(19) DANMARK

(10) **DK/EP 3458083 T5**

Patent- og Varemærkestyrelsen

(12) Rettet oversættelse af europæisk patentskrift

(51) Int.Cl.: ***C 07 K 14/54 (2006.01)*** ***A 61 K 9/51 (2006.01)*** ***A 61 K 38/20 (2006.01)*** ***A 61 P 35/00 (2006.01)*** ***C 12 N 15/67 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2024-10-21**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2022-11-02**

(86) Europæisk ansøgning nr.: **17726458.7**

(86) Europæisk indleveringsdag: **2017-05-18**

(87) Den europæiske ansøgnings publiceringsdag: **2019-03-27**

(86) International ansøgning nr.: **US2017033422**

(87) Internationalt publikationsnr.: **WO2017201350**

(30) Prioritet: **2016-05-18 US 201662338483 P** **2017-01-07 US 201762443693 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **POLYNUKLEOTIDER, DER KODER FOR INTERLEUKIN-12 (IL12), OG ANVENDELSER HERAF**

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Description

BACKGROUND

[0001] Interleukin-12 (IL12) is a pro-inflammatory cytokine that plays an important role in innate and adaptive immunity. Gately, MK et al., Annu Rev Immunol. 16: 495-521 (1998). IL12 functions primarily as a 70 kDa heterodimeric protein consisting of two disulfide-linked p35 and p40 subunits. IL12 p40 homodimers do exist, but other than functioning as an antagonist that binds the IL12 receptor, they do not appear to mediate a biologic response. *Id.* The precursor form of the IL12 p40 subunit (NM_002187; P29460; also referred to as IL12B, natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2) is 328 amino acids in length, while its mature form is 306 amino acids long. The precursor form of the IL12 p35 subunit (NM_000882; P29459; also referred to as IL12A, natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1) is 219 amino acids in length and the mature form is 197 amino acids long. *Id.* The genes for the IL12 p35 and p40 subunits reside on different chromosomes and are regulated independently of each other. Gately, MK et al., Annu Rev Immunol. 16: 495-521 (1998). Many different immune cells (*e.g.*, dendritic cells, macrophages, monocytes, neutrophils, and B cells) produce IL12 upon antigenic stimuli. The active IL12 heterodimer is formed following protein synthesis. *Id.*

[0002] Due to its ability to activate both NK cells and cytotoxic T cells, IL12 protein has been studied as a promising anti-cancer therapeutic since 1994. *See* Nastala, C. L. et al., J Immunol 153: 1697-1706 (1994). But despite high expectations, early clinical studies did not yield satisfactory results. Lasek W. et *al.*, Cancer Immunol Immunother 63: 419-435, 424 (2014). Repeated administration of IL12, in most patients, led to adaptive response and a progressive decline of IL12-induced interferon gamma (IFN-γ) levels in blood. *Id.* Moreover, while it was recognized that IL12-induced anti-cancer activity is largely mediated by the secondary secretion of IFNγ, the concomitant induction of IFN-γ along with other cytokines (*e.g.*, TNF-α) or chemokines (IP-10 or MIG) by IL12 caused severe toxicity. *Id.*

[0003] In addition to the negative feedback and toxicity, the marginal efficacy of the IL12 therapy in clinical settings may be caused by the strong immunosuppressive environment in humans. *Id.* To minimize IFN-γ toxicity and improve IL12 efficacy, scientists tried different approaches, such as different dose and time protocols for IL12 therapy. See Sacco, S. et al., Blood 90: 4473-4479 (1997); Leonard, J. P. et al., Blood 90: 2541-2548 (1997); Coughlin, C. M. et al., Cancer Res. 57: 2460-2467 (1997); Asselin-Paturel, C. et al., Cancer 91: 113-122 (2001); and Saudemont, A. et al., Leukemia 16: 1637-1644 (2002). Nonetheless, these approaches have not significantly impacted patient survival. Kang, W. K., et al., Human Gene Therapy 12: 671-684 (2001).

[0004] Currently, a number of IL12 clinical trials are on-going. Though these multiple clinical trials have been on-going for nearly 20 years since the first human clinical trial of IL12 in 1996, an FDA-approved IL12 product is still not available. Thus, there is a need in the art for an improved therapeutic approach for using IL12 to treat tumors.

[0005] WO 2015/095249 A1 relates to single-chain IL12 polypeptides and isolated nucleic acids encoding the single-chain IL12 polypeptides. Methods of using the single-chain IL12 polypeptides to enhance immune system function are also discussed.

[0006] According to Charoensit et al., 2010. Cancer Gene Ther. 17(7):512-22, the results disclosed therein suggest that intravenous injection of all-trans-retinoic acid-cationic liposome/IL12 plasma DNA complexes is an effective method for the treatment of lung metastasis in mice.

[0007] Suzuki et al., 2010. J Control Release. 142(2):245-50 studied the role of bubble liposomes and ultrasound in cancer gene therapy using IL12 corded plasmid DNA.

[0008] WO 2013/053775 A1 relates to a combination medicament comprising a nucleic acid expression vector comprising a sequence encoding an IL12 polypeptide and a non-agonist CTLA-4 ligand or non-agonist PD-1 ligand for treatment of malignant neoplastic disease.

[0009] WO 2010/126766 A1 relates to a nucleotide sequence encoding a nuclear factor of activated T-cells promoter operatively associated with a nucleotide sequence encoding IL12 for use in the treatment or prevention of cancer.

[0010] WO 2001/52874 A2 describes the use of a nucleic acid molecule encoding an IL12 polypeptide for the preparation of a pharmaceutical composition for the treatment of a tumor.

[0011] Chen et al., 2016. Mol Ther Methods Clin Dev. 3: 16023 provides a review of the clinical development of nanoparticles for gene delivery.

[0012] Anwer et al., 2010. Gene Ther. 17(3):360-9 discloses a phase-I trial to assess the safety and tolerability of human IL12 plasmid formulated with a synthetic lipopolymer, i.e., polyethyleneglycol-polyethyleneimine-cholesterol.

BRIEF SUMMARY

[0013] The present invention provides a composition for use in a method of reducing the size of a tumor or inhibiting growth of a tumor in a subject in need thereof, wherein (a) the composition comprises an mRNA encoding an IL-12 polypeptide, wherein the mRNA comprises an open reading frame ("ORF") encoding an interleukin 12 p40 subunit

("IL12B") polypeptide and an interleukin 12 p35 subunit ("IL12A") polypeptide, (b) the mRNA is formulated as a lipid nanoparticle that comprises a cationic lipid, a phospholipid, a sterol, and a PEG-modified lipid, and (c) the method comprises administering to the subject an effective amount of said composition intratumorally.

[0014] Further, the present invention provides a composition for use in a method of (i) activating T cells in a subject in need thereof, (ii) increasing an effector to suppressor T cell ratio in a tumor of a subject in need thereof, (iii) increasing the number of activated Natural Killer (NK) cells in a subject in need thereof, and/or (vi) increasing cross-presenting dendritic cells in a tumor of a subject in need thereof, wherein (a) the composition comprises an mRNA encoding an IL-12 polypeptide, wherein the mRNA comprises an ORF encoding an IL12B polypeptide and an IL12A polypeptide, (b) the mRNA is formulated as a lipid nanoparticle that comprises a cationic lipid, a phospholipid, a sterol, and a PEG-modified lipid, and (c) the method comprises administering to the subject an effective amount of said composition intratumorally.

[0015] The present invention also provides a lipid nanoparticle comprising an mRNA encoding a human IL12 polypeptide, wherein the mRNA comprises an ORF encoding a human IL12B polypeptide operably linked to a human IL12A polypeptide, and the lipid nanoparticle comprises a cationic lipid, a phospholipid, a sterol, and a PEG-modified lipid.

[0016] Furthers aspects of the invention are provided in the claims.

[0017] The present disclosure provides mRNA therapeutics for the treatment of cancer. The mRNA therapeutics of the disclosure are particularly well-suited for the treatment of cancer as the technology provides for the intracellular delivery of mRNA encoding immune modulating polypeptides (for example, immune stimulating polypeptides, such as IL-12, and the like, useful in immuno-oncology ("IO")), followed by *de novo* synthesis of functional proteins within target cells, e.g., within target cells in tumors. The disclosure features therapeutic mRNAs having modified nucleotides to (1) minimize unwanted immune activation (e.g., the nnate immune response associated with *in vivo* introduction of foreign nucleic acids) and (2) optimize the translation efficiency of mRNA to protein. Exemplary aspects of the disclosure feature therapeutic mRNAs having a combination of nucleotide modifications to reduce the innate immune response and sequence optimization, in particular, within the open reading frame (ORF) of therapeutic mRNAs encoding immune modulating polypeptides (e.g., immune stimulating polypeptides such as IL-12) to enhance protein expression.

[0018] In other aspects, the mRNA therapeutic technology of the disclosure features delivery of mRNA(s) encoding immune modulating (e.g., immune stimulating) polypeptides via a lipid nanoparticle (LNP) delivery system. In exemplary cases, the mRNA therapeutic technology of the disclosure features delivery of mRNA(s) encoding immune modulating polypeptides into tumors via a lipid nanoparticle (LNP) delivery system. The disclosure also features novel ionizable lipid-based LNPs which have improved properties when combined with mRNA(s) encoding immune modulating (e.g., immune stimulating) polypeptides and administered *in vivo,* for example, cellular uptake, intracellular transport and/or endosomal release or endosomal escape. The LNP formulations of the disclosure also demonstrate reduced immunogenicity associated with the *in vivo* administration of LNPs.

[0019] Certain aspects of the present disclosure are directed to a method of reducing the size of a tumor or inhibiting growth of a tumor in a subject in need thereof comprising administering to the subject an effective amount of a composition comprising one or more polynucleotides encoding an IL-12 polypeptide, wherein the polynucleotide comprises an open reading frame ("ORF") encoding an interleukin 12 p40 subunit ("IL12B") polypeptide and an interleukin 12 p35 subunit ("IL12A") polypeptide. In some cases, the method further comprises administering to the subject an effective amount of a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or an effective amount of a composition comprising a checkpoint inhibitor polypeptide. In some cases, the checkpoint inhibitor polypeptide inhibits PD1, PD-L1, CTLA-4, or a combination thereof. In certain cases, the checkpoint inhibitor polypeptide comprises an antibody. In some cases, administering the composition activates T cells in the subject.

[0020] Another aspect of the present disclosure is directed to a method of activating T cells in a subject in need thereof comprising administering to the subject an effective amount of a composition comprising one or more polynucleotides encoding an IL-12 polypeptide, wherein the polynucleotide comprises an ORF encoding an IL12B polypeptide and an IL12A polypeptide. In some cases, the T cell activation comprises inducing T cell proliferation. In some cases, the T cell activation comprises inducing T cell infiltration in the tumor or increasing the number of tumor-infiltrating T cells. In some cases, the T cell activation comprises inducing a memory T cell response. In some cases, the activated T cells comprise $CD4^+$ T cells, $CD8^+$ T cells, or both. In certain cases, administering the composition alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide increases an effector to suppressor T cell ratio in the tumor. In some cases, administering the composition further increases the number of activated NK cells in the subject. In some cases, administering the composition increases cross-presenting dendritic cells in the tumor of the subject. In some cases, administering the composition reduces the size of a distal tumor or inhibits growth of a distal tumor in the subject.

[0021] Another aspect of the present disclosure is directed to a method of increasing an effector to suppressor T cell ratio in a tumor of a subject in need thereof comprising administering to the subject an effective amount of a composition comprising one or more polynucleotides encoding an IL-12 polypeptide, wherein the polynucleotide comprises an ORF encoding an IL12B polypeptide and an IL12A polypeptide. In some cases, the effector to suppressor T cell ratio is a

$CD8^+$ T cells: T regulatory (Treg) cells ratio.

[0022] Another aspect of the present disclosure is directed to a method of increasing the number of activated Natural Killer (NK) cells in a subject in need thereof comprising administering to the subject an effective amount of a composition comprising one or more polynucleotides encoding an IL-12 polypeptide, wherein the polynucleotide comprises an ORF encoding an IL12B polypeptide and an IL12A polypeptide.

[0023] Another aspect of the present disclosure is directed to a method of increasing cross-presenting dendritic cells in a tumor of a subject in need thereof comprising administering to the subject an effective amount of a composition comprising one or more polynucleotides encoding an IL-12 polypeptide, wherein the polynucleotide comprises an ORF encoding an IL12B polypeptide and an IL12A polypeptide. In some cases, the cross-presenting dendritic cells are $CD103^+$ cells.

[0024] Another aspect of the present disclosure is directed to a lipid nanoparticle comprising a polynucleotide encoding a human IL12 polypeptide, wherein the polynucleotide comprises an ORF encoding a human IL12B polypeptide operably linked to a human IL12A polypeptide.

[0025] In some cases, the IL12B polypeptide and the IL12A polypeptide are fused directly or by a nucleic acid encoding a linker. In some cases, the IL12B polypeptide comprises an amino acid sequence at least 80%, at least 90%, at least 95%, or at least 98% identical to amino acids 23 to 328 of SEQ ID NO: 48, wherein the amino acid sequence has IL12B activity. In some cases, the IL12A polypeptide comprises an amino acid sequence at least 80%, at least 90%, at least 95%, or at least 98% identical to amino acids 336 to 532 of SEQ ID NO: 48, wherein the amino acid sequence has IL12A activity. In some cases, the polynucleotide comprises a nucleotide sequence encoding a signal peptide. In some cases, the signal peptide is an IL12B signal peptide.

[0026] In some cases, the composition comprises a polynucleotide comprising an ORF encoding an IL12B polypeptide operably linked via a linker to an IL12A polypeptide. In some cases, the composition comprises a polynucleotide comprising an ORF encoding an IL12B signal peptide, an IL12B polypeptide, a linker and an IL12A polypeptide. In some cases, the linker comprises a Gly/Ser linker.

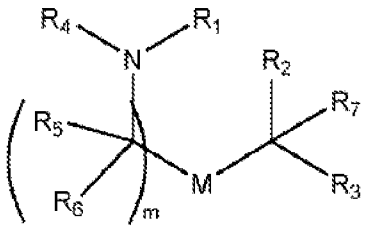
[0027] In some cases, the IL12 polypeptide comprises an amino acid sequence at least 80%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 48. the polynucleotide comprises a nucleotide sequence at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 44, 236, and 237. In some cases, the polynucleotide comprises a nucleotide sequence at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO: 236 or 237.

[0028] In some cases, the polynucleotide comprises an ORF comprising at least one chemically modified nucleoside. In some cases, the at least one chemically modified nucleoside is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5-methoxyuridine, and a combination thereof. In some cases, the chemically modified nucleosides in the ORF are selected from the group consisting of uridine, adenine, cytosine, guanine, and any combination thereof.

[0029] In some cases, the polynucleotide comprises a miRNA binding site. In some cases, the miRNA binding site is a miR-122 binding site. In some cases, the miRNA binding site is a miR-122-3p or miR-122-5p binding site. In certain cases, the miRNA binding site comprises a nucleotide sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% identical to aacgccauua ucacacuaaa ua (SEQ ID NO: 51), wherein the miRNA binding site binds to miR-122. In cerain cases, the miRNA binding site comprises a nucleotide sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% identical to uggaguguga caaugguguu ug (SEQ ID NO: 53), wherein the miRNA binding site binds to miR-122. In certain cases, the miRNA binding site comprises a nucleotide sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% identical to caaacaccau ugucacacuc ca (SEQ ID NO: 54), wherein the miRNA binding site binds to miR-122.

[0030] In some cases, the polynucleotide comprises a 5' untranslated region (UTR). In certain cases, the 5' UTR comprises a nucleic acid sequence at least about 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence listed in Table 3. In some cases, the polynucleotide comprises a 3' untranslated region (UTR). In certain cases, the 3' UTR comprises a nucleic acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence listed in Table 4A or 4B. In some cases, the polynucleotide comprises a miRNA binding site within the 3' UTR. In some cases, the polynucleotide comprises a nucleotide spacer sequence fused to the miRNA binding site. In some cases, the polynucleotide comprises a 5' terminal cap structure. In some cases, the polynucleotide comprises a 3' polyA tail. In some cases, the polynucleotide comprises a codon optimized ORF. In certain cases, the polynucleotide is in vitro transcribed (IVT) polynucleotide. In certain cases, the polynucleotide is circular.

[0031] In certain aspects, the polynucleotide is formulated with a delivery agent. In some cases, the delivery agent comprises a lipidoid, a liposome, a lipoplex, a lipid nanoparticle, a polymeric compound, a peptide, a protein, a cell, a nanoparticle mimic, a nanotube, or a conjugate. In some cases, the delivery agent is a lipid nanoparticle. In some cases, the delivery agent comprises a compound having formula (I)

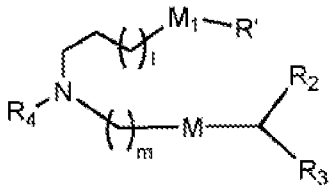


(I),

or a salt or stereoisomer thereof, wherein

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R'; R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2$- an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13; and provided when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

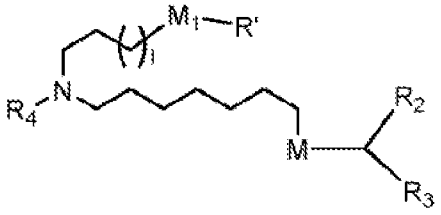
[0032] In some cases, the compound is of Formula (IA):



(IA),

or a salt or stereoisomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which n is 1, 2, 3, 4, or 5 and Q is OH, $-NHC(S)N(R)_2$, or $-NHC(O)N(R)_2$; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

[0033] In some cases, the compound is of Formula (II):

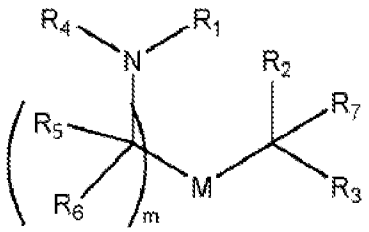


(II),

or a salt or stereoisomer thereof, wherein l is selected from 1, 2, 3, 4, and 5;
M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which n is 2, 3, or 4 and Q is OH, $-NHC(S)N(R)_2$, or

-NHC(O)N(R)$_2$; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

[0034] In some cases, the compound is selected from Compound 1 to Compound 147, and salts and stereoisomers thereof. In some cases, the delivery agent comprises a compound having the formula (I)



(I),

or a salt or stereoisomer thereof, wherein R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -R", and R"M'R'; R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, R*YR", YR", and R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is selected from the group consisting of a C_{3-6} carbocycle, $(CH_2)_nQ$, $-(CH_2)_nCHQR$, CHQR, $CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-(CH_2)_nN(R)_2$, -(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, -N(R)Rs, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, -N(OR)C(O)R, $-N(OR)S(O)_2R$, -N(OR)C(O)OR, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -(O)-, -C(S)-, -C(S)S-, -SC(S)-, -(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle; R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, R*YR", YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13; and provided that when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

[0035] In some cases, the delivery agent further comprises a phospholipid. In some cases, the phospholipid is selected from the group consisting of 1,2 dilinoleoyl sn glycero 3 phosphocholine (DLPC), 1,2 dimyristoyl sn glycero phosphocholine (DMPC), 1,2 dioleoyl sn glycero 3 phosphocholine (DOPC), 1,2 dipalmitoyl sn glycero 3 phosphocholine (DPPC), 1,2 distearoyl sn glycero 3 phosphocholine (DSPC), 1,2 diundecanoyl sn glycero phosphocholine (DUPC), 1 palmitoyl 2 oleoyl sn glycero 3 phosphocholine (POPC), 1,2 di O octadecenyl sn glycero 3 phosphocholine (18:0 Diether PC), 1 oleoyl 2 cholesterylhemisuccinoyl sn glycero 3 phosphocholine (OChemsPC), 1 hexadecyl sn glycero 3 phosphocholine (C16 Lyso PC), 1,2 dilinolenoyl sn glycero 3 phosphocholine, 1,2 diarachidonoyl sn glycero 3 phosphocholine, 1,2 didocosahexaenoyl sn glycero 3 phosphocholine, 1,2 dioleoyl sn glycero 3 phosphoethanolamine (DOPE), 1,2 diphytanoyl sn glycero 3 phosphoethanolamine (ME 16:0 PE), 1,2 distearoyl sn glycero 3 phosphoethanolamine, 1,2 dilinoleoyl sn glycero 3 phosphoethanolamine, 1,2 dilinolenoyl sn glycero 3 phosphoethanolamine, 1,2 diarachidonoyl sn glycero 3 phosphoethanolamine, 1,2 didocosahexaenoyl sn glycero 3 phosphoethanolamine, 1,2 dioleoyl sn glycero 3 phospho rac (1 glycerol) sodium salt (DOPG), sphingomyelin, and mixtures thereof. In some cases, the phospholipid is selected from the group consisting of 1 myristoyl 2 palmitoyl sn glycero 3 phosphocholine (14:0-16:0 PC, MPPC), 1 myristoyl 2 stearoyl sn glycero 3 phosphocholine (14:0-18:0 PC, MSPC), 1 palmitoyl 2 acetyl sn glycero 3 phosphocholine (16:0-02:0 PC), 1 palmitoyl 2 myristoyl sn glycero 3 phosphocholine (16:0-14:0 PC, PMPC), 1 palmitoyl 2 stearoyl sn glycero 3 phosphocholine (16:0-18:0 PC, PSPC), 1 palmitoyl 2 oleoyl sn glycero 3 phosphocholine (16:0-18:1 PC, POPC), 1 palmitoyl 2 linoleoyl sn glycero 3 phosphocholine (16:0-18:2 PC, PLPC), 1 palmitoyl 2 arachidonoyl sn glycero 3 phosphocholine (16:0-20:4 PC), 1 palmitoyl 2 docosahexaenoyl sn glycero 3 phosphocholine (14:0-22:6 PC), 1 stearoyl 2 myristoyl sn glycero 3 phosphocholine (18:0-14:0 PC, SMPC), 1 stearoyl 2 palmitoyl sn glycero 3 phosphocholine (18:0-16:0 PC, SPPC), 1 stearoyl 2 oleoyl sn glycero 3 phosphocholine (18:0-18:1 PC, SOPC), 1 stearoyl 2 linoleoyl sn glycero 3 phosphocholine (18:0-18:2 PC), 1 stearoyl 2 arachidonoyl sn glycero 3 phosphocholine (18:0-20:4 PC), 1 stearoyl 2 docosahexaenoyl sn glycero 3 phosphocholine (18:0-22:6 PC), 1 oleoyl 2 myristoyl sn glycero 3 phosphocholine

(18:1-14:0 PC, OMPC), 1 oleoyl 2 palmitoyl sn glycero 3 phosphocholine (18:1-16:0 PC, OPPC), 1 oleoyl 2 stearoyl sn glycero 3 phosphocholine (18:1-18:0 PC, OSPC), 1 palmitoyl 2 oleoyl sn glycero 3 phosphoethanolamine (16:0-18:1 PE, POPE), 1 palmitoyl 2 linoleoyl sn glycero 3 phosphoethanolamine (16:0-18:2 PE), 1 palmitoyl 2 arachidonoyl sn glycero 3 phosphoethanolamine (16:0-20:4 PE), 1 palmitoyl 2 docosahexaenoyl sn glycero 3 phosphoethanolamine (16:0-22:6 PE), 1 stearoyl 2 oleoyl sn glycero 3 phosphoethanolamine (18:0-18:1 PE), 1 stearoyl 2 linoleoyl sn glycero 3 phosphoethanolamine (18:0-18:2 PE), 1 stearoyl 2 arachidonoyl sn glycero 3 phosphoethanolamine (18:0-20:4 PE), 1 stearoyl 2 docosahexaenoyl sn glycero 3 phosphoethanolamine (18:0-22:6 PE), 1 oleoyl 2 cholesterylhemisuccinoyl sn glycero 3 phosphocholine (OChemsPC), and any combination thereof.

[0036] In some cases, the delivery agent further comprises a structural lipid. In some cases, the delivery agent further comprises a PEG lipid. In some cases, the delivery agent further comprises an ionizable lipid selected from the group consisting of 3 (didodecylamino) N1,N1,4 tridodecyl 1 piperazineethanamine (KL10), N1 [2 (didodecylamino)ethyl] N1,N4,N4 tridodecyl 1,4 piperazinediethanamine (KL22), 14,25 ditridecyl 15,18,21,24-tetraaza-octatriacontane (KL25), 1,2 dilinoleyloxy N,N dimethylaminopropane (DLin-DMA), 2,2 dilinoleyl 4 dimethylaminomethyl [1,3] dioxolane (DLin-K-DMA), heptatriaconta 6,9,28,31 tetraen 19 yl 4 (dimethylamino)butanoate (DLin-MC3-DMA), 2,2 dilinoleyl 4 (2 dimethylaminoethyl) [1,3] dioxolane (DLin-KC2-DMA), 1,2 dioleyloxy N,N dimethylaminopropane (DODMA), 2 ({8 [(3β) cholest 5 en 3 yloxy]octyl}oxy) N,N dimethyl 3 [(9Z,12Z) octadeca 9,12 dien 1 yloxy]propan 1 amine (Octyl-CLinDMA), (2R) 2 ({8 [(3β) cholest 5 en 3 yloxy]octyl}oxy) N,N dimethyl 3 [(9Z,12Z) octadeca 9,12 dien 1 yloxy]propan 1 amine (Octyl-CLinDMA (2R)), and (2S) 2 ({8 [(3β) cholest 5 en 3 yloxy]octyl}oxy) N,N dimethyl 3 [(9Z,12Z) octadeca 9,12 dien 1 yloxy]propan 1 amine (Octyl-CLinDMA (2S)).

[0037] In some cases, the delivery agent further comprises a quaternary amine compound. In certain cases, the quaternary amine compound is selected from the group consisting of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N-(1,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC),

[0038] 1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), 1,2-distearoyl-3-trimethylammonium-propane (DSTAP), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), 1,2-dilinoleoyl-3-trimethylammonium-propane (DLTAP), 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3-ethylphosphocholine (DSePC), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOePC), 1,2-di-(9Z-tetradecenoyl)-sn-glycero-3-ethylphosphocholine (14:1 EPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18:1 EPC), and any combination thereof.

[0039] In certain aspects, the composition is formulated for in vivo delivery. In some aspects, the composition is formulated for intramuscular, subcutaneous, intratumoral, or intradermal delivery.

[0040] In certain aspects, the administration treats a cancer. In some cases, the cancer is selected from the group consisting of adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer, breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, hepatocellular carcinoma (HCC), non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor, secondary cancers caused by cancer treatment, and any combination thereof.

[0041] In certain aspects, the composition is administered by a device comprising a pump, patch, drug reservoir, short needle device, single needle device, multiple needle device, micro-needle device, jet injection device, ballistic powder/particle delivery device, catheter, lumen, cryoprobe, cannula, microcanular, or devices utilizing heat, RF energy, electric current, or any combination thereof. In some aspects, the effective amount is between about 0.10 mg/kg to about 1,000 mg/kg.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0042]

FIG. 1 shows (1) the wild-type IL12B amino acid sequence, (2) the wild-type nucleic acid encoding the wtIL12B, (3) the wild-type IL12A amino acid sequence, (4) the wild-type nucleic acid encoding the wtIL12A, (5) the wild-type IL12B signal peptide amino acid sequence, and (6) the wild-type nucleic acid encoding the wtIL12B signal peptide.

FIGs. 2A-2B. FIG. 2A is a graph depicting the higher AUC and C_{max} for IL12 plasma levels observed following intravenous administration of IL12 mRNA in lipid nanoparticle (LNP) compared to the corresponding IL12 recombinant protein. **FIG. 2B** is a graph depicting the higher AUC and C_{max} for IFNγ plasma levels observed following intravenous administration of IL12 mRNA administered in lipid nanoparticle (LNP) compared to IL12 recombinant protein.

FIG. 3 is a graph depicting the robust efficacy of a single intravenous (IV) dose of IL12 mRNA in lipid nanoparticle (LNP), at doses of 0.1 mg/kg (Group 4) and 0.05 mg/kg (Group 5) (as indicated by lines with the inverted triangles), compared to Groups 1 (PBS), 2 (IL12 protein), 7 and 8 (controls NST-FIX, 0.1 mg/kg and 0.05 mg/kg, respectively).

FIGs. 4A-4F are graphs depicting the mean tumor volume and the number of complete responses (CR) seen following administration of a single intravenous (IV) dose of: IL12 mRNA in lipid nanoparticle (LNP), at doses of 0.1 mg/kg (Group 4) (**FIG. 4F**) and 0.05 mg/kg (Group 5) (**Fig. 4E**), PBS (Group 1) (**FIG. 4A**), IL12 protein (Group 2) (**FIG. 4D**), controls NST-FIX, 0.1 mg/kg and 0.05 mg/kg (Groups 7 and 8, respectively) (**FIG. 4C** and **FIG. 4B**, respectively). Complete responses (CRs) are shown in **FIGs. 4E** and **4F** only. **FIG. 4E** shows that 6 of 8 CRs were seen in Group 5 (IL12 mRNA in lipid nanoparticle (LNP), at a dose of 0.05 mg/kg). **FIG. 4F** shows that 5 of 9 CRs were seen in Group 4 (IL12 mRNA in lipid nanoparticle (LNP), at a dose of 0.1 mg/kg). Aside from the IL12 mRNA groups, all other groups did not observe any CRs.

FIG. 5 is a graph depicting the survival benefit at day 47 post tumor-implantation from a single intravenous (IV) dose of IL12 mRNA in lipid nanoparticle (LNP) at a dose of 0.05 mg/kg (Group 5) and a dose of 0.1 mg/kg (Group 4) compared to a single IV dose of IL12 protein at 1μg (~0.05 mg/kg) (Group 2), NST-FIX at 0.1 mg/kg (Group 7) or 0.05 mg/kg (Group 8), or PBS (Group 1).

FIGs. 6A-6B are graphs showing the *in vivo* anti-tumor efficacy of a single intratumoral dose of IL12 mRNA (4 μg) in a lipid nanoparticle (LNP) administered to mice bearing adenocarcinoma (MC38) tumors. **FIG. 6A** shows the tumor volume means (mm^3), up to day 24, starting at day 10 post implantation. Group 1 (circles) represents mice (n = 7) administered 4 μg IL12 mRNA LNP at day 10 post-implantation; Group 2 (squares) represents mice (n = 7) administered 4 μg of control mRNA encoding non-translated factor IX (NST-FIX LNP); and Group 3 (triangles) represents another control group of mice (n = 7) administered PBS. **FIG. 6B** shows the individual tumor volumes (mm^3) for each group of mice, up to day 47, starting at day 10 post implantation. Complete responses (CR) were achieved in 3 of 7 (44%) animals administered 4 μg IL12 mRNA LNP (circles).

FIGs. 7A-7B are graphs showing the *in vivo* anti-tumor efficacy of an intratumoral dose of IL12 mRNA (5 μg) in MC3-based lipid nanoparticle (LNP) administered to mice bearing A20 B-cell lymphoma tumors. **FIG. 7A** shows the individual tumor volume (mm^3) for mice (n=12) administered 5 μg non-translated control mRNA (NST). **FIG. 7B** shows the individual tumor volumes for mice (n=12) administered 5μg of IL12 (miRless) mRNA. Complete responses (CR) were achieved in 5 of 12 animals that received IL12 mRNA.

FIG. 7C is a graph showing comparable *in vivo* anti-tumor efficacy of IL12 mRNA (5 μg) containing a miR122 binding site (**FIG. 7C**) in a B-cell lymphoma tumor model (A20). Both IL12 mRNAs (with miR122 binding site and without (*i.e.,* miRless)) were formulated in an MC3-based lipid nanoparticle (LNP). The IL12 mRNAs were administered to mice bearing A20 B-cell lymphoma tumors. Complete responses (CR) were achieved in 6 out of 12 mice in the IL12 miR122 group (**FIG. 7C**).

FIGs. 8A-8B are graphs showing *in vivo* anti-tumor efficacy of a single dose of 0.5 μg IL12 mRNA in MC3-based lipid nanoparticle (LNP) administered to mice bearing A20 B-cell lymphoma tumors. Complete responses (CR) were achieved in 4 of 12 mice in the IL12 miRless (0.5 μg) group (**FIG. 8A**) and 3 of 12 mice in the IL12 miR122 (0.5 μg) group (**FIG. 8B**).

FIG. 8C is a graph showing enhanced *in vivo* anti-tumor efficacy in a B-cell lymphoma tumor model (A20) by administering multiple doses of 0.5 μg IL12 mRNA in MC3-based lipid nanoparticle (LNP) to mice bearing A20 tumors. Complete responses (CR) were achieved in 5 out of 12 mice administered *weekly dosing* of 0.5 μg IL12 miR122 for seven (7) days x 6.

FIG. 8D is a graph showing that the *in vivo* anti-tumor efficacy of weekly intratumoral doses of 0.5 μg IL12 mRNA in lipid nanoparticle (LNP) (*i.e.,* Compound 18) administered to mice bearing A20 B-cell lymphoma tumors is similar to the *in vivo* anti-tumor efficacy of 0.5 μg IL12 mRNA in *MC3*-based LNP. **FIG. 14** shows the individual tumor volumes for 12 mice administered 0.5 μg of IL12 mRNA in Compound 18-based LNP for 7 days x 6. Complete responses (CR) were also achieved in 5 out of 12 animals.

FIGs. 8E-8F are graphs showing tumor growth in mice bearing A20 tumors administered weekly dosing (7 days x

6) of 0.5 μg non-translated negative control mRNA (NST) in MC3-based lipid nanoparticle (LNP) (**FIG. 8E**) and 0.5 μg non-translated negative control mRNA (NST) in Compound 18-based LNP (**FIG. 8F**).

FIGs. 9A-9B are graphs showing dose-dependent levels of IL12 in plasma (**FIG. 9A**) and tumor (**FIG. 9B**) at 6 hours and 24 hours following intratumoral administration of the indicated doses of IL12 mRNA in MC3-based LNPs to mice bearing A20 tumors. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST, (iii) 2.5 μg NST, (iv) 5 μg NST, (v) 0.5 μg IL12, (vi) 2.5 μg IL12, (vii) 5 μg IL12, (viii) 0.5 μg IL12 miR122, (ix) 2.5 μg IL12 miR122, and (x) 5 μg IL12 miR122.

FIGs. 9C-9D are graphs showing elevated levels of IL12 in plasma and tumor following administration of indicated doses of IL12 mRNA in Compound 18-based LNPs compared to IL12 mRNA in MC3-based LNPs. **FIG. 9C** shows plasma IL12 levels at 6 hours and 24 hours; **FIG. 9D** shows tumor IL12 levels at 6 hours and 24 hours. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST in MC3, (iii) 2.5 μg NST in MC3, (iv) 0.5 μg IL12 miR122 in MC3, (v) 2.5 μg IL12 miR122 in MC3, (vi) 0.5 μg NST in Compound 18, (vii) 2.5 μg NST in Compound 18, (viii) 5 μg IL12 miR122, (ix) 0.5 μg IL12 miR122 in Compound 18, and (x) 2.5 μg IL12 miR122 in Compound 18.

FIGs. 9E-9F are graphs showing increased levels of IFNγ at 6 hours and 24 hours in plasma (**FIG. 9E**) and in tumor (**FIG. 9F**) following administration of IL12 mRNA to mice bearing A20 tumors. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST in MC3, (iii) 2.5 μg NST in MC3, (iv) 5 μg NST in MC3, (v) 0.5 μg IL12 in MC3, (vi) 2.5 μg IL12 in MC3, (vii) 5 μg IL12 in MC3, (viii) 0.5 μg IL12 miR122 in MC3, (ix) 2.5 μg IL12 miR122 in MC3, (x) 5 μg IL12 miR122 in MC3, (xi) 0.5 μg NST in Compound 18, (xii) 2.5 μg NST in Compound 18, (xiii) 0.5 μg IL12 miR122 in Compound 18, and (xiv) 2.5 μg IL12 miR122 in Compound 18.

FIGs. 9G-9H are graphs showing increased levels of IP10 at 6 hours and 24 hours in plasma (**FIG. 9G**) and in tumor (**FIG. 9H**) following administration of IL12 mRNA to mice bearing A20 tumors. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST in MC3, (iii) 2.5 μg NST in MC3, (iv) 5 μg NST in MC3, (v) 0.5 μg IL12 in MC3, (vi) 2.5 μg IL12 in MC3, (vii) 5 μg IL12 in MC3, (viii) 0.5 μg IL12 miR122 in MC3, (ix) 2.5 μg IL12 miR122 in MC3, (x) 5 μg IL12 miR122 in MC3, (xi) 0.5 μg NST in Compound 18, (xii) 2.5 μg NST in Compound 18, (xiii) 0.5 μg IL12 miR122 in Compound 18, and (xiv) 2.5 μg IL12 miR122 in Compound 18.

FIGs. 9I-9J are graphs showing decreased levels of IL6 at 6 hours and 24 hours in plasma (**FIG. 9I**) and in tumor (**FIG. 9J**) following administration of IL12 mRNA. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST in MC3, (iii) 2.5 μg NST in MC3, (iv) 5 μg NST in MC3, (v) 0.5 μg IL12 in MC3, (vi) 2.5 μg IL12 in MC3, (vii) 5 μg IL12 in MC3, (viii) 0.5 μg IL12 miR122 in MC3, (ix) 2.5 μg IL12 miR122 in MC3, (x) 5 μg IL12 miR122 in MC3, (xi) 0.5 μg NST in Compound 18, (xii) 2.5 μg NST in Compound 18, (xiii) 0.5 μg IL12 miR122 in Compound 18, and (xiv) 2.5 μg IL12 miR122 in Compound 18.

FIGs. 9K-9L are graphs showing decreased levels of G-CSF at 6 hours and 24 hours in plasma (**FIG. 9K**) and in tumor (**FIG. 9L**) following administration of IL12 mRNA. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST in MC3, (iii) 2.5 μg NST in MC3, (iv) 5 μg NST in MC3, (v) 0.5 μg IL12 in MC3, (vi) 2.5 μg IL12 in MC3, (vii) 5 μg IL12 in MC3, (viii) 0.5 μg IL12 miR122 in MC3, (ix) 2.5 μg IL12 miR122 in MC3, (x) 5 μg IL12 miR122 in MC3, (xi) 0.5 μg NST in Compound 18, (xii) 2.5 μg NST in Compound 18, (xiii) 0.5 μg IL12 miR122 in Compound 18, and (xiv) 2.5 μg IL12 miR122 in Compound 18.

FIGs. 9M-9N are graphs showing decreased levels of GROa at 6 hours and at 24 hours in plasma (**FIG. 9M**) and tumor (**FIG. 9N**) following administration of IL12 mRNA. From left to right, the mice were given (i) PBS, (ii) 0.5 μg NST in MC3, (iii) 2.5 μg NST in MC3, (iv) 5 μg NST in MC3, (v) 0.5 μg IL12 in MC3, (vi) 2.5 μg IL12 in MC3, (vii) 5 μg IL12 in MC3, (viii) 0.5 μg IL12 miR122 in MC3, (ix) 2.5 μg IL12 miR122 in MC3, (x) 5 μg IL12 miR122 in MC3, (xi) 0.5 μg NST in Compound 18, (xii) 2.5 μg NST in Compound 18, (xiii) 0.5 μg IL12 miR122 in Compound 18, and (xiv) 2.5 μg IL12 miR122 in Compound 18.

FIGs. 10A-10B are graphs showing individual tumor volumes through day 35 post disease induction with A20 tumor following treatment with IL12_miR122 mRNA (**FIG. 10B**) compared to negative control mRNA (**FIG. 10A**).

FIGs. 10C-10D are graphs showing body weight measurements of mice through day 35 post disease induction with A20 tumor following treatment with IL12_miR122 mRNA (**FIG. 10D**) compared to negative control mRNA (**FIG. 10C**).

FIG. 11A is a graph depicting bioluminescence (BL) as a surrogate for tumor burden at day 22 post disease induction with a luciferase-enabled MC38 colon cancer cell line in mice. From left to right, mice were administered no treatment, 2 μg mIL12_miRless, 2 μg mIL12_miR122, 2 μg NST_OX40L_122, 4 μg mIL12_miRless, 4 μg mIL12_miR122, 4μg NST_OX40L_122, and 1 μg rm IL12.

FIG. 11B is a Kaplan-Meier curve showing the percent survival of mice treated with LNPs carrying IL12 mRNA compared to NST-OX40L negative controls. The graph shows survival to day 150 post implantation with A20 tumor.

FIG. 12A shows uracil (U) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_001 to hIL12AB_020). FIG. 12B shows guanine (G) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_001 to hIL12AB_020). **FIG. 12C** shows cytosine (C) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_001 to hIL12AB_020). **FIG. 12D** shows guanine plus cytosine (G/C) metrics corresponding to wild type IL12B and 20 sequence optimized

IL12B polynucleotides (hIL12AB_001 to hIL12AB_020).

FIG. 13A shows uracil (U) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 13B** shows guanine (G) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 13C** shows cytosine (C) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 13D** shows guanine plus cytosine (G/C) metrics corresponding to wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_021 to hIL12AB_040).

FIG. 14A shows uracil (U) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_001 to hIL12AB_020). **FIG. 14B** shows guanine (G) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_001 to hIL12AB_020). **FIG. 14C** shows cytosine (C) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_001 to hIL12AB_020). **FIG. 14D** shows guanine plus cytosine (G/C) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_001 to hIL12AB_020).

FIG. 15A shows uracil (U) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 15B** shows guanine (G) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 15C** shows cytosine (C) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 15D** shows guanine plus cytosine (G/C) metrics corresponding to wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_021 to hIL12AB_040). The column labeled "G/C Content (%)" corresponds to $\%G/C_{TL}$.

FIG. 16A shows a comparison between the G/C compositional bias for codon positions 1, 2, 3 corresponding to the wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_001 to hIL12AB_020). **FIG. 16B** shows a comparison between the G/C compositional bias for codon positions 1, 2, 3 corresponding to the wild type IL12B and 20 sequence optimized IL12B polynucleotides (hIL12AB_021 to hIL12AB_040). **FIG. 16C** shows a comparison between the G/C compositional bias for codon positions 1, 2, 3 corresponding to the wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_0-1 to hIL12AB_020). **FIG. 16D** shows a comparison between the G/C compositional bias for codon positions 1, 2, 3 corresponding to the wild type IL12A and 20 sequence optimized IL12A polynucleotides (hIL12AB_021 to hIL12AB_040).

FIG. 17A is a graph showing dose-dependent levels of IL12 in plasma at 24 hours following intratumoral administration of the indicated doses of IL12 mRNA to mice bearing tumors. From left to right, the mice were given (i) no treatment, (ii) 5 μg NST, (iii) 0.05 μg IL12 miR122, (iv) 0.5 μg IL12 miR122, (v) 5 μg IL12 miR122, (vi) 5 μg NST, (vii) 0.5 μg IL12 miR122 (4 doses), (viii) 2.5 μg IL12 miR122 (4 doses), and (ix) 5 μg IL12 miR122 (4 doses).

FIG. 17B is a graph showing increased levels of IFNγ in plasma at 24 hours following intratumoral administration of IL12 mRNA to mice bearing tumors. From left to right, the mice were given (i) no treatment, (ii) 5 μg NST, (iii) 0.05 μg IL12 miR122, (iv) 0.5 μg IL12 miR122, (v) 5 μg IL12 miR122, (vi) 5 μg NST, (vii) 0.5 μg IL12 miR122 (4 doses), (viii) 2.5 μg IL12 miR122 (4 doses), and (ix) 5 μg IL12 miR122 (4 doses).

FIGs. 18A-18B are graphs showing increased levels of IL12 in plasma (**FIG. 18A**) and IFNγ in plasma (**FIG. 18B**) over the course of 200 hours following intraperitoneal administration of IL12 mRNA to mice bearing MC38 tumors. Mice were given (i) no treatment, (ii) 2 μg IL12 miRless, (iii) 2 μg IL12 miR122, (iv) 4 μg IL12 miRless, (v) 4 μg miR122, (vi) 1 μg IL12 protein, (vii) 2 μg NST_OX40L_122, or (viii) 4 μg NST_OX40L_122.

FIGs. 19A-19B are graphs showing individual tumor volumes through day 90 post disease induction with A20 tumor following treatment with 0.5 μg IL12_miR122 mRNA in a Compound 18-based lipid nanoparticle (LNP) (**FIG. 19B**) compared to negative control mRNA (**FIG. 19A**).

FIGs. 19C-19D are graphs showing individual tumor volumes through day 60 post disease induction with A20 tumor in naive mice (**FIG. 19C**) or in complete responder mice previously treated with IL12_miR122 mRNA and rechallenged (**FIG. 19D**).

FIGs. 20A-20D are graphs showing individual tumor volumes through 80 days following a single intratumoral dose of IL12 mRNA to mice bearing MC38-S tumors. Mice were given 0.05 μg IL12 mRNA (**FIG. 20A**), 0.5 μg IL12 mRNA (**FIG. 20B**), 5 μg IL12 mRNA (**FIG. 20C**), or NST (**FIG. 20D**).

FIGs. 21A-21F are graphs showing individual tumor volumes through 80 days following a single dose or multiple doses of IL12 mRNA to mice bearing MC38-S tumors. Mice were given a single dose of 0.05 μg IL12 mRNA (**FIG. 21A**), a single dose of 0.5 μg IL12 mRNA (**FIG. 21B**), a single dose of 5 μg IL12 mRNA (**FIG. 21C**), two doses of 0.05 μg IL12 mRNA (**FIG. 21D**), two doses of 0.5 μg IL12 mRNA (**FIG. 21E**), two doses of 5 μg IL12 mRNA (**FIG. 21F**).

FIG. 22 is a Kaplan-Meier curve showing the percent survival of mice treated with LNPs carrying IL12 mRNA compared to NST-FIX negative controls. The graph shows survival to day 80 post implantation with MC38-S tumor.

FIG. 23A-23D are graphs showing individual tumor volumes through 75 days following a single dose of IL12 mRNA to mice bearing MC38-R tumors. Mice were given 0.05 μg IL12 mRNA (**FIG. 23A**), 0.5 μg IL12 mRNA (**FIG. 23B**), 5 μg IL12 mRNA (**FIG. 23C**), or NST (**FIG. 23D**).

FIG. 23E-23J are graphs showing individual tumor volumes through 75 days following a single dose or multiple doses of IL12 mRNA to mice bearing MC38-R tumors. Mice were given a single dose of 0.05 μg IL12 mRNA (**FIG. 23E**), a single dose of 0.5 μg IL12 mRNA (**FIG. 23F**), a single dose of 5 μg IL12 mRNA (**FIG. 23G**), multiple doses of 0.05 μg IL12 mRNA (**FIG. 23H**), multiple doses of 0.5 μg IL12 mRNA (**FIG. 23I**), or multiple doses of 5 μg IL12 mRNA (**FIG. 23J**).

FIG. 24 is a Kaplan-Meier curve showing the percent survival of mice treated with LNPs carrying IL12 mRNA compared to NST-OX40L negative controls. The graph shows survival to day 80 post implantation with MC38-R tumor.

FIG. 25 is a graph showing depletion of CD8+ T cells over the course of 28 days.

FIGs. 26A-26E are graphs showing individual tumor volumes through 90 days following CD8+ T cell depletion and subsequent administration of a single dose of IL12 mRNA to mice bearing MC38-R tumors. Mice were given an antibody control for CD8+ T cell depletion and then 0.5 μg negative control mRNA (**FIG. 26A**), an antibody control for CD8+ T cell depletion and then 0.5 μg IL12 mRNA (**FIG. 26B**), CD8+ T cell-depleting antibody clone 24.3 and then 0.5 μg negative control mRNA (**FIG. 26C**), or CD8+ T cell-depleting antibody clone 24.3 and then 0.5 μg IL12 mRNA (**FIG. 26D**). **FIG. 26E** is a Kaplan-Meier curve showing the percent survival of mice treated with IL12 mRNA absent CD8+ T cell depletion compared to mice treated with IL12 mRNA after CD8+ T cell depletion. The graph shows survival to day 90 post implantation with MC38-R tumor.

FIGs. 27A-27B are graphs showing the percent of CD11b+ myeloid cells staining positive for PDL1 expression in MC38-R (**FIG. 27A**) and B16F10-AP3 (**FIG. 27B**) tumors 24 hours or 72 hours after no treatment, treatment with an NST negative control or two different intratumoral doses of IL12 mRNA. Statistical significance is indicated by asterisks.

FIGs. 28A-28B are graphs showing CD8+ T cells in tumors as a proportion of the immune infiltrate (CD45+ cells) (left panels) and per mg of tumor (right panels) in MC38-R (**FIG. 28A**) and B16F10-AP3 (**FIG. 28B**) tumors 7 days after no treatment, treatment with an NST negative control or two different doses of IL12 mRNA. Statistical significance is indicated by asterisks.

FIGs. 29A-29B are graphs showing ratio of CD8+ T cells to Treg cells in MC38-R (**FIG. 29A**) and B16F10-AP3 (**FIG. 29B**) tumors 7 days after no treatment, treatment with an NST negative control or two different doses of IL12 mRNA. Statistical significance is indicated by asterisks.

FIGs. 30A-30B are graphs showing the percent of cells CD8+ T cells staining positive for the early activation marker CD69 in MC38-R (**FIG. 30A**) and B16F10-AP3 (**FIG. 30B**) tumors 24 hours after no treatment, treatment with an NST negative control or two different doses of IL12 mRNA. Statistical significance is indicated by asterisks.

FIGs. 31A-31B are graphs showing the percent of NK cells staining positive for the early activation marker CD69 in MC38-R (**FIG. 31A**) and B16F10-AP3 (**FIG. 31B**) tumors 24 hours or 72 hours after no treatment, treatment with an NST negative control or two different doses ofIL12 mRNA. Statistical significance is indicated by asterisks.

FIGs. 32A-32B are graphs showing the number of CD103+ classical dendritic cells per mg of MC38-R (**FIG. 32A**) and B16F10-AP3 (**FIG. 32B**) tumors 7 days after no treatment, treatment with an NST negative control or two different doses of IL12 mRNA. **FIG. 32C** is a graph showing the percent of CD8+ classical dendritic cells staining positive for CD86 in the tumor draining lymph node (LN) of a B16F10-AP3 tumor 24 hours, 72 hours, or 7 days after no treatment, treatment with an NST negative control or two different dose of IL12 mRNA. Statistical significance is indicated by asterisks.

FIGs. 33A-33B are graphs showing individual tumor volumes through 55 days following administration of anti-PD-L1 antibody to mice bearing MC38-R tumors. Mice were given an antibody control (**FIG. 33A**) or an anti-PD-L1 antibody (clone 80) (**FIG.** 33B). **FIGs. 33C-33G** are graphs showing individual tumor volumes in mice bearing MC38-R tumors through 90 days following administration of IL12 mRNA alone or in combination with an anti-PD-L1 antibody. Mice were given (i) a single iTu dose of 0.5 μg IL12 mRNA as a monotherapy (**FIG. 33C**), (ii) a single iTu dose of 5.0 μg IL12 miR122 as a monotherapy (**FIG. 33D**), (iii) a single iTu dose of 0.5 μg IL12 miR122 in combination with multiple intraperitoneal doses of anti-PD-L1 antibody (**FIG. 33E**), (iv) a single iTu dose of 5.0 μg IL12 mRNA in combination with multiple intraperitoneal doses of anti-PD-L1 antibody (**FIG. 33F**); or (v) multiple intraperitoneal doses of anti-PD-L1 antibody (**FIG. 33G**).

FIGs. 34A-34C are graphs showing individual tumor volumes through 75 days. Mice bearing MC38-R tumors were treated 10 days post implant with an anti-PD-L1 antibody alone (**FIG. 34A**), 0.5 μg IL12 mRNA alone (**FIG. 34B**), or both an anti-PD-L1 antibody and 0.5 μg IL12 mRNA (**FIG. 34C**). The anti-PD-L1 antibody was administered over 6 doses. Vertical dashed lines indicate dose days.

FIGs. 35A-35D are graphs showing individual tumor volumes through 70 days. Mice bearing B16F10-AP3 tumors were treated 10 days post implant with a negative control (**FIG. 35A**), an anti-PD-L1 antibody alone (**FIG. 35B**), a single dose of 0.5 μg IL12 mRNA alone (**FIG. 35C**), or with both an anti-PD-L1 antibody and 0.5 μg IL12 mRNA (**FIG. 35D**). The anti-PD-L1 antibody was administered over 6 doses. Vertical dashed lines indicate dose days.

FIG. 36A is a drawing of a mouse implanted bilaterally with tumor cells. **FIGs. 36B-36G** are graphs showing individual

tumor volumes in bilaterally implanted MC38-S mice through 60 days in treated (**FIG. 36B, 36D,** and **36F**) and distal (**FIG. 36C, 36E,** and **36G**) tumors following treatment with a negative control (NST mRNA plus isotype antibody control) (**FIGs. 36B-36C**), 0.5 μg IL12 mRNA (**FIGs. 36D-36E**), or 5 μg IL12 mRNA (**FIGs. 36F-36G**). Vertical dashed lines indicate dose days.

FIGs. 37A-37F are graphs showing individual tumor volumes in bilaterally implanted MC38-S mice through 60 days following treatment with no active mRNA (NST mRNA) (**FIGs. 37A-37B**), 0.5 μg IL12 mRNA (**FIGs. 37C-37D**), or 5 μg IL12 mRNA (**FIGs. 37E-37F**), combined with either an isotype control antibody (**FIG. 37A, 37C**, or **37E**) or an anti-PD-L1 antibody (**FIG. 37B, 37D, or 37F**). Vertical dashed lines indicate dose days.

FIG. 38 is a graph showing human IL-12 expression *in vitro* and *in vivo* by wild-type and codon optimized IL-12 mRNA constructs.

DETAILED DESCRIPTION

[0043] The present disclosure provides a new approach to treat cancer involving the prevention or treatment of disease with substances (e.g., mRNAs encoding IL12) that stimulate the immune response, *i.e.,* immunotherapy.

[0044] In one aspect, the disclosure relates to methods of treating cancer using a polynucleotide encoding IL12. An IL12 polypeptide as disclosed herein comprises IL12A, IL12B, or both IL12A and IL12B. In another aspect, the disclosure provides methods of treating cancer using a combination approach that features mRNAs encoding IL12 and an anti-cancer agent, *e.g.,* an immune-checkpoint inhibitor, *e.g.,* anti-PD-1 antibody, anti-PD-L1 antibody, and/or anti-CTLA-4 antibody. Without being bound by any theory, it is believed that priming of an anti-cancer immune response is possible by administering, *e.g.*, intratumorally, mRNAs encoding IL12 in the stimulation of, for example, T-cells and/or natural killer cells. Therefore, an mRNA encoding IL12 is believed to provide a first stimulation signal to the immune system, for example, within the tumor environment, *e.g., via* intratumoral injection of the mRNA. IL12 can also stimulate the production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) from T cells and natural killer (NK) cells. As disclosed herein, IL12, either directly or indirectly through IFN-γ, can also increase expression of PD-L1 in tumor cells, which can impair local tumor immunity. Therefore, in some aspects, the disclosure provides a method of treating tumor comprising administering a polynucleotide (*e.g.*, mRNA) encoding IL12 in combination with an anti-PD-1 antibody or anti-PD-L1 antibody to block the interaction between PD-L1 and its receptor, *i.e.,* PD-1. In other aspects, the disclosure includes a method of treating tumor comprising administering a polynucleotide (*e.g.*, mRNA) encoding IL12 in combination with an anti-CTLA-4 antibody. In further aspects, the disclosure provides a method of treating tumor comprising administering a polynucleotide (*e.g.*, mRNA) encoding IL12 in combination with an anti-PD-1 antibody or anti-PD-L1 antibody and an anti-CTLA-4 antibody. Some aspects of the disclosure also include additional agents, *e.g.,* OX40L, a polynucleotide encoding OX40L, or mRNA encoding an OX40L. In other aspects, the anti-PD-1 antibody or anti-PD-L1 antibody can be administered in the form of a polynucleotide. Similarly, the anti-CTLA-4 antibody can be administered in the form of a polynucleotide. Exemplary aspects feature treatment with lipid nanoparticle-(LNP-) encapsulated mRNAs. Exemplary aspects feature intratumoral administration of mRNAs in cationic lipid-based LNPs.

1. Methods of treating cancer

[0045] Certain aspects of the present disclosure are directed to methods of reducing or decreasing size, mass, and/or volume of a tumor or preventing the growth of a tumor in a subject in need thereof comprising administering a polynucleotide, *e.g.*, mRNA, encoding an IL12B and/or IL12A polypeptide disclosed herein, or a vector or a host cell comprising the polynucleotide, or an IL12B and/or IL12A polypeptide encoded by the polynucleotide. In certain cases, the polynucleotide encodes an IL-12 polypeptide, wherein the polynucleotide comprises an ORF encoding an IL12B and an IL12A polypeptides. In some cases, the methods of reducing the size (including mass and/or volume) of a tumor or inhibiting growth of a tumor in a subject in need thereof comprise administering to the subject an effective amount of a composition comprising one or more polynucleotides. In some cases, the one or more polynucleotides encode an IL-12 polypeptide. In some cases, the one or more polynucleotides comprise an ORF encoding an IL12B polypeptide. In some cases, the one or more polynucleotides comprise an ORF encoding an IL12A polypeptide. In certain cases, the one or more polynucleotides comprise an ORF encoding an IL12B polypeptide and an IL12A polypeptide.

[0046] In some cases, the methods further comprise administering a second agent. In some cases, the second agent comprises an effective amount of a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide. In some cases, the second agent comprises an effective amount of a composition comprising a checkpoint inhibitor polypeptide. In some cases, the checkpoint inhibitor is any checkpoint inhibitor known in the art or described herein. In some cases, the checkpoint inhibitor is an anti-PD-1 antibody. In other cases, the checkpoint inhibitor is an anti-PD-L1 antibody. In other cases, the checkpoint inhibitor is an anti-CTLA-4 antibody. In some cases, the second agent comprises an effective amount of a composition comprising a polynucleotide comprising an ORF encoding OX40L. In some cases, the checkpoint inhibitor is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-

CTLA-4 antibody, or a polynucleotide encoding OX40L or any other agents disclosed herein. In certain cases, the composition comprising a checkpoint inhibitor comprises more than one checkpoint inhibitor. In one particular case, the method comprises administering (i) an mRNA, encoding an IL12B and/or IL12A polypeptide disclosed herein, and (ii) an anti-PD-L1 antibody, an anti-PD-1 antibody, an anti-CTLA-4 antibody, a polynucleotide encoding OX40L, or any combination thereof.

[0047] In some cases, the methods reduce the size of a tumor in a subject as compared to the size of the tumor before the administration. In certain cases, the size of the tumor is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%. In certain cases, the subject exhibits a partial resonse. In certain cases, the subject exhibits a complete response.

[0048] In some cases, the methods of the present disclosure inhibit, stop or delay tumor growth in a subject in need thereof. In certain cases, the tumor growth is inhibited, stopped or delayed for at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 9 months, at least about 12 months, at least about 15 months, at least about 18 months, at least about 24 months, or at least about 36 months. In other cases, the tumor growth is inhibited indefinitely.

[0049] In other cases, the present disclosure provides methods of promoting an anti-tumor effect (*e.g.,* induce T cell proliferation, induce T cell infiltration in a tumor, induce a memory T cell response, increasing the number of NK cells, etc.) by administering the polynucleotide (*e.g.*, mRNA) encoding IL12 alone or the polynucleotide in combination with any agents disclosed herein.

[0050] In one case, the present disclosure provides a method of activating T cells in a subject in need thereof, inducing T cell proliferation in a subject in need thereof, inducing T cell infiltration in a tumor of a subject in need thereof, and/or inducing a memory T cell response in a subject in need thereof, comprising administering to the subject a composition disclosed herein, *e.g.,* a polynucleotide (*e.g.,* mRNA) encoding IL12, alone or in combination with composition comprising a second agent, *e.g.,* a checkpoint inhibitor polypeptide or a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide, *e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, or a polynucleotide encoding OX40L or any other agents disclosed herein. In certain cases, the intratumoral administration of the polynucleotide (*e.g.*, mRNA) encoding IL12 alone or in combination with a second agent can increase the efficacy of the anti-tumor effect (*e.g.,* T cell infiltration in a tumor) compared to other routes of administration.

[0051] In some cases, the administering of the compositions described herein, *e.g.,* a polynucleotide encoding an IL12, alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide, activates T cells in the subject. T cell activation can be characterized in any way known in the art. In some cases, the activated T cells express CD4. In some cases, the activated T cells express CD8. In certain cases the activated T cells express CD4 and CD8. In certain cases, the activated T cells comprise $CD4^+$ T cells, $CD8^+$ T cells, or both $CD4^+$ T cells and $CD8^+$ T cells.

[0052] In one case, activated T cells in the subject reduce the size of a tumor or inhibit the growth of a tumor in the subject. Activation of T cells can be measured using applications in the art such as measuring T cell proliferation; measuring cytokine production with enzyme-linked immunosorbant assays (ELISA) or enzyme-linked immunospot assays (ELISPOT); or detection of cell-surface markers associated with T cell activation (*e.g.*, CD69, CD40L, CD137, CD25, CD71, CD26, CD27, CD28, CD30, CD154, and CD134) with techniques such as flow cytometry.

[0053] In some cases, T cell activation comprises inducing T cell proliferation. In some cases, T cell proliferation is increased by at least about 1.5 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 20 fold, at least about 30 fold, at least about 50 fold, or at least about 100 fold, as compared to the level of T cell proliferation prior to the administration of the IL12 encoding polynucleotide (*e.g.*, mRNA).

[0054] In one case, T cell proliferation in the subject is directed to an anti-tumor immune response in the subject. In another aspect, the T cell proliferation in the subject reduces or decreases the size of a tumor or inhibits the growth of a tumor in the subject. T cell proliferation can be measured using applications in the art such as cell counting, viability staining, optical density assays, or detection of cell-surface markers associated with T cell activation (*e.g.*, CD69, CD40L, CD137, CD25, CD71, CD26, CD27, CD28, CD30, CD154, and CD134) with techniques such as flow cytometry.

[0055] In some cases, T cell activation comprises induction of T cell infiltration of the tumor. In some cases, T cell infiltration in the tumor is increased by at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 20 fold, at least about 30 fold, at least about 50 fold, or at least about 100 fold, as compared to the level of T cell infiltration of the tumor prior to the administration of the IL12 encoding polynucleotide (*e.g.*, mRNA).

[0056] In some cases, T cell activation comprises increasing the number of tumor-infiltrating T cells. In certain cases, the number of tumor-infiltrating T cells in the tumor is increased by at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold,

at least about 10 fold, at least about 20 fold, at least about 30 fold, at least about 50 fold, or at least about 100 fold, as compared to the number of tumor infiltrating T cells in the tumor prior to the administration of the IL12 encoding polynucleotide (*e.g.*, mRNA)

[0057] In one case, T cell infiltration in a tumor of the subject is directed to an anti-tumor immune response in the subject. In another aspect, the T cell infiltration in a tumor of the subject reduces or decreases the size of a tumor or inhibits the growth of a tumor in the subject. T cell infiltration in a tumor can be measured using applications in the art such as tissue sectioning and staining for cell markers, measuring local cytokine production at the tumor site, or detection of T cell-surface markers with techniques such as flow cytometry.

[0058] In some cases, T cell activation comprises inducing a memory T cell response in a subject in need thereof. In certain cases, the memory T cell response is increased by at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 20 fold, at least about 30 fold, at least about 50 fold, or at least about 100 fold, as compared to the memory T cell response prior to the administration of the IL12 encoding polynucleotide (*e.g.*, mRNA).

[0059] In one case, the memory T cell response in the subject is directed to an anti-tumor immune response in the subject. In another aspect, the memory T cell response in the subject reduces or decreases the size of a tumor or inhibits the growth of a tumor in the subject. A memory T cell response can be measured using applications in the art such as measuring T cell markers associated with memory T cells, measuring local cytokine production related to memory immune response, or detecting memory T cell-surface markers with techniques such as flow cytometry.

[0060] In some cases, the administering of the compositions described herein, *e.g.,* a polynucleotide encoding an IL12, alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide, increases an effector to suppressor T cell ratio in the tumor. In certain cases, the effector to suppressor T cell ratio is characterized by the ratio of (i) CD8+, CD4+, or CD8+/CD4+ T cells to (ii) Treg cells in a subject. In certain cases, the increase in the effector to suppressor T cell ratio correlates with an increase in the number of CD8+ T cells. In some cases, the increase in the effector to suppressor T cell ratio correlates with an increase in the number of $CD4^+$ T cells. In some cases, the increase in the effector to suppressor T cell ratio correlates with an increase in the number of CD8+/CD4+ T cells. In some cases, the increase in the effector to suppressor T cell ratio correlates with a decrease in the number of Treg cells.

[0061] In some cases, the effector to suppressor T cell ratio, *e.g.,* the $CD8^+$ T cell to Treg cell ratio, following administration of the IL12 encoding polynucleotide (e.g., mRNA) (alone or in combination with an anti-PD-L1 antibody, an anti-PD-1 antibody, an anti-CTLA-4 antibody, or a polynucleotide encoding OX40L) is at least about 1.5:1, at least about 2:1, at least about 2.5:1, at least about 3:1, at least about 3.5:1, at least about 3.5:1, at least about 4:1, at least about 4.5:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 15:1, at least about 20:1, at least about 25:1, at least about 30:1, at least about 35:1, at least about 40:1, at least about 45:1, at least about 50:1, at least about 60:1, at least about 70:1, at least about 80:1, at least about 90:1, at least about 100:1, at least about 110:1, at least about 120:1, at least about 130:1, at least about 140:1, at least about 150:1, at least about 200:1, at least about 250:1, or at least about 500:1.

[0062] In some cases, the effector to suppressor T cell ratio, *e.g.,* the $CD8^+$ T cell to Treg cell ratio, following administration of the IL12 encoding polynucleotide (e.g., mRNA) (alone or in combination with an anti-PD-L1 antibody, an anti-PD-1 antibody, an anti-CTLA-4 antibody, or a polynucleotide encoding OX40L) is at least about 1.5, at least about 2, at least about 2.5, at least about 3, at least about 3.5, at least about 3.5, at least about 4, at least about 4.5, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about 130, at least about 140, at least about 150, at least about 200, at least about 250, or at least about 500.

[0063] In one case, the increase in the effector to suppressor T cell ratio in the tumor is directed to an anti-tumor immune response in the subject. In another aspect, the increase in the effector to suppressor T cell ratio in the tumor reduces or decreases the size of a tumor or inhibits the growth of a tumor in the subject. The effector to suppressor T cell ratio in the tumor can be measured using applications in the art such as measuring the ratio of CD8+, CD4+, or CD8+/CD4+ T cells to Treg cells, using any methods known in the art including IHC and/or flow cytometry.

[0064] In certain cases, the activated T cells by the present methods are $CD4^+$ cells, $CD8^+$ cells, $CD62^+$ (L-$selectin^+$) cells, $CD69^+$ cel s, $CD40L^+$ cells, $CD137^+$ cells, $CD25^+$ cells, $CD71^+$ cells, $CD26^+$ cells, $CD27^+$ cells, $CD28^+$ cells, $CD30^+$ cells, $CD45^+$ cells, $CD45RA^+$ cells, $CD45RO^+$ cells, $CD11b^+$ cells, $CD154^+$ cells, $CD134^+$ cells, $CXCR3^+$ cells, $CCR4^+$ cells, $CCR6^+$ cells, $CCR7^+$ cells, $CXCR5^+$ cells, $Crth2^+$ cells, gamma delta T cells, or any combination thereof. In some cases, the activated T cells by the present methods are Th_1 cells. In other cases, the T cells activated by the present methods are Th_2 cells. In other cases, the T cells activated by the present disclosure are cytotoxic T cells.

[0065] In some cases, the infiltrating T cells by the present methods are $CD4^+$ cells, $CD8^+$ cells, $CD62^+$ (L-$selectin^+$) cells, $CD69^+$ cel s, $CD40L^+$ cells, $CD137^+$ cells, $CD25^+$ cells, $CD71^+$ cells, $CD26^+$ cells, $CD27^+$ cells, $CD28^+$ cells, $CD30^+$ cells, $CD45^+$ cells, $CD45RA^+$ cells, $CD45RO^+$ cells, $CD11b^+$ cells, $CD154^+$ cells, $CD134^+$ cells, $CXCR3^+$ cells, $CCR4^+$

cells, CCR6$^+$ cells, CCR7$^+$ cells, CXCR5$^+$ cells, Crth2$^+$ cells, gamma delta T cells, or any combination thereof. In some cases, the infiltrating T cells by the present methods are Th_1 cells. In other cases, the infiltrating T cells by the present methods are Th_2 cells. In other cases, the infiltrating T cells by the present disclosure are cytotoxic T cells.

[0066] In some cases, the memory T cells induced by the present methods are CD4$^+$ cells, CD8$^+$ cells, CD62$^+$ (L-selectin$^+$) cells, CD69$^+$ cells, CD40L$^+$ cells, CD137$^+$ cells, CD25$^+$ cells, CD71$^+$ cells, CD26$^+$ cells, CD27$^+$ cells, CD28$^+$ cells, CD30$^+$ cells, CD45$^+$ cells, CD45RA$^+$ cells, CD45RO$^+$ cells, CD11b$^+$ cells, CD154$^+$ cells, CD134$^+$ cells, CXCR3$^+$ cells, CCR4$^+$ cells, CCR6$^+$ cells, CCR7$^+$ cells, CXCR5$^+$ cells, Crth2$^+$ cells, gamma delta T cells, or any combination thereof. In some cases, the memory T cells by the present methods are Th_1 cells. In other cases, the memory T cells by the present methods are Th_2 cells. In other cases, the memory T cells by the present disclosure are cytotoxic T cells.

[0067] In certain cases, the disclosure includes a method of inducing an adaptive immune response, an innate immune response, or both adaptive and innate immune response against tumor comprising administering a polynucleotide, *e.g.*, mRNA, encoding IL12 alone or in combination with a second agent, *e.g.,* a checkpoint inhibitor, *e.g.,* an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, a polynucleotide encoding OX40L, and/or any other agents disclosed herein.

[0068] The present disclosure further provides a method of increasing the number of Natural Killer (NK) cells in a subject in need thereof comprising administering a polynucleotide comprising an mRNA encoding IL12 alone or in combination with a second agent, *e.g.,* a checkpoint inhibitor, *e.g.,* an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, a polynucleotide encoding OX40L, and/or any other agents disclosed herein. In one aspect, the increase in the number of NK cells in the subject is directed to an anti-tumor immune response in the subject. In another aspect, the increase in the number of NK cells in the subject reduces or decreases the size of a tumor or inhibits the growth of a tumor in the subject. Increases in the number of NK cells in a subject can be measured using applications in the art such as detection of NK cell-surface markers (*e.g.*, CD335/NKp46; CD336/NKp44; CD337/NPp30) or intracellular NK cell markers (*e.g.*, perforin; granzymes; granulysin).

[0069] In some cases, the administering of the compositions described herein, *e.g.,* a polynucleotide (*e.g.*, mRNA) encoding an IL12, alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide, increases the number of activated NK cells in the subject as compared to the number of activated NK cells prior to the administration. In some cases, the number of activated NK cells is increased by at least about 1.5 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 15 fold, at least about 20 fold, at least about 25 fold, at least about 30 fold, at least about 35 fold, at least about 40 fold, at least about 45 fold, at least about 50 fold, at least about 60 fold, at least about 70 fold, at least about 80 fold, at least about 90 fold, or at least about 100 fold. In some cases, the increase in activated NK cells is maintained for at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, or at least about 28 days.

[0070] In some cases, the administering of the compositions described herein, *e.g.,* a polynucleotide (*e.g.*, mRNA) encoding an IL12, alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide, increases the number of cross-presenting dendritic cells in the tumor of the subject as compared to the number of cross-presenting dendritic cells in the tumor prior to the administration. In some cases, the number of cross-presenting dendritic cells in the tumor is increased by at least about 1.5 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 15 fold, at least about 20 fold, at least about 25 fold, at least about 30 fold, at least about 35 fold, at least about 40 fold, at least about 45 fold, at least about 50 fold, at least about 60 fold, at least about 70 fold, at least about 80 fold, at least about 90 fold, or at least about 100 fold. In some cases, the increase in cross-presenting dendritic cells in the tumor is maintained for at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, or at least about 28 days.

[0071] In certain cases, the present disclosure is also directed to a method of increasing IFNγ expression in a subject having tumor comprising administering a polynucleotide, *e.g.,* mRNA, encoding IL12 alone or in combination with a second agent, *e.g.,* a checkpoint inhibitor, *e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, a polynucleotide encoding OX40L, and/or any other agents disclosed herein.

[0072] Other cases also include a method of increasing expression of IFNy, TNFα, IL-10, IL-13, IL-15/15R, IL-27, MIP-1β, MIP-1α, MCP-1, MCP-3, M-CSF, IL-4, IL-5, or any combination thereof in a subject having tumor comprising admin-

istering a polynucleotide, *e.g.*, mRNA, encoding IL12 alone or in combination with another agent disclosed herein. In yet other cases, the methods of the present disclosure can include methods of inducing expression of GM-CSF, IL-18, IL-3, RANTES, IL-6, or any combination thereof.

[0073] The polynucleotide encoding IL12 can be formulated as a pharmaceutical composition that is suitable for administration either directly or indirectly to tumors. The term "tumor" is used herein in a broad sense and refers to any abnormal new growth of tissue that possesses no physiological function and arises from uncontrolled usually rapid cellular proliferation. The term "tumor" as used herein relates to both benign tumors and to malignant tumors.

[0074] Certain aspects of the disclosure provide methods of intratumorally administering a single administration dose of a polynucleotide, *e.g.*, mRNA, encoding IL12 alone or in combination with any agents disclosed herein. In such cases, an mRNA encoding IL12 can be administered only once while the other agent can be administered regularly, following its regular dosing schedule. In certain cases, a checkpoint inhibitor, *e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, is administered prior to administration of a polynucleotide, *e.g.*, mRNA, encoding IL12. In some cases, the polynucleotide is formulated in a lipid nanoparticle, *e.g.*, Compound 18, disclosed herein. Not being bound by any theory, in some aspects, the intratumoral delivery of a polynucleotide encoding IL12 and/or the lipid nanoparticle formulation disclosed herein allow single dose administration that is sufficient for the dose to trigger anti-tumor efficacy and treat tumor. Given the potential toxicity of IFNγ induced by IL-12, this single dosing regimen of the disclosed polynucleotide can be beneficial to the subjects in need of the treatment.

[0075] In certain cases, the method comprises administering a single dose of a polynucleotide encoding IL12 in combination with a second agent, *e.g.,* an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, which can be given also in single administration or multiple administrations following its regular (*e.g.,* approved) schedule. In other cases, the method comprises not more than two administrations of a polynucleotide encoding IL12, not more than three administrations of a polynucleotide encoding IL12, not more than four administrations of a polynucleotide encoding IL12, or not more than five administrations of a polynucleotide encoding IL12, optionally in combination with a checkpoint inhibitor, *e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, or a polynucleotide encoding an OX40L polypeptide.

[0076] In other cases, the present methods can result in abscopal effects, *e.g.,* a treatment of tumor where localized treatment of a tumor, *e.g.*, intratumoral delivery, by a polynucleotide, *e.g.*, mRNA, encoding IL12 causes not only a shrinking of the treated tumor, but also a shrinking of tumors outside the scope of the localized treatment ("distal tumor").

[0077] In some cases, the administering of the compositions described herein, *e.g.,* a polynucleotide encoding an IL12 polypeptide, alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide, reduces the size of a distal tumor. In certain cases, the administering is intratumoral to a first tumor, and the administering reduces the size of a second, distal tumor. In some cases, the size of the distal tumor is reduced by at least about 10%, at least about 20% at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100%.

[0078] In certain cases, the administering of the compositions described herein, *e.g.,* a polynucleotide encoding an IL12 polypeptide, alone or in combination with a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide or a composition comprising a checkpoint inhibitor polypeptide, inhibits the growth of a distal tumor. In certain cases, the administering is intratumoral to a first tumor, and the administering inhibits the growth of a second, distal tumor. In some cases, the growth of the distal tumor is inhibited for at least about 7 days, at least about 14 days, at least about 21 days, at least about 28 days, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 18 months, at least about 24 months, at least about 30 months, at least about 36 months, at least about 4 years, or at least about 5 years.

[0079] The delivery of the polynucleotide encoding IL12 to a tumor using a pharmaceutical compositions for intratumoral administration disclosed herein can:

(i) increase the retention of the polynucleotide in the tumor;
(ii) increase the levels of expressed polypeptide in the tumor compared to the levels of expressed polypeptide in peritumoral tissue;
(iii) decrease leakage of the polynucleotide or expressed product to off-target tissue (*e.g.,* peritumoral tissue, or to distant locations, *e.g.,* liver tissue); or,
(iv) any combination thereof,

wherein the increase or decrease observed for a certain property is relative to a corresponding reference composition (*e.g.*, composition in which compounds of formula (I) are not present or have been substituted by another ionizable amino lipid, *e.g.,* MC3).

[0080] In one case, a decrease in leakage can be quantified as decrease in the ratio of polypeptide expression in the tumor to polypeptide expression in non-tumor tissues, such as peritumoral tissue or to another tissue or organ, *e.g.,* liver tissue.

[0081] Delivery of a polynucleotide encoding IL12 to a tumor involves administering a pharmaceutical composition disclosed herein, *e.g.,* in nanoparticle form, including the polynucleotide encoding IL12 to a subject, where administration of the pharmaceutical composition involves contacting the tumor with the composition.

[0082] In the instance that the polynucleotide encoding IL12 is an mRNA, upon contacting a cell in the tumor with the pharmaceutical composition, a translatable mRNA may be translated in the cell to produce a polypeptide of interest. However, mRNAs that are substantially not translatable may also be delivered to tumors. Substantially non-translatable mRNAs may be useful as vaccines and/or may sequester translational components of a cell to reduce expression of other species in the cell.

[0083] The pharmaceutical compositions disclosed herein can increase specific delivery. As used herein, the term "specific delivery," means delivery of more (*e.g.,* at least 1.5 fold more, at least 2-fold more, at least 3-fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more) of a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides by pharmaceutical composition disclosed herein (*e.g.,* in nanoparticle form) to a target tissue of interest (*e.g.,* a tumor) compared to an off-target tissue (*e.g.*, mammalian liver).

[0084] The level of delivery of a nanoparticle to a particular tissue may be measured, for example, by comparing

(i) the amount of protein expressed from a polynucleotide encoding IL12 in a tissue to the weight of the tissue;
(ii) comparing the amount of the polynucleotide in a tissue to the weight of the tissue; or
(iii) comparing the amount of protein expressed from a polynucleotide encoding IL12 in a tissue to the amount of total protein in the tissue.

[0085] Specific delivery to a tumor or a particular class of cells in the tumor implies that a higher proportion of pharmaceutical composition including a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides is delivered to the target destination (*e.g.*, target tissue) relative to other off-target destinations upon administration of a pharmaceutical composition to a subject.

[0086] The present disclosure also provides methods to deliver intratumorally a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides when a pharmaceutical composition comprising the polynucleotides disclosed herein (*e.g.,* in nanoparticle form) are administered to a tumor. The intratumoral administration can show one or more properties selected from the group consisting of:

(i) increased retention of the polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides in the tumor;
(ii) increased levels of expressed polypeptide in the tumor compared to the levels of expressed polypeptide in peritumoral tissue;
(iii) decreased leakage of the polynucleotide or expressed product to off-target tissue (*e.g.*, peritumoral tissue, or to distant locations, *e.g.*, liver tissue); and,
(iv) any combination thereof,

wherein the increase or decrease observed for a certain property is relative to a corresponding reference composition (*e.g.*, composition in which compounds of formula (I) are not present or have been substituted by another ionizable amino lipid, *e.g.,* MC3).

[0087] In one case, a decrease in leakage can be quantified as decrease in the ratio of polypeptide expression in the tumor to polypeptide expression in non-tumor tissues, such as peritumoral tissue or to another tissue or organ, *e.g.,* liver tissue.

[0088] In some cases, another property in delivery caused as a result of using the pharmaceutical compositions disclosed herein is a reduction in immune response with respect to the immune response observed when other lipid components are used to deliver the same a therapeutic agent or polynucleotide encoding a therapeutic agent.

[0089] Accordingly, the present disclosure provides a method of increasing retention of a therapeutic agent (*e.g.,* a polynucleotide or a polypeptide administered as part of the pharmaceutical composition or a polypeptide expressed as a result of the administration) in a tumor tissue in a subject, comprising administering intratumorally to the tumor tissue a pharmaceutical composition disclosed herein, wherein the retention of the therapeutic agent in the tumor tissue is increased compared to the retention of the therapeutic agent in the tumor tissue after administering a corresponding reference composition (*e.g.*, MC3).

[0090] Also provided is a method of increasing retention of a polynucleotide in a tumor tissue in a subject, comprising administering intratumorally to the tumor tissue a pharmaceutical composition disclosed herein, wherein the retention

of the polynucleotide in the tumor tissue is increased compared to the retention of the polynucleotide in the tumor tissue after administering a corresponding reference composition (*e.g.*, MC3).

[0091] Also provided is a method of increasing retention of an expressed polypeptide in a tumor tissue in a subject, comprising administering to the tumor tissue a pharmaceutical composition disclosed herein, wherein the pharmaceutical composition comprises a polynucleotide encoding the expressed polypeptide, and wherein the retention of the expressed polypeptide in the tumor tissue is increased compared to the retention of the polypeptide in the tumor tissue after administering a corresponding reference composition (*e.g.,* MC3).

[0092] The present disclosure also provides a method of decreasing expression leakage of a polynucleotide administered intratumorally to a subject in need thereof, comprising administering said polynucleotide intratumorally to the tumor tissue as a pharmaceutical composition disclosed herein, wherein the expression level of the polypeptide in non-tumor tissue is decreased compared to the expression level of the polypeptide in non-tumor tissue after administering a corresponding reference composition (*e.g.*, MC3).

[0093] Also provided is a method of decreasing expression leakage of a therapeutic agent (*e.g.,* a polypeptide administered as part of the pharmaceutical composition) administered intratumorally to a subject in need thereof, comprising administering the therapeutic agent intratumorally to the tumor tissue as a pharmaceutical composition disclosed herein, wherein the amount of therapeutic agent in non-tumor tissue is decreased compared to the amount of therapeutic in non-tumor tissue after administering a corresponding reference composition (*e.g.*, MC3).

[0094] Also provided is a method of decreasing expression leakage of an expressed polypeptide in a tumor in a subject, comprising administering to the tumor tissue a pharmaceutical composition disclosed herein, wherein the pharmaceutical composition comprises a polynucleotide encoding the expressed polypeptide, and wherein the amount of expressed polypeptide in non-tumor tissue is decreased compared to the amount of expressed polypeptide in non-tumor tissue after administering a corresponding reference composition (*e.g.*, MC3).

[0095] In some cases, the non-tumoral tissue is peritumoral tissue. In other cases, the non-tumoral tissue is liver tissue.

[0096] The present disclosure also provides a method to reduce or prevent the immune response caused by the intratumoral administration of a pharmaceutical composition, *e.g.*, a pharmaceutical composition comprising lipids known in the art, by replacing one or all the lipids in such composition with a compound of Formula (I). For example, the immune response caused by the administration of a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides in a pharmaceutical composition comprising MC3 (or other lipids known in the art) can be prevented (avoided) or ameliorated by replacing MC3 with a compound of Formula (I), *e.g.*, Compound 18.

[0097] In some cases, the immune response observed after a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides is administered in a pharmaceutical composition disclosed herein is not elevated compared to the immune response observed when the therapeutic agent or a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides is administered in phosphate buffered saline (PBS) or another physiological buffer solution (*e.g.*, Ringer's solution, Tyrode's solution, Hank's balanced salt solution, etc.).

[0098] In some cases, the immune response observed after a therapeutic agent or a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides is administered in a pharmaceutical composition disclosed herein is not elevated compared to the immune response observed when PBS or another physiological buffer solution is administered alone.

[0099] In some cases, no immune response is observed when a pharmaceutical composition disclosed herein is administered intratumorally to a subject.

[0100] Accordingly, the present disclosure also provides a method of delivering a therapeutic agent or a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides to a subject in need thereof, comprising administering intratumorally to the subject a pharmaceutical composition disclosed herein, wherein the immune response caused by the administration of the pharmaceutical composition is not elevated compared to the immune response caused by the intratumoral administration of

(i) PBS alone, or another physiological buffer solution (*e.g.*, Ringer's solution, Tyrode's solution, Hank's balanced salt solution, etc.);

(ii) the therapeutic agent or polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides in PBS or another physiological buffer solution; or,

(iii) a corresponding reference composition, *i.e.,* the same pharmaceutical composition in which the compound of Formula (I) is substituted by another ionizable amino lipid, *e.g.,* MC3.

[0101] In certain cases, the administration treats a cancer.

[0102] The polynucleotide (*e.g.*, mRNA) of the present disclosure can be administered in any route available, including, but not limited to, intratumoral, enteral, gastroenteral, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal,

(into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraperitoneal (into the peritoneum), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops. In other cases, the mRNA of the present disclosure is administered parenterally (*e.g.*, includes subcutaneous, intravenous, intraperitoneal, intratumoral, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques), intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. In particular cases, the polynucleotide, composition, or polypeptide is administered subcutaneously, intravenously, intraperitoneally, intratumorally, intramuscularly, intra-articularly, intra-synovially, intrasternally, intrathecally, intrahepatically, intradermally, intralesionally, intracranially, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. In one particular case, the polynucleotide (*e.g.*, mRNA) of the present disclosure is administered intratumorally.

[0103] In some cases, the polynucleotide is delivered by a device comprising a pump, patch, drug reservoir, short needle device, single needle device, multiple needle device, micro-needle device, jet injection device, ballistic powder/particle delivery device, catheter, lumen, cryoprobe, cannula, microcanular, or devices utilizing heat, RF energy, electric current, or any combination thereof.

[0104] Other aspects of the present disclosure relate to transplantation of cells containing polynucleotides to a mammalian subject. Administration of cells to mammalian subjects is known to those of ordinary skill in the art, and includes, but is not limited to, local implantation (*e.g.*, topical or subcutaneous administration), organ delivery or systemic injection (*e.g.*, intravenous injection or inhalation), and the formulation of cells in pharmaceutically acceptable carriers.

[0105] In some cases, the polynucleotide, *e.g.*, mRNA, is administered at an amount between about 0.10 μg/kg and about 1000 mg/kg.

[0106] In some cases, the administration of the polynucleotide, pharmaceutical composition or formulation of the disclosure results in expression of IL12 in cells of the subject. In some cases, administering the polynucleotide, pharmaceutical composition or formulation of the disclosure results in an increase of IL12 activity in the subject. For example, in some cases, the polynucleotides of the present disclosure are used in methods of administering a composition or formulation comprising an mRNA encoding an IL12B polypeptide, IL12A polypeptide, and both IL12B and IL12A polypeptides to a subject, wherein the method results in an increase of IL12 activity in at least some cells of a subject.

[0107] In some cases, the administration of a composition or formulation comprising an mRNA encoding an IL12B polypeptide, IL12A polypeptide, and both IL12B and IL12A polypeptides to a subject results in an increase of IL12 activity in cells subject to a level at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or to 100% or more of the activity level expected in a normal subject.

[0108] Other cases of the disclosure also provide a method of treating a cancer in a subject in need thereof comprising administering (*e.g.*, intratumorally, intraperitoneally, or intravenously) a polynucleotide comprising an mRNA encoding an IL12B polypeptide, IL12A polypeptide, and both IL12B and IL12A polypeptides with one or more anti-cancer agents to the subject.

[0109] In some cases, the polynucleotides (*e.g.*, mRNA) encoding an IL12B polypeptide, IL12A polypeptide, and both IL12B and IL12A polypeptides of the present disclosure can be used to reduce the size of a tumor or inhibit tumor growth in a subject in need thereof.

