such as an antibody or fusion protein, that blocks ligand / receptor signaling in any biological pathway that supports tumor maintenance and/or growth or suppresses the anti-tumor immune response. Classes of biotherapeutic agents include, but are not limited to, antibodies to VEGF, EGFR, Her2/neu, other growth factor receptors, CD20, CD40, CD-40L, CTLA-4, OX-40, 4-1BB, and ICOS.

As used herein, "cationic lipid" refers to any of a number of lipid species which carry a net positive charge at a selective pH, such as physiological pH.

"CpG ODN" or "CpG oligonucleotide" is an oligonucleotide comprising Cytosine nucleotide followed by a Guanine nucleotide.

"A-class CpG ODNs" or "A-class CpG oligonucleotides" are oligonucleotides that comprise a central palindromic phosphodiester sequence containing at least one CpG sequence, flanked on one or both sides by phosphorothioate polyguanosine sequences. In one embodiment, the A-class CpG oligonucleotides induce strong plasmacytoid dendritic cell (PDC) IFN-α secretion and moderate expression of costimulatory molecules, but little B-cell activation.

"B-class CpG ODNs" or "B-class CpG oligonucleotides" are oligonucleotides that comprise at least one CpG sequence. In one embodiment, the B-class CpG oligonucleotides induce strong B-cell proliferation and differentiation, and induces plasmacytoid dendritic cell (PDC) expression of costimulatory molecules and modest IFN-α secretion.

"C-class CpG ODNs" or "C-class CpG oligonucleotides" are oligonucleotides from 12 to 40 bases in length, which have one to two 5'-TCG trinucleotides wherein the 5'-T is positioned 0, 1, 2, or 3 bases from the 5'-end of the oligonucleotide, and at least one palindromic sequence of at least ten bases in length comprising at least two unmethylated CG dinucleotides. In one embodiment, the oligonucleotide is an oligodeoxynucleotide (ODN). In one embodiment, the oligonucleotide is a 2'-oligodeoxynucleotide. In one embodiment, C-class CpG ODNs induce strong B-cell proliferation and differentation, induce plasmacytoid dendritic cell (PDC) maturation and cause secretion of high levels of type I interferons (e.g., IFN-α, IFN-γ, etc.) and expression of costimulatory molecules. In one embodiment, the oligonucleotide is an oligodeoxynucleotide. In one embodiment, one or more of the internucleotide linkages of the C-class CpG ODN are modified linkages. In one embodiment, one or more of the internucleotide linkages of C-class CpG ODN are phosphorothioate (PS) linkages. In one embodiment, all of the internucleotide linkages of CpG-C ODN are phosphorothioate (PS) linkages.

The term "palindromic sequence" or "palindrome" refers to a nucleic acid sequence that is an inverted repeat, e.g., ABCDD'C'B'A', where the bases, e.g., A, and A', B and B', C and C', D and D', are capable of forming Watson-Crick base pairs.

"CDR" or "CDRs" as used herein means complementarity determining region(s) in a immunoglobulin variable region, defined using the Kabat numbering system, unless otherwise indicated.

"Chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, kinase inhibitors, spindle poison plant alkaloids, cytoxic/antitumor antibiotics, topisomerase inhibitors, photosensitizers, anti-estrogens and selective estrogen receptor modulators (SERMs), anti-progesterones, estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, leutinizing hormone-releasing hormone agonists, anti-androgens, aromatase inhibitors, EGFR inhibitors, VEGF inhibitors, and anti-sense oligonucleotides that inhibit expression of genes implicated in abnormal cell proliferation or tumor growth. Chemotherapeutic agents useful in the treatment methods of the present invention include cytostatic and/or cytotoxic agents.

"Chothia" as used herein means an antibody numbering system described in Al-Lazikani *et al.*, *JMB* **273:**927-948 (1997).

"Comprising" or variations such as "comprise", "comprises" or "comprised of" are used throughout the specification and claims in an inclusive sense, i.e., to specify the presence of the stated features but not to preclude the presence or addition of further features that may materially enhance the operation or utility of any of the embodiments of the invention, unless the context requires otherwise due to express language or necessary implication.

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (e.g. charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity or other desired property of the protein, such as antigen affinity and/or specificity. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (*see, e.g.*, Watson *et al.* (1987) *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.)). In addition, substitutions of structurally or functionally similar amino acids

are less likely to disrupt biological activity. Exemplary conservative substitutions are set forth in Table 1 below.

TABLE 1. Exemplary Conservative Amino Acid Substitutions

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys; His
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

"Consists essentially of," and variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, an anti-IL-10 antibody that consists essentially of a recited amino acid sequence may also include one or more amino acids, including

substitutions of one or more amino acid residues, which do not materially affect the properties of the binding compound.

"Framework region" or "FR" as used herein means the immunoglobulin variable regions excluding the CDR regions.

"Kabat" as used herein means an immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat ((1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.).

"Monoclonal antibody" or "mAb" or "Mab", as used herein, refers to a population of substantially homogeneous antibodies, *i.e.*, the antibody molecules comprising the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of different antibodies having different amino acid sequences in their variable domains, particularly their CDRs, which are often specific for different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256: 495, or may be made by recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) *Nature* 352: 624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597, for example. *See also* Presta (2005) *J. Allergy Clin. Immunol.* 116:731.

"Patient" or "subject" refers to any single subject for which therapy is desired or that is participating in a clinical trial, epidemiological study or used as a control, including humans and mammalian veterinary patients such as cattle, horses, dogs, and cats.

"RECIST 1.1 Response Criteria" as used herein means the definitions set forth in Eisenhauer et al., E.A. et al., *Eur. J Cancer* 45:228-247 (2009) for target lesions or nontarget lesions, as appropriate based on the context in which response is being measured.

"Responder patient" when referring to a specific anti-tumor response to treatment with a combination therapy described herein, means the patient exhibited the anti-tumor response.

"Sustained response" means a sustained therapeutic effect after cessation of treatment with a therapeutic agent, or a combination therapy described herein. In some

embodiments, the sustained response has a duration that is at least the same as the treatment duration, or at least 1.5, 2.0, 2.5 or 3 times longer than the treatment duration.

"Tissue Section" refers to a single part or piece of a tissue sample, e.g., a thin slice of tissue cut from a sample of a normal tissue or of a tumor.

"Treat" or "treating" cancer as used herein means to administer a combination therapy of an anti-IL-10 antibody, LNP of the invention and CpG oligonucleotide to a subject having cancer, or diagnosed with cancer, to achieve at least one positive therapeutic effect, such as for example, reduced number of cancer cells, reduced tumor size, reduced rate of cancer cell infiltration into peripheral organs, or reduced rate of tumor metastasis or tumor growth. Positive therapeutic effects in cancer can be measured in a number of ways (See, W. A. Weber, *J. Nucl. Med.* 50:1S-10S (2009)). For example, with respect to tumor growth inhibition, according to NCI standards, a T/C ≦42% is the minimum level of anti-tumor activity. A T/C < 10% is considered a high anti-tumor activity level, with T/C (%) = Median tumor volume of the treated/Median tumor volume of the control × 100. In some embodiments, response to a combination therapy described herein is assessed using RECIST 1.1 criteria or irRC (bidimensional or unidimensional) and the treatment achieved by a combination of the invention is any of PR, CR, OR, PFS, DFS and OS. PFS, also referred to as "Time to Tumor Progression" indicates the length of time during and after treatment that the cancer does not grow, and includes the amount of time patients have experienced a CR or PR, as well as the amount of time patients have experienced SD. DFS refers to the length of time during and after treatment that the patient remains free of disease. OS refers to a prolongation in life expectancy as compared to naive or untreated individuals or patients. In some embodiments, response to a combination of the invention is any of PR, CR, PFS, DFS, OR and OS that is assessed using RECIST 1.1 response criteria. The treatment regimen for a combination of the invention that is effective to treat a cancer patient may vary according to factors such as the disease state, age, and weight of the patient, and the ability of the therapy to elicit an anti-cancer response in the subject. While an embodiment of any of the aspects of the invention may not be effective in achieving a positive therapeutic effect in every subject, it should do so in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

The terms “treatment regimen”, “dosing protocol” and “dosing regimen” are used interchangeably to refer to the dose and timing of administration of each therapeutic agent in a combination of the invention.

"Tumor" as it applies to a subject diagnosed with, or suspected of having, cancer refers to a malignant or potentially malignant neoplasm or tissue mass of any size, and includes primary tumors and secondary neoplasms. A solid tumor is an abnormal growth or mass of tissue that usually does not contain cysts or liquid areas. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors (National Cancer Institute, Dictionary of Cancer Terms).

"Tumor burden" also referred to as "tumor load", refers to the total amount of tumor material distributed throughout the body. Tumor burden refers to the total number of cancer cells or the total size of tumor(s), throughout the body, including lymph nodes and bone marrow. Tumor burden can be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., ultrasound, bone scan, computed tomography (CT) or magnetic resonance imaging (MRI) scans.

The term "tumor size" refers to the total size of the tumor which can be measured as the length and width of a tumor. Tumor size may be determined by a variety of methods known in the art, such as, e.g. by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., bone scan, ultrasound, CT or MRI scans.

“Unidimensional irRC refers to the set of criteria described in Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a Common Language for Tumor Response to Immunotherapy: Immune-related Response Criteria using Unidimensional measurements. *Clin Cancer Res.* 2013;19(14):3936–3943). These criteria utilize the longest diameter (cm) of each lesion.

“Variable regions” or “V region” as used herein means the segment of IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.

"Anti-IL-10 antibody" means an antagonist antibody that binds IL-10 to inhibit the activity of IL-10. Alternative names or synonyms for IL-10 include: Interleukin-

10, cytokine synthesis inhibitor factor or CSIF. Human IL-10 amino acid sequences can be found in US patent 6217857. The amino acid sequence of the mature human IL-10 protein is SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFK GYLGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPC ENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN (SEQ ID NO: 21)

Any IL-10 antibody could be used in the combinations of the invention. In one embodiment, the anti-IL-10 antibodies to be used are the ones described in US 8,226,947 and US 7,662,379, the disclosure of which is hereby incorporated by reference in its entirety. In another embodiment, the anti-IL-10 antibody is anti-IL-10 hum12G8, which comprises two identical light chains with the sequence of SEQ ID NO: 2 and two identical heavy chains with the sequence of SEQ ID NO: 1. Plasmids containing nucleic acids encoding both the heavy and light chains of hum12G8 were deposited with the ATCC on May 6, 2004, as PTA-5922 and PTA-5923, respectively. Anti-IL-10 antibodies useful in any of the treatment method, medicaments and uses of the present invention include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to IL-10. The mAb may be a human antibody, a humanized antibody or a chimeric antibody, and may include a human constant region. In some embodiments, the human constant region is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4 constant regions, and in preferred embodiments, the human constant region is an IgG1 or IgG4 constant region. In some embodiments, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, $F(ab')_2$, scFv and Fv fragments.

In some preferred embodiments of the treatment method, medicaments and uses of the present invention, the anti-IL-10 antibody is a monoclonal antibody, or antigen binding fragment thereof, which comprises: (a) light chain CDRs of SEQ ID NOs: 5, 6 and 7 and heavy chain CDRs SEQ ID NOs: 8, 9 and 10 of anti-IL-10 hum12G8. In other preferred embodiments of the treatment method, medicaments and uses of the present invention, the anti-IL-10 antibody is a monoclonal antibody, or antigen binding fragment thereof, which comprises: (a) light chain CDRs of SEQ ID NOs: 15, 16 and 17 and heavy chain CDRs SEQ ID NOs: 18, 19 and 20 of anti-IL-10 hum11D8.

In other preferred embodiments of the treatment method, medicaments and uses of the present invention, the anti-IL-10 antibody is a monoclonal antibody, or antigen binding fragment thereof, which specifically binds to human IL-10 and comprises (a) a heavy chain variable region comprising SEQ ID NO:11 or a variant thereof, and (b) a light chain

variable region comprising an amino acid sequence of SEQ ID NO:12 or a variant thereof. A variant of a heavy chain variable region sequence is identical to the reference sequence except having up to 17 conservative amino acid substitutions in the framework region (i.e., outside of the CDRs), and preferably has less than ten, nine, eight, seven, six or five conservative amino acid substitutions in the framework region. A variant of a light chain variable region sequence is identical to the reference sequence except having up to five conservative amino acid substitutions in the framework region (i.e., outside of the CDRs), and preferably has less than four, three or two conservative amino acid substitution in the framework region.

Table 2 below provides a list of the amino acid sequences of exemplary anti-IL-10 mAbs for use in the treatment method, medicaments and uses of the present invention, and the sequences are shown in Figures 3-4.

Table 2. EXEMPLARY ANTI-HUMAN IL-10 MONOCLONAL ANTIBODIES	
A. Comprises light and heavy chain CDRs of hum12G8 in US patent 7662379	
CDRL1	SEQ ID NO:5 KTSQNIFENLA
CDRL2	SEQ ID NO:6 YNASPLQA
CDRL3	SEQ ID NO:7 HQYYSGYT
CDRH1	SEQ ID NO:8 GFTFSDYHMA
CDRH2	SEQ ID NO:9 SITLDATYTYYRDSVRG
CDRH3	SEQ ID NO:10 HRGFSVWLDY
B. Comprises the heavy chain variable region and light chain variable regions of hum12G8 in US patent 7662379	
Heavy chain VR	SEQ ID NO:11 QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYHMAWVRQAPGKGLEWVAS ITLDATYTYYRDSVRGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHR GFSVWLDYWGQGTLVTVSSA
Light chain VR	SEQ ID NO:12 DIQMTQSPSSLSASVGDRVTITCKTSQNIFENLAWYQQKPGKAPKLLIYN ASPLQAGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYSGYTFGPG TKLELKRTVAA
C. Comprises the heavy chain and light chain of hum12G8 in US patent 7662379	
Heavy chain	SEQ ID NO:1 QVQLVESGGG VVQPGRSLRL SCAASGFTFS DYHMAWVRQA PGKGLEWVAS ITLDATYTYY RDSVRGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHR GFSVWLDYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK

	DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
Light chain	SEQ ID NO:2 DIQMTQSPSS LSASVGDRVT ITCKTSQNIF ENLAWYQQKP GKAPKLLIYN ASPLQAGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCHQ YYSGYTFGPG TKLELKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC
D. Comprises the heavy chain and light chain of 11D8 in US patent 8226947	
Heavy chain	SEQ ID NO: 14 QVQLVESGGGVVQPGRSLRLSCAASGFSLTNYGVHWVRQAPGKGLEWVAVIWSGGS TDYNAAFISRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNRGYDVYFDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Light chain	SEQ ID NO: 13 EIVLTQSPGTLSLSPGERATLSCRASESVDDYGHSFMHWYQQKPGQAPRLLIYRAS TLESGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGNEDPWTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
E. Comprises light and heavy chain CDRs of hum11D8 in US patent 8226947	
CDRL1	SEQ ID NO: 15: RASESVDDYGHSFMH
CDRL2	SEQ ID NO: 16: RASTLES
CDRL3	SEQ ID NO: 17: QQGNEDPWT
CDRH1	SEQ ID NO: 18: GFSLTNYGVH
CDRH2	SEQ ID NO: 19: VIWSGGSTDYNAAFIS
CDRH3	SEQ ID NO: 20: NRGYDVYFDY

As used herein, an "anti-IL-10 hum 12G8 variant" means a monoclonal antibody which comprises heavy chain and light chain sequences that are identical to those in anti-IL-10 hum 12G8, except for having three, two or one conservative amino acid substitutions at positions that are located outside of the light chain CDRs and six, five, four, three, two or one conservative amino acid substitutions that are located outside of the heavy chain CDRs, e.g, the variant positions are located in the FR regions or the constant region. In other words, anti-IL-10 hum 12G8 and an anti-IL-10 hum 12G8 variant comprise identical CDR sequences, but differ from each other due to having a conservative amino acid

substitution at no more than three or six other positions in their full length light and heavy chain sequences, respectively. An anti-IL-10 hum 12G8 variant is substantially the same as anti-IL-10 hum 12G8 with respect to the following properties: binding affinity to IL-10 and neutralizing effect *in vivo*.

As used herein, "halogen" means Br, Cl, F and I.

