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DESCRIPTION

BACKGROUND

[0001] Antibodies are known to have powerful therapeutic effects and are currently used for the treatment of a range of diseases including cancer, autoimmune diseases, cardiovascular disease, and transplant rejection. Traditionally, therapeutic antibodies are produced by recombinant technology, formulated and then administered to patients in need of antibody therapy. However, antibody production and formulation is highly expensive. In addition, many antibodies only have a very short half-life *in vivo* and therefore, may not reach their target antigen or target tissue before being degraded. To achieve desired efficacy, antibody therapy often requires high doses and frequent administration.

[0002] Gene therapy and genetic vaccination, also known as DNA vaccination, provide alternative approaches for delivery of large amounts of antibodies *in vivo*. However, the use of DNA as an agent in gene therapy and genetic vaccination may cause some safety concerns. For example, DNA is degraded slowly in the bloodstream. Formation of anti-DNA antibodies may occur (Gilkeson et al., J. Clin. Invest. 1995, 95: 1398-1402). The possible persistence of (foreign) DNA in the organism can thus lead to a hyperactivation of the immune system, which was known to result in splenomegaly in mice (Montheith et al., Anticancer Drug Res. 1997, 12(5): 421-432). Furthermore, DNA integration can cause mutations in the host genome by interrupting an intact gene.

[0003] WO 2012/170930 teaches that mRNA encoding a secreted protein can be loaded in lipid nanoparticles and delivered to target cells *in vivo*. Target cells then act as a depot source for production of soluble, secreted protein into the circulatory system at therapeutic levels. WO 2008/083949 teaches that a single mRNA, which encodes for the heavy chain and the light chain of an antibody, can be introduced in the naked form or as a complex with suitable carriers (e.g. liposomes) into an organism. US 2012/237975 describes transfection of Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK) cells with RNA using RNAiMax complexes (see paragraph [0208]). RNAiMax is a lipofectamine™, which is a transfection agent used to increase the transfection efficiency of RNA into *in vitro* cell cultures by lipofection and consists of a cationic liposome formulation (Mamoru Nakanishi et al., Pharmaceuticals Vol. 5, No. 3: 411-420, 2013). Stanley T. Crooke (Chapter 9.1: Liposomal Formulations for Nucleic Acid Delivery, Antisense Drug Technology, 2008, ISBN: 978-0-8493-8796-8) discusses liposomal encapsulation of siRNA for use in antisense therapy. Pisani et al. (Neutral Liposomes and DNA Transfection, Non-Viral Gene Therapy, Prof. Xubo Yuan (Ed.), 2011, ISBN: 978-953-307-538-9) discusses liposomal encapsulation of DNA for *in vitro* transfection of mouse fibroblast cell lines, for use in gene therapy. Martinon et al. (Eur J Immunol. 1993 Jul;23(7):1719-22) discusses liposomal encapsulation of mRNA for use in RNA vaccines. Geall et al. (Proc Natl Acad Sci USA. 2012 Sep 4;109(36):14604-9) discusses liposomal encapsulation of self-amplifying RNA for use in RNA vaccines. Philippot et al. (Biochimica et

Biophysica Acta - Biomembranes Volume 734, Issue 2, 12 October 1983, Pages 137-143) discusses encapsulation of mRNA into very large liposomes (1000nm). Love et al. (Proc Natl Acad Sci USA. 2010 Feb 2;107(5):1864-9) discusses liposomal encapsulation of siRNA for use in RNA interference therapy.

SUMMARY OF THE INVENTION

[0004] The present invention provides a composition for safer and more effective delivery of antibodies *in vivo* based on messenger RNA (mRNA) delivery technology. The present invention is, in part, based on the surprising discovery that production of fully assembled multi-chain antibodies can be accomplished *in vivo* by delivering exogenous mRNAs encoding a heavy chain and a light chain of the antibody, even when the heavy chain and light chain are delivered by separate mRNAs. As illustrated by non-limiting examples described in the Examples section below, when heavy chain and light chain encoding mRNA constructs, encapsulated in liposomes, were injected intravenously into mice, significant amounts of desired mRNA encoded antibody can be detected in mouse serum within six hours post-injection with a peak after 72 or 96 hours. The systemic expression of the antibody persisted even after three weeks post-injection. Thus, the present inventors have successfully demonstrated that multi-chain therapeutic antibodies can be delivered by mRNAs and produced by the patient's body itself, which makes it possible to eliminate the highly expensive recombinant antibody manufacturing process. In addition, contrary to the transient and vulnerable nature of mRNAs, the antibodies produced from the mRNAs are surprisingly long lasting and can achieve systemic distribution efficiently. The transient nature of mRNAs can also minimize the safety concern typically associated with foreign nucleic acids. Thus, the present invention provides a safer, cheaper and more effective antibody delivery approach for therapeutic uses.

[0005] The invention relates to a composition comprising a first mRNA encoding the heavy chain of an antibody and a second mRNA encoding the light chain of the antibody at a molar ratio ranging from 2:1 to 1:2 for use in therapy in a patient, wherein the first mRNA and the second mRNA (i) each comprise a 5' cap structure, a 3' poly-A tail, a 5' untranslated region and a 3' untranslated region and (ii) are encapsulated within liposomes comprising a cationic lipid, a neutral lipid, a cholesterol-based lipid, and a PEG-modified lipid and having a size no greater than 150 nm, wherein the first mRNA encoding the heavy chain and the second mRNA encoding the light chain are encapsulated in the same liposome, wherein the antibody is a tetramer and each tetramer is composed of two identical pairs of polypeptide chains, each pair having one light chain of approximately 25 kD and one heavy chain of approximately 50-70 kD and wherein the composition is administered intravenously to the patient and the antibody is expressed systemically in the patient.

[0006] In some embodiments, a heavy chain or light chain encoding mRNA comprises a sequence encoding a signal peptide. In some embodiments, a heavy chain or light chain encoding mRNA comprises a sequence encoding a human growth hormone (hGH) signal

peptide (e.g, SEQ ID NO: 9 or SEQ ID NO: 10). In some embodiments, the sequence encoding a signal peptide sequence (e.g., SEQ ID NO:9 or SEQ ID NO:10) is linked, directly or indirectly, to the heavy chain or light chain encoding mRNA sequence at the N-terminus.

[0007] In some embodiments, the first mRNA encoding the heavy chain and the second mRNA encoding the light chain are present at a molar ratio ranging from 2:1 to 1:2. In some embodiments, the first mRNA encoding the heavy chain and the second mRNA encoding the light chain are present at a molar ratio of 1:1.

[0008] In some embodiments, the liposomes comprise one or more of cationic lipid, neutral lipid, cholesterol-based lipid, and PEG-modified lipid. In some embodiments, the liposomes comprise cationic lipid, neutral lipid, cholesterol-based lipid, and PEG-modified lipid.

[0009] In some embodiments, the liposomes have a size no greater than about 150 nm (e.g., no greater than about 125 nm, 100 nm, 75 nm, or 50 nm). In some embodiments, the liposomes have a size no greater than about 125 nm. In some embodiments, the liposomes have a size no greater than about 100 nm. In some embodiments, the liposomes have a size no greater than about 75 nm. In some embodiments, the liposomes have a size no greater than about 50 nm.

[0010] In some embodiments, the liposomes have a size ranging from about 150 - 10 nm (e.g., ranging from about 125 - 10 nm, 100 - 10 nm, 75 - 10 nm, or 50 - 10 nm). In some embodiments, the liposomes have a size ranging from about 150 - 100 nm (e.g., ranging from about 125 - 100 nm). In some embodiments, the liposomes have a size ranging from about 100 - 10 nm (e.g., ranging from about 90 - 10 nm, 80 - 10 nm, 70 - 10 nm, 60 - 10 nm, or 50 - 10 nm).

[0011] In some embodiments, the mRNAs are modified to enhance stability. In some embodiments, the mRNAs are modified to include a modified nucleotide.

[0012] In some embodiments, the mRNAs are administered intravenously.

[0013] In some embodiments, the systemic expression of the antibody is detectable at least about 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 96 hours, 120 hours, 144 hours, 156 hours, 168 hours, or 180 hours post-administration (e.g., post single administration). In some embodiments, the systemic expression of the antibody is detectable at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 20 days, 22 days, 25 days, or 30 days post-administration (e.g., post single administration). In some embodiments, the systemic expression of the antibody is detectable at least about 0.5 weeks, 1 week, 1.5 weeks, 2 weeks, 2.5 weeks, 3 weeks, 3.5 weeks, 4 weeks, 4.5 weeks, 5 weeks, 5.5 weeks, 6 weeks, 6.5 weeks, 7 weeks, 7.5 weeks, or 8 weeks post-administration (e.g., post single administration). In some embodiments, the systemic expression of the antibody is detectable at least about 1 month, 2 months, 3 months, or 4 months post-administration (e.g., post single administration).

[0014] In some embodiments, the antibody is an IgG.

[0015] In some embodiments, the antibody is selected from the group consisting of anti-CCL2, anti-lysyl oxidase-like-2 (LOXL2), anti-Fit-1, anti-TNF- α , anti-Interleukin-2R α receptor (CD25), anti-TGF β , anti-B-cell activating factor, anti-alpha-4 integrin, anti-BAGE, anti- β -catenin/m, anti-Bcr-abl, anti-C5, anti-CA125, anti-CAMEL, anti-CAP-1, anti-CASP-8, anti-CD4, anti-CD19, anti-CD20, anti-CD22, anti-CD25, anti-CDC27/m, anti-CD30, anti-CD33, anti-CD52, anti-CD56, anti-CD80, anti-CDK4/m, anti-CEA, anti-CT, anti-CTL4, anti-Cyp-B, anti-DAM, anti-EGFR, anti-ErbB3, anti-ELF2M, anti-EMMPRIN, anti-EpCam, anti-ETV6-AML1, anti-HER2, anti-G250, anti-GAGE, anti-GnT-V, anti-Gp100, anti-HAGE, anti-HER-2/neu, anti-HLA-A*0201-R170I, anti-IGF-IR, anti-IL-2R, anti-IL-5, anti-MCIR, anti-myosin/m, anti-MUC1, anti-MUM-1, -2, -3, anti-proteinase-3, anti-p190 minor bcr-abl, anti-Pml/RAR α , anti-PRAMS, anti-PSA, anti-PSM, anti-PSMA, anti-RAGE, anti-RANKL, anti-RUI or RU2, anti-SAGE, anti-SART-1 or anti-SART-3, anti-survivin, anti-TEL/AML1, anti-TPI/m, anti-TRP-1, anti-TRP-2, anti-TRP-2/INT2, and anti-VEGF or anti-VEGF receptor.

[0016] Among other things, the present invention also provides exemplary mRNAs encoding a heavy chain and a light chain of specific antibodies such as, for example, an anti-CCL2 antibody, and compositions containing the same. In certain embodiments, the present invention provides an mRNA encoding a heavy chain of an anti-CCL2 antibody having a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1 or SEQ ID NO:2, as described herein. In certain specific embodiments, the present invention provides an mRNA encoding a heavy chain of an anti-CCL2 antibody having a sequence of SEQ ID NO:1 or SEQ ID NO:2, as described herein. In certain embodiments, the present invention provides an mRNA encoding a light chain of an anti-CCL2 antibody having a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:3 or SEQ ID NO:4, as described herein. In certain specific embodiments, the present invention provides an mRNA encoding a light chain of an anti-CCL2 antibody having a sequence of SEQ ID NO:3 or SEQ ID NO:4, as described herein.

[0017] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0018] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation.

BRIEF DESCRIPTION OF THE DRAWING

[0019] The following figures are for illustration purposes only and not for limitation.

Figure 1 shows an exemplary bar graph of IgG protein levels, as determined by ELISA, observed after treating HCL1 cells with mRNA using provided methods.

Figure 2 shows an exemplary bar graph of IgG protein levels, as determined by ELISA, observed after treating cells with mRNA using provided methods.

Figure 3 depicts the results of a western blot examining protein levels resulting from introduction of mRNA, according to provided methods, in HCL1 cells after 24 and 48 hours.

Figure 4 shows an exemplary bar graph of CCL2 antibody levels as determined via ELISA in the serum of mice exposed to mRNA according to provided methods for 6, 24, 48, or 72 hours.

Figure 5 shows an exemplary bar graph of α -VEGF antibody levels as determined via ELISA in the serum of mice after single dose of α -VEGF mRNA.

Figure 6 shows an exemplary bar graph of α -VEGF antibody levels as determined via ELISA in the serum of individually identified mice after single dose of α -VEGF mRNA.

Figure 7 shows an exemplary bar graph of *in vivo* production of an anti-human VEGF antibody in wild type mice 24 hours after dosing with α -VEGF mRNA loaded cKK-E12 lipid nanoparticles (LNP). Mice were dosed via either tail vein injection or subcutaneous (SC) injection.

DEFINITIONS

[0020] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0021] *Amino acid*: As used herein, term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some embodiments, an amino acid is an 1-amino acid. "Standard amino acid" refers to any of the twenty standard 1-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, "synthetic amino acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical

groups that can change the peptide's circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (e.g., methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, *etc.*). The term "amino acid" is used interchangeably with "amino acid residue," and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0022] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0023] *Antibody:* As used herein, the term "antibody" refers to an intact "antibody", which is an immunoglobulin that binds specifically to a particular antigen. An antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgE, and IgD. A typical immunoglobulin (antibody) structural unit as understood in the art, is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (approximately 25 kD) and one "heavy" chain (approximately 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain"(VL) and "variable heavy chain" (VH) refer to these light and heavy chains respectively. Each variable region is further subdivided into hypervariable (HV) and framework (FR) regions. The hypervariable regions comprise three areas of hypervariability sequence called complementarity determining regions (CDR 1, CDR 2 and CDR 3), separated by four framework regions (FR1, FR2, FR2, and FR4) which form a beta-sheet structure and serve as a scaffold to hold the HV regions in position. The C-terminus of each heavy and light chain defines a constant region consisting of one domain for the light chain (CL) and three for the heavy chain (CH1, CH2 and CH3). The terms "intact antibody" or "fully assembled antibody" are used in reference to an antibody to mean that it contains two heavy chains and two light chains, optionally associated by disulfide bonds as occurs with naturally-produced antibodies. As used herein, an "antibody fragment" includes a portion of an intact antibody, such as, for example, the antigen-binding or variable region of an antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; and multi specific antibodies formed from antibody fragments. For example, antibody fragments include isolated fragments, "Fv" fragments, consisting of the variable regions of the heavy and light chains.

[0024] *Approximately or about:* As used herein, the term "approximately" or "about," as applied

to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0025] *Bioavailability*: As used herein, the term "bioavailability" generally refers to the percentage of the administered dose that reaches the blood stream of a subject.

[0026] *Biologically active*: As used herein, the phrase "biologically active" refers to a characteristic of any agent that has activity in a biological system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active.

[0027] *Expression*: As used herein, "expression" of a nucleic acid sequence refers to translation of an mRNA into a polypeptide (e.g., heavy chain or light chain of antibody), assemble multiple polypeptides (e.g., heavy chain or light chain of antibody) into an intact protein (e.g., antibody) and/or post-translational modification of a polypeptide or fully assembled protein (e.g., antibody). In this application, the terms "expression" and "production," and grammatical equivalent, are used inter-changeably.

[0028] *Functional*: As used herein, a "functional" biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0029] *GC content*: As used herein, the "GC content" is the fraction or percentage of total nucleobase residues in a nucleic acid sequence that are guanine residues, cytosine residues, or analogs thereof. For example, a 100 nt sequence that contains exactly 30 cytosines, exactly 30 guanines, exactly one cytosine analog, and exactly one guanine analog has a GC richness of 62%.

[0030] *Half-life*: As used herein, the term "half-life" is the time required for a quantity such as protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0031] *Improve, increase, or reduce*: As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein. A "control subject" is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[0032] *In Vitro*: As used herein, the term "*in vitro*" refers to events that occur in an artificial

environment, e.g., in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.

[0033] *In Vivo*: As used herein, the term "*in vivo*" refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0034] *Isolated*: As used herein, the term "isolated" refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (e.g., buffer, solvent, water, *etc.*).

[0035] *Linker*: As used herein, the term "linker" refers to, in a fusion protein, an amino acid sequence other than that appearing at a particular position in the natural protein and is generally designed to be flexible or to interpose a structure, such as an α -helix, between two protein moieties. A linker is also referred to as a spacer. A linker or a spacer typically does not have biological function on its own.

[0036] *Local distribution or delivery*: As used herein, the terms "local distribution," "local delivery," or grammatical equivalent, refer to tissue specific delivery or distribution. Typically, local distribution or delivery requires a protein (e.g., antibody) encoded by mRNAs be translated and expressed intracellularly or with limited secretion that avoids entering the patient's circulation system.

[0037] *messenger RNA (mRNA)*: As used herein, the term "messenger RNA (mRNA)" refers to a polynucleotide that encodes at least one polypeptide. mRNA may contain one or more coding and non-coding regions.

[0038] *Nucleic acid*: As used herein, the term "nucleic acid," in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into a polynucleotide chain via a phosphodiester linkage. "Nucleic acid" refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides). "Nucleic acid" refers to a polynucleotide chain comprising individual nucleic acid residues. "Nucleic acid" encompasses

RNA as well as single and/or double-stranded DNA and/or cDNA. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, *i.e.*, analogs having other than a phosphodiester backbone. "Peptide nucleic acids" have peptide bonds instead of phosphodiester bonds in the backbone. The term "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and/or encode the same amino acid sequence. Nucleotide sequences that encode proteins and/or RNA may include introns. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, *etc.* A nucleic acid sequence is presented in the 5' to 3' direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (*e.g.*, adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated bases; modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5'-*N*-phosphoramidite linkages). In some embodiments, the present invention is specifically directed to "unmodified nucleic acids," meaning nucleic acids (*e.g.*, polynucleotides and residues, including nucleotides and/or nucleosides) that have not been chemically modified in order to facilitate or achieve delivery.

[0039] *Patient:* As used herein, the term "patient" or "subject" refers to any organism to which a provided composition may be administered, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human.

[0040] *Pharmaceutically acceptable:* The term "pharmaceutically acceptable" as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0041] *Systemic distribution or delivery:* As used herein, the terms "systemic distribution," "systemic delivery," or grammatical equivalent, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body's circulation system, *e.g.*, blood stream. Compared to the definition of "local distribution or delivery."

[0042] *Subject:* As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0043] *Substantially:* As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0044] *Target tissues:* As used herein, the term "target tissues" refers to any tissue that is affected by a disease to be treated. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature.

[0045] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0046] *Treating:* As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

DETAILED DESCRIPTION

[0047] The present invention provides a composition for delivering antibodies *in vivo* based on mRNA delivery technology. In particular, the invention provides a composition comprising a first mRNA encoding the heavy chain of an antibody and a second mRNA encoding the light chain of the antibody at a molar ratio ranging from 2:1 to 1:2, for use in treating a patient with a disease or disorder in need of delivery of said antibody, wherein the first mRNA and the second mRNA are encapsulated within liposomes comprising a cationic lipid, a neutral lipid, a cholesterol-based lipid, and a PEG-modified lipid and having a size no greater than 150 nm,

wherein the first mRNA encoding the heavy chain and the second mRNA encoding the light chain are encapsulated in the same liposome, wherein the antibody is a tetramer and each tetramer is composed of two identical pairs of polypeptide chains, each pair having one light chain of approximately 25 kD and one heavy chain of approximately 50-70 kD, and wherein the composition is administered intravenously or subcutaneously to the patient. mRNA encoded antibodies may be expressed locally (e.g., in a tissue specific manner) or systematically in the subject.