[0110] In some cases, the tumor is associated with a disease, disorder, and/or condition. In a particular case, the disease, disorder, and/or condition is a cancer. Thus, in one aspect, the administration of the polynucleotide (*e.g.*, mRNA) encoding an IL12B polypeptide, IL12A polypeptide, and both IL12B and IL12A polypeptides treats a cancer.

[0111] A "cancer" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and can also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor at various stages. In certain cases, the cancer or tumor is stage 0, such that, *e.g.,* the cancer or tumor is very early in development and has not metastasized. In some cases, the cancer or tumor is stage I, such that, *e.g.,* the cancer or tumor is relatively small in size, has not spread into nearby tissue, and has not metastasized. In other cases, the cancer or tumor is stage II or stage III, such that, *e.g.,* the cancer or tumor is larger than in stage 0 or stage I, and it has grown into neighboring tissues but it has not metastasized, except potentially to the lymph nodes. In other cases, the cancer or tumor is stage IV, such that, *e.g.,* the cancer or tumor has metastasized. Stage IV can also be referred to as advanced or metastatic cancer.

[0112] In some aspects, the cancer can include, but is not limited to, adrenal cortical cancer, advanced cancer, anal cancer, aplastic anemia, bileduct cancer, bladder cancer, bone cancer, bone metastasis, brain tumors, brain cancer,

breast cancer, childhood cancer, cancer of unknown primary origin, Castleman disease, cervical cancer, colon/rectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, renal cell carcinoma, laryngeal and hypopharyngeal cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, liver cancer, hepatocellular carcinoma (HCC), non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma in adult soft tissue, basal and squamous cell skin cancer, melanoma, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor, secondary cancers caused by cancer treatment, and any combination thereof.

[0113] In some aspects, the tumor is a solid tumor. A "solid tumor" includes, but is not limited to, sarcoma, melanoma, carcinoma, or other solid tumor cancer. "Sarcoma" refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas include, but are not limited to, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, or telangiectaltic sarcoma.

[0114] The term "melanoma" refers to a tumor arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acra-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, metastatic melanoma, nodular melanoma, subungal melanoma, or superficial spreading melanoma.

[0115] The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas include, *e.g.*, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidernoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, or carcinoma viflosum.

[0116] Additional cancers that can be treated include, *e.g.*, Leukemia, Hodgkin's Disease, Non- Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, papillary thyroid cancer, neuroblastoma, neuroendocrine cancer, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, prostate cancer, Müllerian cancer, ovarian cancer, peritoneal cancer, fallopian tube cancer, or uterine papillary serous carcinoma.

2. Combination Therapy

[0117] The disclosure further includes a polynucleotide comprising an ORF (*e.g.,* mRNA) encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptide or uses thereof as a combination therapy, *i.e.,* with any other anti-cancer agent in combination. In certain cases, the polynucleotide encodes an IL-12 polypeptide, wherein the polynucleotide comprises an ORF encoding an IL12B polypeptide and an IL12A polypeptide or uses thereof as a combination therapy, *i.e.,* with any other anti-cancer agent in combination.

[0118] In certain cases, the disclosure is directed to a polynucleotide comprising an mRNA encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptide in combination with one or more anti-cancer agents or uses of the polynucleotide in combination with one or more anti-cancer agents to the subject. In one case, the combination therapy can be a combination of the polynucleotide encoding IL12 and one or more standard therapy. In another case, the methods of the disclosure include two additional anti-cancer agents, three additional agents, four additional agents, etc. The additional anti-cancer agents can be a protein, *e.g.,* an antibody, or a polynucleotide, *e.g.,* mRNA. In some cases, the one or more anti-cancer agents are an mRNA. In certain cases, the one or more anti-cancer agents are an mRNA encoding a tumor antigen. In other cases, the one or more anti-cancer agents are not a tumor antigen or an mRNA encoding a tumor antigen. In other cases, the one or more anti-cancer agents are a protein, *e.g.,* an antibody.

[0119] In some cases, the one or more anti-cancer agents are an approved agent by the United States Food and Drug Administration. In other cases, the one or more anti-cancer agents are a pre-approved agent by the United States Food and Drug Administration.

[0120] One skilled in the art would also appreciate that alternative aspects of the present disclosure include a combination therapy of IL12 and any other agents, *e.g.,* an anti-PD-1 antibody, an anti-PD-L1 antibody, and/or an anti-CTLA-4 antibody, or OX40L as polynucleotides and/or proteins. For example, the present disclosure encompasses combination therapy of (i) a polynucleotide (*e.g.*, mRNA) encoding IL12 and a protein comprising an anti-PD-1 antibody or an anti-PD-L1 antibody; (iii) a polynucleotide (*e.g.*, mRNA) encoding IL12 and a second protein comprising an anti-CTLA-4 antibody, or (iv) a polynucleotide (*e.g.*, mRNA) encoding IL12 and a second protein comprising OX40L. In other cases, the IL12 can also be administered as a protein.

[0121] In other cases, the additional agents can be formulated together with the polynucleotide encoding IL12, *e.g.,* mRNA, or separately. Moreover, even when formulated separately, the additional agents can be administered concurrently with the polynucleotide encoding IL12 or sequentially. In one case, the polynucleotide encoding IL12 is administered prior to the second agent. In another case, the polynucleotide encoding IL12 is administered after the second agent.

[0122] In certain cases, the additional agents, *e.g.*, any antibody disclosed herein or a polynucleotide encoding OX40L, are also administered intratumorally. In other cases, the second agents, *e.g.*, any antibody disclosed herein or a polynucleotide encoding OX40L, are administered via different routes, *e.g.*, intravenously, subcutaneously, intraperitoneally, etc.

[0123] In some aspects, the subject for the present methods or compositions has been treated with one or more standard of care therapies. In other aspects, the subject for the present methods or compositions has not been responsive to one or more standard of care therapies or anti-cancer therapies. In one aspect, the subject has been previously treated with an IL12 protein or an IL12 DNA gene therapy. In another aspect, the subject is treated with an anti-PD-1 antagonist or an anti-CTLA-4 antagonist prior to the polynucleotide of the present disclosure. In another aspect, the subject has been treated with a monoclonal antibody that binds to PD-1 prior to the polynucleotide of the present disclosure and/or a monoclonal antibody that binds to CTLA-4 prior to the polynucleotide of the present disclosure. In another aspect, the subject has been treated with an anti-PD-1 monoclonal antibody and/or anti-CTLA-4 monoclonal antibody therapy prior to the polynucleotide of the present methods or compositions.

[0124] In recent years, the introduction of immune checkpoint inhibitors for therapeutic purposes has revolutionized cancer treatment. Of interest are therapies featuring combinations of checkpoint inhibitors with other costimulatory or inhibitory molecules.

[0125] T cell regulation, *i.e.,* activation or inhibition is mediated via co-stimulatory or co-inhibitory signals. This interaction is exerted via ligand/receptor interaction. T cells harbor a myriad of both activating receptors, such as OX40, and inhibitory receptors (*i.e.,* immune checkpoints) such as programmed death receptor 1 (PD-1) or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (Mellman et al. 2011 Nature.;480:480-489). Activation of this immune checkpoints results in T cell deactivation and commandeering these pathways by tumor cells contributes to their successful immune escape.

[0126] In some cases, the methods of reducing the size of a tumor or inhibiting growth of a tumor in a subject in need thereof comprise administering to the subject an effective amount of a composition comprising one or more polynucleotides. In some cases, the one or more polynucleotides encode an IL-12 polypeptide. In some cases, the one or more polynucleotides comprise an open reading frame ("ORF") encoding an IL12B polypeptide. In some cases, the one or more polynucleotides comprise an ORF encoding an IL12A polypeptide. In certain cases, the one or more polynucleotides comprise an ORF encoding an IL12B polypeptide and an IL12A polypeptide.

[0127] In some cases, the methods further comprise administering a second agent. In some cases, the second agent

comprises an effective amount of a composition comprising a polynucleotide comprising an ORF encoding a checkpoint inhibitor polypeptide. In some cases, the second agent comprises an effective amount of a composition comprising a checkpoint inhibitor polypeptide. In some cases, the checkpoint inhibitor is any checkpoint inhibitor known in the art or described herein. In some cases, the checkpoint inhibitor is an anti-PD-1 antibody. In other cases, the checkpoint inhibitor is an anti-PD-L1 antibody. In other cases, the checkpoint inhibitor is an anti-CTLA-4 antibody. In some cases, the second agent comprises an effective amount of a composition comprising a polynucleotide comprising an ORF encoding OX40L. In some cases, the checkpoint inhibitor is selected from the group consisting of an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CTLA-4 antibody, a polynucleotide encoding OX40L any other agents disclosed herein, and any combination thereof. In certain cases, the composition comprising a checkpoint inhibitor comprises more than one checkpoint inhibitor. In one particular case, the method comprises administering (i) an mRNA, encoding an IL12B and/or IL12A polypeptide disclosed herein, and (ii) an anti-PD-L1 antibody, an anti-PD-1 antibody, anti-CTLA-4 antibody, a polynucleotide encoding OX40L, or any combination thereof.

[0128] In some cases, the checkpoint inhibitor comprises an antigen binding fragment of an antibody. In some cases, the antibody is an anti-CTLA-4 antibody or antigen-binding fragment thereof that specifically binds CTLA-4, an anti-PD-1 antibody or antigen-binding fragment thereof that specifically binds PD-1, an anti-PD-L1 antibody or antigen-binding fragment thereof that specifically binds PD-L1, or a combination thereof. In one case, the antibody is an anti-CTLA4 antibody or antigen-binding fragment thereof that specifically binds CTLA4. In another case, the antibody is an anti-PD-1 antibody or antigen-binding fragment thereof that specifically binds PD-1. In another case, the antibody is an anti-PD-L1 antibody or antigen-binding fragment thereof that specifically binds PD-L1.

[0129] Immune checkpoint inhibitors such as pembrolizumab or nivolumab, which target the interaction between programmed death receptor 1/programmed death ligand 1 (PD-1/PDL-1) and PDL-2, have been recently approved for the treatment of various malignancies and are currently being investigated in clinical trials for cancers including melanoma, head and neck squamous cell carcinoma (HNSCC). Data available from these trials indicate substantial activity accompanied by a favorable safety and toxicity profile in these patient populations.

[0130] For example, checkpoint inhibitors have been tested in clinical trials for the treatment of melanoma. In particular, phase III clinical trials have revealed that therapies such as ipilimumab and pembrolizumab, which target the CTLA-4 and PD-1 immune checkpoints, respectively, have raised the three-year survival of patients with melanoma to ~70%, and overall survival (>5years) to ~30%.

[0131] Likewise, checkpoint inhibitors have been tested in clinical trials for the treatment of head and neck cancer. In preclinical studies, it had been shown that that 45-80 % of HNSCC tumors express programmed death ligand 1 (PD-L1) (Zandberg et al. (2014) Oral Oncol. 50:627-632). Currently there are dozens of clinical trials evaluating the efficacy and safety of immune checkpoint inhibitors as monotherapy or in combination regimens in HNSCC. For example, clinical trials with PD 1, PD-L1, and CTLA-4 inhibitors are being tested in HNSCC. Data that the PD-1 antibody pembrolizumab might be effective in metastatic/recurrent (R/M) HNSCC patients were generated in the phase 1b Keynote-012 phase I/II trial (Cheng. ASCO 2015, oral presentation). More recently the data of the randomized CheckMate-141 phase III clinical trial were presented (Gillison. AACR 2016, oral presentation). This study investigated the efficacy of the monoclonal PD-1 antibody nivolumab given every 2 weeks in platinum-refractory R/M HNSCC patients. The study was stopped early due to superiority of the nivolumab arm of the study.

[0132] In one aspect, the subject has been previously treated with a PD-1 antagonist prior to the polynucleotide of the present disclosure. In another aspect, the subject has been treated with a monoclonal antibody that binds to PD-1 prior to the polynucleotide of the present disclosure. In another aspect, the subject has been treated with an anti-PD-1 monoclonal antibody therapy prior to the polynucleotide of the present methods or compositions. In other aspects, the anti-PD-1 monoclonal antibody therapy comprises nivolumab, pembrolizumab, pidilizumab, or any combination thereof. In another aspect, the subject has been treated with a monoclonal antibody that binds to PD-L1 prior to the polynucleotide of the present disclosure. In another aspect, the subject has been treated with an anti-PD-L1 monoclonal antibody therapy prior to the polynucleotide of the present methods or compositions. In other aspects, the anti-PD-Ll monoclonal antibody therapy comprises durvalumab, avelumab, MEDI473, BMS-936559, aezolizumab, or any combination thereof.

[0133] In some aspects, the subject has been treated with a CTLA-4 antagonist prior to treatment with the compositions of present disclosure. In another aspect, the subject has been previously treated with a monoclonal antibody that binds to CTLA-4 prior to the compositions of the present disclosure. In another aspect, the subject has been treated with an anti-CTLA-4 monoclonal antibody prior to the polynucleotide of the present disclosure. In other aspects, the anti-CTLA-4 antibody therapy comprises ipilimumab or tremelimumab.

[0134] In some aspects, the disclosure is directed to a method of treating cancer and/or a method of immunotherapy in a subject in need thereof comprising administering to the subject (*e.g.,* intratumorally, intraperitoneally, or intravenously) the polynucleotide (*e.g.,* RNA, *e.g.,* mRNA) encoding an IL12 polypeptide in combination with a PD-L1 antagonist, *e.g.,* an antibody or antigen-binding portion thereof that specifically binds to PD-L1, *e.g.,* an anti-PD-Ll monoclonal antibody, *e.g.,* an anti-PD-Ll monoclonal antibody comprises Durvalumab, Avelumab, MEDI473, BMS-936559, Atezolizumab, or any combination thereof.

[0135] In certain cases, the anti-PD-LI antibody useful for the disclosure is MSB0010718C (also called Avelumab; See US 2014/0341917) or BMS-936559 (formerly 12A4 or MDX-1105) (see, *e.g.,* U.S. Patent No. 7,943,743; WO 2013/173223). In other cases, the anti-PD-LI antibody is atezolizumab (also known as TECENTRIQ®). In other cases, the anti-PD-LI antibody is MPDL3280A (also known as atezolizumab, TECENTRIQ®, and RG7446) (see, *e.g.,* Herbst et al. (2013) J Clin Oncol 31(suppl):3000. Abstract; U.S. Patent No. 8,217,149), MEDI4736 (also called Durvalumab; Khleif (2013) In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands.

[0136] In some aspects, the disclosure is directed to a method of treating cancer and/or a method of immunotherapy in a subject in need thereof comprising administering to the subject (*e.g.,* intratumorally, intraperitoneally, or intravenously) the polynucleotide (*e.g.,* RNA, *e.g.,* mRNA) encoding an IL12 polypeptide, in combination with a PD-1 antagonist, *e.g.,* an antibody or antigen-binding portion thereof that specifically binds to PD-1, *e.g.,* ananti-PD-1 monoclonal antibody.

[0137] In one case, the anti-PD-1 antibody (or an antigen-binding portion thereof) useful for the disclosure is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent No. 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14, 2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma and advanced NSCLC.

[0138] In another case, the anti-PD-1 antibody useful for the disclosure is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang et al., 2014 Cancer Immunol Res. 2(9):846-56). Nivolumab has shown activity in a variety of advanced solid tumors including renal cell carcinoma (renal adenocarcinoma, or hypernephroma), melanoma, and non-small cell lung cancer (NSCLC) (Topalian et al., 2012a; Topalian et al., 2014; Drake et al., 2013; WO 2013/173223.

[0139] In other cases, the anti-PD-1 antibody is MEDI0680 (formerly AMP-514), which is a monoclonal antibody against the PD-1 receptor. MEDI0680 is described, for example, in U.S. Patent No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

[0140] In certain cases, the anti-PD-1 antibody is BGB-A317, which is a humanized monoclonal antibody. BGB-A317 is described in U.S. Publ. No. 2015/0079109.

[0141] In certain cases, a PD-1 antagonist is AMP-224, which is a B7-DC Fc fusion protein. AMP-224 is discussed in U.S. Publ. No. 2013/0017199 or in http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=7005 95 (last accessed July 8, 2015).

[0142] In other cases, the disclosure includes a method of treating cancer and/or a method of immunotherapy in a subject in need thereof comprising administering to the subject (*e.g.,* intratumorally, intraperitoneally, or intravenously) the polynucleotide (*e.g.,* RNA, *e.g.,* mRNA) encoding an IL12 polypeptide together with an antibody or an antigen binding portion thereof that specifically binds to PD-1, *e.g.,* an anti-PD-1 monoclonal antibody, *e.g.,* an anti-PD-1 monoclonal antibody comprises Nivolumab, Pembrolizumab, Pidilizumab, or any combination thereof.

[0143] In other aspects, the disclosure is directed to a method of treating cancer and/or a method of immunotherapy in a subject in need thereof comprising administering to the subject (*e.g.,* intratumorally, intraperitoneally, or intravenously) the polynucleotide (*e.g.,* RNA, *e.g.,* mRNA) encoding an IL12 polypeptide in combination with a CTLA-4 antagonist, *e.g.,* an antibody or antigen-binding portion thereof that specifically binds to CTLA-4, *e.g.,* an anti-CTLA-4 monoclonal antibody, *e.g.,* an anti-CTLA-4 monoclonal antibody comprises Ipilimumab or Tremelimumab, or any combination thereof.

[0144] An exemplary clinical anti-CTLA-4 antibody is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Another anti-CTLA-4 antibody useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

[0145] In one case, a first polynucleotide (e.g. a first mRNA) encoding IL12 and a second polynucleotide (*e.g.,* a second mRNA) encoding an OX40L polypeptide are administered in combination. In another case, the first polynucleotide (e.g. a first mRNA) encoding IL12 and the second polynucleotide (e.g. a second mRNA) encoding an OX40L polypeptide are administered in combination with an antibody or an antigen-binding portion thereof which specifically binds to CTLA-4, an antibody or antigen-binding portion thereof which specifically binds to a PD-1 receptor, or an antibody or antigen-binding portion thereof which specifically binds to a PD-L1 receptor.

[0146] In one case, a first polynucleotide (e.g. a first mRNA) encoding an IL12 polypeptide and a second polynucleotide (e.g. a second mRNA) encoding an OX40L polypeptide are administered in combination with an antibody or an antigen-binding portion thereof that specifically binds to a PD-1 or PD-L1 receptor or a polynucleotide encoding the same.

[0147] In another case, a first polynucleotide (e.g. a first mRNA) encoding an IL12 polypeptide and a second polynucleotide (e.g. a second mRNA) encoding an OX40L polypeptide are administered in combination with an antibody or an antigen-binding portion thereof that specifically binds to a CTLA-4 or a polynucleotide encoding the same.

[0148] In yet another case, a first polynucleotide (e.g. a first mRNA) encoding an IL12 polypeptide and a second polynucleotide (e.g. a second mRNA) encoding an OX40L polypeptide are administered in combination with an antibody or an antigen-binding portion thereof that specifically binds to a PD-1 or PD-L1 receptor and an antibody or an antigen-binding portion thereof that specifically binds to a CTLA-4 (or polynucleotides of the same).

[0149] In some cases, the compositions disclosed herein comprise (i) a first polynucleotide (e.g. a first mRNA) encoding IL12 and (ii) a second polynucleotide (e.g. a second mRNA) encoding an antibody or an antigen binding portion thereof which specifically binds to CTLA-4 in a single formulation.

[0150] In some cases, the compositions disclosed herein comprise a polynucleotide encoding IL12 and a polynucleotide encoding an OX40L protein in a single formulation.

[0151] Human OX40L was first identified on the surface of human lymphocytes infected with human T-cell leukemia virus type-I (HTLV-I) by Tanaka *et al.* (Tanaka et al., International Journal of Cancer (1985), 36(5):549-55). OX40L is the ligand for OX40 (CD134). OX40L has also been designated CD252 (cluster of differentiation 252), tumor necrosis factor (ligand) superfamily, member 4, tax-transcriptionally activated glycoprotein 1, TXGP1, or gp34. Human OX40L is 183 amino acids in length and contains three domains: a cytoplasmic domain of amino acids 1 - 23; a transmembrane domain of amino acids 24 - 50, and an extracellular domain of amino acids 51 - 183.

[0152] In some cases, a polynucleotide encoding OX40L that can be combined with the polynucleotide encoding IL12 comprises an mRNA encoding a mammalian OX40L polypeptide. In some cases, the mammalian OX40L polypeptide is a murine OX40L polypeptide. In some cases, the mammalian OX40L polypeptide is a human OX40L polypeptide. In some cases, the OX40L polypeptide comprises an amino acid sequence set forth in **Table 1.**

[0153] In some cases, each polynucleotide of the disclosure comprises an mRNA, *i.e.,* an mRNA encoding an IL12 polypeptide and an mRNA encoding an OX40L polypeptide. In some cases, the mRNA encoding an IL12 polypeptide encodes a mammalian IL12 polypeptide. In some cases, the mRNA encoding an OX40L polypeptide encodes a mammalian OX40L polypeptide. In some cases, the mRNA encoding an IL12 polypeptide encodes a murine IL12 polypeptide. In some cases, the mRNA encoding an OX40L polypeptide encodes a murine OX40L polypeptide. In some cases, the mRNA encoding an IL12 polypeptide encodes a human IL12 polypeptide. In some cases, the mRNA encoding an OX40L polypeptide encodes a human OX40L polypeptide.

[0154] In some cases, the IL12 polypeptide comprises a human amino acid sequence set forth in **Table 4**. In other cases, the OX40L polypeptide comprises a human amino acid sequence set forth in **Table 1.**

[0155] In some cases, the OX40L polypeptide comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence listed in **Table 1** or an amino acid sequence encoded by a nucleotide sequence listed in **Table 1**, wherein the amino acid sequence is capable of binding to an OX40 receptor.

[0156] In certain cases, the OX40L polypeptide encoded by a polynucleotide of the disclosure comprises an amino acid sequence listed in **Table b**1 with one or more conservative substitutions, wherein the conservative substitutions do not significantly affect the binding activity of the OX40L polypeptide to its receptor, *i.e.,* the OX40L polypeptide binds to the OX40 receptor after the substitutions.

[0157] In other cases, a nucleotide sequence (*i.e.,* mRNA) encoding an OX40L polypeptide comprises a sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to a nucleic acid sequence listed in **Table 1**. One skilled in the art would know that if a sequence is written in DNA form (containing thymidine) in the present application, the corresponding RNA sequence would contain uridine instead of thymidine.

[0158] In some cases, the polynucleotide (*e.g.,* mRNA) useful for the methods and compositions comprises an open reading frame encoding an extracellular domain of OX40L. In other cases, the polynucleotide (*e.g.,* mRNA) comprises an open reading frame encoding a cytoplasmic domain of OX40L. In some cases, the polynucleotide (*e.g.,* mRNA) comprises an open reading frame encoding a transmembrane domain of OX40L. In certain cases, the polynucleotide (*e.g.,* mRNA) comprises an open reading frame encoding an extracellular domain of OX40L and a transmembrane of OX40L. In other cases, the polynucleotide (*e.g.,* mRNA) comprises an open reading frame encoding an extracellular domain of OX40L and a cytoplasmic domain of OX40L. In yet other cases, the polynucleotide (*e.g.,* mRNA) comprises an open reading frame encoding an extracellular domain of OX40L, a transmembrane of OX40L, and a cytoplasmic domain of OX40L.

[0159] **Table 1** presents, *e.g.,* precursor and mature sequences for OX40L as well as constructs comprising the OX40L sequences. Furthermore, a construct comprising a polynucleotide encoding OX40L and further comprising components such 3' UTR and 5' UTR would be considered an OX40L encoding polynucleotide. A person of skill in the art would understand that in addition to the native signal sequences and propeptide sequences disclosed in **Table 1** (sequences present in the precursor for and absent in the mature corresponding form) and the non-native signal peptide disclosed in **Table 1**, other signal sequences can be used. Accordingly, references to an OX40L polypeptide or polynucleotide according to **Table 1** encompass variants in which an alternative signal peptide (or encoding sequence) known in the art has been attached to the OX40L polypeptide (or polynucleotide). It is also understood that references to the sequences

disclosed in **Table 1** through the application are equally applicable and encompass orthologs and functional variants (for example polymorphic variants) and isoforms of those sequences kno n in the art at the time the application was filed.

Table 1: OX40L Polypeptide and Polynucleotide sequences

Encoded Polypeptide	Description	Sequence	SEQ ID NO:
OX40L (TNFSF4)	Tumor necrosis factor ligand superfamily member 4 isoform 1 [Homo sapiens] NP 003317	MERVQPLEENVGNAARPRFERNKLLLVASVIQGLGLLLCFTYICLHFSA LQVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINCD GFYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKD KVYLNVTTDNTSLDDFHVNGGELILIHQNPGEFCVL	SEQ ID NO: 178 183 aa
OX40L (TNFSF4)	TNFSF4 isoform 2 [Homo sapiens] NP_ 00128449 1	MVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINCDG FYLISLKGYFSQEVNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKDK VYLNVTTDNTSLDDFHVNGGELILIHQNPGEFCVL	SEQ ID NO: 179 133 aa
OX40L (TNFSF4)	TNFSF4 [Mus musculus] NP_033478	MEGEGVQPLDENLENGSRPRFKWKKTLRLVVSGIKGAGMLLCFIYVCLQ LSSSPAKDPPIQRLRGAVTRCEDGQLFISSYKNEYQTMEVQNNSVVIKC DGLYIIYLKGSFFQEVKIDLHFREDHNPISIPMLNDGRRIVFTVVASLA FKDKVYLTVNAPDTLCEHLQINDGELIVVQLTPGYCAPEGSYHSTVNQV PL	SEQ ID NO: 180 198 aa
OX40L (TNFSF4)	TNFSF4, ORF [Homo sapiens]	AUGGAAAGGGUCCAACCCCUGGAAGAGAAUGUG GGAAAUGCAGCCAGGCCAAGAUUCGAGAGGAAC AAGCUAUUGCUGGUGGCCUCUGUAAUUCAGGGA CUGGGGCUGCUCCUGUGCUUCACCUACAUCUGC CUGCACUUCUCUGCUCUUCAGGUAUCACAUCGG UAUCCUCGAAUUCAAAGUAUCAAAGUACAAUUU ACCGAAUAUAAGAAGGAGAAAGGUUUCAUCCUC ACUUCCCAAAAGGAGGAUGAAAUCAUGAAGGUG CAGAACAACUCAGUCAUCAUCAACUGUGAUGGG UUUUAUCUCAUCUCCCUGAAGGCUACUUCUCC CAGGAAGUCAACAUUAGCCUUCAUUACCAGAAG	SEQ ID NO: 181 552nts
		GAUGAGGAGCCCCUCUUCCAACUGAAGAAGGUC AGGUCUGUCAACUCCUUGAUGGUGGCCUCUCUG ACUUACAAAGACAAAGUCUACUUGAAUGUGACC ACUGACAAUACCUCCCUGGAUGACUUCCAUGUG AAUGGCGGAGAACUGAUUCUUAUCCAUCAAAAU CCUGGUGAAUUCUGUGUCCUU	

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
OX40L (TNFSF4)	TNFSF4, transcript variant 1, mRNA NM_003326	GGCCCUGGGACCUUUGCCUAUUUUCUGAUUGAU AGGCUUUGUUUUGUCUUUACCUCCUUCUUUCUG GGGAAAACUUCAGUUUUAUCGCACGUUCCCCUU UUCCAUAUCUUCAUCUUCCCUCUACCCAGAUUG UGAAGAUGGAAAGGGUCCAACCCCUGGAAGAGA AUGUGGGAAAUGCAGCCAGGCCAAGAUUCGAGA GGAACAAGCUAUUGCUGGUGGCCUCUGUAAUUC AGGGACUGGGGCUGCUCCUGUGCUUCACCUACA UCUGCCUGCACUUCUCUGCUCUUCAGGUAUCAC AUCGGUAUCCUCGAAUUCAAAGUAUCAAAGUAC AAUUUACCGAAUAUAAGAAGGAGAAAGGUUUCA UCCUCACUUCCCAAAAGGAGGAUGAAAUCAUGA AGGUGCAGAACAACUCAGUCAUCAUCAACUGUG AUGGGUUUUAUCUCAUCUCCCUGAAGGGCUACU UCUCCCAGGAAGUCAACAUUAGCCUUCAUUACC AGAAGGAUGAGGAGCCCCUCUUCCAACUGAAG AAGGUCAGGUCUGUCAACUCCUUGAUGGUGGC CUCUCUGACUUACAAAGACAAAGUCUACUUGA AUGUGACCACUGACAAUACCUCCCUGGAUGAC UUCCAUGUGAAUGGCGGAGAACUGAUUCUUAU CCAUCAAAAUCCUGGUGAAUUCUGUGUCCUUU GAGGGGCUGAUGGCAAUAUCUAAAACCAGGCA CCAGCAUGAACACCAAGCUGGGGGUGGACAGG GCAUGGAUUCUUCAUUGCAAGUGAAGGAGCCU CCCAGCUCAGCCACGUGGGAUGUGACAAGAAG CAGAUCCUGGCCCUCCCGCCCCCACCCCUCAG GGAUAUUUAAAACUUAUUUUAUAUACCAGUUA AUCUUAUUUAUCCUUAUAUUUUCUAAAUUGCC UAGCCGUCACACCCCAAGAUUGCCUUGAGCCU ACUAGGCACCUUUGUGAGAAAGAAAAAAUAGA UGCCUCUUCUUCAAGAUGCAUUGUUUCUAUUG GUCAGGCAAUUGUCAUAAUAAACUUAUGUCAU UGAAAACGGUACCUGACUACCAUUUGCUGGAA AUUUGACAUGUGUGUGGCAUUAUCAAAAUGAA GAGGAGCAAGGAGUGAAGGAGUGGGGUUAUGA AUCUGCCAAAGGUGGUAUGAACCAACCCCUGG AAGCCAAAGCGGCCUCUCCAAGGUUAAAUUGA UUGCAGUUUGCAUAUUGCCUAAAUUUAAACUU UCUCAUUUGGUGGGGGUUCAAAAGAAGAAUCA GCUUGUGAAAAAUCAGGACUUGAAGAGAGCCG UCUAAGAAAUACCACGUGCUUUUUUUCUUUAC CAUUUUGCUUUCCCAGCCUCCAAACAUAGUUA AUAGAAAUUUCCCUUCAAAGAACUGUCUGGGG AUGUGAUGCUUUGAAAAAUCUAAUCAGUGACU UAAGAGAGAUUUUCUUGUAUACAGGGAGAGUG AGAUAACUUAUUGUGAAGGGUUAGCUUUACUG UACAGGAUAGCAGGGAACUGGACAUCUCAGGG UAAAAGUCAGUACGGAUUUUAAUAGCCUGGGG AGGAAAACACAUUCUUUGCCACAGACAGGCAA AGCAACACAUGCUCAUCCUCCUGCCUAUGCUG	SEQ ID NO: 182 3484 nts

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
		AGAUACGCACUCAGCUCCAUGUCUUGUACACA CAGAAACAUUGCUGGGUUUCAAGAAAUGAGGUG AUCCUAUUAUCAAAUUCAAUCUGAUGUCAAAU AGCACUAAGAAGUUAUUGUGCCUUAUGAAAAA UAAUGAUCUCUGUCUAGAAAUACCAUAGACCA UAUAUAGUCUCACAUUGAUAAUUGAAACUAGA AGGGUCUAUAAUCAGCCUAUGCCAGGGCUUCA AUGGAAUAGUAUCCCCUUAUGUUUAGUUGAAA UGUCCCCUUAACUUGAUAUAAUGUGUUAUGCU UAUGGCGCUGUGGACAAUCUGAUUUUUCAUGU CAACUUUCCAGAUGAUUUGUAACUUCUCUGUG CCAAACCUUUUAUAAACAUAAAUUUUUGAGAU AUGUAUUUUAAAAUUGUAGCACAUGUUUCCCU GACAUUUUCAAUAGAGGAUACAACAUCACAGA AUCUUUCUGGAUGAUUCUGUGUUAUCAAGGAA UUGUACUGUGCUACAAUUAUCUCUAGAAUCUC CAGAAAGGUGGAGGGCUGUUCGCCCUUACACU AAAUGGUCUCAGUUGGAUUUUUUUUUCCUGUU UUCUAUUUCCUCUUAAGUACACCUUCAACUAU AUUCCCAUCCCUCUAUUUUAAUCUGUUAUGAA GGAAGGUAAAUAAAAAUGCUAAAUAGAAGAAA UUGUAGGUAAGGUAAGAGGAAUCAAGUUCUGA GUGGCUGCCAAGGCACUCACAGAAUCAUAAUC AUGGCUAAAUAUUUAUGGAGGGCCUACUGUGG ACCAGGCACUGGGCUAAAUACUUACAUUUACA AGAAUCAUUCUGAGACAGAUAUUCAAUGAUAU CUGGCUUCACUACUCAGAAGAUUGUGUGUGUG UUUGUGUGUGUGUGUGUGUGUGUAUUUCACUU UUUGUUAUUGACCAUGUUCUGCAAAAUUGCAG UUACUCAGUGAGUGAUAUCCGAAAAAGUAAAC GUUUAUGACUAUAGGUAAUAUUUAAGAAAAUG CAUGGUUCAUUUUUAAGUUUGGAAUUUUUAUC UAUAUUUCUCACAGAUGUGCAGUGCACAUGCA GGCCUAAGUAUAUGUUGUGUGUGUUGUUUGUC UUUGAUGUCAUGGUCCCCUCUCUUAGGUGCUC ACUCGCUUUGGGUGCACCUGGCCUGCUCUUCC CAUGUUGGCCUCUGCAACCACACAGGGAUAUU UCUGCUAUGCACCAGCCUCACUCCACCUUCCU UCCAUCAAAAAUAUGUGUGUGUGUCUCAGUCC CUGUAAGUCAUGUCCUUCACAGGGAGAAUUAA CCCUUCGAUAUACAUGGCAGAGUUUUGUGGGA AAAGAAUUGAAUGAAAAGUCAGGAGAUCAGAA UUUUAAAUUUUGACUUAGCCACUAACUAGCCAU GUAACCUUGGGAAAGUCAUUUCCCAUUUCUGG GUCUUGCUUUUCUUUCUGUUAAAUGAGAGGAA UGUUAAAUAUCUAACAGUUUAGAAUCUUAUGC UUACAGUGUUAUCUGUGAAUGCACAUAUUAAA UGUCUAUGUUCUUGUUGCUAUGAGUCAAGGAG UGUAACCUUCUCCUUUACUAUGUUGAAUGUAU UUUUUUCUGGACAAGCUUACAUCUUCCUCAGC CAUCUUUGUGAGUCCUUCAAGAGCAGUUAUCA AUUGUUAGUUAGAUAUUUUCUAUUUAGAGAAU GCUUAAGGGAUUCCAAUCCCGAUCCAAAUCAU AAUUUGUUCUUAAGUAUACUGGGCAGGUCCCC UAUUUUAAGUCAUAAUUUUGUAUUUAGUGCUU UCCUGGCUCUCAGAGAGUAUUAAUAUUGAUAU	

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
		UAAUAAUAUAGUUAAUAGUAAUAUUGCUAUUU ACAUGGAAACAAAUAAAAGAUCUCAGAAUUCA CUAAAAAAAAAAA	
OX40L (TNFSF4)	*Mus musculus* Tnfsf4, mRNA NM_ 009452	AUUGCUUUUUGUCUCCUGUUCUGGGACCUUUA UCUUCUGACCCGCAGGCUUGACUUUGCCCUUA UUGGCUCCUUUGUGGUGAAGAGCAGUCUUCCC CCAGGUUCCCCGCCACAGCUGUAUCUCCUCUG CACCCCGACUGCAGAGAUGGAAGGGGAAGGGG UUCAACCCCUGGAUGAGAAUCUGGAAAACGGA UCAAGGCCAAGAUUCAAGUGGAAGAAGACGCU AAGGCUGGUGGUCUCUGGGAUCAAGGGAGCAG GGAUGCUUCUGUGCUUCAUCUAUGUCUGCCUG CAACUCUCUUCCUCUCCGGCAAAGGACCCUCC AAUCCAAAGACUCAGAGGAGCAGUUACCAGAU GUGAGGAUGGGCAACUAUUCAUCAGCUCAUAC AAGAAUGAGUAUCAAACUAUGGAGGUGCAGAA CAAUUCGGUUGUCAUCAAGUGCGAUGGGCUUU AUAUCAUCUACCUGAAGGGCUCCUUUUUCCAG GAGGUCAAGAUUGACCUUCAUUUCCGGGAGGA UCAUAAUCCCAUCUCUAUUCCAAUGCUGAACG AUGGUCGAAGGAUUGUCUUCACUGUGGUGGCC UCUUUGGCUUUCAAAGAUAAAGUUUACCUGAC UGUAAAUGCUCCUGAUACUCUCUGCGAACACC UCCAGAUAAAUGAUGGGGAGCUGAUUGUUGUC CAGCUAACGCCUGGAUACUGUGCUCCUGAAGG AUCUUACCACAGCACUGUGAACCAAGUACCAC UGUGAAUUCCACUCUGAGGGUGGACGGGACAC AGGUUCUUUCUCGAGAGAGAUGAGUGCAUCCU GCUCAUGAGAUGUGACUGAAUGCAGAGCCUAC CCUACUUCCUCACUCAGGGAUAUUUAAAUCAU GUCUUACAUAACAGUUGACCUCUCAUUCCCAG GAUUGCCUUGAGCCUGCUAAGAGCUGUUCUGG GAAUGAAAAAAAAAAUAAAUGUCUCUUCAAGA CACAUUGCUUCUGUCGGUCAGAAGCUCAUCGU AAUAAACAUCUGCCACUGAAAAUGGCGCUUGA UUGCUAUCUUCUAGAAUUUUGAUGUUGUCAAA AGAAAGCAAAACAUGGAAAGGGUGGUGUCCAC CGGCCAGUAGGAGCUGGAGUGCUCUCUUCAAG GUUAAGGUGAUAGAAGUUUACAUGUUGCCUAA AACUGUCUCUCAUCUCAUGGGGGGCUUGGAAA GAAGAUUACCCCGUGGAAAGCAGGACUUGAAG AUGACUGUUUAAGCAACAAGGUGCACUCUUUU CCUGGCCCCUGAAUACACAUAAAAGACAACUU CCUUCAAAGAACUACCUAGGGACUAUGAUACC CACCAAAGAACCACGUCAGCGAUGCAAAGAAA ACCAGGAGAGCUUUGUUUAUUUUGCAGAGUAU ACGAGAGAUUUUACCCUGAGGGCUAUUUUUAU UAUACAGGAUGAGAGUGAACUGGAUGUCUCAG GAUAAAGGCCAAGAAGGAUUUUUCACAGUCUG AGCAAGACUGUUUUUGUAGGUUCUCUCUCCAA AACUUUUAGGUAAAUUUUUGAUAAUUUUAAAA UUUUUAGUUAUAUUUUUGGACCAUUUUCAAUA GAAGAUUGAAACAUUUCCAGAUGGUUUCAUAU CCCCACAAG	SEQ ID NO: 183 1609 nts

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
Human OX40L	mRNA sequence:	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGA AAUAUAAGAGCCACCAUGGAAAGGGUCCAACC	SEQ ID NO: 184
	Human OX40L with 5'-UTR, 3'-UTR, and miR-122 binding site	CCUGGAAGAGAAUGUGGGAAAUGCAGCCAGGC CAAGAUUCGAGAGGAACAAGCUAUUGCUGGUG GCCUCUGUAAUUCAGGGACUGGGGCUGCUCCU GUGCUUCACCUACAUCUGCCUGCACUUCUCUG CUCUUCAGGUAUCACAUCGGUAUCCUCGAAUU CAAAGUAUCAAAGUACAAUUUACCGAAUAUAA GAAGGAGAAAGGUUUCAUCCUCACUUCCCAAA AGGAGGAUGAAAUCAUGAAGGUGCAGAACAAC UCAGUCAUCAUCAACUGUGAUGGGUUUUAUCU CAUCUCCCUGAAGGGCUACUUCUCCCAGGAAG UCAACAUUAGCCUUCAUUACCAGAAGGAUGAG GAGCCCCUCUUCCAACUGAAGAAGGUCAGGUC UGUCAACUCCCUUGAUGGUGGCCUCUCUGACUU ACAAAGACAAAGUCUACUUGAAUGUGACCACU GACAAUACCUCCCCUGGAUGACUUCCAUGUGAA UGGCGGAGAACUGAUUCUUAUCCAUCAAAAUC CUGGUGAAUUCUGUGUCCUUUGAUAAUAGGCU GGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUG GGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGC ACCCGUACCCCCCAAACACCAUUGUCACACUC CAGUGGUCUUUGAAUAAAGUCUGAGUGGGCGG C	
Murine OX40L	mRNA sequence: murine OX40L with 5'-UTR, 3'-UTR, and miR-122 binding site	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAA AUAUAAGAGCCACCAUGGAAGGGGAAGGGGUUC AACCCCUGGAUGAGAAUCUGGAAAACGGAUCAA GGCCAAGAUUCAAGUGGAAGAAGACGCUAAGGC UGGUGGUCUCCUGGGAUCAAGGGAGCAGGGAUGC UUCUGUGCUUCAUCUAUGUCUGCCUGCAACUCU CUUCCUCUCCGGCAAAGGACCCUCCAAUCCAAA GACUCAGAGGAGCAGUUACCAGAUGUGAGGAUG GGCAACUAUUCAUCAGCUCAUACAAGAAUGAGU AUCAAACUAUGGAGGUGCAGAACAAUUCGGUUG UCAUCAAGUGCGAUGGGCUUUAUAUCAUCUACC UGAAGGGCUCCUUUUUCCAGGAGGUCAAGAUUG ACCUUCAUUUCCGGGAGGAUCAUAAUCCCAUCU CUAUUCCAAUGCUGAACGAUGGUCGAAGGAUUG UCUUCACUGUGGUGGCCUCUUUGGCUUUCAAAG AUAAAGUUUACCUGACUGUAAAUGCUCCUGAUA CUCUCUGCGAACACCUCCAGAUAAAUGAUGGGG AGCUGAUUGUUGUCCAGCUAACGCCUGGAUACU GUGCUCCUGAAGGAUCUUACCACAGCACUGUGA ACCAAGUACCACUGUGAUAAUAGGCUGGAGCCU CGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCC CCCAGCCCCUCCUCCCCCUUCCUGCACCCGUACC CCCCAAACACCAUUGUCACACUCCAGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGC	SEQ ID NO: 185

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
hOX40L miR-122	Codon optimized human OX40L sequences	ATGGAAAGGGTCCAACCCCTGGAAGAGAATGTGGGAAATGCAGCCAGGC CAAGATTCGAGAGGAACAAGCTATTGCTGGTGGCCTCTGTAATTCAGGG ACTGGGGCTGCTCCTGTGCTTCACCTACATCTGCCTGCACTTCTCTGCT CTTCAGGTATCACATCGGTATCCTCGAATTCAAAGTATCAAAGTACAAT TTACCGAATATAAGAAGGAGAAAGGTTTCATCCTCACTTCCCAAAAGGA GGATGAAATCATGAAGGTGCAGAACAACTCAGTCATCATCAACTGTGAT GGGTTTTATCTCATCTCCCTGAAGGGCTACTTCTCCCAGGAAGTCAACA TTAGCCTTCATTACCAGAAGGATGAGGAGCCCCTCTTCCAACTGAAGAA	SEQ ID NO: 186
		GGTCAGGTCTGTCAACTCCTTGATGGTGGCCTCTCTGACTTACAAAGAC AAAGTCTACTTGAATGTGACCACTGACAATACCTCCCTGGATGACTTCC ATGTGAATGGCGGAGAACTGATTCTTATCCATCAAAATCCTGGTGAATT CTGTGTCCTT	
mOX40L + miR-122	Codon optimized mouse OX40L sequences	ATGGAAGGGGAAGGGGTTCAACCCCTGGATGAGAATCTGGAAAACGGAT CAAGGCCAAGATTCAAGTGGAAGAAGACGCTAAGGCTGGTGGTCTCTGG GATCAAGGGAGCAGGGATGCTTCTGTGCTTCATCTATGTCTGCCTGCAA CTCTCTTCCTCTCCGGCAAAGGACCCTCCAATCCAAAGACTCAGAGGAG CAGTTACCAGATGTGAGGATGGGCAACTATTCATCAGCTCATACAAGAA TGAGTATCAAACTATGGAGGTGCAGAACAATTCGGTTGTCATCAAGTGC GATGGGCTTTATATCATCTACCTGAAGGGCTCCTTTTTCCAGGAGGTCA AGATTGACCTTCATTTCCGGGAGGATCATAATCCCATCTCTATTCCAAT GCTGAACGATGGTCGAAGGATTGTCTTCACTGTGGTGGCCTCTTTGGCT TTCAAAGATAAAGTTTACCTGACTGTAAATGCTCCTGATACTCTCTGCG AACACCTCCAGATAAATGATGGGGAGCTGATTGTTGTCCAGCTAACGCC TGGATACTGTGCTCCTGAAGGATCTTACCACAGCACTGTGAACCAAGTA CCACTG	SEQ ID NO: 187
OX40L (TNFSF4)	Codon-optimized sequence 1 for ENSP 281834	AUGGAGAGAGUGCAGCCCCUGGAGGAGAACGUG GGCAACGCCGCCAGACCCAGAUUCGAGAGAAAC AAGCUGCUGCUGGUGGCCAGCGUGAUCCAGGGC CUGGGCCUGCUGCUGUGCUUCACCUACAUCUGC CUGCACUUCAGCGCCCUGCAGGUGAGCCACAGA UACCCCAGAAUCCAGAGCAUCAAGGUGCAGUUC ACCGAGUACAAGAAGGAGAAGGGCUUCAUCCUG ACCAGCCAGAAGGAGGACGAGAUCAUGAAGGUG CAGAACAACAGCGUGAUCAUCAACUGCGACGGC UUCUACCUGAUCAGCCUGAAGGGCUACUUCAGC CAGGAGGUGAACAUCAGCCUGCACUACCAGAAG GACGAGGAGCCCCUGUUCCAGCUGAAGAAGGUG AGAAGCGUGAACAGCCUGAUGGUGGCCAGCCUG ACCUACAAGGACAAGGUGUACCUGAACGUGACC ACCGACAACACCAGCCUGGACGACUUCCACGUG AACGGCGGCGAGCUGAUCCUGAUCCACCAGAAC CCCGGCGAGUUCUGCGUGCUG	SEQ ID NO: 188

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
OX40L (TNFSF4)	Codon-optimized sequence 2 for ENSP 281834	AUGGAGCGUGUGCAGCCUCUUGAGGAGAAUGUG GGAAAUGCAGCCCGGCCUCGAUUCGAACGUAAU AAACUCCUGCUCGUGGCCUCCGUGAUCCAGGGU CUCGGUUUAUUGCUGUGUUUUACCUAUAUAUGC UUACACUUUAGUGCAUUACAGGUCUCACACCGG UACCCUCGCAUUCAGUCUAUAAAAGUGCAGUUU ACCGAGUAUAAGAAGGAGAAAGGUUUUAUACUG ACUUCUCAGAAAGAGGACGAGAUCAUGAAGGUG CAGAAUAAUAGCGUCAUUAUCAACUGCGAUGGA UUCUAUCUAAUUUCCCUAAAGGGGUACUUCAGC CAGGAGGUCAAUAUAUCACUGCACUAUCAAAAG GACGAGGAGCCCCUGUUUCAACUGAAGAAAGUG CGAUCAGUUAACUCUCUGAUGGUUGCCUCUCUG ACCUAUAAGGACAAAGUCUACUUGAACGUGACA ACUGACAACACCUCACUGGAUGACUUUCAUGUG AAUGGGGGGGAACUGAUUCUUAUCCAUCAGAAU CCAGGAGAAUUCUGUGUGCUC	SEQ ID NO: 189
OX40L (TNFSF4)	Codon-optimized sequence 3 for ENSP 281834	AUGGAGCGGGUGCAGCCCCUGGAGGAGAAUGUG GGCAAUGCUGCCCGGCCCAGGUUUGAAAGAAAC AAGCUGCUGCUGGUGGCCAGCGUCAUCCAGGGC CUGGGCCUGCUGCUGUGCUUCACCUACAUCUGC CUGCACUUCAGCGCCCUGCAGGUGAGCCACCGC	SEQ ID NO: 190
		UACCCCCGCAUCCAGAGCAUCAAGGUGCAGUUC ACAGAGUACAAGAAGGAGAAGGGCUUCAUCCUG ACCAGCCAGAAGGAGGAUGAGAUCAUGAAGGUG CAGAACAACAGCGUCAUCAUCAACUGUGAUGGC UUCUACCUGAUCAGCCUGAAGGGCUACUUCAGC CAGGAGGUGAACAUCAGCCUGCACUACCAGAAG GAUGAGGAGCCCCUCUUCCAGCUGAAGAAGGUG CGCUCUGUGAACAGCCUGAUGGUGGCCAGCCUG ACCUACAAGGACAAGGUGUACCUGAAUGUGACC ACAGACAACACCAGCCUGGAUGACUUCCACGUG AAUGGAGGAGAGCUGAUCCUGAUCCACCAGAAC CCUGGAGAGUUCUGUGUGCUG	
OX40L (TNFSF4)	Codon-optimized sequence 4 for ENSP 281834	AUGGAGCGGGUGCAGCCCCUGGAGGAGAACGUG GGCAACGCCGCCCGCCCGCGUUUUGAGCGAAAU AAGUUACUGCUUGUUGCAUCUGUGAUACAGGGG UUGGGUUUACUUCUUUGCUUUACAUAUAUUUGU CUCCACUUUAGUGCGCUUCAGGUAUCCCAUCGG UACCCGCGCAUCCAGUCAAUCAAGGUCCAGUUC ACUGAAUAUAAAAAAGGAGAAAGGAUUCAUUCUG ACUUCACAAAAAGAGGACGAAAUCAUGAAAGUG CAGAACAACUCUGUAAUUAUAAACUGCGAUGGG UUCUAUCUGAUCAGUCUGAAGGGAUAUUUUAGC CAGGAAGUAAAUAUUUCACUACAUUAUCAGAAG GACGAAGAACCACUUUUUCAACUGAAGAAAGUC CGGUCCGUGAACUCCCUGAUGGUUGCUAGCCUU ACCUACAAGGAUAAAGUCUAUUUAAACGUCACA ACAGAUAACACUAGCCUCGACGAUUUCCAUGUG AACGGAGGUGAACUGAUAUUGAUCCAUCAAAAC CCCGGCGAGUUCUGCGUUUUA	SEQ ID NO: 191

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
OX40L (TNFSF4)	Codon-optimized sequence 5 for ENSP 281834	AUGGAGCGGGUCCAGCCCCUCGAGGAGAACGUU GGUAAUGCCGCACGUCCCAGGUUUGAACGCAAC AAGCUGCUGUUGGUGGCCAGCGUCAUUCAGGGG CUGGGUUUGUUGCUGUGCUUCACUUACAUCUGU CUGCAUUUUAGUGCACUCCAGGUGUCCCACCGC UACCCCCGUAUCCAAUCCAUUAAAGUCCAAUUU ACCGAAUACAAAAAAGAGAAGGGUUUCAUUCUU ACCUCCCAGAAGGAGGAUGAAAUUAUGAAGGUG CAGAACAAUUCUGUUAUCAUCAACUGUGACGGA UUCUAUCUGAUUUCACUGAAGGGAUACUUUUCC CAGGAGGUGAACAUCAGUCUGCAUUAUCAGAAG GACGAAGAACCGCUUUUUCAACUGAAGAAGGUU AGGAGUGUGAACUCCCUGAAUGGUAGCCAGCCUG ACAUAUAAGGACAAGGUAUAUCUGAACGUCACC ACUGAUAACACCUCUUUAGACGAUUUUCAUGUA AAUGGGGGAGAAUUGAUACUCAUUCACCAGAA UCCGGGUGAGUUUUGUGUUCUG	SEQ ID NO: 192
OX40L (TNFSF4)	Codon-optimized sequence 1 for ENSP 356691	AUGGUGAGCCACAGAUACCCCAGAAUCCAGAGCA UCAAGGUGCAGUUCACCGAGUACAAGAAGGAGAA GGGCUUCAUCCUGACCAGCCAGAAGGAGGACGAG AUCAUGAAGGUGCAGAACAACAGCGUGAUCAUCA ACUGCGACGGCUUCUACCUGAUCAGCCUGAAGGG CUACUUCAGCCAGGAGGUGAACAUCAGCCUGCAC UACCAGAAGGACGAGGAGCCCCUGUUCCAGCUGA AGAAGGUGAGAAGCGUGAACAGCCUGAUGGUGGC CAGCCUGACCUACAAGGACAAGGUGUACCUGAAC GUGACCACCGACAACACCAGCCUGGACGACUUCC	SEQ ID NO: 193
		ACGUGAACGGCGGCGAGCUGAUCCUGAUCCACCA GAACCCCGGCGAGUUCUGCGUGCUG	
OX40L (TNFSF4)	Codon-optimized sequence 2 for ENSP 356691	AUGGUUUCUCACCGUUACCCACGGAUCCAGUCUA UCAAGGUUCAGUUUACCGAGUACAAAAAGGAAAA AGGGUUCAUCCUCACCUCUCAGAAAGAGGACGAA AUCAUGAAGGUGCAGAAUAACUCUGUAAUCAUUA AUUGCGACGGUUUUUAUCUGAUUUCACUGAAGGG CUACUUUAGUCAGGAAGUUAAUAUUAGUUUGCAC UACCAAAAGGACGAGGAGCCUCUCUUCCAACUAA AAAAGGUAAGAUCCGUUAAUUCCCUUAUGGUGGC CUCCUUAACUUAUAAGGACAAGGUGUAUCUGAAU GUGACCACAGAUAACACAUCCCUGGACGACUUUC AUGUAAAUGGCGGCGAGUUAAUUCUGAUACACCA GAACCCUGGCGAGUUCUGCGUGCUG	SEQ ID NO: 194
OX40L (TNFSF4)	Codon-optimized sequence 3 for ENSP 356691	AUGGUGAGCCACCGCUACCCCCCGCAUCCAGAGCA UCAAGGUGCAGUUCACAGAGUACAAGAAGGAGAA GGGCUUCAUCCUGACCAGCCAGAAGGAGGAUGAG AUCAUGAAGGUGCAGAACAACAGCGUCAUCAUCA ACUGUGAUGGCUUCUACCUGAUCAGCCUGAAGGG CUACUUCAGCCAGGAGGUGAACAUCAGCCUGCAC UACCAGAAGGAUGAGGAGCCCCCUCUUCCAGCUGA AGAAGGUGCGCUCUGUGAACAGCCUGAUGGUGGC CAGCCUGACCUACAAGGACAAGGUGUACCUGAAU GUGACCACAGACAACACCAGCCUGGAUGACUUCC ACGUGAAUGGAGGAGAGCUGAUCCUGAUCCACCA GAACCCUGGAGAGUUCUGUGUGCUG	SEQ ID NO: 195

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Encoded Polypeptide	Description	Sequence	SEQ ID NO:
OX40L (TNFSF4)	Codon-optimized sequence 4 for ENSP 356691	AUGGUGAGCCACCGGUACCCCCGGAUCCAGAGCA UCAAGGUGCAGUUCACCGAAUACAAGAAGGAGAA GGGUUUUAUCCUGACGAGCCAGAAGGAAGACGAG AUUAUGAAGGUCCAAAACAACUCAGUCAUCAUAA ACUGCGAUGGAUUUUACCUGAUCUCUCUGAAAGG GUACUUCUCCCAGGAAGUGAAUAUUAGCUUGCAC UAUCAAAAAGAUGAGGAGCCUCUAUUCCAGCUCA AGAAGGUCAGAAGCGUCAAUAGUCUGAUGGUCGC AUCAUUAACCUAUAAAGACAAAGUAUAUCUAAAU GUGACGACAGACAAUACAUCCCUCGAUGAUUUUC ACGUCAACGGAGGCGAACUCAUUCUGAUCCACCA GAAUCCAGGGGAAUUUUGCGUGCUG	SEQ ID NO: 196
OX40L (TNFSF4)	Codon-optimized sequence 5 for ENSP 356691	AUGGUCUCACACCGGUACCCCCGUAUCCAGAGUA UUAAGGUGCAAUUCACGGAGUAUAAAAAAGAAAA GGGAUUCAUUCUGACGUCUCAGAAGGAAGAUGAG AUCAUGAAGGUCCAGAACAAUUCUGUGAUCAUUA AUUGCGAUGGAUUUUAUCUGAUUUCACUUAAAGG AUAUUUUUCCCAGGAGGUUAAUAUCAGUUUGCAC UAUCAGAAAGACGAGGAGCCAUUAUUCCAGCUGA AGAAGGUGAGAUCAGUGAAUAGCCUGAUGGUUGC GUCACUGACGUAUAAAGACAAAGUUUAUCUAAAC GUUACCACUGAUAAUACAUCCCUUGAUGAUUUUC AUGUGAACGGGGGUGAACUGAUCCUUAUACACCA GAACCCCGGAGAGUUCUGUGUGUUG	SEQ ID NO: 197
OX40L (TNFSF4)	Codon-optimized sequence 1 for ENSP 439704	AUGGUGAGCCACAGAUACCCCAGAAUCCAGAGCAU CAAGGUGCAGUUCACCGAGUACAAGAAGGAGAAG GGCUUCAUCCUGACCAGCCAGAAGGAGGACGAGA UCAUGAAGGUGCAGAACAACAGCGUGAUCAUCAA CUGCGACGGCUUCUACCUGAUCAGCCUGAAGGGC UACUUCAGCCAGGAGGUGAACAUCAGCCUGCACU	SEQ ID NO: 198
		ACCAGAAGGACGAGGAGCCCCUGUUCCAGCUGAA GAAGGUGAGAAGCGUGAACAGCCUGAUGGUGGCC AGCCUGACCUACAAGGACAAGGUGUACCUGAACG UGACCACCGACAACACCAGCCUGGACGACUUCCA CGUGAACGGCGGCGAGCUGAUCCUGAUCCACCAG AACCCCGGCGAGUUCUGCGUGCUG	
OX40L (TNFSF4)	Codon-optimized sequence 2 for ENSP 439704	AUGGUGUCACACCGGUACCCUCGGAUCCAGUCUA UUAAAGUUCAAUUUACGGAGUACAAGAAAGAAAA AGGCUUUAUCCUUACAAGCCAAAAGGAAGACGAG AUCAUGAAAGUGCAAAACAACAGUGUGAUUAUAA AUUGUGAUGGCUUCUACCUUAUUAGUCUGAAGGG CUACUUUAGUCAGGAAGUCAAUAUUAGCCUACAC UACCAGAAAGACGAGGAGCCCCUCUUUCAACUGA AAAAGGUGCGCUCCGUGAAUUCGUUGAUGGUCGC CUCUCUGACCUACAAAGAUAAGGUGUAUCUUAAC GUUACUACCGACAAUACUAGUCUGGACGACUUUC ACGUCAACGGAGGCGAACUUAUUCUGAUCCACCA GAACCCCGGCGAAUUCUGCGUGCUG	SEQ ID NO: 199

(continued)

Encoded Polypeptide	Description	Sequence	SEQ ID NO:
OX40L (TNFSF4)	Codon-optimized sequence 3 for ENSP 439704	AUGGUGAGCCACCGCUACCCCCCGCAUCCAGAGCA UCAAGGUGCAGUUCACAGAGUACAAGAAGGAGAA GGGCUUCAUCCUGACCAGCCAGAAGGAGGAUGAG AUCAUGAAGGUGCAGAACAACAGCGUCAUCAUCA ACUGUGAUGGCUUCUACCUGAUCAGCCUGAAGGG CUACUUCAGCCAGGAGGUGAACAUCAGCCUGCAC UACCAGAAGGAUGAGGAGCCCCUCUUCCAGCUGA AGAAGGUGCGCUCUGUGAACAGCCUGAUGGUGGC CAGCCUGACCUACAAGGACAAGGUGUACCUGAAU GUGACCACAGACAACACCAGCCUGGAUGACUUCC ACGUGAAUGGAGGAGAGCUGAUCCUGAUCCACCA GAACCCUGGAGAGUUCUGUGUGCUG	SEQ ID NO: 200
OX40L (TNFSF4)	Codon-optimized sequence 4 for ENSP 439704	AUGGUGAGCCACCGGUACCCCCGGAUCCAGAGCA UCAAGGUGCAGUUCACAGAGUACAAGAAGGAGAA GGGAUUUAUUCUCACAAGUCAGAAAGAAGAUGAG AUCAUGAAGGUUCAGAACAACUCAGUCAUUAUUA AUUGCGACGGAUUCUAUCUCAUUAGCCUCAAAGG CUAUUUCAGCCAGGAGGUCAAUAUCAGCCUGCAC UACCAGAAGGAUGAGGAACCUCUCUUUCAGCUGA AAAAAGUCCGCUCUGUGAAUUCCCUCAUGGUCGC UUCCCUGACCUACAAGGAUAAAGUUUAUUUGAAC GUUACAACAGAUAAUACAUCGCUGGACGACUUCC AUGUGAAUGGUGGCGAACUAAUUCUAAUACACCA AAAUCCAGGCGAAUUUUGUGUCCUU	SEQ ID NO: 201
OX40L (TNFSF4)	Codon-optimized sequence 5 for ENSP 439704	AUGGUAUCCCAUAGAUACCCACGUAUUCAAAGCA UUAAGGUGCAGUUCACAGAGUACAAAAAGGAGAA GGGUUUCAUACUGACGUCACAGAAGGAGGACGAG AUAAUGAAGGUGCAGAAUAAUAGUGUGAUCAUCA AUUGUGAUGGAUUCUAUUUGAUCAGCCUCAAAGG UUAUUUCUCACAGGAAGUCAACAUUUCCCUGCAC UACCAGAAGGACGAAGAGCCUUUGUUUCAGCUGA AGAAGGUGCGCUCAGUGAACAGUUUGAUGGUAGC CUCCCUAACUUAUAAAGAUAAAGUUUAUCUGAAC GUGACAACCGAUAACACAUCCCUGGACGACUUUC ACGUCAAUGGAGGUGAGUUAAUCCUGAUCCAUCA GAAUCCCGGAGAAUUCUGCGUUCUU	SEQ ID NO: 202

3. Interleukin-12 (IL12)

[0160] IL12 (also shown as IL-12) is a pleiotropic cytokine, the actions of which create an interconnection between innate and adaptive immunity. IL12 functions primarily as a 70 kDa heterodimeric protein consisting of two disulfide-linked p35 and p40 subunits. The precursor form of the IL12 p40 subunit (NM_002187; P29460; also referred to as IL12B, natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2) is 328 amino acids in length, while its mature form is 306 amino acids long. The precursor form of the IL12 p35 subunit (NM_000882; P29459; also referred to as IL12A, natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1) is 219 amino acids in length and the mature form is 197 amino acids long. *Id.* The genes for the IL12 p35 and p40 subunits reside on different chromosomes and are regulated independently of each other. Gately, MK et al., Annu Rev Immunol. 16: 495-521 (1998). Many different immune cells (*e.g.*, dendritic cells, macrophages, monocytes, neutrophils, and B cells) produce IL12 upon antigenic stimuli. The active IL12 heterodimer is formed following protein synthesis. *Id.*

[0161] IL12 is composed of a bundle of four alpha helices. It is a heterodimeric cytokine encoded by two separate genes, IL12A (p35) and IL12B (p40). The active heterodimer (referred to as 'p70'), and a homodimer of p40 are formed following protein synthesis.

[0162] Therefore, in some cases, the IL12 polypeptide of the present disclosure comprises a single polypeptide chain comprising the IL12B and IL12A fused directly or by a linker. In other cases, the IL12 polypeptide of the present disclosure

comprises two polypeptides, the first polypeptide comprising IL12B and the second polypeptide comprising IL12A. In certain aspects, the disclosure provides an IL12A polypeptide and an IL12B polypeptide, wherein the IL12A and IL12B polypeptides are on the same chain or different chains. In some cases, the IL12A or IL12B polypeptide of the disclosure is a variant, a peptide or a polypeptide containing a substitution, and insertion and/or an addition, a deletion and/or a covalent modification with respect to a wild-type IL12A or IL12B sequence. In some cases, sequence tags (such as epitope tags, *e.g.,* a V5 tag) or amino acids, can be added to the sequences encoded by the polynucleotides of the disclosure (*e.g.,* at the N-terminal or C-terminal ends), *e.g.,* for localization. In some cases, amino acid residues located at the carboxy, amino terminal, or internal regions of a polypeptide of the disclosure can optionally be deleted providing for fragments.

[0163] In some cases, the IL12A and/or IL12B polypeptide encoded by a polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a substitutional variant of an IL12A and/or IL12B sequence, which can comprise one, two, three or more than three substitutions. In some cases, the substitutional variant can comprise one or more conservative amino acids substitutions. In other cases, the variant is an insertional variant. In other cases, the variant is a deletional variant.

[0164] In other cases, the IL12A and/or IL12B polypeptide encoded the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a linker fusing the IL12A and IL12B polypeptides. Non-limiting examples of linkers are disclosed elsewhere herein.

[0165] Some aspects of the present disclosure are directed to a lipid nanoparticle comprising a polynucleotide (*e.g.,* mRNA) encoding a human IL12 polypeptide, wherein the polynucleotide comprises an ORF encoding a human IL12B polypeptide operably linked to a human IL12A polypeptide. In some cases, the IL12B polypeptide is operably linked to the IL12A polypeptide by a peptide linker. In some cases, the IL12B polypeptide is located at the 5' terminus of the IL12A polypeptide or the peptide linker. In other cases, the IL12A polypeptide is located at the 5' terminus of the IL12B polypeptide or the peptide linker.

[0166] As recognized by those skilled in the art, IL12 protein fragments, functional protein domains, variants, and homologous proteins (orthologs) are also considered to be within the scope of the IL12 polypeptides of the disclosure. Nonlimiting examples of polypeptides encoded by the polynucleotides of the disclosure are shown in FIGs. 1 to 2. For example, Table 2 shows the correlating amino acid numbering in SEQ ID NOs, nucleotide numbering in SEQ ID NOs, and the 5' UTR, IL12B signal peptide, mature IL12A and IL12B peptides, and linker.

Table 2. Domains of IL12.

	Amino acids	**Nucleotides**
Signal Peptide IL12B	1-22 of SEQ ID NO: 48	1-66 of SEQ ID NOs: 5-44
Mature IL12B	23-328 of SEQ ID NO: 48	67-984 of SEQ ID NOs: 5-44
Linker	329-335 of SEQ ID NO: 48	985-1005 of SEQ ID NOs: 5-44
Mature IL12A	336-532 of SEQ ID NO: 48	1006-1596 of SEQ ID NOs: 5-44

4. Polynucleotides and Open Reading Frames (ORFs)

[0167] In certain aspects, the disclosure provides polynucleotides (*e.g.,* a RNA, *e.g.,* an mRNA) that comprise a nucleotide sequence (*e.g.,* an ORF, *e.g.,* mRNA) encoding one or more IL12 polypeptides. In certain cases, the polynucleotides encode an IL-12 polypeptide, wherein the polynucleotides comprise a single ORF encoding an IL12B polypeptide and an IL12A polypeptide. In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure encodes a single IL12 polypeptide chain comprising an IL12B polypeptide and an IL12A polypeptide, which are fused directly or by a linker, wherein the IL12B polypeptide is selected from the group consisting of:

(i) the full-length IL12B polypeptide (*e.g.,* having the same or essentially the same length as wild-type IL12B);
(ii) a functional fragment of the full-length IL12B polypeptide (*e.g.,* a truncated (*e.g.,* deletion of carboxy, amino terminal, or internal regions) sequence shorter than an IL12B wild-type; but still retaining IL12B enzymatic activity);
(iii) a variant thereof (*e.g.,* full length or truncated IL12B proteins in which one or more amino acids have been replaced, *e.g.,* variants that retain all or most of the IL12B activity of the polypeptide with respect to the wild type IL12B polypeptide (such as, *e.g.,* V33I, V298F, or any other natural or artificial variants known in the art); and
(iv) a fusion protein comprising (i) a full length IL12B wild-type, a functional fragment or a variant thereof, and (ii) a heterologous protein; and/or

wherein the IL12A polypeptide is selected from the group consisting of:

(v) the full-length IL12A polypeptide (*e.g.,* having the same or essentially the same length as wild-type IL12A);
(vi) a functional fragment of the full-length IL12A polypeptide (*e.g.,* a truncated (*e.g.,* deletion of carboxy, amino terminal, or internal regions) sequence shorter than an IL12A wild-type; but still retaining IL12A enzymatic activity);
(vii) a variant thereof (*e.g.,* full length or truncated IL12A proteins in which one or more amino acids have been replaced, *e.g.,* variants that retain all or most of the IL12A activity of the polypeptide with respect to the wtIL12A polypeptide (such as natural or artificial variants known in the art); and
(viii) a fusion protein comprising (i) a full length IL12A wild-type, a functional fragment or a variant thereof, and (ii) a heterologous protein.

[0168] In other cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure encodes two polypeptide chains, the first chain comprising an IL12B polypeptide and the second chain comprising an IL12A polypeptide, wherein the IL12B polypeptide is selected from the group consisting of:

(i) the mature IL12B polypeptide (*e.g.,* having the same or essentially the same length as wild-type IL12B) with or without a signal peptide;
(ii) a functional fragment of any of the mature IL12B polypeptide (*e.g.,* a truncated (*e.g.,* deletion of carboxy, amino terminal, or internal regions) sequence shorter than an IL12B wild-type; but still retaining IL12B enzymatic activity);
(iii) a variant thereof (*e.g.,* full length, mature, or truncated IL12B proteins in which one or more amino acids have been replaced, *e.g.,* variants that retain all or most of the IL12B activity of the polypeptide with respect to the wild type IL12B polypeptide (such as, *e.g.,* V33I, V298F, or any other natural or artificial variants known in the art); and
(iv) a fusion protein comprising (i) a mature IL12B wild-type, a functional fragment or a variant thereof, with or without a signal peptide and (ii) a heterologous protein; and/or

wherein the IL12A is selected from the group consisting of:

(v) the mature IL12A polypeptide (*e.g.,* having the same or essentially the same length as wild-type IL12A) with or without a signal peptide;
(vi) a functional fragment of any of the wild-type IL12A polypeptide (*e.g.,* a truncated (*e.g.,* deletion of carboxy, amino terminal, or internal regions) sequence shorter than an IL12A wild-type; but still retaining IL12A enzymatic activity);
(vii) a variant thereof (*e.g.,* full length, mature, or truncated IL12A proteins in which one or more amino acids have been replaced, *e.g.,* variants that retain all or most of the IL12A activity of the polypeptide with respect to a reference isoform (such as natural or artificial variants known in the art); and
(viii) a fusion protein comprising (i) a mature IL12A wild-type, a functional fragment or a variant thereof, with or without a signal peptide and (ii) a heterologous protein.

[0169] In certain cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure encodes a mammalian IL12 polypeptide, such as a human IL12 polypeptide, a functional fragment or a variant thereof.

[0170] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure increases IL12B and/or IL12A protein expression levels and/or detectable IL12 enzymatic activity levels in cells when introduced in those cells, *e.g.,* by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100%, compared to IL12B and/or IL12A protein expression levels and/or detectable IL12 enzymatic activity levels in the cells prior to the administration of the polynucleotide of the disclosure. IL12B and/or IL12A protein expression levels and/or IL12 enzymatic activity can be measured according to methods known in the art. In some cases, the polynucleotide is introduced to the cells *in vitro.* In some cases, the polynucleotide is introduced to the cells *in vivo.*

[0171] In some cases, the polynucleotides (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprise a nucleotide sequence (*e.g.,* an ORF) that encodes a wild-type human IL12B and/or IL12A, (see FIG. 1).

[0172] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a codon optimized nucleic acid sequence, wherein the open reading frame (ORF) of the codon optimized nucleic sequence is derived from a wild-type IL12A and/or IL12B sequence.

[0173] In some cases, the polynucleotides (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprise a nucleotide sequence encoding IL12B and/or IL12A having the full length sequence of human IL12B and/or IL12A (*i.e.,* including the initiator methionine and the signal peptides). In mature human IL12B and/or IL12A, the initiator methionine and/or signal peptides can be removed to yield a "mature IL12B" and/or "mature IL12A" comprising amino acid residues of SEQ ID NO: 1 and SEQ ID NO: 3, respectively. SEQ ID NO: 1 corresponds to amino acids 23 to 328 of SEQ ID NO: 48, and SEQ ID NO: 3 corresponds to amino acids 336 to 532 of SEQ ID NO: 48, respectively. The teachings of the present disclosure directed to the full sequence of human IL12B and/or IL12A are also applicable to the mature form of human IL12B and/or IL12A lacking the initiator methionine and/or the signal peptide. Thus, in some cases, the polynu-

cleotides (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprise a nucleotide sequence encoding IL12B and/or IL12A having the mature sequence of human IL12B and/or IL12A. In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprising a nucleotide sequence encoding IL12B and/or IL12A is sequence optimized.

[0174] In some cases, the polynucleotides (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprise a nucleotide sequence (*e.g.,* an ORF) encoding a mutant IL12B and/or IL12A polypeptide. In some cases, the polynucleotides of the disclosure comprise an ORF encoding an IL12B and/or IL12A polypeptide that comprises at least one point mutation, at least two point mutations, at least three mutations, at least four mutations, at least five mutations, at least six mutations, at least seven mutations, at least eight mutations, at least nine mutations, or at least ten mutations in the IL12B and/or IL12A sequence and retains IL12B and/or IL12A enzymatic activity. In some cases, the mutant IL12B and/or IL12A polypeptide has an IL12B and/or IL12A activity which is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% of the IL12B and/or IL12A activity of the corresponding wild-type IL12B and/or IL12A (*i.e.,* the same IL12B and/or IL12A but without the mutation(s)). In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprising an ORF encoding a mutant IL12B and/or IL12A polypeptide is sequence optimized.

[0175] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) that encodes an IL12B and/or IL12A polypeptide with mutations that do not alter IL12B and/or IL12A enzymatic activity. Such mutant IL12B and/or IL12A polypeptides can be referred to as function-neutral. In some cases, the polynucleotide comprises an ORF that encodes a mutant IL12B and/or IL12A polypeptide comprising one or more function-neutral point mutations.

[0176] In some cases, the mutant IL12B and/or IL12A polypeptide has higher IL12B and/or IL12A enzymatic activity than the corresponding wild-type IL12B and/or IL12A. In some cases, the mutant IL12B and/or IL12A polypeptide has an IL12B and/or IL12A activity that is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% higher than the activity of the corresponding wild-type IL12B and/or IL12A (*i.e.,* the same IL12B and/or IL12A but without the mutation(s)).

[0177] In some cases, the polynucleotides (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprise a nucleotide sequence (*e.g.,* an ORF) encoding a functional IL12B and/or IL12A fragment, *e.g.,* where one or more fragments correspond to a polypeptide subsequence of a wild type IL12B and/or IL12A polypeptide and retain IL12B and/or IL12A enzymatic activity. In some cases, the IL12B and/or IL12A fragment has an IL12B and/or IL12A activity which is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% of the IL12 activity of the corresponding full length IL12B and/or IL12A. In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprising an ORF encoding a functional IL12B and/or IL12A fragment is sequence optimized.

[0178] In some cases, the polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A fragment that has higher IL12B and/or IL12A enzymatic activity than the corresponding full length IL12B and/or IL12A. Thus, in some cases the IL12B and/or IL12A fragment has an IL12B and/or IL12A activity which is at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% higher than the IL12B and/or IL12A activity of the corresponding full length IL12B and/or IL12A.

[0179] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A fragment that is at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% shorter than wild-type IL12B and/or IL12A.

[0180] In other cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B polypeptide, which has:

(i) at least about 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_007, hIL12AB_010, or hIL12AB_012;

(ii) at least about 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_018 or hIL12AB_019;

(iii) at least about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_008;

(iv) at least about 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_005, hIL12AB_013, or hIL12AB_017 or nucleotides 70-987 of

hIL12AB_004;
(v) at least about 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_001 or hIL12AB_009;
(vi) at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_012 or hIL12AB_005;
(vii) at least about 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_022 or hIL12AB_038;
(viii) at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides nucleotides 67-984 of hIL12AB_024, hIL12AB_031, hIL12AB_032, or hIL12AB_036;
(ix) at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_021, hIL12AB_023, hIL12AB_025, hIL12AB_026, hIL12AB_027, hIL12AB_029, hIL12AB_030, hIL12AB_034, hIL12AB_039, or hIL12AB_040;
(x) at least about 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_016, hIL12AB_035, or hIL12AB_037;
(xi) at least about 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_011, hIL12AB_028, or hIL12AB_033;
(xii) at least about 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_015;
(xiii) at least about 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_020; or
(xiv) 100% sequence identity to nucleotides 67-984 of hIL12AB_006.

[0181] In other cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12A polypeptide, which has:

(i) at least about 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_010;
(ii) at least about 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_019;
(iii) at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_013;
(iv) at least about 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_007 or hIL12AB_014;
(v) at least about 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_002, hIL12AB_008;
(vi) at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_012 or hIL12AB_005;
(vii) at least about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_001, or hIL12AB_009 or nucleotides 1009-1589 of hIL12AB_004;
(viii) at least about 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_17;
(ix) at least about 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_029 or hIL12AB_027;
(x) at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_039 or hIL12AB_040;
(xi) at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_036, hIL12AB_034, hIL12AB_016, hIL12AB_023, hIL12AB_030, hIL12AB_031, hIL12AB_025, or hIL12AB_035;
(xii) at least about 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_021, hIL12AB_024, hIL12AB_032, hIL12AB_033, hIL12AB_037, or hIL12AB_022;
(xiii) at least about 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_020, hIL12AB_026, or hIL12AB_038;
(xiv) at least about 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_015, hIL12AB_011, or hIL12AB_028; or
(xv) about 100% sequence identity to nucleotides 1006-1596 of hIL12AB_003.

[0182] In certain cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a single ORF encoding IL12B and IL12A, wherein the ORF comprises a sequence that has:

(i) at least about 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_007, hIL12AB_010, or hIL12AB_012;
(ii) at least about 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_018 or hIL12AB_019;
(iii) at least about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_008;
(iv) at least about 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_005, hIL12AB_013, or hIL12AB_017 or nucleotides 70-987 of hIL12AB_004;
(v) at least about 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_001 or hIL12AB_009;
(vi) at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_012 or hIL12AB_005;
(vii) at least about 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_022 or hIL12AB_038;
(viii) at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_024, hIL12AB_031, hIL12AB_032, or hIL12AB_036;
(ix) at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_021, hIL12AB_023, hIL12AB_025, hIL12AB_026, hIL12AB_027, hIL12AB_029, hIL12AB_030, hIL12AB_034, hIL12AB_039, or hIL12AB_040;
(x) at least about 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_016, hIL12AB_035, or hIL12AB_037;
(xi) at least about 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_011, hIL12AB_028, or hIL12AB_033;
(xii) at least about 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_015;
(xiii) at least about 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_020; or
(xiv) about 100% sequence identity to nucleotides 67-984 of hIL12AB_006; and

a sequence that has:

(i) at least about 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_010;
(ii) at least about 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_019;
(iii) at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_013;
(iv) at least about 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_007 or hIL12AB_014;
(v) at least about 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_002, hIL12AB_008;
(vi) at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_012 or hIL12AB_005;
(vii) at least about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_001, or hIL12AB_009 or nucleotides 1009-1599 of hIL12AB_004;
(viii) at least about 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_17;
(ix) at least about 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_029 or hIL12AB_027;
(x) at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_039 or hIL12AB_040;
(xi) at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_036, hIL12AB_034, hIL12AB_016, hIL12AB_023, hIL12AB_030, hIL12AB_031, hIL12AB_025, or hIL12AB_035;
(xii) at least about 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_021, hIL12AB_024, hIL12AB_032, hIL12AB_033, hIL12AB_037, or hIL12AB_022;
(xiii) at least about 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_020, hIL12AB_026, or hIL12AB_038;

(xiv) at least about 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_015, hIL12AB_011, or hIL12AB_028; or
(xv) about 100% sequence identity to nucleotides 1006-1596 of hIL12AB_003.

[0183] In certain cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a first ORF encoding IL12B and a second ORF encoding IL12A or , wherein the first ORF comprises a sequence that has:

(i) at least about 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_007, hIL12AB_010, or hIL12AB_012;
(ii) at least about 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_018 or hIL12AB_019;
(iii) at least about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_008;
(iv) at least about 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_005, hIL12AB_013, or hIL12AB_017 or nucleotides 70-987 of hIL12AB_004;
(v) at least about 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_001 or hIL12AB_009;
(vi) at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_012 or hIL12AB_005;
(vii) at least about 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_022 or hIL12AB_038;
(viii) at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_024, hIL12AB_031, hIL12AB_032, or hIL12AB_036;
(ix) at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_021, hIL12AB_023, hIL12AB_025, hIL12AB_026, hIL12AB_027, hIL12AB_029, hIL12AB_030, hIL12AB_034, hIL12AB_039, or hIL12AB_040;
(x) at least about 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_016, hIL12AB_035, or hIL12AB_037;
(xi) at least about 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_011, hIL12AB_028, or hIL12AB_033;
(xii) at least about 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_015;
(xiii) at least about 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 67-984 of hIL12AB_020; or
(xiv) about 100% sequence identity to nucleotides 67-984 of hIL12AB_006; and/or

wherein the second ORF comprises a sequence that has:

(i) at least about 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_010;
(ii) at least about 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_019;
(iii) at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_013;
(iv) at least about 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_007 or hIL12AB_014;
(v) at least about 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_002, hIL12AB_008;
(vi) at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_012 or hIL12AB_005;
(vii) at least about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_001, or hIL12AB_009 or nucleotides 1009-1599 of hIL12AB_004;
(viii) at least about 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_17;
(ix) at least about 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_029 or hIL12AB_027;
(x) at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_039 or hIL12AB_040;

(xi) at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_036, hIL12AB_034, hIL12AB_016, hIL12AB_023, hIL12AB_030, hIL12AB_031, hIL12AB_025, or hIL12AB_035;

(xii) at least about 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_021, hIL12AB_024, hIL12AB_032, hIL12AB_033, hIL12AB_037, or hIL12AB_022;

(xiii) at least about 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_020, hIL12AB_026, or hIL12AB_038;

(xiv) at least about 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 1006-1596 of hIL12AB_015, hIL12AB_011, or hIL12AB_028; or

(xv) about 100% sequence identity to nucleotides 1006-1596 of hIL12AB_003.

[0184] In some cases, the polynucleotide sequence comprises an ORF comprising the sequence set forth as hIL12AB_002 (SEQ ID NO: 6) or a nucleotide sequence at least about 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to hIL12AB_002 (SEQ ID NO: 6).

[0185] In one case, the first nucleotide sequence (e.g,. first ORF) encoding the IL12B polypeptide and the second nucleotide sequence (*e.g.,* second ORF) encoding the IL12A polypeptide are fused directly or by a linker. In another case, the first nucleotide sequence (e.g,. first ORF) encoding the IL12B polypeptide and the second nucleotide sequence (*e.g.,* second ORF) encoding the IL12A polypeptide are not fused to each other.

[0186] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B-IL12A fusion polypeptide (*e.g.,* the wild-type sequence, functional fragment, or variant thereof), wherein the nucleotide sequence has at least about 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 5 to 44. *See* Table 4.

[0187] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B-IL12A fusion polypeptide (*e.g.,* the wild-type sequence, functional fragment, or variant thereof), wherein the nucleotide sequence has 70% to 100%, 75% to 100%, 80% to 100%, 85% to 100%, 70% to 95%, 80% to 95%, 70% to 85%, 75% to 90%, 80% to 95%, 70% to 75%, 75% to 80%, 80% to 85%, 85% to 90%, 90% to 95%, or 95% to 100%, sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 5 to 44. *See* Table 4.

[0188] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises from about 900 to about 100,000 nucleotides (*e.g.,* from 900 to 1,000, from 900 to 1,100, from 900 to 1,200, from 900 to 1,300, from 900 to 1,400, from 900 to 1,500, from 1,000 to 1,100, from 1,000 to 1,100, from 1,000 to 1,200, from 1,000 to 1,300, from 1,000 to 1,400, from 1,000 to 1,500, from 1,083 to 1,200, from 1,083 to 1,400, from 1,083 to 1,600, from 1,083 to 1,800, from 1,083 to 2,000, from 1,083 to 3,000, from 1,083 to 5,000, from 1,083 to 7,000, from 1,083 to 10,000, from 1,083 to 25,000, from 1,083 to 50,000, from 1,083 to 70,000, or from 1,083 to 100,000).

[0189] In some cases, the polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B-IL12A fusion polypeptide (*e.g.,* the wild-type sequence, functional fragment, or variant thereof), wherein the length of the nucleotide sequence (*e.g.,* an ORF) is at least 500 nucleotides in length (*e.g.,* at least or greater than about 500, 600, 700, 80, 900, 1,000, 1,050, 1,083, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,100, 2,200, 2,300, 2,400, 2,500, 2,600, 2,700, 2,800, 2,900, 3,000, 3,100, 3,200, 3,300, 3,400, 3,500, 3,600, 3,700, 3,800, 3,900, 4,000, 4,100, 4,200, 4,300, 4,400, 4,500, 4,600, 4,700, 4,800, 4,900, 5,000, 5,100, 5,200, 5,300, 5,400, 5,500, 5,600, 5,700, 5,800, 5,900, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or up to and including 100,000 nucleotides).

[0190] In some cases, the polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide (*e.g.,* the wild-type sequence, functional fragment, or variant thereof) further comprises at least one nucleic acid sequence that is noncoding, *e.g.,* a miRNA binding site.

[0191] In some cases, the polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide that is single stranded or double stranded.

[0192] In some cases, the polynucleotide of the disclosure comprising a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide (*e.g.,* the wild-type sequence, functional fragment, or variant thereof) is DNA or RNA. In some cases, the polynucleotide of the disclosure is RNA. In some cases, the polynucleotide of the disclosure is, or functions as, a messenger RNA (mRNA). In some cases, the mRNA comprises a nucleotide sequence (*e.g.,* an ORF) that encodes at least one IL12B and/or IL12A polypeptide, and is capable of being translated to produce the encoded IL12B and/or IL12A polypeptide *in vitro, in vivo, in situ* or *ex vivo.*

[0193] In some cases, the polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a sequence-optimized nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide (*e.g.,* the wild-type sequence,

functional fragment, or variant thereof), wherein the polynucleotide comprises at least one chemically modified nucleobase, *e.g.,* 5-methoxyuracil. In some cases, the polynucleotide further comprises a miRNA binding site, *e.g.,* a miRNA binding site that binds to miR-122. In some cases, the polynucleotide disclosed herein is formulated with a delivery agent, *e.g.,* a compound having **Formula (I),** *e.g.,* any of Compounds 1-147 or any of Compounds 1-232.

[0194] The polynucleotides (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure can also comprise nucleotide sequences that encode additional features that facilitate trafficking of the encoded polypeptides to therapeutically relevant sites. One such feature that aids in protein trafficking is the signal sequence, or targeting sequence. The peptides encoded by these signal sequences are known by a variety of names, including targeting peptides, transit peptides, and signal peptides. In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) comprises a nucleotide sequence (*e.g.,* an ORF) that encodes a signal peptide operably linked a nucleotide sequence that encodes an IL12B and/or IL12A polypeptide described herein.

[0195] In some cases, the "signal sequence" or "signal peptide" is a polynucleotide or polypeptide, respectively, which is from about 9 to 200 nucleotides (3-70 amino acids) in length that, optionally, is incorporated at the 5' (or N-terminus) of the coding region or the polypeptide, respectively. Addition of these sequences results in trafficking the encoded polypeptide to a desired site, such as the endoplasmic reticulum or the mitochondria through one or more targeting pathways. Some signal peptides are cleaved from the protein, for example by a signal peptidase after the proteins are transported to the desired site.