As used herein, "heterocyclyl" or "heterocycle" means a 4- to 10-membered aromatic or nonaromatic heterocycle containing from 1 to 4 heteroatoms selected from the group consisting of O, N and S, and includes bicyclic groups. "Heterocyclyl" therefore includes, the following: benzoimidazolyl, benzofuranyl, benzofurazanyl, benzopyrazolyl, benzotriazolyl, benzothiophenyl, benzoxazolyl, carbazolyl, carbolinyl, cinnolinyl, furanyl, imidazolyl, indolinyl, indolyl, indolazinyl, indazolyl, isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthpyridinyl, oxadiazolyl, oxazolyl, oxazoline, isoxazoline, oxetanyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridopyridinyl, pyridazinyl, pyridyl, pyrimidyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, tetrahydropyranyl, tetrazolyl, tetrazolopyridyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, azetidinyl, 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, dihydrobenzoimidazolyl, dihydrobenzofuranyl, dihydrobenzothiophenyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrazinyl, dihydropyrazolyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl, dihydrotriazolyl, dihydroazetidinyl, methylenedioxybenzoyl, tetrahydrofuranyl, and tetrahydrothienyl, and N-oxides thereof all of which are optionally substituted with one to three substituents selected from R".

As used herein, "lipid nanoparticle" or "LNP" refers to any lipid composition that can be used to deliver a product, including, but not limited to, liposomes or vesicles, wherein an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), or, in other embodiments, wherein the lipids coat an interior comprising a prophylactic product, or lipid aggregates or micelles, wherein the lipid encapsulated therapeutic product is contained within a relatively disordered lipid mixture. Except where noted, the lipid nanoparticle does not need to have the TLR9 agonist incorporated therein and may be used to deliver a product when in the same formulation.

As used herein, "polyamine" means compounds having two or more amino groups. Examples include putrescine, cadaverine, spermidine, and spermine.

Unless otherwise specified, mole % refers to a mole percent of total lipids.

Generally, the LNPs of the compositions of the invention are composed of one or more cationic lipids (including ionizable cationic lipids) and one or more poly(ethyleneglycol)-lipids (PEG-lipids). In certain embodiments, the LNPs further comprise one or more non-cationic lipids. The one or more non-cationic lipids can include a phospholipid, phospholipid derivative, a sterol, a fatty acid, or a combination thereof.

Cationic lipids and ionizable cationic lipids suitable for the LNPs are described herein. Ionizable cationic lipids are characterized by the weak basicity of their lipid head groups, which affects the surface charge of the lipid in a pH-dependent manner, rendering them positively charged at acidic pH but close to charge-neutral at physiologic pH. Cationic lipids are characterized by monovalent or multivalent cationic charge on their headgroups, which renders them positively charged at neutral pH. In certain embodiments, the cationic and ionizable lipid is capable of complexing with hydrophilic bioactive molecules to produce a hydrophobic complex that partitions into the organic phase of a two-phase aqueous/organic system. It is contemplated that both monovalent and polyvalent cationic lipids may be utilized to form hydrophobic complexes with bioactive molecules.

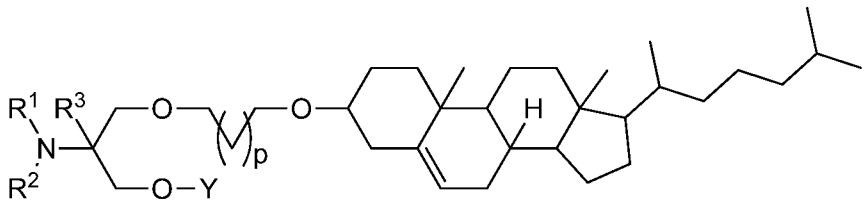
Preferred cationic and ionizable cationic lipids for use in forming the LNPs include, but are not limited to, N,N-dioleyl-N,N-dimethylammonium chloride ("DODAC"); N-(2,3dioleyloxy)propyl)-N,N,Ntrimethylammonium chloride ("DOTMA"); N,NdistearylN,N-dimethylammonium bromide ("DDAB"); N-(2,3dioleoyloxy)propyl)-N,N,N-trimethylamntonium chloride ("DODAP"); 1,2 bis (oleoyloxy)-3-(trimethylammonio) propane (DOTAP); 3-(N-(N,N-dimethylaminoethane)-carbam-oyl)cholesterol ('DC-Chol"); diheptadecylamidoglycylspermidine ("DHGS") and N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydoxyethyl ammonium bromide ("DMRIE"). Additionally, a number of commercial preparations of cationic lipids, as well as other components, are available which can be used in the present invention. These include, for example, LIPOFECTIN® (commercially available cationic lipid nanoparticles comprising DOTMA and 1,2dioleoyl-sn-3-phosphoethanolamine ("DOPE"), from GIBCOBRL, Grand Island, N.Y., USA); and LIPOFECTAMINE® (commercially available cationic lipid nanoparticles comprising N-(1-(2,3dioleyloxy)propyl)N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA') and ("DOPE"), from (GIBCOBRL). The following lipids are cationic and have a positive charge at below physiological pH: DODAP, DODMA,

DMDMA, 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 4-(2,2-diocta-9,12-dienyl-[1,3]dioxolan-4-ylmethyl)-dimethylamine, DLinKDMA (WO 2009/132131 A1), DLin-K-C2-DMA (WO2010/042877), DLin-M-C3-DMA (WO2010/146740 and/or WO2010/105209), DLin-MC3-DMA (heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate; Jayaraman et al., 2012, Angew. Chem. Int. Ed. Engl. 51:8529–8533), 2-{4-[(3β)-cholest-5-en-3-yloxy]butoxy}-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dienlyloxyl]propan-1-amine) (CLinDMA), and the like. Other cationic lipids suitable for use in the invention include, e.g., the cationic lipids described in U.S. Pat. Nos. 5,208,036, 5,264,618, 5,279,833 and 5,283,185, and U.S. Patent Application Publication Nos. 2008/0085870 and 2008/0057080. Other cationic lipids suitable for use in the invention include, e.g., Lipids E0001-E0118 or E0119-E0180 as disclosed in Table 6 (pages 112 - 139) of International Patent Application Publication No. WO2011/076807 (which also discloses methods of making, and methods of using these cationic lipids).

In certain aspects of this embodiment of the invention, the LNPs comprise one or more of the following ionizable cationic lipids: DLinDMA, DlinKC2DMA DLin-MC3-DMA, CLinDMA, or S-Octyl CLinDMA (See International Patent Application Publication No. WO2010/021865).

In certain aspects of this embodiment of the invention, LNPs comprise one or more ionizable cationic lipids described in International Patent Application Publication No. WO2011/022460 A1, or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts therein.

In International Patent Application Publication No. WO2011/022460 A1, the cationic lipids are illustrated by the Formula A1:



A1

wherein:

p is 1 to 8;

R1 and R2 are independently selected from H, (C_1-C_{10})alkyl, heterocyclyl, and a polyamine, wherein said heterocyclyl and polyamine are optionally substituted with one to three substituents selected from R4, or R1 and R2 can be taken together with the

nitrogen to which they are attached to form a monocyclic heterocycle with 4-7 members optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O and S, said monocyclic heterocycle optionally substituted with one to three substituents selected from R^4;

R^3 is selected from H and $(C_1\text{-}C_6)$alkyl, said alkyl optionally substituted with one to three substituents selected from R^4;

R^4 is independently selected from halogen, OR^5, SR^5, CN, CO_2R^5 and $CON(R^5)_2$;

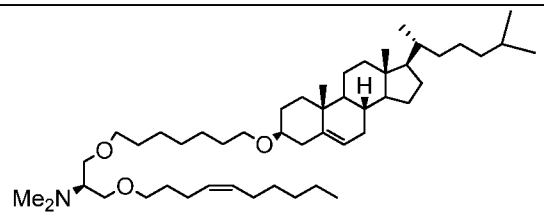
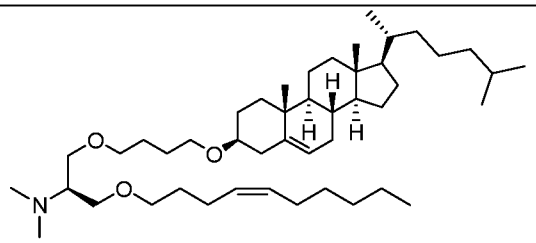
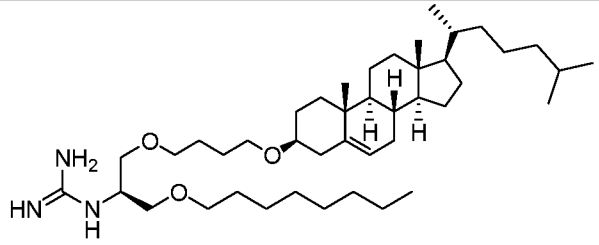
R^5 is independently selected from H, $(C_1\text{-}C_{10})$alkyl and aryl; and

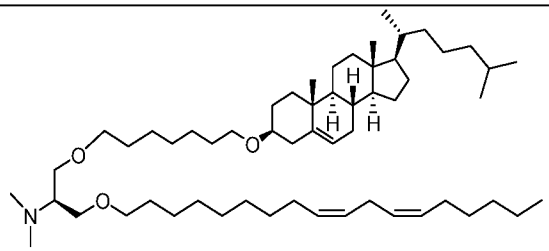
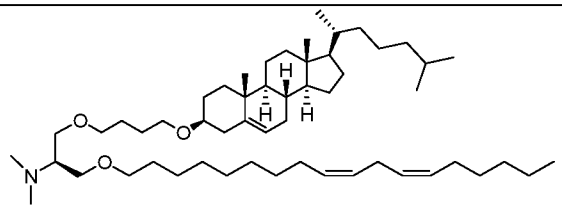
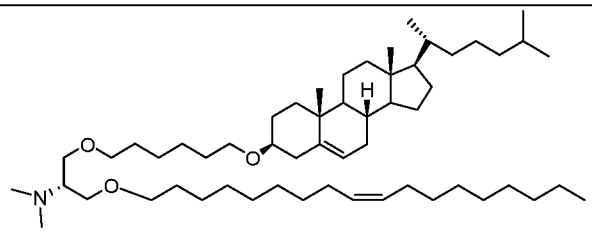
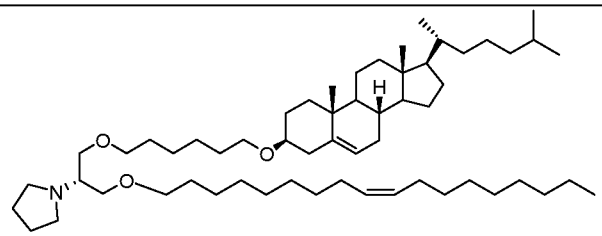
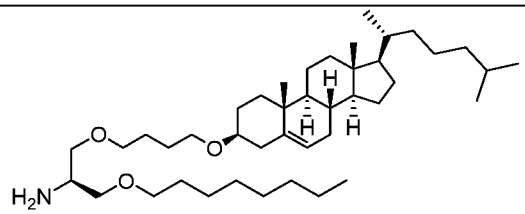
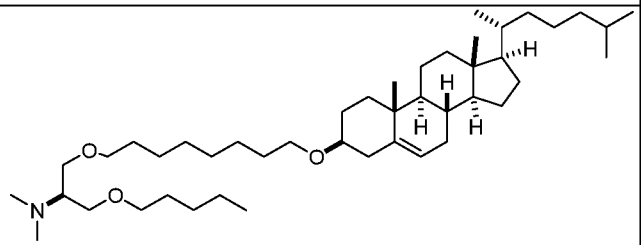
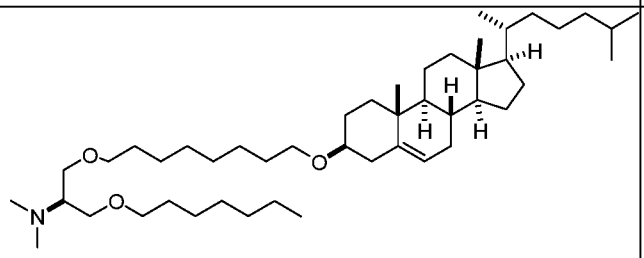
Y is a $(C_4\text{-}C_{22})$alkyl, $(C_4\text{-}C_{22})$perfluoroalkyl, or a $(C_4\text{-}C_{22})$alkenyl;

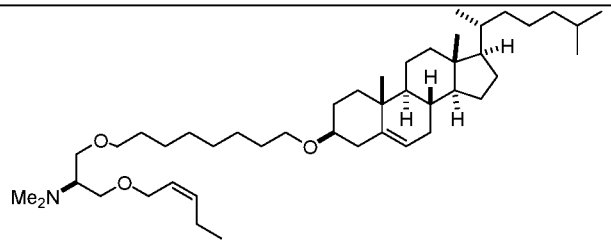
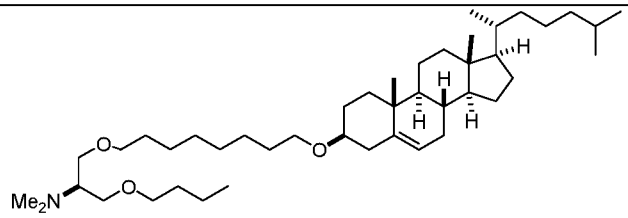
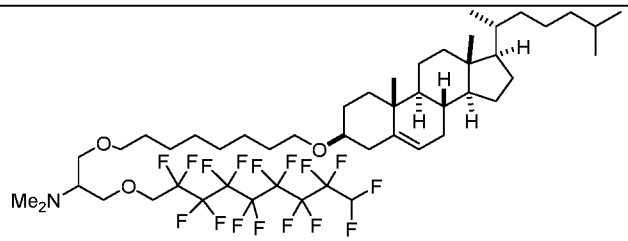
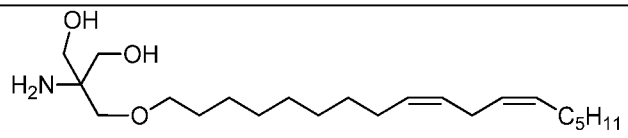
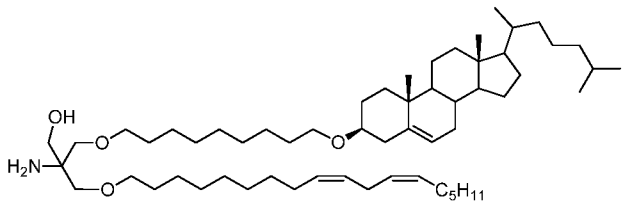
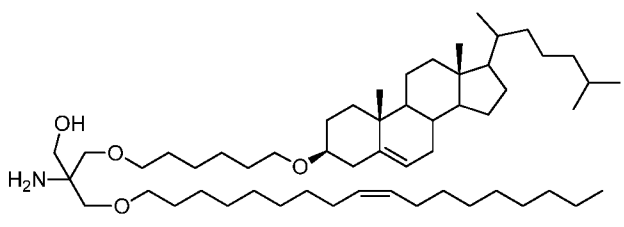
or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts.

Exemplary ionizable cationic lipids include compounds 4-11 and 13-20 described in International Patent Application Publication No. WO2011/022460 A1, as shown in Table 3 (preceded by "1-"), or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts.