[0048] Various aspects of the invention are described in detail in the following sections.

mRNA coded antibodies

[0049] The present invention is used to deliver antibodies. An intact "antibody" is an immunoglobulin that binds specifically to a particular antigen. An antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgE, IgA, and IgD. An intact antibody is a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (approximately 25 kD) and one "heavy" chain (approximately 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain" (VL) and "variable heavy chain" (VH) refer to these corresponding regions on the light and heavy chain respectively. Each variable region can be further subdivided into hypervariable (HV) and framework (FR) regions. The hypervariable regions comprise three areas of hypervariability sequence called complementarity determining regions (CDR 1, CDR 2 and CDR 3), separated by four framework regions (FR1, FR2, FR2, and FR4) which form a beta-sheet structure and serve as a scaffold to hold the HV regions in position. The C-terminus of each heavy and light chain defines a constant region consisting of one domain for the light chain (CL) and three for the heavy chain (CH1, CH2 and CH3). A light chain of immunoglobulins can be further differentiated into the isotypes *kappa* and *lamda*.

[0050] The terms "intact antibody" or "fully assembled antibody" are used in reference to an antibody that contains two heavy chains and two light chains, optionally associated by disulfide bonds as occurs with naturally-produced antibodies.

[0051] As used herein, an "antibody fragment" includes a portion of an intact antibody, such as, for example, the antigen-binding region of an antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; and multi specific antibodies formed from antibody fragments. For example, antibody fragments include isolated fragments, "Fv" fragments, consisting of the variable regions of the heavy and light chains. An antibody fragment contains a sufficient sequence of the parent antibody of which it is a fragment that it binds to the same antigen as does the parent antibody; a fragment binds to the antigen with a comparable affinity to that of the parent antibody and/or competes with the parent antibody for binding to the antigen. Examples of antigen binding fragments of an antibody include, but are not limited to, Fab fragment, Fab' fragment, F(ab')₂ fragment, and Fv fragment.

[0052] Exemplary antibodies include, but are not limited to, anti-chemokine (C-C motif) ligand 2 (CCL2), , anti-lysyl oxidase-like-2 (LOXL2), anti-Fit-1, anti-TNF- α , anti-Interleukin-2R α receptor (CD25), anti-TGF β , anti-B-cell activating factor, anti-alpha-4 integrin, anti-BAGE, anti- β -catenin/m, anti-Bcr-abl, anti-C5, anti-CA125, anti-CAMEL, anti-CAP-1, anti-CASP-8, anti-CD4, anti-CD19, anti-CD20, anti-CD22, anti-CD25, anti-CDC27/m, anti-CD30, anti-CD33, anti-CD52, anti-CD56, anti-CD80, anti-CDK4/m, anti-CEA, anti-CT, anti-CTL4, anti-Cyp-B, anti-DAM, anti-EGFR, anti-ErbB3, anti-ELF2M, anti-EMMPRIN, anti-EpCam, anti-ETV6-AML1, anti-HER2, anti-G250, anti-GAGE, anti-GnT-V, anti-Gp100, anti-HAGE, anti-HER-2/neu, anti-HLA-A*0201-R170I, anti-IGF-IR, anti-IL-2R, anti-IL-5, anti-MCIR, anti-myosin/m, anti-MUC1, anti-MUM-1, -2, -3, anti-proteinase-3, anti-p190 minor bcr-abl, anti-Pm1/RAR α , anti-PRAMS, anti-PSA, anti-PSM, anti-PSMA, anti-RAGE, anti-RANKL, anti-RUI or RU2, anti-SAGE, anti-SART-1 or anti-SART-3, anti-survivin, anti-TEL/AML1, anti-TPI/m, anti-TRP-1, anti-TRP-2, anti-TRP-2/INT2, and anti-VEGF or anti-VEGF receptor.

mRNAs encoding heavy chain and light chain

[0053] According to the present invention, antibodies may be produced in a cell or living organism through exogenous mRNA translation inside the cell and living organism. In particular, according to the present invention, production of fully assembled multi-chain antibodies can be accomplished in a cell or living organism by delivering exogenous mRNAs encoding a heavy chain and a light chain of the antibody. Specifically, a tetramer containing two heavy chains and two light chains is produced.

[0054] As used herein, the term "heavy chain" encompasses all types of naturally-occurring heavy chains of different classes of immunoglobulins, including but not limited to, IgM (μ), IgD (δ), IgG (γ), IgA (α), and IgE (ϵ), and biologically active variants thereof. Typically, a heavy chain according to the present invention contains the N-terminal variable region responsible for antigen recognition, typically including CDR 1, CDR 2 and CDR 3, separated by four framework regions (FR1, FR2, FR2, and FR4). Typically, the N-terminal variable region contains about 100 to 110 or more amino acids. In some embodiments, a heavy chain according to the present invention contains one or more of constant domains (e.g., C_H1, C_H2, and/or C_H3). In some embodiments, an mRNA encoding a heavy chain of an antibody is of or greater than 0.3 kb, 0.5 kb, 0.75 kb, 1.0 kb, 1.25 kb, 1.5 kb, 1.75 kb, 2.0 kb, 2.5 kb, 3.0 kb, 3.5 kb, 4.0 kb in length.

[0055] As used herein, the term "light chain" encompasses all types of naturally-occurring light chains of different classes of immunoglobulins, including but not limited to κ or λ isotypes, and biologically active variants thereof. Typically, a light chain according to the present invention comprises an N-terminal variable domain (V_L). In some embodiments, a light chain according to the present invention contains a C-terminal constant domain (C_L). In some embodiments, an mRNA encoding a light chain of an antibody is of or greater than 0.1 kb, 0.2 kb, 0.3 kb, 0.4 kb,

0.5 kb, 0.6 kb, 0.7 kb, 0.8 kb, 0.9 kb, 1.0 kb, 1.25 kb, 1.5 kb, 1.75 kb, 2.0 kb, 2.5 kb, or 3.0 kb in length.

[0056] Typically, a tetrameric antibody containing two heavy chains and two light chains encoded by mRNAs, each bonded together by a disulfide bridge.

[0057] According to the present invention, a heavy chain and light chain of an antibody are encoded and delivered by separate mRNAs. The heavy chain encoding mRNA (also referred to as the first mRNA) and the light chain encoding mRNA (also referred to as the second mRNA) are delivered at a molar ratio ranging from 2:1 to 1:2. In some embodiments, the heavy chain encoding mRNA (also referred to as the first mRNA) and the light chain encoding mRNA (also referred to as the second mRNA) are delivered at a molar ratio of 2:1 or 1:1. In some embodiments, the heavy chain encoding mRNA (also referred to as the first mRNA) and the light chain encoding mRNA (also referred to as the second mRNA) are delivered at a ratio of approximately 1:1 (i.e., equal molar). In some embodiments, the heavy chain encoding mRNA (also referred to as the first mRNA) and the light chain encoding mRNA (also referred to as the second mRNA) are delivered at a ratio other than 1:1 (equal molar). For example, the heavy chain encoding mRNA (also referred to as the first mRNA) and the light chain encoding mRNA (also referred to as the second mRNA) are delivered at a ratio greater than 1 (e.g., ranging from 2:1 to 1:1). Alternatively, the heavy chain encoding mRNA (also referred to as the first mRNA) and the light chain encoding mRNA (also referred to as the second mRNA) are delivered at a ratio less than 1 (e.g., ranging from 1:1 to 1:2).

Signal Peptide

[0058] An mRNA encoding a heavy chain and/or light chain incorporates a nucleotide sequence encoding a signal peptide. As used herein, the term "signal peptide" refers to a peptide present at a newly synthesized protein that can target the protein towards the secretory pathway. Typically, the signal peptide is cleaved after translocation into the endoplasmic reticulum following translation of the mRNA. Signal peptide is also referred to as signal sequence, leader sequence or leader peptide. Typically, a signal peptide is a short (e.g., 5-30, 5-25, 5-20, 5-15, or 5-10 amino acids long) peptide. A signal peptide may be present at the N-terminus of a newly synthesized protein. Without wishing to be bound by any particular theory, the incorporation of a signal peptide encoding sequence on a heavy chain and/or light chain encoding mRNA may facilitate the secretion and/or production of the antibody produced from the mRNA *in vivo*.

[0059] A suitable signal peptide for the present invention can be a heterogeneous sequence derived from various eukaryotic and prokaryotic proteins, in particular secreted proteins. In some embodiments, a suitable signal peptide is a leucine-rich sequence, as described in Yamamoto Y et al. (1989), Biochemistry, 28:2728-2732. A suitable signal peptide may be derived from a human growth hormone (hGH), serum albumin preproprotein, Ig kappa light chain precursor, Azurocidin preproprotein, cystatin-S precursor, trypsinogen 2 precursor,

potassium channel blocker, alpha conotoxin Ip1.3, alpha conotoxin, alfa-galactosidase, cellulose, aspartic proteinase nepenthesin-1, acid chitinase, K28 prepro-toxin, killer toxin zygocin precursor, and Cholera toxin. Exemplary signal peptide sequences are described in Kober, et al., Biotechnol. Bioeng., 110: 1164-73, 2012.

[0060] In some embodiments, a heavy chain and/or light chain encoding mRNA may incorporate a sequence encoding a signal peptide derived from human growth hormone (hGH), or a fragment thereof. A non-limiting nucleotide sequence encoding a hGH signal peptide is show below.

5' human growth hormone (hGH) sequence (SEQ ID NO:9):

AUGGCCACUGGAUCAAGAACCUCACUGCUCGCUUUUGGACUGCUUUGCCUGC
CCUGGUUGCAAGAAGGAUCGGCUUCCCCGACCAUCCCACUCUCC

Alternative 5' human growth hormone (hGH) sequence (SEQ ID NO:10):

AUGGCAACUGGAUCAAGAACCUCUCCUGCUCGCAUUCGGCCUGCUCUGUCUCC
CAUGGCUCCAAGAAGGAAGCGCGUCCCCACUAUCCCCCUCUCG

[0061] In some embodiments, an mRNA according to the present invention may incorporate a signal peptide encoding sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identity to SEQ ID NO:9 or SEQ ID NO:10.

Synthesis of mRNA

[0062] mRNAs according to the present invention may be synthesized according to any of a variety of known methods. For example, mRNAs according to the present invention may be synthesized via *in vitro* transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNase I, pyrophosphatase, and/or RNase inhibitor. The exact conditions will vary according to the specific application.

[0063] In some embodiments, for the preparation of antibody-coding mRNA according to the invention, a DNA template is transcribed *in vitro*. A suitable DNA template typically has a promoter, for example a T3, T7 or SP6 promoter, for *in vitro* transcription, followed by desired nucleotide sequence for desired antibody encoding (e.g., heavy chain or light chain encoding) mRNA and a termination signal.

[0064] Desired antibody encoding (e.g., heavy chain or light chain encoding) mRNA sequence according to the invention may be determined and incorporated into a DNA template using standard methods. For example, starting from a desired amino acid sequence (e.g., a desired heavy chain or light chain sequence), a virtual reverse translation is carried out based on the degenerated genetic code. Optimization algorithms may then be used for selection of suitable

codons. Typically, the G/C content can be optimized to achieve the highest possible G/C content on one hand, taking into the best possible account the frequency of the tRNAs according to codon usage on the other hand. The optimized RNA sequence can be established and displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. A secondary structure can also be analyzed to calculate stabilizing and destabilizing properties or, respectively, regions of the RNA.

[0065] mRNA according to the present invention may be synthesized as unmodified or modified mRNA. Typically, mRNAs are modified to enhance stability. Modifications of mRNA can include, for example, modifications of the nucleotides of the RNA. A modified mRNA according to the invention can thus include, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, β -D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art e.g. from the U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. Nos. 5,262,530 and 5,700,642, the disclosure of which is included here in its full scope by reference.

[0066] In some embodiments, antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) may contain RNA backbone modifications. Typically, a backbone modification is a modification in which the phosphates of the backbone of the nucleotides contained in the RNA are modified chemically. Exemplary backbone modifications typically include, but are not limited to, modifications from the group consisting of methylphosphonates, methylphosphoramidates, phosphoramidates, phosphorothioates (e.g. cytidine 5'-O-(1-thiophosphate)), boranophosphates, positively charged guanidinium groups etc., which means by replacing the phosphodiester linkage by other anionic, cationic or neutral groups.

[0067] In some embodiments, antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) may contain sugar modifications. A typical sugar modification is a chemical

modification of the sugar of the nucleotides it contains including, but not limited to, sugar modifications chosen from the group consisting of 2'-deoxy-2'-fluoro-oligoribonucleotide

[0068] (2'-fluoro-2'-deoxycytidine 5'-triphosphate, 2'-fluoro-2'-deoxyuridine 5'-triphosphate), 2'-deoxy-2'-deamine-oligoribonucleotide (2'-amino-2'-deoxycytidine 5'-triphosphate, 2'-amino-2'-deoxyuridine 5'-triphosphate), 2'-O-alkyloligoribonucleotide, 2'-deoxy-2'-C-alkyloligoribonucleotide (2'-O-methylcytidine 5'-triphosphate, 2'-methyluridine 5'-triphosphate), 2'-C-alkyloligoribonucleotide, and isomers thereof (2'-aracytidine 5'-triphosphate, 2'-arauridine 5'-triphosphate), or azidotriphosphates (2'-azido-2'-deoxycytidine 5'-triphosphate, 2'-azido-2'-deoxyuridine 5'-triphosphate).

[0069] In some embodiments, antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) may contain modifications of the bases of the nucleotides (base modifications). A modified nucleotide which contains a base modification is also called a base-modified nucleotide. Examples of such base-modified nucleotides include, but are not limited to, 2-amino-6-chloropurine riboside 5'-triphosphate, 2-aminoadenosine 5'-triphosphate, 2-thiocytidine 5'-triphosphate, 2-thiouridine 5'-triphosphate, 4-thiouridine 5'-triphosphate, 5-aminoallylcytidine 5'-triphosphate, 5-aminoallyluridine 5'-triphosphate, 5-bromocytidine 5'-triphosphate, 5-bromouridine 5'-triphosphate, 5-iodocytidine 5'-triphosphate, 5-iodouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate, 5-methyluridine 5'-triphosphate, 6-azacytidine 5'-triphosphate, 6-azauridine 5'-triphosphate, 6-chloropurine riboside 5'-triphosphate, 7-deazaadenosine 5'-triphosphate, 7-deazaguanosine 5'-triphosphate, 8-azaadenosine 5'-triphosphate, 8-azidoadenosine 5'-triphosphate, benzimidazole riboside 5'-triphosphate, N1-methyladenosine 5'-triphosphate, N1-methylguanosine 5'-triphosphate, N6-methyladenosine 5'-triphosphate, O6-methylguanosine 5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate.

[0070] Typically, mRNA synthesis includes the addition of a "cap" on the N-terminal (5') end, and a "tail" on the C-terminal (3') end. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The presence of a "tail" serves to protect the mRNA from exonuclease degradation.

[0071] Antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) include a 5' cap structure. A 5' cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5' nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5'5'5 triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G).

[0072] Antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) include a 3' poly(A) tail structure. A poly-A tail on the 3' terminus of mRNA typically includes about 10 to 300 adenosine nucleotides (e.g., about 10 to 200 adenosine nucleotides, about 10 to 175 adenosine nucleotides, about 10 to 150 adenosine nucleotides, about about 10 to 125

adenosine nucleotides, 10 to 100 adenosine nucleotides, about 10 to 75 adenosine nucleotides, about 20 to 70 adenosine nucleotides, or about 20 to 60 adenosine nucleotides). In some embodiments, antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) include a 3' poly(C) tail structure. A suitable poly-C tail on the 3' terminus of mRNA typically include about 10 to 200 cytosine nucleotides (e.g., about 10 to 150 cytosine nucleotides, about 10 to 100 cytosine nucleotides, about 20 to 70 cytosine nucleotides, about 20 to 60 cytosine nucleotides, or about 10 to 40 cytosine nucleotides). The poly-C tail may be added to the poly-A tail or may substitute the poly-A tail.

[0073] Antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) include a 5' and 3' untranslated region. In some embodiments, a 5' untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5' untranslated region may be between about 50 and 500 nucleotides in length (e.g., about 50 and 400 nucleotides in length, about 50 and 300 nucleotides in length, about 50 and 200 nucleotides in length, or about 50 and 100 nucleotides in length).

[0074] A 5' region of antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) includes a sequence encoding a signal peptide, such as those described herein. In particular embodiments, a signal peptide derived from human growth hormone (hGH) (e.g. SEQ ID NO:9) is incorporated in the 5' region. Typically, a signal peptide encoding sequence (e.g., hGH signal peptide encoding sequence such as SEQ ID NO:9) is linked, directly or indirectly, to the heavy chain or light chain encoding sequence at the N-terminus.

Exemplary mRNAs Encoding Heavy Chain and Light Chain of anti-CCL2

[0075] As a non-limiting example, mRNAs encoding the heavy chain and light chain of an anti-CCL2 antibody are described in Example 1. The heavy chain encoding mRNA without and with the 5' and 3' UTR sequences are shown below as SEQ ID NO:1 and SEQ ID NO:2, respectively. The light chain encoding mRNA without and with the 5' and 3' UTR sequences are shown below as SEQ ID NO:3 and SEQ ID NO:4, respectively.