[0196] In some cases, the polynucleotide of the disclosure comprises a nucleotide sequence encoding an IL12B and/or IL12A polypeptide, wherein the nucleotide sequence further comprises a 5' nucleic acid sequence encoding a native signal peptide. In another case, the polynucleotide of the disclosure comprises a nucleotide sequence encoding an IL12B and/or IL12A polypeptide, wherein the nucleotide sequence lacks the nucleic acid sequence encoding a native signal peptide.

[0197] In some cases, the polynucleotide of the disclosure comprises a nucleotide sequence encoding an IL12B and/or IL12A polypeptide, wherein the nucleotide sequence further comprises a 5' nucleic acid sequence encoding a heterologous signal peptide.

[0198] In some cases, the polynucleotide further comprises a nucleic acid sequence encoding a signal peptide that is located at the 5' terminus of the first ORF.

[0199] In some cases, the first ORF comprises a nucleic acid sequence encoding a signal peptide.

[0200] In some cases, the signal peptide is a human IL12B signal peptide.

[0201] In some cases, the signal peptide comprises a sequence at least about 80%, at least about 90%, at least about 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to amino acids 1 to 22 of SEQ ID NO: 48.

[0202] Based on the RNA sequences provided, a person of ordinary skill in the art would understand the corresponding DNA sequence (*e.g.,* conversion of uracil to thymine). Likewise, based on the DNA sequences provided, a person of ordinary skill in the art would understand the corresponding RNA sequence (*e.g.,* conversion of thymine to uracil).

5. Chimeric Proteins

[0203] In some cases, the polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) can comprise more than one nucleic acid sequence (*e.g.,* more than one ORF) encoding one or more polypeptide of interest. In some cases, polynucleotides of the disclosure comprise a single ORF encoding an IL12B and/or IL12A polypeptide, a functional fragment, or a variant thereof. However, in some cases, the polynucleotide of the disclosure can comprise more than one nucleotide sequence, for example, a first nucleotide sequence encoding an IL12B polypeptide (a first polypeptide of interest), a functional fragment, or a variant thereof, a second nucleotide sequence encoding an IL12A polypeptide (a second polypeptide of interest), a functional fragment, or a variant thereof, and a third nucleotide sequence expressing a third polypeptide of interest (*e.g.,* a polypeptide heterologous to IL12). In one case, the third polypeptide of interest can be fused to the IL12B polypeptide directly or by a linker. In another case, the third polypeptide of interest can be fused to the IL12A polypeptide directly or by a linker. In other cases, the third polypeptide of interest can be fused to both the IL12B polypeptide and the IL12A polypeptide directly or by a linker. In further cases, the polynucleotide of the disclosure can comprise more than three nucleotide sequences, for example, a first nucleotide sequence encoding an IL12B polypeptide (a first polypeptide of interest), a functional fragment, or a variant thereof, a second nucleotide sequence encoding an IL12A polypeptide (a second polypeptide of interest), a functional fragment, or a variant thereof, a third nucleotide sequence expressing a third polypeptide of interest, and a fourth nucleotide sequence expressing a fourth polypeptide of interest. In other cases, the third polypeptide of interest is fused to the IL12A polypeptide directly or by a linker, and the fourth polypeptide of interest is fused to the IL12B polypeptide directly or by a linker. In some cases, two or more polypeptides of interest can be genetically fused, *i.e.,* two or more polypeptides can be encoded by the same nucleotide sequence. In some cases, the polynucleotide can comprise a nucleic acid sequence encoding a linker (*e.g.,* a G_4S peptide linker or another linker known in the art) between two or more polypeptides of interest.

[0204] In some cases, a polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) can comprise two, three, four,

or more nucleotide sequences, each expressing a polypeptide of interest.

[0205] In some cases, the polynucleotide of the disclosure (*e.g.,* a RNA, *e.g.,* an mRNA) can comprise a first nucleic acid sequence (*e.g.,* a first ORF) encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides and a second nucleic acid sequence (*e.g.,* a second ORF) encoding a second polypeptide of interest.

6. Linker

[0206] In one aspect, the IL12B and/or IL12A can be fused directly or by a linker. In other cases, the IL12B and/or IL12A can be fused directly to by a linker to a heterologous polypeptide. The linkers suitable for fusing the IL12B to IL12A or the IL12B and/or IL12A to a heterologous polypeptide can be a polypeptide (or peptide) moiety or a non-polypeptide moiety.

[0207] Some aspects of the present disclosure are directed to a lipid nanoparticle comprising a polynucleotide encoding a human IL12 polypeptide, wherein the polynucleotide comprises an ORF encoding a human IL12B polypeptide operably linked to a human IL12A polypeptide. In some cases, the IL12B polypeptide is operably linked to the IL12A polypeptide by a peptide linker. In some cases, the IL12B polypeptide is located at the 5' terminus of the IL12A polypeptide or the peptide linker. In other cases, the IL12A polypeptide is located at the 5' terminus of the IL12B polypeptide or the peptide linker.

[0208] In some cases, the linker is a peptide linker, including from one amino acid to about 200 amino acids. In some cases, the linker comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, or at least 40 amino acids.

[0209] In some cases, the linker can be GS (Gly/Ser) linkers, for example, comprising $(G_nS)_m$, wherein n is an integer from 1 to 20 and m is an integer from 1 to 20. In some cases, the Gly/Ser linker comprises $(G_nS)_m$(SEQ ID NO: 203), wherein n is 1, 2 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 and m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20. In some cases, the GS linker can comprise $(GGGGS)_o$ (SEQ ID NO: 204), wherein o is an integer from 1 to 5. In some cases, the GS linker can comprise GGSGGGGSGG (SEQ ID NO: 205), GGSGGGGG (SEQ ID NO: 206), or GSGSGSGS (SEQ ID NO: 207). In certain cases, the Gly/Ser linker comprises $(G_nS)_m$, wherein n is 6 and m is 1.

[0210] In some cases, the linker suitable for the disclosure can be a Gly-rich linker, for example, comprising $(Gly)_p$ (SEQ ID NO: 208), wherein p is an integer from 1 to 40. In some cases, a Gly-rich linker can comprise GGGGG, GGGGGG, GGGGGGG or GGGGGGGG

[0211] In some cases, the linker suitable for the disclosure can comprise $(EAAAK)_q$ (SEQ ID NO: 209), wherein q is an integer from 1 to 5. In one case, the linker suitable for the disclosure can comprise $(EAAAK)_3$.

[0212] Further exemplary linkers include, but not limited to, GGGGSLVPRGSGGGGS (SEQ ID NO: 210), GSGSGS (SEQ ID NO: 211), GGGGSLVPRGSGGGG (SEQ ID NO: 212), GGSGGHMGSGG (SEQ ID NO: 213), GGSGGSGGSGG (SEQ ID NO: 214), GGSGG (SEQ ID NO: 215), GSGSGSGS (SEQ ID NO: 216), GGGSEGGGSEGGGSEGGG (SEQ ID NO: 217), AAGAATAA (SEQ ID NO: 218), GGSSG (SEQ ID NO: 219), GSGGGTGGGSG (SEQ ID NO: 220), GSGSGSGSGGSG (SEQ ID NO: 221), GSGGSGSGGSGGSG (SEQ ID NO: 222), and GSGGSGGSGGSGGS (SEQ ID NO: 223).

[0213] The nucleotides encoding the linkers can be constructed to fuse the nucleotide sequences of the present disclosure. Based on the RNA sequences provided, a person of ordinary skill in the art would understand the corresponding DNA sequence (e.g., conversion of uracil to thymine). Likewise, based on the DNA sequences provided, a person of ordinary skill in the art would understand the corresponding RNA sequence (e.g., conversion of thymine to uracil).

7. Sequence Optimization of Nucleotide Sequence Encoding an IL12 Polypeptide

[0214] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure is sequence optimized. In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide, a nucleotide sequence (*e.g.,* an ORF) encoding another polypeptide of interest, a 5'-UTR, a 3'-UTR, a miRNA, a nucleotide sequence encoding a linker, or any combination thereof that is sequence optimized.

[0215] A sequence-optimized nucleotide sequence, *e.g.,* an codon-optimized mRNA sequence encoding an IL12B and/or IL12A polypeptide, is a sequence comprising at least one synonymous nucleobase substitution with respect to a reference sequence (*e.g.,* a wild type nucleotide sequence encoding an IL12B and/or IL12A polypeptide).

[0216] A sequence-optimized nucleotide sequence can be partially or completely different in sequence from the reference sequence. For example, a reference sequence encoding polyserine uniformly encoded by TCT codons can be

sequence-optimized by having 100% of its nucleobases substituted (for each codon, T in position 1 replaced by A, C in position 2 replaced by G, and T in position 3 replaced by C) to yield a sequence encoding polyserine which would be uniformly encoded by AGC codons. The percentage of sequence identity obtained from a global pairwise alignment between the reference polyserine nucleic acid sequence and the sequence-optimized polyserine nucleic acid sequence would be 0%. However, the protein products from both sequences would be 100% identical.

[0217] Some sequence optimization (also sometimes referred to codon optimization) methods are known in the art (and discussed in more detail below) and can be useful to achieve one or more desired results. These results can include, *e.g.,* matching codon frequencies in certain tissue targets and/or host organisms to ensure proper folding; biasing G/C content to increase mRNA stability or reduce secondary structures; minimizing tandem repeat codons or base runs that can impair gene construction or expression; customizing transcriptional and translational control regions; inserting or removing protein trafficking sequences; removing/adding post translation modification sites in an encoded protein (*e.g.*, glycosylation sites); adding, removing or shuffling protein domains; inserting or deleting restriction sites; modifying ribosome binding sites and mRNA degradation sites; adjusting translational rates to allow the various domains of the protein to fold properly; and/or reducing or eliminating problem secondary structures within the polynucleotide. Sequence optimization tools, algorithms and services are known in the art, non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA) and/or proprietary methods.

[0218] Codon options for each amino acid are given in Table 3.

Table 3. Codon Options

Amino Acid	Single Letter Code	Codon Options
Isoleucine	I	ATT, ATC, ATA
Leucine	L	CTT, CTC, CTA, CTG, TTA, TTG
Valine	V	GTT, GTC, GTA, GTG
Phenylalanine	F	TTT, TTC
Methionine	M	ATG
Cysteine	C	TGT, TGC
Alanine	A	GCT, GCC, GCA, GCG
Glycine	G	GGT, GGC, GGA, GGG
Proline	P	CCT, CCC, CCA, CCG
Threonine	T	ACT, ACC, ACA, ACG
Serine	S	TCT, TCC, TCA, TCG, AGT, AGC
Tyrosine	Y	TAT, TAC
Tryptophan	W	TGG
Glutamine	Q	CAA, CAG
Asparagine	N	AAT, AAC
Histidine	H	CAT, CAC
Glutamic acid	E	GAA, GAG
Aspartic acid	D	GAT, GAC
Lysine	K	AAA, AAG
Arginine	R	CGT, CGC, CGA, CGG, AGA, AGG
Selenocysteine	Sec	UGA in mRNA in presence of Selenocysteine insertion element (SECIS)
Stop codons	Stop	TAA, TAG, TGA

[0219] In some cases, a polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a sequence-optimized nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide, a functional fragment, or a variant thereof, wherein the IL12B and/or IL12A polypeptide, functional fragment, or a variant thereof encoded by the sequence-optimized nucleotide sequence has improved properties (*e.g.,* compared to an IL12B and/or IL12A polypeptide, functional

fragment, or a variant thereof encoded by a reference nucleotide sequence that is not sequence optimized), *e.g.,* improved properties related to expression efficacy after administration *in vivo.* Such properties include, but are not limited to, improving nucleic acid stability (*e.g.,* mRNA stability), increasing translation efficacy in the target tissue, reducing the number of truncated proteins expressed, improving the folding or prevent misfolding of the expressed proteins, reducing toxicity of the expressed products, reducing cell death caused by the expressed products, increasing and/or decreasing protein aggregation.

[0220] In some cases, the sequence-optimized nucleotide sequence is codon optimized for expression in human subjects, having structural and/or chemical features that avoid one or more of the problems in the art, for example, features which are useful for optimizing formulation and delivery of nucleic acid-based therapeutics while retaining structural and functional integrity; overcoming a threshold of expression; improving expression rates; half-life and/or protein concentrations; optimizing protein localization; and avoiding deleterious bio-responses such as the immune response and/or degradation pathways.

[0221] In some cases, the polynucleotides of the disclosure comprise a nucleotide sequence (*e.g.,* a nucleotide sequence (*e.g.,* an ORF) encoding an IL12B and/or IL12A polypeptide, a nucleotide sequence (*e.g.,* an ORF) encoding an additional polypeptide of interest, a 5'-UTR, a 3'-UTR, a microRNA, a nucleic acid sequence encoding a linker, or any combination thereof) that is sequence-optimized according to a method comprising:

(i) substituting at least one codon in a reference nucleotide sequence (*e.g.,* an ORF encoding an IL12B and/or IL12A polypeptide) with an alternative codon to increase or decrease uridine content to generate a uridine-modified sequence;
(ii) substituting at least one codon in a reference nucleotide sequence (*e.g.,* an ORF encoding an IL12B and/or IL12A polypeptide) with an alternative codon having a higher codon frequency in the synonymous codon set;
(iii) substituting at least one codon in a reference nucleotide sequence (*e.g.,* an ORF encoding an IL12B and/or IL12A polypeptide) with an alternative codon to increase G/C content; or
(iv) a combination thereof.

[0222] In some cases, the sequence-optimized nucleotide sequence (*e.g.,* an ORF encoding an IL12B and/or IL12A polypeptide) has at least one improved property with respect to the reference nucleotide sequence.

[0223] In some cases, the sequence optimization method is multiparametric and comprises one, two, three, four, or more methods disclosed herein and/or other optimization methods known in the art.

[0224] Features, which can be considered beneficial in some cases of the disclosure, can be encoded by or within regions of the polynucleotide and such regions can be upstream (5') to, downstream (3') to, or within the region that encodes the IL12B and/or IL12A polypeptide. These regions can be incorporated into the polynucleotide before and/or after sequence-optimization of the protein encoding region or open reading frame (ORF). Examples of such features include, but are not limited to, untranslated regions (UTRs), microRNA sequences, Kozak sequences, oligo(dT) sequences, poly-A tail, and detectable tags and can include multiple cloning sites that may have XbaI recognition.

[0225] In some cases, the polynucleotide of the disclosure comprises a 5' UTR, a 3' UTR and/or a miRNA binding site. In some cases, the polynucleotide comprises two or more 5' UTRs and/or 3' UTRs, which can be the same or different sequences. In some cases, the polynucleotide comprises two or more miRNA binding sites, which can be the same or different sequences. Any portion of the 5' UTR, 3' UTR, and/or miRNA binding site, including none, can be sequence-optimized and can independently contain one or more different structural or chemical modifications, before and/or after sequence optimization.

[0226] In some cases, after optimization, the polynucleotide is reconstituted and transformed into a vector such as, but not limited to, plasmids, viruses, cosmids, and artificial chromosomes. For example, the optimized polynucleotide can be reconstituted and transformed into chemically competent *E. coli,* yeast, neurospora, maize, drosophila, etc. where high copy plasmid-like or chromosome structures occur by methods described herein.

8. Sequence-Optimized Nucleotide Sequences Encoding IL12 Polypeptides

[0227] In some cases, the polynucleotide of the disclosure comprises a sequence-optimized nucleotide sequence encoding an IL12B and/or IL12A polypeptide disclosed herein. In some cases, the polynucleotide of the disclosure comprises an open reading frame (ORF) encoding an IL12B and/or IL12A polypeptide, wherein the ORF has been sequence optimized.

[0228] Exemplary sequence-optimized nucleotide sequences encoding human IL12B and/or IL12A are shown in Tables 4A-4D. In some cases, the sequence optimized IL12B and/or IL12A sequences in Table 4A-4D, fragments, and variants thereof are used to practice the methods disclosed herein. In some cases, the sequence optimized IL12B and/or IL12A sequences in Table 4A-4D, fragments and variants thereof are combined with or alternatives to the wild-type sequences disclosed in Figure 1. Based on the RNA sequences provided, a person of ordinary skill in the art would understand the

corresponding DNA sequence (e.g., conversion of uracil to thymine). Likewise, based on the DNA sequences provided, a person of ordinary skill in the art would understand the corresponding RNA sequence (e.g., conversion of thymine to uracil).

Table 4A: Sequence optimized Open Reading Frame sequences for human IL12

> hIL12AB_001 (SEQ ID NO: 5)

ATGTGTCACCAGCAGCTGGTCATTAGCTGGTTTAGCCTTTGTGTTCCTGGCCTCCCCCCTTTGTCGCTATTTGGGAGCTCAAGAAGGA
CGTGTACGTGGTGGAGCTGGACTGGTACCCAGACGCCCCTGGAGAGATGGTAGTTCTGACCTGTGATACCCCAGAGGAGGACGGCA
TCACCTGGACTCTGGACCAAAGCAGCGAGGTTTTGGGCTCAGGGAAAACGCTGACCATCCAGGTGAAGGAATTCGGCGACGCCGGA
CAGTACACCTGCCATAAGGGAGGAGAGGTGCTGAGCCATTCCCTTCTTCTGCTGCACAAGAAAGAGGACGGCATCTGGTCTACCGA
CATCCTGAAAGACCAGAAGGAGCCCAAGAACAAAACCTTCCTGAGGTGCGAGGCCAAGAACTACTCCGGCAGGTTCACTTGTTGGT
GGCTGACCACCATCAGTACAGACCTGACTTTTAGTGTAAAAAGCTCCAGAGGCTCGTCCGATCCCCAAGGGGTGACCTGCGGCGCA
GCCACTCTGAGCGCTGAGCGCGTGCGCGGTGACAATAAAGAGTACGAGTACAGCGTTGAGTGTCAAGAAGACAGCGCTTGCCCTGC
CGCCGAGGAGAGCCTGCCTATCGAGGTGATGGTTGACGCAGTGCACAAGCTTAAGTACGAGAATTACACCAGCTCATTCTTCATTA
GAGATATAATCAAGCCTGACCCACCCAAGAACCTGCAGCTGAAGCCACTGAAAAACTCACGGCAGGTCGAAGTGAGCTGGGAGTAC
CCCGACACCTGGAGCACTCCTCATTCCTATTTCTCTCTTACATTCTGCGTCCAGGTGCAGGGCAAGAGCAAGCGGGAAAAGAAGGA
TCGAGTCTTCACCGACAAAACAAGCGCGACCGTGATTTGCAGGAAGAACGCCAGCATCTCCGTCAGAGCCCAGGATAGATACTATA
GTAGCAGCTGGAGCGAGTGGGCAAGCGTGCCCTGTTCCGGCGGCGGGGCGGGGCAGCCGAAACTTGCCTGTCGCTACCCCGGAC
CCTGGAATGTTTCCGTGTCTGCACCACAGCCAGAACCTGCTGAGAGCCGTGTCGAATATGCTCCAGAAGGCCCGGCAGACCCTTGA
GTTCTACCCCTGTACCAGCGAAGAGATCGATCATGAGGACATCACGAAAGACAAGACTTCCACCGTCGAGGCTTGTCTCCCGCTGG
AGCTGACCAAGAACGAGAGCTGTCTGAATAGCCGGGAGACATCTTTCATCACGAATGGTAGCTGTCTGGCCAGCAGGAAAACTTCC
TTCATGATGGCTCTCTGCCTGAGCTCTATCTATGAAGATCTGAAGATGTATCAGGTGGAGTTTAAGACTATGAACGCCAAACTCCT
GATGGACCCAAAAAGGCAAATCTTTCTGGACCAGAATATGCTGGCCGTGATAGACGAGCTGATGCAGGCACTGAACTTCAACAGCG
AGACAGTGCCACAGAAATCCAGCCTGGAGGAGCCTGACTTTTACAAAACTAAGATCAAGCTGTGTATCCTGCTGCACGCCTTTAGA
ATCCGTGCCGTGACTATCGACAGGGTGATGTCATACCTCAACGCTTCA

> hIL12AB_002(SEQ ID NO: 6)

ATGTGCCACCAGCAGCTGGTGATCAGCTGGTTCAGCCTGGTGTTCCTGGCCAGCCCCCTGGTGGCCATCTGGGAGCTGAAGAAGGA
CGTGTACGTGGTGGAGCTGGACTGGTACCCCGACGCCCCCGGCGAGATGGTGGTGCTGACCTGCGACACCCCCGAGGAGGACGGCA
TCACCTGGACCCTGGACCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCTGACCATCCAGGTGAAGGAGTTCGGCGACGCCGGC
CAGTACACCTGCCACAAGGGCGGCGAGGTGCTGAGCCACAGCCTGCTGCTGCTGCACAAGAAGGAGGACGGCATCTGGAGCACCGA
CATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACCTTCCTGAGATGCGAGGCCAAGAACTACAGCGGCAGATTCACCTGCTGGT
GGCTGACCACCATCAGCACCGACCTGACCTTCAGCGTGAAGAGCAGCAGAGGCAGCAGCGACCCCCAGGGCGTGACCTGCGGCGCC
GCCACCCTGAGCGCCGAGAGAGTGAGAGGCGACAACAAGGAGTACGAGTACAGCGTGGAGTGCCAGGAGGACAGCGCCTGCCCCGC
CGCCGAGGAGAGCCTGCCCATCGAGGTGATGGTGGACGCCGTGCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCA
GAGACATCATCAAGCCCGACCCCCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCAGACAGGTGGAGGTGAGCTGGGAGTAC
CCCGACACCTGGAGCACCCCCCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGCAAGAGCAAGAGAGAGAAGGAAGGA
CAGAGTGTTCACCGACAAGACCAGCGCCACCGTGATCTGCAGAAAGAACGCCAGCATCAGCGTGAGAGCCCAGGACAGATACTACA
GCAGCAGCTGGAGCGAGTGGGCCAGCGTGCCCTGCAGCGGCGGCGGCGGCGGCGGCAGCAGAAACCTGCCCGTGGCCACCCCCGAC
CCCGGCATGTTCCCCTGCCTGCACCACAGCCAGAACCTGCTGAGAGCCGTGAGCAACATGCTGCAGAAGGCCAGACAGACCCTGGA
GTTCTACCCCTGCACCAGCGAGGAGATCGACCACGAGGACATCACCAAGGACAAGACCAGCACCGTGGAGGCCTGCCTGCCCCTGG
AGCTGACCAAGAACGAGAGCTGCCTGAACAGCAGAGAGACCAGCTTCATCACCAACGGCAGCTGCCTGGCCAGCAGAAAGACCAGC
TTCATGATGGCCCTGTGCCTGAGCAGCATCTACGAGGACCTGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCCAAGCTGCT
GATGGACCCCAAGAGACAGATCTTCCTGGACCAGAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAACAGCG
AGACCGTGCCCCAGAAGAGCAGCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCAGA
ATCAGAGCCGTGACCATCGACAGAGTGATGAGCTACCTGAACGCCAGC

> hIL12AB_003(SEQ ID NO: 7)

ATGTGTCACCAGCAGTTGGTCATCTCTTGGTTTTCCCTGGTTTTTCTGGCATCTCCCCTCGTGGCCATATGGGAACTGAAGAAAGA
TGTTTATGTCGTAGAATTGGATTGGTATCCGGATGCCCCTGGAGAAATGGTGGTCCTCACCTGTGACACCCCTGAAGAAGATGGTA
TCACCTGGACCTTGGACCAGAGCAGTGAGGTCTTAGGCTCTGGCAAAACCCTGACCATCCAAGTCAAAGAGTTTGGAGATGCTGGC
CAGTACACCTGTCACAAAGGAGGCGAGGTTCTAAGCCATTCGCTCCTGCTGCTTCACAAAAAGGAAGATGGAATTTGGTCCACTGA
TATTTTAAAGGACCAGAAAGAACCCAAAAATAAGACCTTTCTAAGATGCGAGGCCAAGAATTATTCTGGACGTTTCACCTGCTGGT
GGCTGACGACAATCAGTACTGATTTGACATTCAGTGTCAAAAGCAGCAGAGGCTCTTCTGACCCCCAAGGGGTGACGTGCGGAGCT
GCTACACTCTCTGCAGAGAGAGTCAGAGGGGACAACAAGGAGTATGAGTACTCAGTGGAGTGCCAGGAGGACAGTGCCTGCCCAGC
TGCTGAGGAGAGTCTGCCCATTGAGGTCATGGTGGATGCCGTTCACAAGCTCAAGTATGAAAACTACACCAGCAGCTTCTTCATCA
GGGACATCATCAAACCTGACCCACCCAAGAACTTGCAGCTGAAGCCATTAAAGAATTCTCGGCAGGTGGAGGTCAGCTGGGAGTAC
CCTGACACCTGGAGTACTCCACATTCCTACTTCTCCCTGACATTCTGCGTTCAGGTCCAGGGCAAGAGCAAGAGAGAAAAGAAAGA
TAGAGTCTTCACGGACAAGACCTCAGCCACGGTCATCTGCCGCAAAAATGCCAGCATTAGCGTGCGGGCCCAGGACCGCTACTATA
GCTCATCTTGGAGCGAATGGGCATCTGTGCCCTGCAGTGGCGGAGGGGGCGGAGCGAGCAGAAACCTCCCCGTGGCCACTCCAGAC
CCAGGAATGTTCCCATGCCTTCACCACTCCCAAAACCTGCTGAGGGCCGTCAGCAACATGCTCCAGAAGGCCAGACAAACTTTAGA
ATTTTACCCTTGCACTTCTGAAGAGATTGATCATGAAGATATCACAAAAGATAAAACCAGCACAGTGGAGGCCTGTTTACCATGG
AATTAACCAAGAATGAGAGTTGCCTAAATTCCAGAGAGACCTCTTTCATAACTAATGGGAGTTGCCTGGCCTCCAGAAAGACCTCT
TTTATGATGGCCCTGTGCCTTAGTAGTATTTATGAAGACTTGAAGATGTACCAGGTGGAGTTCAAGACCATGAATGCAAAGCTTCT
GATGGATCCTAAGAGGCAGATCTTTTTAGATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGTG
AGACTGTGCCACAAAAATCCTCCCTTGAAGAACCGGACTTCTACAAGACCAAGATCAAGCTCTGCATACTTCTTCATGCTTTCAGA
ATTCGGGCAGTGACTATTGATAGAGTGATGAGCTATCTGAATGCTTCC

> hIL12AB_004 (SEQ ID NO: 8)

ATGGGCTGCCACCAGCAGCTGGTCATCAGCTGGTTCTCCCTGGTCTTCCTGGCCAGCCCCCTGGTGGCCATCTGGGAGCTGAAGAA
AGATGTCTATGTTGTAGAGCTGGACTGGTACCCAGATGCTCCTGGAGAAATGGTGGTTCTCACCTGTGACACGCCAGAAGAAGATG
GCATCACCTGGACGCTGGACCAGAGCTCAGAAGTTCTTGGCAGTGGAAAAACGCTGACCATACAAGTAAAAGAATTTGGGGATGCT
GGCCAGTACACCTGCCACAAAGGAGGAGAAGTTCTCAGCCACAGCCTGCTGCTGCTGCACAAGAAAGAAGATGGCATCTGGAGCAC
AGACATTTTAAAAGACCAGAAGGAGCCCAAGAACAAAACCTTCCTTCGATGTGAGGCCAAGAACTACAGTGGCCGCTTCACCTGCT
GGTGGCTCACCACCATCAGCACAGACCTCACCTTCTCGGTGAAGAGCAGCCGTGGCAGCTCAGACCCCCAAGGAGTCACCTGTGGG
GCGGCCACGCTGTCGGCAGAAAGAGTTCGAGGGGACAACAAGGAATATGAATACTCGGTGGAATGTCAAGAAGACTCGGCCTGCCC
GGCGGCAGAAGAAAGTCTTCCCATAGAAGTCATGGTGGATGCTGTTCACAAATTAAAATATGAAAACTACACCAGCAGCTTCTTCA
TCAGAGACATCATCAAGCCAGACCCGCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCAGACAAGTGGAAGTTTCCTGGGAG
TACCCAGACACGTGGAGCACGCCGCACAGCTACTTCAGCCTCACCTTCTGTGTACAAGTACAAGGCAAGAGCAAGAGAGAAGAAA
AGATCGTGTGCTTCACAGACAAAACCTCGGCGACGGTCATCTGCAGGAAGAATGCCTCCATCTCGGTTCGAGCCCAGGACCGCTACT
ACAGCAGCAGCTGGAGTGAGTGGGCCTCGGTGCCCTGCAGTGGTGGCGGCGGCGGCGGCAGCAGAAACCTTCCTGTGGCCACGCCG
GACCCTGGCATGTTCCCGTGCCTGCACCACAGCCAAAATTTACTTCGAGCTGTTTCTAACATGCTGCAGAAAGCAAGACAAACTTT
AGAATTCTACCCCTGCACCTCAGAAGAAATAGACCATGAAGACATCACCAAAGATAAAACCAGCACTGTAGAGGCCTGCCTGCCCC
TGGAGCTCACCAAGAATGAATCCTGCCTCAACAGCAGAGAGACCAGCTTCATCACCAATGGCAGCTGCCTGGCCAGCAGGAAAACC
AGCTTCATGATGGCGCTCTGCCTGAGCAGCATCTATGAAGATTTGAAGATGTACCAAGTAGAATTTAAAACCATGAATGCCAAGCT
GCTCATGGACCCCAAGAGACAAATATTTTTGGATCAAAACATGCTGGCTGTCATTGATGAGCTCATGCAAGCATTAAACTTCAACT
CAGAGACGGTGCCCCAGAAGAGCAGCCTGGAGGAGCCAGACTTCTACAAAACCAAGATCAAGCTCTGCATCTTATTACATGCCTTC
CGCATCCGGGCGGTCACCATTGACCGTGTCATGTCCTACTTAAATGCCAGC

> hIL12AB_005 (SEQ ID NO: 9)
ATGTGCCACCAGCAGCTGGTCATCAGCTGGTTCTCCCTGGTCTTCCTGGCCAGCCCCCTGGTGGCCATCTGGGAGCTGAAGAAAGA
TGTCTATGTTGTAGAGCTGGACTGGTACCCAGATGCTCCTGGAGAAATGGTGGTTCTCACCTGTGACACGCCAGAAGAAGATGGCA
TCACCTGGACGCTGGACCAGAGCTCAGAAGTTCTTGGCAGTGGAAAAACGCTGACCATACAAGTAAAAGAATTTGGGGATGCTGGC
CAGTACACCTGCCACAAAGGAGGAGAAGTTCTCAGCCACAGCCTGCTGCTGCTGCACAAGAAAGAAGATGGCATCTGGAGCACAGA
CATTTTAAAAGACCAGAAGGAGCCCAAGAACAAAACCTTCCTTCGATGTGAGGCCAAGAACTACAGTGGCCGCTTCACCTGCTGGT
GGCTCACCACCATCAGCACAGACCTCACCTTCTCGGTGAAGAGCAGCCGTGGCAGCTCAGACCCCCAAGGAGTCACCTGTGGGGCG
GCCACGCTGTCGGCAGAAAGAGTTCGAGGGGACAACAAGGAATATGAATACTCGGTGGAATGTCAAGAAGACTCGGCCTGCCCGGC
GGCAGAAGAAAGTCTTCCCATAGAAGTCATGGTGGATGCTGTTCACAAATTAAAATATGAAAACTACACCAGCAGCTTCTTCATCA
GAGACATCATCAAGCCAGACCCGCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCAGACAAGTGGAAGTTTCCTGGGAGTAC
CCAGACACGTGGAGCACGCCGCACAGCTACTTCAGCCTCACCTTCTGTGTACAAGTACAAGGCAAGAGCAAGAGAGAAGAAAGA
TCGTGTGCTTCACAGACAAAACCTCGGCGACGGTCATCTGCAGGAAGAATGCCTCCATCTCGGTTCGAGCCCAGGACCGCTACTACA
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AGCTCACCAAGAATGAATCCTGCCTCAACAGCAGAGAGACCAGCTTCATCACCAATGGCAGCTGCCTGGCCAGCAGGAAAACCAGC
TTCATGATGGCGCTCTGCCTGAGCAGCATCTATGAAGATTTGAAGATGTACCAAGTAGAATTTAAAACCATGAATGCCAAGCTGCT
CATGGACCCCAAGAGACAAATATTTTTGGATCAAAACATGCTGGCTGTCATTGATGAGCTCATGCAAGCATTAAACTTCAACTCAG
AGACGGTGCCCCAGAAGAGCAGCCTGGAGGAGCCAGACTTCTACAAAACCAAGATCAAGCTCTGCATCTTATTACATGCCTTCCGC
ATCCGGGCGGTCACCATTGACCGTGTCATGTCCTACTTAAATGCCAGC

> hIL12AB_006 (SEQ ID NO: 10)
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CGTGTACGTGGTGGAGCTGGACTGGTACCCCGACGCCCCCGGCGAGATGGTGGTGCTGACCTGTGACACCCCCGAGGAGGACGGCA
TCACCTGGACCCTGGACCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCTGACCATCCAGGTGAAGGAGTTCGGGGACGCCGGC
CAGTACACCTGCCACAAGGGCGGCGAGGTGCTGAGCCACAGCCTGCTGCTGCTGCACAAGAAGGAGGACGGCATCTGGAGCACAGA
TATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACCTTCCTGAGATGCGAGGCCAAGAACTACAGCGGCAGATTCACCTGCTGGT
GGCTGACCACCATCAGCACAGACTTGACCTTCAGCGTGAAGAGCAGCAGAGGCAGCAGCGACCCCCAGGGCGTGACCTGCGGCGCC
GCCACCCTGAGCGCCGAGAGAGTGAGAGGGGACAACAAGGAGTACGAGTACAGCGTGGAGTGCCAGGAGGACAGCGCCTGCCCCGC
CGCCGAGGAGAGCCTGCCCATCGAGGTGATGGTGGACGCCGTGCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCA
GAGACATCATCAAGCCCGACCCGCCGAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCAGACAGGTGGAGGTGAGCTGGGAGTAC
CCCGACACCTGGAGCACCCCCCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGCAAGAGCAAGAGAGAAGAAGGA
CAGAGTGTTCACAGATAAGACCAGCGCCACCGTGATCTGCAGAAAGAACGCCAGCATCAGCGTGAGAGCCCAGGACAGATACTACA
GCAGCAGCTGGAGCGAGTGGGCCAGCGTGCCCTGCAGCGGCGGCGGCGGCGGCAGCAGAAACCTGCCCGTGGCCACCCCCGAC
CCCGGCATGTTCCCCTGCCTGCACCACAGCCAGAACCTGCTGAGAGCCGTGAGCAACATGCTGCAGAAGGCCAGACAGACCCTGGA
GTTCTACCCCTGCACCAGCGAGGAGATCGACCACGAGGACATCACCAAGGACAAGACCAGCACCGTGGAGGCCTGCCTGCCCCTGG
AGCTGACCAAGAATGAAAGCTGCCTGAACAGCAGAGAGACCAGCTTCATCACCAACGGCAGCTGCCTGGCCAGCAGAAAGACCAGC
TTCATGATGGCCCTGTGCCTGAGCAGCATCTACGAGGACCTGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCCAAGCTGCT
GATGGACCCCAAGAGACAGATCTTCCTGGACCAGAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAACAGCG
AGACCGTGCCCCAGAAGAGCAGCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCAGA
ATCAGAGCCGTGACCATCGACAGAGTGATGAGCTACCTGAACGCCAGC

> hIL12AB_007 (SEQ ID NO: 11)
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TGTTTATGTTGTGGAGTTGGACTGGTACCCTGATGCTCCTGGAGAAATGGTGGTTCTCACCTGTGACACTCCTGAGGAGGATGGCA
TCACCTGGACTTTGGACCAGTCTTCTGAGGTTCTTGGCAGTGGAAAAACTCTTACTATTCAGGTGAAGGAGTTTGGAGATGCTGGC

CAGTACACCTGCCACAAGGGTGGTGAAGTTCTCAGCCACAGTTTACTTCTTCTTCACAAGAAGGAGGATGGCATCTGGTCTACTGA
CATTTTAAAAGACCAGAAGGAGCCCAAGAACAAGACTTTCCTTCGTTGTGAAGCCAAGAACTACAGTGGTCGTTTCACCTGCTGGT
GGCTTACTACTATTTCTACTGACCTTACTTTCTCTGTGAAGTCTTCTCGTGGCTCTTCTGACCCTCAGGGTGTCACCTGTGGGGCT
GCTACTCTTTCTGCTGAGCGTGTGCGTGGGGACAACAAGGAGTATGAATACTCGGTGGAGTGCCAGGAGGACTCTGCCTGCCCTGC
TGCTGAGGAGTCTCTTCCTATTGAGGTGATGGTGGATGCTGTGCACAAGTTAAAATATGAAAACTACACTTCTTCTTTCTTCATTC
GTGACATTATAAAACCTGACCCTCCCAAGAACCTTCAGTTAAAACCTTTAAAAAACTCTCGTCAGGTGGAGGTGTCCTGGGAGTAC
CCTGACACGTGGTCTACTCCTCACTCCTACTTCTCTCTTACTTTCTGTGTCCAGGTGCAGGGCAAGTCCAAGCGTGAGAAGAAGGA
CCGTGTCTTCACTGACAAGACTTCTGCTACTGTCATCTGCAGGAAGAATGCATCCATCTCTGTGCGTGCTCAGGACCGTTACTACA
GCTCTTCCTGGTCTGAGTGGGCTTCTGTGCCCTGCTCTGGCGGCGGCGGCGGCGGCAGCAGAAATCTTCCTGTGGCTACTCCTGAC
CCTGGCATGTTCCCCTGCCTTCACCACTCGCAGAACCTTCTTCGTGCTGTGAGCAACATGCTTCAGAAGGCTCGTCAGACTTTAGA
ATTCTACCCCTGCACTTCTGAGGAGATTGACCATGAAGACATCACCAAGGACAAGACTTCTACTGTGGAGGCCTGCCTTCCTTTAG
AGCTGACCAAGAATGAATCCTGCTTAAATTCTCGTGAGACTTCTTTCATCACCAATGGCAGCTGCCTTGCCTCGCGCAAGACTTCT
TTCATGATGGCTCTTTGCCTTTCTTCCATCTATGAAGACTTAAAAATGTACCAGGTGGAGTTCAAGACCATGAATGCAAAGCTTCT
CATGGACCCCAAGCGTCAGATATTTTTGGACCAGAACATGCTTGCTGTCATTGATGAGCTCATGCAGGCTTTAAACTTCAACTCTG
AGACTGTGCCTCAGAAGTCTTCTTTAGAAGAGCCTGACTTCTACAAGACCAAGATAAAACTTTGCATTCTTCTTCATGCTTTCGGC
ATCCGTGCTGTGACTATTGACCGTGTGATGTCCTACTTAAATGCTTCT

> hIL12AB_008 (SEQ ID NO: 12)
ATGTGTCATCAACAACTGGTGATTAGCTGGTTCAGTCTGGTGTTCCTGGCCTCTCCGCTGGTGGCCATCTGGGAGCTTAAGAAGGA
CGTGTACGTGGTGGAGCTCGATTGGTACCCGGATGCTCCTGGCGAGATGGTGGTGCTAACCTGCGATACCCCCGAGGAGGACGGTA
TCACTTGGACCCTGGATCAGAGTAGCGAAGTCCTGGGCTCTGGCAAGACACTCACAATCCAGGTGAAGGAATTCGGAGACGCTGGT
CAGTACACTTGCCACAAGGGGGGTGAAGTCCTGTCTCACAGCCTGCTGTTACTGCACAAGAAGGAGGATGGGATCTGGTCAACCGA
CATCCTGAAGGATCAGAAGGAGCCTAAGAACAAGACCTTTCTGAGGTGTGAAGCTAAGAACTATTCCGGAAGATTCACTTGCTGGT
GGTTGACCACAATCAGCACTGACCTGACCTTTACCGTGAAGTCCAGCAGAGGAAGCAGCGATCCTCAGGGCGTAACGTGCGGCGCG
GCTACCCTGTCAGCTGAGCGGGTTAGAGGCGACAACAAAGAGTATGAGTACTCCGTGGAGTGTCAGGAGGACAGCGCCTGCCCCGC
AGCCGAGGAGAGTCTGCCCATCGAGGTGATGGTGGACGCTGTCCATAAGTTAAAATACGAAAATTACACAAGTTCCTTTTTCATCC
GCGATATTATCAAACCGATCCCCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAATAGCCGACAGGTGGAAGTCTCTTGGGAGTAT
CCTGACACCTGGTCCACGCCTCACAGCTACTTTAGTCTGACTTTCTGTGTCCAGGTCCAGGGCAAGAGCAAGAGAGAGAAAAAGGA
TAGAGTGTTTACTGACAAGACATCTGCTACAGTCATCTGCAGAAAGAACGCCAGTATCTCAGTGAGGGCGCAGGACAGATACTACA
GTAGTAGCTGGAGCGAATGGGCTAGCGTGCCCTGTTCAGGGGGCGGCGGAGGGGGCTCCAGGAATCTGCCCGTGGCCACCCCCGAC
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GATGGACCCCAAGAGACAGATCTTCCTGGACCAGAACATGCTGGCAGTGATCGATGAGCTGATGCAAGCCTTGAACTTCAACTCAG
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> hIL12AB_009 (SEQ ID NO: 13)
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CATTTTAAAAGACCAGAAGGAGCCCAAGAACAAAACCTTCCTTCGATGTGAGGCGAAGAACTACAGTGGCCGCTTCACCTGCTGGT
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> hIL12AB_010 (SEQ ID NO: 14)
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ATCCGTGCTGTCACCATTGACGGTGTCATGTCCTACTTAAATGCTTCT

> hIL12AB_G11 (SEQ ID NO: 15)
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TCACGTGGACGCTGGACCAGAGCAGCGAGGTGCTGGGGAGCGGGAAGACGCTGACGATCCAGGTGAAGGAGTTCGGGGACGCGGGG
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> hIL12AB_G12 (SEQ ID NO: 16)
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> hIL12AB_G13 (SEQ ID NO: 17)
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> hIL12AB_G14 (SEQ ID NO: 18)
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> hIL12AB_G15 (SEQ ID NO: 19)
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> hIL12AB_G16 (SEQ ID NO: 20)
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> hIL12AB_017          (SEQ ID NO: 21)
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> hIL12AB_018                    (SEQ ID NO: 22)
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> hIL12AB_019          (SEQ ID NO: 23)
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GGCTCACCACCATCTCCACTGACCTCACCTTCTCTGTAAAAAGCAGCCGTGGTTCTTCTGACCCCCAAGGAGTCACCTGTGGGGCT
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CCTGGCATGTTCCCGTGCCTTCACCACTCCCAAAATCTTCTTCGTSCTGTTTCTAACATSCTGCAGAASGCGCGCCAAACTTTAGA
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> hIL12AB_020 (SEQ ID NO: 24)
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GCCACCCTGAGCGCCGAAAGAGTGCGCGGCGACAACAAGGAGTACGAGTACTCCGTGGAATGCCAGGAGGACAGCGCCTGCCCCGC
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> hIL12AB_021(SEQ ID NO: 25)
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GCTGCTGATGGACCCCAAGAGGCAAATCTTCCTGGACCAGAATATGCTTGCCGTCATCGACGAGCTCATGCAGGCCCTGAACTTCA
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> hIL12AB_022(SEQ ID NO: 26)
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ACAGCGAGACGGTGCCCCAGAAAAGCTCCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCC
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> hIL12AB_023(SEQ ID NO: 27)

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ACAGCGAGACAGTGCCCCAGAAGTCCTCCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGTATCCTGCTGCACGCC
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> hIL12AB_024(SEQ ID NO: 28)

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> hIL12AB_025(SEQ ID NO: 29)

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ACTCCGAGACCGTGCCGCAGAAGTCATCCCTCGAGGAGCCCGACTTCTATAAGACCAAGATCAAGCTGTGCATCCTGCTCCACGCC
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> hIL12AB_025(SEQ ID NO: 30)

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> hIL12AB_027(SEQ ID NO: 31)

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> hIL12AB_028(SEQ ID NO: 32)

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> hIL12AB_029(SEQ ID NO: 33)

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GCTGCTGATGGATCCCAAGAGGCAGATTTTCCTGGACCAGAACATGCTGGCCGTGATTGACGAGCTGATGCAGGCGCTGAACTTCA
ACAGCGAGACAGTGCCGCAGAAGAGCTCCCTGGAGGAGCCGGACTTTTACAAGACCAAGATAAAGCTGTGCATCCTGCTCCACGCC
TTCAGAATACGGGCCGTCACCATCGATAGGGTGATGTCTTACCTGAACGCCTCC

> hIL12AB_030(SEQ ID NO: 34)
ATGTGCCACCAGCAGCTGGTGATTAGCTGGTTTAGCCTGGTGTTCCTGGCAAGCCCCCTGGTGGCCATCTGGGAACTGAA
AAAGGACGTGTACGTGGTCGAGCTGGATTGGTACCCCGACGCCCCCGGCGAAATGGTGGTGCTGACGTGTGATACCCCCGAGGAGG
ACGGCATCACCTGGACCCTGGATCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCTGACGATCCAGGTCAAGGAGTTCGGCGAC
GCTGGGCAGTACACCTGTCACAAGGGCGGGGAGGTGCTGTCCCACTCCCTGCTGCTCCTGCATAAGAAGGAGGACGGCATCTGGTC
CACCGACATCCTCAAGGACCAGAAGGAGCCCAAGAACAAGACCTTCCTGCGGTGTGAGGCGAAGAACTACAGCGGCCGTTTCACCT
GCTGGTGGCTGACGACAATCAGCACCGACTTGACGTTCTCCGTGAAGTCCTCCAGAGGCAGCTCCGACCCCCAAGGGGTGACGTGC
GGCGCGGCCACCCTGAGCGCCGAGCGGGTGCGGGGGGACAACAAGGAGTACGAGTACTCCGTGGAGTGCCAGGAGGACAGCGCCTG
TCCCGCAGCCGAGGAGTCCCTGCCCATCGAAGTCATGGTGGACGCCGTCAAGAAGCTGAAGTACGAGAACTACACCAGCAGCTTCT
TCATCCGCGATATCATCAAGCCCGATCCCCCCAAAAACCTGCAACTGAAGCCGCTGAAGAATAGCAGGCAGGTGGAGGTGTCCTGG
GAGTACCCGGACACCTGGAGCACGCCCCACAGCTATTTCAGCCTGACCTTTTGCGTGCAGGTCCAGGGGAAGAGCAAGCGGGAGAA
GAAGGACCGCGTGTTTACGGACAAAACCAGCGCCACCGTGATCTGCAGGAAGAACGCCAGCATCAGCGTGAGGGCCCAGGACAGGT
ACTACAGCAGCTCCTGGAGCGAGTGGGCCTCCGTGCCCTGTTCCGGAGGCGGCGGGGGCGGTTCCCGGAACCTCCCGGTGGCCACC
CCCGACCCGGGCATGTTCCCGTGCCTGCACCACTCACAGAATCTGCTGAGGGCCGTGAGCAATATGCTGCAGAAGGCAAGGCAGAC
CCTGGAGTTTTATCCCTGCACCAGCGAGGAGATCGACCACGAAGACATCACCAAGGACAAGACCAGCACAGTGGAGGCCTGCCTGC
CCCTGGAACTGACCAAGAACGAGTCCTGTCTGAACTCCCGGGAAACCAGCTTCATAACCAACGGCTCCTGTCTCGCCAGCAGGAAG
ACCAGCTTCATGATGGCCCTGTGCCTCAGCTCCATCTACGAGGACCTCAAGATGTACCAGGTTGAGTTCAAGACCATGAACGCCAA
GCTCCTGATGGACCCCAAGAGGCAGATCTTCCTGGACCAGAATATGCTGGCCGTGATCGATGAGTTAATGCAGGCGCTGAACTTCA
ACAGCGAGACGGTGCCCCAAAAGTCCTCGCTGGAGGAGCCGGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTCCTGCACGCC
TTCCGAATCCGGGCCGTAACCATCGACAGGGTGATGAGCTATCTCAACGCCTCC

> hIL12AB_031(SEQ ID NO: 35)
ATGTGCCACCAGCAGCTCGTGATCAGCTGGTTCTCGCTTGTGTTCCTGGCCTCCCCCCTCGTCGCCATCTGGGAGCTGAA
GAAAGACGTGTACGTGGTGGAGCTGGACTGGTATCCCGACGCCCCGGGGGAGATGGTGGTGCTGACCTGCGACACCCCCGAAGAGG
ACGGCATCACCTGGACGCTCGACCAGTCGTCCGAAGTGCTGGGGTCGGGCAAGACCCTCACCATCCAGGTGAAGGAGTTCGGAGAC
GCCGGCCAGTACACCTGTCATAAGGGGGGCGAGGTGCTGAGCCACAGCCTCCTGCTCCTGCACAAAAAGGAGGACGGCATCTGGAG
CACCGATATCCTCAAGGACCAGAAGGAGCCCAAGAACAAGACGTTCCTGAGGTGTGAGGCCAAGAACTACAGCGGGCGGTTCACGT
GTTGGTGGCTCACCACCATCTCCACCGACCTCACCTTCTCCGTGAAGTCAAGCAGGGGCAGCTCCGACCCCCAAGGCGTCACCTGC
GGCGCCGCCACCCTGAGCGCCGAGAGGGTCAGGGGGGATAACAAGGAATACGAGTACAGTGTGGAGTGCCAAGAGGATAGCGCCTG
TCCCGCCGCCGAAGAGAGCCTGCCCATCGAAGTGATGGTGGACGCCGTCAAGAAGCTGAAGTACGAGAACTACACCTCCAGCTTCT
TCATCAGGGATATCATCAAGCCCGATCCCCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCAGGCAGGTGGAGGTGAGCTGG
GAGTATCCCGACACGTGGAGCACCCCGCACAGCTACTTCTCGCTGACCTTCTGCGTGCAGGTGCAAGGGAAGTCCAAGAGGGAGAA
GAAGGATAGGGTGTTCACCGACAAAACGAGCGCCACCGTGATCTGCCGGAAGAATGCCAGCATCTCTGTGAGGGCCCAGGACAGGT
ACTATTCCAGCTCCTGGTCGGAGTGGGCCAGCGTGCCCTGTAGCGGCGGGGGCGGGGGCGGCAGCAGGAACCTCCCGGTTGCCACC
CCCGACCCCGGCATGTTCCGTGCCTGCACCACTCGCAAAACCTGCTGCGCCGTCTCCAACATGCTGCAAAAAGCGCGCCAGAC
GCTGGAGTTCTACCCCTGCACCAGCGAGGAGATCGATCATGAAGATATCACCAAAGACAAGACCTCGACCGTGGAGGCCTGCCTGC
CCCTGGAGCTCACCAAGAACGAAAGCTGCCTGAACAGCAGGGAGACAAGCTTCATCACCAACGGCAGCTGCCTGGCCTCCCGGAAG
ACCAGCTTCATGATGGCCCTGTGCCTGTCCAGCATCTACGAGGATCTGAAGATGTACCAAGTGGAGTTTAAGACCATGAACGCCAA
GCTGTTAATGGACCCCAAAAGGCAGATCTTCCTGGATCAGAACATGCTGGCCGTCATCGACGAGCTGATGCAAGCCCTGAACTTCA
ACAGCGAGACGGTGCCCCAGAAGAGCAGCCTCGAGGAGCCCGACTTCTATAAGACCAAGATAAAGCTGTGCATTCTGCTGCACGCC
TTCAGAATCAGGGCCGTGACCATCGATAGGGTGATGAGCTACCTGAACGCCAGC

> hIL12AB_032(SEQ ID NO: 36)
ATGTGTCACCAGCAGCTGGTGATTTCCTGGTTCAGTCTGGTGTTTCTTGCCAGCCCCCTGGTGGCCATCTGGGAGCTGAA
GAAAGACGTATACGTCGTGGAGCTGGACTGGTATCCCGACGCTCCCGGCGAGATGGTGGTCCTCACCTGCGACACCCCAGAGGAGG
ACGGCATCACCTGGACCCTGGACCAGAGCTCCGAGGTCCTGGGCAGCGGTAAGACCCTCACCATCCAGGTGAAGGAGTTTGGTGAT
GCCGGGCAGTATACCTGCCACAAGGGCGGCGAGGTGCTGTCCCACAGCCTCCTGTTACTGCATAAGAAGGAGGATGGCATCTGGAG
CACCGACATCCTCAAGGACCAGAAAGAGCCCAAGAACAAGACCTTTCTGCGGTGCGAGGCGAAAAATTACTCCGGCCGTTTCACCT

GCTGGTGGCTGACCACCATCAGCACGGACCTGACGTTCTCCGTGAAGTCGAGCAGGGGGAGCTCCGATCCCCAGGGCGTGACCTGC
GGCGCGGCCACCCTGAGCGCCGAGCGCGTCCGCGGGGACAATAAGGAATACGAATATAGCGTGGAGTGCCAGGAGGACAGCGCCTG
CCCCAGCGGCCGAGGAGAGCCTCCCGATCGAGGTGATGGTGGATGCCGTCCACAAGCTCAAATACGAAAACTACACCAGCAGCTTCT
TCATTAGGGACATCATCAAGCCCGACCCCCCCAAAAACCTGCAGCTGAAGCCCCTGAAGAACAGCGCCGCCAGGTCGAGGTGTCATGG
GAGTACCCAGACACCTGGAGCACCCCCCACTCCTACTTCAGCCTGACCTTCTGCGTCCAGGTGCAGGGAAAGTCCAAACGGAGAA
GAAGGATAGGGTCTTTACCGATAAGACGTCGGCCACGTCATCTGCAGGAAGAACGCCAGCATAAGCGTGCGGCGCAGGATCGGT
ACTACAGCTCGAGCTGGTCCGAATGGGCCTCCGTGCCCTGTAGCGGAGGGGTGGCGGGGGCAGCAGGAACCTGCCCGTGGCCACC
CCGGACCCGGGCATGTTTCCCTGCCTGCATCACAGTCAGAACCTGCTGAGGGCCGTGAGCAACATGCTCCAGAAGGCCGCCAGAC
CCTGGAGTTTTACCCCTGCACCAGCGAAGAGATCGATCACGAAGACATCACCAAAGACAAGACCTCCACCGTGGAGGCCTGTCTGC
CCCTGGAGCTGACCAAGAACGAGAGCTGTCTGAACAGCAGGGAGACCTCCTTCATCACCAACGGCTCCTGCCTGGCATCCCGGAAG
ACCAGCTTCATGATGGCCCTGTGTCTGAGCTCTATCTACGAGGACCTGAAGATGTACCAGGTCGAGTTCAAGACCATGAACGCCAA
GCTGCTGATGGACCCCAAGCGACAGATATTCCTGGACCAGAACATGCTGGCCGTGATCGATGAACTGATGCAAGCCCTGAACTTCA
ATAGCGAGACCGTGCCCCAGAAAAGCAGCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAACTGTGCATACTGCTGCACGCG
TTCAGGATCCGGGCCGTCACCATCGACCGGGTGATGTCCTATCTGAATGCCAGC

> hIL12AB_033(SEQ ID NO: 37)

ATGTGCCACCAGCAGCTCGTGATTAGCTGGTTTTCGCTGGTGTTCCTGGCCAGCCCTCTCGTGGCCATCTGGGAGCTGAA
AAAAGACGTGTACGTGGTGGAGCTGGACTGGTACCCGGACGCCCCCGGCGAGATGGTGGTGCTGACGTGCGACACCCCGGAAGAGG
ACGGCATCACCTGGACCCTGGACCAGTCATCCGAGGTCCTGGGCAGCGGCAAGACGCTGACCATCCAGGTGAAGGAGTTCGGCGAC
GCCGGCCAGTACACATGCCATAAGGGCGGCGAGGTGCTGAGCCACAGCCTGCTCCTCCACAAGAAGGAGGATGGCATCTGGTC
TACAGACATCCTGAAGGACCAGAAAGAGCCCAAGAACAAGACCTTCCTCCGGTGCGAGGCCAAGAACTACTCCGGCGGTTTACTT
GTTGGTGGCTGACCACCATCAGCACCGACCTCACCTTCAGCGTGAAGAGCTCCCGAGGGAGCTCCGACCCCCAGGGGGTCACCTGC
GGCGCCGCCACCCTGAGCGCCGAGCGGGTGAGGGGCGACAACAAGGAGTATGAATACAGCGTGGAATGCCAAGAGGACAGCGCCTG
TCCCAGCGGCCGAGGAAAGCCTGCCCATCGAGGTGATGGTGGACGCCGTCCACAAACTCAAGTACGAGAACTACACCAGCAGTTTCT
TCATTCGCGACATCATCAAGCCGGACCCCCCCAAAAACCTGCAGCTCAAACCCCTGAAGAACAGCAGGCAGGTGGAGGTCAGCTGG
GAGTACCCGGACACCTGGAGCACCCCCCATAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGCAAGAGCAAACGCGAGAA
GAAGGACCGGGTGTTTACCGACAAGACCAGCGCCACGGTGATCTGCCGAAAGAATGCAAGCATCTCCGTGAGGGCGCAGGACCGCT
ACTACTCTAGCAGCTGGAGCGAGTGGGCCAGCGTGCCCTGCAGCGGTGGCGGCGGAGGCGGCAGCCGTAACCTCCCCGTGGCCACC
CCCGACCCCGGCATGTTCCCGTGTCTGCACCACTCCCAGAACCTGCTGAGGGCCGTCAGCAATATGCTGCAGAAGGCCCGGCAGAC
GCTGGAGTTCTACCCCTGCACCTCCGAGGAGATCGACCATGAGGACATTACCAAGGACAAGACCAGCACTGTGGAGGCCTGCCTGC
CCCTGGAGCTCACCAAAAACGAGAGCTGCCTGAATAGCAGGGAGACCTCCTTCATCACCAACGGCAGCTGTCTGGCCAGCAGGAAG
ACCAGCTTCATGATGGCCCTGTGCCTCTCCTCTATATATGAGGACCTGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCCAA
GCTGCTGATGGATCCCAAGAGGCAGATCTTCCTGGACCAGAATATGCTGGCCGTGATTGACGAGCTGATGCAGGCCCTGAACTTTA
ATAGCGAGACCGTCCCCCAGAAGAGCAGCCTGGAGGAGCCCGACTTCTATAAGACCAAGATCAAGCTGTGCATACTGCTGCACGCG
TTTAGGATAAGGGCCGTCACCATCGACAGGGTGATGAGCTACCTGAATGCCAGC

> hIL12AB_034(SEQ ID NO: 38)

ATGTGCCACCAACAGCTGGTGATCTCCTGGTTCAGCCTGGTGTTCCTCGCCAGCCCCCTGGTGGCCATCTGGGAGCTGAA
GAAAGACGTGTACGTGGTGGAGCTGGACTGGTATCCCGACGCCCCCGGCGAGATGGTCGTGCTGACCTGCGACACCCCGGAGGAGG
ACGGCATCACCTGGACCCTGGATCAGTCCTCCGAGGTGCTGGGCAGCGGGAAGACCCTGACCATCCAGGTGAAAGAGTTCGGAGAT
GCCGGCCAGTATACCTGTCACAAGGGGGGTGAGGTGCTGAGCCATAGCCTCTTGCTTCTGCACAAGGAAGGAGGACGGCATCTGGTC
CACCGACATCCTCAAGGACCAAAAGGAGCCGAAGAATAAAACGTTCCTGAGGTGCGAAGCCAAGAACTATTCCGGACGGTTCACCT
GCTGGTGGCTGACCACCATCAGCACCGACCTCACCTTCTCCGTAAAGTCAAGCAGGGGCAGCTCCGACCCCCAGGGCGTGACCTGC
GGAGCCGCCACCCTGAGCGCAGAGAGGGTGAGGGGCGACAACAAGGAGTACGAATACTCCGTCGAGTGCCAGGAGGACAGCGCCTG
CCCCGCCGCCGAGGAAAGTCTGCCCATCGAGGTGATGGTGGACGCCGTGCACAAGCTCAAATACGAGAACTACACCAGCAGCTTCT
TCATCCGGGATATCATCAAGCCCGACCCTCCAAAGAATCTGCAGCTGAAACCCCTTAAGAACAGCAGGCAGGTGGAGGTCAGCTGG
GAGTACCCCGACACCTGGAGCACGCCCCACTCCTACTTTAGCCTGACCTTTTGCGTGCAGGTGCAGGGGAAAAGCAAGCGGGAGAA
GAAGGACAGGGTGTTCACCGATAAGACCTCCGCTACCGTGATCTGCAGGAAGAACGCCTCAATCAGCGTGAGGGCCCAGGATCGGT
ACTACTCCAGCTCCTGGAGCGAGTGGGCCAGCGTGCCCTGCTCTGGCGGTGGCGGCGGGGGCAGCCGGAACCTGCCGGTGGCCACT
CCCGACCCGGGCATGTTCCCGTGCCTCCACCATTCCCAGAACCTGCTGCGGGCCGTGTCCAATATGCTCCAGAAGGCAAGGCAGAC
CCTGGAGTTCTACCCCTGCACCAGCGAGGAGATCGATCACGAGGACATCACCAAAGACAAAACCAGCACGGTCGAGGCCTGCCTGC
CCCTGGAACTCACCAAGAACGAAAGCTGTCTCAACAGCCGCGAGACCAGCTTCATAACCAACGGTTCCTGTCTGGCCTCCCGCAAG
ACCAGCTTTATGATGGCCCTCTGTCTGAGCTCCATCTATGAAGACCTGAAAATGTACCAGGTGGAGTTCAAAACCATGAACGCCAA
GCTTCTGATGGACCCCAAGAGGCAGATCTTCCTGGATCAGAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTTA
ACTCCGAGACCGTGCCCCAGAAAAGCAGCCTGGAAGAGCCCGATTTCTACAAAACGAAGATCAAGCTGTGCATCCTGCTGCACGCC
TTCCGGATCCGTGCGGTGACCATCGATAGGGTGATGAGCTACCTGAACGCCAGC

> hIL12AB_035(SEQ ID NO: 39)

ATGTGCCACCAACAGCTGGTAATCAGCTGGTTCAGCCTGGTTTTCCTCGCGTCGCCCCTGGTGGCCATCTGGGAGTTAAA
GAAGGACGTGTACGTGGTGGAGCTGGATTGGTACCCCGACGCCCCGGGCGAGATGGTCGTGCTCACCTGCGATACCCCGGAGGAGG
ACGGGATCACCTGGACCCTGGACCAATCCAGCGAGGTGCTGGGCAGCGGCAAGACCCTGACCATACAGGTGAAGGAATTTGGGGAC
GCCGGCCAGTACACCTGCCACAAGGGCGGGGAAGTGCTGTCCCACTCCCTCCTGCTGCTGCATAAGAAGGAGGACGGCATCTGGAG
CACCGACATCCTGAAGGACCAAAAGGAGCCCAAGAACAAGACCTTCCTGAGGTGCGAGGCCAAAAACTATTCCGGCCGCTTTACCT
GTTGGTGGCTGACCACCATCTCCACCGATCTGACCTTCAGCGTGAAGTCGTCTAGGGGCTCCTCCGACCCCCAGGGCGTAACCTGC
GGCGCCGCGACCCTGAGCGCCGAGAGGGTGCGGGGCGATAACAAGGAGTACGAGTACTCGGTGGAGTGCCAGGAGGACAGCGCCTG
TCCGGCGGCCGAGGAGAGCCTGCCCATCGAGGTGATGGTGGACGCCGTCCACAAGCTGAAGTACGAGAACTACACCAGTTCGTTCT

TCATCAGGGACATCATCAAGCCGGACCCCCCCAAGAACCTCCAGCTGAAGCCCCTGAAGAACAGCAGGCAGGTGGAAGTGTCCTGG
GAGTATCCCGACACCTGGAGCACCCCCCACAGCTACTTCAGCCTGACCTTTTGCGTGCAGGTGCAGGGCAAAAGCAAGAGGGAAAA
GAAGGACCGGGTGTTCACCGATAAGACGAGCGCCACCGTTATCTGCAGGAAGAACGCCTCCATAAGCGTGAGGGCGCAGGACCGTT
ACTACAGCAGCAGCTGGAGTGAGTGGGCAAGCGTGCCCTGTAGCGGCGGGGGCGGGGGCGGGTCCCGCAACCTCCCCGTCGCCACC
CCCGACCCAGGCATGTTTCCGTGCCTGCACCACAGCCAGAACCTGCTCCGGGCCGTTACGAACATGCTGCAGAAGGCCAGGCAGAC
CCTCGAGTTCTATCCCTGCACATCTGAGGAGATCGACCACGAAGACATCACTAAGGATAAGACCTCCACCGTGGAGGCCTGTCTGC
CCCTCGAGCTGACCAAGAATGAATCCTGCCTGAACAGCCGAGAGACCAGCTTTATCACCAACGGCTCCTGCCTGGCCAGCAGGAAG
ACCTCCTTCATGATGGCCCTGTGCCTCTCCAGCATCTACGAGGATCTGAAGATGTACCAGGTAGAGTTCAAGACGATGAACGCCAA
GCTCCTGATGGACCCCAAGAGGCAGATATTCCTGGACCAGAACATGCTGGCGGTGATCGACGAGCTGATGCAGGCCCTGAATTTCA
ACAGCGAGACGGTGCCACAGAAGTCCAGCCTGGAGGAGCCAGACTTCTACAAGACCAAGATCAAACTGTGCATCCTCCTGCACGCG
TTCAGGATCCGCGCCGTCACCATAGACAGGGTGATGAGTTATCTGAACGCCAGC

> hIL12AB_036(SEQ ID NO: 40)

ATGTGCCATCAGCAGCTGGTAATCAGCTGGTTTAGCCTGGTGTTCCTGGCCAGCCCACTGGTGGCCATCTGGGAGCTGAA
GAAGGACGTGTACGTGGTGGAACTGGACTGGTACCCCGACGCCCCTGGCGAGATGGTGGTACTGACCTGTGACACCCCGGAGGAAG
ACGGTATCACCTGGACCCTGGATCAGAGCTCCGAGGTGCTGGGCTCCGGCAAGACACTGACCATCCAAGTTAAGGAATTTGGGGAC
GCCGGCCAGTACACCTGCCACAAGGGGGGCGAGGTGCTGTCCCACTCCCTGCTGCTTCTGCATAAGAAGGAGGATGGCATCTGGTC
CACCGACATACTGAAGGACCAGAAGGAGCCCAAGAATAAGACCTTCCTGAGATGCGAGGCCAAGAACTACTCGGGAAGGTTCACCT
GCTGGTGGCTGACCACCATCAGCACCGACCTGACCTTCTCCGTGAAGAGCTCCCGGGGCAGCTCCGACCCCCAGGGCGTAACCTGT
GGGGCCGCTACCCTGTCCGCCGAGAGGGTCCGGGGCGACAACAAGGAATACGAGTACAGCGTGGAGTGCCAGGAGGACTCCGCCTG
CCCCGCCGCCGAGGAGTCGCTGCCCATAGAGGTGATGGTGGACGCCGTGCACAAGCTCAAGTACGAGAATTACACCAGCAGCTTCT
TTATCAGGGACATAATAAGCCGGACCCCCCAAAGAATCTGCAGCTGAAGCCCCTGAAGAATAGCCGGCAGGTGGAAGTGTCCTGG
GAGTACCCGGACACCTGGAGCACCCCCCACTCCTATTTCTCACTGACATTCTGCGTGCAGGTGCAAGGGAAAAGCAAGAGGGAGAA
GAAGGATAGGGTGTTCACCGACAAGACAAGCGCCACCGTGATCTGCCGAAAAATGCCAGCATCAGCGTGAGGGCCCAGGATCGGT
ATTACAGCAGCTCCTGGAGCGAGTGGGCCAGCGTGCCCTGTTCCGGCGGGGGAGGGGGCGGCTCCCGGAACCTGCCGGTGGCCACC
CCCGACCCTGGCATGTTCCCCTGCCTGCATCACAGCCAGAACCTGCTCCGGGCCGTGTCGAACATGCTGCAGAAGGCCCGGCAGAC
CCTCGAGTTTTACCCCTGCACCAGCGAAGAGATCGACCACGAAGACATAACCAAGGACAAGACCAGCACGGTGGAGGCCTGCCTGC
CCCTGGAGCTTACCAAAAACGAGTCCTGCCTGAACAGCCGGGAAACCAGCTTCATAACGAACGGGAGCTGCCTGGCCTCCAGGAAG
ACCAGCTTCATGATGGCGCTGTGTCTGTCCAGCATATACGAGGATCTGAAGATGTATCAGGTGGAATTCAAAACTATGAATGCCAA
GCTCCTGATGGACCCCAAGAGGCAGATCTTCCTGGACCAGAACATGCTAGCCGTGATCGACGAGCTGATGCAGGCCCTCAACTTCA
ACTCGGAGACGGTGCCCCAGAAGTCCAGCCTCGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATACTGCTGCATGCC
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> hIL12AB_037(SEQ ID NO: 41)

ATGTGCCACCAACAACTGGTGATCAGCTGGTTCTCCCTGGTGTTCCTGGCCAGCCCCCTGGTGGCCATCTGGGAGCTCAA
AAAAGACGTGTACGTGGTGGAGCTCGATTGGTACCCAGACGCCCCGGGGGAAATGGTGGTGCTGACCTGCGACACCCCAGAGGAGG
ATGGCATCACGTGGACGCTGGATCAGTCCAGCGAGGTGCTGGGGAGCGGCAAGACGCTCACCATCCAGGTGAAGGAATTTGGCGAC
GCGGGCCAGTATACCTGTCACAAGGGCGGCGAGGTGCTGAGCCACTCCCTGCTGCTGCTGCACAAGAAGGAGGATGGGATCTGGTC
AACCGATATCCTGAAAGACCAGAAGGAGCCCAAGAACAAGACCTTCCTGCGCTGCGAGGCCAAGAACTATAGCGGCAGGTTCACCT
GCTGGTGGCTGACCACCATCAGCACCGACCTGACCTTCAGCGTGAAATCCTCCAGGGGCAGCAGCGACCCCCAGGGCGTGACCTGC
GGTGCCGCCACGCTCTCCGCCGAGCGAGTCAGGGGTGACAACAAGGAGTACGAGTACAGCGTGGAATGTCAGGAGGACAGCGCCTG
TCCCGCCGCCGAGGAGTCGCTGCCCATCGAGGTGATGGTCGACGCGGTGCACAAGCTCAAATACGAGAATTACACCAGCAGCTTCT
TCATCAGGGACATCATCAAGCCCGACCCCCCCAAGAACCTGCAGCTGAAGCCCTTGAAGAACAGCAGGCAGGTGGAGGTGAGCTGG
GAGTACCCGGACACCTGGAGCACCCCCCACTCCTACTTCAGCCTGACGTTCTGTGTGCAGGTGCAGGGGAAGTCCAAGAGGGAGAA
GAAGGACCGGGTGTTCACCGACAAGACCAGCGCCACCGTGATATGCCGCAAGAACGCGTCCATCAGCGTTCGCGCCCAGGACCGCT
ACTACAGCAGCTCCTGGTCCGAATGGGCCAGCGTGCCCTGCAGCGGTGGAGGGGGCGGGGGCTCCAGGAATCTGCCGGTGGCCACC
CCCGACCCCGGGATGTTCCCGTGTCTGCATCACTCCCAGAACCTGCTGCGGGCCGTGAGCAATATGCTGCAGAAGGCCAGGCAGAC
GCTCGAGTTCTACCCCTGCACCTCCGAAGAGATCGACCATGAGGACATCACCAAGGACAAGACCAGCACCGTGGAGGCCTGCCTCC
CCCTGGAGCTGACCAAAAACGAGAGCTGCCTGAACTCCAGGGAGACCAGCTTTATAACCAACGGCAGCTGCCTCGCCTCCAGGAAG
ACCTCGTTTATGATGGCCCTCTGCCTGTCCAGCATCTACGAGGACCTGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCGAA
GTTGCTCATGGACCCCAAGAGGCAGATCTTCCTGGACCAGAACATGCTCGCGGTGATCGACGAGCTGATGCAAGCCCTGAACTTCA
ACAGCGAGACCGTGCCCCAGAAGAGCAGCCTGGAAGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCC
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> hIL12AB_038(SEQ ID NO: 42)

ATGTGCCACCAGCAGCTCGTGATCAGCTGGTTCTCCCTCGTGTTCCTGGCCTCCCCGCTGGTGGCCATCTGGGAGCTGAA
GAAGGACGTGTACGTGGTGGAGCTGGACTGGTATCCCGACGCCCCCGGCGAGATGGTGGTGCTGACGTGCGACACACCAGAAGAGG
ACGGGATCACATGGACCCTGGATCAGTCGTCCGAGGTGCTGGGGAGCGGCAAGACCCTCACCATCCAAGTGAAGGAGTTCGGGGAC
GCCGGCCAGTACACCTGCCACAAGGGCGGGGAGGTGCTCTCCCATAGCCTGCTCCTCCTGCACAAAAAGGAGGATGGCATCTGGAG
CACCGACATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACATTTCTCAGGTGTGAGGCCAAGAACTATTCGGGCAGGTTTACCT
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CCCCGCCGCCGAGGAGTCCCTGCCCATAGAGGTGATGGTGGACGCCGTCCACAAGTTGAAGTACGAAAATTATACCTCCTCGTTCT
TCATTAGGGACATCATCAAGCCTGACCCCCCGAAGAACCTACAACTCAAGCCCCTCAAGAACTCCCGCCAGGTGGAGGTGTCCTGG
GAGTACCCCGACACCTGGTCCACCCCGCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTCCAGGGGAAGAGCAAGCGTGAAAA
GAAAGACAGGGTGTTCACCGACAAGACGAGCGCCACCGTGATCTGCAGGAAAAACGCCTCCATCTCCGTGCGCGCCCAGGACAGGT

ACTACAGTAGCTCCTGGAGCGAATGGGCCAGCGTGCCGTGCAGCGGCGGGGGAGGAGGCGGCAGTCGCAACCTGCCCGTGGCCACC
CCCGACCCCGGCATGTTCCCATGCCTGCACCACAGCCAGAACCTGCTGAGGGCAGTCAGCAATATGCTGCAGAAGGCCAGGCAGAC
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CACTGGAGCTGACCAAAAACGAGAGCTGCCTGAACTCCAGGGAGACCTCCTTCATCACCAACGGGAGCTGCCTGGCCAGCCGGAAG
ACCAGCTTCATGATGGCGCTGTGCCTCAGCAGCATCTACGAGGATCTCAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCGAA
GCTGCTGATGGACCCCAAGCGGCAGATCTTCCTGGACCAGAACATGCTGGCCGTGATTGACGAGCTCATGCAGGCCCTGAACTTCA
ATAGCGAGACCGTGCCCCAAAAGAGCAGCCTGGAGGAACCCGACTTCTACAAAACGAAGATCAAGCTCTGCATCCTGCTGCACGCC
TTCCGGATCCGGGCCGTGACCATCGATCGTGTGATGAGCTACCTGAACGCCTCG

> hIL12AB_039(SEQ ID NO: 43)
ATGTGCCACCAGCAGCTCGTCATCTCCTGGTTTAGCCTGGTGTTTCTGGCCTCCCCCCTGGTCGCCATCTGGGAGCTGAA
GAAAGACGTGTACGTGGTGGAGCTGGACTGGTACCCGGACGCTCCCGGGGAGATGGTGGTGCTGACCTGCGACACCCCCGAGGAGG
ACGGCATCACCTGGACCCTGGACCAGAGCTCCGAGGTGCTGGGGAGCGGCAAGACCCTGACCATTCAGGTGAAAGAGTTCGGCGAC
GCCGGCCAATATACCTGCCACAAGGGGGGGGAGGTCCTGTCGCATTCCCTGCTGCTGCTTCACAAAAAGGAGGATGGCATCTGGAG
CACCGACATCCTGAAGGACCAGAAAGAACCCAAGAACAAGACGTTCCTGCGCTGCGAGGCCAAGAACTACAGCGGCCGGTTCACCT
GTTGGTGGCTGACCACCATCTCCACCGACCTGACTTTCTCGGTGAAGAGCAGCCGCGGGAGCAGCGACCCCCAGGGAGTGACCTGC
GGCGCCGCCACCCTGAGCGCCGAAAGGGTGAGGGGCGACAATAAAGAGTACGAGTATTCCGTGGAGTGCCAGGAGGACAGCGCCTG
TCCGCCGCCGAGGAGTCCCTGCCTATCGAGGTGATGGTCGACGCCGTGCACAAGCTCAAGTACGAAAACTACACCAGCAGCTTTT
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ACTACAGCTCCAGCTGGAGCGAGTGGGCCTCCGTGCCTTGCAGCGGGGGAGGAGGCGGCGGCAGCAGGAATCTGCCCGTCGCAACC
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CCCTGGAGCTCACCAAGAACGAGTCCTGCCTGAATAGCAGGGAGACGTCCTTCATAACCAACGGCAGCTGTCTGGCGTCCAGGAAG
ACCAGCTTCATGATGGCCCTCTGCCTGAGCTCCATCTACGAGGACCTCAAGATGTACCAGGTCGAGTTCAAGACCATGAACGCAAA
ACTGCTCATGGATCCAAAGAGGCAGATCTTTCTGGACCAGAACATGCTGGCCGTGATCGATGAACTCATGCAGGCCCTGAATTTCA
ATTCCGAGACCGTGCCCCAGAAGAGCTCCCTGGAGGAACCCGACTTCTACAAAACAAAGATCAAGCTGTGTATCCTCCTGCACGCC
TTCCGGATCAGGGCCGTCACCATTGACCGGGTGATGTCCTACCTGAACGCCAGC

> hIL12AB_040(SEQ ID NO: 44)
ATGTGCCATCAGCAGCTGGTGATCAGCTGGTTCAGCCTCGTGTTCCTCGCCAGCCCCCTCGTGGCCATCTGGGAGCTGAA
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ACGGCATTACCTGGACACTGGACCAGAGCAGCGAGGTCCTGGGCAGCGGGAAGACCCTGACAATTCAGGTGAAGGAGTTCGGCGAC
GCCGGACAGTACACGTGCCACAAGGGGGGGGAGGTGCTGTCCCACAGCCTCCTGCTGCTGCACAAGAAGGAGGATGGCATCTGGAG
CACCGACATCCTGAAGGATCAGAAGGAGCCCAAGAACAAGACCTTTCTGAGATGCGAGGCCAAGAATTACAGCGGCCGTTTCACCT
GCTGGTGGCTCACCACCATCAGCACCGACCTGACCTTCAGCGTGAAATCCTCCAGGGGCTCCCCGGACCCGCAGGGAGTGACCTGC
GGCGCCGCCACACTGAGCGCCGAGCGGGTCAGAGGGGACAACAAGGAGTACGAGTACAGCGTTGAGTGCCAGGAGGACAGCGCCTG
TCCCGCGGCCGAGGAATCCCTGCCCATCGAGGTGATGGTGGACGCAGTGCACAAGCTGAAGTACGAGAACTATACCTCGAGCTTCT
TCATCCGGGATATCATTAAGCCCGATCCCCCGAAGAACCTGCAGCTCAAACCCCTGAAGAACAGCAGGCAGGTGGAGGTCTCCTGG
GAGTACCCCGACACATGGTCCACCCCCCATTCCTATTTCTCCCTGACCTTTTGCGTGCAGGTGCAGGGCAAGAGCAAGAGGGAGAA
AAAGGACAGGGTGTTCACCGACAAGACCTCCGCCACCGTGATCTGCCGTAAGAACGCTAGCATCAGCGTCAGGGCCCAGGACAGGT
ACTATAGCAGCTCCTGGTCCGAGTGGGCCAGCGTCCCGTGCAGCGGCGGGGGCGGTGGAGGCTCCCCGGAACCTCCCCGTGGCCACC
CCGGACCCCGGCATGTTCCCTGCCTGCATCACAGCCAGAACCTGCTGAGGGCCGTGTCCAACATGCTGCAGAAGGCCAGGCAGAC
ACTCGAGTTTTACCCCTGCACCAGCGAGGAGATCGACCACGAAGACATCACCAAGGACAAGACCTCCACCGTGGAGGCATGCCTGC
CCCTGGAGCTGACCAAAAACGAAAGCTGTCTGAACTCCAGGGAGACCTCCTTTATCACGAACGGCTCATGCCTGGCCTCCAGAAAG
ACCAGCTTCATGATGGCCCTGTGCCTGAGCTCCATCTACGAGGACTTGAAAATGTACCAGGTCGAGTTCAAGACCATGAACGCCAA
GCTGCTCATGGACCCCAAAAGGCAGATCTTTCTGGACCAGAATATGCTGGCCGTGATCGACGAGCTCATGCAAGCCCTGAATTTCA
ACAGCGAGACCGTGCCCCAGAAGTCCTCCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATACTCCTGCACGCG
TTTAGGATCAGGGCGGTGACCATCGATAGGGTGATGAGCTACCTGAATGCCTCC

> hIL12AB_002 (SEQ ID NO: 236)
ATGTGCCACCAGCAGCTGGTGATCAGCTGGTTCAGCCTGGTGTTCCTGGCCAGCCCCCTGGTGGCCATCTGGGAGCTGAAGAAGGA
CGTGTACGTGGTGGAGTTGGATTGGTACCCCGACGCCCCCGGCGAGATGGTGGTGCTGACCTGCGACACCCCCGAGGAGGACGGCA
TCACCTGGACCCTGGACCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCTGACCATCCAGGTGAAGGAGTTCGGCGACGCCGGC
CAGTACACCTGCCACAAGGGCGGCGAGGTGCTGAGCCACAGCCTGCTGCTGCTGCACAAGAAGGAGGACGGCATCTGGAGCACCGA
CATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACCTTCCTGAGATGCGAGGCCAAGAACTACAGCGGCAGATTCACCTGCTGGT
GGCTGACCACCATCAGCACCGACCTGACCTTCAGCGTGAAGAGCAGCAGAGGCAGCAGCGACCCCCAGGGCGTGACCTGCGGCGCC
GCCACCCTGAGCGCCGAGAGAGTGAGAGGCGACAACAAGGAGTACGAGTACAGCGTGGAGTGCCAGGAAGATAGCGCCTGCCCCGC
CGCCGAGGAGAGCCTGCCCATCGAGGTGATGGTGGACGCCGTGCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCA
GAGATATCATCAAGCCCGACCCCCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCCGGCAGGTGGAGGTGAGCTGGGAGTAC
CCCGACACCTGGAGCACCCCCCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGCAAGAGCAAGAGAGAGAAGAAAGA
TAGAGTGTTCACCGACAAGACCAGCGCCACCGTGATCTGCAGAAAGAACGCCAGCATCAGCGTGAGAGCCCAAGATAGATACTACA
GCAGCAGCTGGAGCGAGTGGGCCAGCGTGCCTTGCAGCGGCGGCGGCGGCGGCGGCAGCAGAAACCTGCCCGTGGCCACCCCCGAC
CCCGGCATGTTCCCTTGCCTGCACCACAGCCAGAACCTGCTGAGAGCCGTGAGCAACATGCTGCAGAAGGCCCGGCAGACCCTGGA
GTTCTACCCCTGCACCAGCGAGGAGATCGACCACGAAGATATCACCAAAGATAAGACCAGCACCGTGGAGGCCTGCCTGCCCCTGG

AGCTGACCAAGAACGAGAGCTGCCTGAACAGCAGAGAGACCAGCTTCATCACCAACGGCAGCTGCCTGGCCAGCAGAAAGACCAGC
TTCATGATGGCCCTGTGCCTGAGCAGCATCTACGAGGACCTGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCCAAGCTGCT
GATGGACCCCAAGCGGCAGATCTTCCTGGACCAGAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAACAGCG
AGACCGTGCCCCAGAAGAGCAGCCTGGAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCAGA
ATCAGAGCCGTGACCATCGACAGAGTGATGAGCTACCTGAACGCCAGC