Table 3: Ionizable Cationic Lipids

Cpd	Structure	Name
1-4		(2 S)-1-{7-[(3 β)-cholest-5-en-3-yloxy]heptyloxy}-3-[(4 Z)-dec-4-en-1-yloxy]- *N*, *N* -dimethylpropan-2-amine
1-5		(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-3-[(4 Z)-dec-4-en-1-yloxy]- N, N -dimethylpropan-2-amine
1-6		1-[(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-3-(octyloxy)propan-2-yl]guanidine

1-7		1-[(2 R)-1-{7-[(3 β)-cholest-5-en-3-yloxy]heptyloxy}-N,N-dimethyl-3-[(9 Z, 12 Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine
1-8		1-[(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-N,N-dimethyl-3-[(9 Z, 12 Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine
1-9		(2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine
1-10		(3β)-3-[6-{ [(2S)-3-[(9Z)-octadec-9-en-1-yloxyl]-2-(pyrrolidin-1-yl)propyl]oxy}hexyl)oxy] cholest-5-ene
1-11		(2R)-1-{4-[(3β)-cholest-5-en-3-yloxy]butoxy}-3-(octyloxy)propan-2-amine
1-13		(2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-(pentyloxy)propan-2-amine
1-14		(2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-3-(heptyloxy)-N,N-dimethylpropan-2-amine

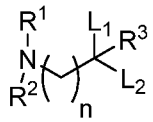
1-15		(2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(2Z)-pent-2-en-1-yloxy]propan-2-amine
1-16		(2S)-1-butoxy-3-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethylpropan-2-amine
1-17		(2S-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-3-[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononyl)oxy]-N,N-dimethylpropan-2-amine
1-18		2-amino-2-{[(9*Z*,12*Z*)-octadeca-9,12-dien-1-yloxy]methyl}propane-1,3-diol
1-19		2-amino-3-((9-(((3S,10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)nonyl)oxy)-2-((((9Z,12Z)-octadeca-9,12-dien-1-yl)oxy)methyl)propan-1-ol
1-20		2-amino-3-((6-(((3S,10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-

		yl)oxy)hexyl)oxy)-2-((((Z)-octadec-9-en-1-yl)oxy)methyl)propan-1-ol

In one embodiment, the ionizable cationic lipid is compound 9 described in International Patent Application Publication No. WO2011/022460 A1 (designated herein as compound 1-9 in Table 3 herewith), or a pharmaceutically acceptable salt thereof, or a stereoisomer of the compound or its salts.

In certain aspects of this embodiment of the invention, LNPs comprise one or more low molecular weight ionizable cationic lipids described in International Patent Application Publication No. WO2012/040184, or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts.

In International Patent Application Publication No. WO2012/040184 A1, the cationic lipids are illustrated by the Formula A2:



A2

wherein:

R^1 and R^2 are independently selected from H, $(C_1\text{-}C_6)$alkyl, heterocyclyl, and polyamine, wherein said alkyl, heterocyclyl and polyamine are optionally substituted with one to three substituents selected from R', or R^1 and R^2 can be taken together with the nitrogen to which they are attached to form a monocyclic heterocycle with 4-7 members optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O and S, said monocyclic heterocycle is optionally substituted with one to three substituents selected from R';

R^3 is independently selected from H and $(C_1\text{-}C_6)$alkyl, said alkyl optionally substituted with one to three substituents selected from R';

R' is independently selected from halogen, R", OR", SR", CN, $CO_2R"$ or $CON(R")_2$;

R" is independently selected from H and $(C_1\text{-}C_6)$alkyl, wherein said alkyl is optionally substituted with halogen and OH;

n is 0, 1, 2, 3, 4 or 5;

L_1 is selected from C_4-C_{24} alkyl and C_4-C_{24} alkenyl, said alkyl and alkenyl are optionally substituted with one or more substituents selected from R'; and

L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl, said alkyl and alkenyl are optionally substituted with one or more substituents selected from R';

or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts.

In certain embodiments, the ionizable cationic lipid is a compound having Formula A2, wherein:

R^1 and R^2 are each methyl;

R^3 is H;

n is 0;

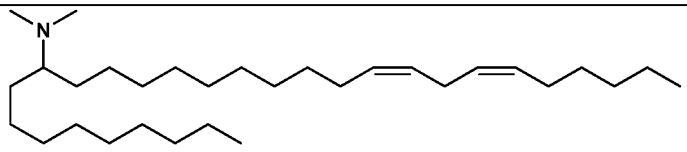
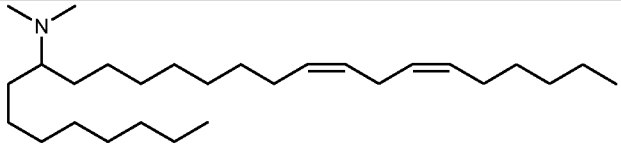
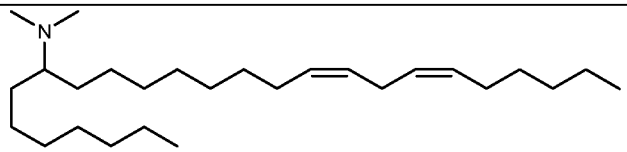
L_1 is selected from C_4-C_{24} alkyl and C_4-C_{24} alkenyl; and

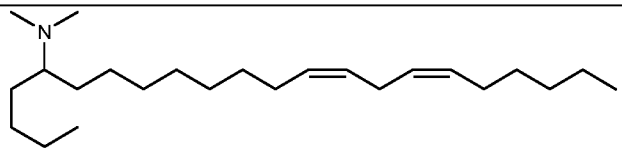
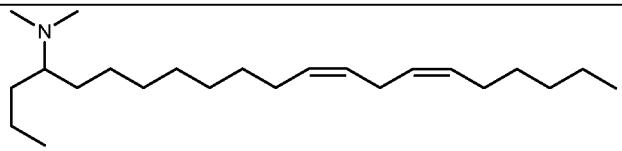
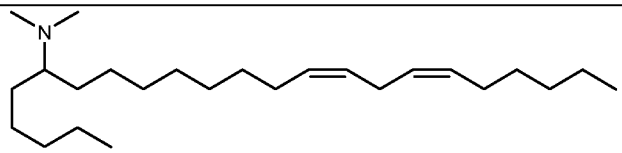
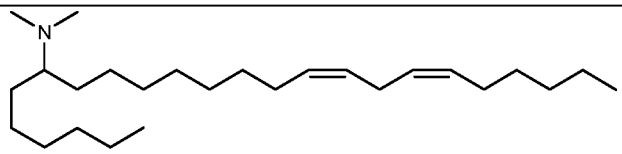
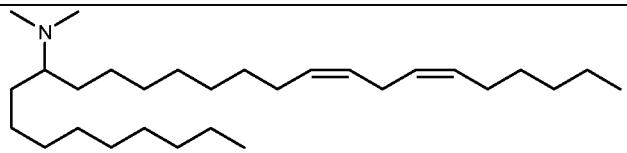
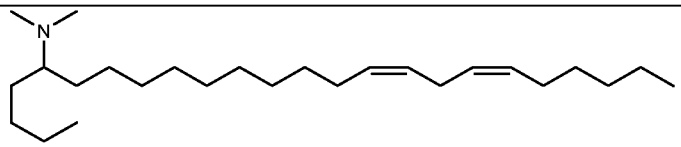
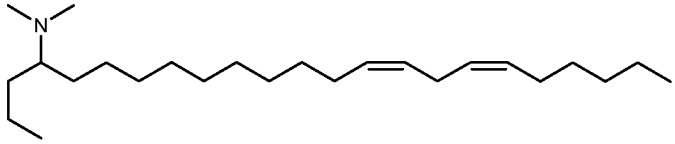
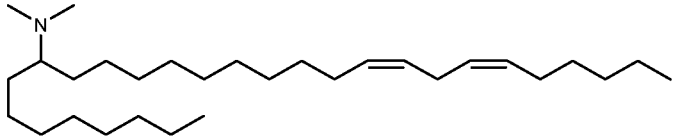
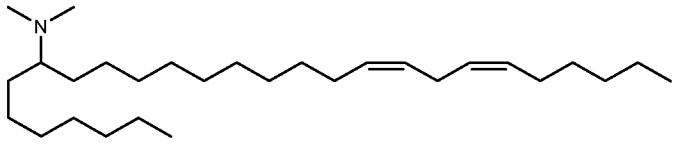
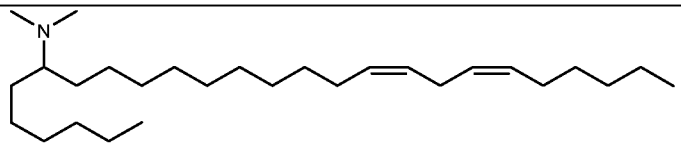
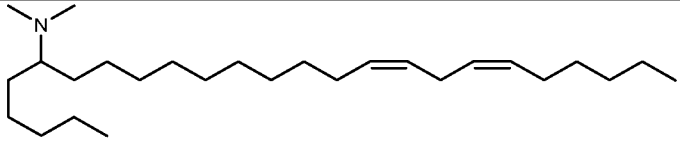
L_2 is selected from C_3-C_9 alkyl and C_3-C_9 alkenyl;

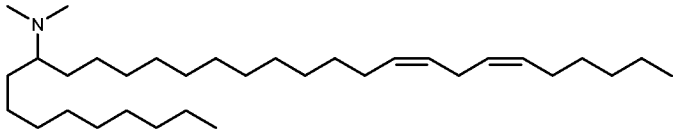
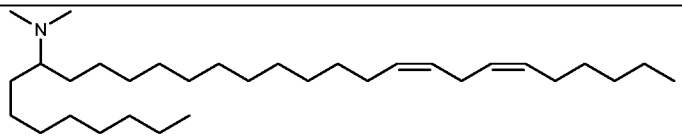
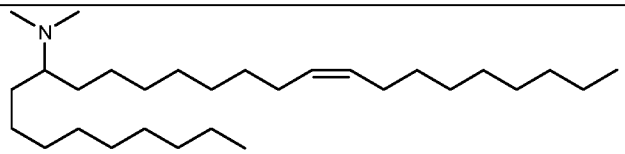
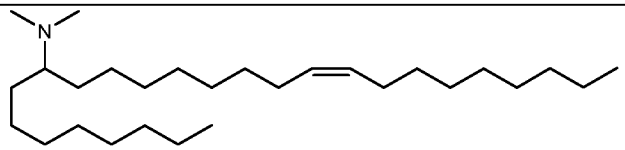
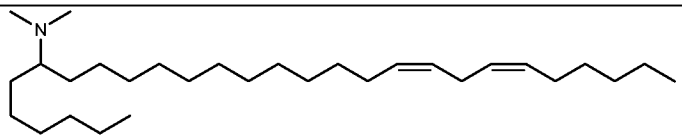
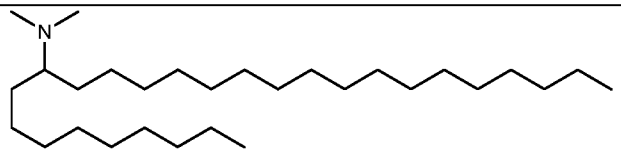
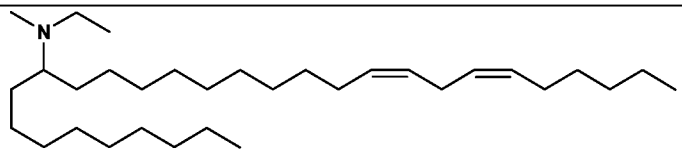
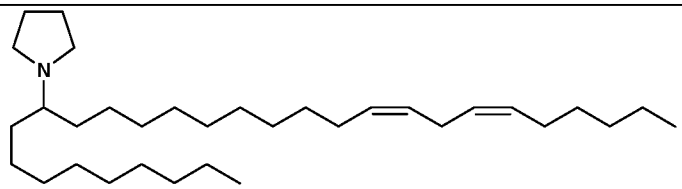
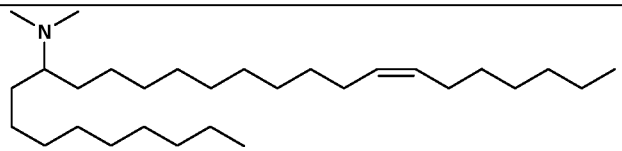
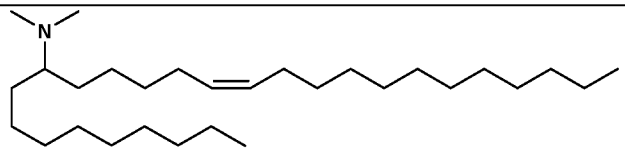
or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts.

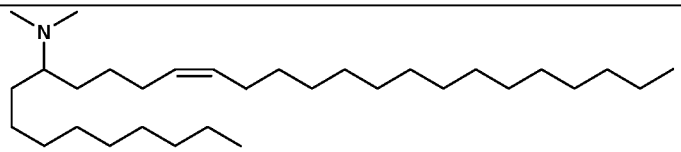
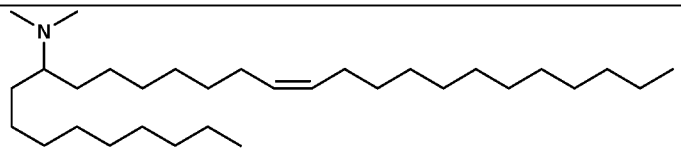
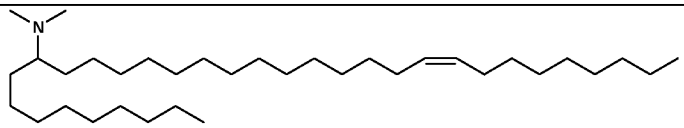
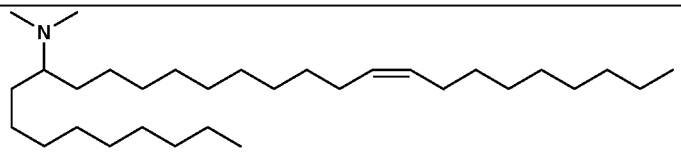
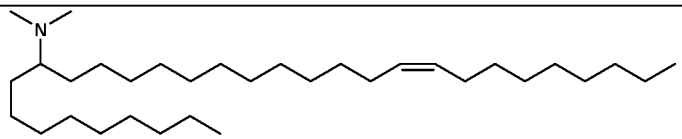
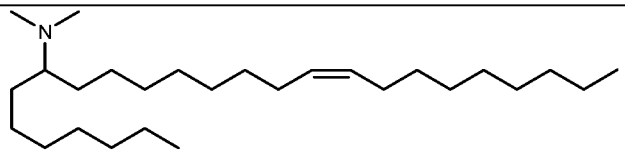
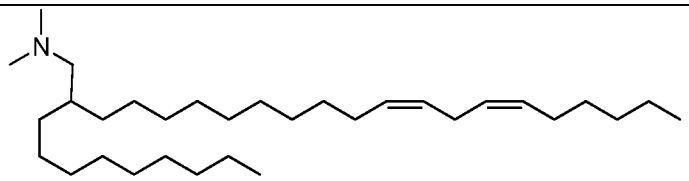
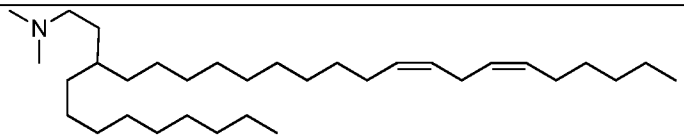
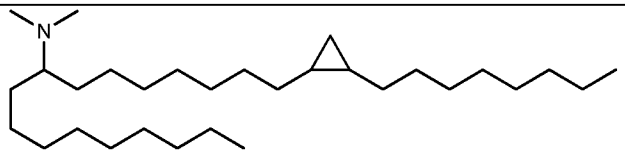
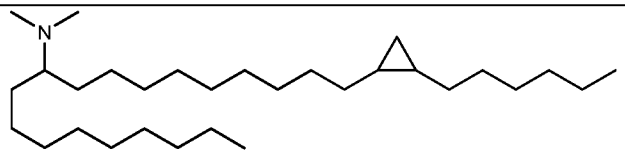
Exemplary ionizable cationic lipids are compounds 1-44 described in International Patent Application Publication No. WO2012/040184, as shown in Table 4 (preceded by "2-" , or any pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or salts.