[0076] *Heavy chain anti-CCL2 (HC- α CCL2) mRNA without 5' and 3' UTR (SEQ ID NO:1):*

AUGGAAUUCGGCCUGAGCUGGCUGUCCUGGUGGCCAUCCUGAAGGGGCGUGCAG
UGCCAGGUCCAGCUGGUGCAGUCUGGGCGCCGAAGUGAAGAAACCCGGCUCUCCG
UGAAGGUGUCCUGCAAGGCCUCCGGCGGCACCUUCUCCAGCUACGGCAUCUCCUG
GGUCCGACAGGCCCCAGGCCAGGGCCUGGAAUGGAUGGGCGGCAUCAUCCCCAUC
UUCGGCACCGCCAACUACGCCCAGAAAUUCCAGGGCAGAGUGACCAUCACCGCCG
ACGAGUCCACCUCACCGCCUACAUGGAACUGUCCUCCUGCGGAGCGAGGACAC
CGCCGUGUACUACUGCGCCAGAUACGACGGCAUCUACGGCGAGCUGGACUUCUGG
GGCCAGGGCACCCUGGUCACCGUGUCCUCUGCCAAGACCACCCCCCCCUCGUGU
ACCCUCUGGGCCCCUGGCUCUGCCGCCCAGACCAACUCUAUGGUCACCCUGGGCUG
CCUGGUCAAGGGGCUACUUCCCCGAGCCCGUGACCGUGACCUUGGAACUCCGGCUC
CUGUCCUCCGGCGUGCACACCUUCCUGCCGUGCUGCAGUCCGACCUCUACACCC
UGUCCAGCAGCGUGACCGUGCCCUCCUCCACCUGGGCCUCCGAGACAGUGACCUG
CAACGUGGGCCACCCCGCCUCCAGCACCAAGGUGGACAAGAAAAUCGUGCCCCGG
GACUGGGGGCUGGAAGGGCCUGCAUCUUGUACCGUGGGCGGAGGUGUCCUCCGUGUUA

[illegible]

[0077] Heavy chain anti-CCL2 (HC- α CCL2) mRNA with 5' and 3' UTR (SEQ ID NO:2):

[0078] (The 5' and 3' UTR sequences are underlined)

GGACAGAU CGCCUGGAGACGCCAUCCACGCUGUUUUGACCUC CAUAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUC CCGG

UGCCAAGAGUGACUCACCGUCCUUGACACGAUGGAAUUCGGCCUGAGCUGGGCUGU
UCCUGGGUGGCCAUCCUGAAGGGGCGUGCAGUGCCAGGUCCAGCUGGUGCAGUCUGG
CGCCGAAGUGAAGAAACCCGGCUCCUCCGUGAAGGUGUCCUGCAAGGCCUCCGGC
GGCACCUUCUCCAGCUACGGCAUCUCCUGGGUCCGACAGGCCCCAGGCCAGGGCC
UGGAAUUGGAUGGGGCGGCAUCAUCCCCAUUCUUCGGCACCGCCAACUACGCCCAGAA
AUUCCAGGGGCAGAGUGACCAUACCCGCCGACGAGUCCACCUCACCGCCUACAUG
GAACUGUCCUCCCUGCGGAGCGAGGACACCGCCGUGUACUACUGCGCCAGAUACG
ACGGCAUCUACGGCGAGCUGGACUUCUGGGGGCCAGGGGCACCCUGGUCACCGUGUC
CUCUGCCAAGACCACCCCCCCCCUCCGUGUACCCUCUGGGCCCCUGGCUCUGCCGCC
AGACCAACUCUAUGGUCACCCUGGGCUGCCUGGUCAAGGGCUACUUCCCCGAGCC
CGUGACCGUGACCUGGAACUCCGGCUCCCUGUCCUCCGGCGUGCACACCUUCCCU
GCCGUGCUGCAGUCCGACCUCUACACCCUGUCCAGCAGCGUGACCGUGCCCUCCU
CCACCUGGGCCCUCCGAGACAGUGACCUGCAACGUGGGCCCACCCCGCCUCCAGCACC
AAGGUGGACAAGAAAUCGUGCCCCGGGACUGCGGCUGCAAGCCCUGCAUCUGUA
CCGUGCCCGAGGUGUCCUCCGUGUUAUCUUCCCACCCAAGCCCAAGGACGUGCU
GACCAUCACACUGACCCCCAAAGUGACCUGCGUGGUGGUGGACAUCUCCAAGGAC
GACCCCGAGGUGCAGUUCAGUUGGUUCGUGGACGACGUGGAAGUGCACACCGCUC
AGACCCAGCCCAGAGAGGAACAGUUAACUCCACCUUCAGAUCCGUGUCCGAGCU
GCCCAUCAUGCACCAGGACUGGCUGAACGGCAAAGAAUUAAGUGCAGAGUGAAC
UCCGCCGCCUUCCCAGCCCCCAUCGAAAAGACCAUCUCCAAGACCAAGGGCAGAC
CCAAGGGCCCCCAGGUCUACACCAUCCCCCCACCCAAGAACAGAUGGCCAAGGA
CAAGGUGUCCCUGACCUGCAUGAUCACCGAUUUCUUCCCAGAGGACAUCACCGUG
GAAUGGCAGUGGAACGGCCAGCCCGCCGAGAACUACAAGAACACCCAGCCCAUCA
UGGACACCGACGGCUCCUACUUCGUGUACUCCAAGCUGAACGUGCAGAAAGUCCAA
CUGGGAGGCCGGCAACACCUUACCCUGUAGCGUGCUGCACGAGGGGCCUGCACAAC
CACCACACCGAGAAGUCCCUGUCCCACUCCCCCGGCAAGUGACGGGUGGCAUCCC
UGUGACCCCUCCCCAGUGCCUCUCCUGGGCCUGGAAGUUGCCACUCCAGUGCCCA
CCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU

[0079] *Light chain anti-CCL2 (LC- α 7CL2) mRNA without 5' and 3' UTR (SEQ ID NO:3):*

AUGGAAACCCUGCCCAGCUGCUGUUCUGCUGCUGCUGUGGCUGCCUGAUACCA
CCGGCGAAAU CGUGCUGACCCAGUCCCCCGCCACCCUGUCUCUGAGCCCUGGCGA
GAGAGCCACCCUGAGCUGCAGAGCCUCCCAGUCCGUGUCCGACGCCUACCUGGCC
UGGU AUCAGCAGAAGCCCGGCCAGGCCCCUCGGCUGCUGAUCUACGACGCCUCCU
CUAGAGCCACCGGCGUGCCCGCCAGAUUCUCCGGCUCUGGCUCUGGCACCGACUU

 CACCCUGACCAUCUCCAGCCUGGAACCCGAGGACUUCGCCGUGUACUACUGCCAC
 CAGUACAUCCAGCUGCACAGCUUCACCUUCGGCCAGGGCACCAAGGUGGAAAUCA
 AGGCCGAUGCCGCCCCUACCGUGUCCAUCUUCCCACCCUCCAGCGAGCAGCUGAC
 CUCUGGCGGCGCUUCCGUCGUGUGCUUCCUGAACAACUUCUACCCCAAGGACAUC
 AACGUGAAGUGGAAGAUCGACGGCUCCGAGCGGCAGAACGGCGUGCUGAACUCCU
 GGACCGACCAGGACUCCAAGGACAGCACCUACUCCAUGUCCUCCACCCUGACCCU
 GACCAAGGACGAGUACGAGCGGCACAACUCCUAUACCUGCGAGGCCACCCACAAG
 ACCUCCACCUCUUUUCAUCGUGAAGUCCUUAACCGGAACGAGUGCUGA

[0080] *Light chain anti-CCL2 (LC- α CCL2) mRNA with 5' and 3' UTR (SEQ ID NO:4): (The 5' and 3' UTR sequences are underlined)*

GGACAGAUCCGCGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUC
UGCCAAGAGUGACUCACCGUCCUUGACACGAUGGAAACCCUCCAGCUGCUGU
 UCCUGCUGCUGCUGUGGCUGCCUGAUACCACCGGCGAAAUCGUGCUGACCCAGUC
 CCCC GCCACCCUGUCUCUGAGCCUGGCGAGAGAGCCACCCUGAGCUGCAGAGCC
 UCCCAGUCCGUGUCCGACGCCUACCUGGCCUGGUAUCAGCAGAAGCCCGGCCAGG
 CCCUCGGCUGCUGAUCUACGACGCCUCCUCUAGAGCCACCGGCGUGCCCGCCAG
 AUUCUCCGGCUCUGGCUCUGGCACCGACUUCACCCUGACCAUCUCCAGCCUGGAA
 CCCGAGGACUUCGCCGUGUACUACUGCCACCAGUACAUCCAGCUGCACAGCUUCA
 CCUUCGGCCAGGGCACCAAGGUGGAAAUCAAGGCCGAUGCCGCCCCUACCGUGUC
 CAUCUUCACCCUCCAGCGAGCAGCUGACCUCUGGCGGCGCUUCCGUCGUGUGC
 UUCUGAACAACUUCUACCCCAAGGACAUAACGUGAAGUGGAAGAUCGACGGCU
 CCGAGCGGCAGAACGGCGUGCUGAACUCCUGGACCGACCAGGACUCCAAGGACAG
 CACCUACUCCAUGUCCUCCACCCUGACCCUGACCAAGGACGAGUACGAGCGGCAC
 AACUCCUAUACCUGCGAGGCCACCCACAAGACCUCACCUCUUUUCAUCGUGAAGU
CCUUAACCGGAACGAGUGCUGACGGGUGGCAUCCUGUGACCCUCCCCAGUGC
CUCUCCUGGCCUGGAAGUUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAA
AUUAAGUUGCAUCAAGCU

[0081] Among other things, the present invention also provides mRNAs encoding a heavy chain and light chain of an anti-CCL2 antibody substantially identical or similar to the sequences described herein. In some embodiments, an mRNA encoding the heavy chain of an anti-CCL2 antibody has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:1 or SEQ ID NO:2 as described herein. In some embodiments, an mRNA encoding the heavy chain of an anti-CCL2 antibody has a nucleotide sequence encoding an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to SEQ ID NO:1 as described herein. In some embodiments, an mRNA encoding the light chain of an anti-CCL2 antibody has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:3 or SEQ ID NO:4 as described herein. In some embodiments, an mRNA encoding the light chain of an anti-CCL2 antibody has a nucleotide sequence encoding an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to SEQ ID NO:3 as described herein.

[0082] In some embodiments, mRNA provided herein contains one or more modified

nucleotides such as those described herein. In some embodiments, an mRNA encoding the heavy chain or light chain of an anti-CCL2 antibody may contain at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of modified nucleotides of all modifiable nucleotides of the sequence.

Exemplary mRNAs Encoding Heavy Chain and Light Chain of anti-VEGF

[0083] Heavy chain anti-VEGF (HC- α VEGF) mRNA without 5' and 3' UTR (SEQ ID NO:5):

AUGGCAACUGGAUCAAGAACCUCUCCUCCUGCUCGCAUUCGGCCUGCUCUGUCUCC
CAUGGCUCCAAGAAGGAAGCGCGUUCUCCACUAUCCCCUCUCGGAGGUUCAGCU
GGUCGAAAGCGGGGGCGGCCUCGUCCAGCCAGGUGGAUCCUCCGCCUGAGCUGC
GCCGCGUCCGGAUACACUUCACCAACUACGGCAUGAACUGGGUCCGCCAGGCGC
CGGGAAAGGGACUGGAAUGGGUCGGCUGGAUCAAUACCUACACUGGAGAGCCUA
CCUACGCCGCUGACUUUAAGAGGGCGGUUCACUUUCACUGGAUACUCCAAGUC
AACCGCUUACCUUCAGAUCAAUUCUCCUGCGCGCCGAGGAUACCGCAGUGUAUUAC
UGCGCCAAAUAACCGCAUUAUACGGCUCCAGCCACUGGUACUUUGACGUGUGGG
GUCAAGGAACCCUGGUGACUGUGUCGUCCGCUUCCACCAAGGGACCAAGCGUGUU
UCCACUCGCCCCGAGCUCAAAAUCGACGUCGGGAGGUACUGCCGCACUGGGGUGC
UUGGUCAAGGACUACUUUCCAGAGCCGGUGACUGUUUCCUGGAACAGCGGAGCGC
UCACCUCGGGCGUGCACACCUUCCUCCUGCGGUGUUGCAGUCAUCUGGACUGUACUC
GCUGUCCAGCGUGGUCACGGUCCCGAGCUUGUCGCUCCGGGACCCAAACCUACA
UGCAAUGUCAACCACAAGCCAUCGAACACCAAAGUCGACAAGAAGGUGGAACCGA
AGUCGUGCGACAAGACUCAUACGUGCCCACCGUGUCCGGCUCGGAACUGUUGGG
GGGCCCCUCCGUGUCCUUUCCCGCCAAAGCCUAAGGACACUCUCAUGAUCUCA
CGGACGCCAGAAGUGACCUGUGUGGUCGUGGAUGUGUCACAUGAGGAUCCGGAA
GUCAAAUUAACUGGUAUGUGGACGGGGUGGAAGUGCAUAAUGCCAAAACCAAA
CCUCGCGAGGAGCAGUACAACUCAACCUACCGGGUGGUGUCCGUGCUGACUGUGC
UGCACCAGGACUGGCUGAAUGGAAAGGAGUACAAAUGCAAGGUCAGCAACAAGG
CCCUUCCCGCCCCAAUCGAAAAGACGAUCUCGAAGGCCAAAGGUCAGCCGCGAGA
GCCUCAAGUGUACACUCUGCCGCCGUGCAAGAGAAGAAAUGACUAAGAACCAAGUU
UCCUCACUUGCCUGGUGAAGGGCUUCUACCCAGCGACAUCGCAGUGGAAUGGG
AGAGCAACGGACAGCCGGAACAACUAUAAGACCACCCUCCUGUGUUGGACUC
GGAUGGUUCCUUCUUCUUAACAGCAAGCUGACCGUGGAUAAAUCGCGGUGGCA
GCAAGGAAAUGUGUUUCAUGCUCAGUCAUGCACGAGGCGCUGCACAAUCACUAC
ACUCAGAAGUCCUGUCGUCGUGCCAGGAAAAUAA

Heavy chain anti-VEGF (HC- α VEGF) mRNA with 5' and 3' UTR (SEQ ID NO:6):

[0084] (The 5' and 3' UTR sequences are underlined, signal peptide sequences are italicized and bolded)

GGACAGAUCCGUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUC
UGCCAAGAGUGACUCACCGUCCUUGACACGAUGGCAACUGGAUCAAGAACC
UCCUGCUCGCAUUCGGCCUGCUCUGUCUCCCAUGGCUCCAAGAAGGAAGCGCGUUC
CCCACUAUCCCCCUCUCGGAGGUUCAGCUGGUCGAAAGCGGGGGCGGCCUCGUCC
AGCCAGGUGGAUCCUCCGCCUGAGCUGCGCCGCGUCCGGAUACACUUUCACCAA
CUACGGCAUGAACUGGGUCCGCCAGGCGCCGGGAAAGGGACUGGAUUGGGUCGGC
UGGAUCAAUACCUACACUGGAGAGCCUACCUACGCCGCUGACUUUAAGAGGCGGU
UCACUUUCUCACUGGAUACUCCAAGUCAACCGCUUACCUUCAGAUCAAUUC

GCGCGCCGAGGAUACCGCAGUGUAUUACUGCGCCAAAUACCCGCAUUACUACGGC
 UCCAGCCACUGGUACUUUGACGUGUGGGGUCAAGGAACCCUGGUGACUGUGUCG
 UCCGCUUCCACCAAGGGACCAAGCGUGUUUCCACUCGCCCCGAGCUCAAAAUCCA
 CGUCGGGAGGUACUGCCGCACUGGGGUGCUUGGUCAAGGACUACUUUCCAGAGCC
 GGUGACUGUUUCCUGGAACAGCGGAGCGCUCACCUCGGGCGUGCACACCUUCCCU
 GCGGUGUUGCAGUCAUCUGGACUGUACUCGCGUGUCCAGCGUGGUCACGGUCCCCGA
 GCUCGUCGUCGCGGGACCCAAACCUACAUUUGCAAUGUCAACCACAAGCCAUCGAA
 CACCAAAGUCGACAAGAAGGUGGAACCGAAGUCGUGCGACAAGACUCAUACGUGC
 CCACCGUGUCCGGCUCGGAACUGUUGGGGGGCCCCUCCGUGUCCUUUUCGGC
 CAAAGCCUAAGGACACUCUCAUGAUCUCACGGACGCCAGAAGUGACCUGUGUGGU
 CGUGGAUGUGUCACAUGAGGAUCCGGAAGUCAAAUUAACUGGUAUGUGGACGG
 GGUGGAAGUGCAUAAUGCCAAAACCAAACCUCGCGAGGAGCAGUACAACUCAACC
 UACCGGGUGGUGUCCGUGCUGACUGUGCUGCACCAGGACUGGCUGAAUGGAAAG
 GAGUACAAAUGCAAGGUCAGCAACAAGGCCCUUCCCGCCCCAAUCGAAAAGACGA
 UCUCGAAGGCCAAAGGUCAGCCGCGAGAGCCUCAAGUGUACACUCUGCCGCCGUC
 AAGAGAAGAAAUGACUAAGAACCAAGUUUCCCUACUUGCCUGGUGAAGGGCUU
 CUACCCAGCGACAUCGCGAGUGGAAUGGGAGAGCAACGGACAGCCGGAAAACAAC
 UAUAAGACCACCCCUCCUGUGUUGGACUCGGAUGGUUCCUUCUUCUUUACAGCA
 AGCUGACCGUGGAUAAAUCGCGGUGGCAGCAAGGAAAUGUGUUUUAUGCUCAG
 UCAUGCACGAGGCGCUGCACAACUACACUCAGAAAGUCCUGUCGUCGUGCGCC
 AGGAAAUAACGGGUGGCAUCCCUUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCCU
 GGAAGUUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAUAAGUUGCAUC
AAGCU

[0085] *Light chain anti-VEGF (HC- α VEGF) mRNA without 5' and 3' UTR (SEQ ID NO:7):*

AUGGCCACUGGAUCAAGAACCUCACUGCUGCUCGCUUUUGGACUGCUUUGCCUGC
 CCUGGUUGCAAGAAGGAUCGGCUUUCGGACCAUCCACUCUCCGACAUAUCAAU
 GACGCAGUCCCCAUUCGAGCCUCUCAGCAUCAGUGGGGGAUCGCGUGACUAUCACU

UGCUCGGCGAGCCAGGAUAUCAGCAAUUACCUGAACUGGUUAUCAGCAAAAGCCUG
 GAAAGGCACCGAAGGUGCUGAUCUACUUCACCUCAAGCCUCCAUUCGGGUGUCCC
 GUCCCGCUUCAGCGGCUCGCGCUCAGGCACUGACUUCACCCUGACUAUCUCCUCG
 CUGCAACCGGAAGAUUUCGCCACUUAUACUGUCAGCAGUACUCCACCGUGCCUU
 GGACGUUCGGACAGGGAACCAAAGUUGAGAUUAAGCGGACGGUCGCGGCCCCCUC
 CGUGUUUAUCUUUCCGCCUUCGGACGAGCAGCUGAAGUCGGGAACCGCCUCUGUC
 GUGUGCCUCCUGAACAACUUCUACCCGCGGGGAAGCCAAGGUGCAGUGGAAAGUGG
 AUAACGCGCUUCAGAGCGGCAAUUCGCAAGAGUCCGUGACCGAAGAGGACUCGAA
 GGACUCAACCUACUCCCUACAGCUCAACCCUCACUUUGUCGAAGGCCGACUACGAG
 AAGCACAAAGUCUACGCAUGCGAAGUCACCCACCAGGGUCUGUCGAGCCCAGUGA
 CUAAAUCCUUCAAUAGGGGGGGAUGUUA

Light chain anti-VEGF (HC- α VEGF) mRNA with 5' and 3' UTR (SEQ ID NO:8):

[0086] *(The 5' and 3' UTR sequences are underlined, signal peptide sequences are italicized and bolded)*

GGACAGAUCCGUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUC
UGCCAAGAGUGACUACCGUCCUUGACACGAUGGCCACUGGAUCAAGAACCUCAC
UGCUGCUCGCUUUUGGACUGCUUUGCCUGCCUGGUUGCAAGAAGGAUCGGCUUU
CCCGACCAUCCACUCUCCGACAUAUCAAUGACGCAGUCCCAUCGAGCCUCUCAG
CAUCAGUGGGGGAUCGCGUGACUAUCACUUGCUCGGCGAGCCAGGAUAUCAGCAA
UUACCUGAACUGGUUAUCAGCAAAAGCCUGGAAAGGCACCGAAGGUGCUGAUCUA

CUUCACCUCAAGCCUCCAUUCGGGUGUCCCGUCCCGCUUCAGCGGCUCCGGCUCA
GGCACUGACUUCACCCUGACUAUCUCCUCGCUGCAACCGGAAGAUUUCGCCACUU
ACUACUGUCAGCAGUACUCCACCGUGCCUUGGACGUUCGGACAGGGAACCAAAGU
UGAGAUUAAGCGGACGGUCGCGGCCCCCUCCGUGUUUAUCUUUCCGCCUUCGGAC
GAGCAGCUGAAGUCGGGAACCGCCUCUGUCGUGUGCCUCCUGAACAAACUUCUACC
CGCGGGAAGCCAAGGUGCAGUGGAAAGUGGAUAACGCGCUUCAGAGCGGCAAUU
CGCAAGAGUCCGUGACCGAAGAGGACUCGAAGGACUCAACCUACUCCCUCAGCUC
AACCCUCACUUUGUCGAAGGCCGACUACGAGAAGCACAAAGUCUACGCAUGCGAA
GUCACCCACCAGGGUCUGUCGAGCCCAGUGACUAAAUCCUUCAAUAGGGGGGAU
GUUAACGGGUGGCAUCCCUGUGACCCCUCUCCAGUGCCUCUCCUGGCCUUGGAAG
UUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAUAAGUUGCAUCAAGCU

[0087] Among other things, the present invention also provides mRNAs encoding a heavy chain and light chain of an anti-VEGF antibody substantially identical or similar to the sequences described herein. In some embodiments, an mRNA encoding the heavy chain of an anti- VEGF antibody has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:5 or SEQ ID NO:6 as described herein. In some embodiments, an mRNA encoding the heavy chain of an anti- VEGF antibody has a nucleotide sequence encoding an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to SEQ ID NO:5 as described herein. In some embodiments, an mRNA encoding the light chain of an anti-VEGF antibody has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:7 or SEQ ID NO:8 as described herein. In some embodiments, an mRNA encoding the light chain of an anti-VEGF antibody has a nucleotide sequence encoding an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical or homologous to SEQ ID NO:7 as described herein.