Table 4B. Sequence Optimized Polynucleotides Comprising 5' UTR, ORF, 3' UTR

hIL12AB_001 (SEQ ID NO: 55)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGCAAATAAAGAGAGAAAAGAAGAGTAAGAAGAAATA TATAAGAGCCACCATGTGTCACCAGCAGTTGGTCATTAGCTGGTTTAGCCTTGTTTCCTGGCCTCCCCCTTGTCGCT ATTTGGGATCTCAAGAAGGACGTGTACGTTGTGGAATTGGATTGGTACCCAGACGCCCCGGAGAAATGGTGTCTCTGA CCTGTGATACCCCAGAGGAGGACGGCATCACCTGGACCCTGGACCAAAGCAGCGAGGTTTTGGGCTCAGGGAAAAGCGT GACCATCCAGGTGAAGGAATTCGGCGACGCCGGCCAGTACACCTGCCATAAGGGAGGAGAGGTGCTGAGCCATTCCCTT CTTCTGCTGCACAAGAAGGAGGACGGCATCTGGTCTACCGACATCCTGAAAGACCAGAAGGAGCCCAAGAACAAAACCT TCCTGAGGTGCGAGGCCAACAATACTACTCCGGCAGGTTCACTTGTTGGCTGGACCACCATCAGTACAGACCTGACTTT TAGTGTAAAAAGCTCCAGAGGCTCGTCGACATCCCCAAGGGGTGACCTGCAGCCACTCTGAGCGCTGAGCGCGTG CGCGGTGACAATATAAGGAGTACGAGTACAGCGTTGAGTGTCAAGAGGATAGCGCTTGCCCTGCCGGAGGAGGAGCCTGC CTATCCAGGTGATGTTTGACGCAGTGCACAAGCTTAAGTACGAGAATTACACCAGCTCATTCTTCATTAGAGATATAAT CAAGCCTGACCCACCCAAGAACTGCAGCTGAAGAAACTCACGGCAGGTCGAAGTGAGCTGGAGTACGCCC GACACCTGGAGGCACTCCTCATTCCTATTCTCTTCTTACATTCTGCGTCCAGGTGCAGGGCAAGAGCGGGAAAAGA AGGATCGAGTCTTCACCGACAAAACAGCCGCCGACCGTGATTTGCAGGAAGAACGCCAGCATCTCCGTCAGAGCCCAGA TAGATACTATAGTAGCAGCTGGAGCGAGTGGGCAAGCGTGCCTGTTCCGGGGGGGCGGGGCAGCCGAAACTTG CCTGTCGCTACCCCCGAACCCTGGAATGTTTCCGTGTCTGCACCACAGCCAGAACCTGCTGAGAGCCGTGTCGAATATGC TCCAGAAGACCCGGCAGACCCTTGAGTTCTACCCCTGTACCAGCGGAAGAGATCGATCATGATCAATATCACGGATAGATAA AACATCCACGTCGAGGCTTGTCTCCCGCCTGGAGCTGACCAAGAACGAGCTGTGTCTGAATAGCCGGGAGAACGTCTTTC ATCACGAATGGTAGCCTGTCTGGCCAGCAGGAAAACTTCCTTCATGATGGTCTCTCTGACCTGAGCTCTAATCTATGAAGATC TGAAGATGTATCAGGTGGAGTTTAAAACAATGAACGGCCAAACTCCTGATGGACCCAAAAAGGCAAATCTTTCTGGACCA GAATATGCTGGCCGTGATAGACGAGCTGATGCAGGCACTGAACTTCAACAGCGAGACGGTGCCACAGAAATCCAGCCTG GAGGAGCCTGACTTTTACAAAACTAAGATCAAGCTGTGTATCCTGCTGCATGCCTTCAGAATCCGTGCCGTGACTATCG ACAGGGTGATGTCATACCTCAACGCTTCAATGATAGGCTTGAGCCTCGGTGGCCATGCCTCTTGCCCCTTGGCCTC CCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_...002 (SEQ ID NO: 56)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGGCACCAGCAGTTGGTGATCATCTGGTTCCAGCTTGTTCCTGGCCAGCCCCTGGTCGCC ATCTGGGAGCTGAAGAAGACGTGTATGTCGTGGATTGGTACCCTGACGCCCCCGGCGAGATGGTGGTGCTCA CCTGCGACACCCCTGAGGAGGATGGCATCACCTGGACCTGGACCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCT GACCATCCAGGTGAAGGAGTTCGGCGACGCCGGCCAGTACACCTGCCACAAGGGCGGCGAGGTGCTGAGCCACAGCCTG CTGCTGCTGCACAAGAAGGAGGACGGCATCTGGAGCACCGACATCCTGAAGGACCAGGAGCCCAAGAACAAGACCT TCCTGAGATGCGAGGCCAAGAACTACAGCGGCAGATTCACCTGCTGGTGGCTGACCACATCAGCACCTGACCTT CAGCGTGAAGAGCAGCAGCAGGGCAGCGACCCCCAGGGCGTGACCTGCGGCGCCGCCACCCTGAGCGCCGAGAGAGTG AGAGGCGACAACAAGGAGTACGAGTACAGCGTGGAGTGCCAGGAGGATAGCGCCCTGCCCGCCGCCGAGGAGAGCCTGC CCATCGAGGTGATGGTGGACGCCGTGCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCAGGGATATCAT CAAGCCCGACCCCCCCAAGAACTTGCAGCTGAAGCCCCTGAAGAACAGCCGGCAGGTGGAGGTGAGCTGGGAGTACCCC GACACCTGGAGCACCCCCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGCAAGAGCAAGAGAGAAGA AAGATAGATTTTCACCGACAAGACCAGCGCCACCGTGATCTGCGCAAAAAGAACGCCAGCATCAGCGTGAGAGCCCAAGA TAGATACTACAGCAGCAGCTGGAGGAGATGGGCCAGGCGTGCCCTGCAGCGGCGGCAGCAGAAACCTG CCCGTGGCCACCCCCGACCCCGGCATGTTCCCCTGCCACAGCCAAAACCTGCTGAGAGCCGTGAGCAACATGC TGCAGAAGGCCGGCAGACCCTGGAGTTCTACCCCTGCACCAGCGAGGATCGACCACGAAGATACCACCAAAGATAA GACCAGCACCGTGGAGGCCTGCCTGCCCCTGGAGCTGACCAAGAACGAGAGCTGCCTGAACAGCAGAGAACCAGCTTC ATCACCAAGGCAGCTACCTGAACAGCGTGGCCAAGCACAGCAAGCTGAGCACCGAGTACGTGGAGAACCACGAGACC TGAAGAATTACCAGAAGCGGAGTTCAAGAACCATGAACAGCCCAAGCTGCTGAAGCAGCGGCAGAGATCTTCCTGGACCA GAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAACAGCGAGACCGTGCCCCAGAAGAGCCAGCCTG GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCAGAATCAGAGCCGTGACCATCG ACAGAGTGATGAGCTACCTGAACGCCAGCCTGGATAATAGGCTGGAGCCTCCGTGTGCCATGTCTTCTTGCCCCTTGGCCTC CCCCCAGCCCCTTCCTCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_...003 (SEQ ID NO: 57)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGGCACCAGCAGTTGGTCATCTCTTGGTTTTCCTTGTTGGTGTTTTCTGGCATCTCCCCCTGGTCGCC ATCTGGGAACTGAAGAAGACGTTTACGTCGTAGATTGGTATCCCGACGCTCCTGGAGAAATGGTGGTCCTCA CCTGTGACACCCCTGAAGAAGATGGCATCACCTGGACCTTGGACCAGAGCAGTGAGGTCTTAGGCTCTGGCAAAACCCT GACCATCCAAGTCAAAGAGTTTGGCGACGCTGGCCAGTACACCTGTCACAAAGGAGGCGAGGTTCTAAGCCATTCGCTC CTGCTGCTTCACAAAAAGGAAGATGGAATTTGGTCCACTGATATTTTAAAGGACCAGAAGAACCCAAAAATAAGACCT TTCTAAGATTCGCGAGGCCAAGAATTATTCTGGACGTTTCACCTGCTGGTGGCTGACGCAATCAGTACTGATTTGACAT CAGTGTCAAAAGCAGCAGAGGCTCTTCTGACCCCCAAGGGGTGACGTTGCGAAAGCTCATCTCTGCAGAGAGAGTC AGAGGTGACAACAAGGAGTATGAGTACTCAGTGGAGTGCCAGGAGGATAGTGCCCTGCCCGCTGCTGAGGAGAGTCTGC

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	CCATTGAGGTCATGCTGGATGCCGTTCACAAGTAAAGCTCAGATTGAAAACTATACAACCAGCAGCTTCTTCATCAGAGATATCAT
	CAAACCTGACCCACCCAAGAACTTGCAGCTGAAGCCATTAAAGAATTCTCGCAAGGTGGAAGGTCAGCTGGGAGTACCCT
	GACACCTGGAGTACTCCACATTCCTACTTCCCTGACATTCTGCGTTCAGGTCCAGGGCAAGAGCAAGAGCAAGAAAGA
	AAGATAGATCTTTACAAATAAGACCTCACGGTCATCTGCCGCAAAAATGCCAGCATTAGCGTGCGGGCCCAGGA
	CCGCTACTATAGCTCATCTTGGAGCGAATGGGCATCTGTGCCTGCAGTGGGCGAGGGGCGGAGCCAGAAGAACCTC
	CCCGTGGCCACTCCAGACCAGAATGTTCCCATGCCTTCACCACTCCCAAAACCTGCTGAGGGCCGTCAGCAACATGC
	TCCAGAAGCCCGCCAAACTTTAGAATTTACCCTTGCACTTCTCGAGAGATTGATCATGAAGATATCACAAAAGATAA
	AACCAGCACAGTGGAGGCCGTGTTACCATTGGAATTAACCAAGAATGAGAGTTGCCTAAATTCCAGAGAGACCTCTTTC
	ATAACTAATTGGGAGTTGCCTGGCCTCCAGAAAGACCTCTTTTATGATGGCCCTGTGCCTTAGTATTATGAAGATCTTTTTAGATCA
	TGAAGATGTACCAGGTGGAGTTCAAGACATGAATGCAAAGCTTCTGATGGATCCTAAGAGGCAGATCTTTTTAGATCA
	AAACATGCTGGCAGTTTATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGTGAGACGGTGCCAGCAAAAATCCTCCCTT
	GAAGAACCAGATTTCTACAAGACCAAGATCAAGCTCTGCATACTTCTTTCATGCTTTCAGAATTCGGGCAGTGACTATTG
	ATAGAGTGATGAGCTATCTGAATGCTTCTGTGATAATAGGCTGGAGCCTCGGTGGCATGCTTCTTGCCCCTTGGGCCTC
	CCCCAGCCCCTCCTCCCCTTCTTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC
	TGAGTGGGCGGC
	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA
	TATAAGAGCCACCATGGCCTGCCCACCAGCAGCTCATCAGTTCTTGCTCCTGCTGATCTTCCACATCAGCTGCCTCCTGTG
	GCCATCTGGGAGCTGAAAAAGACGCTCTACGTAGATTTGGATTCTGCTATCGAGGACCTGAAAGAAGATGGTGTTC
	TCACCTGTGACACCCAGAAGAAGGGTATCACCTGGACGCTGGACCAGAGCTCAGAAGTTCTTGGCAGTGTGGAAAAAC
	GCTGACCATACAAGTAAAGAATTTGGGGATGCTGTGGCCAGTACACCTGCCCACAAAGGAGGAGAAGTTCTCAGCCACAGC
	CTGCTGCTGCTGCACAAGAAAGATGGCATCTGGAGCACAGATATTTTAAAAGACCAGAAGAGCCCAAGAACAAAA
	CCTTCCTTCGATGTGAGCCAAGAACTACAGTGGCCGCTTCACCTGCTGGTGGCTCACCACCATCAGCACAGACCTCAC
	CTTCTCGGTGAAGAGCAGCCGTGGCAGCTCAGACCCCCAAGGAGTCACCTGTGGGGCGCCACGCTGTGGCAGAAAGA
	GTTCGAGGTGACAACAAGGAATATGAATACTCGGTGGAATGTCAAGAAGATTCGGCCTGCCCGGCAGAAGAAGTC
	TTCCATAGAAGTCATGGTGGATGCTGTTCACAAATTAAAATATGAAAACTACACCAGCAGCTTCTTCATCAGAGATAT
	CATCAAGCCAGACCCGCCCAAGAACCTGCAGCTGAAGCCCCTGAAGAACAGCCCGGCAGTGGTGGAAGTTTCCTGGGATGAC
	CCAGATACGTGGAGCACTCCGCAGCTACTTCAGCCTCACCTTCTGTGTACAAGTACAAGGCAAGAGCAAGAGAGA
	AGAAAGATCGTGTCTTCACAGATAAAACCTCGGCGACGGTCATCTGCAGAAGAATGCCTCCATCTCGGTTCGAGCCCA
	GGACCGCTACTACAGCAGCTGGAGTGGGCCTCGGTGCCCTGCAGTGGTGGCGGCGGCAGCAGAAAC
	CTTCCTGTGGCCACGCCTGGACCTGGCATGTGTCCCGTGCCTGCCACCACAAAATTACTTCGAGCTGTTTCTAACA
	TGCTGCAGAAAGCACGGCAAACTTTAGAATTCTACCCCTGCACCTCAGAAAAAATAGACCATGAAGATATCACCAAAGA
	TAAAACCAGCACTGTGAGAGGCCTGCCTGGAGCTCAGCAAGAATGAATCCTGCCTCAACAGCAGAAGACCAG
	TTCATCACCAATGGCAGCTGTGCCTGGCCAGGAAAGCAGCTTCATGATGGCGCTCTGAGCAGCATCTATGAAG
	ATTTGAAGATGTACCAGTAGTTAAAACCATGAATGCCAAGCTGCTCATGGACCCCAAGCGGCAGATATTTTGGA
	TCAAAACATGCTGGCTGTCATTGATGAGCTCATGCAGGCATTAAACTTCAACTCAGAGACGGTGCCCCAGAAGAGCAAC
	CTGGAGGAGCCAGATTTCTACAAAACCAAGATCAAGCTCTGCATACTTCTTTCATGCTTTCAGAATTCGGGCAGTGACTA
	TTGACCGTGTCATGTCCTACTTAAATGCCAGCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGC
	CTCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAA
	GTCTGAGTGGGCGGC

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hIL12AB_ 005 (SEQ ID NO: 59)	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTGGTCATCAGCTGGTTTCCTTGGTCTTCCTGGCCAGCCCCCTGGTGGCC ATCTGGGAGCTGAAGAAGACGTCTACGTAGTAGAGTTGGATTGGTACCCAGACGCCCCTGGAGAAATGGTGGTCCTCA CCTGTGACACGCCCGAGAAGACGGTATGACCTGGACGCTGGACCAGAGCTCAGAAGTTCTTGGCAGTGGAGAAAACGCT GACCATACAAGTAAAAGAATTTGGGGATGCTGGCCAGTACACCTGCCACAAAGGAGGCGAGGTTCTCAGCCACACGCCTG CTGCTGCTGCAAGAAGATGGCATCTGGAGCACAGATATTTTAAAAGACCAGACGAGGGCCCAAGAACAAAAACCT TCCTTCGATTGGAAGCCCAACAACTACAGTGGCGCCTTCACCTGCTGGCTGTCACAACCATCAGCACACGACCTCACCTT GTCGGTGAAGAGCAGCCCGTGGCAGCTCAGACCCCCAAGGAGTCACCTGTGTGGGCGGCCAGCTGTGGCAGAAAGAGTT CGAGGTGACAACAAGGAATATGAATACTGGGTGGAATGTCAAGAAGAATTCGGCCTGCCCGGCGGCAGCATCTATGAAGATT CCATAGAAGTCATGGTTGGATGCTGTTCACAAATTAAAATGAAAACTACCAGCAGCTTCTTCATCAGAGATATTCAT CAAGCCAGACCGGCCAAGAACTGCAGCTGAAGAACAGCGCGGCAGTGGAAGTTCCTGGGAGTACCCA GATACGTGGAGCACGCCGCACAGCTACTTCAGCCTCACCTTCTGTTACAAGTACAAGGCAAGAGAGCAAGAGAAGA AAGATCGTGTCTTCACAGATAAAACCTCGGCGACGGTCATCTGCAAGAAGAATGCCTCCATCTCGGTTCGAGCCCAGGA CCGCTACTACAGCAGCAGCTGGAGTGAGTGGGCCTCGGTGCCCTGCAGTGGTGGCGGCGGCGGCAGCAGAAACCTT CCTGTGGCCAGGCCCGGACCCTGGCATGTTCCCGTGCCTGCACGCACAGCCCAAAATTTACTTCGAGCTGTTTCTAACATGC TGCAGAAAGCACGGCAAACTTTAGAATTCTACCCCTGCACCTCAGAAGAAATAGACCATGAAGATACACCAAAGATAA AACCAGCACTGTAGAGGCCCTGCCTGCCCCTGGAGCTCACCAAGAATGAATCCTGCCTCAACAGCAGAGACCAGCTTC ATCACCAATGGCAGCTGCCTGGCCAGCAGGAAACCAGCTTCAGGATGGGGCTCTGCCTGAGCAGCATCTATGAAGATT TGAAGATGTACCAAGTTAGAATTTAAAACCATGAATGCCAAGCTGCTCATGGACCCCAAGCGGCAGATATTTTGCATCA AACATGCTTGCCTGTCATTGAGCTCAAGCATTAAACTTCAACTCAGAGACGGTGCCCCAGAAGAGCCAGCCTG GAGGAGCCAGATTTCTACAAAACCAAGATCAAGCTCTGCATCTTATTACATGCCTTCCGGCATCCGGGCGGTCACCATTG ACCGTGTCATGTCCTACTTAAATGCCAGCTGATAATAGCCTGGAGCCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 006 (SEQ ID	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTGGTCATCAGCTGGTTTCCTTGGTCTTCCTGGCCAGCCCCCTGGTGGCC ATCTGGGAGCTGAAGAAGACGTCTACGTAGTAGAGTTGGATTGGTACCCAGACGCCCCTGGAGAAATGGTGGTCCTCA

(continued)

NO: 60)	CCTGTGACACCCCCGAGGAGGACGGCATCACCTGGACCTGGACCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCT GACCATCCAGGTGAAGGAGTTCGGGGACGCCGGCCAGTACACCTGCCACAAGGGCGGCGAGGTGCTGAGCCACAGCCTG CTGCTCCTGCACAAGAAGGAGGACGGCATCTGGAGCACAGATATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACCT TCCTGAGATGCGAGGCCAAGAACTACAGCGGCAGATTCACCTGCTGGTGGCTGACCACATCAGCACAGATTGACCTT CAGCGTGAAGAGCAGCAGAGGCAGCGACCCCCAGGGCGTGACCTGCGGCGCCGCCGCCACCCTGAGCGCCGAGAGAGTG AGAGGTCACAACAAGGAGTACGAGTACAGCGTGGAGTGCCAGGAGGATAGCGCCTGCCCCGCCGCCGAGGAGAGCCTGC CCATCGAGGTGATGGTGGACGCCGTGCACAAGCTGAAGGAGTACGAGAACTACATCGACCAGCAGTTCATCAGCGACATCA CAAGCCCGACCCGCCGAAGAACCTGCAGCTGAAGGAGCTGCAGGAGGCCAAGAAGAGCGAGGTGGAGGTGACCGC GACGACCTGGAGCACCCCCCACAGCTACTTCATCGCCGTGGAGGAGGAGGACACCGTGGTGCTGAAGAAGCCCCTGAAG AAGATAGAGGTGTTCATCAATAAGACCGCCTACCTGCAGAAAAAGAACGCCGAGCATCAGCGTGAGAGCCCAAGA TAGATACTACAGCAGCAAGCTGGAGGCCAGCGTGCCTGCAGCCACAGCCAGAACCTGCTGAGCGCCGTGAGCAACATGC CCCGTGGCCACCCCTGACCCCGGCATGTTCCCCTGCTGCACCACAGCCAGAACCTGCTGAGCGCCGTGAGCAACATGC TGCAGAAGGCCGGCGACCCTGGAGTTCTACCCCGTGCACCAGCGAGGAGATCGACCACGAAGATCACCAAAGATAA GACCAGCACCGTGGAGGCCTGCCTGCCCCTGGAGCTGACCAAGAATGAAAGCTGCCTGAACAGCAGAGACCAGCTTC ATCACCAAGGACAGCTGCCTGGCCAGCAGAAGACCAGCTTCATGTGCCTGTGCCTGAGCATCTACGAGGACC TGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCCAAGCTGCTGATGGACCCCAAGCGGCAGATCTTCCTGGACCA GAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAACAGCGAGACCGTGCCCCAGAAGAGCACCCTG GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCAGAATCAGAGCCGTGACCATCG ACAGAGTGATGAGCTACCTGAACGCCAGCCTGATAAIAGGCCTGGAGCATGCTTCTTGCCCCTTGGCCCTC CCCCAGCCCCTCCTCCCCTTCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_007 (SEQ ID NO: 61)	TCAAGCTTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTTGTCATCTCCTGGTTCTCTTTGTCTTCCTCCTTCTGGCC ATCTGGCAGCTGAAGAACGTTTACGTAGATTGGTACCCTGACGCACCTGGAGAATGGTGTTCTCA CCTGTGACACTCCTGAGGACCACGCTATCACCTGGACGTTGGACCAGTCTTCTGAGGTTCTTGGCAAGTGGAAAAACTCT TACTATTCAGGTGAAGGATTTGGAGATGCTGGCCAGATACAGTGCCACAAGGTTGGTGAAGTTCTCAAGCCACAGCTTTA CTTCTACTTCACAAGAAGGATGGCATCTGGTCTACTGACATTTAAAAGACCAGAAGAGCCCAAGAACAAAACAT TCCTTCGTTGTGAAGCCAAGAACTACAGTGGTCGTTCACCTGCTGGTGGCTACTATTCTACTGACCTTACTTT CTCTGTGAAGTCTTCTGGCTCTTCTGACCCTCAGGGTGTCACCTGTGGGGCTGCTACTCTTTCTGTGAGCCTGTG CGTGGTGACAACAAGGAGTATGAATACTCGGTGCAGTGCCAGGAAGATTCTGCCTGCCCTGCTGCTGAGGAGTCTCTTC CTATTGAGGTTGATGGTGCTGTGCACAAGTTAAAATATGAAAACTACACACTTCTTTCTTTCATTCGTGACATTAT AAAACCTGACCCTCCAAGAAGCCTTCAGTTAAAAACCTTTAAAAAAACTCTCGTCAGGTGTCCTGGAGTACCCT GACACGTGGTCTACTCCTCACTCCTACTTCTCTCTTTACTTTCTGTGTCCAGGTGCAGGGCAAGTCCAAGCGTGAGAGA AGGACCGTGTCTTCACTGACAAAACATCTGCTACTGTGTCAGGAGAATGCATCCATCTCTGTGCGTGAGTCAGCA CCGTTACTACAGCTCTTCCTGGTCTGAGTGGGAGTTGTGCCCTGGCTCTGGCGGGGGGCGGCGGCAGCAGAATCTT CCTGTGGCTACTCCTGACCCTGGCATGTTCCCCGTGCCTTCACCACTCCCAGAACCTTCTTCGTGCTGTGAGCAACATGC TTCAGAAGGCTCGTCAAACTTTAGAATTCTACCCCTGCACTTCCTGAGGAGATTGACCATGAAGATACCACCAAAGTAA AACATCTACTGTGGAGGCCTGCCTTCCTTTAGAGCTGACCAAGAATCAATCCTGCTTAAATTCTCGTGAGACGTCTTTC ATCACCAATGGCAGCTGCCTTGCCTCGCGCAAAACATCTTTCATGATGCCTCTTGCCTTTCTTCCATCTATGAAGATT TAAAAATGTACCAGGTGGAGTTCAAGACCATGAATGCTTCTCATGGACCCCAAGCGTCAGATATTTTTGGACCA GAAGATGCTTGCTTCATTGAGCTCATGCAGGCTTTAAACTTCAACTCTGAGACGGTGCCTCAGAAGTCTTCTTTA GAAGAGCCTGACTTCTACAAGACCAAGATAAAACTTTGCATTCTTCATGCTTTCCGCATCCGTGTGTTGACTATTG ACCGTGTGATGTCCTACTTAAATGCTTCTTTGATAAAGCTGTGGAGCCTGGTGGCCAAGCTTCTTGCCCTTGGUCTC CCCCCAGCCCCTCCTCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGGCGGC

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hIL12AB_008 (SEQ ID NO: 62)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGGAGAAAAGAAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGTCATCAACAACTCGTGATTAGCTGGTTCAGTNTGGTGTCCTGGCCTCTCCGCTGGTGGCCC ATCTGGCAGCTTAAAAGAAGAGTGTACGTGGTGGAGCTCGATTGGTACCCTGACGTGCCTGGCGAGATGGTGGTCCTA CCTGCGATACCCCGGAGGAGACGGGATCACTTGCGACCCTGGATCAGAGTAGCGAAGTCCTGGGCTCTGGCAAAACACT CACAATCCAGGTGAAGGAATTCGGAGACGCTGGTCAGTACACTTGCCACACAAGGGGGTGAAGTGCTGTCTCACAGCCTG CTGTTACTGCACAAGCAAGGAGGATGGGATCTGGTCAACCGACATCCTGAAGCATCAGAAGCCTAAGAACAAGACCT TTCTGAGCTTGTGAAGCTTAA(GAACTATTCCGGAAGATTCACTTGCTTGGTTGACCACAATCAGCACTGACCTGACCTT TTCCGTGAAGTCCAGCAGAGGAAGCGAGATCCTCAGGGCGTAACCTGCGGCTACCCTGTCAGCTGAGCGGGTT AGAGGCGACAACAAAGAGTATGAGTACTCCGTGGAGTGTCAGGAAGATAGCGCCTGCCCCGCAGCCGAGGAGAGTCTGC CCATCGAGGTGATGGTGGACGCTGTCCATAAGTTAAAATACGAAAATTACACAAGTTCCTTTTTCATCCGCGATATTAT CAAACCGATCCTCCCCAAGAACTGCAGCTGAAGCCCTTGAAGAATAGCCGAACATGGTCTTCTTGGGAGTATCCT GACACCTGGTCCAGCGCTACTTTAGTCTGACTTTCTGTTGTCCAGTCCAGGGCAAGAGCAAGAGAGAGAAAA AGGATAGATGTTTACTGACAAAACATCTGCTACAGTCATCTGCAGAAGAACGCCAGTATCTCAGTGAGGGCGCAAGA TAGATACTACAGTAGTAGCTGGAGCGGATGGGCGTAGCGTGCCCTTGTTCAGGGGGCGGCGGAGGGGCGCTCCAGGAATCTG CCCCTGGCCAACCCCGACCCTGGGATGTTCCCTTGCCTCCATCACTCACAGAAACCTGCCTCAGCAGTGAGCAACATGC TCCAAAAGAGCCCGCCAGACCCTGGAGTTTTACCCTTGTACTTCAGGAGATCGATCAGAAGATACAACAAAGCATAA AACCAGCACCGTGGAGGCCTGTCTGGCCTCTGGAACTCACAAAGAATGAAAGCTGTCTGAATTCCAGGGAAACCTCCTTC ATTACTAACGGAACCTGTCTGCATCTCGCAAAACATCATTCATGATGGCCCTCTGTCTTCTATCTATGAAGATC TCAAGATGTATCAGTTGGAGTTCAAAACAATGAACGCCAAGCTGCTGATGGACCCCAAGCGGCAGATCTTCCTGGACCA GAACATGCTGGCAGTGATCGATGAGCTGATGCAAGCTTGAACTTCAACTCAGAGACGGTGCCGCAAAAGTCCTCGTTG GAGGAACCAGATTTTTACAAAAGCAAAATCAAGCTGTGTATCCTTCTTCAGCCTTCGGATCAGAGCCGTGACTATCG ACCGGGTGATGTCATACCTGAATGCTTCCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_009 (SEQ ID NO: 63)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTGGTCATCAGCTGGTTTTTAGCCTGGTCTTCCTGGCCAGCCCCCTGGTGGCC ATCTGGGAACTGAAAAAGGACGTCTACGTAGTAGAGTTGGATTGGTATCCAGACGCCCCTGGAGAAATGGTGGTCCTCA CCTGCGACACGCCTGAAGAAGACGGTATCACCTGGACCGCTTGGACCAGAGCAGCGAAGTACTGGCCAGTTGGAAAAACGCT GACCATACAAGTAAAAGAATTTGGCGATGCTTGGCCACTACACCTGCCACAAAGGAGGAGAAGTACTTCAGTCCACAGCCTTG CTGCTGGATTGCACAGAAAAGACATGGCATCTTGGAGCCACCGCATTTTAAAAGACCAGACAAGGAGCCCCAAGAACATAAAGAACCT TCCTTCAGATGTTGAGGCGAAGAACTACAGTTGGCCCTTCACCTGCTGGTGGCTCACCAGCATCAGCCACCGACCTCACCTT CTGGTGTGAAGAGCAGCGTGTCTACAGACCCCGAAGGAGTCACCTGTGGGGCGGCCACGCTGTCTGGCAGAAAGAGTT CGAGGCCAACAAGCAATATCGCTGGAATGTCAAGAAGATTCGGCCTGCCCGGCGGCAGAAGAAAGTCTGC CCATAGAAGTCATGGTGGATGCTGTTCACAAATTAAAATATGAAAACTACACCAGCAGCTTCTTCATCAGAGATATCAT CAAGCCAGACCCCCCAAGAACCTGCAGCTGAAGCCCTGAAGAACAGCCGGCAGGTTGAAGTTTCCTGGGAGTACCCA GATACCTGGAGCACGCCGCACAGCTACTTCAGCCTCACCTTCTGTGTACAGGTCAAGGCAGAGGACAAGAGGAGAAGA AAGATYCGTGTCTTCACCGACAAAACCTCGGCGAACGGTCATCTGTGCAGGAGATGCAAGCATCTCGGTTCGAGCCCAGGA CCGCTACTACAGCAGCTGGAGTGAGTGGGCCTCCGTGCCCTGCAGTGGTGGCGGCGGCGGCAGCAGAAACCTT CCTGTGGCCACGCCCGGACCCTGGCATGTTCCGTGCCTGCAACCACAGCCAAAATTATTACGACCTGTTAGCAACATGC TSCAGAAAGCACGGCAAACTTTAGAATTCTACCCCTGCACCTCAGAAGAATAGACCATGAAGATATCACCAAAGATAA AACCAGCACTGTGAGAGGCCTGCCTGCCCCTGGAGCTCACCAAGAACGAGACGAGCTGCCTCAAATAGCAGAGAGACCAGCTTC ATCAACMATGGCAGCTGCCTGGCCAGCAGGAAAACCAGCTTCATGATGGCGGCTCTGCCTGAGCAGCATCTATGAGATC TGAAGATGTTACCAAGTAGAATTTAAAACCATGAATGCCAAGCTGCCTCATGGACCCCAAGCGGCAGATATTCCTCGACCA AAACATGCTGGCTGTCATTGATGAGCTCATGCAACATTAAACTTCAACTCAGAGAGACGGTGCCCCAGAAGACAGCCTG GAGGAGCCAGATTTCTACAAAACCAAGATCAAGCTCTGCATCTTATTACATGCCTTCCGCATCCGGGCGGTCACCATTG ACCGTGTCATGTCCTACTTAAATGCCAGCTGATAYAGRCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_ 010 (SEQ ID NO: 64)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGGCCACCAGCAGCTTGTCATCTCCTTTTTCTTCTTGCTCCTCGCTTCCTCTGATGCCG ATCTGCCAGTGAAGAAAGACGTCTACGTAGTAGAGTTGGATTGGTACCCGGACGCCCCTGGAGAAATGGTGGTCTCA CCTGCGACACTCCTGAAGAAGACGGTATCACCTGGACCTGGGACTCTAGCTCTGAAGCAGTTTTAGGCTCTGGAAAAACGCT GACCATACAAGTTAAAGAGAATTTCGCGACCTGGCCAGTACACGTCCTGCACAAGGAGACAACTTTCAAGCCACAGTTTA CTTCTTCTTCACAAGAAAGAGATGGCATCTGGAGTACAGATATTTTAAAAGACCAGAAGAGGAGCTAAGAACAAAACCT TCCTCGCTTGTGAAGCTAAGAACTACAGTGGTCGTTTCACCTGCTGGTGGTACCACCATCTCCACTGACCTCACCTT CTCTGTAAAATCAAGCCCGTGGTTCTTCTGACCCCCAAGGAGTCACCTGTGTGGCTGCCAGCGCTGAAGCGCTGAAGAGTT CGAGCCGACAACAAGCAATATGTCTGTGGAATGTCAAGAAGATTCTGCCTGCCCGGCGCCAGAGAAGAAAGTCTTC CCATACAAGTCCATATGGTGGACACGCTGTTTCACAAATTAAAATATGAAAACTACACCAGCAGCTTCCTCATTCGTGACATCAT CAAACCAGACCCTCCTAAGAACTTCAGTTAAAACCGCTGAAGACAGCCCCCACAGTGGAAGTTTCCTGGAGTACCCA GATACGTGGAGATCCGCTACTCCTACAGTTTAACCTTCTGTGTACAAGTACAAGGAAAATCAAAAAGAGAGAAGA AAGATCCGTCTTCAACAGATCTGCCACGGTCAATCTGCCCGTTAAGAACGCTTCCATCTCGGTTCGAGCCCAGGA CCGCTACTACAGCAGCAGCTGGAGTGGGCATCTGTTCCCCTGCAGTGGTGGCGGCGCAGCCCGCAACCTT CCTGTGGCCAGCGCCGACCCTGGCATGTTCCCGTGCCTTCACCACTCTGCCAAAATCTCTTCGTGTGTTCTAACATGC TGCAGAAGGCGCGGCAAACTTTAGAATTCTACCCGTGCACTTCTGAAGAAATAGACCATGAAGATAA AACCAGCACGGTGGAGGCCTGCCTTCCTTTAGAACTTACTAAGAACGAAAGTTGCCTTAACAGCCGTGAGACCAGCTTC ATCACCAATGGCAGCTGCCTTGCTAGCAGGAAGACCAGCTTCAGGATGGGCGCTGTGCCTTTCTTCCATCTATAGAAGATC TTAAGATGTACCAAGTTAGAATTTAAAACCATGAAATTATTAATGGACCCCAAGCGGCAGATATTCCTCGACCA AAACATGCCTGGCTGTCATTGAGCTCAAGCCATTAAACTTCCAACTCAGAAACTGTTCCCCAGAAGTCATCTTTA GAAGAACCAGATTTCTATCAAAACAAAATAAACTCTTCTTCATGCCTTCCGCATCCGTGCTGTCACCATTG ACCGTGTCATGCCTACTTTAAATGCTTCTTTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTTCCTCCCCTTCCTGCACCCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 011 (SEQ ID NO: 65)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGGCCACCAGCAGCTTGTCATCTCCTTTTTCTTCTTGCTCCTCGCTTCCTCTGATGCCG ATCTGCCAGTGAAGAAAGGACGTGTACGTAGTCGAATTGGATTGGTACCCGGACGCCCCTGGAGAAATGGTGGTCTCA CGTGCGACACGCCTCCAGAGGAAGACGGCATCACGTGGACCTGGGACTCTAGCTCCGAAGCAGTTTCTGGGAAAACGCT GACGATCCATAGTTGAAGAGTTCGAGGACGCGGGGCAGTACACGTGCCACAAGGAGATAGCGCTGCCTGCCGGCGCCAGAGAAGAAAGTCTTC CTGCTGCTGCACAAGAAGAGGACGGGATCTGGAGCACAGATATCCTGAAGGACCAGAAGGAGCCGAAAGAACAAGACGTT TCCTGAGGTTGCGAAGCCGAAGAACTACACGTGCTGGTGGCTGACCGATCAGCGACCTGGACTGCACGTTT CAGCGTGAAGAGCACCAGGGGGAGCAGCCCGCCAGGGGGTGACGTGCAGGCGCAAGAGCCAAGAGGCGAAGAGA AGGAGTGACAACAAGCAGTACAGCGTGGAGTGCCAGAAGATAGCGCTGCCCGCGGCGCCAGAGAAGAGCCTGC CGATCGAGGTGATGGTGGACGCGGTGCACAAGCTGAAGTACGAGAACTACACGAGCAGCTTCTTCATCAGAGATATCAT CAAGCCGGACCCGCCGAAGAACCTGCAGCTGAAGCCGCTGAAGAACAGCAGACAGGTGGAGGTGAGCTGGGAGTACCCA GATACGTGGACATCCAGGTGCAGAGCGTGCAGAAGGAGAAGGACGAGATGTTCCTGACCTACTTCATCGGCTGCCAGAGA AAGATAGGGTGTTCACAGATAAGACGGAGGGTGACGTGTGCCCTGAGAATCACATATGGGAGTTGAAAAGCGCCAGCAT TAGGTACTACAGCAGCAGCTGGAGTGGGCGAGCGTGCCGTCTGCAGCCAGAACCTGCTGAGGGCGGTGAGCAACATGC CCGGTGGCGACCCGGACCCGGGGATGTTCCCGTGCCTGCACCACAGCCCAGAACCTGCTGAGGGCGGTGAGCAACATGC

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	TGCAGAAGGCAGGCAGACGCTGGAGTTCTACCCGTGCACGAGCGGCGAGATCACGACCACGGAAGAATACGAGAATAA GACGAGCACGGTGGAGGCGTGCCTGCCGCCTGGAGCTGACGAAGAACGAGAGCTGCCTGAACAGCAGGGAGACGAGCTTC ATCACGAACGGGAAGCTGCCTGGCGAGCAGGAGACGAGCTTCATGATGGCGCTGTGCCTGAGCAGCATCTACGAGGACC TGAAGAATGTACCAGCTTGAAGTTCAAGACGCGAAGCTGCTGAACCCGAACAGAGGCAGATCTTCCTGGACCA GAACATGCTGCCGGTGATCGACGAGCTGATGCAGGCGCTGAACTTCAACAGCGGAGACGGTGCCGCAGAAGAGCAGCCTG GAGGAGCCAGATTCTACAAGACGAAGATCAAGCCTGTGCAGGAAGCGTTCAGGATCAGGGCGGTGACGATCG ACACCGGTGATGACTACCTGAACGCGGAGCTGATAATAGGCTGGACGCCTCGGTGGCCATGCCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 012 (SEQ ID NO: 66)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGGTGCCATCAGCAGCTGGTTGATCAGCCTGGTGTTTCTGGCCAGCCCCCTGGTGGCC ATTTGGGAACTCAAGAAGGACGTTTACGTTGTGGAACTCGACTGGTACCCTGACGCCCCAGGCGAAATGGTGGTCTTAA CCTGGGACACCCCTGAGGAGGACGGAATCACCTGGACCTTGGACCAGAGCTCCGAGGTCCTCGGCAGTGGCAAGACCCT GACCATACAGGTGAAAGAATTTGGAGACGCAGGGCAATACACATGTCACAAGGGCGGGGAGGTTCTTTCTCACTCCCTT CTGCTTCTACATAAAAAGCAAGACGGAATTTGGTCTACCGACATCCTCAAGGACCAAAAGGAGCCTAAGAATAAAACCT TCTTACGCTGTGAACCTAAAAACTACAGCGGCGAGATTGACTTGCTGTACCACGACTTTGTACCGACCTGACCTT CTCGGTGAAGTCTTCAAGGGCTCTAGTGATCCACAGGGAGTGACATGCGGGGCCCACACTGAGCGCTGAACGCGTG AGGGCGATAACAGGTGAAAGAATTTGGAGACGCAGGGCAATACACATGTCACAAGGGCGGGGAGGTTCTTTCTCACTCCCTT CCATAGAGGTTATGGTCGATGCTGTTGCATAAACTGAAGTACGAAAACTACACCAGCAGCTTCTTCATTAGAGATATTAT AAAACCTGACCCCCAAGAACCTGCAACTTAAACCCCTGAAAAACTCTCGGCAGGTCGAAGTTAGCTGGAGTACCCT GATACTTGGTCCACCCCCACTGGTACTTCTCACTGACTTTACTGTGCAGGGCAAGAGCAAGAGAGAAAA AAGATCGTGTATTCACAGATAAGACCTCTGCCACCGTGATCTGCAGAAAAAACGCTTCCATCAGTGTCAGAGCCCAAGA CCGGTACTATAGTAGCTGGAGCGAGTGGGCAAGTGTCCCCTGCTCTGGCGGAGGGGCGGCTCTCGAAACCTC CCCGTCCTACCCCTGATCCAGGATGTTCCCTTGCCTGCATCACTCACAGAATCTGCTGACGCGGTCAGCCACATGC TGCAGAAAGCTAGCCAAACACTGGAGTTTTATCCTTGTACCTCAGAGGACATCGACCACGGAGATATTACCAAAGATAA GACCAGCACGGTGGAGGCCTGCTTGCCCCTGGAACTGACAAAGAATGAATCCTGCCTTAATAGCCGTGAGAACCTCTTT ATAACAAACGGATCCTCTGGCCAGCAGGAAGACCTCCTTCATGATGGCCCTCTGCCTGTCCTCAATCTACGAAGACC TGAAGATGTACCAGGTGGAATTTAAAACTATGAACGCCAAGCTGTTGATGGACCCCAAGCGGCAGATCTTTCTGGATCA AAATATGCTGGTGATCGACGAACTGATGCAGGCCCTCAACTTTAACAGCGAGACCGTGCCACAAAAGAGCAGCCTGT GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCCTGTGCAGGAAGCGTTCAGGATAAGAGGCGTGACGATCG ACAGAGTCATGAGTTACCTGAATGCCAGCCTGGATAATAGGCTGGACGCCTCGGTGGCCATGCCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_...013 (SEQ ID NO: 67)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGTTGGTCATCTCCTTGGTTCTTCCTGGCCTCGCCGCTCGGTGGCC ATCTGGGAGCTGAAGAAAGAGACGTTTACGTAGTAGAGTTGGATTGGTACCCAGATGCGGAAATGGTGGTCCTCA CCTGTGACACGCCAGAAAGAAGACGGTATCACCTGGACCGCTGGACCAGACAGTGAAGTCTTTGGAAGTGCAAAAACGCT GACCATACAAGTAAAAGAATTTGGAGATGCTGGCCAGTACACCTGCCACAAAGGAGGCAGAAGTTCTCAGCCACAGTTA TTATTACTTCACAAGAAAGAGATGGCATCTGGTCCACAGATATTTTAAAAGACCAGAAGGAGGCCAAAAATAAAACAT TTCTTTCGATTGGAGGCCAAGAACTACAGTGGTCGTTTCACCTGCTGGTGGCTGACCACATCCTCCACAGACCTCACCTT CAGTGTAAAAAGCAGCCGTTCTTCTGACCCCCAAGGAGTCACCTGTGGGGCTGCCACGCCTCTCTGCAGAAAGAGTT CGAGGTGACAACAAAGAATATGAGTACTCGGTGGAATGTCAAGAAGATTCTGCCCAGCTGCTGAGGAGAGATCTTTC CCATAGAAGTCATGGTTGGATGCTGTTCACAAATTAAAATGAAAACTACCAGCAGCTTCTTCATCAGAGATATCAT CAAACCTGACCGCCAAGAACTTACAGCTGAAGCCGCTGTGAAAAACAGCCGGCAGGTAGAAGTTCCTGGGAGTACCCA GATACCTGTCACAGCCCGCACTCCTACTTCTCCCTCACCTTCTGTGTACAAGTACAAGGCAAGAGCAAGAGAGAAGA AAGATCGTGTCTTCACAGATAAACATCAGCCACGGTCATCTGCAGAGAAAATGCCAGCATCTCGGTGCGGGCCCAGA CCGCTACAGCAGCAGCTGGAGTGAGTGGGCATCTGTGCCCTGCAGTGGTGGGTGGTGGCAGCAGAAACCTT CCTCTGGCCACTCCAGACCCTGGCATGTTCCCGTGCCTTCACCACTCCCAAAATTTACTTCGAGCTGTTTCTAACATGC TGCAGAAGCACGGCAAACTTTAGAATTCTACCGTGCACTTCTGAGAGAATTGACCATGAGATATCACAAAAGATAA AACCAGCACAGTGGAGGCCTGTCTTCCTTTAGAGCTGACCAAAAATGAATCCCTGCCTCAACAGCAGAGACCAGCTTC ATCACCAATGGCCACCTGCCTGGCCCAGAAAACCAGCTTCATGATGGCCTCTGCCTCAGCTCCATCTATGAAGATT TGAAGATGTACCAAGTTAGAATTTAAAACCATGAATGCCAAATTATTATGAACCCCAAGAGGCAGATATTTTTAGATCA AAACATGCTTGCCAGTTATTGAGGCTCATGCAAGCATTAAACTTCAACAGGTGACCTCAAAAAAGCCAGCCCTT GAAGAGCAGAATTTCTACAAAACCAAGATCAAACTCTGCATTTACTTCATGCCTTCCGCATCCGGGCGGTCACCATTG ACCGTGTCATGTCCTACTTTAAATGCCTCGTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_...014 (SEQ ID NO: 68)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGTTGTGATTCTTGGTTCTTCTTTGTGTTCCTTGCTTCTCTTGTGGCT ATTTGGGAGTTAAAAAGGACGTGTACGTGGTAGAGCTTGACTGGTACCCTGACGCCACCTGGCAGATGGTGGTGCTTA CTTGTGACACTCCTGAGGAGACGGCATTACTTGCAGCCTTCTTCTGAGGTGCTTGGCTCTGGCAAAACACT TACTATTCAGGTGAAGCAGTTCGGGGATGCTGGCCAGTACACTTGCCACAAGGGCGAGGTGCTTTTCTCACTCTCTT CTTCTTCTTCAGAGAAGGAGGACGGCATTTGGTCTACTGACAGTTTTAAAAGACCAGAAGGAGGCCAAGAACAAAACAT TCCTTCGTTTGGAGGCCAAGAACTACTCTGGTGGCGTTTCACTGCTGGTGGCTGACCACATCCTTCCACTCCTTACTTT CTCTTGTGACAACAAGCGAGTACGAGTACTCTGTGGAGTGCCAGGAAGATTCTGCCTTGCCTGCTGAGGAGTCTCTTC CGTGGTGACAACAAGGAGTACGAGTACTCTGTGGAGTGCCAGGAAGATTCTGCCTTGCCTGCTGAGGAGTCTCTTC

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	CTATTGAGGTGATGCTGTGCACAAGTAAATACGAGACTACACTTCTTCTTCATTCGTGACATAT TAAGCCTGACCTCCCAAGAACTTCAGTTAAAACCTTTAAAAACTCTCGTCAGGTGGAGGTGTCTTGGGAGTACCCT GACACTTGGTCTACTCCTCACTTCTTACTTTCTCTTTACTTCTGCGTGCAGGGCAAGTCTAAGCGTGAGAAGA AGGACCGTGTGTTCACAAAACAACCTACTTGTGATTGCAGTTGCAGAAGAATGCATCTATTTCTGCGGCTCAGGA CCGTTACTACTCTTCTTCTTGGTCTGAGTGGGCTTCTGTGCCTTGCTCTGGGGGCGGCGGCTCCAGAAACTCTT CCTGTGCCTACTGCTGACCCTGGCATGTTCCCTTGCCTTCACCACTCTCAGAACCTTCTTCGTGCTGTGTGAGCACATGC TTCAGAAGCCTCGTCAAACTCTTGAGTTCTACCCCTTGCACTTCTTCAGGAGATTGACTTAAATTCTCGTGAGACGTCCTTC AACATCTACTGTGTGGAGGCTTGCCTCCTGAGCTTACCAAGAATGAAACTTGCTTAAATTCTCGTGAGACGTCCTTC ATCACCAACGGCTGTTGCCTTGCGCGCAAAACATCTTTCATGATGGCTGCTTTGCCTTCTTCTATTTACGAGATT TAAAAATGTACCAGGGTGGAGTTCAAAACAATGAATGCAAAGCTTCTTATGGACCCCAAGCGTCAGATTTTCCTTTGACCA GAACATGCTTGCTGTGATTGACGAGCTTTAAATTTCAACTCTGAGACGGTGCCTCAGAAGTCTTCTCTT GAGGAGCCTGACTTCTACAAGACCAAGATTAAGCTTTGCATTCTTTCTTCATGCTTTCCGTATTCGTGACTATTG ACCGTGTGATGTCTTACTTAAATGCTTCTTGATAATAGGCTGGAGCCTCGGTGGCGATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCTTCGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 015 (SEQ ID NO: 69)	TCAAGCTTTTGGACCTCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGTCACCAGCAGCTGGTGATCAGCTGGTTTTAGCCTGGTTCTTTGGCCAGCCCCCTGGTCGCC ATCTGGGAACTGAAGAAAGACGTGTACGTCGTAGAACTGGATTGGTATCCCGACGCCCCTGGCGAAATGGTGGTGCTGA CCTGTGACACCCCTGAAGAGACGGAATCACCTGGACCCTGGAACCAGAGCAGTGCTGGGCAGCGGGCAAACCCT GACCATCCAAGTGAAAGAGTTTGGCGATGCCGGCCAGTACACCTGTCACAAAGGCGGCGAGGTGCTAAGCCATTCGCTG CTGCTGCTGCACAAAAAGGAAGATGGCATCTGGAGCACCGATATCCTGAAGGACCAGAAAGAACCCAAAAATAAGACCT TTCTAAGATGCGAGCCCAAGAATTATAGCGGCCGTTTCACCTGGTGGCTGACCATCAGCACCGATCTGACCTT CAGCGTGAAAAGCAGCAGAGCAGCGACCCCCAGGCGTGACGTGCGGCGCCGCCACCCTGAGCGCCGAGAGATG AGAGCGACAACAAGATGTACAGCGTGTGGAGTGCCAGAAGATAGCGCCTGCCCGCCGCGGAGAAGCCTGC CCATCGAGGTGATGCCCGTGGATGCCGTGCACAAGCTGAAGATGATGCAACCAGCAGCTTCTTCATCAGAGATATCAT CAAACCCGACCCCCCCAAGAACGTGCAGCTGAAGCCCCTGAAGAATAGCCGCCAGGTGGAGGTGAGCTGGGAGTACCCC GACACCTGGAGCACCCCCATAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTCCAAGGGCAAGAGCAAGAGAAAGGA AAGATAGAGTGTTCACAGATAAGACCGCCGCCACGGTGATCTGCAGAAAAAATGCCAGCATCAGCGTGAGAGCCCAAGA TAGATACTATAGCAGCAGCTGGAGCGAATGGGCCAGCGTGCCTGCAGCGGCGGCAGCAGAAACCTG CCCGTGGCCACCCCGACCCCGGCATGTTCCCCTGCCTTCACCACAGCCAAAACCTGCTGAGAGCCGTGAGCAACATGC TGCAGAAGGCCGGCAGACCCTGGAATTTTACCCCTGCACCAGCGAGAGATCGATCATGAAGATCACCACAAGATAA AACCAGCACCGTGGAGGCCTGTCTGGCCCTGGAACTGACCAAGAATGAGGTGCTAAATAGCAGAGACCAGCTTC ATAACCAATGGCAGCTGGCCTGGCCAGCAGAAAGACCAGCTTTATGATGGCCTGTGGCCTGAGCAGCATCTATGAAGACC TGAAGATGTACCAGGTGGAGTTCAAGACCATGAATGCCAAGCTGCCTGATTGGATCCCAAGCGGCAGATCTTTCTGGATCA AAACATGCTGGCCCTGATGACATGCTGATGCAGGCCCTGAATTTCAACAGCGAGACCGTGCCCCAAAAAGCAGCCTG GAGAACCCGGATTTTTATAAAACCAAAATCAAGCTGTGCATTCTTTCATGCTTTCCGTATTCGTGACTATTG ATAGAGTGATGTATCTGAATGCCAGCTGATAATAGGCTGGAGCCTCGGTGGCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCTTCGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_ 016 (SEQ ID NO: 70)	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGGCCACCAGCAGCTTGGTCATCAGCTTGGTTCAGCCTGGTCTTCCTGGCCAGCCCCCTGGTGGCC ATCTGGGAGCTGAAGAAGGACGTATACGTAGTGGAGTTGGATTGGTACCCAGACGCTCCTGGGGAGATGGTGGTGCTGA CCTGTGACACCCCAGAAGAGGACGGTATCACCTGGACCAGAGCTCAGAGTGTCTGGGCAGTGGAAAACCCT GACCATCCAGGTGAAGGAGTTTGGAGATGCTGGCCAGTACACCTGCCACAAGGGTGGTGAAGTGCTGAGCCACAGCCTG CTGCTGCTGCACAAGAAGGAGGATGGCATCTGGAGCACAGATATCCTGAAGGACCAGGAAGAGCCCAAGAACAAGACCT TCCTTCGCCTGTGAAGCCAACAACTACAGTGGCTACACCTGCTGGTGGCCTGACCACATCAGCACAGACCTCACCTT CTCGGTGAAGAGCAGCAGGAGCTCAGACCCCCAGGGTGTCACCTGTGGGGCGGCCACGGCTGTGGCGGAGAGAGTT CGAGGTGACAACAAGGAGTATGAATACTCGGTGGAGTGCCAGAGGAGTTCGGTGTGCCCGGCGGCAGAAGAGGCCTGC CCATAGAAGTGATGGTGTGTGCACAAGCTGAAGTGATGAAAACTACCAGCAGCTTCTTCATCAGAAGATATCAT CAAGCCAGACCGGCCAAGAACTGCAGCTGAAGAACAGCGGCGGCAGTGGAGGTTTCCTGGGAGTACCCA GATACGTGGAGCACCCCCACAGCTACTTCAGCCTGACCTTCTGTGTCCAGTGCAGGGCAAGAGAGCAAGAGAAGA AAGATAGAGTCTTCACAGATAAGACCTCGGCCACGGTCATCTGCAGTAAGAATGCCTCCATCTCGGTTCGAGCCCAAGA TAGATACTACAGCAAGCGTGGTCAGAATGGGCCCTCGGTGCCCTGCAGTGGTGGCGGCGGCAGCAGAAACCTG CCTGTTGCCACCCCAGACCCTGGGATGTTCCCCTGCCTGCACCACAGCCCAGAACTTATTACGAGCTGTTTCTAACATGC TGCAGAAGGCCCGGCAGACCCTGGAGTTCTACCCCTGCACCTCAGAAGAGATTGACCATGAAGATCACCAAAGATAA GACCAGCACTGTAGAGGCCCTGCCTGCCCCTGGAGCTGACCAAGAATGAAAGCTGCCTGAACAGCAGAGACCAGCTTC ATCACCAATGGAAGCTGCCTGGCCAGCAGAAAGACCAGCTTCAGGATGGCCCTGTGCCTGAGCAGCATCTATGAAGACC TGAAGATGTACCAGGTGGAGTTCAAGACCATGAATGCTGCTGATGCAGCCCAAGCGGCACATATTTTGCACCA GAACATGCTTGCTGTCATTGAGCTGATGCAGCCCTGAACTTCAACTCAGAAACTGTACCCCAAGAAGAGCCAGCCCTG GAGGAGCCAGAATTTCTACAAGACCAAGATCAAGCCTGTGCATCCTGCTTCATGCTTTCAGAATCAGAGCTGTCACCATTG ACCGCGTGATGAGCTACCTTAATGCCTCGTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 017 (SEQ ID	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGGCCACCAGCAGCTTGGTTTCCTGTTTTCTGGTCTTTTCTGGCATCACCCCCTGGTGGCC ATCTGGGAGCTGAAGAAGGACGTGTACGTGGTGGAGCTGGATTGGTACCCTGACGCCCCGGGCGAAATGGTGGTGCTGA

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NO: 71)	CCTGCGACAGCGCCTGAGAGGAGACGGCATCACCTGGACCGAGCGAGCAGCGAGGTGCTTGGGTCTGGTAAAACTCT GACTATTCAGGTGAAAGAGATTCGGGGATGCCGGCCAATATACTTGCCACAAGGGTGGCGAGGTGCTTTCTCATTCTCTG CTCCTGCTGCACAGAAAGAAGAATGGCATTTGGGCTACTGATAATTCTGAAAGAGAGCCCAAGAACAAGACCT TTCTGAGATGCGAGGCTAAAAACTACAGCGGAAGATTTACCTGCTGGTGGCTGACCACAATCTCAACCGACCTGACATT TTCAGTGAAGTCCAGCAGAGGGAGCTCCGACCCTCAGGGCGTGACCTGGGAGCCGCCACTCTGTCCGCAGAAAGAGTG AGAGGTGATAATAAGCAGTACGAGTATTCACTCGAGTGCCAAGAAGATTCTGTGCCTGCCAGCCGCCGAGCAGCAGCCTGC CAATCGAGGTGATGGTAGATGCGGTACACAAGCTGAAGTATGAGAACTACACATCCTCCTTCTTCATAAGAGATATTAT CAAGCCTGACCCAACCTAAAAATCTGCAACTCAAGCCTTTGAAAAATTCACGGCAGGTGCAGGGCAAGTCAAAGAGAGAGAGA GATACTTGGAGCACCCCCCATAGCTACTTTTCGCTGGACAGTCTGCGTCCAGGTGCAAGGGCAAGTCAAAGAGAGAGAGA AGGATCGCGTGTTCACTGATAAACAAGCGCCACAGTGATCTGCAGAAAAACGCTAGCATTAGCGTCAGAGCACAGGA CCCGTATACTCCAGCTTCTTGGAGCGAATGGGCAACTCTGTGCCCTCCAGCTCGGTGGAGGCGGAGCCGAACCAGAACCTC CCCGTTGCCACACCTGATCCTGGAATGTTCCCCTGTCTGCACCACAGCCAGAACCTGCTGAGAGCAGTGTCTAACATGC TCCAGAAGGCCAGGCAGACCCTGGAGTTTTACCCCCTGCACCAGCGAGAAATCGATCACGAAGATATCACCAAAGATAA AACCTCCACCGTCGAGGCCTGCCTGGCCCCTGGAACTGACCAAAAACGAGAGCCTGCCTGAATAGCAGGGAGACCTCCTTC ATCACCAACGAGGCTCATGCCTTGCCAGCCGGAAAACTAGCTTCATGAGCCCTGTGCCTGTGTCCTTCGATCTATGAGGACC TGAAAAATGTACCAGGTCCGAATTTAAGACGATGAACGCAAAGCTGCTGATGGACCCCAAGCGGCAGATCTTTCTGGACCA GAACATGCTGGCAGTCATAGATGAGTTGATGCAGCCATTAAACTTCAACAGCGACCGTGCCTCAGAAGTCCAGCCTC GAGAGCCAGATTTTTATAAGACCAAGATCAAACTATGCATCCTGCTGCATGCTTTCAGGATTAGAGCCGTCACCATCG ATCGAGTCATGTCTTACCTCAAYGCTAGCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_ 018 (SEQ ID NO: 72)	TCAAGCTTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGTCACCAACAGTTACTAATCTCCTGGTTTTTCTCTGGTGTTTCTGGCCAGCCCCCTCGTGCCC ATCTGGGAGCTTAAAAAGGACGTTTACGTGGTGGAGTTGGATTGGTATCCCGACGCTCCAGGCGAAATGGTCGTGCTCA CCTGCCATACCCCCTGAAGAAGACGGTATCACCTGGACCAGTCTTCCTGAGGTCGTTGCATCTGGCAAACACT GACAATACAAGTTAAGGAGTTCGGGGACGCACGCGCAGTACACTTGCCACAAAGGCGGGGAGGTCCTGAGTTCACTTCCCTG TTACTGCTTCAAGAAAGAGAGCATTTGTCCACTGGACATTCTTGAAGAACAGGAGCCTAAGAATAAAACTT TCCTGAGATGCGAGGCAAAAAACTATAGCGGCCGCTTTACTTGCTGGTGCTTACAACAATCTCTACCGATTTAACTTT CTGCGTGAAGTCTAGCAGAAGGAGTGACTTGCGGAAGCCGGCCACCTTGAGCGCCGAAAGAGTC CGTGGCGATAAACAAATACGAGTACTCCGTGGAGTGCCAGGAAGATTCCGCCTGCCCAGCTGCCGAGGAGCGTCCCTTGC CCATTGAAGTGATGGTGGATGCCGTCCACAAGCTGAAGTACGAAAACTATACCAGCAGCTTCTTCATCAGGATCTCG TAAGCCCGAGCCTCCTAAAACCTGCAACTTAAGCCCTAAAGAATAGTCGGCAGGTTGAGGTCAGCTGGGAATATCCT GACACATCGAGCACCCCCACTCTTATTTCTCCCTGACTTCTGCGTGCAGGTGCAGGGCAAGAGTAAACGCGAGAAAA AAGATAGGGTCTTTACCGATAAAACCAGCGCTACGTTATCTGTCCGAAGAAGCTTCCATCTCCGTCCGCTCAGGA TCGTTACATTAACTGGTCCTCATGGAGCGAGTGGGCCCAGCGTGCCTGCAGCGGCGGCAGGGTGGCAGGCGGATCCAGAAATCTG CCTGTTGCCACAGACCCTGGCATGTTCCCCTGTCTGCATCATAGCCAGAACCTGCTCAGAGCCGTGAGCAACAGATG TCCAGAAGGCCAAGCAACTTTGGAGTTCTACCCGTGTACATCTGAGGAAATCGATGTATAACCAAAGATGA AACCTCTACAGTAGAGGCTTGTTTGCCCCTGCAGTTGACCAAAAACGAGAGTTGCCTGAACAGTCGCGAGACGTTC ATTACTAACGGCAGCTGTCTCGCCTCCAGAAAAACATCCTTCATGATGGCCCTGTGTCTTTCCAGCAATACGAGAGACC TGAAAATGTACCAGGTCGAGTTCAAACACATGAACGCCAAGCTGCTTATGGACCCCAAGCGGCAGATCTTCCTGGACCA AAACATGCTCCTGTTGATCGATGAGCTGATGCAGTCTCTCAACTTCAATTCCGAAACAGTGCCACAGAAGTCCAGTCTG GAAGAACCCGAATTCTACAAGACCAAGATTAAGCCTGTGCTTCATGCGTTTAGAATCAGAGCCGTGACCATTG ATCGGGTGATGAGCTACCTGAACGCCTCGTGAATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_019 (SEQ ID NO: 73)	TGAGTGGGCGGC TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACAGCTTGTCATCTCCTGGTTTTCTTGTCTTCCTGCCGCCTGGTGGCC ATCTGGGAGCTGAAGAAAGTGTTTACGTAGTGGATTGGTACCCAGACGCCCCTGGAGAAATGGTGGTCTCA CCTGTGACACTCCTGAAGATGACGGTATCACCTGGACCCTGGACCAGAGCTCAGAAGTCTTTGGAGTGGAAAAACGCT GACCATACAAGTAAAGGAAGATGCTGGGCAGTACACGTGCCACAAAGGAGGAGTCTCAGCCACAGTTTA CTTCTTCTTCACAAGAAAGAAGATGGCATCTGGTCCACAGATATTTAAAAGACCAGAAGGAGCCCAAGAACAAAACCT TCCTCCGCCTGTGAGCCAAGAACTACAGTGGTCGTTTCACCTGCTGGTGGACTCACCACTGACCTCACCTT CTCTGTAAAAAGCAGCGTGTTCTTCTGACCCCCAAGGAGTCACCTGTTGGGGCTGCCCACGCTCTCGGCAGAAAGAGTT CGAGGTGACAACAAGGAATATGAATATTCTGTGGAATGTCAAGAAGATTCTGCCTGGCGGCAGAAGAAGGTCTTC CCAATAGAAGTCATTGCTGGATGCTGTTCACAAATTAAAATATGAAACTACACAGCAGCTTCTTCATTCGTGACATCAT CAAACCAGACCCGCCCAAGAACCTTCAGTTAAAAACCTTTAAAAAACAGCCGGCAGGTAGAAGTTCCTGGGAGTACCCA GATACGTGGTCCAGCCGCGACTCCTACTTCAGTTTAACCTTCTGTGTACAAGTACAAGGAAAATCAAAAAGAGAGAAGA AAGATCGTGTCTTCACTGACAAAACATCTGCCACGGTCATCTGCAGGAAGAATGGCCTCCATCGGTTCGAGCCCAGGA CGGCTACTACAGCCAGCAGCTGGAGTGGGCATCTGTTCCCTGCAGTTGGCGGCGGCGGCCAGCCGCAACCTT CCTGTGGCCACGCCCGACCCTGGCAATGTTCCCGTGCCTTCACCACTCCCAAAATCTTCTTCGTGCTGTTTCTAACATGC TGCAGAAGGCGCGGCCAAACTTTAGAATTCTACCCGTGCACTTCTGAAGAAATAGACCATGAAGATATCACCAAAGATAA AACCAGCACGGTTGGAGGCCTTGCCTTCCTTTAGAGCTGACCAAGAATGAATCCTTGCCTCAACAGCAGAGACCAGCTTC ATCACCAATGGCAGCATCCCTGGCCTGGCCAAGAACCAGCTTCATCATGGCGCTGTGCCTTTCTTCCATCTATGAAGATT TAAAGATGTACCAAGTTAGAATTTAAAAGCATGAATTATTAATGGACCCCAAACGGCAGATATTTTTGGATCA AAACATGCTGGTCTATTGATGAGCTGATGCAAGCATTAAACTTCAACTGAGAACTGTTCCCCAGAAGTCATCTTTA GAAGAGCCAGATTTCTACAAAACAAAAATAAAACTCTGCATTCTTTCTTCAATGCCTTCCGCATCGTGCTGTCACCATTG ACCGTGTCATGTCCTACTTAAATGCTTCTTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACACATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_020 (SEQ ID NO: 74)	TCAAGCTTTGGAACCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGTGCCACCAGCAGTTGGTCATCTCTTGGTTTTCCTTGTCGCGATCGCTCCTGGCACTGATGGCCGTTTGTTTGTCAACACAAAAAACGTGAATGCACATTCCTGTTGCCTCAAGATATGATGACGAACTGCTGGACAGCAGGCTCCAGAGCGATGAAATTACAGTTCCCCAGACTCGCTACTATCTGGCTAGAAATGTCCAGCGTCTGTGGTTGAAAAGAGGAGATAAATCTGTTGATCTTCGTGACCTTGGTCTTTGTTATCAGCTATGTGAATAGTGGCTTAACTTTCATTGCTGGATACGTCCACTCCAGTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTCGCCACCATGGACATGAGAGTGCCCGCTCAGCTGCTGGGACTGCTGATGCTGTGGGTGAGCGGCTCCAGCGGCGAGGTGCAGCTGGTGGAGTCTGGCGGCGGCCTGGTGCAGCCTGGCAGATCCCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTCCGACAGGCCCCTGGCAAGGGCCTGGAGTGGGTGTCCGGCATCTCCTGGAACTCCGGCTCCATCGGCTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACATCCAGTACGGCAACCTGTACTACGGCATGGACGTGTGGGGCCAGGGCACCACCGTGACCGTGTCCTCCGCCTCCACCAAGGGCCCCTCCGTGTTCCCCCTGGCCCCCTCCTCCAAGTCCACCTCCGGCGGCACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAACTCCGGCGCCCTGACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGTCCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACCGTGCCCTCCTCCTCCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGTCCTGCGACAAGACCCACACCTGCCCCCCCTGCCCCGCCCCCGAACTGCTGGGCGGCCCCTCCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCCGCCCCCATCGAGAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGGGAGCCCCAGGTGTACACCCTGCCCCCCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGAGTGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCCCCCGGCAAGTGATAAGGATCCGAGCTCGGTACCAAGCTTAAGTTTAAACGCTAGCCAGCTTGGGTCTCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCG

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hIL12AB_021 (SEQ ID NO: 75)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTTGTTCATCTGGTTTCAGCCTCTGGCCAGCCGCTCTGGTTGCC ATCTGGGAGCTCAAAAAGGATGTATACGTTGTCGAACTGGATTGGTACCCAGATGCCCCCGGAGAAATGGTCGTCCTCA CCTGCGACACCCCCGAAGAAGATGGTATCACATGTGACACCCCAGTCGAAGAAGATGGCACTTCTGAAGACATCACA AAGCACAGCTCCCCGTTCAAGCGGATGCTGAAGTCGTCGAGTGCGACTGGAAAGCCCCCATGATCCAGTGCCAGAC GACCTTCCACCGTGGCAAGGACCAGCGCCGACCCGTGAACGTCATCTGCTCCGTGAAGTCCGCCCACGTCGTCACA AGGACAGGGTTTTCACCGACAAGACCAGCGCCGACCCGTGAATCTGGCCGAAGAAGCCACGCATAAGCGTCCGCGCCAAGA TAGGTACTACAGCAGCTCCTGAGCGAGTGGGCTAGCGTGCCTTGTGCCAGTCTGAAGACCGCTC CCAGTGGCGACCCCCGACCCCGGCATGTTCCCCTCGCCTCCAACAGCCAGAACCTGCTGAGGGCCGTCAGCAATATGC TGCAGAAGGCCAGGCAGACCCTGGAATTCTACCCCTGCACCTCGGAGGAGATCGATCACGAGGATATCACAAAAGACAA GACCTTCCACCGTTGGAGGCCTGCCTGCCCCTGAAGCTCACCCCAAGAATGAGTCCTGTCTGAAGCTGACCGTGAG ATCACCAAGGAGTCCTTCCTGAACCACAAGTCCGAGATGGACCTGCAGGCCCTGACGCTGGGCATGTCCGCCATCG TGAAGATGTACCAGATGTCGAGTTCAAGAACACGAATCCACCTGCTGATGGAACCCCAAGAGGCAAATCTTCCTGGACCA GAATATGCCTTGCCGTCATCGACGAGCTCATGCAGGCCCTGAACTTCAACTCCGAGACCGTGCCCCAGAAGAGCAGCCTG GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCGTTCAGGATCCGCGCAGTCACCATCG ACCGTGTGATGAGCTACCTGAACGCCAGCTGAAAGACCTCGGTGAGCCATGCTTCTTTGGACCTTGGAGCCTTC CCCCCAGCCCCTGCCTCCCCTTCCTGCACCCGTACCCCCAAACACGCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_022 (SEQ ID NO: 76)	ATGTGTCACCAGCAGTTGGTCATCTCTTGGTTTTCCCTAGTGTTTCTTGCCTCCCCCTGGTCGCCATCTGGGAGCTG TATAAGAGCCACCATGTGCCATCAGCAGCTGGTGATCAGCTGGTTCAGCCTGTTCCTCGCCTCTCCCCTGGTCGCC ATCTGGGAGCTCAAAAAGGATGTATACGTTGTCGAACTGGATTGGTACCCAGATGCCCCCGGAGAAATGGTCGTCCTGA CCTGCGACACCCCCGAAGAAGATGGTATCACATGTGACACCCCAGTCGAAGAAGATGGCACTTCTGAAGACATCACA GACCATCCAGTCAAGCGGATGCTGAAGTCGTCGAGTGCGACTGGAAAGCCCCCATGATCCAGTGCCAGACCAAGGGC CTCCTGCTGCACAAGAAGCGGAAGAGGATCTGGTCCACAGACATACTGAAGGAACCAGAAGAATAAAAGCCT TTCTCAGGTGCCAGCCACAAGAACTATTCCGGCAGGTTCACGTGCTGGTGGCTTACAACAATCAGCACGACCTGACGTT CAGCGTGAAGTCCAGCCCGCAGCAGCCCCAGGGGGTGACCTTGAGGCGCCGCCAGCCCTGAGGCGCCGGGTG CGCGGGGACAACAAGGAGTACTCCGTGGAGTGCCAGGAAGACAGCGCTGTGTCCGCCGAAGAAGAGTCCCTGCC CTATCGAGTCATGTGTGATGCAGTGCATAAGCTGAAGTACGAGAACTATACGAGCAGTTTTTCATCAGGACATCAT CAAGCCCGACCCCCCAAGAACCTGCAGCTTAAGCCCCTGAAGAATAGCCGGCAGGTGGAGGTCTCCTGGGAGTACCCC GACACCTGGTCAACGCCCACAGCTACTTCTCCCTGACCTTTTGTGTCCAAGTCCAGGTCCGTGGAGGGCCGCAAGA AAGATCGAGGTGTTCACCGACAAGACCTCCGCCACGGTGATCTGCAGGAAGAACGCCAGCATCTCCGTGAGGGCCAAGA CAGGTACTACTCCAGCAGCTGGTCCGAATGGGCCAGCGTGCCCTGCTGCCAGGGGCCGGGGCAGCCGAAACCTA CCCGTGGCCACCCCGAACCCCGGCATGTTTCCCTGCCTGCACCACAGCTCAGAACTCCTGAGGGCCGTGTCCAACATGC

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	TGCAGAAGGCCAGGCAGCACTCTGGAGTTCTACCCCTGCACGAGCGAGGAGATCGATCACGAGGACATCACCAAGGATAA GACCAGCACTGTGGAGGCCTGGCTTCCCCTGGAGCTGACCAAGAACGAGCTGTGTCTGAACTCCAGGGAGACCTCATTC ATCACCAACGGCTCCTGCCTGGCCAGCAGCAAAACCAGCTTCATGATGGCCTTGTGTCTCAGCTCCATCTACGAGGACC TGAAGATGTACCAGGTTCGAGTTCAACAACATGAACGCCAAGCTGCTGATTGACAACCCCAACAAGCGCAGCATCTTCCTGGACCA GAACATGCTGGCCGTCATCGACTTCTACATCCAGGACGGCAAGGACCAGGTGACCGTGATCAACGAGCTGCCCATCGTGAAAAAGCTCCCCAG GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGAGGATCAGGAGTCAGGGCAGTGACCATCG ACCGGGTGATGCATACCTTAACGGCCAGCTGATAATAGGCTGAGAGCCTCCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCCATTGCCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_023 (SEQ ID NO: 77)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCATCAGCAGCTGGTCATCTCCTGGTTTCTGGCCTGGTGTTTCTGGCCCCCTGGTCGCC ATCTGGGAGCTGAAGAAAGACGTGTACGTCGTCGAACTGGACTGGTACCCCGACGCCCCCGGCGAGATGGTGGTGCTGA CCTGCGACACCCCCGAGGAGGACGGCATCACCTGGACCCTGGACCAGAGCAGCGAGGTGCTGGGCAGCGGCAAGACCCT GACCATCCAAGTGAAGGAGTTCGGCGATGCCGGCCAGTACACCTGTCACAAAGGGGGCGAGGTGCTGAGCCACAGCCTG CTGCTGCTGCACAAGAAGGAGGATGGCATCTTGGAGCACCGATATCCTGAAGAACCAGAAGAACCCCACCATCAAGCGT TCCTTCAGCCTGGAGAACGCCGTGATCATCAGCGTGTCGACCTCGCAGCTGCTGCTGCCCGACAGCAAGCAGAGCTTC CAGCGTGAAGACGTTCCTGAGCGGCAAGGAGTTCGCCAGCGGCAGCAGCACCGGCTGGAACATCACCCTGGACAGCGTG CGGGGCCTGGACCAGGTGCTGAACCTGGGCCACAGCCTGAACGACCCCACCCAGCCCCTGGAGCTGCAGGACAGCAAG CCATCGAGGTGAAGGACGCCGTGCATAAGCTGAAGTACGAGAACTACACCAGCTCGTTCTTCATCCGGGACATCATC CAAACCCGAGCCCCCAAAAATCTGCAGCTCAAGCCCCTGAAGAACTCCAGGCAGGTGGAGCTGAGCTGGGAGTACCCC GACACCTGGTCCACCCCCCACAGCTACTTCTCCCTGACATTCTGCGTGCAGGTGCAGGGCAAGAGCAAGAGGGAGAAGA AGGACAGGGTGTTCACCGACAAGACGTCCGCCACCGTGATCTGCCGCAAAAACGCCAGCATCTCGTGGGCCCAGGA TAGGTACTATTCCAGCTCCTGGAGCAGTGGGCCTCGGTACCTCGGCGGGCGGCGGCAGATAGGAATCTG CCCGTGCCTACCCCGACCCGGCATGTTCCCCTGCCTCCACCACAGCCCAAACCTGCTGAGGGCCGTGAGCCACATGC TGCAGAAGGCCAGACAGACGGCTGGAGTTCTACCCCTGCACGAGCGAGGAGATCGACCACGAGGACATCACCAAGGATAA AACTTCCACCGGTCCTGCCTGGCCTTGGAGCTGACCAAGAATGAATCCTGTCTGAACAGCAGGGAGACCTCGTTT ATCACCAATGGCAGCTGCCTCCAGGAAGACCAGCTTCATGATGGCCCTCTGTCTGAGCTCCATCTATGAGGACC TGAAGATGTACCAGGTTGAGTTCAAGACCATGAACGCCAAGCTGCTGATTGACCCCAAGAGGCAGATCTTCCTGGATCA GAATATGCTGGCGATCGACTTCTACATCCAGGACGGCAAGGACCAGGTGACCGTGATCAACGAGCTGCCCATCGTGAAGAAGCTCCCCCTG GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGAGGATCAGGAGTCAGGGCAGTGACCATCG ACCGGGTCATGAGCTACCTCAATGCCAGCCTGATAATAGGCTGAGAGCCTCCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCCATTGCCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_ 024 (SEQ ID NO: 78)	TCAAGCTTCTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTGGTGATCTCCTGGTTTCTCCTGGCCTCGCCCCCTGGTGGCC ATCTGGGAGCTGAAGAAGGACGTGTACGTCGTGGAGCTGGACTGGTACCCGGACGCCCCTGGCGAGATGGTGGTGCTGA CCTGCGACACCCCAGAGGAGGATGGCATCACCTGGACTCAGTCCTCCGAGGAGTGCTGGGCTCCGGCAAGACCCT GACCATCCAAGTGAAGGAGTTCGGTGACGCCGGCAGATATACCTGCCATAAGGGCGGCGAGGTCCTGTCCCACAGCCTC CTCCTCCTGCATAAGAAGGAGGACGGCATCTGGAGCACCGACATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACCT TTCTGAGGTGCGAGGCCAAGAACTACAGCGGCCGATTCACCTGCTGGCTCACCACCATATCCACCGACCTGACCTTT CTGCGTCAAGTCTTCCGGGGGTCCAGCGACCCCCAGGGAGTGACCTGCGGCGCCGTCTCTGCTGAGCGCGTCAGAGG CGGGGGACAACAAGGAGTACGAATACTCCGTCGAGTGCCAGGAGGACTCCGCCTGCCCGGCCGCCGAGGAGAGCCTGC CCATCGAGGTGATGGTCGACGCGGTGCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCAGGGATATCAT CAAGCCAGATCCCCCGAGAATCTGCAACTGAAGCCGCTGAAAGATTCAGTGGAGGTCAGCTGGGAGTACCCC GACACCTGGAGCACCCCACATTCCTACTTCAGCCTGACGTTCTGCGTGCAGGTCCAGGGCAAGAGCAAGAGAGAGA AGGACAGGGTGTTCACGGATAAGACCAGTGCCACCGTGATCTGCAGCAAGAACGCCTCTATTACCGTGAGCGCCAGCA TCGGTATTACTCCTCGAGCTGGAGCGAATGGCCCTCCGTGCCTGCAGTGGGGGGGTGGAGCGGGAGCAGGAACCTG CCCGTAGCAACCCCCGACCCCGGGATGTTCCCCTGTCTGCACACTCCTGCGCCGCGTGAGCAACATGC TCCAAAAGCCCGTCAGACTTTAGAGTTCTACCCCTGCACCAGCGAAGAAATCGACCACGAAGACACATCACCAGACA AACGCACACCTTGGAGCGTGCCTGCCGCTGGAGCTGACCAAGAACGAGGTGGCCTCAACTCCAGGGAGACCAGCTTT ATCACCAAGGACTCGTGCCTAGCCGGAAAAACCAGCTTCATGATGGCCCTGTGCCTGAGCTCCATTTACGAGGACC TGAAGATGTACCAGGTTGAGTTCAAGACCATGAATGCCCAAACTGCCTGGTGGCATGCCTTCTTTGCCCTTGGCCTC GAACATGCCTGCGGTATCGAGTGATTCACAGGCCCTGAACTTTAATAAGCGAGACCGTGCCCCAGAAAAGCAGCCCT GAGGAGCCGGACTTCTACAAGACCAAAATCAAGCTGTGTCATCCTCGCTTCCAGCGCCTTCGTCCCAGCCATCG ACAGGGTGATGAGCTACCTGAACGCCAGCTGAATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGCCTC CCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAACACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGCC TGAGTGGGCGGC
hIL12AB_ 025 (SEQ ID NO: 79)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCATCAGCAGCTGGTGATTTCCTGGTTTCCTGGCCAGCCCCCCTCGTGGCG ATCTGGGAGCTAAAGAAGACGTGTACGTGGTGGAGCTGGACTGGTACCCGGACGCCCCGGCGAGATGGTCGTTCTGA CCTGCCATACCCCAGACGAGGACGGCATCACCTGGACCCTCGATCAGAGCAGGTCCTGGGGAGCGGAAAGACCCT GACCATCCAGGTCAAGGAGTTCGGCGACGCCGGCCAGTACACCTGCCACAAAGGTGGCGAGGTCCTGAGCCACTCGCTG CTGCTCCTGCATAAGAAGGAGGACGGAATCTGGAGCACAGACATCCTGAAAGACCAGAAGGAGCCCAAGAACAAGACCT TCCTGAGGTGCGAGGCCAAGAACTACAGCGGGCGTTCACGTGCTGGCTGACCACCATCAGCACCGACCTCACCTTT CTGCGTCAAGAGCAGCCGGGGATCCAGCGACCCCCAAGGGCGTCACCTGCGGCGCCGTCTGCCTGGCCGAGCGCGTC AGGGGCGATAATAAGGAGTATGAGTACAGCGTGGAGTGCCAGGAGGACAGCGCCTGCCCGGCCGCCGAGGAGAGTCCCTGC

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	CAATCGAAGTGATGCTCGACGCCGTGCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCCGGGATATCAT
	CAAGCCCGATCCCCCCGAAGAACCTGCAGCTGAAGCCCCTCAAGAACAGCCGCGTGGAGTGATTGGGAGTTGGGAGTACCCC
	GACACCTGGTCAAGCGCCCCACAGCTACTTCTCCCTGAGCTTCTGTGCAGGTGCAGGGAAGAGCAAGAGCAAGAGGAAGA
	AAGACCGGATCTTCAACGACCAGGCCCACGTGATCTGCAAGAAGAACGCAATCTCCGTGAGGGCCCAGGA
	CAGGTACTACAGCTCCAGTTGGTCCGAATGGGCCAGCGTGCCTTGTAGCGGGGGCGGTGGCAGCCGCAACCTC
	CCAGTGGCCACCCCGACCCGGCATGTTCCCCTGCCTGCACCACAGCCAGAATCTGCTGAGGGCCGTGAGTAACATGC
	TGCAGAAGTGCAAGCCAAACCCTCGAATTCTATCCCTGCACCTCCCGAGGAATCGACCACGACATACCACCAAGGACAA
	GACCAGCACCGGTCGAGCCTGGTCCCCCTGGAGCTGACCAAGAATGAGAAGTGCCTGAACAGCCGGGAGACCAGCTTC
	ATCACCAACGGGAGCTGCCTGGCCTCCAGGAAGACCTCGTTCATGATGGCGCTGTGCCTCTCAAGCATATACGAGGATC
	TGAAGATGTACCAGGTGGAGTTTAAGACGATGAACGCCAAGCTGCTGATGGACCCGAAGAGGCAGATCTTCCTGGACCA
	GAACATGCTGGCCGTGATAGACGAGCTCATGCAGGCCCTGAACTTCAACTGTGAGACCGTGCCGCAGAAGTCATCCCTC
	GAGGAGCCCGACTTCTATAAGACCAAGATCAAGCTGTGCATCCTGCTCCACGCCTTCCGAATAAGGCCGTGACGATCG
	ACAGGGTGATGAGCTACCTGAACGCCAGCCTGTGGGACCCTCGGTTCGATGCTTCTTGCCCCTTGGCCTC
	CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC
	TGAGTGGGCGGC
	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAA
	TATAAGAGCCACCATGGCCTCCCCGAAGAAGAGGAAAGCTGGAGCTCCACCGCGGTGGCCCAGCTCCCTGTGGTCGAC
	ATCTGGGAGCTGAAGAAGGACGTGTACGTGGTGGAGCTGGACTGGTACCCTGACGCCCCCGGCGAGATGGTCGTGCTGA
	CCTGCGACACCCCCGAAGAGGACGGTATCACCTGGACCCTGGACCAGTCCAGTGAGGTGCTGGGCAGCGGCAAGACCCT
	GACTATTCAAGTCAAGGAGTTCGGAGACGCCGGCCAGTACACCGTGCTCACAAGGGTGGAGAAGGTGTATCACACAGCCTA
	CTGCTGCTGCACAAGAAGGAAGACGGGATCTGGAGCACCGACATCCTGAAGGACCAGAAGGAGCCCAAAAACAAGACCT
	TCCTGCGGTGCGAGAAGCCCAAGAACTATTCGGGCCGCTTTACGTGTGGTGCTGACCACCATCAGCACTGATCTCACCTT
	CAGCGTGAAGTCCTCCCGGGGTCGTCCGACCCCCAGGGGGTGACCTGCGGGGCCGCCACCCTGTCCGCCGAGAGGTC
	AGGGCGATAATAAGGAGTACGAGTACAGCGTTGAGTGCCAGGAGGATAGCGCCTGTCCCGCCGAGGAGAGCCTGC
	CCATCGAGGTGATGGTGGACGCCGTCCACAAGCTGAAGTATGAGAACTACACCTCAAGCTTCTTCATCAGGGACATCAT
	CAAGCCCGATCCGCCCAAGAATCTGCAGCTGAAGCCCCTGAAGAATAGCCGCCAGGTGGAGGTGAGCTGGGAGTACCCC
	GACACCTGGTCCACCCCCATAGCTATTTCTCCCTGACGTTCTGCGTGCAGGTGCAAGGGAAGAGCAAGAGCAAGAGGAAGGA
	AGGACCGGATTTACACGACAAGACCTCCGCCACCGTGATCTGTAGGAAGAACGCGTCGATCTCGGTCAGGGCCCAGGA
	CAGGTATTACAGCAGCTGGAGCGAGTGGGCGAGCGTGCCTTGCTCGGGGGCGGCGGCAGCAGAAACTCG
	CCCGTGGCCACCCCAGACCCCGGAATGTTCCCCTGCCTGCACCATTCGCCAGAACCTCCTGAGGGCCGTGAGCAACATGC
	TGCAGAAGTGCAAGCCGGCAGACGCTGGAGTTCTACCCCTGCACGAGCGAGGAGATCGACCACGAAGACATCACCAAGGACAA
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	ATCACCAACGGCAGCTGCCTGGCCAGCCGAAAGACCTCGTTCATGATGGCCCTGTGCCTGAGCAGCATCTATGAGGATC
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	GAATATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTCAACAGCGAGACCGTGCCCCAGAAGTCCAGCCTG
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	TGAGTGGGCGGC

hIL12AB_026 (SEQ ID NO: 80)

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hIL12AB_027 (SEQ ID NO: 81)	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGTCACCAGCAGTTGGTGATCAGCTGGTTTCCTTGGTGTTCCTGGCCAGCCCCCTGGTCGCC ATCTGGGAGCTCAAGAAGGACGTCTACGTCGTGGAGCTGGATTGGTACCCCGACGCTCCCGGTGAGATGGTGGTGCTGA CCTGCGACACCCCTGAGGAGGATGGCATCACCTGGACCTGGACCAGAGCTCAGAGGTGTCTGGGAAGCGGAAAGACACT GACCATCCAGGTGAAGGAGTTCGGGGATGCCGGCCAGTATACCTGCCACAAGGGCGGCGAAGTGCTGAGCCATTCCCTG CTGCTGCTGCACAAGAAGGAGGACGGCATATGGTCCACCGACATCCTGAAGGATCAGAAGGAGCCCGGAAGAATAAAACCT TCCTGAGAATTACAAGAGCGCCAATTCACCTGCTTGGTTGGCTGTGACCACATCAGCACCGACCTTGACCTTGACCTT CAGTGTGAAGTCCTCACCGGGCAGCTCAGATCCCCAGGGCGTGACCTGCGGGGCCGCGACACTCAGCGCCGAGCGGGTG AGGGGTGATAACAAGGAGTACTATTCTGTGGAGTGCCAGGAAGACTCTGCCGCCGTCCCGGCGCGGAGGAGTCCCTGC CCATCGAGGTGATGGTGGACGCCGTGCATAAACTGAAGTACGAGAACTACACCTCCAGCTTCTTCATCCGGGATATAATC GAAGCCGAGCCTCTGCGAAAAACTGCAGCTGAAGCCCCTTATAAAACAGCGCGGCAGTGTGGAGGTGAGCTGGGAGTACCCC GACACCTGGAGCACCCCCATAGCTATTTCAGCCTGACCTTCTGCGTGCAGGTGCAAGTCCAAGCGCGAGAAAA AGGACCGGGTGTTCACCGACAAGACGGCCACCGTGATCTGCCGGAAGAACGCCAGTATAAGCGTAAGGGCCCAGGA TAGGTACTACAGCTCCTGGAGTGGGCCTCCGTGCCCTGTTCCGGGGGGCGGTGGCAGCAGGAACCTC CCCGTGGCCAGCGCCGACCCCGGCATGTTCCCGTGCCTGCACCACTCCCCAAAACCTCCTGCGGGCCGTCAGCAACATGC TGCAAAAGGCGCGGCAGACCCTGGAGTTTTACCCCTGTACCTCGAAGAGATCGACCACGAGGATATCACCAAGGATAA GACCTCCACGTGGAGGCCTGTCTCCCCCTGGAGCTGACCAAGAACGAGTCTGTCTTAACAGCAGAGACCTCGTTC ATAACGAACGGCTCCTGCCTCGCTTCCAGGAAGACGTCGTTCAGGATGGGCGTGTCCTGTCCAGCATCTACGAGGACC TGAAGATGTATCAGGTCGAGTTCAAAACCATGAACGCCAAGCTGCTGATGGACCCAGAAGGCAGATCTTCCTGGACCA GAACATGCTCGCCGTGATCGACGAGCTGATGCAGTCCTTGAACTTCAACAGCGAAACCGTGCCCCAGAAGTCAACCCTG GAGGAGCCGGAACTTCTATAAGACCAAGATCAAGCTGTGTATCCTGCTACAGGCTTTTCGTATCCGGGCCGTGACCATCG ACAGGGTTATGTCGTACTTTGAACGCCAGCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_028 (SEQ ID	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGTCACCAACAGCTTGGTGATCAGCTGGTTTCAGCTTGGTGTTCCTGGCCAGCCCCCTGGTCGCC ATCTGGGAGCTCAAGAAGGACGTGTACGTCGTGGAGCTGGATTGGTACCCCGACGCTCCCGGCGAGATGGTGGTCCTGA

(continued)