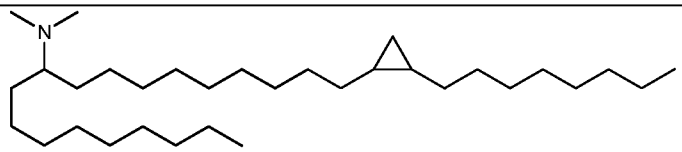
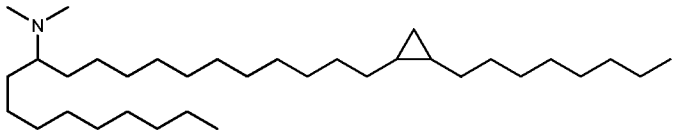
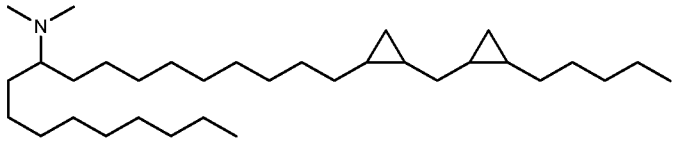
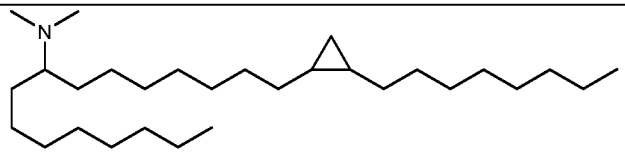
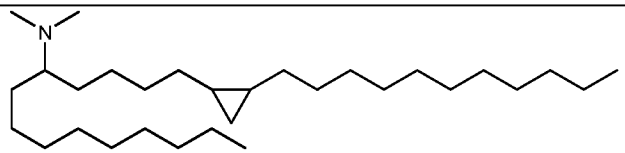
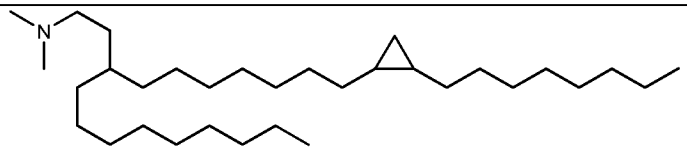
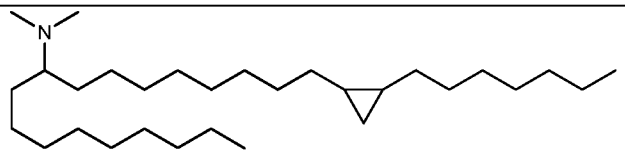
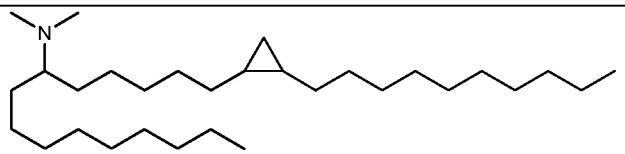
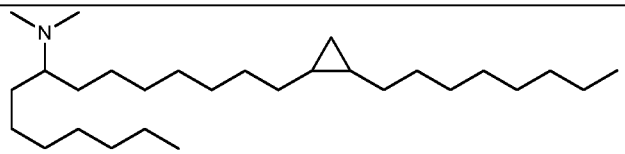
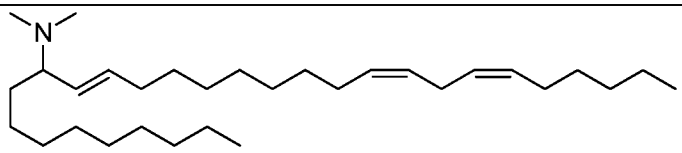
Table 4: Additional Ionizable Cationic Lipids

Cpd.	Structure	Name
2-1		(20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine
2-2		(17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-9-amine
2-3		(16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-8-amine

2-4		(13Z,16Z)-N,N-dimethyldocosa-13,16-dien-5-amine
2-5		(12Z,15Z)-N,N-dimethylhenicosa-12,15-dien-4-amine
2-6		(14Z,17Z)-N,N-dimethyltricosa-14,17-dien-6-amine
2-7		(15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-7-amine
2-8		(18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-10-amine
2-9		(15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-5-amine
2-10		(14Z,17Z)-N,N-dimethyltricosa-14,17-dien-4-amine
2-11		(19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-9-amine
2-12		(18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-8-amine
2-13		(17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-7-amine
2-14		(16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-6-amine

2-15		(22Z,25Z)-N,N-dimethylhentriaconta-22,25-dien-10-amine
2-16		(21Z,24Z)-N,N-dimethyltriaconta-21,24-dien-9-amine
2-17		(18Z)-N,N-dimethylheptacos-18-en-10-amine
2-18		(17Z)-N,N-dimethylhexacos-17-en-9-amine
2-19		(19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-7-amine
2-20		N,N-dimethylheptacosan-10-amine
2-21		(20Z,23Z)-N-ethyl-N-methylnonacosa-20,23-dien-10-amine
2-22		1-[(11Z,14Z)-1-nonylicosa-11,14-dien-1-yl]pyrrolidine
2-23		(20Z)-N,N-dimethylheptacos-20-en-10-amine
2-24		(15Z)-N,N-dimethylheptacos-15-en-10-amine

2-25		(14Z)-N,N-dimethylnonacos-14-en-10-amine
2-26		(17Z)-N,N-dimethylnonacos-17-en-10-amine
2-27		(24Z)-N,N-dimethyltritriacont-24-en-10-amine
2-28		(20Z)-N,N-dimethylnonacos-20-en-10-amine
2-29		(22Z)-N,N-dimethylhentriacont-22-en-10-amine
2-30		(16Z)-N,N-dimethylpentacos-16-en-8-amine
2-31		(12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine
2-32		(13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine
2-33		N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine
2-34		1-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine

2-35		N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]nonadecan-10-amine
2-36		N,N-dimethyl-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine
2-37		N,N-dimethyl-1-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]nonadecan-10-amine
2-38		N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine
2-39		N,N-dimethyl-1-[(1R,2S)-2-undecylcyclopropyl]tetradecan-5-amine
2-40		N,N-dimethyl-3-{7-[(1S,2R)-2-octylcyclopropyl]heptyl}dodecan-1-amine
2-41		1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine
2-42		1-[(1S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine
2-43		N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine
2-44		(11E,20Z,23Z)-N,N-dimethylnonacosa-11,20,23-trien-10-amine

In certain embodiments, the ionizable cationic lipids are compounds 32 and 33 described in International Patent Application Publication No. WO2012/040184 (designated herein as compounds 2-32 and 2-33, respectively), or a pharmaceutically acceptable salt thereof, or a stereoisomer of any of the compounds or its salts.

Stereochemistry

When structures of the same constitution differ in respect to the spatial arrangement of certain atoms or groups, they are stereoisomers, and the considerations that are significant in analyzing their interrelationships are topological. If the relationship between two stereoisomers is that of an object and its nonsuperimposable mirror image, the two structures are enantiomeric, and each structure is said to be chiral. Stereoisomers also include diastereomers, cis-trans isomers and conformational isomers. Diastereoisomers can be chiral or achiral, and are not mirror images of one another. Cis-trans isomers differ only in the positions of atoms relative to a specified plane in cases where these atoms are, or are considered as if they were, parts of a rigid structure. Conformational isomers are isomers that can be interconverted by rotations about formally single bonds. Examples of such conformational isomers include cyclohexane conformations with chair and boat conformers, carbohydrates, linear alkane conformations with staggered, eclipsed and gauche conformers, etc. See J. Org. Chem. 35, 2849 (1970).

Many organic compounds exist in optically active forms having the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, enantiomers are identical except that they are non-superimposable mirror images of one another. A mixture of enantiomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture. Many of the compounds described herein can have one or more chiral centers and therefore can exist in different enantiomeric forms. If desired, a chiral carbon can be designated with an asterisk (*). When bonds to the chiral carbon are depicted as straight lines in the Formulas of the invention, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the Formula. As is used in the art, when it is desired to specify the absolute configuration about a chiral carbon, one of the

bonds to the chiral carbon can be depicted as a wedge (bonds to atoms above the plane) and the other can be depicted as a series or wedge of short parallel lines (bonds to atoms below the plane). The Cahn-Inglod-Prelog system can be used to assign the (R) or (S) configuration to a chiral carbon.

When the compounds of the present invention contain one chiral center, the compounds exist in two enantiomeric forms and the present invention includes both enantiomers and mixtures of enantiomers, such as the specific 50:50 mixture referred to as a racemic mixtures. The enantiomers can be resolved by methods known to those skilled in the art, such as formation of diastereoisomeric salts which may be separated, for example, by crystallization (see, CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation by David Kozma (CRC Press, 2001)); formation of diastereoisomeric derivatives or complexes which may be separated, for example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic esterification; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support for example silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step is required to liberate the desired enantiomeric form. Alternatively, specific enantiomers may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer into the other by asymmetric transformation.

Designation of a specific absolute configuration at a chiral carbon of the compounds of the invention is understood to mean that the designated enantiomeric form of the compounds is in enantiomeric excess (ee) or in other words is substantially free from the other enantiomer. For example, the "R" forms of the compounds are substantially free from the "S" forms of the compounds and are, thus, in enantiomeric excess of the "S" forms. Conversely, "S" forms of the compounds are substantially free of "R" forms of the compounds and are, thus, in enantiomeric excess of the "R" forms. Enantiomeric excess, as used herein, is the presence of a particular enantiomer at greater than 50%. In a particular embodiment when a specific absolute configuration is designated, the enantiomeric excess of depicted compounds is at least about 90%.

When a compound of the present invention has two or more chiral carbons it can have more than two optical isomers and can exist in diastereoisomeric forms. For example, when there are two chiral carbons, the compound can have up to 4 optical isomers

and 2 pairs of enantiomers ((S,S)/(R,R) and (R,S)/(S,R)). The pairs of enantiomers (e.g., (S,S)/(R,R)) are mirror image stereoisomers of one another. The stereoisomers that are not mirror-images (e.g., (S,S) and (R,S)) are diastereomers. The diastereoisomeric pairs may be separated by methods known to those skilled in the art, for example chromatography or crystallization and the individual enantiomers within each pair may be separated as described above. The present invention includes each diastereoisomer of such compounds and mixtures thereof.

The LNPs may also comprise any combination of two or more of the cationic lipids described herein. In certain aspects, the cationic lipid typically comprises from about 0.1 to about 99.9 mole % of the total lipid present in said particle. In certain aspects, the cationic lipid can comprise from about 80 to about 99.9% mole %. In other aspects, the cationic lipid comprises from about 2% to about 70%, from about 5% to about 50%, from about 10% to about 45%, from about 20% to about 99.8%, from about 30% to about 70%, from about 34% to about 59%, from about 20% to about 40%, or from about 30% to about 40% (mole %) of the total lipid present in said particle.

The LNPs described herein can further comprise a noncationic lipid, which can be any of a variety of neutral uncharged, zwitterionic or anionic lipids capable of producing a stable complex. They are preferably neutral, although they can be negatively charged. Examples of noncationic lipids useful in the present invention include phospholipid-related materials, such as natural phospholipids, synthetic phospholipid derivatives, fatty acids, sterols, and combinations thereof. Natural phospholipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI), Phosphatidic acid (phosphatidate) (PA), dipalmitoylphosphatidylcholine, monoacyl-phosphatidylcholine (lyso PC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), N-Acyl-PE, phosphoinositides, and phosphosphingolipids. Phospholipid derivatives include phosphatidic acid (DMPA, DPPA, DSPA), phosphatidylcholine (DDPC, DLPC, DMPC, DPPC, DSPC, DOPC, POPC, DEPC), phosphatidylglycerol (DMPG, DPPG, DSPG, POPG), phosphatidylethanolamine (DMPE, DPPE, DSPE DOPE), and phosphatidylserine (DOPS). Fatty acids include C14:0, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4), C20:0, C22:0 and lethicin.

In certain embodiments of the invention the non-cationic lipid is selected from lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cephalin, cardiolipin, phosphatidic

acid, cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG) , dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC) , palmitoyloleoyl-phosphatidylet-hanolamine (POPE) and dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal). Noncationic lipids also include sterols such as cholesterol, stigmasterol or stigmastanol. Cholesterol is known in the art. See U.S. Patent Application Publication Nos: U.S. 2006/0240554 and U.S. 2008/0020058. In certain embodiments, the LNP comprise a combination of a phospholipid and a sterol.

Where present, the non-cationic lipid typically comprises from about 0.1% to about 65%, about 2% to about 65%, about 10% to about 65%, or about 25% to about 65% expressed as mole percent of the total lipid present in the LNP. The LNPs described herein further include a polyethyleneglycol (PEG) lipid conjugate ("PEG-lipid") which may aid as a bilayer stabilizing component. The lipid component of the PEG lipid may be any non-cationic lipid described above including natural phospholipids, synthetic phospholipid derivatives, fatty acids, sterols, and combinations thereof. In certain embodiments of the invention, the PEG-lipids include, PEG coupled to dialkyloxypropyls (PEG-DAA) as described in, e.g., International Patent Application Publication No. WO 05/026372, PEG coupled to diacylglycerol (PEG-DAG) as described in, e.g., U.S. Patent Publication Nos. 20030077829 and 2005008689; PEG coupled to phosphatidylethanolamine (PE) (PEG-PE), or PEG conjugated to 1,2-Di-O-hexadecyl-sn-glyceride (PEG-DSG), or any mixture thereof (see, e.g., U.S. Pat. No. 5,885,613).

In one embodiment, the PEG-DAG conjugate is a dilaurylglycerol (C 12)-PEG conjugate, a PEG dimyristylglycerol (C14)conjugate, a PEG-dipalmitoylglycerol (C16) conjugate, a PEG-dilaurylglycamide (C12) conjugate, a PEG-dimyristylglycamide (C14) conjugate, a PEG-dipalmitoylglycamide (C16) conjugate, or a PEG-disterylglycamide (C18). Those of skill in the art will readily appreciate that other diacylglycerols can be used in the PEG-DAG conjugates.

In certain embodiments, PEG-lipids include, but are not limited to, PEG-dimyristolglycerol (PEG-DMG), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG- dipalmitoyl phosphatidylethanolamine (PEG-DPPE), and PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA).

In certain embodiments, the PEG-lipid is PEG coupled to dimyristoylglycerol (PEG-DMG), e.g., as described in Abrams *et al.*, 2010, Molecular Therapy 18(1):171, and U.S. Patent Application Publication Nos. US 2006/0240554 and US 2008/0020058.

In certain embodiments, the PEG-lipid, such as a PEG-DAG, PEG-cholesterol, PEG-DMB, comprises a polyethylene glycol having an average molecular weight ranging of about 500 daltons to about 10,000 daltons, of about 750 daltons to about 5,000 daltons, of about 1,000 daltons to about 5,000 daltons, of about 1,500 daltons to about 3,000 daltons or of about 2,000 daltons. In certain embodiments, the PEG-lipid comprises PEG400, PEG1500, PEG2000 or PEG5000.

The acyl groups in any of the lipids described above are preferably acyl groups derived from fatty acids having about C10 to about C24 carbon chains. In one embodiment, the acyl group is lauroyl, myristoyl, palmitoyl, stearoyl or oleoyl.

The PEG-lipid conjugate typically comprises from about 0.1% to about 15%, from about 0.5% to about 20%, from about 1.5% to about 18%, from about 4% to about 15%, from about 5% to about 12%, from about 1% to about 4%, or about 2% expressed as a mole % of the total lipid present in said particle.

In certain embodiments of the invention, the LNPs comprise one or more cationic lipids, cholesterol and 1,2-Dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG).

In certain embodiments the invention, the LNPs comprise one or more cationic lipids, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and 1,2-Dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG).

In certain embodiments of the invention, the LNPs comprise lipid compounds assembled within the following molar ratios:

Cationic Lipid (20-99.8 mole %)

Non-cationic lipid (0.1-65 mole %) and

PEG-DMG (0.1-20 mole %).

In certain embodiments of the invention, the LNPs comprise lipid compounds assembled within the following molar ratios:

Cationic Lipid (30-70 mole %)

Non-cationic lipid (20-65 mole %) and

PEG-DMG (1-15 mole %).

In certain aspects of this embodiment, the non-cationic lipid is cholesterol. Exemplary LNPs may include cationic lipid/cholesterol/PEG-DMG at about the following molar ratios: 58/30/10.

In certain aspects of this embodiment, the non-cationic lipid is cholesterol and DSPC. Exemplary LNPs may include cationic lipid/cholesterol/DSPC/PEG-DMG at about the following molar ratios: 59/30/10/1; 58/30/10/2; 43/41/15/1; 42/41/15/2; 40/48/10/2; 39/41/19/1; 38/41/19/2; 34/41/24/1; and 33/41/24/2.

In one aspect of this embodiment, the TLR9 agonist is selected from A-class CpG ODN, B-class CpG ODN and C-class CpG ODN. In one embodiment, the oligonucleotide is an oligodeoxynucleotide. In one embodiment, one or more of the internucleotide linkages of the CpG ODN are modified linkages. In one embodiment, one or more of the internucleotide linkages of CpG ODN are phosphorothioate (PS) linkages. In one embodiment, all of the internucleotide linkages of C-class and B-class CpG ODNs are phosphorothioate (PS) linkages. Also included are oligonucleotides with mixed internucleotide linkages. See Krieg *et al.*, 1995, Nature 374:546-549; Chu *et al.*, 1997, J. Exp. Med. 186:1623-1631; Lipford *et al.*, 1997, Eur. J. Immunol. 27:2340-2344; Roman *et al.*, 1997, Nat. Med. 3:849-854; Davis *et al.*, 1998, J. Immunol. 160:870-876; Lipford *et al.*, 1998, Trends Microbiol. 6:496-500; and U.S. Pat. Nos. 6,207,646; 7,223,398; 7,250,403; or 7,566,703. Methods for producing phosphorothioate oligonucleotides or phosphorodithioate are described in U.S. Pat. No.5,666,153, U.S. Pat. No. 5,278,302 and International Patent Application Publication No. WO95/26204.