[0088] In some embodiments, mRNA provided herein contains one or more modified nucleotides such as those described herein. In some embodiments, an mRNA encoding the heavy chain or light chain of an anti-VEGF antibody may contain at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of modified nucleotides of all modifiable nucleotides of the sequence.

[0089] As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two nucleic acid sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a

position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

Liposomal Delivery Vehicles

[0090] mRNAs encoding a heavy chain and a light chain of an antibody may be delivered via a single liposomal delivery vehicle.

[0091] As used herein, liposomal delivery vehicles are usually characterized as microscopic vesicles having an interior aqua space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also be formed by amphiphilic polymers and surfactants (e.g., polymerosomes, niosomes, etc.). In the context of the present invention, a liposomal delivery vehicle serves to transport a desired mRNA to a target cell or tissue. The process of incorporation of a desired mRNA into a liposome is often referred to as "loading". Exemplary methods are described in Lasic, et al., FEBS Lett., 312: 255-258, 1992. The liposome-incorporated nucleic acids may be completely or partially located in the interior space of the liposome, within the bilayer membrane of the liposome, or associated with the exterior surface of the liposome membrane. The incorporation of a nucleic acid into liposomes is also referred to herein as "encapsulation" wherein the nucleic acid is entirely contained within the interior space of the liposome. The purpose of incorporating a mRNA into a transfer vehicle, such as a liposome, is often to protect the nucleic acid from an environment which may contain enzymes or chemicals that degrade nucleic acids and/or systems or receptors that cause the rapid excretion of the nucleic acids. Accordingly, in some embodiments, a suitable delivery vehicle is capable of enhancing the stability of the mRNA contained therein and/or facilitate the delivery of mRNA to the target cell or tissue.

[0092] A suitable liposomal delivery vehicle is formulated as a lipid nanoparticle. As used herein, the phrase "lipid nanoparticle" refers to a delivery vehicle comprising a cationic lipid, a non-cationic lipid, a cholesterol-based lipid, and a PEG-modified lipid). The lipid nanoparticles may be prepared by including multi-component lipid mixtures of varying ratios employing the

cationic, non-cationic, cholesterol-based and PEG-modified lipids..

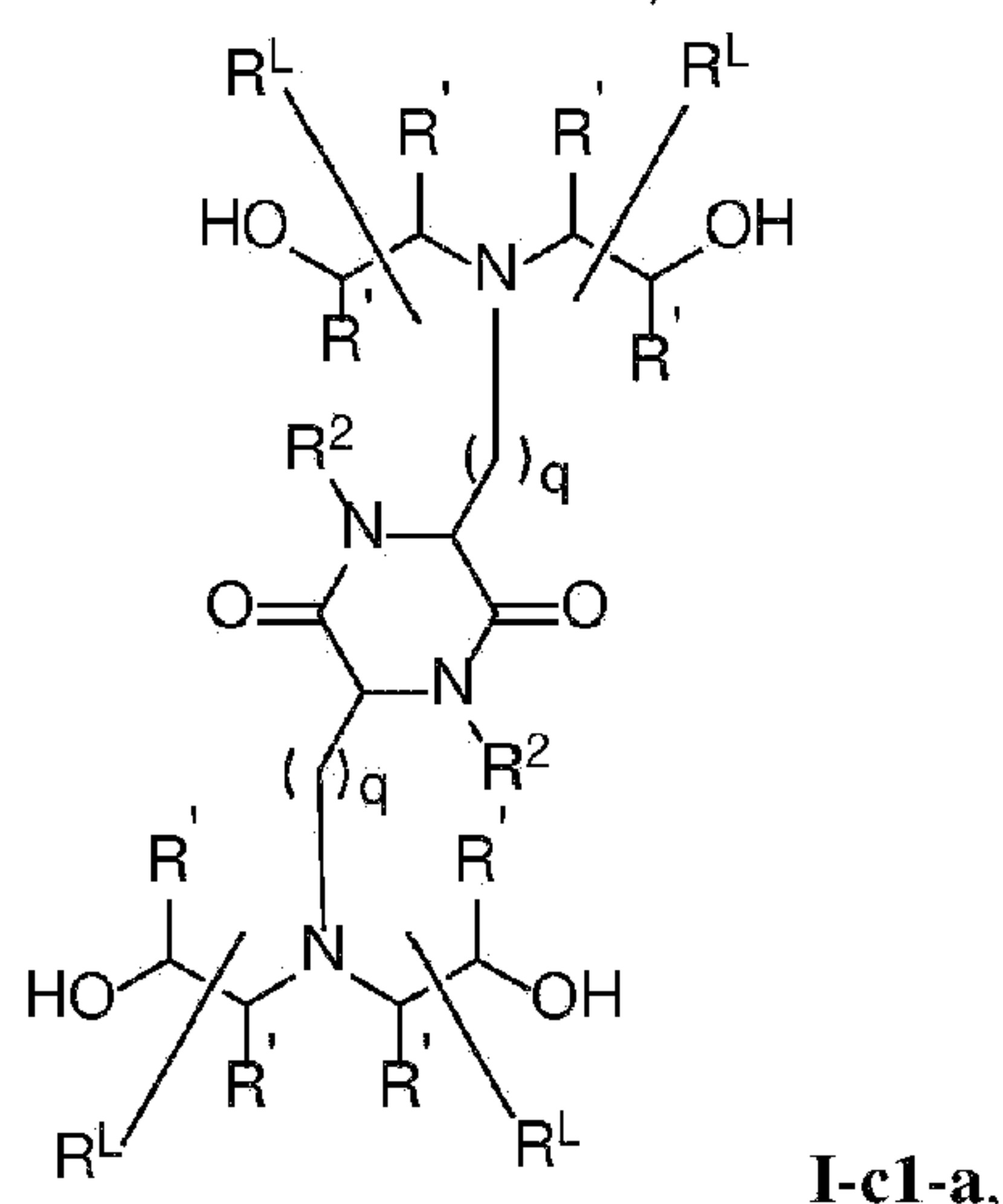
[0093] In some embodiments, a suitable liposomal delivery vehicle contains a cationic lipid. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that have a net positive charge at a selected pH, such as physiological pH. Several cationic lipids have been described in the literature, many of which are commercially available. Particularly suitable cationic lipids for use in the compositions and methods of the invention include those described in international patent publications WO 2010/053572 (and particularly, CI 2-200 described at paragraph [00225]) and WO 2012/170930. In certain embodiments, the compositions of the invention employ a lipid nanoparticles comprising an ionizable cationic lipid described in U.S. provisional patent application 61/617,468, filed March 29, 2012, such as, e.g, (15Z, 18Z)-N,N-dimethyl-6-(9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-15,18-dien-1-amine (HGT5000), (15Z, 18Z)-N,N-dimethyl-6-((9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-4,15,18-trien-1-amine (HGT5001), and (15Z,18Z)-N,N-dimethyl-6-((9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-5, 15, 18-trien-1-amine (HGT5002).

[0094] In some embodiments, the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride or "DOTMA" is used. (Feigner et al. (Proc. Nat'l Acad. Sci. 84, 7413 (1987); U.S. Pat. No. 4,897,355). DOTMA can be formulated alone or can be combined with the neutral lipid, dioleoylphosphatidyl-ethanolamine or "DOPE" or other cationic or non-cationic lipids into a liposomal transfer vehicle or a lipid nanoparticle, and such liposomes can be used to enhance the delivery of nucleic acids into target cells. Other suitable cationic lipids include, for example, 5-carboxyspermylglycinedioctadecylamide or "DOGS," 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium or "DOSPA" (Behr et al. Proc. Nat'l Acad. Sci. 86, 6982 (1989); U.S. Pat. No. 5,171,678; U.S. Pat. No. 5,334,761), 1,2-Dioleoyl-3-Dimethylammonium-Propane or "DODAP", 1,2-Dioleoyl-3-Trimethylammonium-Propane or "DOTAP". Contemplated cationic lipids also include 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane or "DSDMA", 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane or "DODMA", 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane or "DLinDMA", 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane or "DLinDMA", N-dioleoyl-N,N-dimethylammonium chloride or "DODAC", N,N-distearyl-N,N-dimethylammonium bromide or "DDAB", N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide or "DMRIE", 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane or "CLinDMA", 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentoxyl]-3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy)propane or "CpLinDMA", N,N-dimethyl-3,4-dioleoyloxybenzylamine or "DMOBA", 1,2-N,N'-dioleoylcarbonyl-3-dimethylaminopropane or "DOcarbDAP", 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine or "DLinDAP", 1,2-N,N'-Dilinoleoylcarbonyl-3-dimethylaminopropane or "DLincarbDAP", 1,2-Dilinoleoylcarbonyl-3-dimethylaminopropane or "DLinCDAP", 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane or "DLin-DMA", 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane or "DLin-K-XTC2-DMA", and 2-(2,2-di((9Z,12Z)-octadeca-9,12-dien-1-yl)-1,3-dioxolan-4-yl)-N,N-dimethylethanamine (DLin-KC2-DMA)) (See, WO 2010/042877; Semple et al., Nature Biotech. 28: 172-176 (2010)), or mixtures thereof. (Heyes, J., et al., J Controlled Release 107: 276-287 (2005); Morrissey, DV., et al., Nat. Biotechnol. 23(8): 1003-1007 (2005); PCT Publication WO2005/121348A1).

[0095] In some embodiments, one or more of the cationic lipids present in such a composition comprise at least one of an imidazole, dialkylamino, or guanidinium moiety.

[0096] In some embodiments, one or more of the cationic lipids present in such a composition are chosen from XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane), MC3 (((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), ALNY-100 ((3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine)), NC98-5 (4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide), DODAP (1,2-dioleoyl-3-dimethylammonium propane), HGT4003 (WO 2012/170889), ICE (WO 2011/068810), HGT5000 (U.S. Provisional Patent Application No. 61/617,468) or HGT5001 (cis or trans) (Provisional Patent Application No. 61/617,468), aminoalcohol lipidoids such as those disclosed in WO2010/053572, DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DlinDMA (Heyes, J.; Palmer, L.; Bremner, K.; MacLachlan, I. "Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids" J. Contr. Rel. 2005, 107, 276-287), Dlin-KC2-DMA (Semple, S.C. et al. "Rational Design of Cationic Lipids for siRNA Delivery" Nature Biotech. 2010, 28, 172-176), C12-200 (Love, K.T. et al. "Lipid-like materials for low-dose in vivo gene silencing" PNAS 2010, 107, 1864-1869).

[0097] In some embodiments, a cationic lipid present in such a composition is a cationic lipid described in WO 2013063468 and in U.S. provisional application serial number 61/894,299, entitled "Lipid Formulations for Delivery of Messenger RNA" filed on October 22, 2013. In some embodiments, a cationic lipid comprises a compound of formula **I-c1-a**:



or a pharmaceutically acceptable salt thereof, wherein:

each R^2 independently is hydrogen or C_{1-3} alkyl;

each q independently is 2 to 6;

each R' independently is hydrogen or C_{1-3} alkyl;

and each R^L independently is C_{8-12} alkyl.

[0098] In some embodiments, each R^2 independently is hydrogen, methyl or ethyl. In some embodiments, each R^2 independently is hydrogen or methyl. In some embodiments, each R^2 is hydrogen.

[0099] In some embodiments, each q independently is 3 to 6. In some embodiments, each q independently is 3 to 5. In some embodiments, each q is 4.

[0100] In some embodiments, each R' independently is hydrogen, methyl or ethyl. In some embodiments, each R' independently is hydrogen or methyl. In some embodiments, each R' independently is hydrogen.

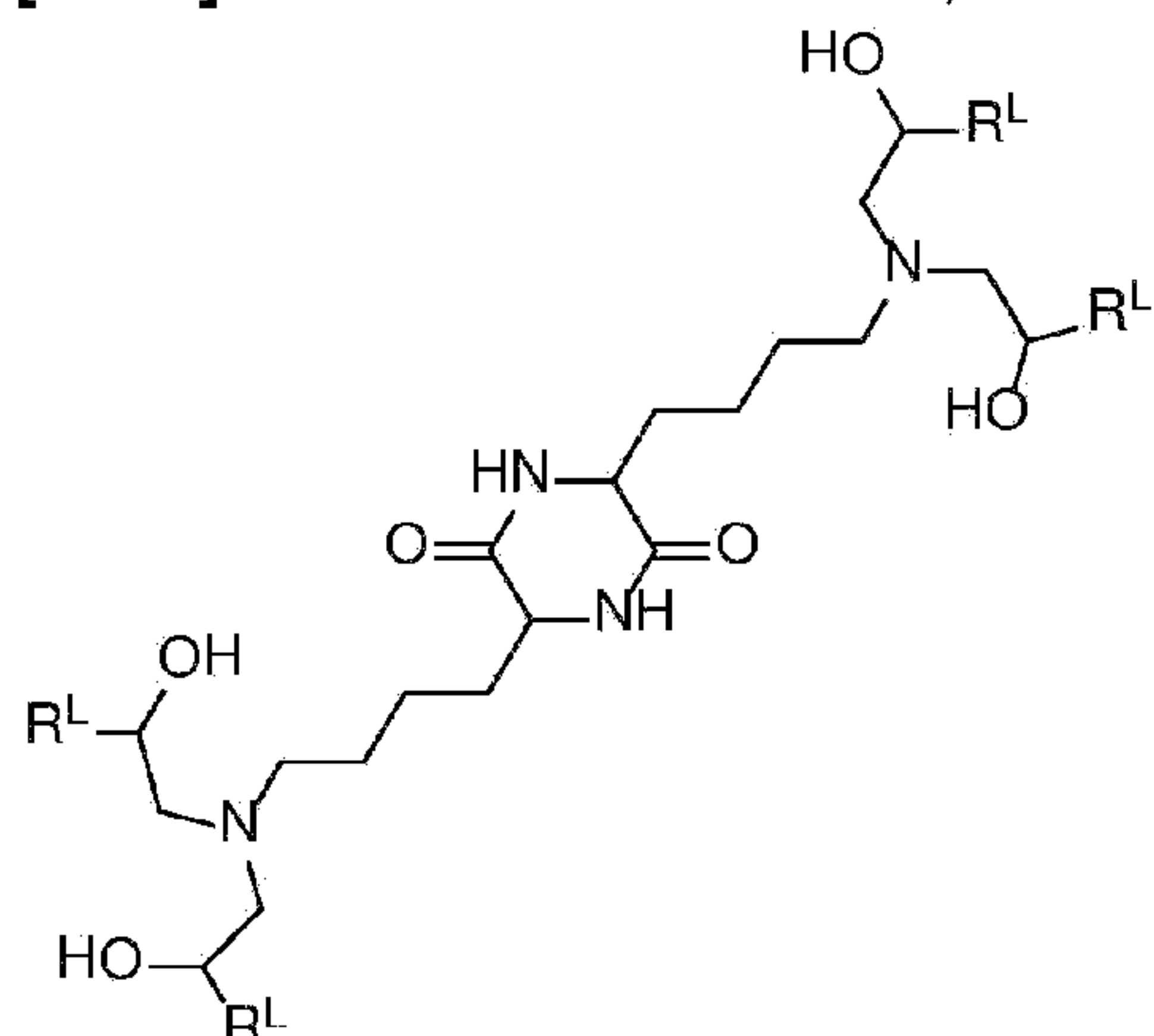
[0101] In some embodiments, each R^L independently is C_{8-12} alkyl. In some embodiments, each R^L independently is n - C_{8-12} alkyl. In some embodiments, each R^L independently is C_{9-11} alkyl. In some embodiments, each R^L independently is n - C_{9-11} alkyl. In some embodiments, each R^L independently is C_{10} alkyl. In some embodiments, each R^L independently is n - C_{10} alkyl.

[0102] In some embodiments, each R^2 independently is hydrogen or methyl; each q independently is 3 to 5; each R' independently is hydrogen or methyl; and each R^L independently is C_{8-12} alkyl.

[0103] In some embodiments, each R^2 is hydrogen; each q independently is 3 to 5; each R' is hydrogen; and each R^L independently is C_{8-12} alkyl.

[0104] In some embodiments, each R^2 is hydrogen; each q is 4; each R' is hydrogen; and each R^L independently is C_{8-12} alkyl.