NO: 82)	CCTGCGACAGCGCCGGAAGAGGACGGCATCACCTGGACCTGGATCAGTCCAGCGAGGTGCTGGGCTCCGGCAAGACCCT GACCATTCAGGTGAAGGAGTTCGGCGACGCCGGTCAGTACACCTGCCACAAGGGCGGCGAGGTGCTGAGCCACAGCCTA CTGCTCCTGCACAAAAAGGAGGATGGAATCGTGGTCCACCGACATCCTCAAGGACCAGAAGGAGCCGAAGAACAAGACCT TCCTCCGGTGCGAGCCCAAGAACTACAGCGGCAGGTTTACCTGCTGGTGGCTGACCACCATCAGCAACCTGACCTGACATT TTCCGTGAAAGCAGCCGCGGCAGCGATCCCCAGGGCGTGACCTGCGGGGCCACCCTGTCCGCCGAGCGCGTGTG AGGGGCCACAACAAGGAGTACAGCGTGGAATGCCAGGAGGACAGCGCCTGTCCCGCCGCCGAGGAGAGCCTGC CAATCGAGGTCATGGTGGACGCCGTGCACAAGCTGAAGTACGAGAACTACATCGACAAGCTTCTTCATCAGGGACATCAT CAAACCGGACATCCGCCCAAGAACCTGCAGCTGAAAGACGGCAAGAGCAGCGTGAAAGTGTCTTTGGAGTACCCC GACACCTGGTCCACCCCCCACAGCTACTTTAGCCTGACCTTCTGTGTGCAGGTCCAGGGCAAGTCCAAGAGGGAGAAGA AGGACAGGGTGTTCACCGACAAACAGGCGCCACCGTGATCTGCATCGAGAATGACGGCTCCATCAGCGTGCGGGCCAGGA CAGCTATTACAGCTCTCTGTGGAGGAGTCGGACGTGCCCTGATCCTGGTGGCGGCGGCGAAGCCGGAAGCCTTA CCCGTGGCCATCCCCGATCCGGCATGTTCCCGTGTCTTGCACCACAGCCAGAACCTGCTGCGGCCGTGAGCAACATGC TGCAGAAGGCCGCCAAACCCTGGAGTTCTACCCCTGTACAAGCGAGGAGATCGACCATGAGGACAATTACCAAGGACAA GACCAGCACCGTGGAGGCCTGCCTGCCCCTCGAGCTCACAAAGAACGAATCCTGCCTGAATAGCCGCGAGACCAGCTTT ATCAGGAACGGGGTCCTGCCTCGCCAGCCGGAAGACAAGCTTCATGATGGCCCCTGTGCCTGAGCAGCATCTACGAGGACC TGAAAATGTACCAAGTGGAGTTCAAAAACGATGAAGCCAAGCTGCTGATGGAACCCCAAGCGCCAGAATCTTCCTGGACCA GAACATGCCTGGCCCATCGACGCTCATGCAGGCCCTGAACTTCAACAACGAGCACCGTGCCCCAGAAGAGCACCTG GAGGAGCCCGACTTCTACAAGACGAAGATCAAGCTCTGCATCCTGCTGCCATCCGCGCGTGACCATCG ACCGGGTGATGAGGCTACCTCAACGCCATCGCTTGATAAIAGGCTGGAGCCTTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCCAGCCCCTCCTCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_...029 (SEQ ID NO: 83)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAACAGCTTGGTGATCAGCTTGGTTCAGCCTGGGTGTTTCTGGCCTCCCCTCTGGTGCCC ATCTGGAGCTGAAGAAGGACGTGTACGTGGTGGAGCTGGATGGTGACCCTGGACGCCCCGGGCGAATGGTGGTGCTGA CGTGCGACACCCCCGAGGAGGATGGCATCACCTGGACCCTGGACCAAAGCAGCGAGGTCCTCGGAAGCGCGCAAGACCCT CACTATCCAAGTGAAGGAGTTCGGGGATGCGGGCCAGTACACCTGCCACAAGGGCGGCGAGGTGCTGTCTCATAGCCTG CTGCTCCTGCATAAGAAGGAAGACGGCATCTGGAGCACCGACATACTGAAGGACCAGAAGGAGCCCAAGAACAAGACCT TCCTGAGGTGCGAGGCCAAGAACTACTCGGGCGCTTCACCTGTTGGTGGACGACATCTCCGACCTGACCTGAACTT CAGCGTGAAGAGCAGCAGCGAGGGGATGACCCCCAGGGGGTGACCTGCGAGCCGCGACTTGTGGGCCGAGCGCGTG AGGGGCGAGAAGTACGAGTACTCGGTCGAATGCCAGGAGGACTCCGCCCCGCCGCGAGGAGTCCCTGCC CCATCGAAGTGATGGTGGACGCCGTCCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCAGGGATATCAT CAAGCCCGACCCCCCCAAGAACCTGCAGCTGAAGCCCCTTGAAGAACTCCAGGTCGAGTTCTCGTGGGAGTACCCC GACACCTGGTCCACCCCGCACTCATACTTCAGCCTGACCTTCTGTGTACAGGTCCAGGGCGAGAGCAAGAGGGAAAAGA AGGATAGGGTGTTCACCGACAAGACCTCCGCCACGGTGATCTGTCGAAAAAACGCCAGCATCTCCGTGCGGGCCCAGGA CAGGTACTATTCCAGCAGCTGGAGCGAGTGGGCCTCCGTCCCCTGCTCCGGCGGTGGCGGGGCAGCAGGAACCTC CCCGTGGCCACCCCCGATCCCGGGATGTTCCCATGCCTGCACCACAGCCCAAAACCTGCTGAGGGCCGTCTCCAATATGC TGCAGAAGGCGAGGCAGACCCTGGAGTTCTACCCCTGTACCTCGAGGAGATCGACCACGAGGATCACCAAGGACAA GACCTCCACGGTGGAGGCGTGCCTGCCCCTGGAGCTCACAAGAACGAGTGCCTTAACTCCAGGGAAACCTCGTTT ATCACGAACGGCAGCTGCCTGGCGTCACGGAAGACCTCCTTTAGATGGCCCTATGTCTGTCCTCGATCTACGAGGACC TGAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCCAAGCTGCTGATGGATCCCAAGAGGCAGATTTCCTGGACCA GAACATGCTGGCCGTGATTGACGAGCTGATGCAGGCGCTGAACTTCAACAGCGAGACAGTGCCGCAGAAGAGCTCCCTG GAGGAGCCGGACTTTTACAAGACCAAGATAAAGCTGTGCATCCTGCTCCACGCCTTCAGAATACGGGCCGTCACCATCG ATAGGGTGATGTCTTACCTGAACGCCTCCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_030 (SEQ ID NO: 84)	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACAGCAGCTGGTGATTAGCTGGTTTAGCCTGGGTGTCCTGGCAAGCCCCCTGGTGGCC ATCTGGGAACTGAAAAAGGATGTGTACGTTGTCGAGCTGGATTGGTACCCTGATGCCCCTGGCGAAATGGTGGTCCTCA CGTGTGATACCCCTGAGGAGGATGACATCACCTGGACCCTGGATCAGAGCAGTCGTGAGGAGCGGGAGAAGAACCGT GACGATCCAGGTCAAGGAGTTCGGCGACGCTGGGCAGTACACCTGTCACAAGGGCGGTGAGGTGCTGTCCCACTCCCTG CTGCTCCTGCATAAGCAAAGAGGACGGCATCTGGTCCACCGACATCCTCAAGGACCAGAAGGAGCCCAAGAACAAGACCT TCCTGCGCTGTGAGGCCAGCAACTACAGCGGCCGTTTCACCTGCTGGTGGCTGACGACAATCAGCACCGACTTGACGTT CTCCGTGAAGTCCTCCAGAGGCAGCTCCGACCCCCAAGGGGTGACGTGCGTGCCACACCTGAGCGCCGAGCGGGATG CGGGGGGACAACGAGTACGAGTACTCCGTGGAGTGCCAGGAGGACAGCGCCTGTCCCGCAGCCGAGGAGTCCCTGC CCATCGAAGTCATGGTGGACGCCGTCCACAAGCTGAAGTACGAGAACTACACCAGCAGCTTCTTCATCCGCGATATCAT CAAGCCCGATCCCCCAAAAACTTGCAACTGAAGCCGCTGAAGAATAGCAGAGGTCGACGGTATCGATAAGCTT GACACCTTGAAGCCAGCCCCACAGCTAATTCAGCCTTGACGTGCAGTCCAAGGGGAAGAGCAAGCGGGAGAAGA AGGACCGCGTGTTTACCGACAAAACCAGCGCCACCGTGATCTGCAGGAAGAACGCCAGCATCAGCGTGAGGGCCAGA CAGGTACTACAGCAGCTGCTGAGCGAGTGGGCCTCCGTGTTCCGGAGCGGGGCGTCCCGGAACCTC CCGCTGGCCACCCCGACCCGGGCATGTTCCCGTGCCTGCACCACTCACAGAAATCTGCTGAGGCCGTGAGCAATATGC TGCAGAAGCGAGACCCTGGAGTTTTATCCCTGCACCAGCGAGGATCGACACGAAGACATCACCAAGGACAA GACCAGCACAGTGGAGGCCTGCTGCCCCTGGAACTGAACAAGAACGAGTCCTGTCTGAACTCCAGCTTC ATAACCAAGGGCTCCTGTCTGCCAGCAGGACCAGCTTCATGATGGCCCTGTGTCTCAGCCTCCATCTACGAGGACC TCAAGATGTACCAGGTTGAGTTCAAGACCATGAACGCCAAGCTCCTGATGGACCCCAAGAGGCAGATCTTCCTAGACCA GAATATGCTGGCGTGATCGATGAGTTAATGCAGGCGCTGAACTTCAACAGCGAGACGGTGCCCCAAAAGTCCTCGCTG GAGGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTCCTGCAGGAATCCGGGCCGTAACCATCG ACAGGGTGATGAGCTATCTCAACGCCTCCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_031 (SEQ ID NO: 85)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGTTGTTGATCATCTGGTTTCTTGTGTTTCCTGGCCTCCCCCTCGTCGCC ATCTGGGAGCTGAAGAAAGACGTGTACGTTGTGGAGCTGGATTGGTATCCCGACGCCCCGGGGGAGATGGTGGTCCTGA CCTGCGACACCCCGAAGAAGATGGCATCACCTGGACCTCGATCGTCCTGGAAGTTCTGGGGTCGGGCAAGACCCT CACCATCCAGGTGAAGGAGTTCGGAGACGCCGGCCAGTACACCGTCTCATAAAGGGGGGAGGTGCTGAGCCACAGCCTC CTGCTCCTGCACAAAAAGGAGGACGGCATCTGGAGCACCGATATCCTCAAGGACCAGGAACCCCAAGAACAAGACGT TCCTGAGGTGTGAGGCCAAGAACTACAGCGGGCGGTTCACGTGGTTGGCTCACCACCATCTCCACCGACCTCACCTT CTCCGTGAAGTCAAGCAGGGGCAGCTCCGACCCCCAAGGGCGTCACCTGCGGCGCCGCCACCCTGAGCGCCGAGAGGGTC AGGCGGGATAACAACGAATACGAGTACAGTGTGGAGTGCCAAGAGGATACGCCCTGTCCCGCCGCCGAAGAGAGCCTGC CCATCGAAGTGATGGTGGACGCCGTGCACAAGCTGAAGTACGAGAACTACACCTCCAGCTTCTTCATCAGGGATATCAT CAAGCCCGATCCTCCCCAAGAACTTGCAGCTGAAGCCCCTGAAGAACAGCAGGCAGGTGGAGGTGAGCTGGGAGTACCC GACACGTGGAGCACCCCGCACAGCTACTTCTCGCTGACCTTCTGCGTGCAGGTCCAGGGCAAGAGCAAGAGAGAAAGA AGGATAGGGTGTTCACGGACAAAACGGCCGCCACGGTGATCTGCCGCAAGAATGCCAGCATCTCTGTGAGGGCCAGAA CAGGTACTATTCCAGTTCCTGGTCGGAGTGGGCCACGCGTGCCCTGTAGCGGGGGCGGGGCGCAGCAGGGAACCTC CCGCTTGCCACCCCGGACCCCGGCATGTTTCCGTGCCTCACCACTCGCAAAACCTGCTGCGCGCGGTCTCCAACATGC TGCAAAAGCGCGCAGACGCTGGAGTTCTACCCCTGCACCAGCGAGGATCGATCATGAAGATATCACCAAAGACAA GACCTCGACCGTGGAGGCCTGCCTGCCCTGGAGCTCACCAAGAACGAAAGCTGCCTGAACAGCAGGGAGACAAGCTTC ATCACCAACGGCAGCTGCCTGGCCTCCCGAAGACCAGCTTCATGATGGCCCTGTGTCTGTCCAGCATCTACGAGGATC TGAAGATGTACCAGGTGGAGTTTAAGACCATGAACGCCAAGCTGTTAATGGACCCCAAAAGGCAGATCTTCCTGGATCA GAACATGCTGGCGTCATCGGAGCTGATGCAAGCCCTGAACTTCAACAGCGAGACGGTGCCCCAGAAGAGCAGCCTC GAGGAGCCCGACTTCTATAAGACCAAGATAAAGCTGTGCATTCTGCTGCACGCCTTCAGAATCAGGGCCGTGACCATCG ATAGGGTGATGAGCTACCTGAACGCCTGAGCCAGCTGGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_ 032 (SEQ ID NO: 86)	TCAAGCTTTGGACCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAA TATAAGAGCCACCATGGTCACCAGCAGTCGTGATTCCTGGTTCAGTCGTCGACCTCCTGGCCATCGTTCTCGCC ATCTGCCAGTTGAAGAAAGACGTATACGTCGTGGAGCTGGACTGGTATCCGGACGCCCCGGGCGAGATGGTGGTCCTCA CCTGCGACACCCCAGAGGAGGACGGCATCACCTGGACCCAGAGCTCCGAGGAGTCCTGGGCAGCGGTAAGACCCT CACCATCCAGGTGAAGGAGTTTGGTGATGCCGGGCAGTATACCTGTCACAAGGGCGGCGAGGTGCTGTCCCACAGCCTC CTGTTACTGCATAAGAAGGAGGATGGCATCTGGAGCACCGAGATTCTTAAGAACCAGAAGAGCCCAAGAACAGACT TTCTGCGGTAGAGCGGAAAATTACTCTGGCCGGTTCACCTGCTGGTGGACCACATCAGCACGGACCTGACGTT CTCCGTGAAGTCGAGCAAGGGGAGCTCCGAATGCCCCAGGGCGTGACCTGCGGCGCCGAGTCCGCTCTGTCCGCC CGCGAGGACAATAAGCGAATACGCAGTGGCAGTGCCACAGCGACAGCGCCTGCCCCGCCGCGAGGAGAGCCTCC CGATCGAGGTGATGGTCGATGCCGTCCACAAGCTCAAATACGAAAACTACACCAGCAGCTTCTTCATTAGGGACATCAT CAAGCCCGACCCCCCAAAAACTGCAGCTGAAGCCCCTGAAGAACAGCGGCCACAGTGTCGAGTGGGAGTACCCA GACACCTGGAGCACCCCCACTCCTACTTCAGCCTGACCTTCTGCGTCCAGGTGCAGGGCAAAAGTCCAAAGAAGA 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CCCCCCGACGTGGACATCCACGACAAGCTGTACATGTACCTGGTGGAGTACGGCAAGGAGGACGTGCACGCCGTG GAGATCGGCGTGTACGGCAAGATCGCCAGCGACCCCACCAAGCAGCCCCTGTACCTGAAGGCCCTGGACAACCAG CGCAGCGAGACCTTCAAGGTGAACATGGAGTACGCCAACTTCGTGCAGATGATCCAGAGCGACTTCGCCCTGGAC GACCTGTCCCTGGACTACATCCACAGCGAGGGCGTGAAGAACTACATCGACTGGTGCTGGAGCGTGGTGAAGAAG CAGTTCGCCGACTTCCTGACCGAGTACCAGCCCCTGGACAAGCTGCAGAACCTGATCATGAAGTTCGGCGACTAC GCCCCCCTGGTGCTGCCCCTGATCGAGATGAAGGCCGACGAGGCCTACCTGCCCCAGTTCAGCGCCGTGTGGGTG TTCCTGGTGAAGAAGCAGGAGGACGGCAAGAGCACCTACTACGCCAGCCCCTTCTGCCTGATCCAGGACGACAGC CTGGAGTTCGTGATCAAGCCCGACGTGTTCGGCAAGGTGGTGGAGGTGAAGAAGGGCACCGAGCAGCTGCTGGGC GTGATCGTGCAGGAGGACGTGGCCAGCCTGGACGAGAACAAGCAGTTCAACGGCACCGTGTGCGTGCTGCCCCTG GAGTACATGGACAAGAACATCGAGCACAGCCCCAACTTCACCGCCAAGTGCAGCCCCATCCCCGTGAGCCCCGAG TTCGTGGTGCCCGACCTGAACCTGGGCGTGTACAAGATCCTGGTGCCCGCCACCTTCCTGCACCAGGAGGACGAG TACGACAAGCTGTGCTTCAGCCCCACCAGCAGCTTCAGCTACCAGAGCGCCAACCTGAGCGTGAGCACCGTGAAG TACAACCAGTGGGAGGAGTTCACCCGCCAGAGCCCCAAGGAGATCAGCAAGAGCGCCGTGGCCTACTTCACCATC 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(continued)

	TGCAGAAGCCCGGCAGACGCTGGAGTTCTACCCCTGCACCTCGGAGCAGATCGACCATGAGGACATACCAAGGACAA GACGAGCACTGTGGAGGCCTGCCTGCCCCGTGGAGCTCACCAAAAACGAGAGCTGCCTGAATAGCAGGGAGACGTCCTTC ATCACCAACGGCAGCTGTGTGGCCAGCAGGAAGACCAGCTTCATGATGGCCCTGTGCCTCTCCTCATATATGAGATC TGAAGATGTACCAGGTTGGAGTTCAAGACCATGAACGCCAAGCTGCTGATGGATCCCAAGAGGCAGATCTTCCTGGACCA GAATATGCTGCCGTGATTGACGAGCTGATGCAGGCCCTGAACTTTAATAGCGAGACCGTCCCCCAGAAGAGCAGCCTG GAGGAGCCCGACTTCTATAAGACCAAGATCAAGCTGTGCATACTGCTGCATGAGATAAGGCCGTCACCATCG ACACCGTGATGACCTACCTGAATGCCAGCCTGGAGCCTCGGTGGCCATGCCTACTCTTGCCCCTTGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 034 (SEQ ID NO: 88)	TCAAGCTTTTGGAGCCTTTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAAGAAGTAAGAAGAAA TATAAGAGCCACCATGGTGCCACCAACAGCTGGTGATCTCCTTGGTTCAGCCTGGTGTTGCCTGCCACGCCCCCTGGTGCC ATCTGGGAGCTGAAGAAAGACGTGTACGTGGTGGAGCTGGATTGGTATCCCGACGCCCCCGGCGAGATGGTCGTGCTGA CCTGCGACACCCCCGAGGAGGACGGCATCACCTGGACCCTGGACCAGTCCTCCGAGGTGCTGGGCAGCGGCGAAACCCT GACCATCCAGGTGAAAGAGTTCGGCGACGCTGGCCAGTATACCTGTCACAAGGGCGGCGAGGTGCTGAGCCATAGCCTC CTGCTGCTGCACAAGAAGGAGGACGGCATCTGGTCCACCGACATCCTGAAGGACCAGAAGGAGCCCAAGAACAAGACC TTCCTGAGGTGCGAGGCCAAGAACTATTCCGGCAGGTTCACCTGCTGGTGGCTGACCACCATCAGCACCGACCTGACCTT CTCCGTGAAAAGTCAAGCAGGGGAGCTCCGGACCCCCAGGGGCGTGACCTGCGGAGCCGCCGACCAGGACGCCGTG AGGGGCGACAACAAGGAGTACGAATACTCCGTGGAGTGCCAGGAGGACAGCGCCTGCCCCGCCGCCGAGGAAGAGCTGC CCATCGAGGTGATGGTGGACGCCGTGCACAAGCTCAAATACGAGAACTACACCAGCAGCTTCTTCATCCGGGATATCAT CAAGCCCGACCCCCCAAGAACTTGCAGCTGAAGCCCCTTAAGAACAGCCGCCAGGTGGAGGTCAGCTGGGAGTACCCC GACACCTGGAGCACCCCCACTCCTACTTCTCCCTGACCTTCTGCGTGCAGGTGCAGGGCAAGAGCAAGAGGGAGAAGA AGGACAGGGTGTTCACCGATAAGACCTCCGCCACCGTGATCTGCAGGAACGCCTCCATCAGCGTGAGGGCCCAGGA TCGGTACTACTCCAGCTCCTGGAGCGAGTGGGCCAGCGTGCCCTGCTGGCGGTGGCGGGCAGCCGGAACCTG CCGGTGCCAACTCCCGACCCGGCATGTTCCCCTGCCTCCACCATTCCCAGAACCTGCTGCGGCCGTGTCCAATATGC TCCAGAAGCCAGCCAGACCCTGGAGTTCTACCCCTGCACCAGCGAGGAGATCGACCATGAGGACATCACCAAGGACAAA AACCAGCACGGCTGCCTGCCCCTGGAACTCACCAAGAACGAGAGCTGTCTCAACAGCCGCGAGACCAGCTTC ATAACCAACGGCTCCTGTCTGGCCTCCCAGCAAGACCAGCTTCATGATGGCCCTCTGCCTGAGCTCCATCTATGAGGACC TGAAAATGTACCAGGTTGAGTTCAAAACCATGAACGCCAAGCTTCTGATGGACCCCAAGAGGCAGATCTTCCTGGATCA GAACATGCTGGCCGTGATCGACGAGCTGATGCAGGCCCTGAACTTTAACTCCGAGACCGTGCCCCAGAAAAGCAGCCTG GAAGAGCCCGATTTCTACAAAACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCCGGATCCGTGCCGTGACCATCG ATAGGGTGATGAGCTACCTGAACGCCAGCCTGGAGCCTCGGTGGCCATGCCTACTCTTGCCCCTTGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

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hIL12AB_...035 (SEQ ID NO: 89)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAACAGCTTGGTTAATCAGCTTCTCAGCCCTGGTTTCCTCGCGTCGCCCTGGTGGCC ATCTGGCAGTTAAAGAAGGACGTGTACGTGGTGGAGCTGGATTGGTACCCCGACGCCCCGGGCGAGATGGTCGTGCTCA CCTGCGATACCCCCGAGGAGGACGGGATCACCTGGACCCTGGACCAATCAAGCGAGGTGCTGGGCAGCGGCAAGACCCT GACCATACAGGTGAAGGAATTTGGGGACGCCGGCAGTACACCTGCCACAAGGGCGGGGAAGTGCTGTCCCACTCCCTC CTGCTGCTTCATAAGAAGGAGGACGGCATCTGGAGCACCGAGATCCTCAAGAACGAGAGCTTCAACAAGACCGTT TCCTTCAGCAATGCGAAGGCCTTTACCTGTTTTGGTCTGAACCATCTGCCACCGATCTTCGACTTCTGACCTT CAGCGTGAAGTCGTCTAGGGGCTCCTCCGACCCCCAGGGCGTAACCTGCGCGCCGACCTGAGCGCCGAGAGGTG CGGGGCGATAACAAAGAGTACGAGTGGCCAGAGGAGCACAGCGCCTGTCCGGCGAGGAGAGCCTGC CCATCGAGGTGATGGTGGACGCCGTCCACAAGCTGAAGTACGAGAACTACACCAGTTCGTTCTTCATCAGGGACATCAT CAAGCCGACCTCCCCAAGAACTCCAGCTGAAGAACAGCAGCAGCAGGTGGAAGTGTCCTGGGAGTATCCC GACACCTGGAGCACCCCCACAGCTACTTCAGCCTGAACGTTTGCGTGCAGGTGCAAGAAGCAAGAGGAAAAGA AGGACCGGGTGTTCACCGATAAGACGAGCGCCACCGTTATCTGCAGGAAGAACGCCTCCATAAGCGTGAGGGCGCAGA CCGTTACTACAGCAGCAGCTGGAGTGAGTGGGCAAGCGTGCCCTGTAGCGGGGGGGGGGGTCCCGCAACCTC CCCGTCGCCACCCGGACCCAGGCATGTTCCGTGCCTGCACCACAGCCAGAAACCTGCTGACGGGCCGTTAGCAACATGC TGCAGAAGGCCAGGCAGACCCTCGAGTTCTATCCCTGCACATCTGGAGCAGATCGACCACGAAGACATCACTAAGGATAA GACCTCCACCGTTGACGAGGCCGTGTCTGGCCCCTCGAGCTGAACAATGAATCCTGCCTGAACAGCCGAGAGACCAGCTTT ATCACCAACGGCTCCTTCCTGGCCAGCAGCAAGACCTCCTTCATGATGGCCCTGTGTCTCTCCAGCATCTACGAGGATC TGAAGATGTACCAGGTTGAGTTCAAGACGATGAACGCCACAGCTCCTTGATGGAACCCCAAGAGGCAGATATTCCTGCACCA GAACATGCTGCGGTGATCGACGAGCTGATCAAGATTGCATCCTCCTGCACAGCGTTCAAGAATCCGGCCGTCACCATAG GAGGAGCCAGAACTTCTACAAGATCAAACTGTGCATCCTCCTGCACAGCGTTCAAGAATCCGGCCGTCACCATAG ACAGGGTGATGAGTTATCTGAACGCCAGCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_...036 (SEQ ID NO: 90)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCATCAGCAGCTGGTTAATCAGCTTCTTAGCCTTGGTTTCCTCGCGTCGCCCTGGTGGCC ATCTGGCAGTTAAAGAAGACGTGTACGTGGTGGAGCTGGATTGGTACCCCGACGCCCCGGGCGAGATGGTCGTGCTCA CCTGTGACACCCCGGAGGAGGACGGCATCACCTGGACCCTGGACCAGTCAAGCGAGGTGCTGGGCAGCGGCAAGACCCT GACCATCCAAGTTAAGGAATTTGGGGACGCCGGCCAGTACACCTGCCACAAGGGCGGCGAAGTGCTGTCCCACTCCCTG CTGCTTCATAAGAAGGAGGATGGCATCTGGAGCACCGACATCCTCAAGAACCAGAAGGAGTTCATCAACAAGACCGTT TCCTTCAGCAATGCGAAGGCCTTTACCTGTTTTGGTCTGAACCATCTGCCACCGATCTTCGACTTCTGACCTT CGGGGCGACAACAAGGAATACGAGTACAGCGTGGAGTGCCAGAGGAGCTCCCCCTGCCCCGCCGAGGAGTCGCTGC

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	CCATAGAGGTGATCGTGGACCGTGCACAAGCTCAAGTACGAGAATTACACCAGCAGCTTCTTTATCAGGGACATAAT TAAGCCGGACCCCCCAAAGAATCTGCAGCTGAAGCCCCTGAAGAATAGCCCAGGTGGAAGTGTCCTGGGAGTACCCC GACACCTGGAGCACCCCCCACTCCTATTTCTCACTGACATTCTGCGTGCAGGTGCAAGGGAAAGCAAGAGGAGGAGAA AGGATAGGGTGTTCACCGACAAGACCGCCACCGTGATCTGCCGCAAAAAATGCCAGCATCAGCGTGAGGGCCCAGGA TCGGTATTACAGCAGCTCCTGGAGCGAGTGGGCCAGCGTGCCTTGTTCCGGGTGAGGGGGCGCTCCCGGAACCTG CCGGTGGCCACCCCGACCCTGGCATGTTCCCTGCCTCACAGCCAGAACCTGCTCCGGGCCGTGTCGAACATGC TGCAGAAGTGCCGCAGACCCTCGAGTTTACCCCTGCACCAGCGAGATCGACTCCTGCCTGAACACAAGGACAA GACCAGCACGGTGGAGGCCTGCTGCCCTGGAGCTTACCAAAAACGAGTCCTGCCTGAACAGCCGGGAAACCAGCTTC ATAACGAACGGGAGCTGCCTGGCCTTCCAGGAAGACCAGCTTCATGATGGCGCTGTGTCTGTCCAGCATATACGAGGATC TGAAGATGTATCAGGTGGAATTCAAAACTATGAATGCCAAGCTCCTGATGGAACCCCAAGAGGCAGATCTTCCTGGACCA GAACATGCTAGCCCTGTGATCGACGAGCTGATGCAGGCCCTCAACTTCAACTCGGAGACGGTGCCCCAGAAGTCCAGCCTC GAGGAGCCCGAACTTCTACAAGACCAAGATCAAGCTGTGCATACTGCTGCATGCTTCAGGATAAGGGCGGTGACTATCG ACAGGGTCATGTCCTACCTGAACGCCAGCTGATAATAGGCTGGAGCCTCGGTGGCATGCTTCTTGCCCCTTGGGCCTC CCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_037 (SEQ ID NO: 91)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGCAGGCCACATGTTGCCACCAACACAGCTGATCAGCTGCTTTCTCCTTGGTGTTCCTGGCCAGCCCCCTGTCGTCA ATCTGGGAGCTCAAAAAGGACGTGTACGTGGTGGAGCTGGATTGGTACCCGGACGCCCCGGGCGAATGGTCGTGCTGA CCTGCGACACCCCAGAGGAGATGGCAICACGTTGGACGGCTGGAATCCATCCAGTCCGGTCAGGTGCTGAGCCACACTC CACCATCCAGGTGAAGGAGTTCGCCGACGCCGGCCAGATATACGTCTACAAGGGCGAGCTGCGTGAGCCACTCCCTG CTGCTGCTGCACAAGAAGGAGGATGGGATCTGGTCCAACGATATCCTGAAAGACCAGAAGGAGCCCCAAGAACAAGACCT TCCTGCGCTGCGAGGCCAAGAACTATAGCGGCAGGTTCACCTGCTGGTGGCTGACCACCATCAGCACCGACCTGACCTT CAGCGTGAAATCCTCCAGAGGGCAGCAGCGACCCCCAGGGCGTGACCTGCGTGCCGCCACGCTCTCCGCCGAGCGCGAGTG AGGGGTGACAACAAGGAGTACGAGTACAGCGTGGAATGTCAGGAGGACAGCGCCTGCCCGCCGCTCGAGGAGTCGCTGC CCATCGAGGTGATGGTCGACGCCGTGCACAAGCTCAAATACGAGAATTACACCAGCAGCTTCTTCATCAGGGACATCAT CAAGCCCGACCCCCCCAAGAACTGCAGCTGAAGCCCCTGAAGAACAGCCAGGTGGAGGTGAGCTGGGAGTACCCCG GACACCTGGAGCACCCCCACTCCTACTTCAGCCTGACATTCTGCGTGCAGGTGCAAGGGAAGTCCAAGAGGAAGAGA AGGACCGGGTGTTCACCGACAAGACCGCCACCGTGATCTGCCGCAAGAACGCCTCCATCAGCGTGCGGGCCCAGGA CCGCTACTACAGCAGCTCCTGGTCCGAATGGGCCAGCGTGCCTTGTTCCGGGTGAGGGGGCGCTCCCAGGAACCTG CCGGTGGCCACCCCGACCCCGGGATGTTCCCCTGCCTCACACAGCCAGAACCTGCTGCGGGCCGTGTCGAACATGC TGCAGAAGTGCCGCAGACGCTCGAGTTTACCCCTGCACCTCCGAAGATCGACTCCTGCCTGAACACAAGGACAA GACCAGCACCGTGGAGGCCTGCTGCCCTGGAGCTGACCAAAAACGAGTCCTGCCTGAACAGCCGGGAAACCAGCTTT ATAACCAACGGCAGCTGCCTGGCCTTCCAGGAAGACCTCGTTATATGATGGCGCTCTGTCCGTCCAGCATCTACGAGGACC TGAAGATGTACCAGGTGGAGTTCAAGAACATGAACGCGAAGTTGCTCATGGACCCCAAGAGGCAGATCTTCCTGGACCA GAACATGCTGGCGGTGATCGACGAGCTGATGCAAGCCCTGAACTTCAACAGCGAGACCGTGCCCCAGAAGAGCAGCCTG GAAGAGCCCGACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGCTGCACGCCTTCCGGATCCGGGCCGTGACCATCG ACAGGGTGATGAGCTACCTGAACGCCTCCATCGGTGAGCCTCCGGTGGCATGCTTCTTGCCCCTTGGGCCTC CCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_ 038 (SEQ ID NO: 92)	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTCGTGATCATCTGGTTTCTTAGTTTCCTGGCCTCCCCGCTGGTGGCC ATCTGGGAGCTGAAGAAGGACGTGTACGTGGTGGAGCTGGATTGGTATCCGGACGCCCCGGGCGAGATGGTGGTGCTGA CGTGCGACACACCAGAAGAGGATGGGATGACATGGACCCTGGATCAGTCGTCCGAGGTGCTGGGGAGCGGCGCAAGACCCT CACCATCCAAGTGAAGGAGTTCGGGGACGCCGGCCAGTACACCTGCCACAAGGGCGGGGAGGTGCTGTCCCATAGCCTG CTCCTCTGCACAAAAAGGAGGATGGCATCTGGAGCACCGACATCCTGAAAGACCAGAAGGAGCCCAAGAACAAGACCT TTCTCAGAGTGTGAGGCCAAGAACTATTCGGGCAGGTTTACCTGTTGGTGGCCTCACCACCATCTCTACCGACCTGGACT GTCCGTCAAGTCAAGCAGGGGAGCTCGGGACCCCCAGGGGGTGACATGTGGGGCCGCCACCCTGAGCGCGGAGCGTGTC CGCGGCGACAACAAGGAGTACGAGTATTCCGTGGAGTGCCAGGAGGACAGCGCCTGCCCCGCCGCCGAGGAGTCCCTGC CCATAGAGGTGATGGTGGACGCCGTCCACAAGTTGAAGTACGAAAATTATACCTCCAGCTTCTTCATCAGGGACATCAT CAAGCCTGACCCCCCGAAGAACTTACAACTCAAGCCCCTCAAGAACTCCCAGGTGGAGGTGTCCTGGGAGTACCCC GACACCTGGTCCACCCCGCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTCCAGGGGAAGAGCAAGCGTGAAAAGA AAGACAGGGTGTTCACCGACAAGACGGCCACCGTGATCTGCAGGAAAAACGCCTCCATCTCCGTGCGCGCCCAGGA CAGGTACTACAGTAGCTCCTGGAGCGAATGGGCCAGCGTGCCGTGCAGCGGGGAGGAGGCGGCAGTCAGCAATCTG CCCGTGGCCACCCCGACCCCGGCATGTTCCCATGCCTGCACCACAGCCAGAACCTGCTGAGGGCAGTCAGCAATATGC TGCAGAAGGCCAGGCAGACCCTGGAGTTTATCCCTGCACCAGCGAGGAGATCGACCACGAGGACCATCACCAAGGACAA GACCTCCACGTCGAGGCCTGGCCTGCCACCTGGAGCTGACCTGCCTGAACTCCAGGGAGACCTCCTTC ATCACCAACGGGAGCTGCCTGGCCAGCCGGAAGACCAGCTTCATGATGGCCCTGTGCCTCAGCAGCATCTACGAGGATC TCAAGATGTACCAGGTGGAGTTCAAGACCATGAACGCGAAGCTGCTGATGGACCCCAAGCGGCAGATCTTCCTGGACCA GAACATGCTGGCCGTGATTGACGAGCTCATGCAGGCCCTGAACTTCAATAGCGAGACCGTGCCCCAAAAGAGCCAGCCTG GAGGAACCGGACTTCTACAAAACGAAGATCAAGCTCTGCATCCTGCTGCACGCGTTCCGGATCCGGGTGACCATCG ATCGTGTGATGAGCTACCTGAACGCCTCGTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTTCCTCCTTCCTGCACCCGTACCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC
hIL12AB_ 039 (SEQ ID	TCAAGCTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAATAAGAGAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCACCAGCAGCTCGTGATCATCTGGTTTCTTAGTTTCCTGGCCTCCCCGCTGGTGGCC ATCTGGGAGCTGAAGAAGGACGTGTACGTGGTGGAGCTGGATTGGTATCCGGACGCCCCGGGCGAGATGGTGGTGCTGA

(continued)

NO: 93)	CCTGCGACACCCCCGAGGAGGACGGCATCACCTGGACCTGGACCAGAGCTCCGAGGTGCTGGGGAGCGGCAAGACCCT GACCATTCAGGTGAAAGAGTTCGGCGACGCCGGCCAATATACCTGCCACAAGGGGGGGAGGTCCTGTCGCATTCCCTG CTGCTCCTTCAAAAAGGATGGCATCTGGAGCACCGACATCCTGAAGGACCAGAAGGAACCCAAGAACAAGACTTT TCCTGCGTGCGAGCCCAAGAACTACAGCGGCGGTTCACCTGTTGGTGGCTGACCACATCTCCACCGACCTGACTTT CTCGGTGAAGCAGCCGGGGAGCAGCCCCAGGGAGTGACCTGCGGCGCCACACCCTGAGCGCCGAAAGGGTG AGGGGCCACAATAAAGAGTACGAGTATTCCGTGGAGTGCCAGGAGGACAGCGCCTGTGCCGCCGAGGAGTCCCTGC CTATCGAGGTGATGGTCGACGCGGTGCACAAGCTCAAGTACGAAAACTACACCAGCAGCTTTTTCATCAGGGATATCAT CAAACCAGACCCCCCAAGAACCTGCAGCTGAAGCCCCTGAAAAACAGCAGGCAGGTGGAAGTGAGCTGGGAATACCCC GATACCTGGTCCACCCCCCACAGCTACTTCAGCCTGACCTTCTGCGTGCAGGTGCAGGGGAAGTCCAAGCGGGAGAGA AAGATCGGGTGTTCACCGACAAGACCAGCGCCACCGTGATCTGCAGGAAAAACGCCAGCATCTCCGTGAGGGCTCAGGA CAGCTACTACAGCTCCAGCTGGAGCTGCGGAGCGTGCAGCTTCGCCGGCAAGGGCGCCGGCAAGGCAGCAGGAAZCTG CCCGTCGCAACCCCGACCCCGGCATGTTCCCCTGCCTGCACCACAGCCAGAATCTGCTGCGAGCCGTGAGCAACATGC TCCAGAAGGCCGGCAGCTGGAGTTCTACCCCTGCACCTCCGAGGAGATCGACCACGAGGACATCACCAAGGATAAA GACGAGCACCGCTCCAGGCCTGTCTCCCCCTGGAGCTCACCAAGAACGAGTCCTGCCTGAATAGCAGGGAGACGTCCTTC ATAACCAAGGGCAGCTGTCTGGCGTCCAGGAGACCAGCTTCATGGCCCCTCTGCCTGAGCTCCATCTACGAGGACC TCAAGATGTACCAGGTCGAGTTCAAGACCATGAACGCCAAACTGCTCATGGATCCAAGAGGCAGATCTTTCTGGACCA GAACATGCTGGCCGTGATCGACTCATGCAGGCCCTGAATTTCAATTCCGACGACCGTGCCCCAGAAGAGCTCCCTG GAGGAACCGACTTCTACAAACAAGATCAAGCTGTGTATCCTGCACGCCTTCCGGATCAGGGCCGTCACCATTG ACCGGGTGATGTCCTACCTGAACGCCAGCCTGATAAIAGGCTGGAGCCTGGTGGCCATGCTTCTTGCCCCTTGGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

(continued)

hIL12AB_...040 (SEQ ID NO: 94)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAAAAGAAGAGTAAGAAGAAA TATAAGAGCCACCATGTGCCATCAGCAGCTTGTTCATCTCTTCCTCGCCATCGCCCTCGTCACCAACTCAGTCGCC ATCTGGGAGCTGAAAAAGGACGTGTACGTTGTGGAGCTGGATTGGTATCCCGACGCCCCGGGCGAGATGGTGGTCCTGA CCTGCGACACCCCCGAAGAGGACGGCATTACCTGGACCAGTGACGAGAGCAGTCCTGGGCAGCGAGGAAGACCCT GACAATTCAGAGTGAAGGAGTTCGGCGACGCCGGACAGTACACGTGCCACAAGGGGGGAGGTGCTGTCCCACAGCCTC CTCCTGCTGCACAAGAAGGAGGATGGCATCTGGAGCACCGACATCCTGAAGGATCAGAAGGAGCCCAAGAACAAGACCT TTCTGAGATGCGAGGCCAAGAATTACAGCGGCGCCGTTTCACCTGCTGGTTCACCACCATCAGCACCGACCTGACCTT CAGCGTGAAATCCTCCAGGGGCTCCTCCGACCGCGGCAGTGACCTGCGGCCGCACACTGAGCGCCGAGCGCGGTC AGAGGGCAGAACAAGCGAGTACGAGTACAGCGTTGAGTGCCAGGAGGACAGCGCCTGTCCCGCCGCCGAGGAATCCCTGC CCATCGAGGTGATGGTGGACGCCGTGCACAAGCTCAAGTACGAGAACTATACCTCCAGCTTCTTCATCCGGGATATCAT TAAGCCCGATCCCCCCAAGAACCTGCAGCTCAAGCCGCTGAAGAACAGCAGGCAGGTCGAGGTCTCCTGGGAGTACCCC GACACATGGTCCACCCCCATTCCTATTTCTCCCTGACCTTTTGCGTGCAGGTGCAGGGCAAGAGCAAGAGGGAGAAAA AGGACAGGGTGTTCACCGACAAGACCTCCGCCACCGTGATCTGCCGTAAGAACGCTAGCATCAGCGTCAGGGCCCAGGA CAGGTACTATAGCAGCTCCTGGTCCGAGTGGGCCAGCGTCCCGTGCAGCGGCGGGGCGGTGGAGGCTCCCGGAACCTC CCCGTGGCCACCCCGGACCCCGGGATGTTTCCCTGCCTGCATCACACGCCAGAACCTGCTGAGGGCCGTGTCCAACATGC TGCAGAAGGCCAGGCAGACACTCGAGTTTTACCCCTGCACCAGCGAGGAGATCGACCACGAAGACATCACCAAGGACAA GACCTCCACGTGGAGGCATGCCTGCCCCTGAAGAACGAAAGCTGTCTGAACTCCAGGGAGACCTCCTTT ATCACGAACGGCTCTAAGGAGCCTCAGAAAGACCAGCTTCATGATGGCCTTGTGCCTGAGCTCCATCTACGAGGACT TGAAAATGTACCAGGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCCAAAAGGCAGATCTTTCTGGACCA GAATATGCTTGCCGTGATCGACGAGCTCATGCAAGCCCTGAATTTCAACAGCGAGACCGTGCCCCAGAAGTCCTCCCCTG GAGGAGCCGGACTTCTACAAGACCAAGATCAAGCTGTGCATACTCCTGCACAGCGTTTAGGATCAGGGCGGTGACCATCG ATAGGGTGATGAGCTACCTGAATGCCTCCTGATAATAGCTCGAGCCTCGAGTTTCCCCTCGTTTCTTGCCCCTTGGCCTC CCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCCAAACACCATTGTCACACTCCAGTGGTCTTTGAATAAAGTC TGAGTGGGCGGC

Table 4C. mRNA Sequences (with T 100 tail)

hIL12AB_ 001 (SEQ ID NO: 95)	G*GGGAAUAAGAGAAAAGAACACGAUAGAUAAAUAUAAGAGCCACCAUGUGUCACCAGCAGUUGGUCAUUAGCUGG UUUAGCCUUGGUGUUUCUGGCCUCCCCCCUUGCCGUCUGGGAGCUCUAAGAAGGAUGUUUACGUUGUAGAACUGGACUGGUAU GGUAGCCCAGAUGCCCCUGGAGAAUGCGUCAACUGUGAUACCCCUGAAGAAGUAGACGACAUCGAUUGGAAGUCAACA CCAAAGCAGUCGAGAUUUUUGGGCUCUAGGGAAAACGCUGACCAUCCAGUUGAAGAAUUUCGUUGGUGGAAGUUACAC UGCCAUAAGGGAGGAGAGGUGCUGGAGCCAUUCCCUUCUGCUGCACAAGAAAGAGACGACAUCUUGGUCUACCGAACA UCCUGAAAGACCAGAAGUCCCAAGAACAUUUCCUGCAGGUGGCGACCCCAAGAACUUACUUCCGGCAGGUUCACUCG UUGGUGGCUGACCACCAUCAGAACUGACUUUUAGUGUAAAAAGCUUCAGAGUUCCCCAAGGGCUG ACCUGCGGCAGCCAUCUUGAGCGCUGAGCGCGUGCGGGUGACAAUAAAGAGUACGAGUACAGCGUGGAGUGUCAAG AAGAUAGCGCUUGCCCUGGCCCCGAGGUAGAGCCUGCCUAUCGAGGUGAUGGUUGACGCCCACGCCAGUGCCACAAGCUUAAGUACGA GAAUUACACCAGCUCAUUCUUCAUUAGAUAUAAUCAAGCCUGACCCCACCAACAGCUUGUAGGCUGAGCUGCUCUUACAUUCU AACUCACGGGCAAGGUCGAAGUGGAGUUGGGAGUUACCCCGACACCUGGAGCACAUCCUCCUUAUUCCUAUUCUCCUUACAUUCU GCGUCCAGGUGCAGGUGGCAAGAGCAAGCGUUGGGAAAGAAGGAUCGAGUCUUCACCGACAAAACAAGCGCGACCGUGAUUUG CAGGAAGAACGCCAGCAUCUCCCGUCACAGGCCCAGGAUAGAUACUAUAGUAGCAGCUGGAGCAGUGGGCCAGCGUGCCC UGUUCCGGCGCGGGGCUGAGCCCAUUGCCCUGCUACCCCGGACCCUUGGAAUGGUUUCCGGUGUCUGCAC ACAGCCAGAACCUGCUGAAGACCCGUGUCGAAUAUGCUCCAGAAGGCCUGGCAGACCCCUGAGUUCCUACCCCUGUACCAG CGAAGAGAUCGAUCAUAAAGAUAUCACGAAAGAUAAAACACAUCCACCGUCAGGCUUGUUCCCCGCUGGAGCCAAG AACGAGAGCUGUCUGAAUAGCCGCGAGACGUCUUUCAUCACGAAUGGUAGCUGUGACUCAGCAGAAAACUUCCUUCA UGAUGGCUCUCUGCCUGAGCUCUCAUCUAUGAAGAUCUGAAGAUGUAUCAGUUGGAGUUUUAAACAUAAUGAACCCAAACU CCUGAUGGACCCAAAAGCUAAUCAUGUCCUGGACCAGAAUAUGCUGUGGCCGUGAUAGACAUGAUGCAGGCACUGAACAC UUCAACAGCGACGAGUUGCCACAGAAAUCCAGCCUGGAGGAGCUUGACUUUUACAAAACUAAGAUCAAGCUGUGUAUCC UGCUGCACGCCUUUAGAAUCCGUGUCGUGACAUAUCGACAGGGUGAUGUCAUAACGCUUCAUGAUAAUAGGCUGG AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGCCUCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCAAAC ACCAUUGUCACACUCCAGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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hIL12AB_ 002 (SEQ ID NO: 96)	G*GGAAAUAAGAGAGAAAAGAAGAGUAGAAGAAAUAAAGAGCCACCAUGUGUCAUCAGCAGCUGGUGAUCAGCUGG UUCAGCCUGGUGUUCCUGGCCAGCCCCCUGGUGGCCAUCUGGGAGCUGAAGAAGGACGUGUACGUGGUGGAGCUGGAUU GGUACCCCGACGCCCCCGGCGAGAUGGUGGUGCUGACCUGCGACACCCCCGAGGAGGACGGCAUCACCUGGACCCUGGA CCAGAGCAGCGAGGUGCUGGGCAGCGGCAAGACCCUGACCAUCCAGGUGAAGGAGUUCGGCGACGCCGGCCAGUACACC UGCCACAAGGGCGGCGAGGUGCUGAGCCACAGCCUGCUGCUGCUGCACAAGAAGGAGGACGGCAUCUGGAGCACCGACA UCCUGAAGGACCAGAAGGAGCCCAAGAACAAGACCUUCCUGAGAUGCGAGGCCAAGAACUACAGCGGCAGAUUCACCUG CUGGUGGCUGACCACAUCAGCACCGACCUGACCUUCAGCGUGAAGAGCAGCAGAGGCAGCGACCCCCAGGGCGUG ACCUGCGGCGCCGCCACCCUGAGCGCCGAGAGAGUCAGAGGGACAACAAGGAGUACGAGUACAGCGUGGAGUGCCAGG AAGAUAGCGCCUGCCCCGCCGCCCUGGAGGAGCCCGACGACUUCGUGAUGAAGUUCAAGAACUACCUGGACAGCAACG GAACUACACCAGCGCCGUGAUCUGCAUCAAGAAGAUCAAGAGCGCCGAGCCCGUGGAGUGCAGCUGAAGAGCCCCCUGAAG AACAGCCGCGCCGAGGUGGAGUACCCCGACACCCUGGAGCACCCCGUGGCCCCCACAGCCUACUUCAGCCUGGACCUGU GCGUGCAGGUGCAGGGCAAGAGCAAGAGAAAGAUAGAGUGUCACCGACAAGACCAGCGCCACCGUGAUCUG CAGAAAGAACGCCAUCAGCCUGGAGAGCCCUAAGAUACUACAGCAGCUGCAGAGUGGGCCAGCGUGCCC UGCAGCGGCGGCGGCUGGGCGCAGAAAACCUGCCCGUGGCCACCCCCGACCCCGGCAUGUUCCCCUGCCUGCACC ACAGCCAGAACCUGCUGAGAGCCGUGAGCAACAUGCUGCAGAAGGCCCGGCAGACCUUGGAGUUCUACCCCUGCACCAG CGAGGAGAUCGACCACGAGGAUCACCAAAGAUAAGACCAGCACCGUGGAGGCCUGCCUGCCCCUGGAGCUGACCAAG AACGAGAGCUGCCUGAACAGCAGAGACCAGCUUCAUCACCAACGGCAGCUGCCUGGCCAGCAGAAAGACCAGCUUCA UGAUGGCCCUGUGCCUGAGCAGCAUCGAGGACCUGAACAUGCUGCAGAGCGGCAUGUUCAACGUGAAGCUGAAGGCU GCUGAUGGACCCCAACGCCAAGCCUACCUUCCUGGACGCCCUGCAUGUCCUGCCCGAGGAGGUGGGCGAGCGGGAAC UUCAACAGCGGAGACCCGUGCCCCAGAAGAGCAGCAGCCUGAUGAGCCCGGACUUCAAGAUCAAGGUCUGCAUCUCC UGCUGCACGCCUUCAGAAUCAGAGCCGUGACCAUUGACAGAGUGAUGAGCUACCUGAACGCCAGCCUGAUAAUAGGCUGG AGCCUGGUGGCCAUCUCUUGGCCCUUGGGCCUCCCCCCAGCCCCUUCCUCCCCUUCCUGCACCCCCAAAC ACCAUUGUCACACUCCAGUGGUCCUUUGAAUAAAGUCUGAGUGGGCGGCAAAA

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hIL12AB_ 003 (SEQ ID NO: 97)	G*GGGAAAUAAAGAGAGAAAAAGAAAUAAGAAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCACAGCAGUUGGUCAUCUCUUGG UUUUCCCUGGUUAUUCUUCCCCUCGUGGCCAUCUGGGAACUGAAGAAAGACGUUUACGUUGUAGAAUUGGAUU GGUAUCCGGACGCUCCUGGAGAAAUGGUGGUCCUCACCUGUGACACCCCUGAAGAAGACGGAUCACCUGGAACUUGGA CCAGAGCAGUGACGUCUUAGCCUUGGCAAAACCCUGACCAUCCAAGUCAAAGAGUUUGGAGAUGCUGGCCAGUACACC UGUCACAAAGGAGGCGAGGUUCUAAGCCAUCGCUCCUGCUCACAAAAAGGAAGAUGGAAUUUGGUCCACUGAUA UUUUAAAGGACCAGAAACCCAAAAAUAAGACCUUUUUAGAGUGCGAGCCAAGAAUAUGCUGGACGUUUCACCUG CUGGUGGCUGACCACAAUCAGUUUGACAUUUCAGUGUCAAAAGCAGAGGAAGCUCCUUCUCCCUGACCCCAAGGGGGG ACGUGCGGAGCUGCUACACUCCUGCAGAGAGUCAGAGGUGACACAAGGAGUAUGAGUACUCAGUGGAGUGCCAGG AAGAUAGUGCUGCCAGCUGCCUGGAGAGAGUCUGCCCAUUGAGGUCAUGGUGGAUGCCGUUCACAAGCUCAAGUAUGA AACUACACCAGCAGUUUCUUCAUCAGAUAUCAUCAACCUGACCCCAAGAACUUGCAGCUGAAGCCAUUAAAG AAUUCUCGGCAGGUGGAGUACCUGGACACCUGGAGUACUCCACAUUCCUACUUCUCCUGACAUUCU GCGUUCAGGUCCAGGCAAGAGAAAAGAAAGAUAGAGAUCUUCACACGAUAAGACCUCAGCCACGGUCAUCUG CCGCAAAAUGCCAGCAUUACGCCUGUCGGGCCCAGGACCGCUACUAUAGCUCAUCUUGGAGCGAAUGGGCAUCUGUGCCC UGCAGUGGCGGAGGGGCGGAGGGAGCAGAAACCUCCCCGGUGGCCACUCCAGACCCAGGAAUGGAUCCCAUGCCUUCAC ACUCCCAAAACCUGUGAGCCCCGUCAGCAACAUGCUCCACAAGGCCCGGCAAACUUUAGAAUUUACCCUUGCACUUC UGAAGAGAUUGAUCAUGAAGAUAUCACAAAAGAUAAACCAGCACAGUGGAGCCCUGUUACCAUUGGAAUUAACCAAG AAUGAGAGUUGCCUAAAUUCCAGAGAGACUCUCUUCAUAAGCUUCAUCUUGAAUGGGAGUUGCUUCCUGAUAUGAC UGAUGGCCCUGGUCCUUAGUAUAUUAGAAGUUGAACAUGUACCAGGUUCAAAGUGCAAAGCU UCUGAUGGAUCCUAAGAGGCAGAUCUUUUAGAUCAAACAUGCUGGCAGUUAUGAGCUGAUGCAGGCCUGAU UUCAACAGUGGAGACGGUGCCACAAAAAUCCUCCCUUGAAGAACCAGAUUUCUACAAGAUCAAGCUCUGCAUAC UUCUUCAUGCUUUCAGAAUUCGGGCAGUGACUAUUGAUAGAGUGAUGAGUCUAUCUGAUUCCUGAUAUAGGCUGG AGCCUGGGUGGCCAUGAUUCUCUGCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUUUGUCCAGACCCCCAAAC ACCAUUGUCACACUCCAGUUGGUCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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	CUGCAGGAAGAUGCCUUCAUCUUCGGUCGAGCCCAGCCGCUACUACAGCAGCUGGAGUGAGUGGGCCUCGGUG
	CCCUGCAGUGGGCGGCGGCAGCAGAACCUUCUGUGGCCACGCCGGACCCUGGCAUGUUCCGUGCCUGC
	ACCACAGCCAAAAUUACUUCCAGCUGUUCUACACAUGCUGCAGAAAGCACGGCCAAACUUUAGAAUCCUACCCUGCAC
	CUCAGAAGAAAUAGACCAUGAACAUACACCAAGAUAAACCAGCACUCUGAGAGGCCUGCCUGGCCCUGGAGCUCACC
	AAGAAUGAAUCUGGCCUCAACACGCAGAGACCAGCUUCAUCACCAAUGGCAGCUUCCUGUCCCUCCCCUUCCUGCACCCCCCA
	UCAUGAUGGCGCUCUGCCUGAGCAGCAUCUCAUGAAGAUUUGAAGAUGUACCAAGUAGAAUUUAAAACCAUGAAUGCCAA
	GCUGCUCAUGGACCCCAAGCGGCAGAUAUUUUUGGAUCAAAACAUGCUGUCCUCAUUGAUGAGCUCAUGCAAGCAUUA
	AACUUCAACUCAGAGACGGUGUCCCAGAAGAGCAGCCUGGAGGAGCCAGAUUUCUACAAAACCAAGAUCAAGCUCUGCA
	UCUUAUUACAUGCCUUCCGCGAUCGGGGCGGUCACCAUUGACCAUAAAUGCCAGCUGAUCCUGGAGCACAGAUA
	UGGAGCCUCGGUGGCCAUGCUUCUUGCCCCCUUGGGCCUCCCCCCAGCCCCCUCCUCCCCUUCCUGCACCCGUACCCCCCA
	AACACCAUGUGUCACACUCCAGAGUCUGUUUAAAUAAAGUCUGAGUGGGCGGCAGACAAAAAAAAAAAAAAAAAAAAAAAAAA
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hIL12AB_ 006 (SEQ ID NO: 100)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAGAAGAAAUAAUAAGAGCCACCAUGUGCCACCAGCAGCUGGUGAUCAGCUGG UUCAGCCUGGUGUUCCUGGCCAGCCCCCUGGUGGCCAUCUGGGAGCUGAAGAAGGACGUGUACGUGGUGGAGCUGGAUUGGA GGUACCCCGACGCCCCCGGCGAGAUGGUGGUGCUGUCCUGCGACACCCCCGAGGAGGACGGCAUCACCUGGACCCUGGA CCAGAGCAGCGAGGUGCUGGGCAGCGGCAAGACCCUGACCAUCCAGGUGAAGGAGUUCGGCGACGCCGGCCAGUACACC UGCCACAAGGGCGGCGAGGUGCUGAGCCACAGCCUGCUGCUGCUGCACAAGAAGGAGGACGGCAUCUGGAGCACAGAUA UCCUGAAGGACCAGAAGGAGCCCAAGAACAAGACCUUCCUGAGAUGCGAGGCCAAGAACUACAGCGGCAGAUUCACCUG CUGGUGGCUGACCACAUCAGCACCAUCAGCGUGCAGGCCGAGUGGGAGAGCAACGGCCAGCCCGAGAACAACUACAAG ACCACCCCCCCUGUGCUGGACAGCGACGGCAGCUUCUUCCUGUACAGCAAGCUGACCGUGGACAAGAGCCGGUGGCAGC AAGGGCAACGUGUUCAGCUGCAGCGUGAUGCACGAGGCCCUGCACAACCACUACACCCAGAAGAGCCUGAGCCUGAGC GAACUGGGCAAGAUGAUCAUCGUGCUGGGCGGCCUGGUGUUCAUCGGCCUGAUCGCCUUCGUGGUGCAGAGAAUGAAG AACAGCCGGGCAGGUGGAGUGCUGGGAGUACCCCGACACCUGGAGCACCCCCACAGCUACCUGAGCCUGAUCAUCAAG GCGUGCAGGGUGCAGGCCAAGAGAGAAGAAAGAAUAGAGUGUCACAGAAUAAGACCAGCGCCACCGUGAUCUG CAGAAAGAACGCCAGAUCACCCUGAGAGCCCAAGAUAGAUACUACAGCAGCAGCGAGUGGGCCAGCGUGCCC UGCAGCGGCGGCGGCGGCAGCAGAAACCUGCCCGUGGCCACCCCCGACCCCGGCAUGAUCCCCUGGCCUGCACC ACAGCCAGAACCUGUCUGAGAUGCCCGUGAGCAACAUGCUGCAGAAGGCCCGGCAGACCCUGGAGUUCUACCCCUGCACCAG CGAGGAGAUCGACCACGAGGAUAUCACCAAAGAUAAGACCAGCACCGUGGAGGCCUGCCUGCCCCUGGAGCUGACCAAG AAUGAAAGCUGCCUGAACAGCAGAGACCAGCUUCAUCACCAACGGCAGUUGCCUGGCCAGCAAGAGCUGCAUCAGCGUG UGAUGGCCCUGGUGCUGAGCAUCUACGAGGACCUGAAGAUGUACCAGGUGGAGUUCAAGACCAUGAACGCCAAGCUG GCUGAUGACCCCAACCAGAUCACCAAGAACAUGCUGCAGAUCGACGCCGGCAAGCUGGCCGCCGUGGACCUGGAAC UUCAACAGCGAGACCGUGCCCCAGAAGAGCCUGGAGGAGCCCGACUUCUACAAGACCAAGAUCAAGCUGUGCAUCC UGCUGCACGCCUUCAGAAUCAGAGCCGUGACCAUCGACAGAGUGAUGAGCUACCUGAACGCCAGCUGAUAAGGCUGG AGCCUGGUGGCCAUGAUCUUCUUGGCCUCCCUGGCCUUCAUCCUGACCAGAAAGAUGAAGGUGAAGGUGUAA ACCAUUGUCACACUCCAGUGGGCUUUGAUAAGUCUGAGUGGGCGGCAAAA

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	UUUAAAAGACCAGAAGAGCCCAAGAACAAAACAUUCCUUCGUGUGAAGCCAACAGAACUACAGUGGUCGUUUCACCUG
	CUGGUGGCUUACUACUAUUUCCUACUGACUUUCUCUCUGUGAAGUCUUCUCGUGGCUCUUCUGACCCUCAGGGUGUC
	ACCUGUGGGGCUGCUUACUUUCUGCUGAGCGUGUGCGUGGUGAACACAAGGAGUAUGAAUACUCGGUGGAGUGCCAGG
	AAGAUUCUGCCUGGCCCUGGAGGAGUCUCUUCCUAUUGAGGUGAUGGUGCAUGCUGUGGCACAGUUAAAUGAUGA
	AAACUACACUUCUUUCUUUCAUUCGUGACAUUAUAAACCUGACCCUCCCAGAAACCUUCAGUUAAACCUUUAAAA
	AACUCUCGUUCAGUGUGGAGUACCUGACACGUGGUUCUACUCCUCACUUCGCUCUUACUUUCU
	GUGUCCAGGUGCAGUCCAAGCGUGAGAAGAAGGACCGUGUCUUCACUGACAAAACAUCUGCUACUCGUCAUCUG
	CAGGAAGAAUGCAUCCUUCUGUGCUCAGGACCGUUACUACAGCUCUCUUCCUGGUGGCUUCUGUGCCC
	UGCUCUGGCGGCUGGCUUCGUUCGUGCUGAGCAGAAAUCUUCCUGUGGCUACUCCUUCCCUGCCUUCACC
	ACUCGCAGAACCUUCUUCGUGCCUUGAGCAACAUGCUUCAGAAGCUCGUCGUCAACUUUAGAAUUCUACCCCUGCACUUC
	UGAGGAGAUUGACCAUGAAGAUAUCACCGUAAGAUAAACACAUCUACUGUGGAGCCUGCUUCCUUUAGAGCUGACCAAG
	AAUGAAUCCUGCUUAAAUUCUCGUGAUCUUCAUCACCAAUGGCAGCAUGCCUUUGCUGCGCAAAACAUCUUUCA
	UGAUGGCUCUUUGCCUUUCUUCCAUCUAUGAAGAUUUAAAAUGUACCAGGUUCAAGACCAUGAAUGCAAGCU
	UCUCAUGGACCCCAAGCUCAGAUAUUUUGGACCAGAACAUGCUUGCUUGCAUUGAUGAGCUCAUGCAGGCUUUAAAC
	UUCAACUCUGAGACGGUGCCUCAGAAGUCUUCUUUAGAGAGCCUGACUUCUACAAGAUAAACUUUGCAUUC
	UUCUUCAUGCUUUCCGAAUCCGUGUGAUCGACUAUUGACCGUCCUACUUAAAUGCUCCUUGAUAUAGGCUGG
	AGCCUCGGUGGACCAAGAUUCUUGGCCUCCCCCAGCCCCUCCUCCCCCUUCCUGCACCCGUACCCCCAAAC
	ACCAUUGUCACACUCCAGUGGUCUUUGAUAAGGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAA

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hIL12AB_ 009 (SEQ ID NO: 103)	G*GGGAAUAAGAGAAAAGAACAGAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCACCAGCAGCUGGUCAUCAGCUGG UUUAGCCUGGUCUUCCUGGCCAGCCCCCUGGUGGCCAUCUGGGAGCUGAAGAAAGACGUCUACGUAGUAGAGUUGGAUU GGUACCCAGACCCCACCCUGGAGAAAUGUGUGUUCACCUGCGACACCCCAGAACGAGACGGUAACACCUGGACGCUGCA CCAGAGCACGCGAAGUACUGGCCAGUGGAAAACGCUUGACCAUACAAGUAAAGAAUUUGGGAUGCUGGCCAGUACACC UGCCACAAAGGAGGACGAUGACUUCCCAGCCCUGCUGCUGCGCACACAAGAAAGAAGAUGGGCAUCUGGAGCACCCGACA UUUUAAAGACCAGAAGCAGCCCCAGAACACAAAACCUUCCUUCGAUGUGGAGGCGAAGUACAGCGUGGCCGUCUCACCUG CUGGUGGCUCACCACCAGCAGCUCGACCUUCUCGGUGAAGAGCAGCCGCGUGUAGCUCAGACCCCCAAGGAGUC ACCUGUGGGGCUCAGCUCUCUGGCAGAAGAUGCGAGGCGACAGCAAGGAAUAUGAUUACGUGCCGGUGGAAUGUGCAAG AAGAUUCCCCUCCCCUGGCCCAUAGAAGUCAUUCCUUUUGCCGUGGGAUGCCUGUUCACAAAUUAAAUUAUGA AAACUACACCAGCAGCUUUCUUCAUCAGAUAUCAAGCCCAGAACCCCCCCAAGAACCUGCAGCUGAAGCCCCUGAAG AACAGCGCGGCAAGGUGGAAGUUUGCUGGGAGUACCCAGAUACGUGGAGCACGCCGCCAGCUACUUCAGCCUCACCUUCU GUGUACAAGUACAAGCCAAGAGAGAAGAAAGAUCGUGUCUUCACGGACAAAACCUCGGGCGACGGUCAUCUG CAGGAAGAUGCAAGCAUCUCCGAGCCCAGGACCGCUACUACAGCAGCAGCUGGAGUGAGUGGGCCUCGGUGCCC UGCAGUGGUGGCGGCGGCAGCAGAAACCUUCCUGUGGCCACGCCCGACCCUGGCAUGUUCCGUGCCUGCACC ACAGCCAAAAUUUAUUACGAGCUGUUAGCAACAUGCUGCAGAAGCACGGCAAACUUUAGAAUUCUACCCCUGCACCUC AGAAGAAUAGACCAUGAAGAUUAAAACCAGCACACUGUACAGAGGCCUGCCCUGGAGCUCACCAAG AACGAGAGCUGCCUCAAUAGCAGAGACCAGCUUCAUCACCAAUGGCAGCUGCCUGGCCAGGAAAACCAGCUUCA UGAUGGCGCUCUGCCUGAGCAUCUAUGAAGAUCUGAAGAUGUACCAAGUGGAGUUCAAGAUGCCAAUGCCAAGCU GCUCAUGGACCCCAAGCGGCAGAUAUUCCUGGACCAAAACAUGCUGGCUGUCAUUGAUGAGCUCAUGCAAGCAUUAAAC UUCAACUCAGAGACGGUGCCCCAGAAGAGCAGCCUGGAGGAGCCAGAUUUUUAUAAAACUAAAAUCAAGCUCUGCAUCCU UAUUAGAUGCCUUCGAGAUCGGGCGGUCACCAUUGAGGGUGUCAUGUCCUAUUUAAUGCCAGCUGAUAAUAGGCUGG AGCCUCGGUGGCCAUGCUUCUUUGCCUCCUCUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCCAAAC ACCAUUGUCACACUCCAGUUGGCCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG

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hIL12AB_ 010 (SEQ ID NO: 104)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCACCAGCAGUUGGUCAUCUCCUGG UUUUCUCCUGGUCGCCUCCCCGUCGUCCUUGGGCCAUCGUGGGAAGCUGAAGAAGACGUCUACGUUGUAGAUUGGACGUGGA GCUACCCGGACGCCCCCGGAGAAAUGGUGGUUCUCACCUGCGACACCCCUGAAGAUGACAUCACCUGGACAGCUGCA CCAAAGCAGCGAAGUUUUAGAGCUUGGAAAACGCUGACCAUACAAGUUACGUUGAAACGUGGCCAGUACACG UGCCACAAAGGAGAGAAGUUUUAAGCCACAGUUUACUCUCGUCUUCACACAAGAAAGAAGAUGGCAUCGUGGAGUACAGAUA UUUUAAAGACCAGAAGCAGCCUAAGAACACAAAACGCUUCCUCGCCGUGUCAAGCUAACAGUGGCUCGUUUCACCUG CUGGUGGCUCACCACCAUCUCCACUGACUUCUCUGUAAAAUCAAGCCGUGUUUCUGACCCCCAAGGAGUC ACCGUGUGGGCUGCUACGCUGCUGAAAGAGUCCGAGGCGACACAAGAAUAUGAAUACUCGUGUGGAAUGUCAAG AAGAUUCUGCCUGGCCAGGAAGAAAGUCUUCCCAUAGAAGUCAUGGUGGACGCUGUCCACAAAUUAAAAUAUGA AAACUACACCAGCAGCAGUUCGUGACAUCAAACCAGACCUUCCUUAAGAACCUUCAGUUAAAACCGCUGAAG AACAGCCGGCAGGUGGAAGUUUCCUGGGAGUACCCAGAUACGUGGAGUACGCCGCACUCCUACUUCAGUUAACCUUCU GUGUACAAGUACGAGAAAAUCAAAGAAGAGAAGAUCGUGUCUUCACUGACAAAACAUCUGCCACGGUCAUCUG CCGUAAGAACGCUUCCAUCUUCGUUCCAGCCCAGGACCGCCUACUUACAGCACAGCGUGGAGUCAUCUGUUCCC UGCAGUGGUGGCGGCGGCAGCCGCAACCUUCCUGUGGCCACGCCGGACCCUGGCAUGUUCCCGUGCCUUCACC ACUCGCAAAAUCUUUCGUGCUAUUCUUUCUAACAUGCUGCACAAGGCGCGGCAAACUUUAGAUUCUACCCGUGCACUUC UGAAGAAUAGACCAUGAUAUCACCAAAGAUAAAACCAGCACGGUGGAGGCCUGCCUUCUUAGAACUUACUAAG AACGAAAGUUGCCUUAACAGCCGUGAGACUCAGCUUCAUCACCAAUGGCAGCUUCCUUAGCAGGAAGACCAGCUUCA UGAUGGCGCUGUGUCCUUUCUUCCAUCUUAGAAGAUCUUAAGAUGUACCAAGUAGAAUUUAAAACCAUGAAUGCCAAAUU AUUAAUGGACCCCAAGCGGCAGAUAUUCCUCGACCAAAACAUGCUGGCUGUCAUUGAUGAGCUCAUGCAAGCAUUAAAC UUCAACUCAGGAAACUGUUCCCCAGAAGUCAUCUUUAGAAGACCAGAUUUCUUAAAUAAACUCUGCAUUC UUCUUCAUGCCUUCCGCAUCCGUGGCUGUCACCAUUGACCGUUCAUGUCCUACUUAAAUGCUUCUUGAUAAUAGGCUGG AGCCUCGGUGGCCAUGAUUCUUUGGCCUCCCCCCAGCCCCUCCUCCCCCUUCCUCCACCCGUACCCCCCAAAC ACCAUUGUCACACUCCAGUGGUCCUUUGAAUAAAGUCUGAGUGGGCGGCAAAA

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hIL12AB_ 011 (SEQ ID NO: 105)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCCACAGCUGGUUGAUCAGCUGG UUCAGCCCUGGUGUCCUGGCCAGCCCCCUGGUGGCCAUCUGGGAGCUGAAGAAGGACGUGUACGUGGUGGAGUUGGAUU GGUACCCGGACGCCCCUGGAGAUGGUGUCCUGACCGUGCUACACGAGGACGAGGACGGUCACGUGGAACGCUGGA CCAGAGCUCCGAGGUGUCUUCGGCGAGGGCAAGACCCUGACCGUGAACGUGAGCAGCACCGUGAACGACGUGCAG UGCCACAAGGCCGGCAGCACCCUGGUGCUGCAAGAAGGAGGAGGACGGCGUGCGGCAGAUCCUGGGCCACAAGA UCCUGGAAGGAGCCAGAAGAACAAGACGUUCCUGAGGUGCGAGGCCGAAGAACUACAGCGGGAGGUUCACGUG CUGGUGGCCGAGAUCGAGCACGGCACCAGGGUGAAGGCCCACUACAAGAAGGCCCUGGAGCUGUUCAAGCAG ACGUGCGGCGGCGAGCUGGUGAGCUCCGAGGUGUUCCUGCCCCUGGUGGCCGGCCUGGUGAUCCUGGCCGUG AAGAUAGCCGUGGUGCCCGGCGGGGAGGAGCUGGCCGAUCCGAGGUUAUGGUGGGACGCCGGUGCACAAGCUGAUGAAGCUGAA GAACUACGCCGAGGAGGUGUUCUACCUGCAGAUAUCAUCAAGCCCGACCUGCAGCUGAAGCCCCUGAAG AACGGCGGCUGGACGUGCAGCUGGGAGUUCCCCGCCACCGGCAACCUGAACGGCCUGGAGCUGGCCUUCU GCGUGCAGGUGCUGCCCGAGGAGGAGGAUAGGGUGUUCAAGGGCAAGCAGGCCUUCAACGUGGAGAUCGUG CAGGAAGAACGCCGAGGCCUUCAGCCCUGAAGAUAGCGUACUACAGCAGCUUCGUGCCCGUGGCCGCCGAGCAGUCCG UGCAGCGGGGGGGGGGGGGGGAGCAGGAACCUGCCCUGGUGGCGACGCCCGGACCCGGGGAUGUUCCCGUGCACC ACAGCCCAGAACCUGCUGAGGGCGGUGAGCAGAUGCUGCAGAAGGCGAGGCAGACGCUGGAGUUCUACCCGUGCACGAG CGAGGAGAUCGACCACGAAUAUCACGAAAGAUAGACGGCGUUGCGACCGUGAACGUGAGCUGCUGCCGCUGGACGAAG AACGAGACGUGCCUGGAAUCAGGCGAGCACGGAUUCAUCACAGCAACCGGGACUGCGAGCCUGGCCAGCGAGGACGAGCUUCA UGAUGGCGCUGUGCCUGAGCACAUCUACGAGAAGAUGGUACCAGGUGGAGUUCAAGACGAUGAACGCCGAAGCU GCUGAUGGACCCGAAGAGGCAGAUCUUCCUGGACCAGAACAUGCUGGCCGUGAUCGACGAGCUGAUGCAGGCCCUGAAC UUCAACAGCGAGACGGUGACCCAGAAGAGCAGCCUGGAGGAGCCAGAUUUCUACAAGACGAUCAAGCUGUGCAUCC UGCUGCACGCCUUCAGGAUCAGGGUGACGAUCGACAGGGUGAUGGCCUCCCUGAACGUGGCCAGCUUCGUGAAAAAG AGCCUCGAGUGGCCAUUCUUCUGGCCCUGGUGGCCCUCCUCCUCCUCUUCUUCUGCACCCGUACCCCCAAAC ACCAUUGUCACACUCCAGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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	UGAUGGCCCUCUGCCUGAUCCUCAAUCUACGAAGACCUGAAGUGUACCAGGUCGAAUUUAAAACUAUCAACGCCAAGCU
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	UUUAACAGCGAGACCGUGCCACAAAAGAGCAGCUUGAGGAGCCGACUUCUACAAGACCAAGAUCAAGCUGUGCAUCC
	UCCUUCAUGCCUUCAGGAUAAGAGCGUGUCACCAUCGACAGAGUCAUGAGUUACCUGAAUGCAUCCUGAUAAUAGGCUGG
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hIL12AB_013 (SEQ ID NO: 107)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCCACAGCAGUUGGUCAUCUCCUGG
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	GGUACCCAGACGCCCCUGGAGAAAUGGUGGUCCUCACCUGUGACACCCCUGAAGAAGAUGGUAUCACCUGGACCUUGGAC
	CCAGAGCAGUGUGAAGAUCUUAGCAGUGACCAUACAUCAAGUAUUUGGAGAUUGCUGGUCCAGUAUACACC
	UGCCACAAAGGAGGAGAAGUUCCACAGUUUAUUACUGCACAAAGAAAGAAGAUGGGUCCACAGAA
	UUUUAAAAGACCAGAAGGAGCCCCAAAAUAAAACAUUUCUUCGAGUGUGAGGCCAAGAACUACAGUGGUCGUUUCACCUG
	CUGGUGGCUGACCACAUCUCCACGACCUCACCUUCAGUGUAAAAAGCAGGCCGAGUGGAUUCUUCUGACCCCCAAGAGUC
	ACCUGCGUGGCCUGCUCUCUUGCAGAAAGAGUCGAGCGUGACAACAAAGAAUAUGAGUACUCAGUGGAGUGCCAAG
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	AAACUACACCAGCAGCUUCUUCAUCAGAGAUAUCAUCAAACUGAACCUGACCUGGCCCAAGAAACUUACAGAGCUGAAA
	AACAGCCGGCAGGUAGAAGUUUCCUGGGAGUACCCAGAUACCUGGUCCACGCCGCUACCUUCGUCCUCCUGACCGUUCU
	GUGUACAAGUACAAGGCAAGAGAGAGAGAAGAUCGUGUGUCUUCACAGAUAAACAUCAGCCACGGAUCAUCUG
	CAGGAAAAAUGCCAGCAUCUCGGUGCGGGCCAGGACCGCCUACUACAGCAGCAGCUGGAGUGAGUGGGCCAUCUGUGCCC
	UGCAGUGGUGGGGGUGGUGCCAGCAGAAACCUUCCUGGGCCACUCCAGACCCUGGCAUCCCGUGCCUUCACC
	ACUCCCAAAAUUUACUUCGAGCUCGUUUCUUAACAUGCUGCAGAAAGCACGGCCAAACUUUAGAAUUCUACCCGUGCACUUC
	UGAAGAAAUUGACCAUGAAGAUAUCACACAAAGAUAAACCAGCACACAGUGGAGACCCUCAAACAUUCAUUGAGCGCUCA
	AAUGAAUCCUGCCUCACAGAGAGACCAGCUUCAUCACCAAUGGCAGCUGCCUGGCCUCCAGGAAAACCAGCUUCA
	UGAUGGCGCUCUGCUCCAGCUCCAUCUAUGAAGAUUUGAAGAUGUACCAAGUAGAAUUCAAGAUGCCAAAAU
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	UACUUCAUGCCUUUCGCAUCCGGGCUGUCACCAUUGACCAGGUCAUGUCCUUAAAUGCCUCGUGAUAAUAGGCUGG
	AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCCUCCUCCCCUUCCUGCACCGUACCCCAAAC
	ACCAUUGUCACACUCCAGUGGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAG

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hIL12AB_ 014 (SEQ ID NO: 108)	G*GGGAAAUAAGAGAGAAAAGAAGUAAGAAAUAUAAGAGCCACCAUGUGUCACCAGCAGUUGGUCAUCUGGUUUUUCUUGG UUCUCUCGUUGGUCCUUUGCCUCCUCUUGGUGGCUAUUGGGAGUUAUAUGAUAUCCGUCAGCUCCCAAAUCUGUGCAACUGGAGCUU GGUACCCUGACGCACCGGCAGAUGGUGCUACUUGUGACACUCCUGAGCAGGACGGCACAUUACUUGCACGCUUGA CCAGUCUUCUGACGUGCUUGCCUUGCCAAAACACUUACUAUUCAGGUGAAGCAGUUCAGGUAUGCUGGCCAGUACACU UGCCACAAGGGCGCGAGGUCCUUUCUCACUCUCUUCUUCUCUUCACAGAAGGAUGAACGGCAUUUGGGUCUACUGACA UUUUAAAGACCAGAAGCAGCCCAUAACACAAAACAUUCCUCGUUGCGAGCCAGAACUACUCUGGCCGUUUCACUUG CUGGUGGCUUACUAUUUCUACUUUCUCUGUGAAGUCUUCUCGUGACCCUCAGAGGCGUG ACUUGUGGGGCUCUACUCUUUCUGCUGAGCGUGUCGUGUCACUGACAAAACAUCCUGUGGAGUGCCAGG AAGAUUCUGCUUGCCUUGCUAUGGAGUCUCUUCCUAUUGAGGUGAUGCUGUCCACAAGUUAAAUACGA GAACUACACUUCCUUCUUCAUUAUUAAGCCUGACCUUCCCAAGAACCUUCAGUUAAAACCUUUAAA AACUCUCGUCAGGUGGAGGUGCCUUGGGAGUACCCUGACACUUGGUUCUACUCCUCCUUACUUCCU GCGUGCAGGUGCAGGCAUGUCUAAGCGUGAAGAGGACCGUGUGUUCACUGACAAAACAUCCUGUGAUUUG CAGGAAGAAUGCAUCUAUUUCUGUCUCGCUCAGGACCGUUACUACUCUUCCUUGAGUGGCUUCUGUGCCU UGCUCUGGGCGGCUGGCUCCAGAAAUCUUCCUGUGGCUCAUCCUGACCCUGCCCUUCCUUCACC ACUCUCAGAACCUUCUUCGUGCUGAGCAACAUGCUUCAGAAGGCUCGUCAAUUGACGAGCUUGAAU UGAGGAGAUUGACCACAGAUUCACCAAAGAUAAAACACUACUGUGGAGCCUGCCUUCCUUACCAAG AAUGAAUCUGUGCUUAAAUUCCGUAGAGACCGUCUUCAUCACCAACGGCUCUUUGCCUGCAAAACAUCUUUCA UGAUGGCUCUUUGCCUUUCUUUACAGAAGAUUUAAAAAUGUACCAGGUGCAGUUCAAAACAAUGAAGCAAGCU UCUUAUGGACCCCAAUCUUUCCUUGACCAGAACAUGCUUGCUGUGAUUGACGAGCUUAUGCAGGCUUAAAU UUCAACUCGGAGACGGUGCCUCAGAAAGUCUUCUCUGAGGAGCCUGACUUCUACAAGAUCAAGACCAUUC UUCUUCAUGCUUUCCGUAUUCGUUGACUAUUCGAGUGUCUUACUUAAAUGCUUCUAUCAUAUAGGCUGG AGCCUCGGUGGCCAUAGUUCUUUGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCAAC ACCAUUGUCACACUCCAGUUGGCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAUAUAAAAAAAAAA AAAAAAAAAAA

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	GCGGUGAGGUGCAGGGCAAAGCAGAAAGAGAAAGAAAGAAGAGAUAGAGUGUUCACAGAUAAAGACCAGGCCACGGUGAUCUG CAGAAAAAAUGCCAGCAUCAGCUGAGAGCCCAGAUAGAUACUAUAGCAGAGAUGGCGAAUGGGCCAGCGUGCCC UGCAGCGGCGGCGCGGCAGCAGAACCUGCCCGUGGCCACCCCCGACCCCGGCAUGUUCCCCUGCCUGCACC ACAGCCAAAACCUGCUGAGACCCGUGAGCAACAUGCCUGCAGAAGGCCCGGCAGACCCUGGAAUUUUACCCCUGCACCAG CGAAGAGAUCGAUCAUGAAGAUAUCACCAAAGAUAAAACCAGCACCGUGGCAGGCCUGUCUGCCCCUGGAACUGACCAAG AAUGAGAGCUGCCUAAAUAGCAGAGACCAGCUUCAUAACCAAUGGCAGUGCAGUGGAGUUCAAGACCAUGAUGCCAAGCU UGAUGGCCCUGAGCAGCAUCUAUGAAGACCUGAAGAUGUACCAGACGGAAGAUCAGCUGGCCUGUGAUCGAGCCUGAUCAAU GCUGAUGGAUCCCAAGGGCAGAUCAUCAUCUUCUGGAUCAAACAUGCUGGCCUGUGAUCGAUGCAGGCCCUGAAU UUCAACAGCGAGACCGUGCCCCAAAAAAGCAGCCUGGAAGAACCGGAUUUUUUAUAAAACAAAAAUCAAGCUGUGCAUAC UGCUGCAUGCCUUCAGAAUCGAGAGCCGUGACCAUCGAAUAGAGUGAUGAGCUAUCUGAAUAGCAUAAGGCUGG AGCCUCGGUGGCCAUAUCUUCUUCCCCUGGGCCUCCCCCCAGCCCCCUCCUUCCUGCACCCGUACCCCCCAAAC ACCAUUGUCACACUCCAGUGGGCUCUGAGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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hIL12AB_ 017 (SEQ ID NO: 111)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAGAAGAAAUAAUAAGAGCCACCAUGUGCCACCAGCUGGUAAUCAGCUGG UUUUCCUCGUCUUUCUUGGCAUCACCCUGGUGGCUAUCUGGGAAGAAGAACUGUAUGUACGUGGUGGAGCUGGAUU GGUACCCUGACGCCCCCGGGGAAAUGGUGGUGUUACCUGCGACACGCCUGAGGAGGACGGCAUCACCUGGACGCUGGA CCAGAGCAGCGAGGUGCUUGGGUCUGAAAACUCUGACUAUCUCAGGUGAAAGAGUUGGGAAUGCCUGGCCAAUAUACU UGCCACAAGGGGUGCAGGUCCUUUCUCGUUGCUCCUGCUCUGCCUGCCUGCUUCUGGCCUACUGAUA UUCUGAAAGACCAGAAGGAACCCCAUGAACACAAGACCUUUCUCUGAGAUGCGAGGCUAAAAACUACAGCGGAAGAUUUACCUG CUGGUGGCUGACCACAAUCUCUGAACAUUUUUCAGUGAAGUCCAGAGAAAAACUCUCCCACCUCAGGGCCUG ACCUGCGGAGCCGCCACAUCUGGUCGGAAGAGGUGAUAAUAAGUGGUACGAGUUACAGUCCGAGUUGCCAAG AAGAUUCUGCCUGGCCAGCCCCGAGGAGCCUGCCAAUCGAGGUGAUGCUGGGUACACAAGCUGAAGUAUGA GAACUACACAUCCUCUUCUUCAUAAGAUAUAUCAAGCCUGAACCUACCUAAAAAUCUGCAACUCAAGCCUUUGAAA AAUUCACGGCAGGUGGAGUACCCUGAUACUUGGAGCACCCCCAUAGCUACUUUCGCUGAACUUCU GCGUCCAGGUGCAGCGUCAAAGAGAAGAAGGAUCGCGUGUCACUGAUAAAACAAGCGCCACAGUGAUCUG CAGAAAAACGCUAGCAUACCGUCAGAGCACAGGACCGGCUAUACUCCAGCUCCUGGAGCGAUGGGCAUCUGUGCCC UGCAGCGGUGGGGCGGAGGCGCAUCCAGAAACCUCCCCGGUGCCACACCUGAUCCUGGAAUGUUCCCCUGUCUGCACC ACAGCCAGAACCUGCUGAGCCAGUGUCUACAUGCUCCAGAAGCCAGGCGACCCUGGAGUUGAUCGACAAGACUGAAC CGAGGAAAUCGAUCACGAAGAUAUCACCAAAGAUAAAACCAGCACCGUGGAGGCCUGCCUGCCUUGAACAAUGAC AACGAGAGCUGCUUCCUGGAAUAGCAGGGAGACCUCCUUCAUCACCAACGGCUCUAUGCCUGCCAGGAAACUAGCUUCA UGAUGGCCCUGGUGCUCUGUCUGAUCUAUGAGGACCUGAAAAUGUACCAGGUCGAAUUUAAGACGAUGAACGCAAAGCU GCUGAUGGACCCCAAGCGGCAGAUCUUCCUGGACCAGAACAUGCUGGCAGUCAUAGAUGAGUUGAUGCAGGCCCUGAAC UUCAACAGCGAGACCGUGCCUCAGAAGUCCAGCCUCGAGGAGCCAGAUUUUUAUAAGACCAAGAUCAAACUAUGCAUCC UGCUGCAUGCUUUCAGAUUUAGAGCGUCACAUCGAUCGAGUCAUGUCUUACCUGAAUGCUAGCUGAUAAUAGGCUGG AGCCUCGGUGGCCAUGAUUCUUCUUGGGCCUCCUCCCCCAGCGCCUCUCCUCCCCUUCCUCGCACCCCGUACCCCAAAC ACCAUUGUCACACUCCAGUUGGCUCUUUGAUAAGGUCUGAGGGUGGCGCAAAAA