In certain embodiments of the invention, the invention formulation features LNP compositions formulated or complexed with TLR9 agonist compounds, and assembled within the following molar ratios:

Cationic Lipid (20-99.7 mole %)

Non-cationic lipid (0.1-65 mole %)

PEG-DMG (0.1-15 mole %)

TLR9 agonists (0.1-50 mole %).

In other embodiments of the invention, the TLR9 agonist is provided at a wt/wt% of agonist to total lipids in a range of 1% to 20% or 4% to 15%.

In certain embodiments of the invention, the one or more TLR9 agonist is physically encapsulated in the LNP before or after LNP preparation. TLR9 agonists physically encapsulated in the LNP can be prepared via confined-volume ethanol desolvation

method as described, or via alternative techniques known in the art, including, but not limited to thin-film hydration, emulsion diffusion, or homogenization.

In certain embodiments of the invention, one or more TLR9 agonist is adsorbed, covalently coupled, ionically-interacted or formulated onto surfaces of the LNP. See, e.g., Li *et al.*, 2002, Vaccine 20:148-157; Wilson *et al.*, 2009, J. Gene Med. 11:14-25; Goldinger *et al.*, 2012, Eur. J. Immunol. 42:3049-3061; Gursel *et al.*, 2001, J. Immunol. 167:3324-3328; and Chikh *et al.*, 2009, Int. Immunol. 7:757-767.

Preparation of LNPs

LNPs can be formed, for example, by a rapid precipitation process which entails micro-mixing the lipid components dissolved in ethanol with an aqueous solution using a confined volume mixing apparatus such as a confined volume T-mixer, a multi-inlet vortex mixer (MIVM), or a microfluidics mixer device as described below. The lipid solution contains one or more cationic lipids, one or more noncationic lipids (e.g., DSPC), PEG-DMG, and optionally cholesterol, at specific molar ratios in ethanol. The aqueous solution consists of a sodium citrate or sodium acetate buffered salt solution with pH in the range of 2-6, preferably 3.5-5.5. The two solutions are heated to a temperature in the range of 25°C-45°C, preferably 30°C-40°C, and then mixed in a confined volume mixer thereby instantly forming the LNP. When a confined volume T-mixer is used, the T-mixer has an internal diameter (ID) range from 0.25 to 1.0 mm. The alcohol and aqueous solutions are delivered to the inlet of the T-mixer using programmable syringe pumps, and with a total flow rate from 10-600 mL/minute. The alcohol and aqueous solutions are combined in the confined-volume mixer with a ratio in the range of 1:1 to 1:3 vol:vol, but targeting 1:1.1 to 1:2.3. The combination of ethanol volume fraction, reagent solution flow rates and t-mixer tubing ID utilized at this mixing stage has the effect of controlling the particle size of the LNPs between 30 and 300 nm. The resulting LNP suspension is twice diluted into higher pH buffers in the range of 6-8 in a sequential, multi-stage in-line mixing process. For the first dilution, the LNP suspension is mixed with a buffered solution at a higher pH (pH 6-7.5) with a mixing ratio in the range of 1:1 to 1:3 vol:vol, but targeting 1:2 vol:vol. This buffered solution is at a temperature in the range of 15-40°C, targeting 30-40°C. The resulting LNP suspension is further mixed with a buffered solution at a higher pH, e.g., 6-8 and with a mixing ratio in the range of 1:1 to 1:3 vol:vol, but targeting 1:2 vol:vol. This later buffered solution is at a temperature in the range of 15-40°C, targeting 16-25°C. The mixed LNPs are

held from 30 minutes to 2 hours prior to an anion exchange filtration step. The temperature during incubation period is in the range of 15-40°C, targeting 30-40°C. After incubation, the LNP suspension is filtered through a 0.8 μm filter containing an anion exchange separation step. This process uses tubing IDs ranging from 1 mm ID to 5 mm ID and a flow rate from 10 to 2000 mL/minute. The LNPs are concentrated and diafiltered via an ultrafiltration process where the alcohol is removed and the buffer is exchanged for the final buffer solution such as phosphate buffered saline or a buffer system suitable for cryopreservation (for example containing sucrose, trehalose or combinations thereof). The ultrafiltration process uses a tangential flow filtration format (TFF). This process uses a membrane nominal molecular weight cutoff range from 30-500 KD, targeting 100 KD. The membrane format can be hollow fiber or flat sheet cassette. The TFF processes with the proper molecular weight cutoff retains the LNP in the retentate and the filtrate or permeate contains the alcohol and final buffer wastes. The TFF process is a multiple step process with an initial concentration to a lipid concentration of 20-30 mg/mL. Following concentration, the LNP suspension is diafiltered against the final buffer (for example, phosphate buffered saline (PBS) with pH 7-8, 10 mM Tris, 140 mM NaCl with pH 7-8, or 10 mM Tris, 70 mM NaCl, 5 wt% sucrose, with pH 7-8) for 5-20 volumes to remove the alcohol and perform buffer exchange. The material is then concentrated an additional 1-3 fold via ultrafiltration. The final steps of the LNP manufacturing process are to sterile filter the concentrated LNP solution into a suitable container under aseptic conditions. Sterile filtration is accomplished by passing the LNP solution through a pre-filter (Acropak 500 PES 0.45/0.8 μm capsule) and a bioburden reduction filter (Acropak 500 PES 0.2/0.8 μm capsule). Following filtration, the vialed LNP product is stored under suitable storage conditions (2°C-8°C, or -20°C if frozen formulation).

In some embodiments, the LNPs of the compositions provided herein have a mean geometric diameter that is less than 1000 nm. In some embodiments, the LNPs have mean geometric diameter that is greater than 50 nm but less than 500 nm. In some embodiments, the mean geometric diameter of a population of LNPs is about 60 nm, 75 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 225 nm, 250 nm, 275 nm, 300 nm, 325 nm, 350 nm, 375 nm, 400 nm, 425 nm, 450 nm, or 475 nm. In some embodiments, the mean geometric diameter is between 100-400 nm, 100-300 nm, 100-250 nm, or 100-200 nm. In some embodiments, the mean geometric diameter is between 60-400 nm, 60-350 nm, 60-300 nm, 60-250 nm, or 60-200 nm. In some embodiments, the mean geometric diameter is

between 75-250 nm. In some embodiments, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the LNPs of a population of LNPs have a diameter that is less than 500 nm. In some embodiments, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the LNPs of a population of LNPs have a diameter that is greater than 50 nm but less than 500 nm. In some embodiments, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the LNPs of a population of LNPs have a diameter of about 60 nm, 75 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 225 nm, 250 nm, 275 nm, 300 nm, 325 nm, 350 nm, 375 nm, 400 nm, 425 nm, 450 nm, or 475 nm. In some embodiments, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the LNPs of a population of LNPs have a diameter that is between 100-400 nm, 100-300 nm, 100-250 nm, or 100-200 nm. In some embodiments, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more of the LNPs of a population of LNPs have a diameter that is between 60-400 nm, 60-350 nm, 60-300 nm, 60-250 nm, or 60-200 nm.

In a particular embodiment, the size of the LNPs ranges between about 1 and 1000 nm, preferably between about 10 and 500 nm, more preferably between about 100 to 300 nm, and preferably 100 nm.

Method of Treatment

The present invention is also directed to methods of treating cancer in the subject by co-administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) an anti-IL-10 antibody. In another embodiment, the invention provides a medicament comprising the LNP of the invention and a TLR9 agonist for use in combination with an anti-IL-10 antibody for treating cancer in the subject by co-administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) an anti-IL-10 antibody. In a another embodiment, the invention provides a medicament comprising the LNP of the invention and a TLR9 agonist for use in combination with an anti-IL-10 antibody for treating cancer in the subject by intravenously administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) intravenously administering an anti-IL-10 antibody. In a further embodiment, the invention provides a medicament comprising the LNP of the invention and a TLR9 agonist for use in combination with an anti-IL-10 antibody for treating cancer in the subject by intratumorally administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) intratumorally administering an anti-IL-10 antibody. In yet a further embodiment, the invention provides a medicament comprising the LNP of the

invention and a TLR9 agonist for use in combination with an anti-IL-10 antibody for treating cancer in the subject by intratumorally administering to the subject 1) an effective amount of a composition comprising the LNP of the invention and TLR9 agonist; and 2) intravenously administering an anti-IL-10 antibody.

Examples of cancers include, but are not limited to breast cancer; biliary tract cancer; bladder cancer; brain cancer including glioblastomas and meduUoblastomas; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms including acute lymphocytic and myelogenous leukemia, e.g., B Cell CLL; T-cell acute lymphoblastic leukemia/lymphoma; hairy cell leukemia; chronic myelogenous leukemia, multiple myeloma; AIDS-associated leukemias and adult T-cell leukemia/lymphoma; intraepithelial neoplasms including Bowen's disease and Paget's disease; liver cancer; lung cancer; lymphomas including Hodgkin's disease and lymphocytic lymphomas; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; skin cancer including melanoma, Merkel cell carcinoma, Kaposi's sarcoma, basal cell carcinoma, and squamous cell cancer; testicular cancer including germinal tumors such as seminoma, non-seminoma (teratomas, choriocarcinomas), stromal tumors, and germ cell tumors; thyroid cancer including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms tumor.

The combination therapy may also comprise one or more additional therapeutic agents. The additional therapeutic agent may be, e.g., a chemotherapeutic other than a CpG oligonucleotide, a biotherapeutic agent, immunotherapeutic agent, an immunogenic agent (for example, attenuated cancerous cells, tumor antigens, antigen presenting cells such as dendritic cells pulsed with tumor derived antigen or nucleic acids, immune stimulating cytokines (for example, IL-2, IFNα2, GM-CSF), cells transfected with genes encoding immune stimulating cytokines such as but not limited to GM-CSF), and radiation. In some embodiments, the immunotherapeutic agent comprises one or more of a cytokine, a small molecule adjuvant, and an antibody. In some embodiments, the cytokine comprises one or more of a chemokine, an interferon, an interleukin, a lymphokine, and a tumour necrosis factor. The specific dosage and dosage schedule of the additional therapeutic agent can further vary, and the optimal dose, dosing schedule and route of

administration will be determined based upon the specific therapeutic agent that is being used.

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin gamma1I and calicheamicin phiI1, see, e.g., Agnew, Chem. Intl. Ed. Engl., 33:183-186 (1994); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide

glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2, 2′,2″-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel and docetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane, formestane, fadrozole, vorozole, letrozole, and anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Each therapeutic agent in a combination therapy of the invention may be administered either alone or in a medicament (also referred to herein as a pharmaceutical composition) which comprises the therapeutic agent and one or more pharmaceutically acceptable carriers, excipients and diluents, according to standard pharmaceutical practice.

Each therapeutic agent in a combination therapy of the invention may be administered simultaneously (i.e., in the same medicament), concurrently (i.e., in separate medicaments administered one right after the other in any order) or sequentially in any order. Sequential administration is particularly useful when the therapeutic agents in the combination therapy are in different dosage forms (one agent is a tablet or capsule and another agent is a sterile liquid) and/or are administered on different dosing schedules, e.g., a

chemotherapeutic that is administered at least daily and a biotherapeutic that is administered less frequently, such as once weekly, once every two weeks, or once every three weeks.

In some embodiments, the CpG oligonucleotide and LNP of the invention is administered before administration of the anti-IL-10 antibody, while in other embodiments, the CpG oligonucleotide and LNP of the invention is administered after administration of the anti-IL-10 antibody. In another embodiment, the CpG oligonucleotide is administered concurrently with the anti-IL-10 antibody.

In some embodiments, the CpG oligonucleotide and LNP of the invention is administered intratumorally or intravenously. In another embodiment, the anti-IL-10 antibody is administered intratumorally or intravenously.

In some embodiments, at least one of the therapeutic agents in the combination therapy is administered using the same dosage regimen (dose, frequency and duration of treatment) that is typically employed when the agent is used as monotherapy for treating the same cancer. In other embodiments, the patient receives a lower total amount of at least one of the therapeutic agents in the combination therapy than when the agent is used as monotherapy, e.g., smaller doses, less frequent doses, and/or shorter treatment duration.

Each small molecule therapeutic agent in a combination therapy of the invention can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal, topical, and transdermal routes of administration.

A combination therapy of the invention may be used prior to or following surgery to remove a tumor and may be used prior to, during or after radiation therapy.

In some embodiments, a combination therapy of the invention is administered to a patient who has not been previously treated with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-naïve. In other embodiments, the combination therapy is administered to a patient who failed to achieve a sustained response after prior therapy with a biotherapeutic or chemotherapeutic agent, i.e., is treatment-experienced.

A combination therapy of the invention is typically used to treat a tumor that is large enough to be found by palpation or by imaging techniques well known in the art, such as MRI, ultrasound, or CAT scan.

Selecting a dosage regimen (also referred to herein as an administration regimen) for a combination therapy of the invention depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells, tissue or organ in the individual being treated.

Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the patient consistent with an acceptable level of side effects. Accordingly, the dose amount and dosing frequency of each biotherapeutic and chemotherapeutic agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated, and patient characteristics. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available. *See, e.g.*, Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert *et al.* (2003) *New Engl. J. Med.* 348:601-608; Milgrom *et al.* (1999) *New Engl. J. Med.* 341:1966-1973; Slamon *et al.* (2001) *New Engl. J. Med.* 344:783-792; Beniaminovitz *et al.* (2000) *New Engl. J. Med.* 342:613-619; Ghosh *et al.* (2003) *New Engl. J. Med.* 348:24-32; Lipsky *et al.* (2000) *New Engl. J. Med.* 343:1594-1602; Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002). Determination of the appropriate dosage regimen may be made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment, and will depend, for example, on the patient's clinical history (e.g., previous therapy), the type and stage of the cancer to be treated and biomarkers of response to one or more of the therapeutic agents in the combination therapy.

Biotherapeutic agents in a combination therapy of the invention may be administered by continuous infusion, or by doses at intervals of, *e.g.*, daily, every other day, three times per week, or one time each week, two weeks, three weeks, monthly, bimonthly, etc. A total weekly dose is generally at least 0.05 μg/kg, 0.2 μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. *See, e.g.*, Yang *et al.* (2003) *New Engl. J. Med.* 349:427-434; Herold *et al.* (2002) *New Engl. J. Med.* 346:1692-1698; Liu *et al.* (1999) *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji *et al.* (20003) *Cancer Immunol. Immunother.* 52:133-144.

In one embodiment of the invention, the anti-IL-10 antibody in the combination therapy is anti-IL-10 hum 12G8, which is administered intravenously at a dose selected from the group consisting of: 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 4 mg/kg Q3W, 5 mg/kg Q3W, 6 mg/kg Q3W, 7 mg/kg Q3W, 8 mg/kg Q3W, 9 mg/kg Q3W, 10 mg/kg Q3W, 11 mg/kg Q3W, 12 mg/kg Q3W, 13 mg/kg Q3W, 14 mg/kg Q3W and 15 mg/kg Q3W. In another embodiment of the invention, the anti-IL-10 antibody in the combination therapy is anti-IL-10 hum 12G8, which is administered intravenously at a dose of 1 mg/kg Q3W. In

a futher embodiment of the invention, the anti-IL-10 antibody in the combination therapy is anti-IL-10 hum 12G8, which is administered intravenously at a dose of 3 mg/kg Q3W. In yet another embodiment of the invention, the anti-IL-10 antibody in the combination therapy is anti-IL-10 hum 12G8, which is administered intravenously at a dose of 10 mg/kg Q3W.