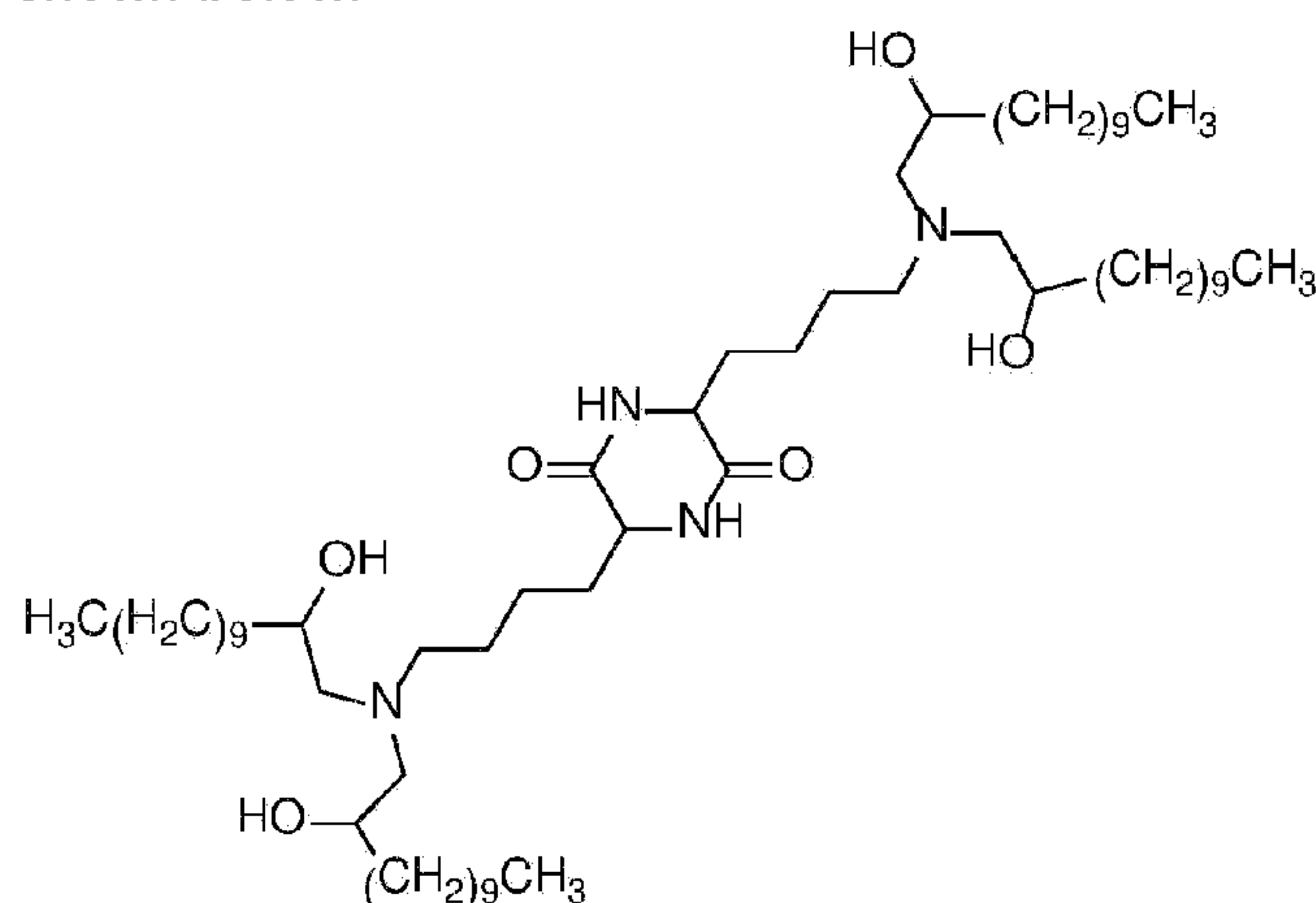
[0105] In some embodiments, a cationic lipid comprises a compound of formula I-g:



I-g,

or a pharmaceutically acceptable salt thereof, wherein each R^L independently is C_{8-12} alkyl. In some embodiments, each R^L independently is n - C_{8-12} alkyl. In some embodiments, each R^L independently is C_{9-11} alkyl. In some embodiments, each R^L independently is n - C_{9-11} alkyl. In some embodiments, each R^L independently is C_{10} alkyl. In some embodiments, each R^L is n - C_{10} alkyl.

[0106] In particular embodiments, provided compositions include a cationic lipid cKK-E12, or (3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine-2,5-dione). Structure of cKK-E12 is shown below:



[0107] In some embodiments, a suitable liposomal delivery vehicle contains a non-cationic lipids. In some embodiments, a non-cationic lipid is a neutral lipid, i.e., a lipid that does not carry a net charge in the conditions under which the composition is formulated and/or administered. Such exemplary non-cationic or neutral lipids can be chosen from DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), and cholesterol.

[0108] Suitable cholesterol-based cationic lipids include, for example, DC-Choi (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, et al. Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf et al. BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335), or ICE.

[0109] In other embodiments, suitable lipid nanoparticles comprising one or more cleavable lipids, such as, for example, one or more cationic lipids or compounds that comprise a cleavable disulfide (S-S) functional group (e.g., HGT4001, HGT4002, HGT4003, HGT4004 and HGT4005), as further described in U.S. Provisional Application No: 61/494,745.

[0110] In addition, several reagents are commercially available to enhance transfection efficacy. Suitable examples include LIPOFECTIN (DOTMA:DOPE) (Invitrogen, Carlsbad, Calif.),

LIPOFECTA INE (DOSPA:DOPE) (Invitrogen), LIPOFECTAMINE2000. (Invitrogen), FUGENE, TRANSFECTAM (DOGS), and EFFECTENE.

[0111] In some embodiments, the cationic lipid may comprise a molar ratio of about 1% to about 90%, about 2% to about 70%, about 5% to about 50%, about 10% to about 40% of the total lipid present in the transfer vehicle, or preferably about 20% to about 70% of the total lipid present in the transfer vehicle.

[0112] The use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-Octanoyl-Sphingosine-1-[Succinyl(Methoxy Polyethylene Glycol)-2000] (C8 PEG-2000 ceramide) is in combination with other lipids together which comprise the liposomal transfer vehicle. Contemplated PEG-modified lipids include, but is not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C6-C20 length. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target cell, (Klibanov et al. (1990) FEBS Letters, 268 (1): 235-237), or they may be selected to rapidly exchange out of the formulation in vivo (see U.S. Pat. No. 5,885,613).

[0113] Particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C14 or C18). The PEG-modified phospholipid and derivitized lipids of the present invention may comprise a molar ratio from about 0% to about 20%, about 0.5% to about 20%, about 1% to about 15%, about 4% to about 10%, or about 2% of the total lipid present in the liposomal transfer vehicle.

[0114] As used herein, the phrase "non-cationic lipid" refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase "anionic lipid" refers to any of a number of lipid species that carry a net negative charge at a selected H, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), cholesterol, or a mixture thereof. Such non-cationic lipids may be used alone, but are preferably used in combination with other excipients, for example, cationic lipids. When used in combination with a cationic lipid, the non-cationic lipid may comprise a molar ratio of 5% to about 90%, or preferably about 10 % to about 70% of the total lipid present in the transfer vehicle.

[0115] In particular embodiments, a suitable liposomal transfer vehicle is prepared by combining multiple lipid and/or polymer components. For example, a liposomal transfer vehicle

may be prepared using C 12-200, DOPE, chol, DMG-PEG2K at a molar ratio of 40:30:25:5, or DODAP, DOPE, cholesterol, DMG-PEG2K at a molar ratio of 18:56:20:6, or HGT5000, DOPE, chol, DMG-PEG2K at a molar ratio of 40:20:35:5, or HGT5001, DOPE, chol, DMG-PEG2K at a molar ratio of 40:20:35:5. The selection of cationic lipids, non-cationic lipids and PEG-modified lipids which comprise the lipid nanoparticle, as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s), the nature of the intended target cells, the characteristics of the mRNA to be delivered. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus the molar ratios may be adjusted accordingly. For example, in embodiments, the percentage of cationic lipid in the lipid nanoparticle may be greater than 10%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, or greater than 70%. The percentage of non-cationic lipid in the lipid nanoparticle may be greater than 5%, greater than 10%, greater than 20%, greater than 30%, or greater than 40%. The percentage of cholesterol in the lipid nanoparticle may be greater than 10%, greater than 20%, greater than 30%, or greater than 40%. The percentage of PEG-modified lipid in the lipid nanoparticle may be greater than 1%, greater than 2%, greater than 5%, greater than 10%, or greater than 20%.

[0116] In certain embodiments, suitable lipid nanoparticles of the invention comprise at least one of the following cationic lipids: C12-200, Dlin-KC2-DMA, DODAP, HGT4003, ICE, HGT5000, or HGT5001. In some embodiments, suitable liposomal transfer vehicle comprises cholesterol and/or a PEG-modified lipid. In some embodiments, suitable transfer vehicles comprises DMG-PEG2K. In some embodiments, suitable liposomal transfer vehicle comprises one of the following lipid combinations: C12-200, DOPE, cholesterol, DMG-PEG2K; DODAP, DOPE, cholesterol, DMG-PEG2K; HGT5000, DOPE, cholesterol, DMG-PEG2K; HGT5001, DOPE, cholesterol, DMG-PEG2K; XTC, DSPC, cholesterol, PEG-DMG; MC3, DSPC, cholesterol, PEG-DMG; and ALNY-100, DSPC, cholesterol, PEG-DSG.

[0117] The liposomal transfer vehicles for use in the compositions of the invention can be prepared by various techniques which are presently known in the art. Multilamellar vesicles (MLV) may be prepared conventional techniques, for example, by depositing a selected lipid on the inside wall of a suitable container or vessel by dissolving the lipid in an appropriate solvent, and then evaporating the solvent to leave a thin film on the inside of the vessel or by spray drying. An aqueous phase may then added to the vessel with a vortexing motion which results in the formation of MLVs. Uni-lamellar vesicles (ULV) can then be formed by homogenization, sonication or extrusion of the multi-lamellar vesicles. In addition, unilamellar vesicles can be formed by detergent removal techniques.

[0118] In certain embodiments of this invention, the compositions of the present invention comprise a liposomal transfer vehicle wherein the mRNA is associated on both the surface of the liposomal transfer vehicle and encapsulated within the same liposomal transfer vehicle. For example, during preparation of the compositions of the present invention, cationic liposomal transfer vehicles may associate with the mRNA through electrostatic interactions.

[0119] Suitable liposomal delivery vehicles according to the present invention may be made in various sizes. Selection of an appropriate size may take into consideration the site of the target cell or tissue and to some extent the application for which the liposome is being made. In some embodiments, an appropriate size of liposomal delivery vehicles is selected to facilitate systemic distribution of antibody encoded by the mRNA. In some embodiments, it may be desirable to limit transfection of the mRNA to certain cells or tissues. For example, to target hepatocytes a liposomal transfer vehicle may be sized such that its dimensions are smaller than the fenestrations of the endothelial layer lining hepatic sinusoids in the liver; accordingly the liposomal transfer vehicle can readily penetrate such endothelial fenestrations to reach the target hepatocytes. Alternatively, a liposomal transfer vehicle may be sized such that the dimensions of the liposome are of a sufficient diameter to limit or expressly avoid distribution into certain cells or tissues. For example, a liposomal transfer vehicle may be sized such that its dimensions are larger than the fenestrations of the endothelial layer lining hepatic sinusoids to thereby limit distribution of the liposomal transfer vehicle to hepatocytes.

[0120] In some embodiments, a suitable liposomal delivery vehicle has a size no greater than about 150 nm (e.g., no greater than about 125 nm, 100 nm, 75 nm, or 50 nm). In some embodiments, a suitable liposomal delivery vehicle has a size ranging from about 150 - 10 nm (e.g., ranging from about 125 - 10 nm, 100 - 10 nm, 75 - 10 nm, or 50 - 10 nm). In some embodiments, a suitable liposomal delivery vehicle has a size ranging from about 150 - 100 nm (e.g., ranging from about 125 - 100 nm). In some embodiments, a suitable liposomal delivery vehicle has a size ranging from about 100 - 10 nm (e.g., ranging from about 90 - 10 nm, 80 - 10 nm, 70 - 10 nm, 60 - 10 nm, or 50 - 10 nm).

[0121] A variety of alternative methods known in the art are available for sizing of a population of liposomal transfer vehicles. One such sizing method is described in U.S. Pat. No. 4,737,323. Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small ULV less than about 0.05 microns in diameter. Homogenization is another method that relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, MLV are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. The size of the liposomal vesicles may be determined by quasi-electric light scattering (QELS) as described in Bloomfield, Ann. Rev. Biophys. Bioeng., 10:421-150 (1981). Average liposome diameter may be reduced by sonication of formed liposomes. Intermittent sonication cycles may be alternated with QELS assessment to guide efficient liposome synthesis.

Expression of RNA Coded Antibodies in vivo

[0122] According to the present invention, antibody encoding mRNAs (i.e., heavy chain and light chain encoding mRNAs) are delivered via liposomal delivery vehicles to a subject in need of delivery, such that a fully assembled desired antibody is expressed *in vivo*.

[0123] A desired antibody encoded by mRNAs is expressed systemically in the subject. This can be achieved by secreting fully assembled antibodies from a cell or tissue in which the antibody is initially expressed into the circulation system of the subject. Compositions of the invention containing antibody encoding mRNAs and liposomal vehicles distribute into the cells of the liver to facilitate the delivery and the subsequent expression of the mRNA comprised therein by the cells of the liver (e.g., hepatocytes). The targeted hepatocytes may function as a biological "reservoir" or "depot" capable of producing, and excreting a fully assembled desired antibody, resulting in systemic distribution of the antibody. Cells other than hepatocytes (e.g., lung, spleen, heart, ocular, or cells of the central nervous system) can serve as a depot location for protein production. Typically, sustained production and secretion of fully assembled antibodies from the reservoir or depot cells results in effective systemic distribution.

[0124] In some embodiments, systemic expression of a desired antibody encoded mRNAs in the patient serum (i.e., blood) is detectable for more than 1 hour, more than 4 hours, more than 6 hours, more than 12 hours, more than 18 hours, more than 24 hours, more than 30 hours, more than 36 hours, more than 42 hours, more than 48 hours, more than 54 hours, more than 60 hours, more than 66 hours, more than 72 hours, more than 96 hours, more than 120 hours, more than 144 hours, more than 168 hours, more than 2 weeks, more than 3 weeks, more than 1 month or more than 2 months after administration. In some embodiments, the serum concentration of the antibody encoded by mRNAs reaches a peak level about 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, 72 hours, 78 hours, 84 hours, 90 hours, or 96 hours after administration. In some embodiments, sustained circulation of the desired antibody encoded by mRNAs are observed. For example, the systemic expression of the antibody encoded by mRNAs in the patient serum (i.e., blood) may be detected for more than 1 day, 2 days, 3 days, 4 days, 5 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months or more after administration.

[0125] mRNAs encoding heavy chain and light chain of an antibody may be delivered to target cells or tissues for intracellular expression or local distribution of the antibody. Typically, local distribution results when a fully assembled antibody is produced and secreted from a target cell to the surrounding extracellular fluid without entering the circulation system, such as blood stream. As used herein, the term "target cell" or "target tissue" refers to a cell or tissue to which antibody encoding mRNA(s) is to be directed or targeted. For example, where it is desired to deliver an mRNA to a hepatocyte, the hepatocyte represents the target cell. Antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) described herein may be delivered to a variety of target cells or tissues including, but not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells, mesenchymal cells, neural cells (e.g., meninges, astrocytes, motor neurons, cells of the dorsal root ganglia and anterior horn motor neurons), photoreceptor cells (e.g., rods and cones), retinal pigmented epithelial cells, secretory cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

[0126] Delivery of mRNAs to target cells and tissues may be accomplished by both passive and active targeting means. The phenomenon of passive targeting exploits the natural distributions patterns of a transfer vehicle *in vivo* without relying upon the use of additional excipients or means to enhance recognition of the transfer vehicle by target cells. For example, transfer vehicles which are subject to phagocytosis by the cells of the reticulo-endothelial system are likely to accumulate in the liver or spleen, and accordingly may provide means to passively direct the delivery of the compositions to such target cells.

[0127] Alternatively, delivery of mRNAs to target cells and tissues may be accomplished by active targeting, which involves the use of additional excipients, referred to herein as "targeting ligands" that may be bound (either covalently or non-covalently) to the transfer vehicle to encourage localization of such transfer vehicle at certain target cells or target tissues. For example, targeting may be mediated by the inclusion of one or more endogenous targeting ligands (e.g., apolipoprotein E) in or on the transfer vehicle to encourage distribution to the target cells or tissues. Recognition of the targeting ligand by the target tissues actively facilitates tissue distribution and cellular uptake of the transfer vehicle and/or its contents in the target cells and tissues (e.g., the inclusion of an apolipoprotein-E targeting ligand in or on the transfer vehicle encourages recognition and binding of the transfer vehicle to endogenous low density lipoprotein receptors expressed by hepatocytes). As provided herein, the composition can comprise a ligand capable of enhancing affinity of the composition to the target cell. Targeting ligands may be linked to the outer bilayer of the lipid particle during formulation or post-formulation. These methods are well known in the art. In addition, some lipid particle formulations may employ fusogenic polymers such as PEAA, Hemagglutinin, other lipopeptides (see U.S. Patent Application Ser. Nos. 08/835,281, and 60/083,294) and other features useful for *in vivo* and/or intracellular delivery. Contemplated are compositions which comprise one or more ligands (e.g., peptides, aptamers, oligonucleotides, a vitamin or other molecules) that are capable of enhancing the affinity of the compositions and their nucleic acid contents for the target cells or tissues. Suitable ligands may optionally be bound or linked to the surface of the transfer vehicle. In some embodiments, the targeting ligand may span the surface of a transfer vehicle or be encapsulated within the transfer vehicle. Suitable ligands are selected based upon their physical, chemical or biological properties (e.g., selective affinity and/or recognition of target cell surface markers or features). Cell-specific target sites and their corresponding targeting ligand can vary widely. Suitable targeting ligands are selected such that the unique characteristics of a target cell are exploited, thus allowing the composition to discriminate between target and non-target cells. For example, compositions may include surface markers (e.g., apolipoprotein-B or apolipoprotein-E) that selectively enhance recognition of, or affinity to hepatocytes (e.g., by receptor-mediated recognition of and binding to such surface markers). Additionally, the use of galactose as a targeting ligand would be expected to direct the compositions to parenchymal hepatocytes, or alternatively the use of mannose containing sugar residues as a targeting ligand would be expected to direct the compositions to liver endothelial cells (e.g., mannose containing sugar residues that may bind preferentially to the asialoglycoprotein receptor or mannose receptor present in hepatocytes). (See Hillery AM, et al. "Drug Delivery and Targeting: For Pharmacists and Pharmaceutical Scientists" (2002) Taylor & Francis, Inc.) The presentation of such targeting ligands that have been conjugated to

moieties present in the transfer vehicle (e.g., a lipid nanoparticle) may facilitate recognition and uptake of the compositions in target cells and tissues. Examples of suitable targeting ligands include one or more peptides, proteins, aptamers, vitamins and oligonucleotides.

[0128] As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, to which the mRNAs and compositions of the present invention are administered. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

Pharmaceutical Composition and Administration

[0129] To facilitate expression of antibodies *in vivo*, antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) and delivery vehicles can be formulated in combination with one or more additional nucleic acids, carriers, targeting ligands or stabilizing reagents, or in pharmacological compositions where it is mixed with suitable excipients. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

[0130] Antibody encoding mRNAs and compositions containing the same may be administered and dosed in accordance with current medical practice, taking into account the clinical condition of the subject, the site and method of administration, the scheduling of administration, the subject's age, sex, body weight and other factors relevant to clinicians of ordinary skill in the art. The "effective amount" for the purposes herein may be determined by such relevant considerations as are known to those of ordinary skill in experimental clinical research, pharmacological, clinical and medical arts. In some embodiments, the amount administered is effective to achieve at least some stabilization, improvement or elimination of symptoms and other indicators as are selected as appropriate measures of disease progress, regression or improvement by those of skill in the art. For example, a suitable amount and dosing regimen is one that causes at least transient antibody production.

[0131] In one embodiment, the compositions are formulated such that they are suitable for extended-release of the mRNA contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. For example, in one embodiment, the compositions are administered to a subject twice day, daily or every other day. In a preferred embodiment, the compositions are administered to a subject twice a week, once a week, every ten days, every two weeks, every three weeks, or more preferably every four weeks, once a month, every six weeks, every eight weeks, every other month, every three months, every four months, every six months, every eight months, every nine months or annually. Also contemplated are compositions and liposomal vehicles which are formulated for depot administration to either deliver or release a mRNA over extended periods of time. Preferably, the extended-release means employed are combined with modifications made to the mRNA to enhance stability.