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	UUCUGAAGGACCAGAAGGAGCCUAAGAAUAAACUUUCCUGAGAUGCGAGGCAAAAAACUAUAGCGGCCGCUUUACUUG
	CUGGUGGCUUACAACAACAAUCUCCUACCGAUUAACUUCUCCGUGAAGUCUAGCAGAGAAUCCUCUGACCGCAAGGAGUG
	ACUUGCGGAGCCGCCACCUUGAGUGCCGAAAGAGUCCGUGGCGAUAACAAAGAAUACGAGUACUCCGUGGAGUGCCAGG
	AAGAUUCCGCCUGCCUGGCCCAGCUGCCCGCCCAUUGAAGUGAUGGUGCAUGCCGUCCACAAGCUGAAGUUACGA
	AAACUAUACCAGCAGCUUCUUCAUCGGGAUAUCAUUAAGCCCGACCCUCCUAAAAACCUGCAACUUAAGCCCCUAAAG
	AAUAGUCGGCAGGUGAGGUCAGCUGGGAAUAUCCUGACACAUGGAGCACCCCCCACUCUUAUUUCUCCCCUGACCUUCU
	GCGUGCAGUGGCAAGAUAAACGGGAGAAAAAGAUAGGGUCUUUACCGAUAAAACCAGCGACUACGCUAUUCUG
	UCGGAAGAACGCUUCCAUCUCCGCCCAGGAUCGUACUACUCGUCCCUCAUGGAGCGAGUGGGCCAGCGUGCCC
	UGCAGCGGCGGCUGUGGAGGCGGAUCCAGAAAUCUGCCUGUGCCACACCAGACCCUUGGCAUGUUCCCCUGUCUGCAUC
	AUAGCCAGAACCUGCUCAGAGCCCGUGAGCAACAUGCUCCAGAAGGCCAGGCAAACUUUGGAGAUUCUACCCGUGUACAUC
	UGAGGAAAUCGAUCACGAAGAUAUAACCAAAGAUAAAACCUCUACAGUGAGAGGCUUGUUUGGCCCCUGGAGUUGACCAAA
	AACGAGAGUUGCCUGAACAGUCCGUGAUCGAGCUUCAUUACUAACGGCAGCUUCGGCCAGAAAACAUCCUUCA
	UGAUGGCCCUGUGUCUUUCCAGCAUAUACGAAGACCUGAAAAUGUACCAGGUCGAGUUCAAAACAUGAACGCCAAGCU
	GCUUAUGGACCCCAAGCGGCAGAUCUUCCUCGACCAAAACAUGCUCGCGUUGAUCGAUGAGCUGAUGCAGGCUCUCAAC
	UUCAAUUCCGAAACAGUGCCAGAGAAGUCCAGUCUGGAGAAGACCCGACUUCUACAAGAUUAAGCUGUGUAUUU
	UGCUGCAUGCGCUUUAGAAUCAGAGCCGUGACCAUUGAUCGGGUGAUGAGCUACCUGAACGCCUCGUGAUAUAGGCUGG
	AGCCUCGGUGGCCAUUUUUGGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUUCCUGCACCCGUACCCCCAAAC
	ACCAUUGUCACACUCCAGUGGUCUUUGAUAAAGUCUGAGUGGGCGGCAAAAA

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hIL12AB_ 019 (SEQ ID NO: 113)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGCCACCAGCUGGUCAUCUCCUGG UUUUCUCCUGGUCGCCAGCCGCUGGGUGAGCUGAAGAAAGACGUUUACGUCGUAGUAGAGUUGGAUU GGUACCCAGACGCCCCUGGAAUGGGUGGUCUCACCGUGUGACACUCCUGAAGAUGACGGUACUCACCUGGACUGUGA CCAAAGCUCAGAAGUUCUUGGGCAGUUGGUAAAAACGCUGACCAUACAUAUCAAGAGUUUGGGAUGCUGGCCAGUACACG UGCCACAAAGGAGGUAGAAGUUCUCAGCCACAGUUUACUUCUUCUUCACAAGAAGAGAUGGCAUCUGGUCCACAGAUA UUUUAAAAGACUACAAGGAGCCCAAGAACAAAACCUUCCUCCGCUGUGAGGCCAAGAACUACAGUGGUCGUUCACCUG CUGGUUGCUCACCACCAUCUCACCUUCUCUGUAAAAGCAGCGCGUGUUCUGACCCCCAAGGGUC ACCUGUGGGCUGACCAGCUCUCGGCAGAAAGAGUUCGAGGUGAUAUUCUGUGGAAUGUCAAG AAGAUUCUGCCUUGCCCGGGCAGAAGAUGCUUCCCAUAGAAGUCAUGGUGGAUGCUUGUCACAAAUUAAAAUGA AAACUACACCAGCAGCUUCUUCAUUCUGUCAUCAUCAAACCAGACCCGCCAGCUAAAUUCAGAUAUCCUUUAAA AACAGCCGCGAGAAGUUUCCUGGGAGUACCCAGAUACGUGGUCCACGCCGCACUCCUACUUCAGUUUAACCUUCU GUGUACAAGUACAAGCAAAAAGAGAUAAGAUCGUGUCUUCACUGACAAAACAUCGUCCACGGUCAUCUG CAGGAAGAAUGCUUCCAUUCGAGCCCAGGACCGCUACUACAGCAGCUGGAGUGAGUGGGCAUCUGUUCC UGCAGUGGUGUCGCUGCAACCUUCCUGUGUCCACGUCCUGGACCCUGGCAUGUUCCCGUGCCUUCACC ACUCCCAAAAUCUUUCGUGCUGUUCUUAACAUGCUGCAGAAGGGCGCCACAAACUUUAGAAUUCUACCGUGCACUUC UGAAGAAUUAGACCAUGAAUAUCACGCAAUAAAACCAGCACGGUGGAGUCCUGCUUCCUUUAGAGCUGACGAG AAUGAAUCCUGCCUUAACAGAGAGACCAGCUUCAUCACCAAGCAUUCGCUGGCCUCGCGCAAGACCAGCUUCA UGAUGGCGCUGUGUCUUCCAUCUAUGAAGAUUUAAAGAUGUACCAAGUAGAAUUUAAAACCAUGAAUGCCAAAUU AUUAAUGGACCCCAAACGGCAGAUAUUUUUGGAUCAAAACAUGCUGGCUGAUGAGCUCAUGCAAGCAUUAAAC UUCAACUCAGAAACUGUUCCCCAGAAGUCAUCUUUAGAAGAGCCAGAUUUCUGCAAAGAAUAAACUCUGCAUUC UUCUUCAUGGCCUUUCCAUCCUUGCUGUCACAUUGACCGUGUCAUUGAAAUGCUUCUUGAUAUAGGCUUG AGCCUCGGUGGCGAAUGCUUCUUGCCCUUGGGCUCCCCCCAGCUCCCUCCCCUUCCUGCACCCGUACCCCCAAC ACCAUUGUCACACUCCAGUGGUCUUGAAUAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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hIL12AB_ 020 (SEQ ID NO: 114)	G*GGGAAAUAAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCACCAGCAGCUGGUGAUCAGCUGG UUCAGCCUGGUGUUCCUGGCCAUCUUUCAUGAGGGCCAUCUGGGCAAGAAGAACGAUGUUACGUGUGGGUGGAGUGGACU GCUACCCCGACGCCCUCCCGGCAGAUGUGGUGCUGACCUGCAACACCCCCGAGAAGGACGGCACCACCUGUACCUGA UCAGUCAAGCGACGUUGCUGGCAAAGCGGCAAGACCCUGACCAUCCAGGUGAAGGAGUUCGGCGACGCCGGCCAAUACACU UGCCACAAGGGAGGCGAGGUUCUUAGCCACGCCCUCCUGCCUGCUGCACAAAAAGGAAGAUGGCAUCUGGAGCACCGACA UCCUGAAAGACCAGAAGGAGCCUAAGAACAAAACAUUCCUCAGAUGCGAGGCCAAGAAUUACGUCCGGGAGAUUCACCUG UUUGUGGCUGACCACCAGACAGCUUGACCUUCAGCGUGAAGAGCAGCAGGAGCAGCGACCCCCAGGGCGUG ACCUGUGGCGCCGCUGAGUACGCCGCCGUGUCUGCACAGAUGUACCGUGUGGAAUGCCAGG AAGAUAGCGCCUUCCCCGCCGAGGAGCUGUGCCCAUCGAGCUGAUGGUGCUCCACGAGCUGCAGUACGA GAACUACACCUCUAGCUUCUUCAUCAGAUAUCAUCAAGCCCGAUCCCCCAAGAACCUGCAGCUGAAAGCCCUGAAG AACAGCGCGGAGGUGGAGGUGUCGUGGGAGUAUCCCGACACCUGGUCCACCCCCACAGCUAUUUUAGCCUGACCUUCU GCGUGCAAGUGCAGGGUCAAGAGAGAAGAAGGAGAAAGAUAGAGUUUUCACCGACAAAACCGCGGAUGAUGUCUCG CAGAAAGAACGCCAGCAUCAGCGUGAGGCCAGAAUGAUACUACAGUUUCAGAUCUGGAGCGAAGUGGGCCAGCGUGCCC UGCAGCGGCGGCGGGAGGCUCGAGAAACCUGCCCGUGGCUACCCCGGAUCCCGAAUGGUUCCCCUGCCUGCACC ACAGCCAGAACCUGCUGAGGGCCGUGUCCAACAUGCUUCAGAAGGCCGUGGCAGUUAUCGAGUCCAAGCCUGAC UGAGGAGAUCGAUCAUGAAGAUAUCACAAAAGAUAAAACCAGCACCGUGGAGGCCUGCCUGCCCCUGGAGCUGACCAAG AACGAGAGCUGCCUGAACGCCGAGAGCUUCAUCACGAACGGCAGCUGCGCCUCCCAGGAAGACCUCCUUCA UGAUGCCCUGGCCUGAAGCAGCAUCUACGAGGACCUGAAAAUGUACCAGGUCGAGUUUAAGACCAUGAACGCCAAGCU GCUGAUGCCCAAGCGGCAAAUCUUCCUGGACCAGAACAUGCUGGCAGUGAUCGACGAGCUCAUGCAGGCCUGAAC UUCAAUAGCGAGACGGUCCCCCAGAAGAGCAGCCUGGAGGAGCCCGACUUUUACAAGACCAAGAUCAAGCUGUGCAUCCU UGCUGCACGCCUUUAGAAUCCGUGCCGUGACCAUUGACAGAGUGAUGAGCUACCUGAAUGCCAGCUGAUAAUAGGCUGG AGCCUCGGUGGCCAUGCUUCUUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCAAC ACCAUUGUCACACUCCAGUGGCCUUUGAUAAAGCUGAGUUGGCUGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG

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hIL12AB_ 021 (SEQ ID NO: 115)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAAGAGCCACCAUGUGUCACCAGCAGCUGGUGAUCAGCUGG UUCAGCCUGGUGUUCCUGGCCAGCCCCUCUGGUUGCCAUCUGGGAGCUGAAGAAAGACGUGUACGUCGUGGAACUGGACU GGUAUCCGGACGCCCCCGGGAGAUGGUGGUCCUGACCUGCGACACCCCCGAGGAGGACGGCAUCACCUGGACGCUGGA CCAAUCCGCCGAGGUGCUGGGAAAGGACCCUGACCAUCCAGGUGAAGGAAUUCGGCGACGCUGGCCAGGUACACC UGCCACAAGGGGGGCGAAGUGCUGUCCCACAGCGUGCUGCUGCUGCACAAGAAGGAAGAUGGAAUCUGGUCCACCGACA UCCUCAAAGAUCAGAAGGAGCCCCAAGAACACAAGACGUUCCUGCGCUGUGAAGCCAAGAAUUACUCUGGGCGUUUCACCUGC CUGGUGGCUGACAACCAUCAGGACUGGACUUUUAGCGUGAGAGCAGAUAGGUCCAGCGACCCCCAGGGCGUG ACGUGCGCCGCUCACCCUCCUCCUGCCGGGGGGACAAUAAGGAGUACAGUGUACAGCGUGGAAUGCCAGG AGGACAGCGCCUGCCCUCCGCUGAAGAAGCUCCCGAUAGAGGUGAUGGUGGACGCCGUGCACAAGCUCAAGUAUGA GAAUUACACCAGCAGCUUUUUCAUCCGGGACAUUAUCAAGCCCGACCCCCCGAAGAACCUCCAGCUGAAGCCCCUGAAG AACAGCCGGCAGGUGGAAGUCUCCUGGGAGUAUCCCGACACCUGGAGCACCCCGCACAGCUACUUCUCCCUGACCUUCU GUGUGCAGGUGCAGGGCAAGUCCAAGAGAGGGAAAAGAAGGACAGAGUUUUCACCGACAAGACCAGCGCGACCGUGAUCUG CCGGAAGAACGCCAGCAUAAGCCUCCGCCCAAGAUAGGCUACUACAGCAGCUCCUGGAGCGCAGUGGGCUAGCGUGCCC UGCAGCGGGGGCGGGGUGGGGCUCCAGGAACCUGCCAGUGGCGACCCCCGGCAUGUUCCCCCUGCCUCCAGC ACAGCCAGAACCCUGAGGCCCGUCAGCAAUAUGCUGGCACAAGGCCAGGACCCUGGAAUUCUACCCCCUGCACGUC GGAGGAGUCGAUCAUGAGGAUAUCACAAAAGACAAGACUUCCACCGUGGAGGCCUGCCUGCCCCUGGAGCUCACCAAG AAUGAGUCCUGUCUGAACUCCAGGAAACCAGCUUCAUCACCAACGGGUCCUGCCUGGCCAGCAGGAAGACCAGCUUUA UGAUGGCCUGAUGCCUGUCGAGGACAUCUACGAAGAUGUACCAGGUCGAGUUCAAGACAAUGAACGCCAAGCU GCUGAUGGACCCCAAGAGGCAAAUCUUCCUGGACCAGAAUAUGCUUGCCGUCAUCGACGAGCUCAUGCAGGCCCUGAAC UUCAACUCCGAGACCGUGCCCCAGAAGAGCAGCCUGGAGGAGCCCGACUUUUACAAGACCAAGAUCAAGCUGUGCAUCC UGCUGCACGCGUUCAGAAUCCGCGCAGUCACCAUUGACCGUGUGAUGUCCUACCUGAACGCUAGCUGAUAAUAGGCUGG AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCCAAAC ACCAUUGCCACACUCCAGUGGGCCUUUCUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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hIL12AB_ 022 (SEQ ID NO: 116)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGCCAUCAGCAGCUGGUGAUCAGCUGG UUCAGCCUGGUGUUCCUGGCCUCUCCCCUGGUGGCCAUCUGGGAGCUCAAGAAAGACGUGUACGUGGUGGAGCUGGACU GGUACCCAGACGCCCCCGGAGAUGGUGGUCCUGACCUGCGACACCCCCGAAGAAGACGGCAUCACGUGGACCUUGGACCAGUCG CCAGUCCAGCGAGGUGCUGGGCAGCGGCAAGACCCUGACCAUCCAGGUCAAGGAGUUCGGCGACGCUGGCCAGUACACCUGC UGCCACAAGGGCGGCGAAGUCCUGAGCCACAGCCUGCUGCUGCUGCACAAGAAGGAAGACGGCAUCUGGUCCACAGACAUC UACUGAAGGACCAGAAGGAGCCCAAGAAUAAGACCUUUCUGAGAUGCGAGAGCAAGAAUUAUUCGGCAGUUUCACGUG CUGGUGGCUUACAACAAUCAGCACAGACUUGACGUUCACGCUGUGAAGUCCAACCCCGGCAGCAGCGACCCCCAGGGGGUG ACCUGCGGCGCCGCCACCCUCGAGCGCCGAGCGGGGGAGACAAGGAGUACGAGUACUCGGUGGAGUGCCAGG AAGACAGCGCUGUCCCCGCCGAAGAGGCCUGCCUAUCCGAGGUCAUGGUAGCAUGCAUUAAGCUGAAGUACGA GAACUAUACGAGCAGCUUUUUCAUACGCGACAUCAUCAAGCCCGACCCCCCUAAGAACCUGCAGCUUAAGCCCCUGAAG AAUAGCCGGCAGGUGGAAGUCUCUUGGGAGUACCCCGACACCUGGUCAACCCCCACAGCUACUUCUCCCUCCUGACCUUUU GUGUCCAAGUCCAGGAAAAGAGCAAGAGGGAGAAGAAGGAUCGGGUGUUCACGGACAAGACCUCCGCCACGGUGAUCUG CAGGAAGAACGCCAGCAUCUCCGUGAGGGCGAGAUCUACUCCACGCUGGUCGGUCCGAAUGGGCCAGCGUGCCC UGCUCCGGCGGCGGCGGCAGCCUGAAACCUACCCGUGGCCACGCCGUUCCCCUGCCUGCACC ACAGCCAGAACCCUCUGAGGCCCGUGUUCCAACAUGCUGCAGAAGGCCAGGCAGACUCUGGAUUCCAGAGGCAGCGAG CGAGGAGAUCGAUCACGAGGACAUCACCAAGGAUAAGACCAGCACUGUGGAGGCCUGCCUGCCUGGAGCUGACCAAG AACGAGAGCUGUCUGAACUCCAGGAGACCUCAUCACCAACGGCUCCUUGCCUGAGCAGGAAACCAGCUCU UGAUGGCCUUGAGUCUCCAGUCCAUCUACGAGGACCUGAAGAUGUACCAGGUCGAGUUCAAGACCAUGAACGCCAAGCU GCUGAUGGACCCCAAAAGGCAGAUCUUCCUGGACCAGAACAUGCUGGCCGUCAUCGACGAGCUGAUGCAGGCCCUGAAC UUCAACAGCGAGACGGUGCCCCAGAAAUCCUCCCUGGAGGAGCCGGACUUUUACAAGACCAAGAUCAAGCUGUGCAUCC UGCUGCACGCCUUCAGGAUCAGGGCAGUGACGACCGGGUGAUGUCAUACCUUAACGCCAGCUGAUAAUAGGCUGG AGCCUGGGUGGCCAUGUCUUCUUGGCCCUCCUCCCCCCAGCCCCUCCUCCCCUUCCUUGCACCCCCCAAAC ACCAUUGUCACACUCCAGUGGGCUUUGAUAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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	UGAUGGCCCUUGUCUGAGCUCCAUCUAUGAGAGACCUGAAGAGAUGUACCAGGUCGAGUUCAAGACCAUGAACGCGAAGCCU
	GCUGAUGGAACCCCAAGAGCGCAUGAUCUUCUUGGAUCAGAUAUGCUGGCGGUGGAGCUCAUGCAGGCCCUCAAU
	UUCAAUAGCGAGACAGUGCCCCAGAAGUCCUCCCUGGAGGAGCCCGACUUUUACAAGACCAAGAUCAAGCUGUGUAUCCC
	UGCUGCACGCCUUCCGGAUCCGCGCCGUCACCGACCGGUCAUGACCUCAAUGCCAGCUGAUUAUAUGGCUGG
	AGCCUCCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCGCCCCCUCCUCCCCGUCCCCUUCCUUGCACCCCGUACCCCAAC
	ACCAUUGUCACACUCCAGUGGCCUUUGAUAUAAGUCUGAGUGGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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hIL12AB_025 (SEQ ID NO: 119)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGCCAUCAGCAGCUGGUGAUUUCCUGG UUCUCCCUGGUGAUCCUGGCCAGCCCCCUCGUGGCGAUCUGGGAGCUAAAGAAGGACGUGUACGUGGUGGAGCUGGACU GGUACCCGGACGCCCCGGGCGAGAUGGUCGUCCUGACCUGCGAUACGCCUGAGGAGGACGGCAUCACCUGGACCCUCGA UCAGAGCAGCGAGGUCCUGGGCAGCGGCAAGACCCUGACCAUCCAGGUCAAGGAGUUCGGCGACGCCGGCCAGUACACC UGCCACAAGGGUGGCGAGGUCCUGAGCCACUCGCUGCUGCUGCACAAGAAGGAGGACGGCAUCUGGAGCACAGACA UCCUGAAGGACCAGAAGGAGCCCAAGAACACGUUCCUGAGGUGCGAGGCCAAGAACUACAGCGGCCGCUUCACGUGC CUGGUGACCUACAUCACGGACUUGACCUUCAGCGUCAAAAGCAGCAGAGGCUCUGCCGACCCCCAGGGCGUGACCUGC ACCGCUGCGCCGCCAGCCUGAGCGUCGGAGAGGGCCAGGGGCGAUACGUACGAGUGGAGCUGGAGCGUGGAGUGCCAGGG AGGACAGCGCCUGCCUGCCCGCCGAGGAGUCCCUGCCCAUCGAGGUGAUGGUGGACGCCGUGCACAAGCUGAAGUACGA GAACUACACCAGCAGCUUCUUCAUCAGGGAUAUCAUCAAGCCCGACCCCCCGAAGAACCUGCAGCUGAAGCCCCUGAAG AACAGCCGGCAGGUCGAGGUGUCGUGGGAGUACCCGGACACCUGGUCGACCCCGCACAGCUACUCUCUCUCGUCACCUUCU GUGUGCAGGUGCAGGGAAAGAGCAAGAGAGGAAAGAUCCGGGUCUUCACCGACAAGACCAGCGCCACGGUGAUCUG CAGGAAGAACGCAUCUCCCGUGAGGCCCAGGUACUACAGCUCCCAGUCGUCCGAUGGGCCACGUGCCCC UGUAGCGGCGGGCGGGGCUGGUGCCAGCCGCAACACCUCCCAGUGGCCACCCCGGACCCCGAUCCCCUGCCUGCACC ACAGCCAGAAUCUGUGAGGCCCGUGAUAACAUGCCUGCACAAGGCAAGGUCAUGGCCGUGAUAGACGAGCCCUGAAC CGAGGAGAUCGACCAGAGGACAUCUUCCUGGACCAGAACAUGCUGGCCGUGAUAGACGAGCUCAUGCAGGCCCUGAAC AAUGAGAGCUGCCUGAACAGCCGGGAGACCAGCUUCAUCACCAACGGGAGCUGCCCUGGCCUGCAGGAAGACCUCGUGUCA UGAUGGCCUGGGCCUCCUCAAGCCAUAUACGAGGAUCUGAAGCAUGUACCAGGUUGAAAUGAACGAUCCAAGCCCAAGCU GCUGAUGGACCCGAAGAGGCAGAUCUUCCUGGACCAGAACAUGCUGGCCGUGAUAGACGAGCUCAUGCAGGCCCUGAAC UUCAACUCCGAGACCGUGCCGCAGAAGUCAUCCCUCGAGGAGCCCGAGUUCUUCAAGAUCAAGUCAAGUCAAGCUGUGCAUCC UGCUCCACGCCUUCCGGAUAAGGGCCGUGACGAUGGAUGACAGGGUGAUGAGCUACCUUAACGCCAGCUGAUAAUAGGCUGG AGCCUGGGUGGCCAUGAUUCUUCUGCCCCUGUGGGCCUCCCCCCAGCCCCUCCCCCUUCCUUCCUUUGUCCCAAAC ACCAUUGUCACACUCCUGAGUGGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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	GCGGUGAGGUGCAAGGGAAAGGCAAAGGGGAGAAGAGGAAGGACUGGGGUGUUCACCAACAAGACCGCCGCCACCGCCGUGAUCUG
	UAGGAAGAACGCCGUCGAUCUCGAUCUCCGUCAGGGCCACGGACAGGUAUUACAGCAGCAGGUGGAAGUCGAGUGGGCGAGCGUGCCC
	UGCUCGGGCGGCGGCGGCGGCGGCAGGAGCAGAAAUCUGCCCGUGGCCACCCCGACCCCCGGAAUCUUCCCCUGGCCUGCACC
	AUUCGCAGAACCCUCUGAGGCCCGUGAGCAACAUGCCUGCAGAAGGCCCGGCCAAGACGCCUGGAGUUCUACCCCUGCACGAG
	CGAGGAGAUCGACCACGAAGACAUCACCAAGGACAAAACCAGCACCGUGGCAGGCCUGCCCGCCCCUGGAGCCUGACCAAA
	AACGAAUCCUGCCUCAACAGCCGGGAGACCAGCUUCAUCACCAACGGCAGUUGCCUGGCCAGCCAGUGGCCGAAGAACCUCCUUCA
	UGAUGCCCUCUGCUUGAGCAGCAUCUUAUGAGGAUCUGAAGAUGUACAGGUGGAGUUCAAGACCAUGAUGCCAAGCU
	GCUGAUGGACCCCAAGAGGCAGAUUAUCCUGGACCAGAUUAUGCCUGGCCUGAUCGACGCCUGAUGCAGCCCCUGAAC
	UUCAACAGCGAGACCGUCCCCCAGAAUCCGGAGCCUGGAGGAGCCGGACUUUUACAAAACGAAGAUCAAGCUGUGCAUAC
	UGCUGCACGCCUUCAGGAUCCGGGCGUGACAAUCGACAGGUGAUGUCCUACCUGAACGCCAGCUGACUAAUAAGGCUGG
	AGCCUCGGUGGCCAUUCUUCUGCCCCUCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCCAAC
	ACCAUUGUCACACUCCAGUGGCUCUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAA

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hIL12AB_ 028 (SEQ ID NO: 122)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGCCACCAACAGCUCGUGAUCAGCUGG UUCAGCCUGGUGUUCCUGGCCAGCCCGCUGGUGGCCAUCUGGGAGCUGAAGAAGGACGUGUACGUUGUGGAGCUGGACU GGUACCCCGACGCCCCCGGCGAGAUGGUGGUCCUGACCUGCGACACGCCUGAAGAGGACGGCAUCACCUGGACCCUGGA UCAGUCCAGCGAGGUGCUGGGCUCCGGCAAGACCCUGACCAUUCAGGUGAAGGAGUUCGGCGACGCCGGCCAGUACACC UGCCACAAGGGCGGCGAGGUGCUGAGCCACAGCCUGCUGCUGCUGCACAAAAAGGAAGAUGGAAUUUGGUCCACCGACA UCCUCAAGGACCAGAAGGAACCGAGAGAGUACCUGGCCUCCGGUGCGAGGCCAAGAACUACAGCGGCGAGUUUACCUUC CUGGUGGACUGGACCACCCCUACAUCAGCGAGAGCAUCGACAUCUUCUGCUGCGAGACCAUCGAGAACGAGUGGGUGG ACCUGCGGCGGCCACCCUGUGCUGGAGGGCGUGUCCGACAAGAAGGAGUACAGCGUGGAAUGCCAGG AGGACAGGCGCCUGUGCCCCGCAGAGAGCCUGCCAAUCGAGGUCAUGGUGGACGCCGUGCACAAGCUGAAGUACGA GAACUACACGAGCAGCUUCUUCAUCAGGACAUCAAGAAGCCUGCCAAGAACCUGCAGCUGAAAACCCUUGAAA AACAGGCAGGUGGAAGUGCUUUGGGAGUACCCCGACACCUGGUCCACCCCCACAGCUUAGCCUGACCUUCU GUGUGCAGGUCCAGGGCGAAGAGGACAAGGCAGGGUGUCACCGACAAAACCGCGCCACCGUGAUCUG CAGGAAGAACGCCUCCAUCAGCCUGUCGGGCCCAGGACAGGUAUUACAGCUCGUGGGCGAGCAGUGGCCCC UGCUCCGGGGAGGCGGCGUGGCGAAGCCGGAAAUCUGCCCGGUGGCCACCCCCGGAUCCCGGCAUGAUCCCGUGUCUGCACC ACAGCCAGAACCUGCUGCGCCGUGAGCAACAUGCUGCAGAAGGCCCGCCAAACCCUGGAGUUCUACCCCUGUACCAAG CGAGGAGAUCGACCAUGAGGACAUUACCAAGGACAAGACCAGCACCGUGGAGGCCUGCCUGCCGUGAGCUCACAAAG AACGAAUCCUGCCUGGAUUAGCCAGCUUUAUCACGAACGGGGUCCUUGCCUCCAGCCGGAAGACAAGCUUCA UGAUGGCCUGUGCCUGGAGCAGCAUCUACAAGAAGUACCAAAAUGUGGAGUUCAAAACGAUGAACGCCAAGCU GCUGAUCGACCCCAAGCGCCAGAUCUUCCUGGACCAGAACAUGCUGGCCGUCAUCGACGAGCUCAUGCAGGCCCUGAAC UUCAACAGCGAGACCGUGCCCCAGAAGAGCUCCCUGGAGGAGCCCGACUUCUACAAGAGCACCAUCAAGCUCUGCAUCC UGCUGCACGCUUUCCGCACAUCCGCGCGGUGACCAUCGACCGGGUGAUGAGCUACCUGAACGCCAGUUGAUAAGGCUGG AGCCUGGUGGCCAUGAUCUUCUUGGCCUCCUUCCCCAGCGCCUUCGCCCUCAAAGACGAGACCAUCAAGGUCCAAAAC ACCAUUGUCACACUCCAGUUGAUAAGUCUGAGUGGGCGGCAAAAA

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	UACUGAAGGAUCAGAAGAGAGCCCAAGAACACAAGACCUUCCUGAGGUGCAGGGCCAAGAACUACUCCGGCGGCAUCACCUG
	UUGGUGGCUGACCACCAUCUCCACCGACCUUCAGCGUGAAGAGCAGCAGGGGCAGCAGCGACCCCCAGGGGGUG
	ACCUGCGGAGCCGUGACCUUGCUGGCCGAGGGGCGAGAAGGCGACAAUAAGGAGUACUGGGUCGAAUGCCAGG
	AGGACUCCGCCUGCCCGCCCCGGAGGAGUCCCUCCCAUCGAAGUGAUGGUGCACCCGUCCACAAGCUGAAGUACGA
	GAACUACCAGCAGCUUCUUCAUACGGGAUAUCAUCAAGCCCGACCCCCCCGAAGAACCUGCAGCUGAAGCCCUUGAAG
	AACUCCAGGGCAGUGUGGAGUACCCGGACACCUGGUCCACCCGGACAUACAUUCAGCCUCGACCUUCU
	GUGUACAGUGUCCAGUGCAAGAGCAAGAAAGAAGAAGUGUUCACCGACAAGACUCCGGCCACCGGUGAUCUG
	UCGGAAAACGCCAGCAUCUCCGUGCGGGCCCAGGACAGGUACUAUUCCAGCGACUGGAGCGAGUGGGCCUCCGUGCCC
	UGCUCCGGCGCGGUGGCUGGAAGGCAGCAGGAACCUCCCCGGGUGGCCACCCUGGAUCCCAUGCCUGCACC
	ACAGCCAAAACCGGCUGAGCCCCGUCUCCAAUAUGCUGCAGCAAGGCGAGGCAGACCCUGGAGAUUCUACCCCGUGUACCC
	CGAGGAGAUCGAACCACUGAGGAUAUCAUCAAGGACAAGACCUCCACGGUUCGAGGUGUCCUUGAGCCCCUGGAGCUCACGAAG
	AACGAGAGCUGCCUUAACUCCAGGGAAACUCUCGUUUAUCACGAACGGCAGCUUGCCUUGGCGUCAGCGAAGACCUCCUUUGA
	UGAUGGCCCUAUGUCUGUCUCGAUCGAGGAGCUGAAGAUGUACCAGGUGGAGUUCAAGACCAUGAAGCCAAGCU
	GCUGAUGGAUCCCAAGAGGCAGAUUUUCCUGGACAGAACAUGCUGGCCGUGAUUGACGAGCUGAUGCAGGCUGAAC
	UUCAACAGCGAGACGAGUGCCGCGAAGAGGCUCCCUGGAGGAGCCCGACUUUUACAAGAUAAGCUUGUGACAUCC
	UGCUCCACGCCCUUCAGAAUACGGCCCGUCACCAUCGAUGGUGAUGCUUACCUGACCUUCGACAUCUCCGGUUGG
	AGCCUCGGUGGCCAUGCUUCUUGGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCAAAC
	ACCAUUGUCACACUCCAGUGGCUCUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
hIL12AB_030 (SEQ ID NO: 124)	G*GGGAAUUAAGAGAGAAAACGAACAGUAAGAAGAAUAUAAGAGCCACCAUGUGCCACCAGCACUGGUUGAUUAGCUGG
	UUUAGCCUGGUGGUCCUGGCAACCCUCCUGGUGGCCAUCUGGGAACUGAAAAAGGACGUGUACGUGGUUGAGCUGGAUU
	GGUACCCCGACGCCCCCGGCGAAAUGGUGGUGCUCACGUGUGACACCCCCGAGGAGGACGGCAUCACCUGGACCCUGGA
	UCAGAGCAGCGAGGUGCUGGGCAGCGGCAAACACCCUGACCAUCCAGGUCAAGGAGUUCGGCGACGCUGGCCAGUACACC
	UGUCACAAGGGCGGCGAGGUGCUGAGCCACUCCCUGCUGCUCCACAAGAAGGAGGACGGCAUCUGGUCCACCGACAUCC
	UCCUCAAGGACCAGAAGGAGCCCAAGAACAAGACCUUCCUGCGCUGCGAGGCCAAGAACUACAGCGGCCGUUUCACCUGG
	CUGCCUCAGUGGUGCACAGCAUCGACUUCAGCUUCAAGUCCGGCGCCGAGGACCCUGCCGUGACCCUGGGCCCCAAGGGCGUG
	ACGUGGCCGCCGUGAGCGUGGGCUCUGCCUGCGUGCUGCUGCACAAGAAGGAGGACGGCAUCUGGUCCACCGACAUCCUG
	AGGGACCAGAAGGAGCCCAAGAACAAGACCUUCCUGAGAUGCGAGGCCAAGAACUACUCCGGCAGAUUCACCUGCUGG
	GAACUACACCAGCAGCUUCUUCAUCAGCGACAUCAAGCCCGACCCCCCCGAAGAACCUGCAGCUGAAGCCCCUGAAG
	AAUAGCAGGCAGGUGGAGGUGUCCUGGGAGUACCCGGACACCUGGAGCACCCCCACAGCUACUUCAGCCUGGACCUUCU
	GCGUGCAGGUCCAGGGCAAGAGCAAGCGGGAGAAGAAGGACCGCGUGUUCACGGACAAGACCAGCGCCACCGUGAUCUG
	CAGGAAGAACGCCAGCAUCAGCGUGAGGGCCCAGGAUAGGUACUACAGCAGCUGGAGCGAGUGGGCCUCCGUGCCC
	UGUUCCGGAGGCGGCUGGAAGGCAGCAGGAACCUCCCCGGGUGGCCACCCUGGAUCCCAUGCCUGCACC
	ACUCACAGAAUCUGCUGAGGCCCGUGACAAUAUGCUGCAGCAAGGCGAGGCAGACCCUGGAGAUUCUACCCCGUGUACCCC
	CGAGGAGAUCGAACCACUGAGGACAUCAUCAAGGACAAGACCUCCACGGUUCGAGGUGUCCUUGAGCCCCUGGAGCUCACGAAG
	AACGAGAGCUGCCUGAACUCCAGGGAAACUCUCGUUUAUCACGAACGGCAGCUUGCCUUGGCGUCAGCGAAGACCUCCUUCGA
	UGAUGGCCCUAUGUCUGUCUCGAUCACGAGGAGCUGAAGAUGUACCAGGUGGAGUUCAAGACCAUGAAGCCAAGCU
	CCUGAUGGACCCCAAGAGGCAGAUUUUCCUGGACCAGAACAUGCUGGCCGUGAUCGAUGAGUUAAUGCAGGCUGAAC
	UUCAACAGCGAGACGAGUGCCGCGAAGAGGCUCCUGGAGGAGCCCGACUUUUACAAGAUAAGCUUGUGACAUCC
	UCCUGCACGCCUUCCGAAUACGGCCGUCACCAUCGACGGUGAUGCUUACCUGACCUUCGACAUCUCCGGUUGG
	AGCCUCGGUGGCCAUGCUUCUUGGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCAAAC
	ACCAUUGUCACACUCCAGUGGCUCUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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hIL12AB_031 (SEQ ID NO: 125)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCACCAGCUCGUGAUCAGCUGG UUCUUGCCUGGUGUUUCUGGGCCUCCCCCUCGUCGCCAUCUGGGAAGCUGAAGAAAGACGUGUACGUGGUGGAGCUGGACU GGUAUCCCGACGCCCCCCCGGGGAGAUGUGGUGCUGACCUGCGACACCCCCGAAGAGGACGGCAUCACCUGGACGCUGGA CCAGUCGUCCGAAGUGCUGGGCUCUGGCAAGACCCUGACCAUCCAGGUGAAGGAGUUCGGAGACGCCGGCCAGUACACC UGUCAUAAGGGGGGUGAGGUCCUGAGCCACAGGCUCCUGCUGCCCCUGCACAAAAAGGAAGAUGGCAUCGUGGAAGCACCGAUA UCCUCAAGGACCAGAAGCAGCCCCAUGAACACAAGACGGUCCUCGAGGUGUGAGGCCAAGAACUACACAGCGGGCGGUCACGUG UUUGUGGCUCACCACCAUCUCCACCUUCUCCGUGAAGUCAAGCAGGGGCAGCUCCCGACCCCCCAAGGCGUC ACCUGCGGCGCCGCUCCAGCUGCCGAAGAGGGGCAGAUAACAAGGAAUACCAGUACAGUGUGGAGUGCCAAG ACGAUACGCCCUCUCCUGCCCAUCGAGGUCAUGGUGGACGCCGUGCACAAGCUCAAGUACGA GAACUACACCUCCAGCUUCUUCAUCAGGAUAUCAUCAAGCCCGAUCCCCCCAAGAACCUGCAGCUGAAGCCCCUGAAG AACAGCAGGUGGAGGUGAGCUGGGAGUAUCCCGACACGUGGAGCACCCCUCGUACUUCUCGCUGACCUUCU GCGUGCAGGUGCAAGGGAAGAGGAAGAGGAUAGGGUGUUCACGGACAAAACGGCCACGGUGAUCUGC CGGAAGAAUGCCAGCAUCUCCGUGAGGCCAGGCAUCUAUUCCAGCUCCUGGUCGCCAUGGGCCACGUGCCC UGUAGCGGCGGGGGCGGGGCCGCAGCAGGAACCUCCCGGUGCCACCCCCGGACCCCGGAAGAACCAGCUUCA ACUCGCAAAACCUGCUGCGCGGCUCUCCAACAUGCUGCAAAAAGCGCGCCAGACGCGUGGAGUUCUACCCCUGCAGCCAG CGAGGAGAUCGAUCAUGAAGAUCACCAAAGACAAGACCUCGUGCCAGGCCUGCCUGCCCCUGGAGCUCACCAAG AACGAAAGCUGCCUGAACAGCAAGGAGACAAGCUUCAUCACCAACGGCAGCUGCCUGCCCUCCGGAAGAACCAGCUUCA UGAUGCCCUGUGCCUUCAGCAUCUACGAGGAUCUGAAGAUGUACCAAGUGGAGUUCAAGACCAUGAACGCCAAGCU GUUAAUGGACCCCAAAAGGCAGAUCUUCCUGGAUCAGAACAUGCUGGCCGUCAUCGACGAGCUGAUGCAAGCCCUGAAC UUCAACAGCGAGACGGUGCCCCAGAAGAGCAGCCUCGAGGAGCCGGACUUCUAUAAGACCAAGAUAAAGCUCUGCAUCC UGCUGCACGCCUUCAGAAUCAGGGCCGUGACCAUCGAUAGGGUGAUGAGCGUACCUGAACGCCAGCUGAUAAGGCUGG AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCCAAAC ACCAUUGUCACACUCCAGUUGGCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG

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hIL12AB_ 032 (SEQ ID NO: 126)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCACCAGCAGCUGGUGAUUUCCUGG UUCAGUUCUGGUGAUUUCUUGGCAGCCCCCUGGUGGCCAUCUGGGAGCUGAAGAAAGACGUUAUACGUCGUGGAGCUGGACU GGUAUCCCGACGCCUCCCCGGAGAUGGUGGUCCUCACCUGCGACACCCCAGAGGAGGACGGCAUCACCUGGACCCUGGA CCAGAGCUCCGAGGUCCUGGGCAGCGGUAAGACCCUCACCAUCCAGGUGAAGGAGUUUGGCGAUGCCGGGCAGUAUACC UGCCACAAGGGCGGCGAGGUGCUGUCCCACAGCCUCCUGCUGCUACACUGCAUAAGAAGGAGGAUGGCAUCCUGGAGCACCGACA UCCUCAAGGACCAGAAGCAGCCCCAAGAACACAAGACCUUUCUGCGGUGCGAGCCAAAAAUUACGUCGGCCGGGUCACCUG CUGGUGGCUGACCACCAUCAGCAGUGACUGGACGUUCUCCGUGAAGUCGAGCAGGUGGAGCCCGAUCCCCAGGGCGUG ACCUGCGGCGCGCUCACCCUGAGCGCCGUCCGGGGGACAAUAAGGAAUACGAAUAUAGCGUGGAGUGCCAGGG AGGACAGCGCCUGCCCUGCGUCCCGAGGAGUCGAGGUGAGGUGAUGGUGCCGUCCCACAAGCUCAAAUACGA AAACUACACCAGCAGCUUCUUCAUUAGGGACAUCAUCAAGCCCGACCCCCCCAAAAACCUGCAGCUGAAGCCCCUGAAG AACAGCCGCCAGGUCGAGGUGUCGUGGGAGUACCCAGACACCUGGAGCACCCCCACUUCAGCCUGACCUUCU GCGUCCAGGUGCAGGGAAAGUCCAAACGGGAGAAGAAUAGGGUCUUUACCGAUAAGACGUCGCCACCGUCAUCUG CAGGAAGAACGCCAGAUAACGCUGUCGGGCGCAGGAUCGGCUACAGCUCGAGCUUCGAUGGGCCUCCCGUGCCC UGUAGCGAGGGGUGGCUGGGGCAGCCAGGAACCUGCCCGUGGCCACCCCGGACCCCGAGGUUUCCCUGCCUGCAUC ACAGUCAGAACCCUGAGGCCCGUGAGCAACAUGCUCCCAGAAGGCCCGCCAGACCCCGGGAGUUUUACCCCUGCACCAG CGAAGAGAUCGAUCACGAAGACAUCACCAAAGACAAGACCGUCCACCGCUGGAGGCCUGUCGCCCCUGGAGCUGACCAAG AACGAGAGCUGUCUGAACAGCAGGAGACCGUCCUCCAUCACCAACGGCUCCUGCCUGGCCAGCCGGAAGACCAGCUUCA UGAUGGCCCUGUGUCUGAGCUCUAUCUACGAGGACCUGAAGAUGUACCAGGUCGAGUUCAAGACCAUGAACGCCAAGCU GCUGAUGGACCCCAAGCGACAGAUAUUCCUGGACCAGAACAUGCUCGCCGUGAUCGAUGAACUGAUGCAAGCCCUGAAC UUCAACAGCGAGACCGUGCCCCAGAAAUCCUCGCUGGAGGAGCCGGACUUCUACAAGACCAAGAUCAAGCUGUGCAUAC UGCUGCACGCGUUCAGAUCCGCGCCGUCACCAUUGACCGUGUGAUGUCCUACCUGAACGCCUCCACCUGGAAUAAGGCUUCG AGCCUGUGGUGCCAUGAUUCUUCUUGGCCUCCUCCCCCAGCCCCUUCCUCCCCUUCCUGCACCCCCCAAAC ACCAUUGUCACACUCCAGUGGGCCUUUGAAAAGGUCUGAGGGCGGCAAAAA

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hIL12AB_ 033 (SEQ ID NO: 127)	G*GGGAAAUAAAGAGAGAAAAGAAGAGUAGAAGAAAUAUAAGAGCCACCAUGUGCCACCAGCAGCUCGUGAUUAGCUGGUUUUCCGCCUGGUUCCUGGCCAUCGUGGGCCAGCCACUCGCCAUCGCCCUGUCCGGCGAAGCCGCCGUGAUCCAGGACUCCGCCGUGUCCGUGCAGGAGGGCCUGUGCCACCUGCAGUGCACCUACCAGCACCUGAGGGGCUACCUGGCCGUGGUCCACUCCCAGGCCCGGGACCUGGCCGACGACCAGGCCGAGCCCGAGCCCCUGCCCGACGUGCACACCGUGACCGGCCUGAGCGCCGUGACCCUGACCUGGUACGAGCCCCUGGUGCUGGGCGCCGAGGACCUGGUGUACGAGGUGACCGUGCUGAGGGAGGACAAGCGGGGCAACAGGCUGGAGUUCGCCCUGCCCGACGGCACCGCCUUCAGCGUGCGGGGCCUGAAGCCCGAGAAGGAGUACCACGUGCAGGCCCGGGCCUACGUGAGGGAGGGCGAGCGGAGCGCCCAGGUGGUGUUCAAGACCCAGCCCCAGGCCGCCGAGGCCGAGGCCGCCGCCGCCGCCGACCUGAGCGGCGCCGUGCUGAGCGUGCCCGGCCCCAGCCUGCCCGGCGAGAAGCCCGUGAUCCUGGACCUGCCCGAGCAGCCCGGCGGCCAGCGGCCCGCCUGCCCCGCCGCCGACGCCGCCGCCAAGGACGUGGCCUGCACCGAGUUCGCCGCCGACAUCUACGCCGCCCUGGCCGAGUACAACAGCGUGAGCGACCAGGAGAUCAGCCUGGACAACCACGUGAUCCCCCUGGAGAACAGCUACACCUUCACCGUGGACAACGGCAAGAACCUGGAGCUGAUCGAGGCCAACAUCGAGCCCUUCCUGGCCUUCGCCAGAGACAUCGAGAGCAUGGACUUCGCCAAGAACCCCCUGGGCGAGUUCGCCAAGCUGAUCGGCCAGCCCGCCCUGCCCUUCGUGACCCCCGGCAAGCCCCAGGCCGGCCAGGCCACCCCCAACAGCAGAGGCAAGAGAAACAAGGGCCCCUACCCCGACGCCCUGAUCCCCGACCUGGCCCCCGAGGCCCCCGAGAUCGUGCACGUGCAGAGCGGCCUGACCCCCGGCCUGCAGUACAGCGUGGGCCAGGAGGACGAGAGCUUCGUGCUGCGGCUGCACGGCCUGUUCGCCUGGCUGGCCAAGGAGGGCAGCCUGGCCACCGAGUGGGCCGGCAGCGGCCCCCAGCCCGCCGAGAGCGCCGAGCCCGAGAGCGCCUUCCCCGAGGUGCCCCCCCUGGCCAGCCUGUCCGGCCACCACCUGGCCUACGUGACCUUCCUGCUGCCCGAGGCCAAGUACAUCCCCGAGUUCAAGCUGAGCAACGAGAUCAUCAAGGCCGGCAGGCUGGAGGACCUGGUGCCCGGCAGCAUCGCCGAGGUGCUGACCCAGCCCCUGCACGAGUGCAUCACCGGCACCACCGUGAACGCCCCCCUGUUCAAGGAGGGCGACUACGCCGUGCGGAUGAAGCUGAGCAUCAACGACGAGGUGAGCUUCAGCUACCAGGUGACCGUGAACGACGGCCCCAGGUUCCACCAGGGCAGCCUGAGCGCCGGCGUGCAGGACGAGACCGCCGCCGCCAGCGCCAGCGGCGACGUGGAGCUGCUGAUCAACGUGGACGACAGCGGCAGCUUCUGCGUGAGCCUGGCCGAGGCCAGCGGCAUGAUCGCCCCCAUCGGCAAGGUGAUCGACAGCAUCACCCAGAGCGCCAAGGCCGUGUGCAAGGUGUUCCACAAGUG

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	UGAUGGCCCUUGUCUGAGCUCCAUCUAUGAGACCUGAAAAUGUACCAGGUCGAGUUCAAAACCAUGACGCCAAGCCU
	UCUGAUGGAACCCCAAGAGCGCAGUCUUCCUUGGAUCAGAUGACAUGCUGGCCGUGAUCGAUGCCAGGCCCUGAAC
	UUUAACGUCCGAGACCGUGCCCCAGAAAAGCAGCCUGGAAGAGCCCGAUUUUUACAAAACGAAGAUCAAGCUGUGCAUCC
	UGCUGCACGCCUUCCGGAUCCUUGCGGUGACAUCGAUAGGGUGAUGAGCUACCUGAACGCCAGCUGAUAUAAUAGGCUGG
	AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCGCCCCCUCCUGCACCGAGAGUGCACCAUACCACDUCAAC
	ACCAUUGUCACACUCCAGUGGGCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG
hIL12AB_ 035 (SEQ ID NO: 129)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAUAAGAGCCACCAUGUGCCACCAACAGCUGGUAAUCAGCUGG
	UUCAGCCUGGUUUUCCUGCUUCGCGCCCCUGGUGGCCAUCUGGGAGUUAAGAAGGAAGUGUACGUGGUGGAGCUGGAUU
	GCUACCCCGACGCCCCCGGCGAGAUGGUCGUGCUCACCUGCGAUACCCCUGAGGAAGAUGACCACAUCGAGUGGACCCUGGA
	CCAAUCCAGCGAGGUGCUGGGCAGCGGCAAGACCCUGACCAUACAGGUGAAGGAGUUCGGCGACGCCGGCCAGUACACC
	UGCCACAAGGGCGGCGAAGUGCUGUCCCACUCCCUGCUGCUGCACAAGAAGGAAGACGGCAUCUGGAGCACCGACA
	UCCUGAAGGACCAGAAGGAACCCAAGAACAAGACCUUCCUGAGAUGCGAAGCCAAGAACUACUCCGGCCGCUUCACCUG
	UGGUGGCUGGACCACCAUCUCCACCGAUCUGACCUUCAGCGUGAAGAGCAGCAGAGGCUCUCCCGACCCCCAGGGCGUG
	ACCUGCGGCGCCGCCGACCUGAAGGGCGUGCGGAGCUUCCAGAUCAGCAGCCUGGAGAACUACAAGGACCUGCAGC
	AGGACAGCGCCGGCUACGGCAGCGCCUGCGCCAUCGAGGAGGACGACCUGCCCAAGGCUACGAAGGUACGA
	GAACUACACCAGCUUCCAUCAGGGACAUCAUCAAGCCGGAACCCCCCCAAGAACGGCAGCUGAAGCCCCUGAAG
	AACAGCGUGGCCGAAGAGGCCUGGGAGUACUCCGACACCUGGAAGCCCUACAGCAUGUCCUACCUGAGCCUGUGGUU
	GCGUGCAGGUUCAGUGCCAAGAGGGAAAAGAAGAGGACCGGGUGUUCACCGAUAAGACGAGCGCCACCGUGAUCUG
	CAGGAAGAACGCCUCCAUAAGCGUGAGGGCGAGGACCGUUACUACAGCAGCAGCUGGAGAGUGGGGCAAGCGUGCCC
	UGUAGCGGCGGGGCCAGGGCGGUCCCGCAACCUCCCCGUCGCCACCCCUGACCUCAGGUUUCCGUGGCUGCACC
	ACAGCCAGAACCUGCUGGCCCCGUUAGCAACAUGCUGCAGAAGGCCAGACGACCCUCGAGUUCCUGCACAUC
	UGAGGAGAUCGACCACAUAAGACAUCACUUAAGGAUAAGACCUCCACCGUGCAGGCCUUGCCCCUCGAGCUGACCAAG
	AAUGAAUCCUGCCUGAACAGCCCAGAGACCAGCUUUAUCACCAACGGCUCCUGCCUUGCCUCCAGAAGACUCCUUCA
	UGAUGGCCCUGUGCCUCUCCAGCAUCUACGAGGAUCUGAAGAUGUACCAGGUAGAGUUCAAGACCAUGAACGCCAAGCU
	CCUGAUGGACCCCAAGAGGCAGAUAUUCCUGGACCAGAACAUGCUGGCGGUGAUCGACGAGCUGAUGCAGGCCCUGAAU
	UUCAACAGCGAGACGGUGCCACAGAAGUCCAGCCUGGAGGAGCCAGAACUUUAUAAGACCAAGAUCAAGCUGUGCAUCC
	UCCUGCACGCGUUCAGGAUCCGCGCCGUCACCAUAGACAGAGUGAUGAGCUACCUGAACGCCAGCUGAUAAUAGGCUGG
	AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCGCCCCCUCCUGCACCGAGAGUGCACCAUACCACDUCAAC
	ACCAUUGUCACACUCCAGUGGGCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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hIL12AB_036 (SEQ ID NO: 130	G*GGGAAAUAAAGAGAGAAAAGAAGUAAGAAGAAAUAUAAGAGCCACCAUGUGCCAUCAGCAGCUGGUAAUCAGCUGG UUUAGCCUGGUGUUCCUGGCCAGCCCCACUGGUGGCCAUCUGGGAGCUGAAGAAGGACGUGUACGUGGUGGAACUGGACU GGUACCCCGACGCCCCUGGCCGAGAUGGUGUCCUUCGACACCCGCGAGCCGAGACUGGCAUCACCUGGACCCUGGA UCAGAGCUCCGAGCUUGCCUGGCCAAGACACUGACCAUCCAAGUUAAGCAAUUUGGGACGCCGGCCAGUACACC UGCCACAAGGGGCGAGGUCCUGUCCCACUCCCUGCUGCUUCUGCAUAAGAAGGAAGAUGGCAUCCUGUCCACCGACA UACUGAAGGACCAGAAGCAGUCCACAUAAUAAGACCUUCCUGAGAUGCCAGGCUACGUACCGGGAGAGGCUUCACCUG CUGGUGGCUGACCACAUAAUCAGCUUUCCUCCGUGAAGAGCUCUUCCGGCCCGAUCCCCAGGGCCUA AGCUGUGGGGCGCUACCCUGCUGCCGAGAGGGUCCGGGGCGACAACAAGGAAUACGAGUACAGCGUGGAGUGCCAGGG AGGACUCCGCGCCUGCCCCGAGGAGUCGCUGCCCAUAGAGGUGAUGGUGGACGCCGUGCACAAGCUCAAGUACGA GAAUUACACCAGCAGCUUCUUCAUCAGGACAUAAUUAAGCCUGACCCCCCAAAGAAUCGCAGCUGAAGCCCCUGAAG AAUAGCCGGCAGGUGGAAGUGCUGGAGUACCCCGACACCUGGAGCACCCCCACUCCUUCUCUCACUGACAUUCU GCGUGCAGGUGCAAGGGAAAGGCAAGAGAAGGAUAGGGUGUCACCGACAAGACAAGCGCCACCGUGAUCUG CCGAAAAAAUGCCAGCAUCAGCCUGAGCGCCCAGGAUCGGCUAUUACAGCAGCUCCUGGAGCGACAGUGGCCCC UGUUCCGGCGAGGGGCGCUCCCGGAACCUGCCGGUGGCCACCCCCGACCCUGGCAUGAUUCCAAUGCCAAGCU ACAGCCAGAACCCUGCCGCCCGUGUUCGAACAUCGCUGGCACAAGGCCCGGACACCCCUGCACCAG CGAAGAGAUCGACCACAUAACCAAGGACAAGACCAGCACGGUGGAGGCCCUGCCUGGCCUUACCAAA AACGAGUCCUGCCUGAACAGCCGGGAAACCAGCUUCAUAACGAACGGGAGCUUGCCUAUCCCAGGAAGACCAGCUUC UGAUGGCGCUGUGUCUCCAGCCAUAUACGAAGAUCUGAACAUGUACAGCUUCGAAUGCCAAGCU CCUGAUGGACCCCCAAGAGGCAGAUCUUCCUGGACCAGAACAUGCUAGCCGUGAUCGACGAGCUCUUCAAC UUCAACUCGGAAGACGUGUCCCAGAAGUCCAGCCUCGAGGAGCCCGAGUUCUUCUACAAGAUCAAGCCAAGAUCAAGCUGAUAC UGCUGCAUGCCUUCAGGAUAGGGCGGUGACUAUCGACAGGGUCAUGUCCUACCUGAACGCCAGCUGAUAUAGGCUGG AGCCUGGGUGGCCAUGAUCUUCUUGGCCUCCUCCCCCAGCCCCUCCUCCCCUUCCUUGCACCCGUACCCCCAAAC ACCAUUGUCACACUCCAGUGGGCUUUGAUAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAA

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	GUGUGCAGGUGCAGGGGAAGUCCAAGAGGAAGGAGACUGGGGUGUCACCGACAAGACCAGGGCCACCGUGAUG
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	UGCAGCGGUGGAGGGGGCUCCAGGAAUCUGCCGGUGGCCACCCCCGGACCCCGGGAUCUUCCCGUGUCUGCAUC
	ACUCCCAGAACCUGCUGGCCGUGAGCAAUAUGCUGCAGAGCCAGGCGACAGCCUCCAGAGUUCUACCCCUGCACCUC
	CGAAGAGAUCGACCAUGAGGACAAUCAGACCAAGGACAAGACCAGCACCGUGGUGCAGGCCUACCUCGACGCCCUC
	AACGAGAGCUGCCUGAACUCCAGGGAGACCAGCUUUAUAACCAACGGCAGCUGCCUCGCCCAGGGAAGACCUCGUUA
	UGAUGGCCCUCUGCUCCAGCAUCUACGAGGACCUGAAGAUGUACCAGGUGGAGUUCAAGACCAUGAACGCGAAGCU
	GCUCAUGGACCCCAAGAGGCAGAUCUUCCUGGACCAGAACAUGCUGGCGGUGAUCGACGAGCUGAUGCAAGCCCUGAAC
	UUCAACAGCGAGACCGUGCCCCAGAAGAGCCCGGACUUUUACAAGACCAAGAUCAAGCUGUGCAUCC
	UGCUGCACGCCUUCCGGAUCCGGGCCGUGACCAUCGACAGGGUGAUGAGCUACCUCAACGCCUCCCUGAUAAUAGGCUGG
	AGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCUUCCUGCACCCCCCAAAC
	ACCAUUGUCACACUCCAGUGGAUCUUUGAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA
	AAAAAAAAAAAAAAAAAAAA

(continued)

hIL12AB_ 039 (SEQ ID NO: 133)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAGAAGAAAUAAUAAGAGCCACCAUGUGCCAUCAGCAGCUCGUCAUCUCCUGG UUUAGCCUGGUGUUUCUGGCCUCCCCCCUGGUCGCCAUCUGGGAGCUGAAGAAAGACGUGUACGUUGUGGAGCUGGACU GGUACCCGGACGCCCCCGGGGAGAUGGUGGUCCUCACCUGCGACACCCCCGAGGAGGACGGCAUCACCUGGACCUGGA CCAGAGCUCCGAGGUCCUGGGAGCUGCAAGAACCCUGACCAUUCAGGUGAAAGAGUUCGGCGACGCCGGCCAAUAUACC UGCCACAAGGGAGGCGAGGUCCUUAGCCAUUCCCUGCUGCUGCUUCACAAAAAGGAAGAUGGCAUCCUGGAGCAGCCGA UCCUGAAGAACCAGAAAGAAUCCCUAGACAAGAGCGUUCCUGCGCUGCGAGGCCAAGAGUACUCUGCGCGGUUCACCUG UGGCUGACCGUGGACCACCUUGACUUCUCCUCGAAGAGCAGCUAGUCUCCUGGAAGCCGUGAAUGAUCUGUUCCUCGU ACCUGCGGCGGCCAGCCUGAAGGGUGAGGGCGACAAUAAAGAGUACGAGUAUCCGUGGAGUGCCAGGAGGCCAGG AGGACAGCGCCUGUCUUCGCCUCGAGGUGAGGUGAUGGUCGACGCGGUGCACAAGCUCAAGUUCGCGAGCUACGA AAACUACACCAGCAGCUUUUUCAUCAGGAUAUCAUCAAACCAGAACCCCCCCAAGAACCUGCAGCUGAAGCCCCUGAAA AACAGCGAGGCAGAGUUGGAAGUCGACUGGAGUAAAGAUCGGGUGUCACGCGACAAGCCAGCGCCGACCGCUUCCU GCGUGCAGGUGCAGGGGAAGUCCAAGGAGAAAGAUCGGGUGUCACGCGACAAGCCAGCGCCGACCGCUUCCUGAUUUG CAGGAAAACGCCAGCAUCUCCCUGAGGCUCAGGACAGGCUACUACAGCUCCCAGCCGGGAGCGAGUGGGCUUCCGUGCCU UGCAGCGGGGAGGAGCGGCGCCAGCAGGAAUCUGCCCGGUCGCAACCCCCGGACCCCGGCAUGAUUCCCCUGCCUGCACC ACAGCCAGAAUCUGUCUGACCCGUGAGCAACAUGCUCCUCCAGAAGCCCCGGCAGACGCCUGGAGUUCUACCCCUGCACCUC CGAGGAGAUCGACCACGAGGACAUCACCAAGGAUAAGACGAGCACCGUCGAGGCCUGUCUGCCCCUGGAGCUCACCAAG AACGAGUCCUGCCUGGCUGAAUAGCAGGAGACGGUCCUUCAUAACCAACGGCAGGUUGUUGGCCAGGAAGACCAGCUUCA UGAUGGCCCUCUGCCUGAGCUCCAUCUACGAGGACCUCAAGAUGUACCAGGUCGAGUUCAAGACCAUGAACGCAAACUU GCUCAUGGAUCCAAAGAGGCAGAUCUUCUUGGACCACAUGCUGGCCGUGAUCGAACUCAUGCAGGCCCUGAAU UUCAAUUCCGAGACCGUGCCCCAGAAGAGCUCCCUGGAGGAACCCGACUUUUACAAAACAAGAUCAAGCUGAUAAGGCUG UCCUGCACGCCUUCGGAUCAGGGCGUCACAUUGACCAUUGAGGUGAUGUCCUACCUGAACGGCGAGCUGAUAAGGCUG AGCCUGGUGGCCAUGCUUCUUCUGGCCUCCCCCCAGCCCCUCCUCCCCCUUCCUGCACCCCCAAAC ACCAUUGUCACACUCCAGUUGGUCUUUGAUAAGUCUGAGUGGGCGGCAAAAA

(continued)

	UCCUGAAGGAUCAGAAGGAGCCCAAGAACACAAGACCUUUCUGAGAUGCGAGGCCAAGAAUUACAGCGGCCGGUUCACCUG
	CUGGUGGCUCACCACCAUCAGCAUCCGACCUUCAGCGUGAAAAUCCUCCAGGGGCUCCUCCGACCCGCAGGGAGUG
	ACCUGCGGCGCCACACUGAGUGCCGAGGGGUCAGAGGGGACACAAGGAGUACAGCGUUUGAGUGCCAGG
	AGGACAGCGCCUGUCCUGCCCCGAGGAAUCCCUGCCCAUCGAGGUGAUGGUCGACGCCAGUGCACAAGCUGAAGUACGA
	GAACUAUACCUCGAGCUUCUUCAUCGGGAUAUCAUUAAGCCCGAUCCUCCCGAAGAACCUGCAGCUCAAACCCCUGAAG
	AACAGCAGGGCAGGUGGAGUACCCUGGGAGUACCCGACACAUGGUCCACCCCCCAUUUCGCCCCUGACGUUU
	GCGUGCAGGUGCAGCCAACAGAGGAAAAAGGACAGGGUGUUCACCGACAAGACUCCGGCCAGCGUGAUCUG
	CCGUAAGAACGCUACGUCAGCGUCAGGGCCAGGACAGGUACUAUAGCAGCUCCUGGUCCGAGUGGGCCAGCGUCCCG
	UGCAGCGGCGGGUGGAGGGCUCCCGGAACCUCCCGGGCCACCCCGGGAUGUUCCCUGCCUGCAUC
	ACAGCCAGAACCUGCUGAGGCCCGUGUCCAACAUGCUGCAGAAGGCCAGACGCACAUCGAGUUUACCCCGUGCACCAG
	CGAGGAGAUCGACCACUGAAGACAUCACCAAGGACAAGACCGUCCACCGUGGAGGCAUGCCUGGAGCUGACCAAA
	AACGAAAGCUGCCUGAACUCCACGGAUGACCUGCCUUUAUCACGAACGGCUCAUGCCUUGGCCUCAGAAAGACCAGCUUCA
	UGAUGGCCCUGUGCCUGAGCUCCAUCUACGAGGACUUGAAAAUGUACCAGGUCGAGUUCAAGACCAUGAACGCCAACU
	GCUCAUGGACCCCAAAAGGCAGAUCUUCUGGACCAGAAUAUGCUGGCUGUGAUCGACUGAGCUCAUGCAAGCCCUGAAU
	UUCAACAGCGAGACCGUGCCCCAGAAGUCCUCCCUGGAGGAGCCCGACUUCUACAAGACCAAGAUCAAGCUGUGCAUAC
	UCCUGCACGCGCUUUAGGAUCAGCGGCGGUGACCAUCGAUAGGGUGAUGAGCUACCUGAAUGCCUCGAUAUAGGCUGG
	AGCCUCGGUGGCCAUAUUCUUGGCCUCCUUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCAAAC
	ACCAUUGUCACACUCCUGAGUGGUCCUUUGAUAAAGUCUGAGUGGGCGGCAAAAA

Table 4D. mRNA ORF Sequence

> hIL12AB_002(SEQ ID NO: 237)

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AUGUGCCACCAGCAGCUGGUGAUCAGCUGGUUCAGCCUGGUGUUCCUGGCCAGCCCCCUGGUGGCCAUCUGGGAGCUGAAGAAGGACG
GUACGUGGUGGAGUUGGAUUGGUACCCCGACGCCCCCGGCGAGAUGGUGGUGCUGACCUGCGACACCCCCGAGGAGGACGGCAUCACC
GGACCCUCGGACCAGAGCAGCGAGCUGCUGGGCAGCGGCAAGACCCUGACCAUCCAGGUGAAGGAGUUCGGCGACGCCGGCCAGUACAC
UGCCACAAGGGCGGCGAGGUGCUGAGCCACAGCCUGCUGCUGCACAAGAAGGAGGACGGCAUCUGGAGCACCGACAUCCUGAAGG
CCAGAAGGAGCCCAAGAACAAGACCUUCCUGAGAUGCGAGGCCAAGAACUACAGCGGCAGAUUCACCUGCUGGUGGCUGACCACCAUC
GCACCGACCUGACCUUCAGCGUGAAGAGCAGCAGAGGCAGCAGCGACCCCCAGGGCGUGACCUGCGGCGCCGCCACCCUGAGCGCCGA
AGAGUGAGAGGCGACAACAAGGAGUACGAGUACAGCGUGGAGUGCCAGGAAGAUAGCGCCUGCCCCGCCGCCGAGGAGAGCCUGCCCA
CGAGGUGAUGGUGGACGCCGUGCACAAGCUGAAGUACGAGAACUACACCAGCAGCUUCUUCAUCAGAGAUAUCAUCAAGCCCGACCCC
CCAAGAACCUGCAGCUGAAGCCCCUGAAGAACAGCCGGCAGGUGGAGGUGAGCUGGGAGUACCCCGACACCUGGAGCACCCCCCACAG
UACUUCAGCCUGACCUUCUGCGUGCAGGUGCAGGGCAAGAGCAAGAGAGAAGAAAGAUAGAGUGUUCACCGACAAGACCAGCGCCA
CGUGAUCUGCAGAAAGAAGCCAGCAUCAGCGUGAGAGCCCAAGAUAGAUACUACAGCAGCGCAGCUGGAGCGAGUGGCCAGCGUGCC
GCAGCGGCGGCGGCGGCGGCGGCAGCAGAAACCUGCCCGUGGCCACACCCCGACCCCGGCAUGUUCCCCUGCCUGCACCACAGCCAGAA
CUGCUGAGAGCCGUGAGCAACAUGCUGCAGAAGGCCCGGCAGACCCUGGAGUUCUACCCCUGCACCAGCGAGGAGAUCGACCACGAAG
UAUCACCAAAGAUAAGACCAGCACCGUGGAGGCCUGCCUGCCCCUGGAGCUGACCAAGAACGAGAGCUGCCUGAACAGCAGAGAGACC
GCUUCAUCACCAACGGCAGCUGCCUGGCCAGCAGAAAGACCAGCUUCAUGAUGGCCCUGUGCCUGAGCAGCAUCUACGAGGACCUGAA
AUGUACCAGGUGGAGUUCAAGACCAUGAACGCCAAGCUGCUGAUGGACCCCAAGCGGCAGAUCUUCCUGGACCAGAACAUGCUGGCCG
GAUCGACGAGCUGAUGCAGGCCCUGAACUUCAACAGCGAGACCGUGCCCCAGAAGAGCAGCCUGGAGGAGCCCGACUUCUACAAGACC
AGAUCAAGCUGUGCAUCCUGCUGCACGCCUUCAGAAUCAGAGCCGUGACCAUCGACAGAGUGAUGAGCUACCUGAACGCCAGC
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[0229] The sequence-optimized nucleotide sequences disclosed herein are distinct from the corresponding wild type nucleotide acid sequences and from other known sequence-optimized nucleotide sequences, *e.g.*, these sequence-optimized nucleic acids have unique compositional characteristics.

[0230] In some cases, the percentage of uracil or thymine nucleobases in a sequence-optimized nucleotide sequence (*e.g.*, encoding an IL12B and/or IL12A polypeptide, a functional fragment, or a variant thereof) is modified (*e.g.*,. reduced) with respect to the percentage of uracil or thymine nucleobases in the reference wild-type nucleotide sequence. Such a sequence is referred to as a uracil-modified or thymine-modified sequence. The percentage of uracil or thymine content in a nucleotide sequence can be determined by dividing the number of uracils or thymines in a sequence by the total number of nucleotides and multiplying by 100. In some cases, the sequence-optimized nucleotide sequence has a lower uracil or thymine content than the uracil or thymine content in the reference wild-type sequence. In some cases, the uracil or thymine content in a sequence-optimized nucleotide sequence of the disclosure is greater than the uracil or thymine content in the reference wild-type sequence and still maintain beneficial effects, *e.g.*, increased expression and/or reduced Toll-Like Receptor (TLR) response when compared to the reference wild-type sequence.

[0231] In some cases, the optimized sequences of the present disclosure contain unique ranges of uracils or thymine (if DNA) in the sequence. The uracil or thymine content of the optimized sequences can be expressed in various ways, *e.g.*, uracil or thymine content of optimized sequences relative to the theoretical minimum ($\%U_{TM}$ or $\%T_{TM}$), relative to the wild-type ($\%U_{WT}$ or $\%T_{WT}$), and relative to the total nucleotide content ($\%U_{TL}$ or $\%T_{TL}$). For DNA it is recognized that thymine is present instead of uracil, and one would substitute T where U appears. Thus, all the disclosures related to, *e.g.*, $\%U_{TM}$, $\%U_{WT}$, or $_{\%}U_{TL}$, with respect to RNA are equally applicable to $\%T_{TM}$, $\%T_{WT}$, or $_{\%}T_{TL}$ with respect to DNA.

[0232] Uracil- or thymine- content relative to the uracil or thymine theoretical minimum, refers to a parameter determined by dividing the number of uracils or thymines in a sequence-optimized nucleotide sequence by the total number of uracils or thymines in a hypothetical nucleotide sequence in which all the codons in the hypothetical sequence are replaced with synonymous codons having the lowest possible uracil or thymine content and multiplying by 100. This parameter is abbreviated herein as $\%U_{TM}$ or $\%T_{TM}$

[0233] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12B polypeptide of the disclosure is below 196%, below 195%, below 190%, below 185%, below 180%, below 175%, below 170%, below 165%, below 160%, below 155%, below 150%, below 145%, below 140%, below 139%, below 138%, below 137%, below 136%, below 135%, below 134%, below 133%, below 132%, below 131%, below 130%, below 129%, below 128%, below 127%, below 126%, below 125%, below 124%, below 123%, below 122%, below 121%, below 120%, below 119%, below 118%, below 117%, below 116%, or below 115%.

[0234] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12B polypeptide of the disclosure is below 196% and above 100%, above 101%, above 102%, above 103%, above 104%, above 105%, above 106%, above 107%, above 108%, above 109%, above 110%, above 111%, above 112%, above 113%, above 114%, above 115%, above 116%, above 117%, above 118%, above 119%, above 120%, above 121%, above 122%, above 123%, above 124%, above 125%, above 126%, above 127%, above 128%, above 129%, or above 130%, above 135%, above 130%, above 131%, or above 132%.

[0235] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12B polypeptide of the disclosure is between 132% and 150%, between 133% and 150%, between 134% and 150%, between 135% and 150%, between

136% and 150%, between 137% and 150%, between 138% and 150%, between 139% and 150%, between 140% and 150%, between 132% and 151%, between 132% and 152%, between 132% and 153%, between 132% and 154%, between 132% and 155%, between 132% and 156%, between 132% and 157%, between 132% and 158%, between 132% and 159%, between 132% and 160%, between 133% and 151%, between 134% and 152%, between 135% and 153%, between 136% and 154%, between 137% and 155%, between 138% and 156%, between 138% and 157%, between 139% and 158%, between 140% and 159%, or between 141% and 160%.

[0236] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12B polypeptide of the disclosure is between about 133% and about 152%, *e.g.,* between 132.32% and 150.51%.

[0237] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12A polypeptide of the disclosure is below 198%, below 195%, below 190%, below 185%, below 180%, below 175%, below 170%, below 165%, below 160%, below 155%, below 150%, below 145%, below 140%, below 139%, below 138%, below 137%, below 136%, below 135%, below 134%, below 133%, below 132%, below 131%, below 130%, below 129%, below 128%, below 127%, below 126%, below 125%, below 124%, below 123%, below 122%, below 121%, below 120%, below 119%, below 118%, below 117%, below 116%, or below 115%.

[0238] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12A polypeptide of the disclosure is below 198% and above 100%, above 101%, above 102%, above 103%, above 104%, above 105%, above 106%, above 107%, above 108%, above 109%, above 110%, above 111%, above 112%, above 113%, above 114%, above 115%, above 116%, above 117%, above 118%, above 119%, above 120%, above 121%, above 122%, above 123%, above 124%, or above 125%.

[0239] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12A polypeptide of the disclosure is between 125% and 143%, between 126% and 143%, between 127% and 143%, between 128% and 143%, between 129% and 143%, between 130% and 143%, between 131% and 132%, between 133% and 134%, between 135% and 143%, between 125% and 144%, between 125% and 145%, between 125% and 146%, between 125% and 147%, between 125% and 148%, between 125% and 149%, between 125% and 150%, between 125% and 151%, between 125% and 152%, between 125% and 153%, between 125% and 154%, between 125% and 155%, between 126% and 144%, between 127% and 145%, between 128% and 146%, between 129% and 147%, between 130% and 148%, between 131% and 149%, between 132% and 150%, or between 133% and 151%.

[0240] In some cases, the $\%U_{TM}$ of a uracil-modified sequence encoding an IL12A polypeptide of the disclosure is between about 124% and about 145%, e.g., between 125% and 144.42%.

[0241] A uracil- or thymine-modified sequence encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides of the disclosure can also be described according to its uracil or thymine content relative to the uracil or thymine content in the corresponding wild-type nucleic acid sequence ($\%U_{WT}$ or $\%T_{WT}$).

[0242] The phrases "uracil or thymine content relative to the uracil or thymine content in the wild type nucleic acid sequence," refers to a parameter determined by dividing the number of uracils or thymines in a sequence-optimized nucleic acid by the total number of uracils or thymines in the corresponding wild-type nucleic acid sequence and multiplying by 100. This parameter is abbreviated herein as $\%U_{WT}$ or $\%T_{WT}$.

[0243] In some cases, the $\%U_{WT}$ or $\%T_{WT}$of a uracil- or thymine-modified sequence encoding an IL12B polypeptide of the disclosure is above 50%, above 55%, above 60%, above 65%, above 70%, above 75%, above 80%, above 85%, above 90%, or above 95%.

[0244] In some cases, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine modified sequence encoding an IL12B polypeptide of the disclosure is between 55% and 88%, between 56% and 87%, between 57% and 86%, between 58% and 85%, between 59% and 84%, between 60% and 83%, between 61% and 82%, between 62% and 81%, between 63% and 80%, between 64% and 79%, between 65% and 78%, or between 65% and 77%.

[0245] In some cases, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine-modified sequence encoding an IL12B polypeptide of the disclosure is between 66% and 78%, between 66% and 77%, between 67% and 77%, between 67% and 76%, or between 65% and 77%.

[0246] In a particular case, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine-modified sequence encoding an IL12B polypeptide of the disclosure is between about 66% and about 77%, e.g., between 67% and 76%.

[0247] In some cases, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine-modified sequence encoding an IL12A polypeptide of the disclosure is above 50%, above 55%, above 60%, above 65%, above 70%, above 75%, above 80%, above 85%, above 90%, or above 95%.

[0248] In some cases, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine modified sequence encoding an IL12A polypeptide of the disclosure is between 50% and 85%, between 51% and 84%, between 52% and 83%, between 53% and 82%, between 54% and 81%, between 55% and 80%, between 56% and 79%, between 57% and 78%, between 58% and 77%, between 59% and 76%, between 60% and 75%, between 61% and 74%, or between 62% and 73%.

[0249] In some cases, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine-modified sequence encoding an IL12A polypeptide of the disclosure is between 61% and 74%, between 61% and 73%, between 61% and 72%, between 61% and 73%, between 62% and 73%, between 62% and 72%, between 62% and 74%, or between 63% and 72%.

[0250] In a particular case, the $\%U_{WT}$ or $\%T_{WT}$ of a uracil- or thymine-modified sequence encoding an IL12A polypeptide of the disclosure is between about 62% and about 73%, *e.g.,* between 63% and 72%.

[0251] The uracil or thymine content of wild-type IL12B relative to the total nucleotide content (%) is about 21%. In some cases, the uracil or thymine content of a uracil- or thymine- modified sequence encoding an IL12B polypeptide relative to the total nucleotide content (%) ($\%U_{TL}$ or $\%T_{TL}$) is less than 21%. In some cases, the $\%U_{TL}$ or $\%T_{TL}$ is less than 20%, less than 19%, less that 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, or less than 10%. In some cases, the $\%U_{TL}$ or $\%T_{TL}$ is not less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

[0252] In some cases, the uracil or thymine content of a uracil- or thymine-modified sequence encoding an IL12B polypeptide of the disclosure relative to the total nucleotide content ($\%U_{TL}$ or $\%T_{TL}$) is between 10% and 20%, between 11% and 20%, between 11.5% and 19.5%, between 12% and 19%, between 12% and 18%, between 13% and 18%, between 13% and 17%, between 13% and 16%, between 13% and 16%, between 14% and 16%, between 14% and 17%, or between 13% and 17%.

[0253] In a particular case, the uracil or thymine content ($\%U_{TL}$ or $\%T_{TL}$) of a uracil- or thymine modified sequence encoding an IL12B polypeptide of the disclosure is between about 13% and about 17%, *e.g.,* between 14% and 16%

[0254] The uracil or thymine content of wild-type IL12A relative to the total nucleotide content (%) is about 26%. In some cases, the uracil or thymine content of a uracil- or thymine- modified sequence encoding an IL12A polypeptide relative to the total nucleotide content (%) ($\%U_{TL}$ or $\%T_{TL}$) is less than 25%. In some cases, the $\%U_{TL}$ or $\%T_{TL}$ is less than 25%, less than 24%, less than 23%, less than 22%, less than 21%, less than 20%, less than 19%, less that 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, or less than 10%. In some cases, the $\%U_{TL}$ or $\%T_{TL}$ is not less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

[0255] In some cases, the uracil or thymine content of a uracil- or thymine-modified sequence encoding an IL12A polypeptide of the disclosure relative to the total nucleotide content ($\%U_{TL}$ or $\%T_{TL}$) is between 10% and 25%, between 11% and 25%, between 12% and 25%, between 13% and 25%, between 14% and 25%, between 15% and 25%, between 16% and 25%, between 10% and 24%, between 10% and 23%, between 11% and 22%, between 11% and 21%, between 11% and 20%, between 11% and 19%, between 11% and 18%, between 12% and 24%, between 12% and 23%, between 13% and 22%, between 14% and 21%, between 13% and 20%, between 15% and 19%, between 15% and 20%, between 16% and 19%, between 16% and 18%, or between 13% and 17%.

[0256] In a particular case, the uracil or thymine content ($\%U_{TL}$ or $\%T_{TL}$) of a uracil- or thymine modified sequence encoding an IL12A polypeptide of the disclosure is between about 15% and about 19%, e.g., between 16% and 18% In some cases, a uracil-modified sequence encoding an IL12B polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure has a reduced number of consecutive uracils with respect to the corresponding wild-type nucleic acid sequence. For example, two consecutive leucines can be encoded by the sequence CUUUUG, which includes a four uracil cluster. Such a subsequence can be substituted, e.g., with CUGCUC, which removes the uracil cluster.

[0257] Phenylalanine can be encoded by UUC or UUU. Thus, even if phenylalanines encoded by UUU are replaced by UUC, the synonymous codon still contains a uracil pair (UU). Accordingly, the number of phenylalanines in a sequence establishes a minimum number of uracil pairs (UU) that cannot be eliminated without altering the number of phenylalanines in the encoded polypeptide.

[0258] In some cases, a uracil-modified sequence encoding an IL12B and/or IL12A polypeptide of the disclosure has a reduced number of uracil triplets (UUU) with respect to the wild-type nucleic acid sequence. In some cases, a uracil-modified sequence encoding an IL12B and/or IL12A polypeptide of the disclosure contains 4, 3, 2, 1 or no uracil triplets (UUU).

[0259] In some cases, a uracil-modified sequence encoding an IL12B and/or IL12A polypeptide has a reduced number of uracil pairs (UU) with respect to the number of uracil pairs (UU) in the wild-type nucleic acid sequence. In some cases, a uracil-modified sequence encoding an IL12B and/or IL12A polypeptide of the disclosure has a number of uracil pairs (UU) corresponding to the minimum possible number of uracil pairs (UU) in the wild-type nucleic acid sequence.

[0260] In some cases, a uracil-modified sequence encoding an IL12B polypeptide of the disclosure has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 uracil pairs (UU) less than the number of uracil pairs (UU) in the wild-type nucleic acid sequence. In some cases, a uracil-modified sequence encoding an IL12B polypeptide of the disclosure has between 7 and 13, between 8 and 14, between 9 and 15, between 10 and 16, between 11 and 7, between 12 and 18 uracil pairs (UU).

[0261] In some cases, a uracil-modified sequence encoding an IL12B polypeptide of the disclosure has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 uracil pairs (UU) less than the number of uracil pairs (UU) in the wild-type nucleic acid sequence. In some cases, a uracil-modified sequence encoding an IL12A polypeptide of the disclosure has between 7 and 13, between 8 and 14, between 9 and 15, between 10 and 16, between 11 and 7, between 12 and 18 uracil pairs (UU).

[0262] The phrase "uracil pairs (UU) relative to the uracil pairs (UU) in the wild type nucleic acid sequence," refers to

a parameter determined by dividing the number of uracil pairs (UU) in a sequence-optimized nucleotide sequence by the total number of uracil pairs (UU) in the corresponding wild-type nucleotide sequence and multiplying by 100. This parameter is abbreviated herein as $\%UU_{wt}$.

[0263] In some cases, a uracil-modified sequence encoding an IL12A or IL12B polypeptide of the disclosure has a $\%UU_{wt}$ less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 65%, less than 60%, less than 55%, less than 50%, less than 40%, less than 30%, or less than 20%.

[0264] In some cases, a uracil-modified sequence encoding an IL12B polypeptide has a $\%UU_{wt}$ between 24% and 59%. In a particular case, a uracil-modified sequence encoding an IL12B polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure has a $\%UU_{wt}$ between 29% and 55% .

[0265] In some cases, a uracil-modified sequence encoding an IL12A polypeptide has a $\%UU_{wt}$ between 14% and 57%. In a particular case, a uracil-modified sequence encoding an IL12B polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure has a $\%UU_{wt}$ between 19% and 52% .

[0266] In some cases, the polynucleotide of the disclosure comprises a uracil-modified sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides disclosed herein. In some cases, the uracil-modified sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides comprises at least one chemically modified nucleobase, e.g., 5-methoxyuracil. In some cases, at least 95% of a nucleobase (e.g., uracil) in a uracil-modified sequence encoding an IL 12A polypeptide, an IL 12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure are modified nucleobases. In some cases, at least 95% of uracil in a uracil-modified sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is 5-methoxyuracil. In some cases, the polynucleotide comprising a uracil-modified sequence further comprises a miRNA binding site, *e.g.,* a miRNA binding site that binds to miR-122. In some cases, the polynucleotide comprising a uracil-modified sequence is formulated with a delivery agent, *e.g.,* a compound having **Formula (I),** *e.g.,* any of Compounds 1-147 or any of Compounds 1-232.

[0267] In some cases, the "guanine content of the sequence optimized ORF encoding IL12B and/or IL12A with respect to the theoretical maximum guanine content of a nucleotide sequence encoding the IL12B and/or IL12A polypeptide," abbreviated as $\%G_{TMX}$ is at least 69%, at least 70%, at least 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%. In some cases, the $\%G_{TMX}$ is between about 70% and about 80%, between about 71% and about 79%, between about 71% and about 78%, or between about 71% and about 77%.

[0268] In some cases, the "cytosine content of the ORF relative to the theoretical maximum cytosine content of a nucleotide sequence encoding the IL12B and/or IL12A polypeptide," abbreviated as $\%C_{TMX}$, is at least 59%, at least 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%. In some cases, the $\%C_{TMX}$ is between about 60% and about 80%, between about 62% and about 80%, between about 63% and about 79%, or between about 68% and about 76%.

[0269] In some cases, the "guanine and cytosine content (G/C) of the ORF relative to the theoretical maximum G/C content in a nucleotide sequence encoding the IL12B and/or IL12A polypeptide," abbreviated as $\%G/C_{TMX}$ is at least about 81%, at least about 85%, at least about 90%, at least about 95%, or about 100%. The $\%G/C_{TMX}$ is between about 80% and about 100%, between about 85% and about 99%, between about 90% and about 97%, or between about 91% and about 96%.

[0270] In some cases, the "G/C content in the ORF relative to the G/C content in the corresponding wild-type ORF," abbreviated as$\%G/C_{WT}$ is at least 102%, at least 103%, at least 104%, at least 105%, at least 106%, at least 107%, at least 110%, at least 115%, or at least 120%.

[0271] In some cases, the average G/C content in the 3rd codon position in the ORF is at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, or at least 30% higher than the average G/C content in the 3rd codon position in the corresponding wild-type ORF.

[0272] In some cases, the polynucleotide of the disclosure comprises an open reading frame (ORF) encoding an IL12B and/or IL12A polypeptide, wherein the ORF has been sequence optimized, and wherein each of$\%U_{TL}$, $\%U_{WT}$, %UTM, $\%G_{TL}$, $\%G_{WT}$, $\%G_{TMX}$, $\%C_{TL}$, $\%C_{WT}$, $\%C_{TMX}$, $\%G/C_{TL}$, $\%G/C_{WT}$, or $\%G/C_{TMX}$, alone or in a combination thereof is in a range between (i) a maximum corresponding to the parameter's maximum value (MAX) plus about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 standard deviations (STD DEV), and (ii) a minimum corresponding to the parameter's minimum value (MIN) less 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 standard deviations (STD DEV).

9. Methods for Sequence Optimization

[0273] In some cases, a polynucleotide of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence, e.g., an ORF, encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides (*e.g.,* the wild-type sequence, functional fragment, or variant thereof)) is sequence optimized. A sequence optimized nucleotide sequence (nucleotide sequence is also referred to as "nucleic acid" herein) comprises at least one codon modification

with respect to a reference sequence *(e.g.,* a wild-type sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides). Thus, in a sequence optimized nucleic acid, at least one codon is different from a corresponding codon in a reference sequence *(e.g.,* a wild-type sequence).

[0274] In general, sequence optimized nucleic acids are generated by at least a step comprising substituting codons in a reference sequence with synonymous codons (*i.e.,* codons that encode the same amino acid). Such substitutions can be effected, for example, by applying a codon substitution map (*i.e.,* a table providing the codons that will encode each amino acid in the codon optimized sequence), or by applying a set of rules *(e.g.,* if glycine is next to neutral amino acid, glycine would be encoded by a certain codon, but if it is next to a polar amino acid, it would be encoded by another codon). In addition to codon substitutions (*i.e.,* "codon optimization") the sequence optimization methods disclosed herein comprise additional optimization steps which are not strictly directed to codon optimization such as the removal of deleterious motifs (destabilizing motif substitution). Compositions and formulations comprising these sequence optimized nucleic acids *(e.g.,* a RNA, *e.g.,* an mRNA) can be administered to a subject in need thereof to facilitate *in vivo* expression of functionally active IL12.