In a preferred embodiment of the invention, the anti-IL-10 antibody in the combination therapy is anti-IL-10 hum 12G8, an anti-IL-10 hum 12G8 variant, which is administered in a liquid medicament at a dose selected from the group consisting of 1 mg/kg Q3W, 2 mg/kg Q3W, 3 mg/kg Q3W, 4 mg/kg Q3W, 5 mg/kg Q3W, 6 mg/kg Q3W, 7 mg/kg Q3W, 8 mg/kg Q3W, 9 mg/kg Q3W, 10 mg/kg Q3W, 11 mg/kg Q3W, 12 mg/kg Q3W, 13 mg/kg Q3W, 14 mg/kg Q3W and 15 mg/kg Q3W.

In certain embodiments of the invention, one or more of the TLR9 agonist is adsorbed, covalently coupled, ionically-interacted, or formulated onto surfaces of the LNP. In an alternative embodiment, the LNP may be co-administered with one or more TLR9 agonists.

The compositions of the invention can be administered to cells by a variety of methods known to those of skill in the art. In one embodiment, delivery systems of the invention include, for example, aqueous and nonaqueous gels, multiple emulsions, microemulsions, aqueous and nonaqueous solutions, aerosols, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrolidone). In one embodiment, the pharmaceutically acceptable carrier is a transdermal enhancer.

In one embodiment, delivery systems of the invention include patches, suppositories, and gels, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

In one embodiment, the invention features a composition comprising one or more formulated TLR9 agonists in an acceptable carrier, such as a stabilizer, buffer, and the like. The compositions of the invention can be administered and introduced to a subject by any standard means, with or without stabilizers, buffers, and the like, to form a composition. The compositions of the present invention can also be formulated and used as gels, sprays, oils and other suitable compositions for topical, dermal, or transdermal administration as is known in the art.

In one embodiment, compositions of the invention are administered to a subject by systemic administration in a pharmaceutically acceptable composition or formulation. By "systemic administration" is meant in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular.

By "pharmaceutically acceptable formulation" or "pharmaceutically acceptable composition" is meant, a composition or formulation suitable for administration in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the formulated molecular compositions of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich *et al*, 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate.

The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired lipid nanoparticles in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A. R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the formulated composition.

Aqueous suspensions contain the lipid nanoparticles in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose,

hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the lipid nanoparticles in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the lipid nanoparticles in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable

solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Formulated compositions of the invention can be administered parenterally in a sterile medium. The lipid nanoparticles, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

For administration to non-human animals, the composition can also be added to water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein, as presently representative of preferred embodiments, are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

EXAMPLES

EXAMPLE 1: PREPARATION OF LIPID NANOPARTICLE COMPOSITIONS

The lipid nanoparticle (LNP) composition, LNP A, comprised compound 1-9 in Table 3 (amino lipid 9 from WO2011/022460), cholesterol, polyethylene glycol-lipid (PEG-DMG), and phospholipid (DSPC) at a specified molar composition of 58:30:10:2 amino lipid:cholesterol:DSPC:PEG-DMG.

The LNP composition was prepared by a rapid precipitation process which entailed micromixing lipids dissolved in ethanol with an aqueous solution using a confined volume mixing apparatus (Gindy et al., Molecular Pharmaceutics, 2014, 11 (11), pp 4143–4153). The lipid solution contained amino lipid, cholesterol, PEG-DMG, and DSPC at the specified molar ratios in ethanol. The aqueous solution consisted of a sodium citrate buffered

salt solution [20 mM] with pH in the range of 5-5.5. The two solutions were heated to a temperature in the range of 35°C-40°C and then mixed in a confined volume mixer (T-mixer with ID of 0.5 mm) instantly forming the LNP. The alcohol and aqueous solutions were delivered to the inlet of the T-mixer using programmable syringe pumps, and with a total flow rate from 100-150 mL/minute. The alcohol and aqueous solutions were combined in the confined-volume mixer with a ratio in the range of 11:1.5 to 2.1:3.8 to produce 40-55 vol:vol % alcohol in the mixed solution. The combination of ethanol volume fraction, reagent solution flow rates and t-mixer tubing ID utilized at this mixing stage had the effect of controlling the particle size of the LNPs between 30 and 300 nm. The resulting LNP suspension was twice diluted into higher pH buffers [pH range 6-8] in a sequential, multi-stage in-line mixing process. For the first dilution, the LNP suspension was mixed with a 20 mM sodium citrate, 300 mM sodium chloride buffered solution with pH 6 with a mixing ratio of 1:1 vol:vol. This buffered solution was at a temperature in the range of 35-40°C. The resulting LNP suspension was further mixed with a buffered solution (phosphate buffered saline (PBS), pH of 7.5) and with a mixing ratio of 1:1 vol:vol. This later buffered solution was at a temperature in the range 16-25°C. The mixed LNPs were held from 30 minutes prior to an anion exchange filtration step. The temperature during incubation period was in the range of 30-40°C. After incubation, the LNPs were concentrated and diafiltered via an ultrafiltration process where the alcohol was removed and the buffer was exchanged for the final buffer solution. The ultrafiltration process used a tangential flow filtration format (TFF). This process used a PES membrane nominal molecular weight cutoff of 100 KD. The membrane format was hollow fiber or flat sheet cassette. Ultrafiltration with a 100 kDa PES membrane is first used to concentrate the LNP solution 8-fold by volume, targeting a total lipids concentration of 20-30 mg/mL. Ethanol removal is effected by subsequent diafiltration using 10mM Tris, 140 mM sodium chloride, pH 7-7.5 (5-10 diavolumes). A final buffer exchange into a buffer solution comprising 10 mM Tris, 70 mM NaCl and 5 wt% sucrose is performed. The LNP solution is then sterile filtered into sterile vials under asceptic conditions via Pall 0.45 μm PES, and a Pall 0.2 PES μm syringe filters. The LNP solution is stored under refrigeration (2-8°C) or as a frozen image (-20°C).

Analytical Procedure:

Particle size and polydispersity LNPs were diluted to a final volume of 3 ml with 1 × phosphate buffered saline (PBS). The particle size and polydispersity of the samples was measured by a dynamic light scattering method using ZetaPALS instrument

(Brookhaven Instruments Corporation, Holtsville, NY). The scattered intensity was measured with He–Ne laser at 25°C with a scattering angle of 90°.

Zeta Potential Measurement

LNPs were diluted to a final volume of 2 ml with 1 mM Tris buffer (pH 7.4). Electrophoretic mobility of samples was determined using ZetaPALS instrument (Brookhaven Instruments Corporation, Holtsville, NY) with a disposable Zeta cell DTS 1060C (Malvern Instruments Ltd, Worcestershire, UK) and He–Ne laser as a light source. The Smoluchowski limit (Z. Phys. Chem., 93 (1918), p. 129) was assumed in the calculation of zeta potentials.

Lipids analysis

Individual lipid concentrations were determined by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) using Waters 2695 Alliance system (Water Corporation, Milford MA) with a Corona charged aerosol detector (CAD) (ESA Biosciences, Inc, Chelmsford, MA). Individual lipids in RDVs were analyzed using an Agilent Zorbax SBC18 (50 × 4.6 mm, 1.8 μm particle size) column with CAD at 60°C. The mobile phase was composed of A: 0.1% TFA in H2O and B: 0.1% TFA in IPA. The gradient changed from 60% mobile phase A and 40% mobile phase B from time 0 to 40% mobile phase A and 60% mobile phase B at 1.00 min; 40% mobile phase A and 60% mobile phase B from 1.00 to 5.00 min; 40% mobile phase A and 60% mobile phase B from 5.00 min to 25% mobile phase A and 75% mobile phase B at 10.00 min; 25% mobile phase A and 75% mobile phase B from 10.00 min to 5% mobile phase A and 95% mobile phase B at 15.00 min; and 5% mobile phase A and 95% mobile phase B from 15.00 to 60% mobile phase A and 40% mobile phase B at 20.00 min with a flow rate of 1 ml/minute. The individual lipid concentration was determined by comparing to the standard curve with all the lipid components in the RDVs with a quadratic curve fit. The molar percentage of each lipid was calculated based on its molecular weight. Analytic analysis confirmed the correct molar ratios.

EXAMPLE 2: PREPARATION OF LIPID NANOPARTICLE COMPOSITIONS ENCAPSULATING IMMUNOSTIMULATORY OLIGODEOXYNUCLEOTIDES

Lipid nanoparticles (LNP) formulations encapsulating immunostimulatory oligonucleotides were prepared as described in Example 1 with the following modifications.

The LNP composition, LNP B, comprised compound 1-9, cholesterol, DSPC and PEG-DMG at a molar composition of 58:30:10:2, respectively, and encapsulated

Cytidine phospho-guanosine (CpG)-based phosphorothioate oligodeoxynucleotide, ODN 1826, (InvivoGen, San Diego, CA). ODN 1826 (5'-tccatgacgttcctgacgtt-3') (SEQ ID NO: 22) is a mouse TLR9 specific agonist and is a class B type sequence.

The LNP composition, LNP C, comprised compound 1-9, cholesterol, DSPC and PEG-DMG at a molar composition of 58:30:10:2, respectively, and encapsulated a negative control ODN 1826 sequence. ODN 1826 control sequence contains GpC dinucleotides instead of CpGs and is used as a negative control ODN.

LNPs encapsulating immunostimulatory molecules (LNP B or LNP C) were formed by micro-mixing lipids dissolved in ethanol with an aqueous solution containing the immunostimulatory molecule using a confined volume mixing apparatus. The aqueous solution containing ODN consisted of a sodium citrate solution [20 mM sodium citrate] with pH in the range of 5-5.5.

Analytical Procedure:

IMO concentration

The ODN 1826 concentrations were determined by Strong Anion-Exchange High-Performance Liquid Chromatography (SAX-HPLC) using Waters 2695 Alliance system (Water Corporation, Milford MA) with a 2996 PDA detector. The LNPs were treated with 0.5% Triton X-100 to free total ODN 1826 and analyzed by SAX separation using a Dionex BioLC DNAPac PA 200 (4 × 250 mm) column with UV detection at 254 nm. Mobile phase was composed of A: 25 mM NaClO4, 10 mM Tris, 20% EtOH, pH 7.0 and B: 250 mM NaClO4, 10 mM Tris, 20% EtOH, pH 7.0 with a linear gradient from 0-15 min and a flow rate of 1 ml/minute. The ODN 1826 amount was determined by comparing to the ODN 1826 standard curve.

Encapsulation rate

Fluorescence reagent SYBR Gold was employed for ODN 1826 quantitation to monitor the encapsulation of oligonucleotide in RDVs. RDVs with or without Triton X-100 were used to determine the free ODN 1826 and total ODN 1826 amount. The assay was performed using a SpectraMax M5e microplate spectrophotometer from Molecular Devices (Sunnyvale, CA). Samples were excited at 485 nm and fluorescence emission was measured at 530 nm. The ODN 1826 amount is determined by comparing to an ODN 1826 standard curve. Encapsulation rate = (1- free ODN/total ODN) ×100%

EXAMPLE 3: PREPARATION OF LIPID NANOPARTICLE COMPOSITIONS CO-FORMULATED WITH IMMUNOSTIMULATORY OLIGODEOXYNUCLEOTIDES

Lipid nanoparticles (LNP) composition co-formulated with immunostimulatory ODN 1826 (InvivoGen, San Diego), LNP D, was prepared by co-mixing LNP composition of Example 1, LNP A, with ODN 1826 (InvivoGen, San Diego, CA), in buffer solution comprising 10 mM Tris, 70 mM NaCl and 5 wt% sucrose. The solution was mixed gently at room temperature for 15 minutes and stored at 4-8°C for further use. Lipid Nanoparticle composition co-formulated with ODN 1826 control sequence, LNP E, was similarly prepared. ODN 1826 control sequence (InvivoGen, San Diego) contains GpC dinucleotides instead of CpGs and is used as a negative control ODN.

Table 5

Reagent	**Composition**
LNP A	Lipid Nanoparticle (LNP) composition comprising compound 1-9, cholesterol, DSPC and PEG-DMG at a molar ratio of 58:30:10:2
LNP B	LNP A encapsulating ODN 1826 (LNP A/ODN 1826)
LNP C	LNP A encapsulating ODN 1826 control (LNP A/ODN 1826 control)
LNP D	LNP A co-formulated with ODN 1826 (LNP A + ODN 1826)
LNP E	LNP A co-formulated with ODN 1826 control (LNP A + ODN 1826 control)

EXAMPLE 4: EFFECTS OF LIPID NANOPARTICLES ON ANTI-TUMOR ACTIVITY OF ANTI-IL-10 AND PERITUMORAL CpG IN ANIMAL MODEL

Antibodies

TC40.11D8 is a mouse IgG1/kappa monoclonal antibody targeted against mouse IL-10. The mouse IgG1 isotype control is a mouse monoclonal antibody specific for adenoviral hexon 25. Both antibodies were obtained from internal sources as frozen (-80°C) stocks.

Formulations of Antibodies

The formulation buffer is specific for each antibody to stabilize proteins and prevent precipitation. The formulations for both TC40.11D8 and mouse IgG1 isotype control were 75 mM sodium chloride, 10 mM sodium phosphate, 3% sucrose, pH7.3.

Oligodeoxynucleotides

Cytidine phospho-guanosine (CpG)-based phosphorothioate oligodeoxynucleotide ODN 1826 (InvivoGen, San Diego, CA) is a mouse TLR9 specific

agonist. ODN 1826 has CpG class B type sequence. ODN 1826 control contains GpC dinucleotides instead of CpGs and is used as a negative control ODN.

Formulations of Oligodeoxynucleotides, Lipid Nanoparticle, and Lipid-Nanoparticle Encapsulated Oligodeoxynucleotides

The formulation buffer for Lipid Nanoparticle of Example 1 (LNP A), Lipid Nanoparticle encapsulating ODN 1826 (Example 2, LNP B), Lipid Nanoparticle encapsulating ODN 1826 control (Example 2, LNP C), or free ODN 1826 and ODN 1826 control, is 10 mM Tris, 5 wt% sucrose, pH 7.5.

Animals

Approximately seven to eight week old female C57BL/6J mice were obtained from Jackson Laboratory (Sacramento, CA). Conventional animal chow and water were provided ad libitum. Animals were housed for one week prior to the start of the study. The average weight of the animals at the start of the study (i.e. tumor implantation) was 19 grams.

Tumor Cell Line Preparation and Implantation

The TC-1 cell line, provided by Johns Hopkins University (Baltimore, MD) is derived from mouse primary lung epithelial cells that were cotransformed with human papilloma virus (HPV-16) E6 and E7 and c-Ha.ras oncogene (Lin et al., *Cancer Res.*, 1996 Jan 1, 56(1):21-6). TC-1 cells are syngeneic to C57BL6/J strain.

The TC-1 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 0.4 mg/mL Geneticin. 1×10^5 TC-1 cells were injected subcutaneously (SC) in 0.1 mL of serum-free DMEM in lower dorsal right and left flanks of each animal. Animals were first shaved with electronic clippers in the areas that were used for the implantation.

Tumor measurements and body weights

Tumors were measured the day before the first dose and twice a week thereafter. Tumor length and width were measured using electronic calipers and tumor volume determined using the formula Volume (mm^3) = 0.5 × Length × $Width^2$ where length is the longer dimension. Animals were weighed the day before the first dose and twice a week thereafter. To prevent bias, any outliers by weight or tumor volume were removed and the remaining mice were grouped into various treatment groups based on the tumor volume in the right flank (referred to as injected tumor).

Dosing Solution Preparation

Frozen stocks of the antibodies were thawed on ice. To avoid repeated freeze thaw, each vial of stock was thawed once and aliquots made in volumes sufficient for one

time use. The aliquots were stored at -80°C. Before each dosing, one aliquot was thawed on ice and diluted to nominal concentration in appropriate formulation buffer.

Stocks of ODN 1826 and ODN 1826 control were stored at 4°C. Stocks of empty Lipid Nanoparticles (LNP A) and Lipid Nanoparticle-encapsulated ODN 1826 (LNP B) and Lipid Nanoparticle-encapsulated ODN 1826 (LNP C) control were stored at -20°C, and stocks were thawed on ice and diluted to nominal concentration in the appropriate formulation buffer. If vial of thawed stock was not used in entirety, it was stored at 4°C for next dosing.