[0132] Also contemplated herein are lyophilized pharmaceutical compositions comprising one or more of the liposomal nanoparticles disclosed herein and related methods for the use of such lyophilized compositions as disclosed for example, in United States Provisional Application No. 61/494,882, filed June 8, 2011. For example, lyophilized pharmaceutical compositions according to the invention may be reconstituted prior to administration or can be reconstituted *in vivo*. For example, a lyophilized pharmaceutical composition can be formulated in an appropriate dosage form (e.g., an intradermal dosage form such as a disk, rod or membrane) and administered such that the dosage form is rehydrated over time *in vivo* by the individual's bodily fluids.

Expression of RNA Coded Antibodies in vitro

[0133] Antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) may be used to produce antibodies *in vitro*. For example, cells may be transfected by antibody encoding mRNAs (e.g., heavy chain and light chain encoding mRNAs) and cultured under cell culture conditions that allow the production of the antibody by the cells. Alternatively, the antibody is expressed intracellularly. Alternatively, the antibody is secreted by the cells such that the antibody may be harvested from the supernatant.

[0134] Mammalian cells may be used. Non-limiting examples of mammalian cells include BALB/c mouse myeloma line (NSO/1, ECACC No: 85110503); human retinoblasts (PER.C6, CruCell, Leiden, The Netherlands); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (HEK293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59,1977); human fibrosarcoma cell line (e.g., HT1080); baby hamster kidney cells (BHK21, ATCC CCL 10); Chinese hamster ovary cells +/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216, 1980); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251, 1980); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci., 383:44-68, 1982); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0135] Standard cell culture media and conditions may be used to cultivate transfected cells and produce desired antibodies encoded by mRNAs.

EXAMPLES

Example 1. Production of mRNA

[0136] Heavy chain anti-chemokine (C-C motif) ligand 2 (HC- α CCL2, SEQ ID NO: 1) and light chain anti-CCL2 (LC- α CCL2, SEQ ID NO: 2) were synthesized by *in vitro* transcription from a plasmid DNA template encoding the gene, which was followed by the addition of a 5' cap structure (Cap1) according to known methods (see Fechter, P.; Brownlee, G.G. "Recognition of mRNA cap structures by viral and cellular proteins" J. Gen. Virology 2005, 86, 1239-1249) and a 3' poly(A) tail of approximately 200 nucleotides in length as determined by gel electrophoresis. The sequences for HC- α CCL2 and LC- α CCL2 were as shown below, and 5' and 3' untranslated regions present in each mRNA product are represented as X and Y, respectively, and defined below:

[0137] *Heavy chain anti-CCL2 (HC- α CCL2) mRNA:*

X₁AUGGAAUUCGGCCUGAGCUGGCUGUUCCUGGUGGCCAUCCUGAAGGGCGUGCA
GUGCCAGGUCCAGCUGGUGCAGUCUGGCGCCGAAGUGAAGAAACCCGGCUCCUCC
GUGAAGGUGUCCUGCAAGGCCUCCGGCGGCACCUUUCUCCAGCUACGGCAUCUCCU
GGGUCCGACAGGCCCCAGGCCAGGGCCUGGAAUGGAUGGGCGGCAUCAUCCCCAU
CUUCGGCACCGCCAACUACGCCCAGAAAUUCAGGGCAGAGUGACCAUCACCGCC
GACGAGUCCACCUCACCGCCUACAUGGAACUGUCCUCCUUGCGGAGCGAGGACA
CCGCCGUGUACUACUGCGCCAGAUACGACGGCAUCUACGGCGAGCUGGACUUCUG
GGGCCAGGGCACCCUGGUCACCGUGUCCUCUGCCAAGACCACCCCCCUCCGUG
UACCCUCUGGGCCCCUGGCUCUGCCGCCAGACCAACUCUAUGGUCACCCUGGGCU
GCCUGGUCAAGGGCUACUCCCCGAGCCCGUGACCGUGACCUGGAACUCCGGCUC

CCUGUCCUCCGGCGUGCACACCUUCCCUGCCGUGCUGCAGUCCGACCUCUACACCC
UGUCCAGCAGCGUGACCGUGCCCUCCUCCACCUGGCCCUCGAGACAGUGACCUG

CAACGUGGGCCACCCCGCCUCCAGCACCAAGGUGGACAAGAAAAUCGUGCCCCGG
GACUGCGGCUGCAAGCCCUUGCAUCUGUACCGUGCCCGAGGUGUCCUCCGUGUUA
UCUUCCACCCAAGCCCAAGGACGUGCUGACCAUCACACUGACCCCCAAAGUGAC
CUGCGUGGUGGUGGACAUCUCCAAGGACGACCCCGAGGUGCAGUUCAGUUGGUUC
GUGGACGACGUGGAAGUGCACACCGCUCAGACCCAGCCCAGAGAGGAACAGUUA
ACUCCACCUUCAGAUCCGUGUCCGAGCUGCCCAUCAUGCACCAGGACUGGCUGAA
CGGCAAAGAAUUAAGUGCAGAGUGAACUCCGCCGCCUUCCCAGCCCCCAUCGAA
AAGACCAUCUCCAAGACCAAGGGCAGACCCAAGGCCCCCAGGUCUACACCAUCC
CCCCACCAAAGAACAGAUGGCCAAGGACAAGGUGUCCUUGACCUGCAUGAUCAC
CGAUUUCUUCCCAGAGGACAUCACCGUGGAAUGGCAGUGGAACGGCCAGCCCGCC
GAGAACUACAAGAACACCCAGCCCAUCAUGGACACCGACGGCUCCUACUUCGUGU
ACUCCAAGCUGAACGUGCAGAAGUCCAACUGGGAGGCCGGCAACACCUUCACCUG
UAGCGUGCUGCACGAGGGCCUGCACAACCACACACCGAGAAGUCCUGUCCAC
UCCCCCGGCAAGUGAY₁

[0138] *Light chain anti-CCL2 (LC- α CCL2) mRNA:*

X₁AUGGAAACCCCUGCCAGCUGCUGUUCCUGCUGCUGCUGUGGCUGCCUGAUAC
CACCGGCGAAAUCGUGCUGACCCAGUCCCCCGCCACCCUGUCUCUGAGCCUGGC
GAGAGAGCCACCCUGAGCUGCAGAGCCUCCAGUCCGUGUCCGACGCCUACCUGG
CCUGGUAUCAGCAGAAGCCCGGCCAGGCCCCUCGGCUGCUGAUCUACGACGCCUC
CUCUAGAGCCACCGGCGUGCCCGCCAGAUUCUCCGGCUCUGGCUCUGGCACCGAC
UUCACCCUGACCAUCUCCAGCCUGGAACCCGAGGACUUCGCCGUGUACUACUGCC
ACCAGUACAUCAGCUGCACAGCUUCACCUUCGGCCAGGGCACCAAGGUGGAAAU
CAAGGCCGAUGCCGCCCCUACCGUGUCCAUCUUCCCACCCUCCAGCGAGCAGCUG
ACCUUCUGGCGGCGCUUCCGUCGUGUGCUUCCUGAACAACUUCUACCCCAAGGACA
UCAACGUGAAGUGGAAGAUCGACGGCUCCGAGCGGCAGAACGGCGUGCUGAACUC
CUGGACCGACCAAGGACAGCACCUCUCCAUGUCCUCCACCCUGACC

CUGACCAAGGACGAGUACGAGCGGCACAACUCCUAUACCUGCGAGGCCACCCACA
AGACCUCCACCUCUCCCCCAUCGUGAAGUCCUUAACCGGAACGAGUGCUGAY₁

[0139] 5' and 3' UTR Sequences:

$X_I =$

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUCUCCCG
UGCCAAGAGUGACUCACCGUCCUUGACACG

$Y_I =$

CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCUGGAAGUUGCC
ACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU

Example 2. *In Vitro* mRNA Transfection Materials and Conditions

A. Exemplary Lipid Materials

[0140] The lipid formulations used for transfection in the examples herein consisted of one or more lipids or a multi-component lipid mixture of varying ratios employing one or more cationic lipids, helper lipids and PEGylated lipids designed to encapsulate various nucleic acid-based materials. Cationic lipids can include (but not exclusively) DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DODAP (1,2-dioleoyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA (see Heyes, J.; Palmer, L.; Bremner, K.; MacLachlan, I. "Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids" J. Contr. Rel. 2005, 107, 276-287), DLin-KC2-DMA (see Semple, S.C. et al. "Rational Design of Cationic Lipids for siRNA Delivery" Nature Biotech. 2010, 28, 172-176), C12-200 (Love, K.T. et al. "Lipid-like materials for low-dose in vivo gene silencing" PNAS 2010, 107, 1864-1869), HGT4003, ICE, dialkylamino-based, imidazole-based, guanidinium-based, etc. Helper lipids can include (but not exclusively) DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), cholesterol, etc. The PEGylated lipids can include (but not exclusively) a poly(ethylene) glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C₆-C₂₀ length.

B. Experimental Formulations

[0141] In these experiments, aliquots of 50 mg/mL ethanolic solutions of C12-200, DOPE,

cholesterol and DMG-PEG2K were mixed and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of HC- α CCL2 mRNA and LC- α CCL2 mRNA (1:1 wt:wt) mRNA was prepared by addition of 500 microgram of each construct from a 1 mg/mL stock. The lipid solution was injected rapidly into the aqueous mRNA solution and shaken to yield a final suspension in 20% ethanol. The resulting nanoparticle suspension was filtered, diafiltrated with 1x PBS (pH 7.4), concentrated and stored at 2-8°C. Final concentration = 1.35 mg/mL α CCL2 mRNA (encapsulated). Z_{ave} = 89.2 nm ($Dv_{(50)}$ = 64.0 nm; $Dv_{(90)}$ = 115 nm).

Example 3. Detection of Antibody After *In Vitro* mRNA Transfection

A. Via ELISA

[0142] In this example, F96 MaxiSorp Nunc-Immuno Plates were coated with 100 μ l of 1 μ g/ml of goat anti mouse IgG1 (Invitrogen A10538) in sodium carbonate buffer, pH 9.6 and incubated 1hr at 37°C. After washing 3x with wash buffer (1xPBS, 0.05% Tween 20), the wells were blocked with 320 μ l blocking buffer (1xPBS, 0.05% Tween 20, 2% BSA) for 1hr at 37°C. Serial dilutions of monoclonal IgG standards were prepared in blocking buffer in the range from 250-0 ng/ml. Serial dilutions of the samples were prepared in blocking buffer to be in the range of the standard curve (1:100 to 1:10,000). Both samples and standards were incubated 1hr at 37°C. After washing 3x with wash buffer, goat anti mouse IgG Fc HRP conjugated secondary antibody (Pierce 31439) was used at 1:40,000 dilution and incubated at 37°C for 1hr. After washing 3x with wash buffer TMB EIA substrate reagent was prepared according to manufactures instructions. After 15 min incubation at 37°C, the reaction was stopped by adding 2N H₂SO₄ and the plate read at 450nm.

B. Via Western Blot

[0143] In this example, conditioned medium from transfected 293T cells or electroporated HCL2 cells were fractionated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane using a transfer apparatus according to the manufacturer's instructions (Invitrogen). After incubation with 5% non-fat dry milk in TBST (10mM Tris, pH 8.0, 150mM NaCl, 0.5% Tween 20) for 1hr, the membrane was washed three times with PBST and incubated with goat anti mouse IgG1 (Invitrogen A10538) for 1hr at RT. Membranes were washed three times in PBST and incubated with 1:5000 dilution of horseradish peroxidase conjugated anti-mouse secondary antibody (Promega W4021) for 1hr at RT. Blots were washed in PBST three more times and developed with the ECL system (Amersham Bioscience) according to manufacturer's instructions.

Example 4. *In Vitro* Analysis of α CCL2 Antibody Produced from Transfection of HC-

αCCL2 and LC-αCCL2

[0144] Both HC-αCCL2 and LC-αCCL2 mRNA were produced as described above in Example 1. Subsequently, in accordance with Example 2A and B, HC-αCCL2 and LC-αCCL2 mRNA was transfected into either HCL1 (i.e., human cell line 1) cells or HCL2 cells in various ratios (wt:wt) according to known methods.

[0145] The results of these studies in HCL1 cells and HCL2 cells are demonstrated in **Figures 1 and 2**, respectively. Various mixtures of α-CCL2 (chemokine (C-C motif) ligand 2) heavy chain and light chain mRNA constructs were mixed (wt:wt) and transfected into either HCL1 cells (**Figure 1**) or HCL2 cells (**Figure 2**). Cell supernatants were harvested at select time points post-transfection and analyzed for the presence of anti-mouse IgG using ELISA-based methods as described above in **Example 3A**.

[0146] As shown in **figures 1 and 2**, varying the ratio of heavy chain to light chain (wt:wt) produced a significant difference in protein production as determined via ELISA. While a 1:1 (wt:wt) mixture of heavy chain:light chain α-CCL2 mRNA provided strong signal in HCL2 cells, a 4:1 (wt:wt) ratio provided higher protein production in HCL1 cells. While there were differences among the varying ratios, strong protein production was observed for all ratios tested. Further, in both cases, 48 hr post-transfection (Day 2, or D2) gave the strongest signal of desired protein in this example.

[0147] To further confirm the presence of the exogenous mRNA-derived antibody, immunoblot (Western) techniques were employed for additional characterization (see **Figure 3**) of HCL1-derived samples. Heavy chain and light chain fragments were successfully detected in the supernatant of mRNA transfected HCL1 cells using western blot methods as described in example 3B. Samples were analyzed 24 and 48 hours post-transfection of various mixtures of α-CCL2 heavy chain and light chain mRNA constructs (wt:wt). Band intensities observed were reflective of the mRNA ratios employed in each example.

Example 5. *In Vivo* Analysis of αCCL2 Antibody Produced from Intravenously Administered**mRNA-Loaded Nanoparticles**

[0148] In this example, production of fully processed antibody was accomplished *in vivo* via delivery of exogenous messenger RNA, specifically, α-CCL2 heavy chain and light chain mRNA constructs (HC-αCCL2:LC-αCCL2 mRNA, 1:1 (wt:wt)) were encapsulated in cationic lipid nanoparticles as described in Example 2A and delivered to mice as a single bolus, intravenous injection.

[0149] Briefly, male CD-1 mice of approximately 6-8 weeks of age at the beginning of each experiment were used. Samples of encapsulated HC- α CCL2 mRNA and LC- α CCL2 mRNA (1:1 wt:wt) were introduced by a single bolus tail-vein injection of an equivalent total dose of 30 micrograms. Mice were sacrificed and perfused with saline at the designated time points.

[0150] The liver and spleen of each mouse was harvested, apportioned into three parts, and stored in either 10% neutral buffered formalin or snap-frozen and stored at -80°C for analysis.

[0151] All animals were euthanized by CO₂ asphyxiation at given time points post dose administration followed by thoracotomy and terminal cardiac blood collection. Whole blood (maximal obtainable volume) was collected via cardiac puncture on euthanized animals into serum separator tubes, allowed to clot at room temperature for at least 30 minutes, centrifuged at 22°C $\pm$ 5°C at 9300 g for 10 minutes, and the serum was extracted. For interim blood collections, approximately 40-50 μ L of whole blood was collected via facial vein puncture or tail snip. Samples collected from non-treatment animals were used as baseline levels for comparison to study animals.

[0152] For ELISA analysis of α CCL2 antibody production, F96 MaxiSorp Nunc-Immuno Plate were coated with 100ml of 1mg/ml of MCP-1 recombinant rabbit purified monoclonal antibody in carbonate buffer, pH 9.6 and incubated 1hr at 37°C. After washing 3x with wash buffer (1xPBS, 0.05% Tween 20), the wells were blocked with 320 ml blocking buffer (1xPBS, 0.05% Tween 20, 2% BSA) for 1hr at 37°C. Approximately 100ng/ml MCP-1 human or mouse recombinant protein was added to each well and incubated for 1hr at 37°C. After washing 3x with wash buffer, serial dilutions of the samples (1:5 to 1:200) were added and incubated for 1hr at 37°C. After washing 3x with wash buffer, goat anti-mouse IgG Fc HRP conjugated secondary antibody was used at 1:40,000 dilution and incubated at 37°C for 1hr. After washing 3x with wash buffer TMB EIA substrate reagent was prepared according to manufactures instructions. After 15 min incubation at 37°C, the reaction was stopped by adding 2N H₂SO₄ and the plate read at 450nm.

[0153] Serum levels of treated mice were monitored at select time points post-administration (6 hr, 24 hr, 48 hr, 72 hr). The levels of fully formed α -human CCL2 antibody present in mouse serum were quantified using ELISA-based methods (see **Figure 4**). A significant increase in the desired, exogenous α -CCL2 mRNA derived antibody can be observed within six hours post-administration with a peak after 24 hours.

Example 6. *In Vivo* α -VEGF Antibody Production

[0154] In this example, production of fully processed α -VEGF antibody was accomplished *in vivo* via delivery of exogenous messenger RNA.