[0275] The recombinant expression of large molecules in cell cultures can be a challenging task with numerous limitations (*e.g.,* poor protein expression levels, stalled translation resulting in truncated expression products, protein misfolding, etc.) These limitations can be reduced or avoided by administering the polynucleotides (*e.g.*, a RNA, *e.g.,* an mRNA), which encode a functionally active IL12 or compositions or formulations comprising the same to a patient suffering from cancer, so the synthesis and delivery of the IL12 polypeptide to treat cancer takes place endogenously.

[0276] Changing from an *in vitro* expression system (*e.g.,* cell culture) to *in vivo* expression requires the redesign of the nucleic acid sequence encoding the therapeutic agent. Redesigning a naturally occurring gene sequence by choosing different codons without necessarily altering the encoded amino acid sequence can often lead to dramatic increases in protein expression levels (Gustafsson et al., 2004, Journal/Trends Biotechnol 22, 346-53). Variables such as codon adaptation index (CAI), mRNA secondary structures, cis-regulatory sequences, GC content and many other similar variables have been shown to somewhat correlate with protein expression levels (Villalobos et al., 2006, "Journal/BMC Bioinformatics 7, 285). However, due to the degeneracy of the genetic code, there are numerous different nucleic acid sequences that can all encode the same therapeutic agent. Each amino acid is encoded by up to six synonymous codons; and the choice between these codons influences gene expression. In addition, codon usage (*i.e.,* the frequency with which different organisms use codons for expressing a polypeptide sequence) differs among organisms (for example, recombinant production of human or humanized therapeutic antibodies frequently takes place in hamster cell cultures).

[0277] In some cases, a reference nucleic acid sequence can be sequence optimized by applying a codon map. The skilled artisan will appreciate that the T bases in the codon maps disclosed below are present in DNA, whereas the T bases would be replaced by U bases in corresponding RNAs. For example, a sequence optimized nucleic acid disclosed herein in DNA form, *e.g.,* a vector or an in-vitro translation (IVT) template, would have its T bases transcribed as U based in its corresponding transcribed mRNA. In this respect, both sequence optimized DNA sequences (comprising T) and their corresponding RNA sequences (comprising U) are considered sequence optimized nucleic acid of the present disclosure. A skilled artisan would also understand that equivalent codon-maps can be generated by replaced one or more bases with non-natural bases. Thus, *e.g.,* a TTC codon (DNA map) would correspond to a UUC codon (RNA map), which in turn may correspond to a ΨΨC codon (RNA map in which U has been replaced with pseudouridine).

[0278] In one case, a reference sequence encoding IL12A, IL12B, or both IL12A and IL12B can be optimized by replacing all the codons encoding a certain amino acid with only one of the alternative codons provided in a codon map. For example, all the valines in the optimized sequence would be encoded by GTG or GTC or GTT.

[0279] Sequence optimized polynucleotides of the disclosure can be generated using one or more codon optimization methods, or a combination thereof. Sequence optimization methods which may be used to sequence optimize nucleic acid sequences are described in detail herein. This list of methods is not comprehensive or limiting.

[0280] It will be appreciated that the design principles and rules described for each one of the sequence optimization methods discussed below can be combined in many different ways, for example high G/C content sequence optimization for some regions or uridine content sequence optimization for other regions of the reference nucleic acid sequence, as well as targeted nucleotide mutations to minimize secondary structure throughout the sequence or to eliminate deleterious motifs.

[0281] The choice of potential combinations of sequence optimization methods can be, for example, dependent on the specific chemistry used to produce a synthetic polynucleotide. Such a choice can also depend on characteristics of the protein encoded by the sequence optimized nucleic acid, *e.g.,* a full sequence, a functional fragment, or a fusion protein comprising IL12, etc. In some cases, such a choice can depend on the specific tissue or cell targeted by the sequence optimized nucleic acid (*e.g.,* a therapeutic synthetic mRNA).

[0282] The mechanisms of combining the sequence optimization methods or design rules derived from the application and analysis of the optimization methods can be either simple or complex. For example, the combination can be:

(i) *Sequential*: Each sequence optimization method or set of design rules applies to a different subsequence of the

overall sequence, for example reducing uridine at codon positions 1 to 30 and then selecting high frequency codons for the remainder of the sequence;
(ii) *Hierarchical*: Several sequence optimization methods or sets of design rules are combined in a hierarchical, deterministic fashion. For example, use the most GC-rich codons, breaking ties (which are common) by choosing the most frequent of those codons.
(iii) *Multifactorial / Multiparametric*: Machine learning or other modeling techniques are used to design a single sequence that best satisfies multiple overlapping and possibly contradictory requirements. This approach would require the use of a computer applying a number of mathematical techniques, for example, genetic algorithms.

[0283] Ultimately, each one of these approaches can result in a specific set of rules which in many cases can be summarized in a single codon table, *i.e.,* a sorted list of codons for each amino acid in the target protein (*i.e.,* an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides), with a specific rule or set of rules indicating how to select a specific codon for each amino acid position.

a. Uridine Content Optimization

[0284] The presence of local high concentrations of uridine in a nucleic acid sequence can have detrimental effects on translation, *e.g.*, slow or prematurely terminated translation, especially when modified uridine analogs are used in the production of synthetic mRNAs. Furthermore, high uridine content can also reduce the *in vivo* half-life of synthetic mRNAs due to TLR activation.
[0285] Accordingly, a nucleic acid sequence can be sequence optimized using a method comprising at least one uridine content optimization step. Such a step comprises, *e.g.*, substituting at least one codon in the reference nucleic acid with an alternative codon to generate a uridine-modified sequence, wherein the uridine-modified sequence has at least one of the following properties:

(i) increase or decrease in global uridine content;
(ii) increase or decrease in local uridine content (*i.e.,* changes in uridine content are limited to specific subsequences);
(iii) changes in uridine distribution without altering the global uridine content;
(iv) changes in uridine clustering (*e.g.*, number of clusters, location of clusters, or distance between clusters); or
(v) combinations thereof.

[0286] In some cases, the sequence optimization process comprises optimizing the global uridine content, *i.e.,* optimizing the percentage of uridine nucleobases in the sequence optimized nucleic acid with respect to the percentage of uridine nucleobases in the reference nucleic acid sequence. For example, 30% of nucleobases may be uridines in the reference sequence and 10% of nucleobases may be uridines in the sequence optimized nucleic acid.
[0287] In other cases, the sequence optimization process comprises reducing the local uridine content in specific regions of a reference nucleic acid sequence, *i.e.,* reducing the percentage of uridine nucleobases in a subsequence of the sequence optimized nucleic acid with respect to the percentage of uridine nucleobases in the corresponding subsequence of the reference nucleic acid sequence. For example, the reference nucleic acid sequence may have a 5'-end region (*e.g.,* 30 codons) with a local uridine content of 30%, and the uridine content in that same region could be reduced to 10% in the sequence optimized nucleic acid.
[0288] In specific cases, codons can be replaced in the reference nucleic acid sequence to reduce or modify, for example, the number, size, location, or distribution of uridine clusters that could have deleterious effects on protein translation. Although as a general rule it is desirable to reduce the uridine content of the reference nucleic acid sequence, in certain cases the uridine content, and in particular the local uridine content, of some subsequences of the reference nucleic acid sequence can be increased.
[0289] The reduction of uridine content to avoid adverse effects on translation can be done in combination with other optimization methods disclosed here to achieve other design goals. For example, uridine content optimization can be combined with ramp design, since using the rarest codons for most amino acids will, with a few exceptions, reduce the U content.
[0290] In some cases, the uridine-modified sequence is designed to induce a lower Toll-Like Receptor (TLR) response when compared to the reference nucleic acid sequence. Several TLRs recognize and respond to nucleic acids. Double-stranded (ds)RNA, a frequent viral constituent, has been shown to activate TLR3. See Alexopoulou et al. (2001) Nature, 413:732-738 and Wang et al. (2004) Nat. Med., 10:1366-1373. Single-stranded (ss)RNA activates TLR7. See Diebold et al. (2004) Science 303 :1529-1531. RNA oligonucleotides, for example RNA with phosphorothioate internucleotide linkages, are ligands of human TLR8. See Heil et al. (2004) Science 303:1526-1529. DNA containing unmethylated CpG motifs, characteristic of bacterial and viral DNA, activate TLR9. See Hemmi et al. (2000) Nature, 408: 740-745.
[0291] As used herein, the term "TLR response" is defined as the recognition of single-stranded RNA by a TLR7

receptor, and in some cases encompasses the degradation of the RNA and/or physiological responses caused by the recognition of the single-stranded RNA by the receptor. Methods to determine and quantitate the binding of an RNA to a TLR7 are known in the art. Similarly, methods to determine whether an RNA has triggered a TLR7-mediated physiological response (*e.g.*, cytokine secretion) are well known in the art. In some cases, a TLR response can be mediated by TLR3, TLR8, or TLR9 instead of TLR7.

[0292] Suppression of TLR7-mediated response can be accomplished via nucleoside modification. RNA undergoes over hundred different nucleoside modifications in nature (see the RNA Modification Database, available at mods.rna.albany.edu). Human rRNA, for example, has ten times more pseudouridine (Ψ) and 25 times more 2'-O-methylated nucleosides than bacterial rRNA. Bacterial mRNA contains no nucleoside modifications, whereas mammalian mRNAs have modified nucleosides such as 5-methylcytidine (m5C), N6-methyladenosine (m6A), inosine and many 2'-O-methylated nucleosides in addition to N7-methylguanosine (m7G).

[0293] Uracil and ribose, the two defining features of RNA, are both necessary and sufficient for TLR7 stimulation, and short single-stranded RNA (ssRNA) act as TLR7 agonists in a sequence-independent manner as long as they contain several uridines in close proximity. See Diebold et al. (2006) Eur. J. Immunol. 36:3256-3267. Accordingly, one or more of the optimization methods disclosed herein comprises reducing the uridine content (locally and/or locally) and/or reducing or modifying uridine clustering to reduce or to suppress a TLR7-mediated response.

[0294] In some cases, the TLR response (*e.g.,* a response mediated by TLR7) caused by the uridine-modified sequence is at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% lower than the TLR response caused by the reference nucleic acid sequence.

[0295] In some cases, the TLR response caused by the reference nucleic acid sequence is at least about 1-fold, at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold higher than the TLR response caused by the uridine-modified sequence.

[0296] In some cases, the uridine content (average global uridine content) (absolute or relative) of the uridine-modified sequence is higher than the uridine content (absolute or relative) of the reference nucleic acid sequence. Accordingly, in some cases, the uridine-modified sequence contains at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% more uridine that the reference nucleic acid sequence.

[0297] In other cases, the uridine content (average global uridine content) (absolute or relative) of the uridine-modified sequence is lower than the uridine content (absolute or relative) of the reference nucleic acid sequence. Accordingly, in some cases, the uridine-modified sequence contains at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% less uridine that the reference nucleic acid sequence.

[0298] In some cases, the uridine content (average global uridine content) (absolute or relative) of the uridine-modified sequence is less than 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the total nucleobases in the uridine-modified sequence. In some cases, the uridine content of the uridine-modified sequence is between about 10% and about 20%. In some particular cases, the uridine content of the uridine-modified sequence is between about 12% and about 16%.

[0299] In some cases, the uridine content of the reference nucleic acid sequence can be measured using a sliding window. In some cases, the length of the sliding window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleobases. In some cases, the sliding window is over 40 nucleobases in length. In some cases, the sliding window is 20 nucleobases in length. Based on the uridine content measured with a sliding window, it is possible to generate a histogram representing the uridine content throughout the length of the reference nucleic acid sequence and sequence optimized nucleic acids.

[0300] In some cases, a reference nucleic acid sequence can be modified to reduce or eliminate peaks in the histogram that are above or below a certain percentage value. In some cases, the reference nucleic acid sequence can be modified to eliminate peaks in the sliding-window representation which are above 65%, 60%, 55%, 50%, 45%, 40%, 35%, or 30% uridine. In another case, the reference nucleic acid sequence can be modified so no peaks are over 30% uridine in the sequence optimized nucleic acid, as measured using a 20 nucleobase sliding window. In some cases, the reference nucleic acid sequence can be modified so no more or no less than a predetermined number of peaks in the sequence

optimized nucleic sequence, as measured using a 20 nucleobase sliding window, are above or below a certain threshold value. For example, in some cases, the reference nucleic acid sequence can be modified so no peaks or no more than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 peaks in the sequence optimized nucleic acid are above 10%, 15%, 20%, 25% or 30% uridine. In another case, the sequence optimized nucleic acid contains between 0 peaks and 2 peaks with uridine contents 30% of higher.

[0301] In some cases, a reference nucleic acid sequence can be sequence optimized to reduce the incidence of consecutive uridines. For example, two consecutive leucines could be encoded by the sequence CUUUUG, which would include a four uridine cluster. Such subsequence could be substituted with CUGCUC, which would effectively remove the uridine cluster. Accordingly, a reference nucleic sequence can be sequence optimized by reducing or eliminating uridine pairs (UU), uridine triplets (UUU) or uridine quadruplets (UUUU). Higher order combinations of U are not considered combinations of lower order combinations. Thus, for example, UUUU is strictly considered a quadruplet, not two consecutive U pairs; or UUUUUU is considered a sextuplet, not three consecutive U pairs, or two consecutive U triplets, etc.

[0302] In some cases, all uridine pairs (UU) and/or uridine triplets (UUU) and/or uridine quadruplets (UUUU) can be removed from the reference nucleic acid sequence. In other cases, uridine pairs (UU) and/or uridine triplets (UUU) and/or uridine quadruplets (UUUU) can be reduced below a certain threshold, *e.g.,* no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 occurrences in the sequence optimized nucleic acid. In a particular case, the sequence optimized nucleic acid contains less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 uridine pairs. In another particular case, the sequence optimized nucleic acid contains no uridine pairs and/or triplets.

[0303] Phenylalanine codons, *i.e.,* UUC or UUU, comprise a uridine pair or triples and therefore sequence optimization to reduce uridine content can at most reduce the phenylalanine U triplet to a phenylalanine U pair. In some cases, the occurrence of uridine pairs (UU) and/or uridine triplets (UUU) refers only to non-phenylalanine U pairs or triplets. Accordingly, in some cases, non-phenylalanine uridine pairs (UU) and/or uridine triplets (UUU) can be reduced below a certain threshold, *e.g.,* no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 occurrences in the sequence optimized nucleic acid. In a particular case, the sequence optimized nucleic acid contains less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 non-phenylalanine uridine pairs and/or triplets. In another particular case, the sequence optimized nucleic acid contains no non-phenylalanine uridine pairs and/or triplets.

[0304] In some cases, the reduction in uridine combinations (*e.g.*, pairs, triplets, quadruplets) in the sequence optimized nucleic acid can be expressed as a percentage reduction with respect to the uridine combinations present in the reference nucleic acid sequence.

[0305] In some cases, a sequence optimized nucleic acid can contain about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of the total number of uridine pairs present in the reference nucleic acid sequence. In some cases, a sequence optimized nucleic acid can contain about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of the total number of uridine triplets present in the reference nucleic acid sequence. In some cases, a sequence optimized nucleic acid can contain about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of the total number of uridine quadruplets present in the reference nucleic acid sequence.

[0306] In some cases, a sequence optimized nucleic acid can contain about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of the total number of non-phenylalanine uridine pairs present in the reference nucleic acid sequence. In some cases, a sequence optimized nucleic acid can contain about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of the total number of non-phenylalanine uridine triplets present in the reference nucleic acid sequence.

[0307] In some cases, the uridine content in the sequence optimized sequence can be expressed with respect to the theoretical minimum uridine content in the sequence. The term "theoretical minimum uridine content" is defined as the uridine content of a nucleic acid sequence as a percentage of the sequence's length after all the codons in the sequence have been replaced with synonymous codon with the lowest uridine content. In some cases, the uridine content of the sequence optimized nucleic acid is identical to the theoretical minimum uridine content of the reference sequence (*e.g.,* a wild type sequence). In some aspects, the uridine content of the sequence optimized nucleic acid is about 90%, about 95%, about 100%, about 105%, about 110%, about 115%, about 120%, about 125%, about 130%, about 135%, about 140%, about 145%, about 150%, about 155%, about 160%, about 165%, about 170%, about 175%, about 180%, about 185%, about 190%, about 195% or about 200% of the theoretical minimum uridine content of the reference sequence (*e.g.,* a wild type sequence).

[0308] In some cases, the uridine content of the sequence optimized nucleic acid is identical to the theoretical minimum uridine content of the reference sequence (*e.g.,* a wild type sequence).

[0309] The reference nucleic acid sequence (*e.g.,* a wild type sequence) can comprise uridine clusters which due to their number, size, location, distribution or combinations thereof have negative effects on translation. As used herein,

the term "uridine cluster" refers to a subsequence in a reference nucleic acid sequence or sequence optimized nucleic sequence with contains a uridine content (usually described as a percentage) which is above a certain threshold. Thus, in certain cases, if a subsequence comprises more than about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% or 65% uridine content, such subsequence would be considered a uridine cluster.

[0310] The negative effects of uridine clusters can be, for example, eliciting a TLR7 response. Thus, in some implementations of the nucleic acid sequence optimization methods disclosed herein it is desirable to reduce the number of clusters, size of clusters, location of clusters (*e.g.*, close to the 5' and/or 3' end of a nucleic acid sequence), distance between clusters, or distribution of uridine clusters (*e.g.,* a certain pattern of cluster along a nucleic acid sequence, distribution of clusters with respect to secondary structure elements in the expressed product, or distribution of clusters with respect to the secondary structure of an mRNA).

[0311] In some cases, the reference nucleic acid sequence comprises at least one uridine cluster, wherein said uridine cluster is a subsequence of the reference nucleic acid sequence wherein the percentage of total uridine nucleobases in said subsequence is above a predetermined threshold. In some cases, the length of the subsequence is at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, or at least about 100 nucleobases. In some cases, the subsequence is longer than 100 nucleobases. In some cases, the threshold is 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% uridine content. In some cases, the threshold is above 25%.

[0312] For example, an amino acid sequence comprising A, D, G, S and R could be encoded by the nucleic acid sequence GCU, GAU, GGU, AGU, CGU. Although such sequence does not contain any uridine pairs, triplets, or quadruplets, one third of the nucleobases would be uridines. Such a uridine cluster could be removed by using alternative codons, for example, by using GCC, GAC, GGC, AGC, and CGC, which would contain no uridines.

[0313] In other cases, the reference nucleic acid sequence comprises at least one uridine cluster, wherein said uridine cluster is a subsequence of the reference nucleic acid sequence wherein the percentage of uridine nucleobases of said subsequence as measured using a sliding window that is above a predetermined threshold. In some cases, the length of the sliding window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleobases. In some cases, the sliding window is over 40 nucleobases in length. In some cases, the threshold is 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24% or 25% uridine content. In some cases, the threshold is above 25%.

[0314] In some cases, the reference nucleic acid sequence comprises at least two uridine clusters. In some cases, the uridine-modified sequence contains fewer uridine-rich clusters than the reference nucleic acid sequence. In some cases, the uridine-modified sequence contains more uridine-rich clusters than the reference nucleic acid sequence. In some cases, the uridine-modified sequence contains uridine-rich clusters with are shorter in length than corresponding uridine-rich clusters in the reference nucleic acid sequence. In other cases, the uridine-modified sequence contains uridine-rich clusters which are longer in length than the corresponding uridine-rich cluster in the reference nucleic acid sequence.

[0315] See, Kariko et al. (2005) Immunity 23:165-175; Kormann et al. (2010) Nature Biotechnology 29:154-157; or Sahin et al. (2014) Nature Reviews Drug Discovery | AOP, published online 19 September 2014m doi:10.1038/nrd4278.

b. Guanine/Cytosine (G/C) Content

[0316] A reference nucleic acid sequence can be sequence optimized using methods comprising altering the Guanine/Cytosine (G/C) content (absolute or relative) of the reference nucleic acid sequence. Such optimization can comprise altering (e.g., increasing or decreasing) the global G/C content (absolute or relative) of the reference nucleic acid sequence; introducing local changes in G/C content in the reference nucleic acid sequence (e.g., increase or decrease G/C in selected regions or subsequences in the reference nucleic acid sequence); altering the frequency, size, and distribution of G/C clusters in the reference nucleic acid sequence, or combinations thereof.

[0317] In some cases, the sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides comprises an overall increase in G/C content (absolute or relative) relative to the G/C content (absolute or relative) of the reference nucleic acid sequence. In some cases, the overall increase in G/C content (absolute or relative) is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% relative to the G/C content (absolute or relative) of the reference nucleic acid sequence.

[0318] In some cases, the sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides comprises an overall decrease in G/C content (absolute or relative) relative

to the G/C content of the reference nucleic acid sequence. In some cases, the overall decrease in G/C content (absolute or relative) is at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% relative to the G/C content (absolute or relative) of the reference nucleic acid sequence.

[0319] In some cases, the sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides comprises a local increase in Guanine/Cytosine (G/C) content (absolute or relative) in a subsequence (*i.e.,* a G/C modified subsequence) relative to the G/C content (absolute or relative) of the corresponding subsequence in the reference nucleic acid sequence. In some cases, the local increase in G/C content (absolute or relative) is by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% relative to the G/C content (absolute or relative) of the corresponding subsequence in the reference nucleic acid sequence.

[0320] In some cases, the sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides comprises a local decrease in Guanine/Cytosine (G/C) content (absolute or relative) in a subsequence (*i.e.,* a G/C modified subsequence) relative to the G/C content (absolute or relative) of the corresponding subsequence in the reference nucleic acid sequence. In some cases, the local decrease in G/C content (absolute or relative) is by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100% relative to the G/C content (absolute or relative) of the corresponding subsequence in the reference nucleic acid sequence.

[0321] In some cases, the G/C content (absolute or relative) is increased or decreased in a subsequence which is at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleobases in length.

[0322] In some cases, the G/C content (absolute or relative) is increased or decreased in a subsequence which is at least about 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 nucleobases in length.

[0323] In some cases, the G/C content (absolute or relative) is increased or decreased in a subsequence which is at least about 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, or 10000 nucleobases in length.

[0324] The increases or decreases in G and C content (absolute or relative) described herein can be conducted by replacing synonymous codons with low G/C content with synonymous codons having higher G/C content, or vice versa. For example, L has 6 synonymous codons: two of them have 2 G/C (CUC, CUG), 3 have a single G/C (UUG, CUU, CUA), and one has no G/C (UUA). So if the reference nucleic acid had a CUC codon in a certain position, G/C content at that position could be reduced by replacing CUC with any of the codons having a single G/C or the codon with no G/C.

[0325] See, U.S. Publ. Nos. US20140228558, US20050032730 A1; Gustafsson et al. (2012) Protein Expression and Purification 83: 37-46.

c. Codon Frequency - Codon Usage Bias

[0326] Numerous codon optimization methods known in the art are based on the substitution of codons in a reference nucleic acid sequence with codons having higher frequencies. Thus, in some cases, a nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides disclosed herein can be sequence optimized using methods comprising the use of modifications in the frequency of use of one or more codons relative to other synonymous codons in the sequence optimized nucleic acid with respect to the frequency of use in the non-codon optimized sequence.

[0327] As used herein, the term "codon frequency" refers to codon usage bias, *i.e.,* the differences in the frequency of occurrence of synonymous codons in coding DNA/RNA. It is generally acknowledged that codon preferences reflect a balance between mutational biases and natural selection for translational optimization. Optimal codons help to achieve

faster translation rates and high accuracy. As a result of these factors, translational selection is expected to be stronger in highly expressed genes. In the field of bioinformatics and computational biology, many statistical methods have been proposed and used to analyze codon usage bias. See, *e.g.,* Comeron & Aguadé (1998) J. Mol. Evol. 47: 268-74. Methods such as the 'frequency of optimal codons' (Fop) (Ikemura (1981) J. Mol. Biol. 151 (3): 389-409), the Relative Codon Adaptation (RCA) (Fox & Eril (2010) DNA Res. 17 (3): 185-96) or the 'Codon Adaptation Index' (CAI) (Sharp & Li (1987) Nucleic Acids Res. 15 (3): 1281-95) are used to predict gene expression levels, while methods such as the 'effective number of codons' (Nc) and Shannon entropy from information theory are used to measure codon usage evenness. Multivariate statistical methods, such as correspondence analysis and principal component analysis, are widely used to analyze variations in codon usage among genes (Suzuki et al. (2008) DNA Res. 15 (6): 357-65; Sandhu et al., In Silico Biol. 2008;8(2): 187-92).

[0328] The nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides disclosed herein (*e.g.,* a wild type nucleic acid sequence, a mutant nucleic acid sequence, a chimeric nucleic sequence, etc. which can be, for example, an mRNA), can be codon optimized using methods comprising substituting at least one codon in the reference nucleic acid sequence with an alternative codon having a higher or lower codon frequency in the synonymous codon set; wherein the resulting sequence optimized nucleic acid has at least one optimized property with respect to the reference nucleic acid sequence.

[0329] In some cases, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100% of the codons in the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides are substituted with alternative codons, each alternative codon having a codon frequency higher than the codon frequency of the substituted codon in the synonymous codon set.

[0330] In some cases, at least one codon in the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is substituted with an alternative codon having a codon frequency higher than the codon frequency of the substituted codon in the synonymous codon set, and at least one codon in the reference nucleic acid sequence is substituted with an alternative codon having a codon frequency lower than the codon frequency of the substituted codon in the synonymous codon set.

[0331] In some cases, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75% of the codons in the reference nucleic acid sequence encoding IL12 are substituted with alternative codons, each alternative codon having a codon frequency higher than the codon frequency of the substituted codon in the synonymous codon set.

[0332] In some cases, at least one alternative codon having a higher codon frequency has the highest codon frequency in the synonymous codon set. In other cases, all alternative codons having a higher codon frequency have the highest codon frequency in the synonymous codon set.

[0333] In some cases, at least one alternative codon having a lower codon frequency has the lowest codon frequency in the synonymous codon set. In some cases, all alternative codons having a higher codon frequency have the highest codon frequency in the synonymous codon set.

[0334] In some specific cases, at least one alternative codon has the second highest, the third highest, the fourth highest, the fifth highest or the sixth highest frequency in the synonymous codon set. In some specific cases, at least one alternative codon has the second lowest, the third lowest, the fourth lowest, the fifth lowest, or the sixth lowest frequency in the synonymous codon set.

[0335] Optimization based on codon frequency can be applied globally, as described above, or locally to the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides. In some cases, when applied locally, regions of the reference nucleic acid sequence can modified based on codon frequency, substituting all or a certain percentage of codons in a certain subsequence with codons that have higher or lower frequencies in their respective synonymous codon sets. Thus, in some cases, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100% of the codons in a subsequence of the reference nucleic acid sequence are substituted with alternative codons, each alternative codon having a codon frequency higher than the codon frequency of the substituted codon in the synonymous codon set.

[0336] In some cases, at least one codon in a subsequence of the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is substituted with an alternative codon having a codon frequency higher than the codon frequency of the substituted codon in the synonymous codon set, and at least one codon in a subsequence of the reference nucleic acid sequence is substituted with an alternative codon

having a codon frequency lower than the codon frequency of the substituted codon in the synonymous codon set.

[0337] In some cases, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75% of the codons in a subsequence of the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides are substituted with alternative codons, each alternative codon having a codon frequency higher than the codon frequency of the substituted codon in the synonymous codon set.

[0338] In some cases, at least one alternative codon substituted in a subsequence of the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides and having a higher codon frequency has the highest codon frequency in the synonymous codon set. In other cases, all alternative codons substituted in a subsequence of the reference nucleic acid sequence and having a lower codon frequency have the lowest codon frequency in the synonymous codon set.

[0339] In some cases, at least one alternative codon substituted in a subsequence of the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides and having a lower codon frequency has the lowest codon frequency in the synonymous codon set. In some cases, all alternative codons substituted in a subsequence of the reference nucleic acid sequence and having a higher codon frequency have the highest codon frequency in the synonymous codon set.

[0340] In specific cases, a sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides can comprise a subsequence having an overall codon frequency higher or lower than the overall codon frequency in the corresponding subsequence of the reference nucleic acid sequence at a specific location, for example, at the 5' end or 3' end of the sequence optimized nucleic acid, or within a predetermined distance from those region *(e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 codons from the 5' end or 3' end of the sequence optimized nucleic acid).

[0341] In some cases, an sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides can comprise more than one subsequence having an overall codon frequency higher or lower than the overall codon frequency in the corresponding subsequence of the reference nucleic acid sequence. A skilled artisan would understand that subsequences with overall higher or lower overall codon frequencies can be organized in innumerable patterns, depending on whether the overall codon frequency is higher or lower, the length of the subsequence, the distance between subsequences, the location of the subsequences, etc.

[0342] See, U.S. Pat. Nos. US5082767, US8126653, US7561973, US8401798; U.S. Publ. No. US 20080046192, US 20080076161; Int'l. Publ. No. WO2000018778; Welch et al. (2009) PLoS ONE 4(9): e7002; Gustafsson et al. (2012) Protein Expression and Purification 83: 37-46; Chung et al. (2012) BMC Systems Biology 6:134.

d. Destabilizing Motif Substitution

[0343] There is a variety of motifs that can affect sequence optimization, which fall into various non-exclusive categories, for example:

(i) *Primary sequence based motifs:* Motifs defined by a simple arrangement of nucleotides.
(ii) *Structural motifs:* Motifs encoded by an arrangement of nucleotides that tends to form a certain secondary structure.
(iii) *Local motifs:* Motifs encoded in one contiguous subsequence.
(iv) *Distributed motifs:* Motifs encoded in two or more disjoint subsequences.
(v) *Advantageous motifs:* Motifs which improve nucleotide structure or function.
(vi) *Disadvantageous motifs:* Motifs with detrimental effects on nucleotide structure or function.

[0344] There are many motifs that fit into the category of disadvantageous motifs. Some examples include, for example, restriction enzyme motifs, which tend to be relatively short, exact sequences such as the restriction site motifs for *Xba1* (TCTAGA (SEQ ID NO: 224)), *EcoRI* (GAATTC (SEQ ID NO: 225)), *EcoRII* (CCWGG (SEQ ID NO: 226), wherein W means A or T, per the IUPAC ambiguity codes), or *HindIII* (AAGCTT (SEQ ID NO: 227)); enzyme sites, which tend to be longer and based on consensus not exact sequence, such in the T7 RNA polymerase (GnnnnWnCRnCTCnCnnWnD (SEQ ID NO: 228), wherein n means any nucleotide, R means A or G, W means A or T, D means A or G or T but not C); structural motifs, such as GGGG (SEQ ID NO: 229) repeats (Kim et al. (1991) Nature 351(6324):331-2); or other motifs such as CUG-triplet repeats (Querido et al. (2014) J. Cell Sci. 124:1703-1714).

[0345] Accordingly, the nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides disclosed herein can be sequence optimized using methods comprising substituting at least one destabilizing motif in a reference nucleic acid sequence, and removing such disadvantageous motif or replacing it with an advantageous motif.

[0346] In some cases, the optimization process comprises identifying advantageous and/or disadvantageous motifs in the reference nucleic sequence, wherein such motifs are, *e.g.,* specific subsequences that can cause a loss of stability in the reference nucleic acid sequence prior or during the optimization process. For example, substitution of specific bases during optimization may generate a subsequence (motif) recognized by a restriction enzyme. Accordingly, during the optimization process the appearance of disadvantageous motifs can be monitored by comparing the sequence optimized sequence with a library of motifs known to be disadvantageous. Then, the identification of disadvantageous motifs could be used as a post-hoc filter, *i.e.,* to determine whether a certain modification which potentially could be introduced in the reference nucleic acid sequence should be actually implemented or not.

[0347] In some cases, the identification of disadvantageous motifs can be used prior to the application of the sequence optimization methods disclosed herein, *i.e.,* the identification of motifs in the reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides and their replacement with alternative nucleic acid sequences can be used as a preprocessing step, for example, before uridine reduction.

[0348] In other cases, the identification of disadvantageous motifs and their removal is used as an additional sequence optimization technique integrated in a multiparametric nucleic acid optimization method comprising two or more of the sequence optimization methods disclosed herein. When used in this fashion, a disadvantageous motif identified during the optimization process would be removed, for example, by substituting the lowest possible number of nucleobases in order to preserve as closely as possible the original design principle(s) (*e.g.*, low U, high frequency, etc.).

[0349] See, *e.g.,* U.S. Publ. Nos. US20140228558, US20050032730, or US20140228558.

e. Limited Codon Set Optimization

[0350] In some particular cases, sequence optimization of a reference nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides can be conducted using a limited codon set, *e.g.,* a codon set wherein less than the native number of codons is used to encode the 20 natural amino acids, a subset of the 20 natural amino acids, or an expanded set of amino acids including, for example, non-natural amino acids.

[0351] The genetic code is highly similar among all organisms and can be expressed in a simple table with 64 entries which would encode the 20 standard amino acids involved in protein translation plus start and stop codons. The genetic code is degenerate, *i.e.*, in general, more than one codon specifies each amino acid. For example, the amino acid leucine is specified by the UUA, UUG, CUU, CUC, CUA, or CUG codons, while the amino acid serine is specified by UCA, UCG, UCC, UCU, AGU, or AGC codons (difference in the first, second, or third position). Native genetic codes comprise 62 codons encoding naturally occurring amino acids. Thus, in some cases of the methods disclosed herein optimized codon sets (genetic codes) comprising less than 62 codons to encode 20 amino acids can comprise 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, or 20 codons.

[0352] In some cases, the limited codon set comprises less than 20 codons. For example, if a protein contains less than 20 types of amino acids, such protein could be encoded by a codon set with less than 20 codons. Accordingly, in some cases, an optimized codon set comprises as many codons as different types of amino acids are present in the protein encoded by the reference nucleic acid sequence. In some cases, the optimized codon set comprises 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or even 1 codon.

[0353] In some cases, at least one amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, and Val, *i.e.,* amino acids which are naturally encoded by more than one codon, is encoded with less codons than the naturally occurring number of synonymous codons. For example, in some cases, Ala can be encoded in the sequence optimized nucleic acid by 3, 2 or 1 codons; Cys can be encoded in the sequence optimized nucleic acid by 1 codon; Asp can be encoded in the sequence optimized nucleic acid by 1 codon; Glu can be encoded in the sequence optimized nucleic acid by 1 codon; Phe can be encoded in the sequence optimized nucleic acid by 1 codon; Gly can be encoded in the sequence optimized nucleic acid by 3 codons, 2 codons or 1 codon; His can be encoded in the sequence optimized nucleic acid by 1 codon; Ile can be encoded in the sequence optimized nucleic acid by 2 codons or 1 codon; Lys can be encoded in the sequence optimized nucleic acid by 1 codon; Leu can be encoded in the sequence optimized nucleic acid by 5 codons, 4 codons, 3 codons, 2 codons or 1 codon; Asn can be encoded in the sequence optimized nucleic acid by 1 codon; Pro can be encoded in the sequence optimized nucleic acid by 3 codons, 2 codons, or 1 codon; Gln can be encoded in the sequence optimized nucleic acid by 1 codon; Arg can be encoded in the sequence optimized nucleic acid by 5 codons, 4 codons, 3 codons, 2 codons, or 1 codon; Ser can be encoded in the sequence optimized nucleic acid by 5 codons, 4 codons, 3 codons, 2 codons, or 1 codon; Thr can be encoded in the sequence optimized nucleic acid by 3 codons, 2 codons, or 1 codon; Val can be encoded in the sequence optimized nucleic acid by 3 codons, 2 codons, or 1 codon; and, Tyr can be encoded in the sequence optimized nucleic acid by 1 codon.

[0354] In some cases, at least one amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, and Val, *i.e.,* amino acids which are naturally encoded by more than one

codon, is encoded by a single codon in the limited codon set.

[0355] In some specific cases, the sequence optimized nucleic acid is a DNA and the limited codon set consists of 20 codons, wherein each codon encodes one of 20 amino acids. In some cases, the sequence optimized nucleic acid is a DNA and the limited codon set comprises at least one codon selected from the group consisting of GCT, GCC, GCA, and GCG; at least a codon selected from the group consisting of CGT, CGC, CGA, CGG, AGA, and AGG; at least a codon selected from AAT or ACC; at least a codon selected from GAT or GAC; at least a codon selected from TGT or TGC; at least a codon selected from CAA or CAG; at least a codon selected from GAA or GAG; at least a codon selected from the group consisting of GGT, GGC, GGA, and GGG; at least a codon selected from CAT or CAC; at least a codon selected from the group consisting of ATT, ATC, and ATA; at least a codon selected from the group consisting of TTA, TTG, CTT, CTC, CTA, and CTG; at least a codon selected from AAA or AAG; an ATG codon; at least a codon selected from TTT or TTC; at least a codon selected from the group consisting of CCT, CCC, CCA, and CCG; at least a codon selected from the group consisting of TCT, TCC, TCA, TCG, AGT, and AGC; at least a codon selected from the group consisting of ACT, ACC, ACA, and ACG; a TGG codon; at least a codon selected from TAT or TAC; and, at least a codon selected from the group consisting of GTT, GTC, GTA, and GTG.

[0356] In other cases, the sequence optimized nucleic acid is an RNA (*e.g.,* an mRNA) and the limited codon set consists of 20 codons, wherein each codon encodes one of 20 amino acids. In some cases, the sequence optimized nucleic acid is an RNA and the limited codon set comprises at least one codon selected from the group consisting of GCU, GCC, GCA, and GCG; at least a codon selected from the group consisting of CGU, CGC, CGA, CGG, AGA, and AGG; at least a codon selected from AAU or ACC; at least a codon selected from GAU or GAC; at least a codon selected from UGU or UGC; at least a codon selected from CAA or CAG; at least a codon selected from GAA or GAG; at least a codon selected from the group consisting of GGU, GGC, GGA, and GGG; at least a codon selected from CAU or CAC; at least a codon selected from the group consisting of AUU, AUC, and AUA; at least a codon selected from the group consisting of UUA, UUG, CUU, CUC, CUA, and CUG; at least a codon selected from AAA or AAG; an AUG codon; at least a codon selected from UUU or UUC; at least a codon selected from the group consisting of CCU, CCC, CCA, and CCG; at least a codon selected from the group consisting of UCU, UCC, UCA, UCG, AGU, and AGC; at least a codon selected from the group consisting of ACU, ACC, ACA, and ACG; a UGG codon; at least a codon selected from UAU or UAC; and, at least a codon selected from the group consisting of GUU, GUC, GUA, and GUG.

[0357] In some specific cases, the limited codon set has been optimized for *in vivo* expression of a sequence optimized nucleic acid (*e.g.,* a synthetic mRNA) following administration to a certain tissue or cell.

[0358] In some cases, the optimized codon set (*e.g.,* a 20 codon set encoding 20 amino acids) complies at least with one of the following properties:

(i) the optimized codon set has a higher average G/C content than the original or native codon set; or,
(ii) the optimized codon set has a lower average U content than the original or native codon set; or,
(iii) the optimized codon set is composed of codons with the highest frequency; or,
(iv) the optimized codon set is composed of codons with the lowest frequency; or,
(v) a combination thereof.

[0359] In some specific cases, at least one codon in the optimized codon set has the second highest, the third highest, the fourth highest, the fifth highest or the sixth highest frequency in the synonymous codon set. In some specific cases, at least one codon in the optimized codon has the second lowest, the third lowest, the fourth lowest, the fifth lowest, or the sixth lowest frequency in the synonymous codon set.

[0360] As used herein, the term "native codon set" refers to the codon set used natively by the source organism to encode the reference nucleic acid sequence. As used herein, the term "original codon set" refers to the codon set used to encode the reference nucleic acid sequence before the beginning of sequence optimization, or to a codon set used to encode an optimized variant of the reference nucleic acid sequence at the beginning of a new optimization iteration when sequence optimization is applied iteratively or recursively.

[0361] In some cases, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of codons in the codon set are those with the highest frequency. In other cases, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of codons in the codon set are those with the lowest frequency.

[0362] In some cases, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of codons in the codon set are those with the highest uridine content. In some cases, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of codons in the codon set are those with the lowest uridine content.

[0363] In some cases, the average G/C content (absolute or relative) of the codon set is 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% higher than the average G/C content (absolute or relative) of the original codon set. In some cases, the average G/C content (absolute or relative) of

the codon set is 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% lower than the average G/C content (absolute or relative) of the original codon set.

[0364] In some cases, the uracil content (absolute or relative) of the codon set is 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% higher than the average uracil content (absolute or relative) of the original codon set. In some cases, the uracil content (absolute or relative) of the codon set is 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% lower than the average uracil content (absolute or relative) of the original codon set.

[0365] See also U.S. Appl. Publ. No. 2011/0082055, and Int'l. Publ. No. WO2000018778.

10. Characterization of Sequence Optimized Nucleic Acids

[0366] In some cases of the disclosure, the polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) comprising a sequence optimized nucleic acid *(e.g.,* an ORF) disclosed herein encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides can be can be tested to determine whether at least one nucleic acid sequence property (*e.g.*, stability when exposed to nucleases) or expression property has been improved with respect to the non-sequence optimized nucleic acid.

[0367] As used herein, "expression property" refers to a property of a nucleic acid sequence either *in vivo* (*e.g.,* translation efficacy of a synthetic mRNA after administration to a subject in need thereof) or *in vitro* (*e.g.,* translation efficacy of a synthetic mRNA tested in an *in vitro* model system). Expression properties include but are not limited to the amount of protein produced by an mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides after administration, and the amount of soluble or otherwise functional protein produced. In some cases, sequence optimized nucleic acids disclosed herein can be evaluated according to the viability of the cells expressing a protein encoded by a sequence optimized nucleic acid sequence (*e.g.,* a RNA, *e.g.,* an mRNA) encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides disclosed herein.

[0368] In a particular case, a plurality of sequence optimized nucleic acids disclosed herein (*e.g.,* a RNA, *e.g.,* an mRNA) containing codon substitutions with respect to the non-optimized reference nucleic acid sequence can be characterized functionally to measure a property of interest, for example an expression property in an *in vitro* model system, or *in vivo* in a target tissue or cell.

a. Optimization of Nucleic Acid Sequence Intrinsic Properties

[0369] In some cases of the disclosure, the desired property of the polynucleotide is an intrinsic property of the nucleic acid sequence. For example, the nucleotide sequence *(e.g.,* a RNA, *e.g.,* an mRNA) can be sequence optimized for *in vivo* or *in vitro* stability. In some cases, the nucleotide sequence can be sequence optimized for expression in a particular target tissue or cell. In some cases, the nucleic acid sequence is sequence optimized to increase its plasma half by preventing its degradation by endo and exonucleases.

[0370] In other cases, the nucleic acid sequence is sequence optimized to increase its resistance to hydrolysis in solution, for example, to lengthen the time that the sequence optimized nucleic acid or a pharmaceutical composition comprising the sequence optimized nucleic acid can be stored under aqueous conditions with minimal degradation.

[0371] In other cases, the sequence optimized nucleic acid can be optimized to increase its resistance to hydrolysis in dry storage conditions, for example, to lengthen the time that the sequence optimized nucleic acid can be stored after lyophilization with minimal degradation.

b. Nucleic Acids Sequence Optimized for Protein Expression

[0372] In some cases of the disclosure, the desired property of the polynucleotide is the level of expression of an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides encoded by a sequence optimized sequence disclosed herein. Protein expression levels can be measured using one or more expression systems. In some cases, expression can be measured in cell culture systems, *e.g.*, CHO cells or HEK293 cells. In some cases, expression can be measured using *in vitro* expression systems prepared from extracts of living cells, *e.g.,* rabbit reticulocyte lysates, or *in vitro* expression systems prepared by assembly of purified individual components. In other cases, the protein expression is measured in an *in vivo* system, *e.g.,* mouse, rabbit, monkey, etc.

[0373] In some cases, protein expression in solution form can be desirable. Accordingly, in some cases, a reference sequence can be sequence optimized to yield a sequence optimized nucleic acid sequence having optimized levels of expressed proteins in soluble form. Levels of protein expression and other properties such as solubility, levels of aggregation, and the presence of truncation products (*i.e.,* fragments due to proteolysis, hydrolysis, or defective translation) can be measured according to methods known in the art, for example, using electrophoresis (*e.g.*, native or SDS-PAGE) or chromatographic methods (*e.g.,* HPLC, size exclusion chromatography, etc.).

c. Optimization of Target Tissue or Target Cell Viability

[0374] In some cases, the expression of heterologous therapeutic proteins encoded by a nucleic acid sequence can have deleterious effects in the target tissue or cell, reducing protein yield, or reducing the quality of the expressed product (*e.g.,* due to the presence of protein fragments or precipitation of the expressed protein in inclusion bodies), or causing toxicity.

[0375] Accordingly, in some cases of the disclosure, the sequence optimization of a nucleic acid sequence disclosed herein, *e.g.,* a nucleic acid sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides, can be used to increase the viability of target cells expressing the protein encoded by the sequence optimized nucleic acid.

[0376] Heterologous protein expression can also be deleterious to cells transfected with a nucleic acid sequence for autologous or heterologous transplantation. Accordingly, in some cases of the present disclosure the sequence optimization of a nucleic acid sequence disclosed herein can be used to increase the viability of target cells expressing the protein encoded by the sequence optimized nucleic acid sequence. Changes in cell or tissue viability, toxicity, and other physiological reaction can be measured according to methods known in the art.

d. Reduction of Immune and/or Inflammatory Response

[0377] In some cases, the administration of a sequence optimized nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides or a functional fragment thereof may trigger an immune response, which could be caused by (i) the therapeutic agent (*e.g.,* an mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides), or (ii) the expression product of such therapeutic agent (*e.g.,* the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides encoded by the mRNA), or (iv) a combination thereof. Accordingly, in some cases of the present disclosure the sequence optimization of nucleic acid sequence (*e.g.*, an mRNA) disclosed herein can be used to decrease an immune or inflammatory response triggered by the administration of a nucleic acid encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides or by the expression product of IL12A, IL12B, and/or IL12A and IL12B fusion encoded by such nucleic acid.

[0378] In some aspects, an inflammatory response can be measured by detecting increased levels of one or more inflammatory cytokines using methods known in the art, *e.g.,* ELISA. The term "inflammatory cytokine" refers to cytokines that are elevated in an inflammatory response. Examples of inflammatory cytokines include interleukin-6 (IL-6), CXCL1 (chemokine (C-X-C motif) ligand 1; also known as GROα, interferon-γ (IFNγ), tumor necrosis factor α (TNFα), interferon γ-induced protein 10 (IP-10), or granulocyte-colony stimulating factor (G-CSF). The term inflammatory cytokines includes also other cytokines associated with inflammatory responses known in the art, *e.g.*, interleukin-1 (IL-1), interleukin-8 (IL-8), interleukin-13 (11-13), interferon α (IFN-α), etc.

11. Modified Nucleotide Sequences Encoding IL12 Polypeptides

[0379] In some cases, the polynucleotide (*e.g.,* a RNA, *e.g.,* an mRNA) of the disclosure comprises a chemically modified nucleobase, *e.g.*, 5-methoxyuracil. In some cases, the mRNA is a uracil-modified sequence comprising an ORF encoding an IL12B polypeptide, IL12A polypeptide, and/ IL12B and IL12A fusion polypeptides, wherein the mRNA comprises a chemically modified nucleobase, *e.g.*, 5-methoxyuracil.

[0380] In certain aspects of the disclosure, when the 5-methoxyuracil base is connected to a ribose sugar, as it is in polynucleotides, the resulting modified nucleoside or nucleotide is referred to as 5-methoxyuridine. In some cases, uracil in the polynucleotide is at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least 90%, at least 95%, at least 99%, or about 100% 5-methoxyuracil. In one case, uracil in the polynucleotide is at least 95% 5-methoxyuracil. In another case, uracil in the polynucleotide is 100% 5-methoxyuracil.

[0381] In cases where uracil in the polynucleotide is at least 95% 5-methoxyuracil, overall uracil content can be adjusted such that an mRNA provides suitable protein expression levels while inducing little to no immune response. In some cases, the uracil content of the ORF ($\%U_{TM}$)is between about 105% and about 145%, about 105% and about 140%, about 110% and about 140%, about 110% and about 145%, about 115% and about 135%, about 105% and about 135%, about 110% and about 135%, about 115% and about 145%, or about 115% and about 140%. In other cases, the uracil content of the ORF is between about 117% and about 134% or between 118% and 132% of the $\%U_{TM}$. In some cases, the $\%U_{TM}$ is about 115%, about 120%, about 125%, about 130%, about 135%, about 140%, about 145%, or about 150%. In this context, the term "uracil" can refer to 5-methoxyuracil and/or naturally occurring uracil.

[0382] In some cases, the uracil content in the ORF of the mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure is less than about 50%, about 40%, about 30%, or about

20% of the total nucleobase content in the ORF. In some cases, the uracil content in the ORF is between about 15 % and about 25% of the total nucleobase content in the ORF. In other cases, the uracil content in the ORF is between about 20% and about 30% of the total nucleobase content in the ORF. In one case, the uracil content in the ORF of the mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is less than about 20% of the total nucleobase content in the open reading frame. In this context, the term "uracil" can refer to 5-methoxyuracil and/or naturally occurring uracil.

[0383] In further cases, the ORF of the mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides having 5-methoxyuracil and adjusted uracil content has increased Cytosine (C), Guanine (G), or Guanine/Cytosine (G/C) content (absolute or relative). In some cases, the overall increase in C, G, or G/C content (absolute or relative) of the ORF is at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 10%, at least about 15%, at least about 20%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 100% relative to the G/C content (absolute or relative) of the wild-type ORF. In some cases, the G, the C, or the G/C content in the ORF is less than about 100%, less than about 90%, less than about 85%, or less than about 80% of the theoretical maximum G, C, or G/C content of the corresponding wild type nucleotide sequence encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides ($\%G_{TMX}$; $\%C_{TMX}$, or $\%G/C_{TMX}$). In other cases, the G, the C, or the G/C content in the ORF is between about 70% and about 80%, between about 71% and about 79%, between about 71% and about 78%, or between about 71% and about 77% of the $\%G_{TMX}$, $\%C_{TMX}$, or $\%G/C_{TMX}$. In some cases, the increases in G and/or C content (absolute or relative) described herein can be conducted by replacing synonymous codons with low G, C, or G/C content with synonymous codons having higher G, C, or G/C content. In other cases, the increase in G and/or C content (absolute or relative) is conducted by replacing a codon ending with U with a synonymous codon ending with G or C.

[0384] In further cases, the ORF of the mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure comprises 5-methoxyuracil and has an adjusted uracil content containing less uracil pairs (UU) and/or uracil triplets (UUU) and/or uracil quadruplets (UUUU) than the corresponding wild-type nucleotide sequence encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides. In some cases, the ORF of the mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure contains no uracil pairs and/or uracil triplets and/or uracil quadruplets. In some cases, uracil pairs and/or uracil triplets and/or uracil quadruplets are reduced below a certain threshold, *e.g.,* no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 occurrences in the ORF of the mRNA encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides. In a particular case, the ORF of the mRNA encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure contains less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 non-phenylalanine uracil pairs and/or triplets. In another case, the ORF of the mRNA encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides contains no non-phenylalanine uracil pairs and/or triplets.

[0385] In further cases, the ORF of the mRNA encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure comprises 5-methoxyuracil and has an adjusted uracil content containing less uracil-rich clusters than the corresponding wild-type nucleotide sequence encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides. In some cases, the ORF of the mRNA encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides of the disclosure contains uracil-rich clusters that are shorter in length than corresponding uracil-rich clusters in the corresponding wild-type nucleotide sequence encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides.

[0386] In further cases, alternative lower frequency codons are employed. At least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100% of the codons in the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides -encoding ORF of the 5-methoxyuracil-comprising mRNA are substituted with alternative codons, each alternative codon having a codon frequency lower than the codon frequency of the substituted codon in the synonymous codon set. The ORF also has adjusted uracil content, as described above. In some cases, at least one codon in the ORF of the mRNA encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is substituted with an alternative codon having a codon frequency lower than the codon frequency of the substituted codon in the synonymous codon set.

[0387] In some cases, the adjusted uracil content, IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides -encoding ORF of the 5-methoxyuracil-comprising mRNA exhibits expression levels of IL12 when administered to a mammalian cell that are higher than expression levels of IL12 from the corresponding wild-type mRNA. In other cases, the expression levels of IL12 when administered to a mammalian cell are increased relative to a corresponding mRNA containing at least 95% 5-methoxyuracil and having a uracil content of about 160%, about 170%, about

180%, about 190%, or about 200% of the theoretical minimum. In yet other cases, the expression levels of IL12 when administered to a mammalian cell are increased relative to a corresponding mRNA, wherein at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100% of uracils are 1-methylpseudouracil or pseudouracils. In some cases, the mammalian cell is a mouse cell, a rat cell, or a rabbit cell. In other cases, the mammalian cell is a monkey cell or a human cell. In some cases, the human cell is a HeLa cell, a BJ fibroblast cell, or a peripheral blood mononuclear cell (PBMC). In some cases, IL12 is expressed when the mRNA is administered to a mammalian cell *in vivo.* In some cases, the mRNA is administered to mice, rabbits, rats, monkeys, or humans. In one case, mice are null mice. In some cases, the mRNA is administered to mice in an amount of about 0.01 mg/kg, about 0.05 mg/kg, about 0.1 mg/kg, or about 0.15 mg/kg. In some cases, the mRNA is administered intravenously or intramuscularly. In other cases, the IL12 polypeptide is expressed when the mRNA is administered to a mammalian cell *in vitro.* In some cases, the expression is increased by at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 50-fold, at least about 500-fold, at least about 1500-fold, or at least about 3000-fold. In other cases, the expression is increased by at least about 10%, about 20%, about 30%, about 40%, about 50%, 60%, about 70%, about 80%, about 90%, or about 100%.

[0388] In some cases, adjusted uracil content, IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides -encoding ORF of the 5-methoxyuracil-comprising mRNA exhibits increased stability. In some cases, the mRNA exhibits increased stability in a cell relative to the stability of a corresponding wild-type mRNA under the same conditions. In some cases, the mRNA exhibits increased stability including resistance to nucleases, thermal stability, and/or increased stabilization of secondary structure. In some cases, increased stability exhibited by the mRNA is measured by determining the half-life of the mRNA (*e.g.,* in a plasma, cell, or tissue sample) and/or determining the area under the curve (AUC) of the protein expression by the mRNA over time (*e.g., in vitro* or *in vivo*). An mRNA is identified as having increased stability if the half-life and/or the AUC is greater than the half-life and/or the AUC of a corresponding wild-type mRNA under the same conditions.

[0389] In some cases, the mRNA of the present disclosure induces a detectably lower immune response (*e.g.*, innate or acquired) relative to the immune response induced by a corresponding wild-type mRNA under the same conditions. In other cases, the mRNA of the present disclosure induces a detectably lower immune response (*e.g.*, innate or acquired) relative to the immune response induced by an mRNA that encodes for an IL12B polypeptide, IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides but does not comprise 5-methoxyuracil under the same conditions, or relative to the immune response induced by an mRNA that encodes for an IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides and that comprises 5-methoxyuracil but that does not have adjusted uracil content under the same conditions. The innate immune response can be manifested by increased expression of pro-inflammatory cytokines, activation of intracellular PRRs (RIG-I, MDA5, etc), cell death, and/or termination or reduction in protein translation. In some cases, a reduction in the innate immune response can be measured by expression or activity level of Type 1 interferons (*e.g.*, IFN-α, IFN-β, IFN-κ, IFN-δ, IFN-ε, IFN-τ, IFN-ω, and IFN-ζ) or the expression of interferon-regulated genes such as the toll-like receptors (*e.g.*, TLR7 and TLR8), and/or by decreased cell death following one or more administrations of the mRNA of the disclosure into a cell.

[0390] In some cases, the expression of Type-1 interferons by a mammalian cell in response to the mRNA of the present disclosure is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or greater than 99.9% relative to a corresponding wild-type mRNA, to an mRNA that encodes an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides but does not comprise 5-methoxyuracil, or to an mRNA that encodes an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides and that comprises 5-methoxyuracil but that does not have adjusted uracil content. In some cases, the interferon is IFN-β. In some cases, cell death frequency caused by administration of mRNA of the present disclosure to a mammalian cell is 10%, 25%, 50%, 75%, 85%, 90%, 95%, or over 95% less than the cell death frequency observed with a corresponding wild-type mRNA, an mRNA that encodes for an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides but does not comprise 5-methoxyuracil, or an mRNA that encodes for an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides and that comprises 5-methoxyuracil but that does not have adjusted uracil content. In some cases, the mammalian cell is a BJ fibroblast cell. In other cases, the mammalian cell is a splenocyte. In some cases, the mammalian cell is that of a mouse or a rat. In other cases, the mammalian cell is that of a human. In one case, the mRNA of the present disclosure does not substantially induce an innate immune response of a mammalian cell into which the mRNA is introduced.

[0391] In some cases, the polynucleotide is an mRNA that comprises an ORF that encodes an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides, wherein uracil in the mRNA is at least about 95% 5-methoxyuracil, wherein the uracil content of the ORF is between about 115% and about 135% of the theoretical minimum uracil content in the corresponding wild-type ORF, and wherein the uracil content in the ORF encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is less than about 30% of the total nucleobase content in the ORF. In some cases, the ORF that encodes the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is further modified to increase G/C content of the ORF (absolute or relative) by at least about

40%, as compared to the corresponding wild-type ORF. In yet other cases, the ORF encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides contains less than 20 non-phenylalanine uracil pairs and/or triplets. In some cases, at least one codon in the ORF of the mRNA encoding the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides is further substituted with an alternative codon having a codon frequency lower than the codon frequency of the substituted codon in the synonymous codon set. In some cases, the expression of the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides encoded by an mRNA comprising an ORF wherein uracil in the mRNA is at least about 95% 5-methoxyuracil, and wherein the uracil content of the ORF is between about 115% and about 135% of the theoretical minimum uracil content in the corresponding wild-type ORF, is increased by at least about 10-fold when compared to expression of the IL12A polypeptide, IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides from the corresponding wild-type mRNA. In some cases, the mRNA comprises an open ORF wherein uracil in the mRNA is at least about 95% 5-methoxyuracil, and wherein the uracil content of the ORF is between about 115% and about 135% of the theoretical minimum uracil content in the corresponding wild-type ORF, and wherein the mRNA does not substantially induce an innate immune response of a mammalian cell into which the mRNA is introduced.

12. Methods for Modifying Polynucleotides

[0392] The disclosure includes modified polynucleotides comprising a polynucleotide described herein (*e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides). The modified polynucleotides can be chemically modified and/or structurally modified. When the polynucleotides of the present disclosure are chemically and/or structurally modified the polynucleotides can be referred to as "modified polynucleotides."

[0393] The present disclosure provides for modified nucleosides and nucleotides of a polynucleotide (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides. A "nucleoside" refers to a compound containing a sugar molecule (*e.g.,* a pentose or ribose) or a derivative thereof in combination with an organic base (*e.g.,* a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase"). A "nucleotide" refers to a nucleoside including a phosphate group. Modified nucleotides may by synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides. Polynucleotides may comprise a region or regions of linked nucleosides. Such regions may have variable backbone linkages. The linkages may be standard phosphodiester linkages, in which case the polynucleotides would comprise regions of nucleotides.

[0394] The modified polynucleotides disclosed herein can comprise various distinct modifications. In some cases, the modified polynucleotides contain one, two, or more (optionally different) nucleoside or nucleotide modifications. In some cases, a modified polynucleotide, introduced to a cell may exhibit one or more desirable properties, *e.g.*, improved protein expression, reduced immunogenicity, or reduced degradation in the cell, as compared to an unmodified polynucleotide.

a. Structural Modifications

[0395] In some cases, a polynucleotide of the present disclosure (*e.g.*, a polynucleotide comprising a nucleotide sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides) is structurally modified. As used herein, a "structural" modification is one in which two or more linked nucleosides are inserted, deleted, duplicated, inverted or randomized in a polynucleotide without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different sequence of nucleotides. For example, the polynucleotide "ATCG (SEQ ID NO: 230)" can be chemically modified to "AT-5meC-G". The same polynucleotide can be structurally modified from "ATCG (SEQ ID NO: 230)" to "ATCCCG (SEQ ID NO: 231)". Here, the dinucleotide "CC" has been inserted, resulting in a structural modification to the polynucleotide.

b. Chemical Modifications

[0396] In some cases, the polynucleotides of the present disclosure (*e.g.*, a polynucleotide comprising a nucleotide sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides) are chemically modified. As used herein in reference to a polynucleotide, the terms "chemical modification" or, as appropriate, "chemically modified" refer to modification with respect to adenosine (A), guanosine (G), uridine (U), thymidine (T) or cytidine (C) ribo- or deoxyribonucleosides in one or more of their position, pattern, percent or population, including, but not limited to, its nucleobase, sugar, backbone, or any combination thereof. Generally, herein, these terms are not intended to refer to the ribonucleotide modifications in naturally occurring 5'-terminal mRNA cap moieties.

[0397] In some cases, the polynucleotides of the disclosure (*e.g.*, a polynucleotide comprising a nucleotide sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides) can have a uniform chemical modification of all or any of the same nucleoside type or a population of modifications produced by downward titration of the same starting modification in all or any of the same nucleoside type, or a measured percent of a chemical modification of all any of the same nucleoside type but with random incorporation, such as where all uridines are replaced by a uridine analog, *e.g.*, 5-methoxyuridine. In another case, the polynucleotides can have a uniform chemical modification of two, three, or four of the same nucleoside type throughout the entire polynucleotide (such as all uridines and/or all cytidines, etc. are modified in the same way).

[0398] Modified nucleotide base pairing encompasses not only the standard adenosine-thymine, adenosine-uracil, or guanosine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures. One example of such non-standard base pairing is the base pairing between the modified nucleotide inosine and adenine, cytosine or uracil. Any combination of base/sugar or linker may be incorporated into polynucleotides of the present disclosure.

[0399] The skilled artisan will appreciate that, except where otherwise noted, polynucleotide sequences set forth in the instant application will recite "T"s in a representative DNA sequence but where the sequence represents RNA, the "T"s would be substituted for "U"s.

[0400] Modifications of polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) that are useful in the composition of the present disclosure include, but are not limited to the following: 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine; 2-methylthio-N6-methyladenosine; 2-methylthio-N6-threonyl carbamoyladenosine; N6-glycinylcarbamoyladenosine; N6-isopentenyladenosine; N6-methyladenosine; N6-threonylcarbamoyladenosine; 1,2'-O-dimethyladenosine; 1-methyladenosine; 2'-O-methyladenosine; 2'-O-ribosyladenosine (phosphate); 2-methyladenosine; 2-methylthio-N6 isopentenyladenosine; 2-methylthio-N6-hydroxynorvalyl carbamoyladenosine; 2'-O-methyladenosine; 2'-O-ribosyladenosine (phosphate); Isopentenyladenosine; N6-(cis-hydroxyisopentenyl)adenosine; N6,2'-O-dimethyladenosine; N6,2'-O-dimethyladenosine; N6,N6,2'-O-trimethyladenosine; N6,N6-dimethyladenosine; N6-acetyladenosine; N6-hydroxynorvalylcarbamoyladenosine; N6-methyl-N6-threonylcarbamoyladenosine; 2-methyladenosine; 2-methylthio-N6-isopentenyladenosine; 7-deaza-adenosine; N1-methyl-adenosine; N6, N6 (dimethyl)adenine; N6-cis-hydroxy-isopentenyl-adenosine; α-thio-adenosine; 2 (amino)adenine; 2 (aminopropyl)adenine; 2 (methylthio) N6 (isopentenyl)adenine; 2-(alkyl)adenine; 2-(aminoalkyl)adenine; 2-(aminopropyl)adenine; 2-(halo)adenine; 2-(halo)adenine; 2-(propyl)adenine; 2'-Amino-2'-deoxy-ATP; 2'-Azido-2'-deoxy-ATP; 2'-Deoxy-2'-a-aminoadenosine TP; 2'-Deoxy-2'-a-azidoadenosine TP; 6 (alkyl)adenine; 6 (methyl)adenine; 6-(alkyl)adenine; 6-(methyl)adenine; 7 (deaza)adenine; 8 (alkenyl)adenine; 8 (alkynyl)adenine; 8 (amino)adenine; 8 (thioalkyl)adenine; 8-(alkenyl)adenine; 8-(alkyl)adenine; 8-(alkynyl)adenine; 8-(amino)adenine; 8-(halo)adenine; 8-(hydroxyl)adenine; 8-(thioalkyl)adenine; 8-(thiol)adenine; 8-azido-adenosine; aza adenine; deaza adenine; N6 (methyl)adenine; N6-(isopentyl)adenine; 7-deaza-8-aza-adenosine; 7-methyladenine; 1-Deazaadenosine TP; 2'Fluoro-N6-Bz-deoxyadenosine TP; 2'-OMe-2-Amino-ATP; 2'O-methyl-N6-Bz-deoxyadenosine TP; 2'-a-Ethynyladenosine TP; 2-aminoadenine; 2-Aminoadenosine TP; 2-Amino-ATP; 2'-a-Trifluoromethyladenosine TP; 2-Azidoadenosine TP; 2'-b-Ethynyladenosine TP; 2-Bromoadenosine TP; 2'-b-Trifluoromethyladenosine TP; 2-Chloroadenosine TP; 2'-Deoxy-2',2'-difluoroadenosine TP; 2'-Deoxy-2'-a-mercaptoadenosine TP; 2'-Deoxy-2'-a-thiomethoxyadenosine TP; 2'-Deoxy-2'-b-aminoadenosine TP; 2'-Deoxy-2'-b-azidoadenosine TP; 2'-Deoxy-2'-b-bromoadenosine TP; 2'-Deoxy-2'-b-chloroadenosine TP; 2'-Deoxy-2'-b-fluoroadenosine TP; 2'-Deoxy-2'-b-iodoadenosine TP; 2'-Deoxy-2'-b-mercaptoadenosine TP; 2'-Deoxy-2'-b-thiomethoxyadenosine TP; 2-Fluoroadenosine TP; 2-Iodoadenosine TP; 2-Mercaptoadenosine TP; 2-methoxy-adenine; 2-methylthio-adenine; 2-Trifluoromethyladenosine TP; 3-Deaza-3-bromoadenosine TP; 3-Deaza-3-chloroadenosine TP; 3-Deaza-3-fluoroadenosine TP; 3-Deaza-3-iodoadenosine TP; 3-Deazaadenosine TP; 4'-Azidoadenosine TP; 4'-Carbocyclic adenosine TP; 4'-Ethynyladenosine TP; 5'-Homo-adenosine TP; 8-Aza-ATP; 8-bromo-adenosine TP; 8-Trifluoromethyladenosine TP; 9-Deazaadenosine TP; 2-aminopurine; 7-deaza-2,6-diaminopurine; 7-deaza-8-aza-2,6-diaminopurine; 7-deaza-8-aza-2-aminopurine; 2,6-diaminopurine; 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine; 2-thiocytidine; 3-methylcytidine; 5-formylcytidine; 5-hydroxymethylcytidine; 5-methylcytidine; N4-acetylcytidine; 2'-O-methylcytidine; 2'-O-methylcytidine; 5,2'-O-dimethylcytidine; 5-formyl-2'-O-methylcytidine; Lysidine; N4,2'-O-dimethylcytidine; N4-acetyl-2'-O-methylcytidine; N4-methylcytidine; N4,N4-Dimethyl-2'-OMe-Cytidine TP; 4-methylcytidine; 5-aza-cytidine; Pseudo-iso-cytidine; pyrrolo-cytidine; α-thio-cytidine; 2-(thio)cytosine; 2'-Amino-2'-deoxy-CTP; 2'-Azido-2'-deoxy-CTP; 2'-Deoxy-2'-a-aminocytidine TP; 2'-Deoxy-2'-a-azidocytidine TP; 3 (deaza) 5 (aza)cytosine; 3 (methyl)cytosine; 3-(alkyl)cytosine; 3-(deaza) 5 (aza)cytosine; 3-(methyl)cytidine; 4,2'-O-dimethylcytidine; 5 (halo)cytosine; 5 (methyl)cytosine; 5 (propynyl)cytosine; 5 (trifluoromethyl)cytosine; 5-(alkyl)cytosine; 5-(alkynyl)cytosine; 5-(halo)cytosine; 5-(propynyl)cytosine; 5-(trifluoromethyl)cytosine; 5-bromo-cytidine; 5-iodo-cytidine; 5-propynyl cytosine; 6-(azo)cytosine; 6-aza-cytidine; aza cytosine; deaza cytosine; N4 (acetyl)cytosine; 1-methyl-1-deaza-pseudoisocytidine; 1-methyl-pseudoisocytidine; 2-methoxy-5-methyl-cytidine; 2-methoxy-cytidine; 2-thio-5-methyl-cytidine; 4-methoxy-1-methyl-pseudoisocytidine; 4-methoxy-pseudoisocytidine; 4-

thio-1-methyl-1-deaza-pseudoisocytidine; 4-thio-1-methyl-pseudoisocytidine; 4-thio-pseudoisocytidine; 5-aza-zebularine; 5-methyl-zebularine; pyrrolo-pseudoisocytidine; Zebularine; (E)-5-(2-Bromo-vinyl)cytidine TP; 2,2'-anhydro-cytidine TP hydrochloride; 2'Fluor-N4-Bz-cytidine TP; 2'Fluoro-N4-Acetyl-cytidine TP; 2'-O-Methyl-N4-Acetyl-cytidine TP; 2'O-methyl-N4-Bz-cytidine TP; 2'-a-Ethynylcytidine TP; 2'-a-Trifluoromethylcytidine TP; 2'-b-Ethynylcytidine TP; 2'-b-Trifluoromethylcytidine TP; 2'-Deoxy-2',2'-difluorocytidine TP; 2'-Deoxy-2'-a-mercaptocytidine TP; 2'-Deoxy-2'-a-thiomethoxycytidine TP; 2'-Deoxy-2'-b-aminocytidine TP; 2'-Deoxy-2'-b-azidocytidine TP; 2'-Deoxy-2'-b-bromocytidine TP; 2'-Deoxy-2'-b-chlorocytidine TP; 2'-Deoxy-2'-b-fluorocytidine TP; 2'-Deoxy-2'-b-iodocytidine TP; 2'-Deoxy-2'-b-mercaptocytidine TP; 2'-Deoxy-2'-b-thiomethoxycytidine TP; 2'-O-Methyl-5-(1-propynyl)cytidine TP; 3'-Ethynylcytidine TP; 4'-Azidocytidine TP; 4'-Carbocyclic cytidine TP; 4'-Ethynylcytidine TP; 5-(1-Propynyl)ara-cytidine TP; 5-(2-Chloro-phenyl)-2-thiocytidine TP; 5-(4-Amino-phenyl)-2-thiocytidine TP; 5-Aminoallyl-CTP; 5-Cyanocytidine TP; 5-Ethynylara-cytidine TP; 5-Ethynylcytidine TP; 5'-Homo-cytidine TP; 5-Methoxycytidine TP; 5-Trifluoromethyl-Cytidine TP; N4-Amino-cytidine TP; N4-Benzoyl-cytidine TP; Pseudoisocytidine; 7-methylguanosine; N2,2'-O-dimethylguanosine; N2-methylguanosine; Wyosine; 1,2'-O-dimethylguanosine; 1-methylguanosine; 2'-O-methylguanosine; 2'-O-ribosylguanosine (phosphate); 2'-O-methylguanosine; 2'-O-ribosylguanosine (phosphate); 7-aminomethyl-7-deazaguanosine; 7-cyano-7-deazaguanosine; Archaeosine; Methylwyosine; N2,7-dimethylguanosine; N2,N2,2'-O-trimethylguanosine; N2,N2,7-trimethylguanosine; N2,N2-dimethylguanosine; N2,7,2'-O-trimethylguanosine; 6-thio-guanosine; 7-deaza-guanosine; 8-oxo-guanosine; N1-methyl-guanosine; α-thio-guanosine; 2 (propyl)guanine; 2-(alkyl)guanine; 2'-Amino-2'-deoxy-GTP; 2'-Azido-2'-deoxy-GTP; 2'-Deoxy-2'-a-aminoguanosine TP; 2'-Deoxy-2'-a-azidoguanosine TP; 6 (methyl)guanine; 6-(alkyl)guanine; 6-(methyl)guanine; 6-methyl-guanosine; 7 (alkyl)guanine; 7 (deaza)guanine; 7 (methyl)guanine; 7-(alkyl)guanine; 7-(deaza)guanine; 7-(methyl)guanine; 8 (alkyl)guanine; 8 (alkynyl)guanine; 8 (halo)guanine; 8 (thioalkyl)guanine; 8-(alkenyl)guanine; 8-(alkyl)guanine; 8-(alkynyl)guanine; 8-(amino)guanine; 8-(halo)guanine; 8-(hydroxyl)guanine; 8-(thioalkyl)guanine; 8-(thiol)guanine; aza guanine; deaza guanine; N (methyl)guanine; N-(methyl)guanine; 1-methyl-6-thio-guanosine; 6-methoxy-guanosine; 6-thio-7-deaza-8-aza-guanosine; 6-thio-7-deaza-guanosine; 6-thio-7-methyl-guanosine; 7-deaza-8-aza-guanosine; 7-methyl-8-oxo-guanosine; N2,N2-dimethyl-6-thio-guanosine; N2-methyl-6-thio-guanosine; 1-Me-GTP; 2'Fluoro-N2-isobutyl-guanosine TP; 2'O-methyl-N2-isobutyl-guanosine TP; 2'-a-Ethynylguanosine TP; 2'-a-Trifluoromethylguanosine TP; 2'-b-Ethynylguanosine TP; 2'-b-Trifluoromethylguanosine TP; 2'-Deoxy-2',2'-difluoroguanosine TP; 2'-Deoxy-2'-a-mercaptoguanosine TP; 2'-Deoxy-2'-a-thiomethoxyguanosine TP; 2'-Deoxy-2'-b-aminoguanosine TP; 2'-Deoxy-2'-b-azidoguanosine TP; 2'-Deoxy-2'-b-bromoguanosine TP; 2'-Deoxy-2'-b-chloroguanosine TP; 2'-Deoxy-2'-b-fluoroguanosine TP; 2'-Deoxy-2'-b-iodoguanosine TP; 2'-Deoxy-2'-b-mercaptoguanosine TP; 2'-Deoxy-2'-b-thiomethoxyguanosine TP; 4'-Azidoguanosine TP; 4'-Carbocyclic guanosine TP; 4'-Ethynylguanosine TP; 5'-Homo-guanosine TP; 8-bromo-guanosine TP; 9-Deazaguanosine TP; N2-isobutyl-guanosine TP; 1-methylinosine; Inosine; 1,2'-O-dimethylinosine; 2'-O-methylinosine; 7-methylinosine; 2'-O-methylinosine; Epoxyqueuosine; galactosyl-queuosine; Mannosylqueuosine; Queuosine; allyamino-thymidine; aza thymidine; deaza thymidine; deoxy-thymidine; 2'-O-methyluridine; 2-thiouridine; 3-methyluridine; 5-carboxymethyluridine; 5-hydroxyuridine; 5-methyluridine; 5-taurinomethyl-2-thiouridine; 5-taurinomethyluridine; Dihydrouridine; Pseudouridine; (3-(3-amino-3-carboxypropyl)uridine; 1-methyl-3-(3-amino-5-carboxypropyl)pseudouridine; 1-methylpseduouridine; 1-methyl-pseudouridine; 2'-O-methyluridine; 2'-O-methylpseudouridine; 2'-O-methyluridine; 2-thio-2'-O-methyluridine; 3-(3-amino-3-carboxypropyl)uridine; 3,2'-O-dimethyluridine; 3-Methyl-pseudo-Uridine TP; 4-thiouridine; 5-(carboxyhydroxymethyl)uridine; 5-(carboxyhydroxymethyl)uridine methyl ester; 5,2'-O-dimethyluridine; 5,6-dihydro-uridine; 5-aminomethyl-2-thiouridine; 5-carbamoylmethyl-2'-O-methyluridine; 5-carbamoylmethyluridine; 5-carboxyhydroxymethyluridine; 5-carboxyhydroxymethyluridine methyl ester; 5-carboxymethylaminomethyl-2'-O-methyluridine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; 5-carboxymethylaminomethyluridine; 5-Carbamoylmethyluridine TP; 5-methoxycarbonylmethyl-2'-O-methyluridine; 5-methoxycarbonylmethyl-2-thiouridine; 5-methoxycarbonylmethyluridine; 5-methyluridine,), 5-methoxyuridine; 5-methyl-2-thiouridine; 5-methylaminomethyl-2-selenouridine; 5-methylaminomethyl-2-thiouridine; 5-methylaminomethyluridine; 5-Methyldihydrouridine; 5-Oxyacetic acid- Uridine TP; 5-Oxyacetic acid-methyl ester-Uridine TP; N1-methyl-pseudo-uridine; uridine 5-oxyacetic acid; uridine 5-oxyacetic acid methyl ester; 3-(3-Amino-3-carboxypropyl)-Uridine TP; 5-(iso-Pentenylaminomethyl)- 2-thiouridine TP; 5-(iso-Pentenylaminomethyl)-2'-O-methyluridine TP; 5-(iso-Pentenylaminomethyl)uridine TP; 5-propynyl uracil; α-thio-uridine; 1 (aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-4 (thio)pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-pseudouracil; 1 (aminocarbonylethylenyl)-2(thio)-pseudouracil; 1 (aminocarbonylethylenyl)-2,4-(dithio)pseudouracil; 1 (aminocarbonylethylenyl)-4 (thio)pseudouracil; 1 (aminocarbonylethylenyl)-pseudouracil; 1 substituted 2(thio)-pseudouracil; 1 substituted 2,4-(dithio)pseudouracil; 1 substituted 4 (thio)pseudouracil; 1 substituted pseudouracil; 1-(aminoalkylamino-carbonylethylenyl)-2-(thio)-pseudouracil; 1-Methyl-3-(3-amino-3-carboxypropyl) pseudouridine TP; 1-Methyl-3-(3-amino-3-carboxypropyl)pseudo-UTP; 1-Methyl-pseudo-UTP; 2 (thio)pseudouracil; 2' deoxy uridine; 2' fluorouridine; 2-(thio)uracil; 2,4-(dithio)psuedouracil; 2' methyl, 2'amino, 2'azido, 2'fluro-guanosine; 2'-Amino-2'-deoxy-UTP; 2'-Azido-2'-deoxy-UTP; 2'-Azido-deoxyuridine TP; 2'-O-methylpseudouridine; 2' deoxy uridine; 2' fluorouridine; 2'-Deoxy-2'-a-aminouridine TP; 2'-Deoxy-2'-a-azidouridine TP; 2-methylpseudouridine; 3 (3 amino-3 carboxypropyl)uracil; 4 (thio)pseu-

douracil; 4-(thio)pseudouracil; 4-(thio)uracil; 4-thiouracil; 5 (1,3-diazole-1-alkyl)uracil; 5 (2-aminopropyl)uracil; 5 (aminoalkyl)uracil; 5 (dimethylaminoalkyl)uracil; 5 (guanidiniumalkyl)uracil; 5 (methoxycarbonylmethyl)-2-(thio)uracil; 5 (methoxycarbonyl-methyl)uracil; 5 (methyl) 2 (thio)uracil; 5 (methyl) 2,4 (dithio)uracil; 5 (methyl) 4 (thio)uracil; 5 (methylaminomethyl)-2 (thio)uracil; 5 (methylaminomethyl)-2,4 (dithio)uracil; 5 (methylaminomethyl)-4 (thio)uracil; 5 (propynyl)uracil; 5 (trifluoromethyl)uracil; 5-(2-aminopropyl)uracil; 5-(alkyl)-2-(thio)pseudouracil; 5-(alkyl)-2,4 (dithio)pseudouracil; 5-(alkyl)-4 (thio)pseudouracil; 5-(alkyl)pseudouracil; 5-(alkyl)uracil; 5-(alkynyl)uracil; 5-(allylamino)uracil; 5-(cyanoalkyl)uracil; 5-(dialkylaminoalkyl)uracil; 5-(dimethylaminoalkyl)uracil; 5-(guanidiniumalkyl)uracil; 5-(halo)uracil; 5-(1,3-diazole-1-alkyl)uracil; 5-(methoxy)uracil; 5-(methoxycarbonylmethyl)-2-(thio)uracil; 5-(methoxycarbonyl-methyl)uracil; 5-(methyl) 2(thio)uracil; 5-(methyl) 2,4 (dithio)uracil; 5-(methyl) 4 (thio)uracil; 5-(methyl)-2-(thio)pseudouracil; 5-(methyl)-2,4 (dithio)pseudouracil; 5-(methyl)-4 (thio)pseudouracil; 5-(methyl)pseudouracil; 5-(methylaminomethyl)-2 (thio)uracil; 5-(methylaminomethyl)-2,4(dithio)uracil; 5-(methylaminomethyl)-4-(thio)uracil; 5-(propynyl)uracil; 5-(trifluoromethyl)uracil; 5-aminoallyl-uridine; 5-bromo-uridine; 5-iodo-uridine; 5-uracil; 6 (azo)uracil; 6-(azo)uracil; 6-aza-uridine; allyamino-uracil; aza uracil; deaza uracil; N3 (methyl)uracil; P seudo-UTP-1-2-ethanoic acid; Pseudouracil; 4-Thio-pseudo-UTP; 1-carboxymethyl-pseudouridine; 1-methyl-1-deaza-pseudouridine; 1-propynyl-uridine; 1-taurinomethyl-1-methyl-uridine; 1-taurinomethyl-4-thio-uridine; 1-taurinomethyl-pseudouridine; 2-methoxy-4-thio-pseudouridine; 2-thio-1-methyl-1-deaza-pseudouridine; 2-thio-1-methyl-pseudouridine; 2-thio-5-aza-uridine; 2-thio-dihydropseudouridine; 2-thio-dihydrouridine; 2-thio-pseudouridine; 4-methoxy-2-thio-pseudouridine; 4-methoxy-pseudouridine; 4-thio-1-methyl-pseudouridine; 4-thio-pseudouridine; 5-aza-uridine; Dihydropseudouridine; (±)1-(2-Hydroxypropyl)pseudouridine TP; (2R)-1-(2-Hydroxypropyl)pseudouridine TP; (2S)-1-(2-Hydroxypropyl)pseudouridine TP; (E)-5-(2-Bromo-vinyl)ara-uridine TP; (E)-5-(2-Bromo-vinyl)uridine TP; (Z)-5-(2-Bromo-vinyl)ara-uridine TP; (Z)-5-(2-Bromo-vinyl)uridine TP; 1-(2,2,2-Trifluoroethyl)-pseudo-UTP; 1-(2,2,3,3,3-Pentafluoropropyl)pseudouridine TP; 1-(2,2-Diethoxyethyl)pseudouridine TP; 1-(2,4,6-Trimethylbenzyl)pseudouridine TP; 1-(2,4,6-Trimethyl-benzyl)pseudo-UTP; 1-(2,4,6-Trimethyl-phenyl)pseudo-UTP; 1-(2-Amino-2-carboxyethyl)pseudo-UTP; 1-(2-Amino-ethyl)pseudo-UTP; 1-(2-Hydroxyethyl)pseudouridine TP; 1-(2-Methoxyethyl)pseudouridine TP; 1-(3,4-Bis-trifluoromethoxybenzyl)pseudouridine TP; 1-(3,4-Dimethoxybenzyl)pseudouridine TP; 1-(3-Amino-3-carboxypropyl)pseudo-UTP; 1-(3-Amino-propyl)pseudo-UTP; 1-(3-Cyclopropyl-prop-2-ynyl)pseudouridine TP; 1-(4-Amino-4-carboxybutyl)pseudo-UTP; 1-(4-Amino-benzyl)pseudo-UTP; 1-(4-Amino-butyl)pseudo-UTP; 1-(4-Amino-phenyl)pseudo-UTP; 1-(4-Azidobenzyl)pseudouridine TP; 1-(4-Bromobenzyl)pseudouridine TP; 1-(4-Chlorobenzyl)pseudouridine TP; 1-(4-Fluorobenzyl)pseudouridine TP; 1-(4-Iodobenzyl)pseudouridine TP; 1-(4-Methanesulfonylbenzyl)pseudouridine TP; 1-(4-Methoxybenzyl)pseudouridine TP; 1-(4-Methoxy-benzyl)pseudo-UTP; 1-(4-Methoxy-phenyl)pseudo-UTP; 1-(4-Methylbenzyl)pseudouridine TP; 1-(4-Methyl-benzyl)pseudo-UTP; 1-(4-Nitrobenzyl)pseudouridine TP; 1-(4-Nitro-benzyl)pseudo-UTP; 1(4-Nitro-phenyl)pseudo-UTP; 1-(4-Thiomethoxybenzyl)pseudouridine TP; 1-(4-Trifluoromethoxybenzyl)pseudouridine TP; 1-(4-Trifluoromethylbenzyl)pseudouridine TP; 1-(5-Amino-pentyl)pseudo-UTP; 1-(6-Amino-hexyl)pseudo-UTP; 1,6-Dimethyl-pseudo-UTP; 1-[3-(2-{2-[2-(2-Aminoethoxy)-ethoxy]-ethoxy}-ethoxy)-propionyl]pseudouridine TP; 1-{3-[2-(2-Aminoethoxy)-ethoxy]-propionyl } pseudouridine TP; 1-Acetylpseudouridine TP; 1-Alkyl-6-(1-propynyl)-pseudo-UTP; 1-Alkyl-6-(2-propynyl)-pseudo-UTP; 1-Alkyl-6-allyl-pseudo-UTP; 1-Alkyl-6-ethynyl-pseudo-UTP; 1-Alkyl-6-homoallyl-pseudo-UTP; 1-Alkyl-6-vinyl-pseudo-UTP; 1-Allylpseudouridine TP; 1-Aminomethyl-pseudo-UTP; 1-Benzoylpseudouridine TP; 1-Benzyloxymethylpseudouridine TP; 1-Benzyl-pseudo-UTP; 1-Biotinyl-PEG2-pseudouridine TP; 1-Biotinylpseudouridine TP; 1-Butyl-pseudo-UTP; 1-Cyanomethylpseudouridine TP; 1-Cyclobutylmethyl-pseudo-UTP; 1-Cyclobutyl-pseudo-UTP; 1-Cycloheptylmethyl-pseudo-UTP; 1-Cycloheptyl-pseudo-UTP; 1-Cyclohexylmethyl-pseudo-UTP; 1-Cyclohexyl-pseudo-UTP; 1-Cyclooctylmethyl-pseudo-UTP; 1-Cyclooctyl-pseudo-UTP; 1-Cyclopentylmethyl-pseudo-UTP; 1-Cyclopentyl-pseudo-UTP; 1-Cyclopropylmethyl-pseudo-UTP; 1-Cyclopropyl-pseudo-UTP; 1-Ethyl-pseudo-UTP; 1-Hexyl-pseudo-UTP; 1-Homoallylpseudouridine TP; 1-Hydroxymethylpseudouridine TP; 1-iso-propyl-pseudo-UTP; 1-Me-2-thio-pseudo-UTP; 1-Me-4-thio-pseudo-UTP; 1-Me-alpha-thio-pseudo-UTP; 1-Methanesulfonylmethylpseudouridine TP; 1-Methoxymethylpseudouridine TP; 1-Methyl-6-(2,2,2-Trifluoroethyl)pseudo-UTP; 1-Methyl-6-(4-morpholino)-pseudo-UTP; 1-Methyl-6-(4-thiomorpholino)-pseudo-UTP; 1-Methyl-6-(substituted phenyl)pseudo-UTP; 1-Methyl-6-amino-pseudo-UTP; 1-Methyl-6-azido-pseudo-UTP; 1-Methyl-6-bromo-pseudo-UTP; 1-Methyl-6-butyl-pseudo-UTP; 1-Methyl-6-chloro-pseudo-UTP; 1-Methyl-6-cyano-pseudo-UTP; 1-Methyl-6-dimethylamino-pseudo-UTP; 1-Methyl-6-ethoxy-pseudo-UTP; 1-Methyl-6-ethyl-carboxylate-pseudo-UTP; 1-Methyl-6-ethyl-pseudo-UTP; 1-Methyl-6-fluoro-pseudo-UTP; 1-Methyl-6-formyl-pseudo-UTP; 1-Methyl-6-hydroxyamino-pseudo-UTP; 1-Methyl-6-hydroxy-pseudo-UTP; 1-Methyl-6-iodo-pseudo-UTP; 1-Methyl-6-iso-propyl-pseudo-UTP; 1-Methyl-6-methoxy-pseudo-UTP; 1-Methyl-6-methylamino-pseudo-UTP; 1-Methyl-6-phenyl-pseudo-UTP; 1-Methyl-6-propyl-pseudo-UTP; 1-Methyl-6-tert-butyl-pseudo-UTP; 1-Methyl-6-trifluoromethoxy-pseudo-UTP; 1-Methyl-6-trifluoromethyl-pseudo-UTP; 1-Morpholinomethylpseudouridine TP; 1-Pentyl-pseudo-UTP; 1-Phenyl-pseudo-UTP; 1-Pivaloylpseudouridine TP; 1-Propargylpseudouridine TP; 1-Propyl-pseudo-UTP; 1-propynyl-pseudouridine; 1-p-tolyl-pseudo-UTP; 1-tert-Butyl-pseudo-UTP; 1-Thiomethoxymethylpseudouridine TP; 1-Thiomorpholinomethylpseudouridine TP; 1-Trifluoroacetylpseudouridine TP; 1-Trifluoromethyl-pseudo-UTP; 1-Vinylpseudouridine TP; 2,2'-anhydro-uridine TP; 2'-bromo-deoxyuridine TP; 2'-F-5-Methyl-2'-deoxy-UTP; 2'-OMe-5-Me-UTP; 2'-OMe-pseudo-UTP; 2'-a-Ethynyluridine TP; 2'-a-Trifluoromethyluridine TP; 2'-b-Ethynyluridine TP; 2'-b-Trifluoromethyluridine

TP; 2'-Deoxy-2',2'-difluorouridine TP; 2'-Deoxy-2'-a-mercaptouridine TP; 2'-Deoxy-2'-a-thiomethoxyuridine TP; 2'-Deoxy-2'-b-aminouridine TP; 2'-Deoxy-2'-b-azidouridine TP; 2'-Deoxy-2'-b-bromouridine TP; 2'-Deoxy-2'-b-chlorouridine TP; 2'-Deoxy-2'-b-fluorouridine TP; 2'-Deoxy-2'-b-iodouridine TP; 2'-Deoxy-2'-b-mercaptouridine TP; 2'-Deoxy-2'-b-thiomethoxyuridine TP; 2-methoxy-4-thio-uridine; 2-methoxyuridine; 2'-O-Methyl-5-(1-propynyl)uridine TP; 3-Alkyl-pseudo-UTP; 4'-Azidouridine TP; 4'-Carbocyclic uridine TP; 4'-Ethynyluridine TP; 5-(1-Propynyl)ara-uridine TP; 5-(2-Furanyl)uridine TP; 5-Cyanouridine TP; 5-Dimethylaminouridine TP; 5'-Homo-uridine TP; 5-iodo-2'-fluoro-deoxyuridine TP; 5-Phenylethynyluridine TP; 5-Trideuteromethyl-6-deuterouridine TP; 5-TrifluoromethylUridine TP; 5-Vinylarauridine TP; 6-(2,2,2-Trifluoroethyl)-pseudo-UTP; 6-(4-Morpholino)-pseudo-UTP; 6-(4-Thiomorpholino)-pseudo-UTP; 6-(Substituted-Phenyl)-pseudo-UTP; 6-Amino-pseudo-UTP; 6-Azido-pseudo-UTP; 6-Bromo-pseudo-UTP; 6-Butyl-pseudo-UTP; 6-Chloro-pseudo-UTP; 6-Cyano-pseudo-UTP; 6-Dimethylamino-pseudo-UTP; 6-Ethoxy-pseudo-UTP; 6-Ethylcarboxylate-pseudo-UTP; 6-Ethyl-pseudo-UTP; 6-Fluoro-pseudo-UTP; 6-Formyl-pseudo-UTP; 6-Hydroxyamino-pseudo-UTP; 6-Hydroxy-pseudo-UTP; 6-Iodo-pseudo-UTP; 6-iso-Propyl-pseudo-UTP; 6-Methoxy-pseudo-UTP; 6-Methylamino-pseudo-UTP; 6-Methyl-pseudo-UTP; 6-Phenyl-pseudo-UTP; 6-Phenyl-pseudo-UTP; 6-Propyl-pseudo-UTP; 6-tert-Butyl-pseudo-UTP; 6-Trifluoromethoxy-pseudo-UTP; 6-Trifluoromethyl-pseudo-UTP; Alpha-thio-pseudo-UTP; Pseudouridine 1-(4-methylbenzenesulfonic acid) TP; Pseudouridine 1-(4-methylbenzoic acid) TP; Pseudouridine TP 1-[3-(2-ethoxy)]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-(2-ethoxy)-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-{2(2-ethoxy)-ethoxy}-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-ethoxy)-ethoxy}] propionic acid; Pseudouridine TP 1-methylphosphonic acid; Pseudouridine TP 1-methylphosphonic acid diethyl ester; Pseudo-UTP-N1-3-propionic acid; Pseudo-UTP-N1-4-butanoic acid; Pseudo-UTP-N1-5-pentanoic acid; Pseudo-UTP-N1-6-hexanoic acid; Pseudo-UTP-N1-7-heptanoic acid; Pseudo-UTP-N1-methyl-p-benzoic acid; Pseudo-UTP-Nl-p-benzoic acid; Wybutosine; Hydroxywybutosine; Isowyosine; Peroxywybutosine; undermodified hydroxywybutosine; 4-demethylwyosine; 2,6-(diamino)purine;1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl: 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl;1,3-(diaza)-2-(oxo)-phenoxazin-1-yl; 1,3,5-(triaza)-2,6-(dioxa)-naphthalene;2 (amino)purine;2,4,5-(trimethyl)phenyl;2' methyl, 2'amino, 2'azido, 2'fluro-cytidine;2' methyl, 2'amino, 2'azido, 2'fluro-adenine;2'methyl, 2'amino, 2'azido, 2'fluro-uridine;2'-amino-2'-deoxyribose; 2-amino-6-Chloro-purine; 2-aza-inosinyl; 2'-azido-2'-deoxyribose; 2'fluoro-2'-deoxyribose; 2'-fluoro-modified bases; 2'-O-methyl-ribose; 2-oxo-7-aminopyridopyrimidin-3-yl; 2-oxo-pyridopyrimidine-3-yl; 2-pyridinone; 3 nitropyrrole; 3-(methyl)-7-(propynyl)isocarbostyrilyl; 3-(methyl)isocarbostyrilyl; 4-(fluoro)-6-(methyl)benzimidazole; 4-(methyl)benzimidazole; 4-(methyl)indolyl; 4,6-(dimethyl)indolyl; 5 nitroindole; 5 substituted pyrimidines; 5-(methyl)isocarbostyrilyl; 5-nitroindole; 6-(aza)pyrimidine; 6-(azo)thymine; 6-(methyl)-7-(aza)indolyl; 6-chloropurine; 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl; 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl;7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl; 7-(aza)indolyl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazinl-yl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl; 7-(guanidiniumalkyl-hydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl; 7-(propynyl)isocarbostyrilyl; 7-(propynyl)isocarbostyrilyl, propynyl-7-(aza)indolyl; 7-deaza-inosinyl; 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-substituted 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl; 9-(methyl)-imidizopyridinyl; Aminoindolyl; Anthracenyl; bis-ortho-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; bis-ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Difluorotolyl; Hypoxanthine; Imidizopyridinyl; Inosinyl; Isocarbostyrilyl; Isoguanisine; N2-substituted purines; N6-methyl-2-aminopurine; N6-substituted purines; N-alkylated derivative; Napthalenyl; Nitrobenzimidazolyl; Nitroimidazolyl; Nitroindazolyl; Nitropyrazolyl; Nubularine; O6-substituted purines; O-alkylated derivative; ortho-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Oxoformycin TP; para-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; para-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Pentacenyl; Phenanthracenyl; Phenyl; propynyl-7-(aza)indolyl; Pyrenyl; pyridopyrimidin-3-yl; pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl; pyrrolopyrimidin-2-on-3-yl; Pyrrolopyrimidinyl; Pyrrolopyrizinyl; Stilbenzyl; substituted 1,2,4-triazoles; Tetracenyl; Tubercidine; Xanthine; Xanthosine-5'-TP; 2-thio-zebularine; 5-aza-2-thio-zebularine; 7-deaza-2-amino-purine; pyridin-4-one ribonucleoside; 2-Amino-riboside-TP; Formycin A TP; Formycin B TP; Pyrrolosine TP; 2'-OH-ara-adenosine TP; 2'-OH-ara-cytidine TP; 2'-OH-ara-uridine TP; 2'-OH-ara-guanosine TP; 5-(2-carbomethoxyvinyl)uridine TP; and N6-(19-Amino-pentaoxanonadecyl)adenosine TP.