Administration of Antibodies, Oligodeoxynucleotides, Lipid Nanoparticle, and Lipid Nanoparticle-encapasulated Oligodeoxynucleotides

Isotype control mIgG1 and anti-IL-10 mIgG1 were administered intraperitoneally (IP) at 10 mg/kg on Days 0, 3, 7, and 12. ODN 1826 control (0.5 mg/kg), ODN 1826 (0.5 mg/kg), Lipid Nanoparticle, LNP A (4.2 mg/kg), Lipid Nanoparticle (LNP)-encapsulating ODN 1826, LNP B (4.2 mg/kg LNP /0.5 mg/kg ODN 1826), and Lipid Nanoparticle (LNP)-encapsulating ODN 1826 control, LNP C (3.8 mg/kg LNP /0.5 mg/kg ODN 1826 control) were administered peritumoral (PT) only in right tumors on Days 0, 3, 7 , and 12. "/" designates encapsulation. Dosing was withheld if an animal lost body weight ≥ 15% and less than 20% based on measurement prior to dosing.

Statistical Methods

Comparisons between treatments were made at each day of follow-up, using tumor volume at that day.

Analysis of tumor volumes. Follow-up of individual animals could be terminated early because of excessive tumor burden or other reasons. Depending on the reason and tumor size at the last measurement (see below), the last observed tumor volume was treated as a lower bound on volume at all later days for that animal (right-censored data).

To compare two treatment groups on a given day, a generalization of the nonparametric Mann-Whitney (or Wilcoxon rank sum) test that allows for right-censored data was used: the Peto and Peto version of the Gehan-Breslow test. Two-sided p-values were estimated from 20,000 random reassignments of animals between the two treatments being compared. To control the familywise error rate across all time points for a given pair of treatments, p-values were multiplicity adjusted by Holm's method. A p-value of less than 0.05 was used to define statistical significance.

For descriptive purposes, volumes for each day and treatment group were summarized by their median. To allow for censoring, a distribution function for each day and

treatment group was estimated by the Kaplan-Meier method, with confidence band obtained using the beta product confidence procedure. The median was estimated as the 50th percentile of the distribution function, with confidence interval obtained by inverting the confidence band. A 68% confidence level was used, to be comparable to the common "mean ± SE" format for summarizing data, since the latter is approximately a 68% confidence interval for the mean.

When follow-up of an animal was terminated early, the reason was categorized and the animal's data were handled as follows: (1) tumor burden: right-censor at last measured value; (2) tumor ulceration: right-censor at last measured value, provided this exceeded a threshold (1000 mm^3); otherwise omit animal at later times; (3) weight loss/ill: omit animal at later times; (4) found dead; and (5) unrelated to treatment (e.g., accident, administrative termination): right-censor at last measured value, provided this exceeded a threshold (1000 mm^3); otherwise omit animal at later times.

Table 6

Group	**Treatment**	**Animals remaining at Day 11**	**Early Terminations by Day 11**				
			Tumor burden	Tumor ulcer-ation	Weight loss/ill	Found dead	Unrelated to treatment
1	mIgG1 isotype control + ODN 1826 control	5 of 10	1	0	4	0	0
2	anti-IL-10 + ODN 1826	10 of 10	0	0	0	0	0
3	mIgG1 isotype control + LNP A/ODN 1826 control	9 of 10	0	0	1	0	0
4	mIgG1 isotype control + LNP A + ODN 1826	9 of 10	0	0	1	0	0
5	anti-IL-10 + LNP A + ODN 1826 control	7 of 10	0	0	0	3	0
6	anti-IL-10 + LNP A + ODN 1826	8 of 10	0	0	1	1	0
7	anti-IL-10 + LNP A/ ODN	9 of 10	0	0	0	1	0

Group	Treatment	Animals remaining at Day 11	Early Terminations by Day 11				
			Tumor burden	Tumor ulcer-ation	Weight loss/ill	Found dead	Unrelated to treatment
	1826 control						
8	anti-IL-10 + LNP A/ ODN 1826)	9 of 10	0	0	1	0	0

The threshold for using the last measured volume as a lower bound on later volumes was based on a judgment that once a tumor's volume exceeded that threshold, it was unlikely to shrink.

Treatment Results

TC-1 tumor-bearing C57BL/6J mice were grouped into 8 treatment groups of ten animals when the mean volume of tumors on right flank reached approximately 98 mm^3 (51 mm^3 – 196 mm^3): (1) mIgG1 isotype control + ODN 1826 control; (2) anti-IL-10 + ODN 1826; (3) mIgG1 isotype control + LNP A/ODN 1826 control; (4) mIgG1 isotype control + LNP A/ODN 1826; and (5) anti-IL-10 + LNP A + ODN 1826 control; (6) anti-IL-10 + LNP A + ODN 1826; (7) anti-IL-10 + LNP A/ODN 1826 control; and (8) anti-IL-10 + LNP A/ODN 1826. The range of volumes of tumors on left was 18 mm^3 – 489 mm^3. Complete regression (CR) of a tumor was defined as the absence of a measurable tumor at the time measurement was conducted, given that a tumor was measurable on the day that animals were grouped. The results are shown in Figures 1 and 2.

Administration of LNP A in combination with anti-IL-10 and free ODN 1826 (Group 6) or administration of LNP A encapsulated ODN 1826 (LNP B) in combination with anti-IL-10 (Group 8) resulted in 9 and 8 Complete Regressions (CRs), respectively, of injected tumors (Figure 1A). In contrast, administration of anti-IL-10 in combination with free ODN 1826 (Group 2) resulted in 2 CRs of injected tumors (Figure 1A). Median tumor volumes of injected tumors with 68% confidence intervals for Days 0, 3, 7, and 11 are shown in Figure 1B. There is a significant difference in tumor volumes of injected tumors in Groups 6 and 8 compared to Group 2 by Day 11 ($p < 0.05$, multiplicity adjusted across time points) (Figure 1C). There is not a significant difference in tumor volumes of injected tumors between Groups 6 and 8 on any day (Figure 1C). No treatments resulted in CRs or in

significant difference in tumor volumes in non-injected tumors compared to control treatment (Group 1) (Figures 2A-2C).

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. The contents of the priority U.S. provisional application 62/331,092 is also incorporated herein by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents. To the extent that the references provide a definition for a claimed term that conflicts with the definitions provided in the instant specification, the definitions provided in the instant specification shall be used to interpret the claimed invention.

WHAT IS CLAIMED IS:

1. A composition comprising:

a) a Lipid Nanoparticle (LNP) comprising one or more cationic lipids and a poly(ethyleneglycol)-lipid (PEG-lipid);

b) a Toll-Like Receptor 9 (TLR9) agonist CpG oligonucleotide; and

c) an anti-IL-10 antibody or antigen binding fragment thereof.

2. The composition of claim 1, wherein the one or more cationic lipids are ionizable cationic lipids.

3. The composition of claim 2 wherein the ionizable cationic lipids are selected from DLinDMA; DlinKC2DMA; DLin-MC3-DMA; CLinDMA; S-Octyl CLinDMA;

(2 S)-1-{7-[(3 β)-cholest-5-en-3-yloxy]heptyloxy}-3-[(4 Z)-dec-4-en-1-yloxy]- *N*, *N* -dimethylpropan-2-amine;

(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-3-[(4 Z)-dec-4-en-1-yloxy]-N, N -dimethylpropan-2-amine;

1-[(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-3-(octyloxy)propan-2-yl]guanidine;

1-[(2 R)-1-{7-[(3 β)-cholest-5-en-3-yloxy]heptyloxy}-N,N-dimethyl-3-[(9 Z, 12 Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine;

1-[(2 R)-1-{4-[(3 β)-cholest-5-en-3-yloxy]butoxy}-N,N-dimethyl-3-[(9 Z, 12 Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine;

(2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine;

(3β)-3-[6-{ [(2S)-3-[(9Z)-octadec-9-en-1-yloxyl]-2-(pyrrolidin-1-yl)propyl]oxy}hexyl)oxy] cholest-5-ene;

(2R)-1-{4-[(3β)-cholest-5-en-3-yloxy]butoxy}-3-(octyloxy)propan-2-amine;

(2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-(pentyloxy)propan-2-amine;

(2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-3-(heptyloxy)-N,N-dimethylpropan-2-amine;

(2R)-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(2Z)-pent-2-en-1-yloxy]propan-2-amine;

(2S)-1-butoxy-3-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethylpropan-2-amine;

(2S-1-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-3-[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononyl)oxy]-N,N-dimethylpropan-2-amine;

2-amino-2-{[(9*Z*,12*Z*)-octadeca-9,12-dien-1-yloxy]methyl}propane-1,3-diol;

2-amino-3-((9-(((3S,10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)nonyl)oxy)-2-((((9Z,12Z)-octadeca-9,12-dien-1-yl)oxy)methyl)propan-1-ol;

2-amino-3-((6-(((3S,10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)hexyl)oxy)-2-((((Z)-octadec-9-en-1-yl)oxy)methyl)propan-1-ol;

(20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine;

(17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-9-amine;

(16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-8-amine;

(13Z,16Z)-N,N-dimethyldocosa-13,16-dien-5-amine;

(12Z,15Z)-N,N-dimethylhenicosa-12,15-dien-4-amine;

(14Z,17Z)-N,N-dimethyltricosa-14,17-dien-6-amine;

(15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-7-amine;

(18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-10-amine;

(15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-5-amine;

(14Z,17Z)-N,N-dimethyltricosa-14,17-dien-4-amine;

(19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-9-amine;

(18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-8-amine;

(17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-7-amine;

(16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-6-amine;

(22Z,25Z)-N,N-dimethylhentriaconta-22,25-dien-10-amine;

(21Z,24Z)-N,N-dimethyltriaconta-21,24-dien-9-amine;

(18Z)-N,N-dimethylheptacos-18-en-10-amine;

(17Z)-N,N-dimethylhexacos-17-en-9-amine;

(19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-7-amine;

N,N-dimethylheptacosan-10-amine;

(20Z,23Z)-N-ethyl-N-methylnonacosa-20,23-dien-10-amine;

1-[(11Z,14Z)-1-nonylicosa-11,14-dien-1-yl]pyrrolidine;

(20Z)-N,N-dimethylheptacos-20-en-10-amine;

(15Z)-N,N-dimethylheptacos-15-en-10-amine;

(14Z)-N,N-dimethylnonacos-14-en-10-amine;

(17Z)-N,N-dimethylnonacos-17-en-10-amine;

(24Z)-N,N-dimethyltritriacont-24-en-10-amine;

(20Z)-N,N-dimethylnonacos-20-en-10-amine;

(22Z)-N,N-dimethylhentriacont-22-en-10-amine;

(16Z)-N,N-dimethylpentacos-16-en-8-amine;

(12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine;

(13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine;

N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine;

1-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine;

N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]nonadecan-10-amine;

N,N-dimethyl-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine;

N,N-dimethyl-1-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]nonadecan-10-amine;

N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine;

N,N-dimethyl-1-[(1R,2S)-2-undecylcyclopropyl]tetradecan-5-amine;

N,N-dimethyl-3-{7-[(1S,2R)-2-octylcyclopropyl]heptyl}dodecan-1-amine;

1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine;

1-[(1S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine;

N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine; and

(11E,20Z,23Z)-N,N-dimethylnonacosa-11,20,23-trien-10-amine;

or a pharmaceutically acceptable salt thereof, or a stereoisomer of any of the foregoing.

4. The composition of claim 3, wherein the ionizable cationic lipids are selected from the group consisting of (2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine; (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine; and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine; or a pharmaceutically acceptable salt thereof, or a stereoisomer of any of the foregoing.

5. The composition of any one of claims 2-4, which comprises 30-75 mole % ionizable cationic lipid and 0.1-20 mole % PEG-lipid.

6. The composition of any one of claims 1 to 5, further comprising one or more non-cationic lipids selected from a phospholipid, a phospholipid derivative, a fatty acid, a sterol, or a combination thereof.

7. The composition of claim 6, wherein the sterol is cholesterol, stigmasterol or stigmastanol.

8. The composition of claim 6, wherein the phospholipid is selected from phosphatidylserine, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), dilauroylphosphatidylcholine (DLPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine, and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC).

9. The composition of any one of claims 1 to 8, wherein the PEG-lipid is 1,2-Dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG- dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA).

10. The composition of any one of claims 6 to 9, which comprises 20-99.8 mole % ionizable cationic lipids, 0.1-65 mole % non-cationic lipids, and 0.1-20 mole % PEG-lipid.

11. The composition of claim 10, wherein the non-cationic lipids comprise a mixture of cholesterol and DSPC.

12. The composition of claim 1, which comprises 34-59 mole % ionizable cationic lipids selected from the group consisting of (2S)-1-({6-[(3β))-cholest-5-en-3-yloxy]hexyl}oxy)-N,N-dimethyl-3-[(9 Z)-octadec-9-en-1-yloxy]propan-2-amine; (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine; and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, 30-48 mole % cholesterol, 10-24% DSPC and 1-2 mole % PEG-DMG.

13. The composition of any one of claims 1 to 12, wherein the TLR9 agonist CpG oligonucleotide is a B-class or C-class CpG oligonucleotide.

14. The composition of any one of claims 1 to 12, wherein the TLR9 agonist CpG oligonucleotide is a B-class or C-class CpG oligonucleotide and the anti-IL-10 antibody or antigen binding fragment comprises: (a) light chain CDRs of SEQ ID NOs: 5, 6 and 7 and heavy chain CDRs SEQ ID NOs: 8, 9 and 10.

15. The composition of any one of claims 1 to 12, wherein the TLR9 agonist CpG oligonucleotide is a B-class or C-class CpG oligonucleotide and the anti-IL-10 antibody or antigen binding fragment comprises a light chain variable region sequence of SEQ ID NO: 12 and a heavy chain variable region sequence of SEQ ID NO: 11.

16. The composition of any one of claims 1 to 15, wherein the TLR9 agonist CpG oligonucleotide is physically encapsulated in the Lipid Nanoparticle (LNP).

17. The composition of any one of claims 1 to 15, wherein the TLR9 agonist is adsorbed, covalently coupled, ionically-interacted or formulated onto the surface of the LNP.

18. A method of treating cancer in a subject comprising administering to the subject an effective amount of the composition of any one of claims 1 to 17.

19. A method of treating cancer in a subject comprising co-administering to the subject 1) an effective amount of a lipid nanoparticle comprising one or more cationic lipids and a poly(ethyleneglycol)-lipid (PEG-lipid); 2) a TLR9 agonist CpG oligonucleotide; and 3) an anti-IL-10 antibody or antigen binding fragment thereof.

20. The method of claim 19, wherein the lipid nanoparticle, TLR9 agonist and anti-IL-10 antibody or antigen binding fragment thereof are administered intratumorally or intravenously.

21. The method of claim 19, wherein the lipid nanoparticle and TLR9 agonist are administered intratumorally and the anti-IL-10 antibody or antigen binding fragment thereof is administered intravenously.