[0155] The sequences for HC- α VEGF and LC- α VEGF are as shown below, and 5' and 3' untranslated regions present in each mRNA product are represented as X and Y, respectively, and defined below:

[0156] *Heavy chain anti-VEGF (HC- α VEGF) mRNA:*

X₁AUGGCAACUGGAUCAAGAACCUCUCCUCCUGCUCGCAUUCGGCCUGCUCUGUCUC
CCAUGGCUCCAAGAAGGAAGCGCGUUCUCCACUAUCCCCCUCUCGGAGGUUCAGC
UGGUCGAAAGCGGGGGCGGCCUCGUCCAGCCAGGUGGAUCCCUCGCGCUGAGCUG
CGCCGCGUCCGGAUACACUUUCACCAACUACGGCAUGAACUGGGUCCGCCAGGCG
CCGGGAAAGGGACUGGAAUGGGUCGGCUGGAUCAAUACCUACACUGGAGAGCCU
ACCUACGCCGCUGACUUUAAGAGGGCGGUUCACUUUCUCACUGGAUACUCCAAGU
CAACCGCUUACCUUCAGAUGAAUUCUCCUGCGCGCCGAGGAUACCGCAGUGUAUUA
CUGCGCCAAAUACCCGCAUUACUACGGCUCCAGCCACUGGUACUUUGACGUGUGG
GGUCAAGGAACCCUGGUGACUGUGUCGUCCGCUUCCACCAAGGGACCAAGCGUGU
UCCACUCGCCCCGAGCUCAAAAUCGACGUCGGGAGGUACUGCCGCACUGGGGUG
CUUGGUCAAGGACUACUUUCCAGAGCCGGUGACUGUUUCCUGGAACAGCGGAGCG
CUCACCUCGGGCGUGCACACCUUCCCUGCGGUGUUGCAGUCAUCUGGACUGUACU
CGCUGUCCAGCGUGGUCACGGUCCCGAGCUCGUCGUCGGGACCCAAACCUACAU
UUGCAAUGUCAACCACAAGCCAUCGAACACCAAGUCGACAAGAAGGUGGAACCG
AAGUCGUGCGACAAGACUCAUACGUGCCCACCGUGUCCGGCUCGGAACUGUUGG

GGGGCCCCUCCGUGUUCUUUUUCCCGCCAAAGCCUAAGGACACUCUCAUGAUCUC
ACGGACGCCAGAAGUGACCUGUGUGGUCGUGGAUGUGUCACAUGAGGAUCCGGA
AGUCAAAUUCACUGGUAUGUGGACGGGGUGGAAGUGCAUAAUGCCAAAACCAA
ACCUCGCGAGGAGCAGUACAACUCAACCUACCGGGUGGUGUCCGUGCUGACUGUG
CUGCACCAGGACUGGCUGAAUGGAAAGGAGUACAAAUGCAAGGUCAGCAACAAG
GCCCCUCCCGCCCCAAUCGAAAAGACGAUCUCGAAGGCCAAAGGUCAGCCGCGAG
AGCCUCAAGUGUACACUCUGCCGCGGUCAGAGAAAGAAUUGACUAAGAACCAAGU
UUCCCUACAUUGCCUGGUGAAGGGCUUCUACCCAGCGACAUCGCAGUGGAAUGG
GAGAGCAACGGACAGCCGGAACAACUAUAAGACCACCCCUCCUGUGUUGGACU
CGGAUGGUUCCUUCUCCUUUACAGCAAGCUGACCGUGGAUAAAUCGCGGUGGCA
GCAAGGAAAUGUGUUUUAUGCUCAGUCAUGCACGAGGCGCUGCACAACUACUAC
ACUCAGAAGUCCCUUGUCGUCGUCGCCAGGAAAAUAAY₁

[0157] *Light chain anti-VEGF (LC- α VEGF) mRNA:*

X₁AUGGCCACUGGAUCAAGAACCUCACUGCUCGCUUUUGGACUGCUUUGCCU
GCCUGGUUGCAAGAAGGAUCGGCUUUCUCCGACCAUCCACUCUCCGACAUAUCAA
AUGACGCAGUCCCCAUCGAGCCUCUCAGCAUCAGUGGGGGGAUCGCGUGACUAUCA
CUUGCUCGGCGAGCCAGGAUAUCAGCAAUUAACCUGAACUGGUAUCAGCAAAAGCC
UGGAAAGGCACCGAAGGUGCUGAUCUACUUCACCUCAAGCCUCCAUUCGGGUGUC
CCGUCCCGCUUCAGCGGCUCGCGCUCAGGCACUGACUUCACCCUGACUAUCUCCU
CGCUGCAACCGGAAGAUUUCGCCACUUAUACUACUGUCAGCAGUACUCCACCGUGCC
UUGGACGUUCGGACAGGGAACCAAGUUGAGAUUAAGCGGACGGUCGCGGCCCCC
UCCGUGUUUAUCUUUCCGCCUUCGGACGAGCAGCUGAAGUCGGGAACCGCCUCUG
UCGUGUGCCUCCUGAACAACUUCUACCCGCGGGAAGCCAAGGUGCAGUGGAAAGU
GGAUAACGCGCUUCAGAGCGGCAAUUCGCAAGAGUCCGUGACCGAAGAGGACUCG
AAGGACUCAACCUACUCCCUCAGCUCACCCUCACUUUGUCGAAGGCCGACUACG
AGAAGCACAAAGUCUACGCAUGCGAAGUCACCCACCAAGGGUCUGUCGAGCCCAGU
GACUAAAUCCUUCAAUAGGGGGGGAUGUUAY₁

[0158] *5' and 3' UTR Sequences:*

[0159] X_1 (5' UTR Sequence) =

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUCCTCCG
UGCCAAGAGUGACUCACCGUCCUUGACACG

[0160] Y_1 (3' UTR Sequence) =

CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUUGGAAGUUGCC
ACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU

[0161] α -VEGF heavy chain and light chain mRNA constructs (HC- α -VEGF:LC- α -VEGF mRNA, 1:1 (wt:wt)) were encapsulated in cationic lipid nanoparticles as described below:

[0162] In these experiments, aliquots of 50 mg/mL ethanolic solutions of cKK-E12, DOPE, cholesterol and DMG-PEG2K were mixed and diluted with ethanol to 3 mL final volume. Separately, an aqueous buffered solution (10 mM citrate/150 mM NaCl, pH 4.5) of HC- α VEGF mRNA and LC- α VEGF mRNA (1:1 wt:wt) mRNA was prepared by addition of 500 microgram of each construct from a 1 mg/mL stock. The lipid solution was injected rapidly into the aqueous mRNA solution and shaken to yield a final suspension in 20% ethanol. The resulting nanoparticle suspension was filtered, diafiltrated with 1x PBS (pH 7.4), concentrated and stored at 2-8°C. Final concentration = 0.20 mg/mL α VEGF mRNA (encapsulated). Z_{ave} = 81.0 nm (PDI = 0.16).

[0163] HC- α -VEGF and LC- α -VEGF mRNA loaded lipid nanoparticles were delivered to wild type mice either by a single intravenous tail vein injection or subcutaneous injection at a dosage of 1.0 mg/kg and the production of anti-VEGF antibody was monitored over time in serum via ELISA.

[0164] Briefly, male CD-1 mice of approximately 6-8 weeks of age at the beginning of each experiment were used. Samples of encapsulated HC- α -VEGF mRNA and LC- α -VEGF mRNA (1:1 wt:wt) were introduced by a single bolus tail-vein injection of an equivalent total dose of 1.0 mg/kg (-30 micrograms). Mice were sacrificed and perfused with saline at the designated time points (0.50 hour, 3 hours, six hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks post-administration).

[0165] All animals were euthanized by CO₂ asphyxiation at given time points post dose administration followed by thoracotomy and terminal cardiac blood collection. Whole blood (maximal obtainable volume) was collected via cardiac puncture on euthanized animals into serum separator tubes, allowed to clot at room temperature for at least 30 minutes, centrifuged at 22°C $\pm$ 5°C at 9300 g for 10 minutes, and the serum was extracted. For interim blood collections, approximately 40-50 μ L of whole blood was collected via facial vein puncture or tail snip. Samples collected from non-treatment animals were used as baseline levels for comparison to study animals

[0166] For ELISA analysis of α -VEGF antibody production, F96 MaxiSorp Nunc-Immuno Plate were coated with 100 microliters of 0.50 microgram/mL/well of recombinant human VEGF protein (Invitrogen #PHC9391) in coating buffer (50mM NaHCO₃, pH9.6). After washing 3x with wash buffer, wells were blocked using a blocking buffer (1X DPBS, 2% BSA, 0.05% Tween-20) for one hour at 37°C. Upon further washing as described above, mouse serum collected from injected mice were added to each well and rabbit anti-human IgG Fc HRP (Pierce #PA-28587) conjugated secondary antibody was used at 1:10,000 dilution and incubated at 37°C for 1hr. After washing 3x with wash buffer TMB EIA substrate reagent was prepared according to manufactures instructions. After 10 min incubation at 37°C, the reaction was stopped by adding 2N H₂SO₄ and the plate read at 450nm. Serum levels of treated mice were monitored at select time points post-administration (e.g., 0.5 hr, 3 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 8 days and 15 days). **Figure 5** depicts exemplary results illustrating α -VEGF antibody detected in serum of wild type mice after single dose of HC- α -VEGF mRNA and LC- α -VEGF mRNA loaded nanoparticles. A significant increase in the desired, exogenous α -VEGF mRNA derived antibody can be observed within six hours post-administration with a peak after 24 hours and continued out to 2 weeks after a single dose of α -VEGF mRNA. **Figure 6** depicts the same exemplary results as **Figure 5**, but plotted by specific mouse number. **Figure 7** shows a comparison of the levels of α -VEGF antibody present in the serum of mice injected either intravenously or subcutaneously after 24 hours.

[0167] This example provides further confirmation that mRNA based therapy can be used for effective *in vivo* antibody production.

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Patentkrav

1. Sammensætning omfattende et første mRNA, som koder for den tunge kæde af et antistof, og et andet mRNA som koder for den lette kæde af antistoffet i et molforhold i området fra 2:1 til 1:2 til anvendelse i terapi hos en patient, hvor
5 det første mRNA og det andet mRNA (i) hver omfatter en 5'-cap-struktur, en 3'-poly-A-hale, en 5'-ikke-translateret region og en 3'-ikke-translateret region og (ii) er indkapslet inde i liposomer, som omfatter et kationisk lipid, et neutralt lipid, et kolesterol-baseret lipid og et PEG-modificeret lipid og har en størrelse, som ikke er mere end 150 nm, hvor det første mRNA, som koder for den tunge kæde, og
10 det andet mRNA som koder for den lette kæde, er indkapslet i det samme liposom, hvor antistoffet er en tetramer, og hver tetramer er sammensat af to identiske par af polypeptidkæder, idet hvert par har en let kæde på omtrent 25 kD og en tung kæde på omtrent 50-70 kD, og hvor sammensætningen indgives intravenøst til patienten, og antistoffet fordeles systemisk i patienten.
15
2. Sammensætningen til anvendelse ifølge krav 1, hvor det første mRNA og det andet mRNA er i et molforhold på 1:1.
3. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående
20 krav, hvor liposomerne har en størrelse, som ikke er mere end ca. 100 nm eller 75 nm.
4. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor liposomerne har en størrelse i området fra 10 nm - 100 nm.
25
5. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor mRNA'erne er (i) modificeret for at forstærke stabilitet, hvor mRNA'erne er modificeret for at inkludere nukleosidanaloger, kemisk eller biologisk modificerede baser, interkalerede baser, modificerede phosphatgrupper
30 og/eller modificerede sukre eller (ii) kemisk umodificerede.
6. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor mRNA'erne er syntetiseret fra (i) de naturligt forekommende nukleotider adenin (A), guanin (G), thymin (T), cytosin (C) og uracil (D), eller

modificerede nukleotidanaloger valgt fra gruppen bestående af 1-methyladenin, 2-methyl-adenin, 2-methylthio-N-6-isopentenyl-adenin, N-6-methyl-adenin, N-6-isopentenyl-adenin, 2-thio-cytosin, 3-methyl-cytosin, 4-acetyl-cytosin, 5-methyl-cytosin, 2,6-diaminopurin, 1-methyl-guanin, 2-methyl-guanin, 2,2-dimethyl-guanin, 7-methylguanin, inosin, 1-methyl-inosin, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thiouracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoruracil, 5-brom-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyluracil, N-uracil-5-oxyeddikesyremethylester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyeddikesyremethylester, uracil-5-oxyeddikesyre (v), 1-methyl-pseudouracil, queosin, β -D-mannosyl-queosin, wybutoxosin og phosphoramidater, phosphorthioater, peptidnukleotider, methylphosphonater, 7-deazaguanosin, 5-methylcytosin og inosin.

15

7. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet er en IgG.

8. Sammensætningen til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet er valgt fra gruppen bestående af anti-CCL2, anti-lysyl oxidase-like-2 (LOXL2), anti-Flt-1, anti-TNF- α , anti-interleukin-2R α -receptor (CD25), anti-TGF β , anti-B-celle-aktiveringsfaktor, anti-alpha-4 integrin, anti-BAGE, anti- β -catenin/m, anti-Bcr-abl, anti-C5, anti-CA125, anti-CAMEL, anti-CAP-1, anti-CASP-8, anti-CD4, anti-CD19, anti-CD20, anti-CD22, anti-CD25, anti-CDC27/m, anti-CD 30, anti-CD33, anti-CD52, anti-CD56, anti-CD80, anti-CDK4/m, anti-CEA, anti-CT, anti-CTL4, anti-Cyp-B, anti-DAM, anti-EGFR, anti-ErbB3, anti-ELF2M, anti-EMMPRIN, anti-EpCam, anti-ETV6-AML1, anti-HER2, anti-G250, anti-GAGE, anti-GnT-V, anti-Gp100, anti-HAGE, anti-HER-2/neu, anti-HLA-A*0201-R170I, anti-IGF-IR, anti-IL-2R, anti-IL-5, anti-MC1R, anti-myosin/m, anti-MUC1, anti-MUM-1, -2, -3, anti-proteinase-3, anti-p190 minor bcr-abl, anti-Pml/RAR α , anti-PRAMS, anti-PSA, anti-PSM, anti-PSMA, anti-RAGE, anti-RANKL, anti-RUI eller RU2, anti-SAGE, anti-SART-1 eller anti-SART-3, anti-survivin, anti-TEL/AML1, anti-TPI/m, anti-TRP-1, anti-TRP-2, anti-TRP-2/INT2, anti-VEGF og anti-VEGF receptor.