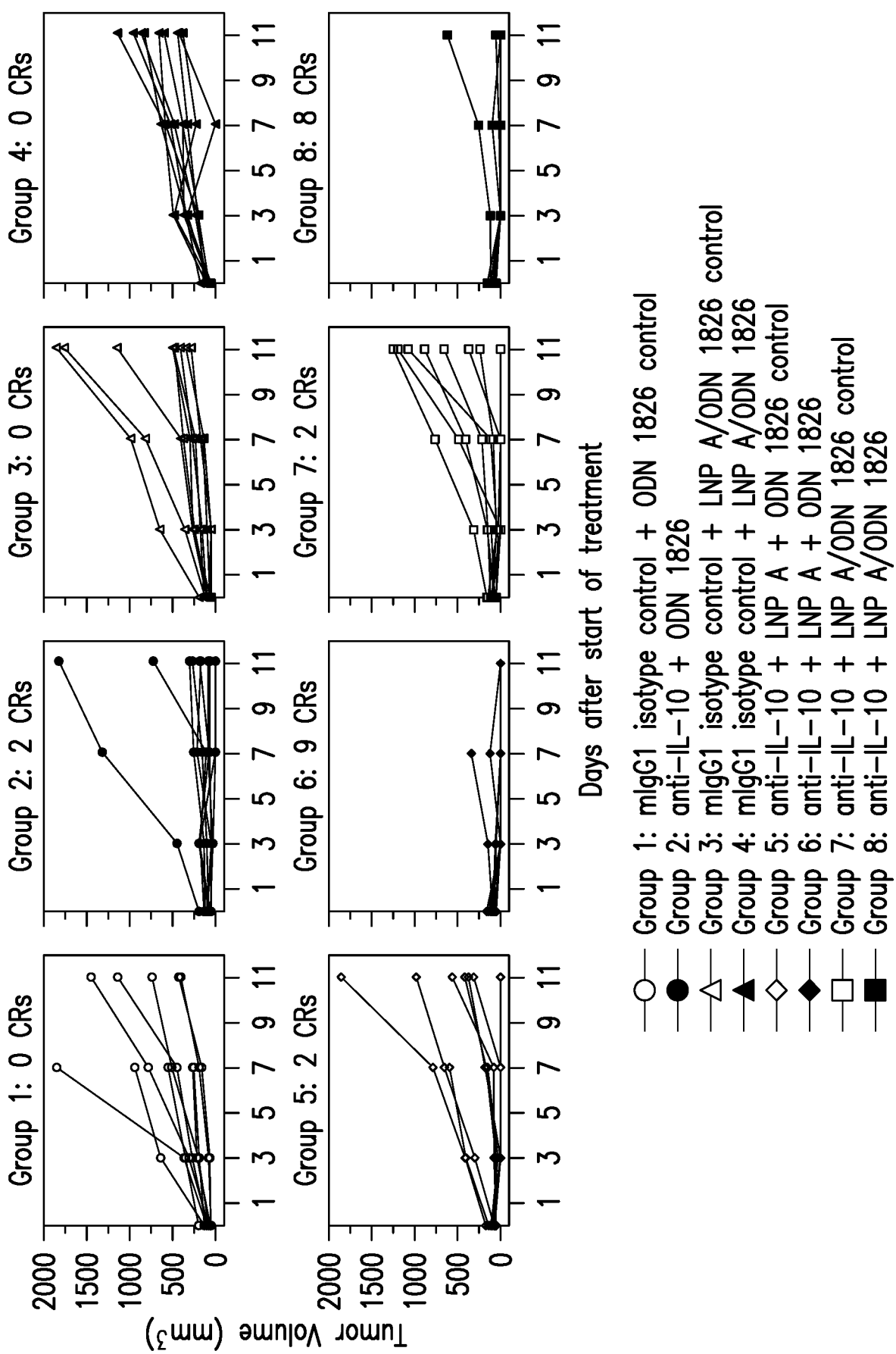

Group 1: 0 CRs
Group 2: 2 CRs
Group 3: 0 CRs
Group 4: 0 CRs
Group 5: 2 CRs
Group 6: 9 CRs
Group 7: 2 CRs
Group 8: 8 CRs
Tumor Volume (mm3)
Days after start of treatment
Group 1: mIgG1 isotype control + ODN 1826 control
Group 2: anti-IL-10 + ODN 1826
Group 3: mIgG1 isotype control + LNP A/ODN 1826 control
Group 4: mIgG1 isotype control + LNP A/ODN 1826
Group 5: anti-IL-10 + LNP A + ODN 1826 control
Group 6: anti-IL-10 + LNP A + ODN 1826
Group 7: anti-IL-10 + LNP A/ODN 1826 control
Group 8: anti-IL-10 + LNP A/ODN 1826

FIG.1A

	Day 0				Day 3				Day 7				Day 11			
Group	N	Median	CI, lower	CI, upper	N	Median	CI, lower	CI, upper	N	Median	CI, lower	CI, upper	N	Median	CI, lower	CI, upper
1	10	80.65	62.15	130.75	10	216.05	188.88	350.07	10	450.59	254.07	784.23	6	739.84	427.51	1449.63
2	10	81.11	63.96	123.71	10	102.40	49.49	184.96	10	88.03	69.40	214.30	10	169.64	63.87	302.30
3	10	85.77	66.12	120.33	10	177.89	98.90	267.49	10	269.42	174.82	405.19	9	488.43	408.51	1143.84
4	10	86.40	66.15	119.48	10	323.56	209.17	363.99	10	384.61	322.38	583.25	9	654.93	437.40	857.40
5	10	88.22	68.09	116.96	10	32.77	0.00	300.37	10	148.76	0.00	594.37	7	416.58	313.61	982.96
6	10	88.40	68.88	116.23	10	0.00	0.00	53.30	9	0.00	0.00	0.00	8	0.00	0.00	0.00
7	10	90.06	69.52	116.15	10	40.50	0.00	147.82	9	114.04	0.00	407.93	9	658.32	240.05	1078.73
8	10	91.73	73.07	115.55	10	0.00	0.00	0.00	10	0.00	0.00	18.00	9	0.00	0.00	0.00

FIG. 1B

Comparison	Unadjusted p-value*			Multiplicity-adjusted p-value**		
	Day 3	Day 7	Day 11	Day 3	Day 7	Day 11
Group 1 vs. Group 2	0.02345	0.00415	0.01610	0.03220	0.01245	0.03220
Group 1 vs. Group 3	0.44090	0.32065	0.50015	0.96195	0.96195	0.96195
Group 1 vs. Group 4	0.35285	0.79570	0.40225	1.00000	1.00000	1.00000
Group 1 vs. Group 5	0.05950	0.05245	0.18175	0.15735	0.15735	0.18175
Group 1 vs. Group 6	0.00005	0.00035	0.00050	0.00015	0.00070	0.00070
Group 1 vs. Group 7	0.00455	0.02800	0.23215	0.01365	0.05600	0.23215
Group 1 vs. Group 8	0.00005	0.00010	0.00175	0.00015	0.00020	0.00175
Group 2 vs. Group 6	0.00465	0.05115	0.00145	0.00930	0.05115	0.00435
Group 2 vs. Group 8	0.0005	0.03565	0.01555	0.00015	0.03565	0.03110
Group 6 vs. Group 8	0.45035	0.96185	0.47130	1.00000	1.00000	1.00000
Group 5 vs. Group 6	0.15360	0.03875	0.00095	0.15360	0.07750	0.00285
Group 7 vs. Group 8	0.01380	0.06090	0.00975	0.02925	0.06090	0.02925

*Two-sided p-values based on the Peto & Peto version of the Gehan-Breslow nonparametric test statistic for right-censored data. P-values were estimated from 20,000 random reassignments of animals between the two treatments being compared.

**P-values adjusted to control the family wise error rate across all the time points for a given pair of treatments. Adjustment was by Holm's method.

FIG.1C

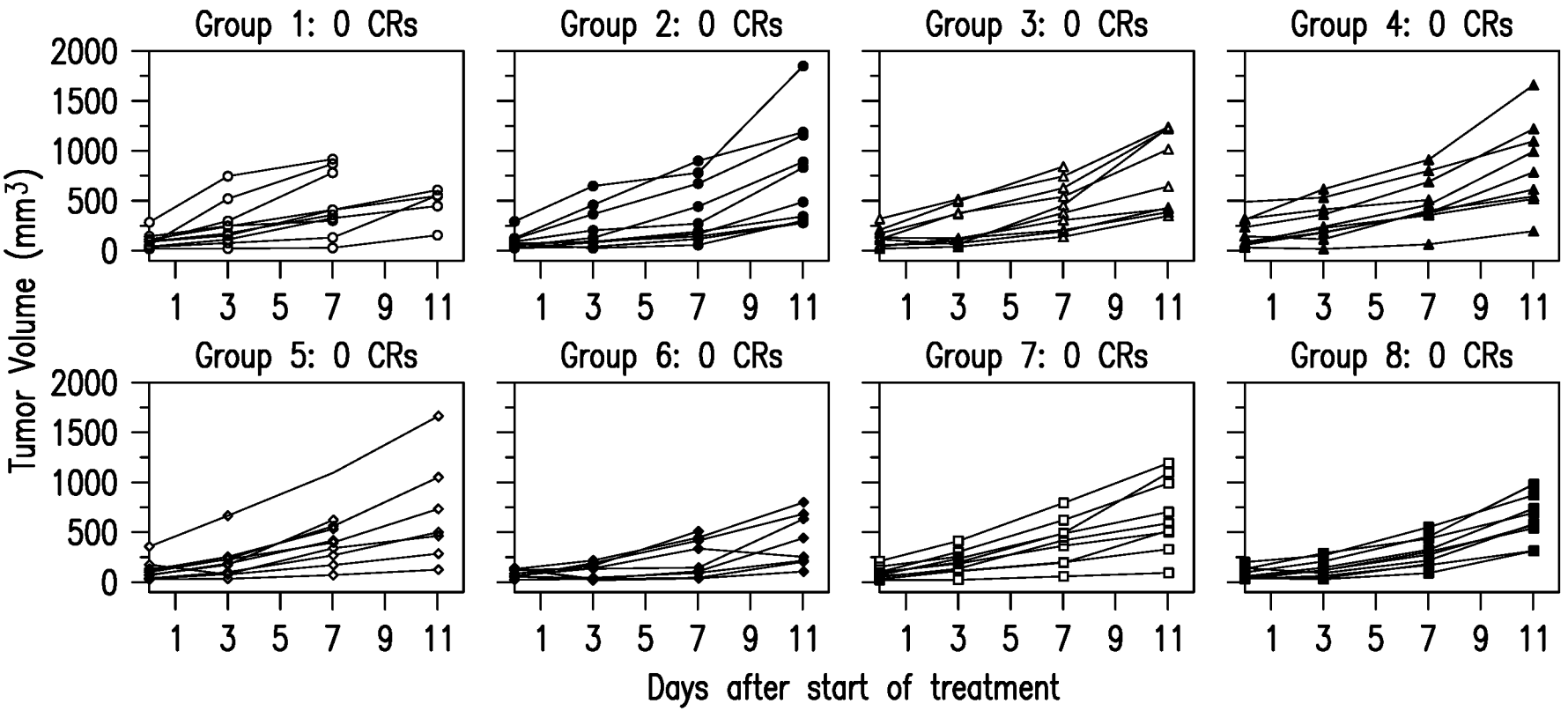

Group 1: 0 CRs
Group 2: 0 CRs
Group 3: 0 CRs
Group 4: 0 CRs
Group 5: 0 CRs
Group 6: 0 CRs
Group 7: 0 CRs
Group 8: 0 CRs
Tumor Volume (mm3)
2000
1500
1000
500
0
1 3 5 7 9 11
Days after start of treatment
Group 1: mIgG1 isotype control + ODN 1826 control
Group 2: anti-IL-10 + ODN 1826
Group 3: mIgG1 isotype control + LNP A/ODN 1826 control
Group 4: mIgG1 isotype control + LNP A/ODN 1826
Group 5: anti-IL-10 + LNP A + ODN 1826 control
Group 6: anti-IL-10 + LNP A + ODN 1826
Group 7: anti-IL-10 + LNP A/ODN 1826 control
Group 8: anti-IL-10 + LNP A/ODN 1826

FIG.2A

	Day 0				Day 3				Day 7				Day 11			
Group	N	Median	CI, lower	CI, upper	N	Median	CI, lower	CI, upper	N	Median	CI, lower	CI, upper	N	Median	CI, lower	CI, upper
1	10	90.18	41.61	112.88	10	174.10	111.86	297.67	10	360.14	301.69	780.39	6	548.25	446.28	607.37
2	10	72.37	45.59	117.89	10	92.71	82.00	366.08	10	191.90	144.52	673.30	10	486.39	301.01	1155.40
3	10	113.84	46.48	171.40	10	122.81	76.48	38.07	10	379.41	206.91	628.89	9	642.40	421.48	1222.69
4	10	85.17	69.16	309.87	10	228.53	180.11	413.40	10	403.58	377.42	690.52	9	786.73	542.82	1095.38
5	10	75.05	39.29	133.69	10	174.24	82.92	231.52	10	398.73	270.61	561.71	7	501.70	286.64	1051.02
6	10	66.17	54.78	132.10	10	45.87	33.50	165.65	9	147.14	94.70	417.19	8	255.09	217.49	633.78
7	10	72.77	50.99	102.79	10	187.63	114.10	256.88	9	421.33	200.00	493.15	9	591.03	502.51	991.14
8	10	55.64	41.19	132.13	10	117.44	63.30	208.74	10	276.95	182.86	436.38	9	585.93	540.53	738.31

FIG.2B

Comparison	Unadjusted p-value*			Multiplicity-adjusted p-value**		
	Day 3	Day 7	Day 11	Day 3	Day 7	Day 11
Group 1 vs. Group 2	0.52885	0.52960	0.72750	1.00000	1.00000	1.00000
Group 1 vs. Group 3	0.62950	1.00000	0.65655	1.00000	1.00000	1.00000
Group 1 vs. Group 4	0.63440	0.58065	0.20850	1.00000	1.00000	0.62550
Group 1 vs. Group 5	0.57930	0.91375	0.97610	1.00000	1.00000	1.00000
Group 1 vs. Group 6	0.05270	0.27575	0.55410	0.15810	0.55150	0.55410
Group 1 vs. Group 7	0.85285	0.96620	0.69910	1.00000	1.00000	1.00000
Group 1 vs. Group 8	0.22120	0.31480	0.48345	0.66360	0.66360	0.66360
Group 2 vs. Group 6	0.31750	0.27770	0.06630	0.55540	0.55540	0.19890
Group 2 vs. Group 8	0.79510	0.91215	0.96550	1.00000	1.00000	1.00000
Group 6 vs. Group 8	0.43140	0.40220	0.11455	0.80440	0.80440	0.34365
Group 5 vs. Group 6	0.06160	0.09325	0.28165	0.18480	0.18650	0.28165
Group 7 vs. Group 8	0.31445	0.31280	0.79660	0.93840	0.93840	0.93840

*Two-sided p-values based on the Peto & Peto version of the Gehan-Breslow nonparametric test statistic for right-censored data. P-values were estimated from 20,000 random reassignments of animals between the two treatments being compared.

**P-values adjusted to control the family wise error rate across all the time points for a given pair of treatments. Adjustment was by Holm's method.

FIG.2C

Humanized x [IL-10_H] mAb (JES3.12G8) IgG1 / Kappa (CK)

Amino acid sequence of hu12G8 light chain. CDRs are underlined.

```
  1  DIQMTQSPSS LSASVGDRVT ITCKTSQNIF ENLAWYQQKP GKAPKLLIYN
 51  ASPLQAGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCHQ YYSGYTFGPG
101  TKLELKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD
151  NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
201  SSPVTKSFNR GEC (SEQ ID NO: 2)
```

Amino acid sequence of hu12G8 heavy chain. CDRs are underlined.

```
  1  QVQLVESGGG VVQPGRSLRL SCAASGFTFS DYHMAWVRQA PGKGLEWVAS
 51  ITLDATYTYY RDSVRGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARHR
101  GFSVWLDYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
151  YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
201  ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK
251  DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
301  TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
351  YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
401  DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
     (SEQ ID NO: 1)
```

FIG.3

Mouse x IL-10_M mAb (TC40.11D8) IgG1/ Kappa (HY)

Amino acid sequence of 11D8 variable region of light chain

```
  1  DIVLTQSPAS LAVSLGQRAT ISCRASESVD DYGHSFMHWY QQKPGQPPKL
 51  LIWRASTLES GIPARFSGSG SRTDFTLTIN PVEADDVATY YCQQGNEDPW
101  TFGGGTKLEI K  (SEQ ID NO: 3)
```

Amino acid sequence of 11D8 variable region of heavy chain.

```
  1  QVQLKQSGPG LVQPSQSLSI TCTVSGFSLT NYGVHWVRQS PGKGLEWLGV
 51  IWSGGSTDYN AAFISRLSIN KDNSKSQVFF KMNSLQANDT AIYYCARNRG
101  YDVYFDYWGQ GTTLTVSS  (SEQ ID NO: 4)
```

FIG.4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/30484

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00 (2017.01)
CPC - A61K 2039/55561, A61K 31/7088, A61K 47/48246, A61K 2039/55572

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/130584 A2 (MERCK SHARP & DOHME CORP) 03 September 2015 (03.09.2015) abstract, pg 2, para 2, para 3, pg 3, para 1, pg 5, para 1, pg 23, para 8, pg 27, para 3	1-5, 12, 19-21
Y	US 2009/0087440 A1 (VICARI et al.) 02 April 2009 (02.04.2009) para [0011]-[0014], [0041], [0061], [0089]	1-5, 12, 19-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 2017	14 AUG 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/30484

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/30484

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-11, 13-18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.