35

DRAWINGS

IgG ELISA on cells transfected with CCL2 HC and LC mRNA

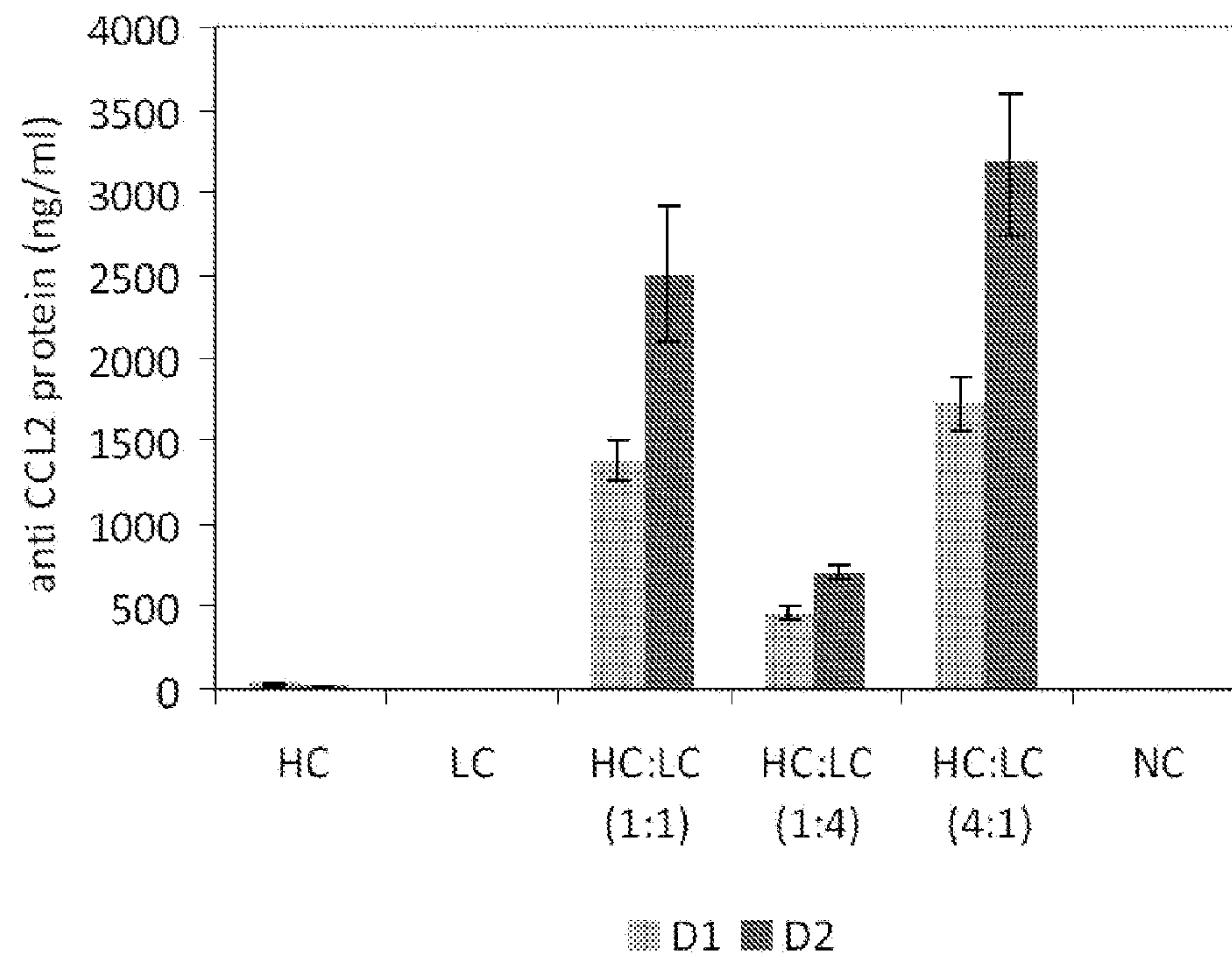


Figure 1

IgG ELISA on electroporated cells with anti-CCL2 HC and anti CCL2
LC mRNA

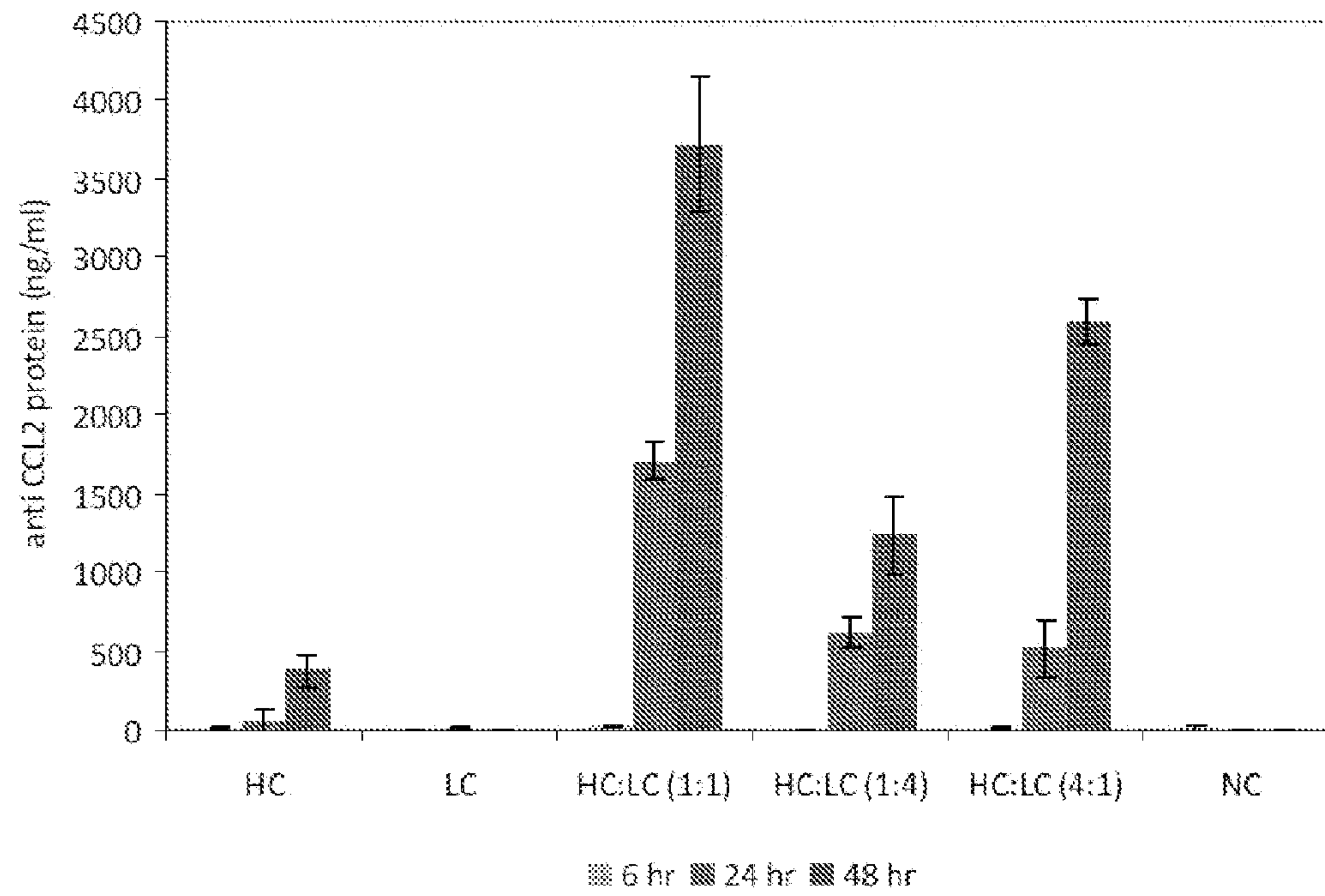


Figure 2

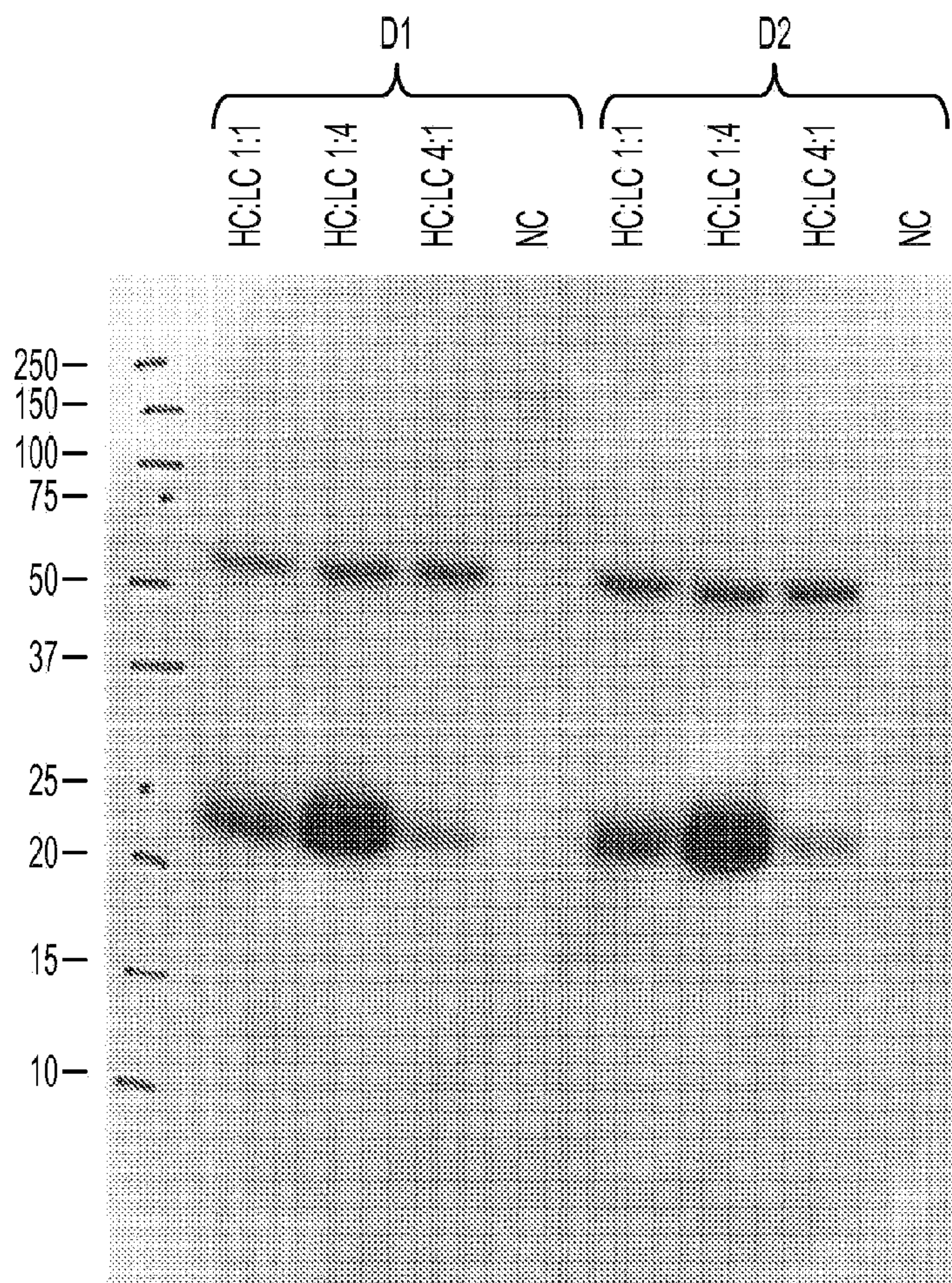


Figure 3

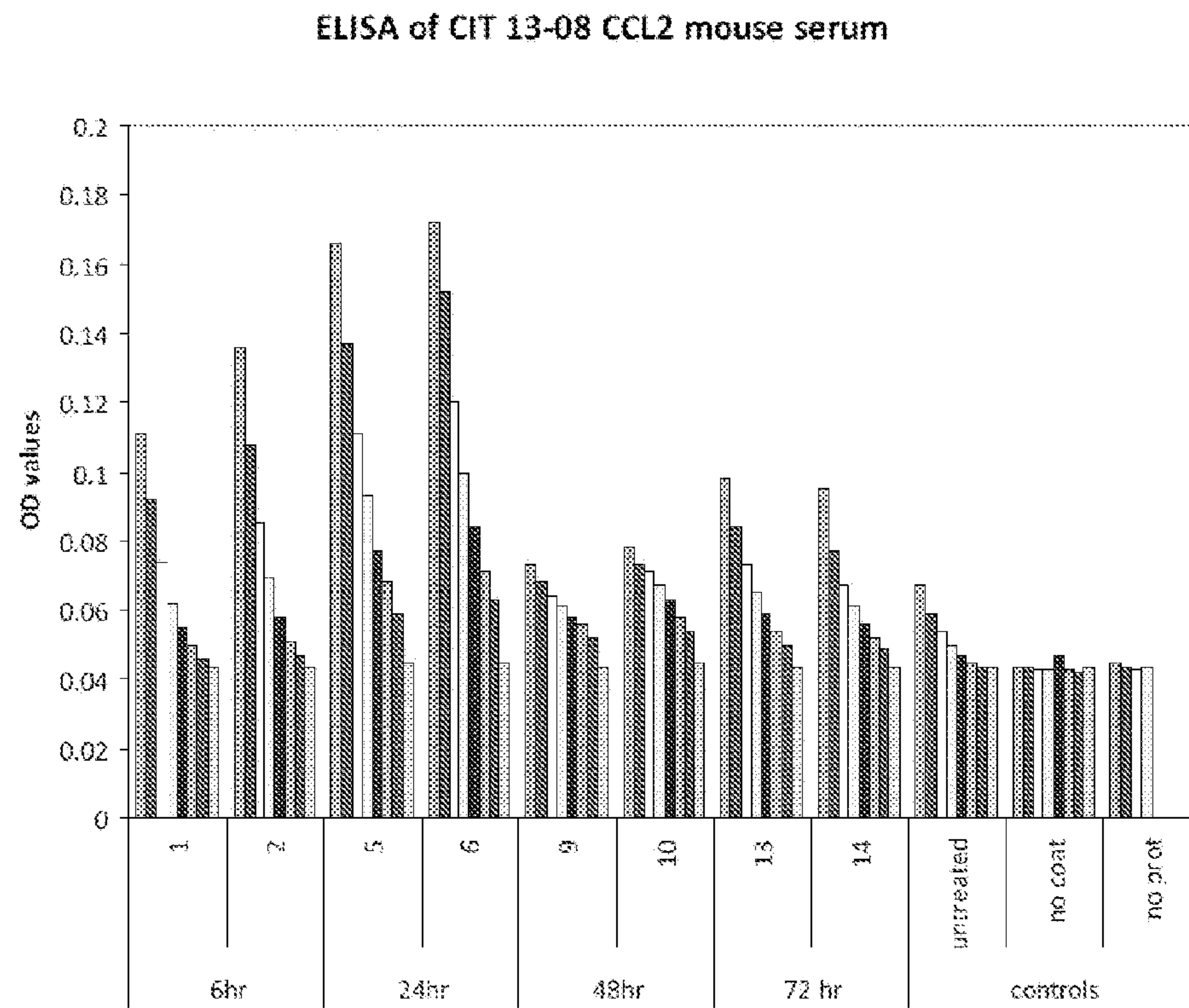


Figure 4

α -VEGF Antibody Detected in Serum of WT Mice After
Treatment of α -VEGF MRT

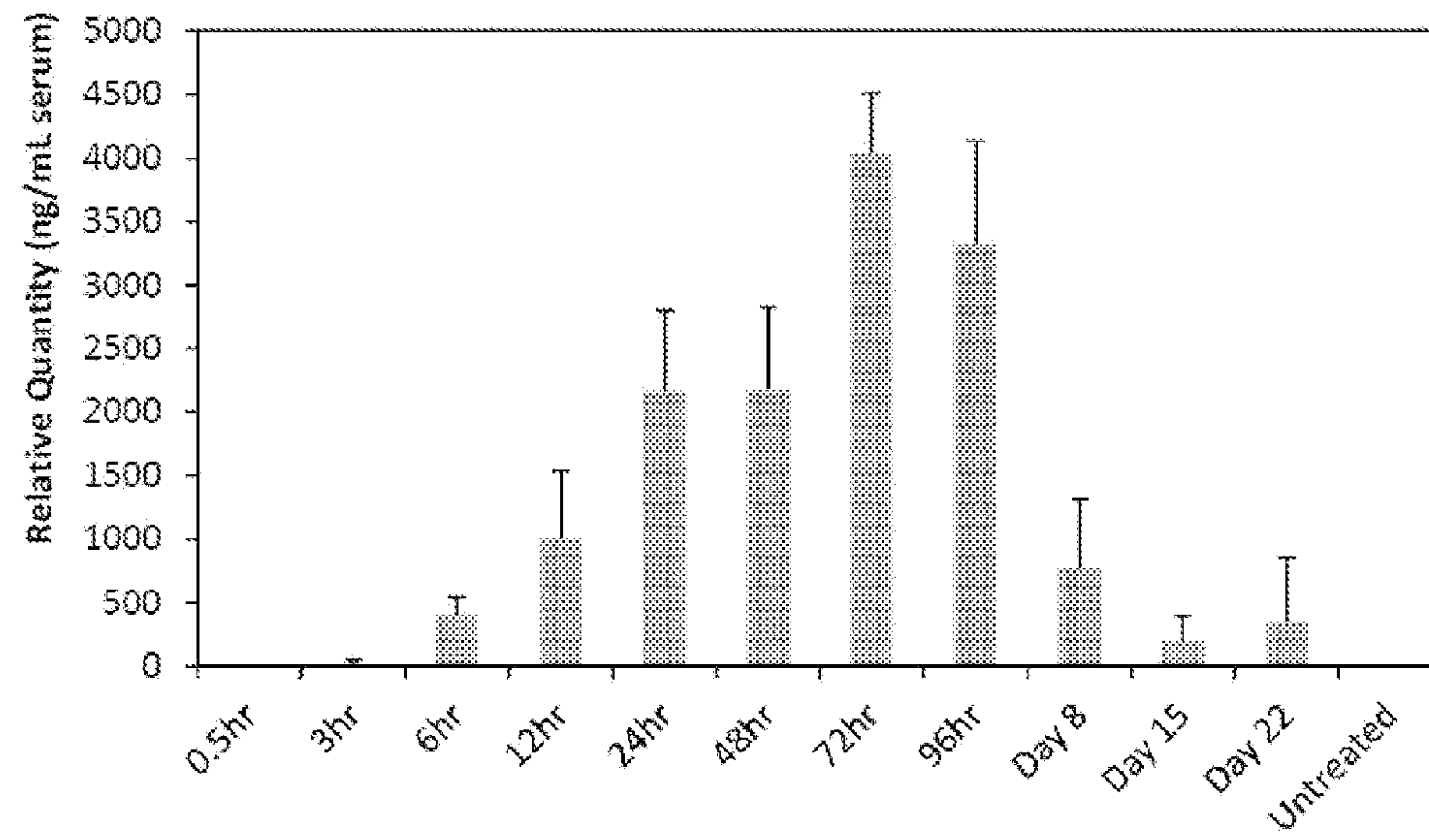


Figure 5

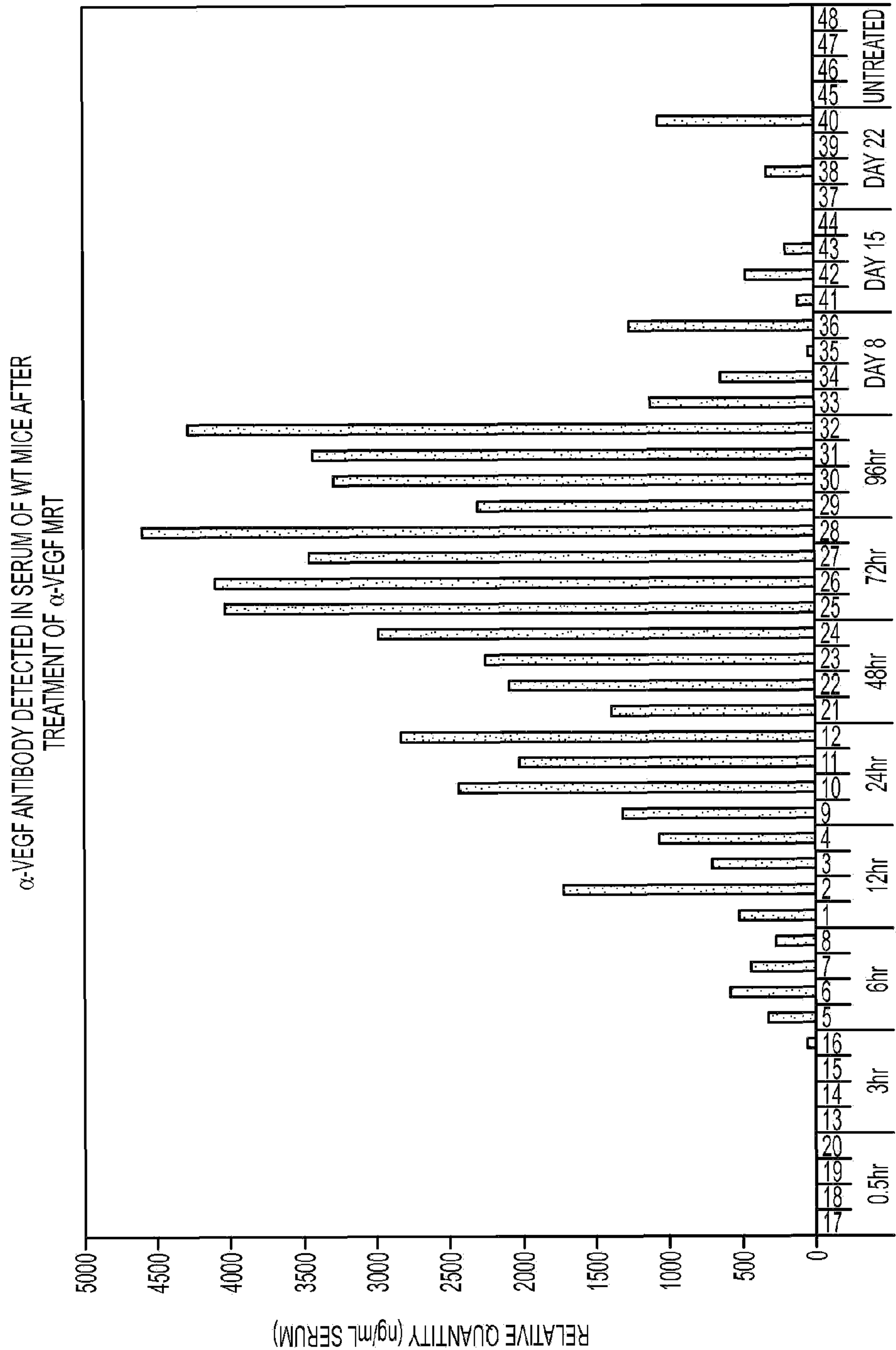


Figure 6

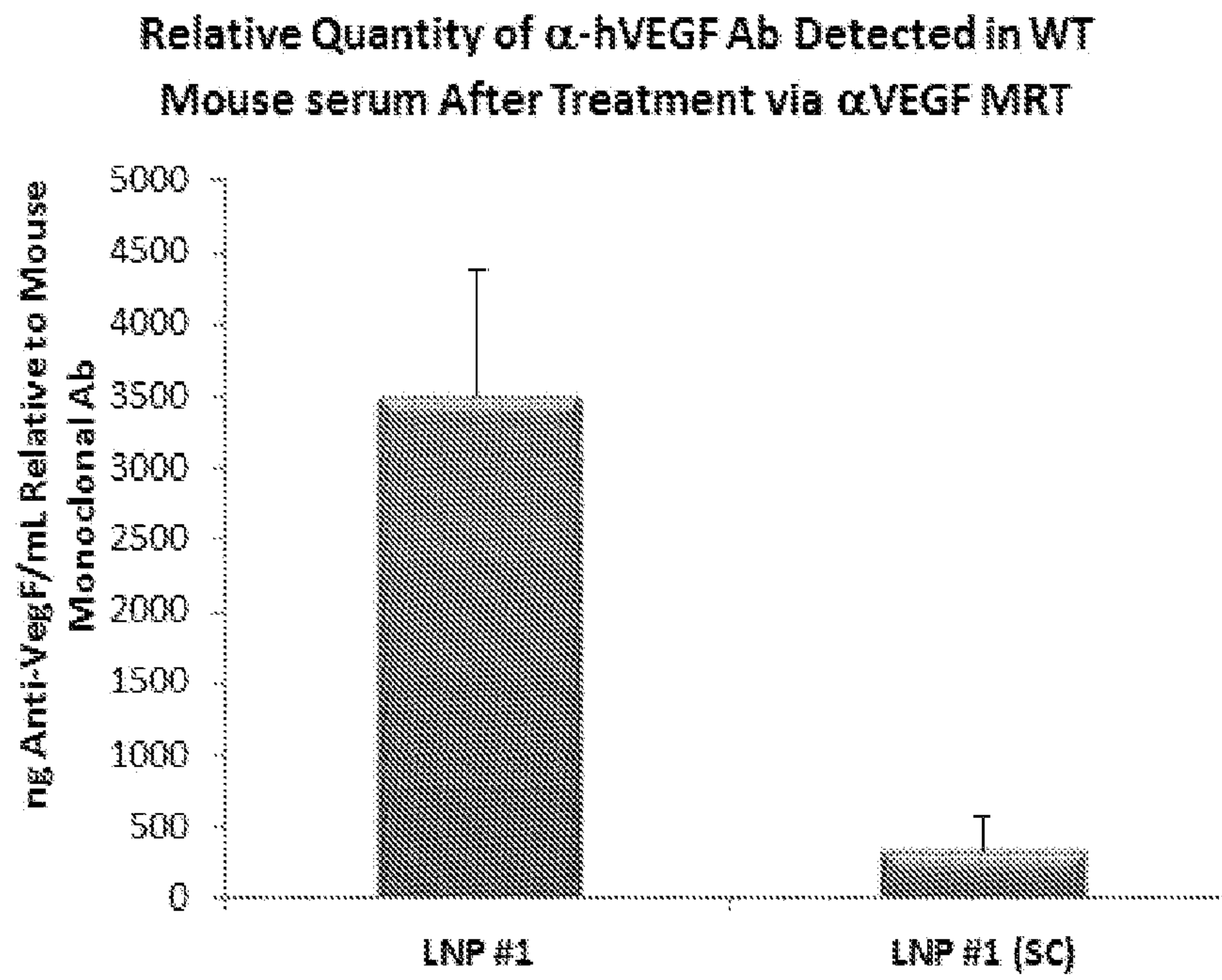


Figure 7