[0401] In some cases, the polynucleotide (e.g., RNA polynucleotide, such as mRNA polynucleotide) includes a combination of at least two *(e.g.,* 2, 3, 4 or more) of the aforementioned modified nucleobases.

[0402] In some cases, the mRNA comprises at least one chemically modified nucleoside. In some cases, the at least one chemically modified nucleoside is selected from the group consisting of pseudouridine (ψ), N1-methylpseudouridine ($m1\psi$), 2-thiouridine (s2U), 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-urid-

ine, dihydropseudouridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, 1-methyl-pseudouridine (m1ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), α-thio-guanosine, α-thio-adenosine, 5-cyano uridine, 4'-thio uridine 7-deaza-adenine, 1-methyl-adenosine (m1A), 2-methyl-adenine (m2A), N6-methyl-adenosine (m6A), and 2,6-Diaminopurine, (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-guanosine (preQO), 7-aminomethyl-7-deaza-guanosine (preQ1), 7-methyl-guanosine (m7G), 1-methyl-guanosine (m1G), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 2,8-dimethyladenosine, 2-geranylthiouridine, 2-lysidine, 2-selenouridine, 3-(3-amino-3-carboxypropyl)-5,6-dihydrouridine, 3-(3-amino-3-carboxypropyl)pseudouridine, 3-methylpseudouridine, 5-(carboxyhydroxymethyl)-2'-O-methyluridine methyl ester, 5-aminomethyl-2-geranylthiouridine, 5-aminomethyl-2-selenouridine, 5-aminomethyluridine, 5-carbamoylhydroxymethyluridine, 5-carbamoylmethyl-2-thiouridine, 5-carboxymethyl-2-thiouridine, 5-carboxymethylaminomethyl-2-geranylthiouridine, 5-carboxymethylaminomethyl-2-selenouridine, 5-cyanomethyluridine, 5-hydroxycytidine, 5-methylaminomethyl-2-geranylthiouridine, 7-aminocarboxypropyl-demethylwyosine, 7-aminocarboxypropylwyosine, 7-aminocarboxypropylwyosine methyl ester, 8-methyladenosine, N4,N4-dimethylcytidine, N6-formyladenosine, N6-hydroxymethyladenosine, agmatidine, cyclic N6-threonylcarbamoyladenosine, glutamyl-queuosine, methylated undermodified hydroxywybutosine, N4,N4,2'-O-trimethylcytidine, geranylated 5-methylaminomethyl-2-thiouridine, geranylated 5-carboxymethylaminomethyl-2-thiouridine, Qbase , preQ0base, preQ1base, and two or more combinations thereof. In some cases, the at least one chemically modified nucleoside is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5-methoxyuridine, and a combination thereof. In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) includes a combination of at least two (*e.g.,* 2, 3, 4 or more) of the aforementioned modified nucleobases. In one particular case, the at least one chemically modified nucleoside is N1-methylpseudouridine.

(i) Base Modifications

[0403] In certain cases, the chemical modification is at nucleobases in the polynucleotides (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide).

[0404] In some cases, a polynucleotide as disclosed herein comprises at least one chemically modified nucleobase.

[0405] In some cases, the at least one chemically modified nucleobase is selected from the group consisting of pseudouracil (ψ), N1-methylpseudouracil (m1ψ), 2-thiouracil (s2U), 4'-thiouracil, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouracil, 2-thio-1-methyl-pseudouracil, 2-thio-5-aza-uracil, 2-thio-dihydropseudouracil, 2-thio-dihydrouracil, 2-thio-pseudouracil, 4-methoxy-2-thio-pseudouracil, 4-methoxy-pseudouracil, 4-thio-1-methyl-pseudouracil, 4-thio-pseudouracil, 5-aza-uracil, dihydropseudouracil, 5-methyluracil, 5-methoxyuracil, 2'-O-methyl uracil, 1-methyl-pseudouracil (m1ψ), 5-methoxy-uracil (mo5U), 5-methyl-cytosine (m5C), α-thio-guanine, α-thio-adenine, 5-cyano uracil, 4'-thio uracil, 7-deaza-adenine, 1-methyl-adenine (m1A), 2-methyl-adenine (m2A), N6-methyl-adenine (m6A), and 2,6-Diaminopurine, (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanine, 7-cyano-7-deaza-guanine (preQO), 7-aminomethyl-7-deaza-guanine (preQ1), 7-methyl-guanine (m7G), 1-methyl-guanine (m1G), 8-oxo-guanine, 7-methyl-8-oxo-guanine, and two or more combinations thereof.

[0406] In some cases, the nucleobases in a polynucleotide as disclosed herein are chemically modified by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 95%, at least 99%, or 100%.

[0407] In some cases, the chemically modified nucleobases are selected from the group consisting of uracil, adenine, cytosine, guanine, and any combination thereof.

[0408] In some cases, the uracils in a polynucleotide disclosed herein are chemically modified by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 95%, at least 99%, or 100%.

[0409] In some cases, the adenines in a polynucleotide disclosed herein are chemically modified by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 95%, at least 99%, or 100%.

[0410] In some cases, the cytosines in a polynucleotide disclosed herein are chemically modified by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 95%, at least 99%, or 100%.

[0411] In some cases, the guanines in a polynucleotide disclosed herein are chemically modified by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 95%, at least 99%, or 100%.

[0412] In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) includes a combination of at least two (*e.g.,* 2, 3, 4 or more) of modified nucleobases.

[0413] In some cases, modified nucleobases in the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) are selected from the group consisting of 1-methyl-pseudouridine (m1ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), pseudouridine (ψ), α-thio-guanosine and α-thio-adenosine. In some cases, the polynucleotide includes

a combination of at least two (*e.g.,* 2, 3, 4 or more) of the aforementioned modified nucleobases.

[0414] In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises pseudouridine (ψ) and 5-methyl-cytidine (m5C). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 1-methyl-pseudouridine (m1ψ). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 1-methyl-pseudouridine (m1ψ) and 5-methyl-cytidine (m5C). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 2-thiouridine (s2U). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 2-thiouridine and 5-methyl-cytidine (m5C). In some cases, the polynucleotide (*e.g.,* RNA polynucleotide, such as mRNA polynucleotide) comprises methoxy-uridine (mo5U). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 5-methoxy-uridine (mo5U) and 5-methyl-cytidine (m5C). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 2'-O-methyl uridine. In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 2'-O-methyl uridine and 5-methyl-cytidine (m5C). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises N6-methyl-adenosine (m6A). In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises N6-methyl-adenosine (m6A) and 5-methyl-cytidine (m5C).

[0415] In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) is uniformly modified (*e.g.,* fully modified, modified throughout the entire sequence) for a particular modification. For example, a polynucleotide can be uniformly modified with 5-methyl-cytidine (m5C), meaning that all cytosine residues in the mRNA sequence are replaced with 5-methyl-cytidine (m5C). Similarly, a polynucleotide can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as any of those set forth above.

[0416] In some cases, the chemically modified nucleosides in the open reading frame are selected from the group consisting of uridine, adenine, cytosine, guanine, and any combination thereof.

[0417] In some cases, the modified nucleobase is a modified cytosine. Examples of nucleobases and nucleosides having a modified cytosine include N4-acetyl-cytidine (ac4C), 5-methyl-cytidine (m5C), 5-halo-cytidine (*e.g.*, 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm5C), 1-methyl-pseudoisocytidine, 2-thio-cytidine (s2C), 2-thio-5 -methyl-cytidine.

[0418] In some cases, a modified nucleobase is a modified uridine. Example nucleobases and nucleosides having a modified uridine include 5-cyano uridine or 4'-thio uridine.

[0419] In some cases, a modified nucleobase is a modified adenine. Example nucleobases and nucleosides having a modified adenine include 7-deaza-adenine, 1-methyl-adenosine (m1A), 2-methyl-adenine (m2A), N6-methyl-adenine (m6A), and 2,6-Diaminopurine.

[0420] In some cases, a modified nucleobase is a modified guanine. Example nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-guanosine (preQ0), 7-aminomethyl-7-deaza-guanosine (preQ1), 7-methyl-guanosine (m7G), 1-methyl-guanosine (m1G), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine.

[0421] In some cases, the nucleobase modified nucleotides in the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) are 5-methoxyuridine.

[0422] In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) includes a combination of at least two (*e.g.,* 2, 3, 4 or more) of modified nucleobases.

[0423] In some cases, at least 95% of a type of nucleobases (*e.g.*, uracil) in a polynucleotide of the disclosure (*e.g.*, an mRNA polynucleotide encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides) are modified nucleobases. In some cases, at least 95% of uracil in a polynucleotide of the present disclosure (*e.g.*, an mRNA polynucleotide encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides) is 5-methoxyuracil.

[0424] In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) comprises 5-methoxyuridine (5mo5U) and 5-methyl-cytidine (m5C).

[0425] In some cases, the polynucleotide (*e.g.*, RNA polynucleotide, such as mRNA polynucleotide) is uniformly modified (*e.g.,* fully modified, modified throughout the entire sequence) for a particular modification. For example, a polynucleotide can be uniformly modified with 5-methoxyuridine, meaning that substantially all uridine residues in the mRNA sequence are replaced with 5-methoxyuridine. Similarly, a polynucleotide can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as any of those set forth above.

[0426] In some cases, the modified nucleobase is a modified cytosine.

[0427] In some cases, a modified nucleobase is a modified uracil. Example nucleobases and nucleosides having a modified uracil include 5-methoxyuracil.

[0428] In some cases, a modified nucleobase is a modified adenine.

[0429] In some cases, a modified nucleobase is a modified guanine.

[0430] In some cases, the nucleobases, sugar, backbone, or any combination thereof in the open reading frame encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides are chemically modified by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least

80%, at least 90%, at least 95%, at least 99%, or 100%.

[0431] In some cases, the uridine nucleosides in the open reading frame encoding IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides are chemically modified by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100%.

[0432] In some cases, the adenosine nucleosides in the open reading frame encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides are chemically modified by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100%.

[0433] In some cases, the cytidine nucleosides in the open reading frame encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides are chemically modified by at least at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100%.

[0434] In some cases, the guanosine nucleosides in the open reading frame encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides are chemically modified by at least at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100%.

[0435] In some cases, the polynucleotides can include any useful linker between the nucleosides, including subunit and heterologous polypeptide linkers as disclosed elsewhere herein. Such linkers, including backbone modifications, that are useful in the composition of the present disclosure include, but are not limited to the following: 3'-alkylene phosphonates, 3'-amino phosphoramidate, alkene containing backbones, aminoalkylphosphoramidates, aminoalkylphosphotriesters, boranophosphates, $-CH_2-ON(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$, $-CH_2-NH-CH_2-$, chiral phosphonates, chiral phosphorothioates, formacetyl and thioformacetyl backbones, methylene (methylimino), methylene formacetyl and thioformacetyl backbones, methyleneimino and methylenehydrazino backbones, morpholino linkages, $-N(CH_3)-CH_2-CH_2-$, oligonucleosides with heteroatom internucleoside linkage, phosphinates, phosphoramidates, phosphorodithioates, phosphorothioate internucleoside linkages, phosphorothioates, phosphotriesters, PNA, siloxane backbones, sulfamate backbones, sulfide sulfoxide and sulfone backbones, sulfonate and sulfonamide backbones, thionoalkylphosphonates, thionoalkylphosphotriesters, and thionophosphoramidates.

(ii) Sugar Modifications

[0436] The modified nucleosides and nucleotides (*e.g.*, building block molecules), which can be incorporated into a polynucleotide (*e.g.*, RNA or mRNA, as described herein), can be modified on the sugar of the ribonucleic acid. For example, the 2' hydroxyl group (OH) can be modified or replaced with a number of different substituents. Exemplary substitutions at the 2'-position include, but are not limited to, H, halo, optionally substituted C_{1-6} alkyl; optionally substituted C_{1-6} alkoxy; optionally substituted C_{6-10} aryloxy; optionally substituted C_{3-8} cycloalkyl; optionally substituted C_{3-8} cycloalkoxy; optionally substituted C_{6-10} aryloxy; optionally substituted C_{6-10} aryl-C_{1-6} alkoxy, optionally substituted C_{1-12} (heterocyclyl)oxy; a sugar (*e.g.*, ribose, pentose, or any described herein); a polyethyleneglycol (PEG), $-O(CH_2CH_2O)_nCH_2CH_2OR$, where R is H or optionally substituted alkyl, and n is an integer from 0 to 20 (*e.g.,* from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20); "locked" nucleic acids (LNA) in which the 2'-hydroxyl is connected by a C_{1-6} alkylene or C_{1-6} heteroalkylene bridge to the 4'-carbon of the same ribose sugar, where exemplary bridges included methylene, propylene, ether, or amino bridges; aminoalkyl, as defined herein; aminoalkoxy, as defined herein; amino as defined herein; and amino acid, as defined herein

[0437] Generally, RNA includes the sugar group ribose, which is a 5-membered ring having an oxygen. Exemplary, non-limiting modified nucleotides include replacement of the oxygen in ribose (*e.g.,* with S, Se, or alkylene, such as methylene or ethylene); addition of a double bond (*e.g.,* to replace ribose with cyclopentenyl or cyclohexenyl); ring contraction of ribose (*e.g.,* to form a 4-membered ring of cyclobutane or oxetane); ring expansion of ribose (*e.g.,* to form a 6- or 7-membered ring having an additional carbon or heteroatom, such as for anhydrohexitol, altritol, mannitol, cyclohexanyl, cyclohexenyl, and morpholino that also has a phosphoramidate backbone); multicyclic forms (*e.g.,* tricyclo; and "unlocked" forms, such as glycol nucleic acid (GNA) (*e.g.,* R-GNA or S-GNA, where ribose is replaced by glycol units attached to phosphodiester bonds), threose nucleic acid (TNA, where ribose is replace with α-L-threofuranosyl-(3'$\rightarrow$2')), and peptide nucleic acid (PNA, where 2-amino-ethyl-glycine linkages replace the ribose and phosphodiester backbone). The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a polynucleotide molecule can include nucleotides containing, *e.g.,* arabinose, as the sugar. Such sugar modifications are taught International Patent Publication Nos. WO2013052523 and WO2014093924.

(iii) Combinations of Modifications

[0438] The polynucleotides of the disclosure (*e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12A polypeptide, an IL12B polypeptide, and/or IL12A and IL12B fusion polypeptides or a functional fragment or variant thereof) can include a combination of modifications to the sugar, the nucleobase, and/or the internucleoside linkage. These combinations can include any one or more modifications described herein.

[0439] Examples of modified nucleotides and modified nucleotide combinations are provided below in **Table 5**. Combinations of modified nucleotides can be used to form the polynucleotides of the disclosure. Unless otherwise noted, the modified nucleotides can be completely substituted for the natural nucleotides of the polynucleotides of the disclosure. As a non-limiting example, the natural nucleotide uridine can be substituted with a modified nucleoside described herein. In another non-limiting example, the natural nucleotide uridine can be partially substituted or replaced (*e.g.,* about 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99.9%) with at least one of the modified nucleoside disclosed herein. Any combination of base/sugar or linker can be incorporated into the polynucleotides of the disclosure and such modifications are taught in International Patent Publications WO2013052523 and WO2014093924, and U.S. Publ. Nos. US 20130115272 and US20150307542.

Table 5. Combinations

Uracil	Cytosine	Adenine	Guanine
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	N4Ac-CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Trifluoromethyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Hydroxymethyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Bromo-CTP	ATP	GTP
5-Methoxy-UTP	N4Ac-CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Trifluoromethyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Hydroxymethyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Bromo-CTP	ATP	GTP
5-Methoxy-UTP	N4-Ac-CTP	ATP	GTP
5-Methoxy-UTP	5-Iodo-CTP	ATP	GTP
5-Methoxy-UTP	5-Bromo-CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	5-Methyl-CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	5-Methyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
5-Methoxy-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
50 % 5-Methoxy-UTP + 50 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	CTP	Alpha-thio-ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	Alpha-thio-ATP	GTP
5-Methoxy-UTP	CTP	ATP	Alpha-thio-GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	Alpha-thio-GTP
5-Methoxy-UTP	CTP	N6-Me-ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	N6-Me-ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	5-Methyl-CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	5-Methyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
5-Methoxy-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 %	50 % 5-Methyl-CTP +	ATP	GTP
UTP	50 % CTP		
50 % 5-Methoxy-UTP + 50 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
75 % 5-Methoxy-UTP + 25 % UTP	CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Ethyl-CTP	ATP	GTP
5-Methoxy-UTP	5-Methoxy-CTP	ATP	GTP
5-Methoxy-UTP	5-Ethynyl-CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % 1-Methyl-pseudo-UTP	5-Methyl-CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % 1-Methyl-pseudo-UTP	5-Methyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % 1-Methyl-pseudo-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
5-Methoxy-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % 1-Methyl-pseudo-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % 1-Methyl-pseudo-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % 1-Methyl-pseudo-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % 1-Methyl-pseudo-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % 1-Methyl-pseudo-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % 1-Methyl-pseudo-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % 1-Methyl-pseudo-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % 1-Methyl-pseudo-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % 1-Methyl-pseudo-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % 1-Methyl-pseudo-UTP	CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % 1-Methyl-pseudo-UTP	CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
25 % 5-Methoxy-UTP + 75 % 1-Methyl-pseudo-UTP	CTP	ATP	GTP
5-methoxy-UTP	CTP	ATP	GTP
5-methoxy-UTP	CTP	ATP	GTP
5-methoxy-UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	5-Methyl-CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	5-Methyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
5-Methoxy-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 %	75 % 5-Methyl-CTP +	ATP	GTP
UTP	25 % CTP		
75 % 5-Methoxy-UTP + 25 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
5-Methoxy-UTP	5-Methyl-CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	5-Methyl-CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	5-Methyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	5-Methyl-CTP	ATP	GTP
5-Methoxy-UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
5-Methoxy-UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
50 % 5-Methoxy-UTP + 50 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	CTP	ATP	GTP
50 % 5-Methoxy-UTP + 50 % UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 %	75 % 5-Methyl-CTP +	ATP	GTP
UTP	25 % CTP		
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	50 % 5-Methyl-CTP + 50 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Fluoro-CTP	ATP	GTP
5-Methoxy-UTP	5-Phenyl-CTP	ATP	GTP
5-Methoxy-UTP	N4-Bz-CTP	ATP	GTP
5-Methoxy-UTP	CTP	N6-Isopentenyl-ATP	GTP
5-Methoxy-UTP	N4-Ac-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % N4-Ac-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % N4-Ac-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % N4-Ac-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % N4-Ac-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Hydroxymethyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Hydroxymethyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Hydroxymethyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Hydroxymethyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Hydroxymethyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	N4-Methyl CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % N4-Methyl CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % N4-Methyl CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 %	25 % N4-Methyl CTP +	ATP	GTP
UTP	75 % CTP		
75 % 5-Methoxy-UTP + 25 % UTP	75 % N4-Methyl CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Trifluoromethyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Trifluoromethyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Trifluoromethyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Trifluoromethyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Trifluoromethyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Bromo-CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Bromo-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Bromo-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Bromo-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Bromo-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Iodo-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Iodo-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Iodo-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Iodo-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Iodo-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Ethyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Ethyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Ethyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Ethyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Ethyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Methoxy-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Methoxy-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Methoxy-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Methoxy-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Methoxy-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Ethynyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Ethynyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Ethynyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Ethynyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Ethynyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Pseudo-iso-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Pseudo-iso-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Pseudo-iso-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Pseudo-iso-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Pseudo-iso-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Formyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Formyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Formyl-CTP + 25 % CTP	ATP	GTP

(continued)

Uracil	Cytosine	Adenine	Guanine
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Formyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Formyl-CTP + 25 % CTP	ATP	GTP
5-Methoxy-UTP	5-Aminoallyl-CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	25 % 5-Aminoallyl-CTP + 75 % CTP	ATP	GTP
25 % 5-Methoxy-UTP + 75 % UTP	75 % 5-Aminoallyl-CTP + 25 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	25 % 5-Aminoallyl-CTP + 75 % CTP	ATP	GTP
75 % 5-Methoxy-UTP + 25 % UTP	75 % 5-Aminoallyl-CTP + 25 % CTP	ATP	GTP

13. Untranslated Regions (UTRs)

[0440] Untranslated regions (UTRs) are nucleic acid sections of a polynucleotide before a start codon (5'UTR) and after a stop codon (3'UTR) that are not translated. In some cases, a polynucleotide (*e.g.,* a ribonucleic acid (RNA), *e.g.,* a messenger RNA (mRNA)) of the disclosure comprising an open reading frame (ORF) encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12A and IL12B fusion polypeptides further comprises UTR (*e.g.,* a 5'UTR or functional fragment thereof, a 3'UTR or functional fragment thereof, or a combination thereof).

[0441] A UTR can be homologous or heterologous to the coding region in a polynucleotide. In some cases, the UTR is homologous to the ORF encoding the IL12 polypeptide. In some cases, the UTR is heterologous to the ORF encoding the IL12 polypeptide. In some cases, the polynucleotide comprises two or more 5'UTRs or functional fragments thereof, each of which have the same or different nucleotide sequences. In some cases, the polynucleotide comprises two or more 3'UTRs or functional fragments thereof, each of which have the same or different nucleotide sequences.

[0442] In some cases, the 5'UTR or functional fragment thereof, 3' UTR or functional fragment thereof, or any combination thereof is sequence optimized.

[0443] In some cases, the 5'UTR or functional fragment thereof, 3' UTR or functional fragment thereof, or any combination thereof comprises at least one chemically modified nucleobase, *e.g.,* 5-methoxyuracil.

[0444] UTRs can have features that provide a regulatory role, *e.g.,* increased or decreased stability, localization and/or translation efficiency. A polynucleotide comprising a UTR can be administered to a cell, tissue, or organism, and one or more regulatory features can be measured using routine methods. In some cases, a functional fragment of a 5'UTR or 3'UTR comprises one or more regulatory features of a full length 5' or 3' UTR, respectively.

[0445] Natural 5'UTRs bear features that play roles in translation initiation. They harbor signatures like Kozak sequences that are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG (SEQ ID NO: 232), where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5'UTRs also have been known to form secondary structures that are involved in elongation factor binding.

[0446] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production of a polynucleotide. For example, introduction of 5'UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, can enhance expression of polynucleotides in hepatic cell lines or liver. Likewise, use of 5'UTR from other tissue-specific mRNA to improve expression in that tissue is possible for muscle (*e.g.,* MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (*e.g.,* Tie-1, CD36), for myeloid cells (*e.g.,* C/EBP, AML1, G-CSF, GM-CSF, CD11b, MSR, Fr-1, i-NOS), for leukocytes (e.g., CD45, CD18), for adipose tissue (*e.g.,* CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (*e.g.,* SP-A/B/C/D).

[0447] In some cases, UTRs are selected from a family of transcripts whose proteins share a common function, structure, feature or property. For example, an encoded polypeptide can belong to a family of proteins (*i.e.,* that share at least one function, structure, feature, localization, origin, or expression pattern), which are expressed in a particular cell, tissue or at some time during development. The UTRs from any of the genes or mRNA can be swapped for any other UTR of the same or different family of proteins to create a new polynucleotide.

[0448] In some cases, the 5'UTR and the 3'UTR can be heterologous. In some cases, the 5'UTR can be derived from

a different species than the 3'UTR. In some cases, the 3'UTR can be derived from a different species than the 5'UTR.

[0449] Co-owned International Patent Application No. PCT/US2014/021522 (Publ. No. WO/2014/164253) provides a listing of exemplary UTRs that can be utilized in the polynucleotide of the present disclosure as flanking regions to an ORF.

[0450] Exemplary UTRs of the application include, but are not limited to, one or more 5'UTR and/or 3'UTR derived from the nucleic acid sequence of: a globin, such as an α- or β-globin (*e.g.,* a *Xenopus*, mouse, rabbit, or human globin); a strong Kozak translational initiation signal; a CYBA (*e.g.,* human cytochrome b-245 α polypeptide); an albumin (*e.g.,* human albumin7); a HSD17B4 (hydroxysteroid (17-β) dehydrogenase); a virus (*e.g.,* a tobacco etch virus (TEV), a Venezuelan equine encephalitis virus (VEEV), a Dengue virus, a cytomegalovirus (CMV) (*e.g.,* CMV immediate early 1 (IE1)), a hepatitis virus (*e.g.,* hepatitis B virus), a sindbis virus, or a PAV barley yellow dwarf virus); a heat shock protein (*e.g.,* hsp70); a translation initiation factor (*e.g.,* eIF4G); a glucose transporter (*e.g.,* hGLUT1 (human glucose transporter 1)); an actin (*e.g.,* human α or β actin); a GAPDH; a tubulin; a histone; a citric acid cycle enzyme; a topoisomerase (*e.g.,* a 5'UTR of a TOP gene lacking the 5' TOP motif (the oligopyrimidine tract)); a ribosomal protein Large 32 (L32); a ribosomal protein (*e.g.,* human or mouse ribosomal protein, such as, for example, rps9); an ATP synthase (*e.g.,* ATP5A1 or the β subunit of mitochondrial H^+-ATP synthase); a growth hormone e (*e.g.,* bovine (bGH) or human (hGH)); an elongation factor (*e.g.,* elongation factor 1 α1 (EEF1A1)); a manganese superoxide dismutase (MnSOD); a myocyte enhancer factor 2A (MEF2A); a β-F1-ATPase, a creatine kinase, a myoglobin, a granulocyte-colony stimulating factor (G-CSF); a collagen (*e.g.,* collagen type I, alpha 2 (Col1A2), collagen type I, alpha 1 (Coll A1), collagen type VI, alpha 2 (Col6A2), collagen type VI, alpha 1 (Col6A1)); a ribophorin (*e.g.,* ribophorin I (RPNI)); a low density lipoprotein receptor-related protein (*e.g.,* LRP1); a cardiotrophin-like cytokine factor (*e.g.,* Nnt1); calreticulin (Calr); a procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (Plod1); and a nucleobindin (*e.g.,* Nucb1).

[0451] Other exemplary 5' and 3' UTRs include, but are not limited to, those described in Karikó et al., Mol. Ther. 2008 16(11): 1833-1840; Karikó et al., Mol. Ther. 2012 20(5):948-953; Karikó et al., Nucleic Acids Res. 2011 39(21):e142; Strong et al., Gene Therapy 1997 4:624-627; Hansson et al., J. Biol. Chem. 2015 290(9):5661-5672; Yu et al., Vaccine 2007 25(10):1701-1711; Cafri et al., Mol. Ther. 2015 23(8):1391-1400; Andries et al., Mol. Pharm. 2012 9(8):2136-2145; Crowley et al., Gene Ther. 2015 Jun 30, doi:10.1038/gt.2015.68; Ramunas et al., FASEB J. 2015 29(5):1930-1939; Wang et al., Curr. Gene Ther. 2015 15(4):428-435; Holtkamp et al., Blood 2006 108(13):4009-4017; Kormann et al., Nat. Biotechnol. 2011 29(2):154-157; Poleganov et al., Hum. Gen. Ther. 2015 26(11):751-766; Warren et al., Cell Stem Cell 2010 7(5):618-630; Mandal and Rossi, Nat. Protoc. 2013 8(3):568-582; Holcik and Liebhaber, PNAS 1997 94(6):2410-2414; Ferizi et al., Lab Chip. 2015 15(17):3561-3571; Thess et al., Mol. Ther. 2015 23(9):1456-1464; Boros et al., PLoS One 2015 10(6):e0131141; Boros et al., J. Photochem. Photobiol. B. 2013 129:93-99; Andries et al., J. Control. Release 2015 217:337-344; Zinckgraf et al., Vaccine 2003 21(15): 1640-9; Garneau et al., J. Virol. 2008 82(2):880-892; Holden and Harris, Virology 2004 329(1):119-133; Chiu et al., J. Virol. 2005 79(13):8303-8315; Wang et al., EMBO J. 1997 16(13):4107-4116; Al-Zoghaibi et al., Gene 2007 391(1-2):130-9; Vivinus et al., Eur. J. Biochem. 2001 268(7):1908-1917; Gan and Rhoads, J. Biol. Chem. 1996 271(2):623-626; Boado et al., J. Neurochem. 1996 67(4):1335-1343; Knirsch and Clerch, Biochem. Biophys. Res. Commun. 2000 272(1):164-168; Chung et al., Biochemistry 1998 37(46):16298-16306; Izquierdo and Cuevza, Biochem. J. 2000 346 Pt 3:849-855; Dwyer et al., J. Neurochem. 1996 66(2):449-458; Black et al., Mol. Cell. Biol. 1997 17(5):2756-2763; Izquierdo and Cuevza, Mol. Cell. Biol. 1997 17(9):5255-5268; US8278036; US8748089; US8835108; US9012219; US2010/0129877; US2011/0065103; US2011/0086904; US2012/0195936; US2014/020675; US2013/0195967; US2014/029490; US2014/0206753; WO2007/036366; WO2011/015347; WO2012/072096; WO2013/143555; WO2014/071963; WO2013/185067; WO2013/182623; WO2014/089486; WO2013/185069; WO2014/144196; WO2014/152659; 2014/152673; WO2014/152940; WO2014/152774; WO2014/153052; WO2014/152966, WO2014/152513; WO2015/101414; WO2015/101415; WO2015/062738; and WO2015/024667.

[0452] In some cases, the 5'UTR is selected from the group consisting of a β-globin 5'UTR; a 5'UTR containing a strong Kozak translational initiation signal; a cytochrome b-245 α polypeptide (CYBA) 5'UTR; a hydroxysteroid (17-β) dehydrogenase (HSD17B4) 5'UTR; a Tobacco etch virus (TEV) 5'UTR; a Venezuelen equine encephalitis virus (TEEV) 5'UTR; a 5' proximal open reading frame of rubella virus (RV) RNA encoding nonstructural proteins; a Dengue virus (DEN) 5'UTR; a heat shock protein 70 (Hsp70) 5'UTR; a eIF4G 5'UTR; a GLUT1 5'UTR; functional fragments thereof and any combination thereof.

[0453] In some cases, the 3'UTR is selected from the group consisting of a β-globin 3'UTR; a CYBA 3'UTR; an albumin 3'UTR; a growth hormone (GH) 3'UTR; a VEEV 3'UTR; a hepatitis B virus (HBV) 3'UTR; α-globin 3'UTR; a DEN 3'UTR; a PAV barley yellow dwarf virus (BYDV-PAV) 3'UTR; an elongation factor 1 α1 (EEF1A1) 3'UTR; a manganese superoxide dismutase (MnSOD) 3'UTR; a β subunit of mitochondrial H(+)-ATP synthase (β-mRNA) 3'UTR; a GLUT1 3'UTR; a MEF2A 3'UTR; a β-F1-ATPase 3'UTR; functional fragments thereof and combinations thereof.

[0454] Other exemplary UTRs include, but are not limited to, one or more of the UTRs, including any combination of UTRs, disclosed in WO2014/164253. Shown in Table 21 of U.S. Provisional Application No. 61/775,509 and in Table 22 of U.S. Provisional Application No. 61/829,372, is a listing start and stop sites for 5'UTRs and 3'UTRs. In Table 21, each 5'UTR (5'-UTR-005 to 5'-UTR 68511) is identified by its start and stop site relative to its native or wild-type (ho-

mologous) transcript (ENST; the identifier used in the ENSEMBL database).

[0455] Wild-type UTRs derived from any gene or mRNA can be incorporated into the polynucleotides of the disclosure. In some cases, a UTR can be altered relative to a wild type or native UTR to produce a variant UTR, *e.g.*, by changing the orientation or location of the UTR relative to the ORF; or by inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. In some cases, variants of 5' or 3' UTRs can be utilized, for example, mutants of wild type UTRs, or variants wherein one or more nucleotides are added to or removed from a terminus of the UTR.

[0456] Additionally, one or more synthetic UTRs can be used in combination with one or more non-synthetic UTRs. *See, e.g.*, Mandal and Rossi, Nat. Protoc. 2013 8(3):568-82, and sequences available at www.addgene.org/Derrick_Rossi/, last accessed April 16, 2016. UTRs or portions thereof can be placed in the same orientation as in the transcript from which they were selected or can be altered in orientation or location. Hence, a 5' and/or 3' UTR can be inverted, shortened, lengthened, or combined with one or more other 5'UTRs or 3'UTRs.

[0457] In some cases, the polynucleotide comprises multiple UTRs, *e.g.*, a double, a triple or a quadruple 5'UTR or 3'UTR. For example, a double UTR comprises two copies of the same UTR either in series or substantially in series. For example, a double beta-globin 3'UTR can be used (see US2010/0129877).

[0458] In certain cases, the polynucleotides of the disclosure comprise a 5'UTR and/or a 3'UTR selected from any of the UTRs disclosed herein. In some cases, the 5'UTR comprises:

5'UTR-001 (Upstream UTR)
(GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 135);
5'UTR-002 (Upstream UTR)
(GGGAGATCAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 136);
5'UTR-003 (Upstream UTR)

(GGAATAAAAGTCTCAACACAACATATACAAAACAAACGAATCTCAAGCAAT
CAAGCATTCTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAAG
CAATTTTCTGAAAATTTTCACCATTTACGAACGATAGCAAC) (SEQ ID NO. 137);

5'UTR-004 (Upstream UTR)
(GGGAGACAAGCUUGGCAUUCCGGUACUGUUGGUAAAGCCACC) (SEQ ID NO. 138);
5'UTR-005 (Upstream UTR)
(GGGAGATCAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 139);
5'UTR-006 (Upstream UTR)

(GGAATAAAAGTCTCAACACAACATATACAAAACAAACGAATCTCAAGCAAT
CAAGCATTCTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAAG
CAATTTTCTGAAAATTTTCACCATTTACGAACGATAGCAAC) (SEQ ID NO. 140);

5'UTR-007 (Upstream UTR)
(GGGAGACAAGCUUGGCAUUCCGGUACUGUUGGUAAAGCCACC) (SEQ ID NO. 141);
5'UTR-008 (Upstream UTR)
(GGGAATTAACAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 142);
5'UTR-009 (Upstream UTR)
(GGGAAATTAGACAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 143);
5'UTR-010, Upstream
(GGGAAATAAGAGAGTAAAGAACAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 144);
5UTR-011 (Upstream UTR)
(GGGAAAAAAGAGAGAAAAGAAGACTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 145);
5'UTR-012 (Upstream UTR)
(GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGATATATAAGAGCCACC) (SEQ ID NO. 146);
5'UTR-013 (Upstream UTR)
(GGGAAATAAGAGACAAAACAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 147);
5'UTR-014 (Upstream UTR)
(GGGAAATTAGAGAGTAAAGAACAGTAAGTAGAATTAAAAGAGCCACC)(SEQ ID NO. 148);
5'UTR-15 (Upstream UTR)

(GGGAAATAAGAGAGAATAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 149);
5'UTR-016 (Upstream UTR)
(GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAAATTAAGAGCCACC) (SEQ ID NO. 150);
5'UTR-017 (Upstream UTR)
(GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATTTAAGAGCCACC) (SEQ ID NO. 151);
5'UTR-018 (Upstream UTR)

(TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAAT AAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC) (SEQ ID NO. 152);

142-3p 5'UTR-001 (Upstream UTR including miR142-3p)

(TGATAATAGTCCATAAAGTAGGAAACACTACAGCTGGAGCCTCGGTGGCCAT GCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTA CCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC) (SEQ ID NO. 153);

142-3p 5'UTR-002 (Upstream UTR including miR142-3p)

(TGATAATAGGCTGGAGCCTCGGTGGCTCCATAAAGTAGGAAACACTACACAT GCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTA CCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC) (SEQ ID NO. 154);

142-3p 5'UTR-003 (Upstream UTR including miR142-3p)

(TGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTCCATAAAGT AGGAAACACTACATGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGT ACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC) (SEQ ID NO. 155);

142-3p 5'UTR-004 (Upstream UTR including miR142-3p)

(TGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCC CCAGTCCATAAAGTAGGAAACACTACACCCCTCCTCCCCTTCCTGCACCCGTA CCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC) (SEQ ID NO. 156);

142-3p 5'UTR-005 (Upstream UTR including miR142-3p)

(TGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCC CCAGCCCCTCCTCCCCTTCTCCATAAAGTAGGAAACACTACACTGCACCCGTA CCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC) (SEQ ID NO. 157);

142-3p 5'UTR-006 (Upstream UTR including miR142-3p)

(TGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCC
CCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCTCCATAAAGTAGGAAACA
CTACAGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC) (SEQ ID NO. 158); or

142-3p 5'UTR-007 (Upstream UTR including miR142-3p)

(TGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCC
CCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTT
CCATAAAGTAGGAAACACTACACTGAGTGGGCGGC) (SEQ ID NO. 159).

[0459] In some cases, the 3'UTR comprises:

3'UTR-001 (Creatine Kinase UTR)

(GCGCCTGCCCACCTGCCACCGACTGCTGGAACCCAGCCAGTGGGAGGGCCTG
GCCCACCAGAGTCCTGCTCCCTCACTCCTCGCCCCGCCCCCTGTCCCAGAGTC
CCACCTGGGGGCTCTCTCCACCCTTCTCAGAGTTCCAGTTTCAACCAGAGTTC
CAACCAATGGGCTCCATCCTCTGGATTCTGGCCAATGAAATATCTCCCTGGCA
GGGTCCTCTTCTTTTCCCAGAGCTCCACCCCAACCAGGAGCTCTAGTTAATGG
AGAGCTCCCAGCACACTCGGAGCTTGTGCTTTGTCTCCACGCAAAGCGATAA
ATAAAAGCATTGGTGGCCTTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTA
GA) (SEQ ID NO. 160);

3'UTR-002 (Myoglobin UTR)

(GCCCCTGCCGCTCCCACCCCCACCCATCTGGGCCCCGGGTTCAAGAGAGAGC
GGGGTCTGATCTCGTGTAGCCATATAGAGTTTGCTTCTGAGTGTCTGCTTTGTT
TAGTAGAGGTGGGCAGGAGGAGCTGAGGGGCTGGGGCTGGGGTGTTGAAGTT
GGCTTTGCATGCCCAGCGATGCGCCTCCCTGTGGGATGTCATCACCCTGGGAA
CCGGGAGTGGCCCTTGGCTCACTGTGTTCTGCATGGTTTGGATCTGAATTAAT
TGTCCTTTCTTCTAAATCCCAACCGAACTTCTTCCAACCTCCAAACTGGCTGTA
ACCCCAAATCCAAGCCATTAACTACACCTGACAGTAGCAATTGTCTGATTAAT
CACTGGCCCCTTGAAGACAGCAGAATGTCCCTTTGCAATGAGGAGGAGATCT
GGGCTGGGCGGGCCAGCTGGGGAAGCATTTGACTATCTGGAACTTGTGTGTG
CCTCCTCAGGTATGGCAGTGACTCACCTGGTTTTAATAAAACAACCTGCAACA
TCTCATGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGA) (SEQ ID NO. 161);

3'UTR-003 (α-actin UTR)

(ACACACTCCACCTCCAGCACGCGACTTCTCAGGACGACGAATCTTCTCAATG GGGGGGCGGCTGAGCTCCAGCCACCCCGCAGTCACTTTCTTTGTAACAACTTC CGTTGCTGCCATCGTAAACTGACACAGTGTTTATAACGTGTACATACATTAAC TTATTACCTCATTTTGTTATTTTTCGAAACAAAGCCCTGTGGAAGAAAATGGA AAACTTGAAGAAGCATTAAAGTCATTCTGTTAAGCTGCGTAAATGGTCTTTGA ATAAAGCCTGAGTAGGAAGTCTAGA) (SEQ ID NO. 162);

3'UTR-004 (Albumin UTR)

(CATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAAT GAAGATCAAAAGCTTATTCATCTGTTTTTCTTTTTCGTTGGTGTAAAGCCAAC ACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGC

TTCAATTAATAAAAAATGGAAAGAATCTAATAGAGTGGTACAGCACTGTTAT TTTTCAAAGATGTGTTGCTATCCTGAAAATTCTGTAGGTTCTGTGGAAGTTCC AGTGTTCTCTCTTATTCCACTTCGGTAGAGGATTTCTAGTTTCTTGTGGGCTAA TTAAATAAATCATTAATACTCTTCTAATGGTCTTTGAATAAAGCCTGAGTAGG AAGTCTAGA) (SEQ ID NO. 163);

3'UTR-005 (α-globin UTR)

(GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCAC CTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGAGC ATGCATCTAGA) (SEQ ID NO. 164);

3'UTR-006 (G-CSF UTR)

(GCCAAGCCCTCCCCATCCCATGTATTTATCTCTATTTAATATTTATGTCTATTT
AAGCCTCATATTTAAAGACAGGGAAGAGCAGAACGGAGCCCCAGGCCTCTGT
GTCCTTCCCTGCATTTCTGAGTTTCATTCTCCTGCCTGTAGCAGTGAGAAAAA
GCTCCTGTCCTCCCATCCCCTGGACTGGGAGGTAGATAGGTAAATACCAAGTA
TTTATTACTATGACTGCTCCCCAGCCCTGGCTCTGCAATGGGCACTGGGATGA
GCCGCTGTGAGCCCCTGGTCCTGAGGGTCCCCACCTGGGACCCTTGAGAGTAT
CAGGTCTCCCACGTGGGAGACAAGAAATCCCTGTTTAATATTTAAACAGCAG
TGTTCCCCATCTGGGTCCTTGCACCCCTCACTCTGGCCTCAGCCGACTGCACA
GCGGCCCCTGCATCCCCTTGGCTGTGAGGCCCCTGGACAAGCAGAGGTGGCC
AGAGCTGGGAGGCATGGCCCTGGGGTCCCACGAATTTGCTGGGGAATCTCGT
TTTTCTTCTTAAGACTTTTGGGACATGGTTTGACTCCCGAACATCACCGACGC
GTCTCCTGTTTTTCTGGGTGGCCTCGGGACACCTGCCCTGCCCCCACGAGGGT
CAGGACTGTGACTCTTTTTAGGGCCAGGCAGGTGCCTGGACATTTGCCTTGCT
GGACGGGGACTGGGGATGTGGGAGGGAGCAGACAGGAGGAATCATGTCAGG
CCTGTGTGTGAAAGGAAGCTCCACTGTCACCCTCCACCTCTTCACCCCCCACT
CACCAGTGTCCCCTCCACTGTCACATTGTAACTGAACTTCAGGATAATAAAGT
GTTTGCCTCCATGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGA
GCATGCATCTAGA) (SEQ ID NO. 165);

3'UTR-007 (Col1a2; collagen, type I, alpha 2 UTR)

(ACTCAATCTAAATTAAAAAAGAAAGAAATTTGAAAAAACTTTCTCTTTGCCA
TTTCTTCTTCTTCTTTTTTAACTGAAAGCTGAATCCTTCCATTTCTTCTGCACAT
CTACTTGCTTAAATTGTGGGCAAAAGAGAAAAAGAAGGATTGATCAGAGCAT

TGTGCAATACAGTTTCATTAACTCCTTCCCCCGCTCCCCCAAAAATTTGAATTT
TTTTTTCAACACTCTTACACCTGTTATGGAAAATGTCAACCTTTGTAAGAAAA
CCAAAATAAAAATTGAAAAATAAAAACCATAAACATTTGCACCACTTGTGGC
TTTTGAATATCTTCCACAGAGGGAAGTTTAAAACCCAAACTTCCAAAGGTTTA
AACTACCTCAAAACACTTTCCCATGAGTGTGATCCACATTGTTAGGTGCTGAC
CTAGACAGAGATGAACTGAGGTCCTTGTTTTGTTTTGTTCATAATACAAAGGT
GCTAATTAATAGTATTTCAGATACTTGAAGAATGTTGATGGTGCTAGAAGAAT
TTGAGAAGAAATACTCCTGTATTGAGTTGTATCGTGTGGTGTATTTTTTAAAA
AATTTGATTTAGCATTCATATTTTCCATCTTATTCCCAATTAAAAGTATGCAGA
TTATTTGCCCAAATCTTCTTCAGATTCAGCATTTGTTCTTTGCCAGTCTCATTTT
CATCTTCTTCCATGGTTCCACAGAAGCTTTGTTTCTTGGGCAAGCAGAAAAAT
TAAATTGTACCTATTTTGTATATGTGAGATGTTTAAATAAATTGTGAAAAAAA
TGAAATAAAGCATGTTTGGTTTTCCAAAAGAACATAT) (SEQ ID NO. 166);

3'UTR-008 (Col6a2; collagen, type VI, alpha 2 UTR)

(CGCCGCCGCCCCGGGCCCCGCAGTCGAGGGTCGTGAGCCCACCCCGTCCATGG
TGCTAAGCGGGCCCGGGTCCCACACGGCCAGCACCGCTGCTCACTCGGACGA
CGCCCTGGGCCTGCACCTCTCCAGCTCCTCCCACGGGGTCCCCGTAGCCCCGG
CCCCCGCCCAGCCCCAGGTCTCCCCAGGCCCTCCGCAGGCTGCCCGGCCTCCC
TCCCCCTGCAGCCATCCCAAGGCTCCTGACCTACCTGGCCCCTGAGCTCTGGA
GCAAGCCCTGACCCAATAAAGGCTTTGAACCCAT) (SEQ ID NO. 167);

3'UTR-009 (RPN1; ribophorin I UTR)

(GGGGCTAGAGCCCTCTCCGCACAGCGTGGAGACGGGGCAAGGAGGGGGGTT
ATTAGGATTGGTGGTTTTGTTTTGCTTTGTTTAAAGCCGTGGGAAAATGGCAC
AACTTTACCTCTGTGGGAGATGCAACACTGAGAGCCAAGGGGTGGGAGTTGG
GATAATTTTTATATAAAAGAAGTTTTTCCACTTTGAATTGCTAAAAGTGGCAT
TTTTCCTATGTGCAGTCACTCCTCTCATTTCTAAAATAGGGACGTGGCCAGGC
ACGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCAGGCGGC
TCACGAGGTCAGGAGATCGAGACTATCCTGGCTAACACGGTAAAACCCTGTC
TCTACTAAAAGTACAAAAAATTAGCTGGGCGTGGTGGTGGGCACCTGTAGTC
CCAGCTACTCGGGAGGCTGAGGCAGGAGAAAGGCATGAATCCAAGAGGCAG
AGCTTGCAGTGAGCTGAGATCACGCCATTGCACTCCAGCCTGGGCAACAGTG

TTAAGACTCTGTCTCAAATATAAATAAATAAATAAATAAATAAATAAATAAA
TAAAAATAAAGCGAGATGTTGCCCTCAAA) (SEQ ID NO. 168);

3'UTR-010 (LRP1; low density lipoprotein receptor-related protein 1 UTR)

(GGCCCTGCCCCGTCGGACTGCCCCCAGAAAGCCTCCTGCCCCCTGCCAGTGA
AGTCCTTCAGTGAGCCCCTCCCCAGCCAGCCCTTCCCTGGCCCCGCCGGATGT
ATAAATGTAAAAATGAAGGAATTACATTTTATATGTGAGCGAGCAAGCCGGC
AAGCGAGCACAGTATTATTTCTCCATCCCCTCCCTGCCTGCTCCTTGGCACCC
CCATGCTGCCTTCAGGGAGACAGGCAGGGAGGGCTTGGGGCTGCACCTCCTA
CCCTCCCACCAGAACGCACCCCACTGGGAGAGCTGGTGGTGCAGCCTTCCCCT
CCCTGTATAAGACACTTTGCCAAGGCTCTCCCCTCTCGCCCCATCCCTGCTTGC
CCGCTCCCACAGCTTCCTGAGGGCTAATTCTGGGAAGGGAGAGTTCTTTGCTG
CCCCTGTCTGGAAGACGTGGCTCTGGGTGAGGTAGGCGGGAAAGGATGGAGT
GTTTTAGTTCTTGGGGGAGGCCACCCCAAACCCCAGCCCCAACTCCAGGGGC
ACCTATGAGATGGCCATGCTCAACCCCCCTCCCAGACAGGCCCTCCCTGTCTC
CAGGGCCCCCACCGAGGTTCCCAGGGCTGGAGACTTCCTCTGGTAAACATTCC
TCCAGCCTCCCCTCCCCTGGGGACGCCAAGGAGGTGGGCCACACCCAGGAAG
GGAAAGCGGGCAGCCCCGTTTTGGGGACGTGAACGTTTTAATAATTTTTGCTG
AATTCCTTTACAACTAAATAACACAGATATTGTTATAAATAAAATTGT) (SEQ
ID NO. 169);

3'UTR-011 (Nnt1; cardiotrophin-like cytokine factor 1 UTR)

(ATATTAAGGATCAAGCTGTTAGCTAATAATGCCACCTCTGCAGTTTTGGGAA
CAGGCAAATAAAGTATCAGTATACATGGTGATGTACATCTGTAGCAAAGCTC
TTGGAGAAAATGAAGACTGAAGAAAGCAAAGCAAAAACTGTATAGAGAGAT
TTTTCAAAAGCAGTAATCCCTCAATTTTAAAAAAGGATTGAAAATTCTAAATG
TCTTTCTGTGCATATTTTTTGTGTTAGGAATCAAAAGTATTTTATAAAAGGAG
AAAGAACAGCCTCATTTTAGATGTAGTCCTGTTGGATTTTTTATGCCTCCTCA
GTAACCAGAAATGTTTTAAAAAACTAAGTGTTTAGGATTTCAAGACAACATT
ATACATGGCTCTGAAATATCTGACACAATGTAAACATTGCAGGCACCTGCATT
TTATGTTTTTTTTTTCAACAAATGTGACTAATTTGAAACTTTTATGAACTTCTG
AGCTGTCCCCTTGCAATTCAACCGCAGTTTGAATTAATCATATCAAATCAGTT
TTAATTTTTTAAATTGTACTTCAGAGTCTATATTTCAAGGGCACATTTTCTCAC
TACTATTTTAATACATTAAAGGACTAAATAATCTTTCAGAGATGCTGGAAACA

AATCATTTGCTTTATATGTTTCATTAGAATACCAATGAAACATACAACTTGAA
AATTAGTAATAGTATTTTTGAAGATCCCATTTCTAATTGGAGATCTCTTTAATT
TCGATCAACTTATAATGTGTAGTACTATATTAAGTGCACTTGAGTGGAATTCA
ACATTTGACTAATAAAATGAGTTCATCATGTTGGCAAGTGATGTGGCAATTAT
CTCTGGTGACAAAAGAGTAAAATCAAATATTTCTGCCTGTTACAAATATCAAG
GAAGACCTGCTACTATGAAATAGATGACATTAATCTGTCTTCACTGTTTATAA
TACGGATGGATTTTTTTTCAAATCAGTGTGTGTTTTGAGGTCTTATGTAATTGA
TGACATTTGAGAGAAATGGTGGCTTTTTTTAGCTACCTCTTTGTTCATTTAAGC
ACCAGTAAAGATCATGTCTTTTTATAGAAGTGTAGATTTTCTTTGTGACTTTGC
TATCGTGCCTAAAGCTCTAAATATAGGTGAATGTGTGATGAATACTCAGATTA
TTTGTCTCTCTATATAATTAGTTTGGTACTAAGTTTCTCAAAAAATTATTAACA
CATGAAAGACAATCTCTAAACCAGAAAAAGAAGTAGTACAAATTTTGTTACT
GTAATGCTCGCGTTTAGTGAGTTTAAAACACACAGTATCTTTTGGTTTTATAA
TCAGTTTCTATTTTGCTGTGCCTGAGATTAAGATCTGTGTATGTGTGTGTGTT
GTGTGTGCGTTTGTGTGTTAAAGCAGAAAAGACTTTTTTAAAAGTTTTAAGTG
ATAAATGCAATTTGTTAATTGATCTTAGATCACTAGTAAACTCAGGGCTGAAT
TATACCATGTATATTCTATTAGAAGAAAGTAAACACCATCTTTATTCCTGCCC
TTTTTCTTCTCTCAAAGTAGTTGTAGTTATATCTAGAAAGAAGCAATTTTGATT
TCTTGAAAAGGTAGTTCCTGCACTCAGTTTAAACTAAAAATAATCATACTTGG
ATTTTATTTATTTTTGTCATAGTAAAAATTTTAATTTATATATATTTTTATTTAG
TATTATCTTATTCTTTGCTATTTGCCAATCCTTTGTCATCAATTGTGTTAAATG
AATTGAAAATTCATGCCCTGTTCATTTTATTTTACTTTATTGGTTAGGATATTT
AAAGGATTTTTGTATATATAATTTCTTAAATTAATATTCCAAAAGGTTAGTGG
ACTTAGATTATAAATTATGGCAAAAATCTAAAAACAACAAAAATGATTTTTAT
ACATTCTATTTCATTATTCCTCTTTTTCCAATAAGTCATACAATTGGTAGATAT
GACTTATTTTATTTTTGTATTATTCACTATATCTTTATGATATTTAAGTATAAA
TAATTAAAAAAAATTTATTGTACCTTATAGTCTGTCACCAAAAAAAAAAAATTA
TCTGTAGGTAGTGAAATGCTAATGTTGATTTGTCTTTAAGGGCTTGTTAACTA
TCCTTTATTTTCTCATTTGTCTTAAATTAGGAGTTTGTGTTTAAATTACTCATCT
AAGCAAAAAATGTATATAAATCCCATTACTGGGTATATACCCAAAGGATTAT
AAATCATGCTGCTATAAAGACACATGCACACGTATGTTTATTGCAGCACTATT
CACAATAGCAAAGACTTGGAACCAACCCAAATGTCCATCAATGATAGACTTG

ATTAAGAAAATGTGCACATATACACCATGGAATACTATGCAGCCATAAAAAA
GGATGAGTTCATGTCCTTTGTAGGGACATGGATAAAGCTGGAAACCATCATTC
TGAGCAAACTATTGCAAGGACAGAAAACCAAACACTGCATGTTCTCACTCAT
AGGTGGGAATTGAACAATGAGAACACTTGGACACAAGGTGGGGAACACCAC
ACACCAGGGCCTGTCATGGGGTGGGGGGAGTGGGGAGGGATAGCATTAGGA
GATATACCTAATGTAAATGATGAGTTAATGGGTGCAGCACACCAACATGGCA
CATGTATACATATGTAGCAAACCTGCACGTTGTGCACATGTACCCTAGAACTT
AAAGTATAATTAAAAAAAAAAAGAAAACAGAAGCTATTTATAAAGAAGTTAT
TTGCTGAAATAAATGTGATCTTTCCCATTAAAAAAATAAAGAAATTTTGGGGT
AAAAAAACACAATATATTGTATTCTTGAAAAATTCTAAGAGAGTGGATGTGA
AGTGTTCTCACCACAAAAGTGATAACTAATTGAGGTAATGCACATATTAATTA
GAAAGATTTTGTCATTCCACAATGTATATATACTTAAAAATATGTTATACACA
ATAAATACATACATTAAAAAATAAGTAAATGTA) (SEQ ID NO: 170);

3'UTR-012 (Col6a1; collagen, type VI, alpha 1 UTR)

(CCCACCCTGCACGCCGGCACCAAACCCTGTCCTCCCACCCCTCCCCACTCATC
ACTAAACAGAGTAAAATGTGATGCGAATTTTCCCGACCAACCTGATTCGCTA
GATTTTTTTTAAGGAAAAGCTTGGAAAGCCAGGACACAACGCTGCTGCCTGCT
TTGTGCAGGGTCCTCCGGGGCTCAGCCCTGAGTTGGCATCACCTGCGCAGGGC
CCTCTGGGGCTCAGCCCTGAGCTAGTGTCACCTGCACAGGGCCCTCTGAGGCT
CAGCCCTGAGCTGGCGTCACCTGTGCAGGGCCCTCTGGGGCTCAGCCCTGAG
CTGGCCTCACCTGGGTTCCCCACCCCGGGCTCTCCTGCCCTGCCCTCCTGCCC
GCCCTCCCTCCTGCCTGCGCAGCTCCTTCCCTAGGCACCTCTGTGCTGCATCCC
ACCAGCCTGAGCAAGACGCCCTCTCGGGGCCTGTGCCGCACTAGCCTCCCTCT
CCTCTGTCCCCATAGCTGGTTTTTCCCACCAATCCTCACCTAACAGTTACTTTA
CAATTAAACTCAAAGCAAGCTCTTCTCCTCAGCTTGGGGCAGCCATTGGCCTC
TGTCTCGTTTTGGGAAACCAAGGTCAGGAGGCCGTTGCAGACATAAATCTCG
GCGACTCGGCCCCGTCTCCTGAGGGTCCTGCTGGTGACCGGCCTGGACCTTGG
CCCTACAGCCCTGGAGGCCGCTGCTGACCAGCACTGACCCCGACCTCAGAGA
GTACTCGCAGGGGCGCTGGCTGCACTCAAGACCCTCGAGATTAACGGTGCTA
ACCCCGTCTGCTCCTCCCTCCCGCAGAGACTGGGGCCTGGACTGGACATGAG
AGCCCCTTGGTGCCACAGAGGGCTGTGTCTTACTAGAAACAACGCAAACCTC
TCCTTCCTCAGAATAGTGATGTGTTCGACGTTTTATCAAAGGCCCCCTTTCTAT

GTTCATGTTAGTTTTGCTCCTTCTGTGTTTTTTTCTGAACCATATCCATGTTGCT GACTTTTCCAAATAAAGGTTTTCACTCCTCTC) (SEQ ID NO. 171);

3'UTR-013 (Calr; calreticulin UTR)

(AGAGGCCTGCCTCCAGGGCTGGACTGAGGCCTGAGCGCTCCTGCCGCAGAGC TGGCCGCGCCAAATAATGTCTCTGTGAGACTCGAGAACTTTCATTTTTTTCCA GGCTGGTTCGGATTTGGGGTGGATTTTGGTTTTGTTCCCCTCCTCCACTCTCCC CCACCCCCTCCCCGCCCTTTTTTTTTTTTTTTTTTAAACTGGTATTTTATCTTTG ATTCTCCTTCAGCCCTCACCCCTGGTTCTCATCTTTCTTGATCAACATCTTTTCT TGCCTCTGTCCCCTTCTCTCATCTCTTAGCTCCCCTCCAACCTGGGGGGCAGTG GTGTGGAGAAGCCACAGGCCTGAGATTTCATCTGCTCTCCTTCCTGGAGCCCA GAGGAGGGCAGCAGAAGGGGGTGGTGTCTCCAACCCCCCAGCACTGAGGAA GAACGGGGCTCTTCTCATTTCACCCCTCCCTTTCTCCCCTGCCCCCAGGACTGG GCCACTTCTGGGTGGGGCAGTGGGTCCCAGATTGGCTCACACTGAGAATGTA AGAACTACAAACAAAATTTCTATTAAATTAAATTTTGTGTCTCC) (SEQ ID NO. 172);

3'UTR-014 (Col1a1; collagen, type I, alpha 1 UTR)

(CTCCCTCCATCCCAACCTGGCTCCCTCCCACCCAACCAACTTTCCCCCCAACC
CGGAAACAGACAAGCAACCCAAACTGAACCCCCTCAAAAGCCAAAAAATGG
GAGACAATTTCACATGGACTTTGGAAAATATTTTTTTCCTTTGCATTCATCTCT
CAAACTTAGTTTTTATCTTTGACCAACCGAACATGACCAAAAACCAAAAGTGC
ATTCAACCTTACCAAAAAAAAAAAAAAAAAAAAGAATAAATAAATAACTTTTT
AAAAAAGGAAGCTTGGTCCACTTGCTTGAAGACCCATGCGGGGGTAAGTCCC
TTTCTGCCCGTTGGGCTTATGAAACCCCAATGCTGCCCTTTCTGCTCCTTTCTC
CACACCCCCCTTGGGGCCTCCCCTCCACTCCTTCCCAAATCTGTCTCCCCAGA
AGACACAGGAAACAATGTATTGTCTGCCCAGCAATCAAAGGCAATGCTCAAA
CACCCAAGTGGCCCCCACCCTCAGCCCGCTCCTGCCCGCCCAGCACCCCCAGG
CCCTGGGGGACCTGGGGTTCTCAGACTGCCAAAGAAGCCTTGCCATCTGGCG
CTCCCATGGCTCTTGCAACATCTCCCCTTCGTTTTTGAGGGGGTCATGCCGGG
GGAGCCACCAGCCCCTCACTGGGTTCGGAGGAGAGTCAGGAAGGGCCACGAC
AAAGCAGAAACATCGGATTTGGGGAACGCGTGTCAATCCCTTGTGCCGCAGG
GCTGGGCGGGAGAGACTGTTCTGTTCCTTGTGTAACTGTGTTGCTGAAAGACT
ACCTCGTTCTTGTCTTGATGTGTCACCGGGGCAACTGCCTGGGGGCGGGGATG

GGGGCAGGGTGGAAGCGGCTCCCCATTTTATACCAAAGGTGCTACATCTATG
TGATGGGTGGGGTGGGGAGGGAATCACTGGTGCTATAGAAATTGAGATGCCC
CCCCAGGCCAGCAAATGTTCCTTTTTGTTCAAAGTCTATTTTTATTCCTTGATA
TTTTTCTTTTTTTTTTTTTTTTTTTGTGGATGGGGACTTGTGAATTTTTCTAAAG
GTGCTATTTAACATGGGAGGAGAGCGTGTGCGGCTCCAGCCCAGCCCGCTGC
TCACTTTCCACCCTCTCTCCACCTGCCTCTGGCTTCTCAGGCCTCTGCTCTCCG
ACCTCTCTCCTCTGAAACCCTCCTCCACAGCTGCAGCCCATCCTCCCGGCTCC
CTCCTAGTCTGTCCTGCGTCCTCTGTCCCCGGGTTTCAGAGACAACTTCCCAA
AGCACAAAGCAGTTTTTCCCCCTAGGGGTGGGAGGAAGCAAAAGACTCTGTA
CCTATTTTGTATGTGTATAATAATTTGAGATGTTTTTAATTATTTTGATTGCTG
GAATAAAGCATGTGGAAATGACCCAAACATAATCCGCAGTGGCCTCCTAATT
TCCTTCTTTGGAGTTGGGGGAGGGGTAGACATGGGGAAGGGGCTTTGGGGTG
ATGGGCTTGCCTTCCATTCCTGCCCTTTCCCTCCCCACTATTCTCTTCTAGATC
CCTCCATAACCCCACTCCCCTTTCTCTCACCCTTCTTATACCGCAAACCTTTCT
ACTTCCTCTTTCATTTTCTATTCTTGCAATTTCCTTGCACCTTTTCCAAATCCTC
TTCTCCCCTGCAATACCATACAGGCAATCCACGTGCACAACACACACACACA
CTCTTCACATCTGGGGTTGTCCAAACCTCATACCCACTCCCCTTCAAGCCCAT
CCACTCTCCACCCCCTGGATGCCCTGCACTTGGTGGCGGTGGGATGCTCATGG
ATACTGGGAGGGTGAGGGGAGTGGAACCCGTGAGGAGGACCTGGGGGCCTC
TCCTTGAACTGACATGAAGGGTCATCTGGCCTCTGCTCCCTTCTCACCCACGC
TGACCTCCTGCCGAAGGAGCAACGCAACAGGAGAGGGGTCTGCTGAGCCTGG
CGAGGGTCTGGGAGGGACCAGGAGGAAGGCGTGCTCCCTGCTCGCTGTCCTG
GCCCTGGGGGAGTGAGGGAGACAGACACCTGGGAGAGCTGTGGGGAAGGCA
CTCGCACCGTGCTCTTGGGAAGGAAGGAGACCTGGCCCTGCTCACCACGGAC
TGGGTGCCTCGACCTCCTGAATCCCCAGAACACAACCCCCCTGGGCTGGGGT
GGTCTGGGGAACCATCGTGCCCCCGCCTCCCGCCTACTCCTTTTTAAGCTT)
(SEQ ID NO. 173);

3'UTR-015 (Plod1; procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 UTR)

(TTGGCCAGGCCTGACCCTCTTGGACCTTTCTTCTTTGCCGACAACCACTGCCC
AGCAGCCTCTGGGACCTCGGGGTCCCAGGGAACCCAGTCCAGCCTCCTGGCT
GTTGACTTCCCATTGCTCTTGGAGCCACCAATCAAAGAGATTCAAAGAGATTC
CTGCAGGCCAGAGGCGGAACACACCTTTATGGCTGGGGCTCTCCGTGGTGTTC

TGGACCCAGCCCCTGGAGACACCATTCACTTTTACTGCTTTGTAGTGACTCGT
GCTCTCCAACCTGTCTTCCTGAAAAACCAAGGCCCCCTTCCCCCACCTCTTCC
ATGGGGTGAGACTTGAGCAGAACAGGGGCTTCCCCAAGTTGCCCAGAAAGAC
TGTCTGGGTGAGAAGCCATGGCCAGAGCTTCTCCCAGGCACAGGTGTTGCAC
CAGGGACTTCTGCTTCAAGTTTTGGGGTAAAGACACCTGGATCAGACTCCAA
GGGCTGCCCTGAGTCTGGGACTTCTGCCTCCATGGCTGGTCATGAGAGCAAAC
CGTAGTCCCCTGGAGACAGCGACTCCAGAGAACCTCTTGGGAGACAGAAGAG
GCATCTGTGCACAGCTCGATCTTCTACTTGCCTGTGGGGAGGGGAGTGACAG
GTCCACACACCACACTGGGTCACCCTGTCCTGGATGCCTCTGAAGAGAGGGA
CAGACCGTCAGAAACTGGAGAGTTTCTATTAAAGGTCATTTAAACCA) (SEQ
ID NO. 174);

3'UTR-016 (Nucb1; nucleobindin 1 UTR)

(TCCTCCGGGACCCCAGCCCTCAGGATTCCTGATGCTCCAAGGCGACTGATGG
GCGCTGGATGAAGTGGCACAGTCAGCTTCCCTGGGGGCTGGTGTCATGTTGG
GCTCCTGGGGCGGGGGCACGGCCTGGCATTTCACGCATTGCTGCCACCCCAG
GTCCACCTGTCTCCACTTTCACAGCCTCCAAGTCTGTGGCTCTTCCCTTCTGTC
CTCCGAGGGGCTTGCCTTCTCTCGTGTCCAGTGAGGTGCTCAGTGATCGGCTT
AACTTAGAGAAGCCCGCCCCCTCCCCTTCTCCGTCTGTCCCAAGAGGGTCTGC
TCTGAGCCTGCGTTCCTAGGTGGCTCGGCCTCAGCTGCCTGGGTTGTGGCCGC
CCTAGCATCCTGTATGCCCACAGCTACTGGAATCCCCGCTGCTGCTCCGGGCC
AAGCTTCTGGTTGATTAATGAGGGCATGGGGTGGTCCCTCAAGACCTTCCCCT
ACCTTTTGTGGAACCAGTGATGCCTCAAAGACAGTGTCCCCTCCACAGCTGGG
TGCCAGGGGCAGGGGATCCTCAGTATAGCCGGTGAACCCTGATACCAGGAGC
CTGGGCCTCCCTGAACCCCTGGCTTCCAGCCATCTCATCGCCAGCCTCCTCCT
GGACCTCTTGGCCCCCAGCCCCTTCCCCACACAGCCCCAGAAGGGTCCCAGA
GCTGACCCCACTCCAGGACCTAGGCCCAGCCCCTCAGCCTCATCTGGAGCCCC
TGAAGACCAGTCCCACCCACCTTTCTGGCCTCATCTGACACTGCTCCGCATCC
TGCTGTGTGTCCTGTTCCATGTTCCGGTTCCATCCAAATACACTTTCTGGAACA
AA) (SEQ ID NO. 175);

3'UTR-017 (α-globin)

(GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCT CCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGG CGGC) (SEQ ID NO. 176);

or
3'UTR-018

(TGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCC CCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGT CTGAGTGGGCGGC) (SEQ ID NO. 177).

[0460] In certain cases, the 5'UTR and/or 3'UTR sequence of the disclosure comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting of 5'UTR sequences comprising any of the 5'UTR sequences disclosed herein and/or 3'UTR sequences comprises any of the 3'UTR sequences disclosed herein, and any combination thereof.

[0461] In certain cases, the 3' UTR sequence comprises one or more miRNA binding sites, *e.g.*, miR-122 binding sites, or any other heterologous nucleotide sequences therein, without disrupting the function of the 3' UTR. Some examples of 3' UTR sequences comprising a miRNA binding site are listed in **TABLE 6.** In some cases, the 3' UTR sequence comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 238-240. In certain cases, the 3' UTR sequence comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 238. In certain cases, the 3' UTR sequence comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 239. In certain cases, the 3' UTR sequence comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to SEQ ID NO: 240.

Table 6. Exemplary 3' UTR with miRNA Binding Sites

3'UTR identifier/ miRNA BS	Name/ Description	Sequence	SEQ ID NO.
3UTR-018 + miR-122-5p binding site	Downstream UTR	UAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCC CCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUG CACCCGUACCCCC**CAAACACCAUUGUCACACUCCA**G UGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	SEQ ID NO: 238
3UTR-018 + miR-122-3p binding site	Downstream UTR	UAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCC CCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUG CACCCGUACCCCC**UAUUUAGUGUGAUAAUGGCGUU**G UGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	SEQ ID NO: 239
3UTR-019 + miR-122 binding site	Downstream UTR	UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUU GCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUC CUGCACCCGUACCCCCCAAACACCAUUGUCACACUC CAGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	SEQ ID NO: 240
*miRNA binding site is boxed or underlined.			

[0462] The polynucleotides of the disclosure can comprise combinations of features. For example, the ORF can be flanked by a 5'UTR that comprises a strong Kozak translational initiation signal and/or a 3'UTR comprising an oligo(dT)

sequence for templated addition of a poly-A tail. A 5'UTR can comprise a first polynucleotide fragment and a second polynucleotide fragment from the same and/or different UTRs (see, *e.g.,* US2010/0293625).

[0463] It is also within the scope of the present disclosure to have patterned UTRs. As used herein "patterned UTRs" include a repeating or alternating pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than 3 times. In these patterns, each letter, A, B, or C represent a different UTR nucleic acid sequence.

[0464] Other non-UTR sequences can be used as regions or subregions within the polynucleotides of the disclosure. For example, introns or portions of intron sequences can be incorporated into the polynucleotides of the disclosure. Incorporation of intronic sequences can increase protein production as well as polynucleotide expression levels. In some cases, the polynucleotide of the disclosure comprises an internal ribosome entry site (IRES) instead of or in addition to a UTR (see, *e.g.,* Yakubov et al., Biochem. Biophys. Res. Commun. 2010 394(1): 189-193). In some cases, the polynucleotide of the disclosure comprises 5' and/or 3' sequence associated with the 5' and/or 3' ends of rubella virus (RV) genomic RNA, respectively, or deletion derivatives thereof, including the 5' proximal open reading frame of RV RNA encoding nonstructural proteins (*e.g.,* see Pogue et al., J. Virol. 67(12):7106-7117). Viral capsid sequences can also be used as a translational enhancer, *e.g.,* the 5' portion of a capsid sequence, (*e.g.,* semliki forest virus and sindbis virus capsid RNAs as described in Sjöberg et al., Biotechnology (NY) 1994 12(11):1127-1131, and Frolov and Schlesinger J. Virol. 1996 70(2):1182-1190). In some cases, the polynucleotide comprises an IRES instead of a 5'UTR sequence. In some cases, the polynucleotide comprises an ORF and a viral capsid sequence. In some cases, the polynucleotide comprises a synthetic 5'UTR in combination with a non-synthetic 3'UTR.

[0465] In some cases, the UTR can also include at least one translation enhancer polynucleotide, translation enhancer element, or translational enhancer elements (collectively, "TEE," which refers to nucleic acid sequences that increase the amount of polypeptide or protein produced from a polynucleotide. As a non-limiting example, the TEE can include those described in US2009/0226470, and others known in the art. As a non-limiting example, the TEE can be located between the transcription promoter and the start codon. In some cases, the 5'UTR comprises a TEE.

[0466] In one aspect, a TEE is a conserved element in a UTR that can promote translational activity of a nucleic acid such as, but not limited to, cap-dependent or cap-independent translation. The conservation of these sequences has been shown across 14 species including humans. See, *e.g.,* Panek et al., "An evolutionary conserved pattern of 18S rRNA sequence complementarity to mRNA 5'UTRs and its implications for eukaryotic gene translation regulation," Nucleic Acids Research 2013, doi:10.1093/nar/gkt548.

[0467] In one non-limiting example, the TEE comprises the TEE sequence in the 5'-leader of the Gtx homeodomain protein. See Chappell et al., PNAS 2004 101:9590-9594.

[0468] In another non-limiting example, the TEE comprises a TEE having one or more of the sequences of SEQ ID NOs: 1-35 in US2009/0226470, US2013/0177581, and WO2009/075886; SEQ ID NOs: 1-5 and 7-645 in WO2012/009644; and SEQ ID NO: 1 WO1999/024595, US6310197, and US6849405.

[0469] In some cases, the TEE is an internal ribosome entry site (IRES), HCV-IRES, or an IRES element such as, but not limited to, those described in: US7468275, US2007/0048776, US2011/0124100, WO2007/025008, and WO2001/055369. The IRES elements can include, but are not limited to, the Gtx sequences (*e.g.,* Gtx9-nt, Gtx8-nt, Gtx7-nt) as described by Chappell et al., PNAS 2004 101:9590-9594, Zhou et al., PNAS 2005 102:6273-6278, US2007/0048776, US2011/0124100, and WO2007/025008.

[0470] "Translational enhancer polynucleotide" or "translation enhancer polynucleotide sequence" refer to a polynucleotide that includes one or more of the TEE provided herein and/or known in the art (see. *e.g.,* US6310197, US6849405, US7456273, US7183395, US2009/0226470, US2007/0048776, US2011/0124100, US2009/0093049, US2013/0177581, WO2009/075886, WO2007/025008, WO2012/009644, WO2001/055371, WO1999/024595, EP2610341A1, and EP2610340A1), or their variants, homologs, or functional derivatives. In some cases, the polynucleotide of the disclosure comprises one or multiple copies of a TEE. The TEE in a translational enhancer polynucleotide can be organized in one or more sequence segments. A sequence segment can harbor one or more of the TEEs provided herein, with each TEE being present in one or more copies. When multiple sequence segments are present in a translational enhancer polynucleotide, they can be homogenous or heterogeneous. Thus, the multiple sequence segments in a translational enhancer polynucleotide can harbor identical or different types of the TEE provided herein, identical or different number of copies of each of the TEE, and/or identical or different organization of the TEE within each sequence segment. In one case, the polynucleotide of the disclosure comprises a translational enhancer polynucleotide sequence.

[0471] In some cases, a 5'UTR and/or 3'UTR of a polynucleotide of the disclosure comprises at least one TEE or portion thereof that is disclosed in: WO1999/024595, WO2012/009644,WO2009/075886, WO2007/025008, WO1999/024595, WO2001/055371, EP2610341A1, EP2610340A1, US6310197, US6849405, US7456273, US7183395, US2009/0226470, US2011/0124100, US2007/0048776, US2009/0093049, or US2013/0177581.

[0472] In some cases, a 5'UTR and/or 3'UTR of a polynucleotide of the disclosure comprises a TEE that is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,

at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a TEE disclosed in: US2009/0226470, US2007/0048776, US2013/0177581, US2011/0124100, WO1999/024595, WO2012/009644, WO2009/075886, WO2007/025008, EP2610341A1, EP2610340A1, US6310197, US6849405, US7456273, US7183395, Chappell et al., PNAS 2004 101:9590-9594, Zhou et al., PNAS 2005 102:6273-6278, and Supplemental Table 1 and in Supplemental Table 2 of Wellensiek et al., "Genome-wide profiling of human cap-independent translation-enhancing elements," Nature Methods 2013, DOI:10.1038/NMETH.2522.

[0473] In some cases, a 5'UTR and/or 3'UTR of a polynucleotide of the disclosure comprises a TEE which is selected from a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, or a 5-10 nucleotide fragment (including a fragment of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides) of a TEE sequence disclosed in: US2009/0226470, US2007/0048776, US2013/0177581, US2011/0124100, WO1999/024595, WO2012/009644, WO2009/075886, WO2007/025008, EP2610341A1, EP2610340A1, US6310197, US6849405, US7456273, US7183395, Chappell et al., PNAS 2004 101:9590-9594, Zhou et al., PNAS 2005 102:6273-6278, and Supplemental Table 1 and in Supplemental Table 2 of Wellensiek et al., "Genome-wide profiling of human cap-independent translation-enhancing elements," Nature Methods 2013, DOI:10.1038/NMETH.2522.

[0474] In some cases, a 5'UTR and/or 3'UTR of a polynucleotide of the disclosure comprises a TEE which is a transcription regulatory element described in any of US7456273, US7183395, US2009/0093049, and WO2001/055371. The transcription regulatory elements can be identified by methods known in the art, such as, but not limited to, the methods described in US7456273, US7183395, US2009/0093049, and WO2001/055371.

[0475] In some cases, a 5'UTR and/or 3'UTR comprising at least one TEE described herein can be incorporated in a monocistronic sequence such as, but not limited to, a vector system or a nucleic acid vector. As non-limiting examples, the vector systems and nucleic acid vectors can include those described in US7456273, US7183395, US2007/0048776, US2009/0093049, US2011/0124100, WO2007/025008, and WO2001/055371.

[0476] In some cases, a 5'UTR and/or 3'UTR of a polynucleotide of the disclosure comprises a TEE or portion thereof described herein. In some cases, the TEEs in the 3'UTR can be the same and/or different from the TEE located in the 5'UTR.

[0477] In some cases, a 5'UTR and/or 3'UTR of a polynucleotide of the disclosure can include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. In one case, the 5'UTR of a polynucleotide of the disclosure can include 1-60, 1-55, 1-50, 1-45, 1-40, 1-35, 1-30, 1-25, 1-20, 1-15, 1-10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 TEE sequences. The TEE sequences in the 5'UTR of the polynucleotide of the disclosure can be the same or different TEE sequences. A combination of different TEE sequences in the 5'UTR of the polynucleotide of the disclosure can include combinations in which more than one copy of any of the different TEE sequences are incorporated. The TEE sequences can be in a pattern such as ABABAB or AABBAABBAABB or ABCA-BCABC or variants thereof repeated one, two, three, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE nucleotide sequence.

[0478] In some cases, the TEE can be identified by the methods described in US2007/0048776, US2011/0124100, WO2007/025008, WO2012/009644.

[0479] In some cases, the 5'UTR and/or 3'UTR comprises a spacer to separate two TEE sequences. As a non-limiting example, the spacer can be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 5'UTR and/or 3'UTR comprises a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, at least 10 times, or more than 10 times in the 5'UTR and/or 3'UTR, respectively. In some cases, the 5'UTR and/or 3'UTR comprises a TEE sequence-spacer module repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times.

[0480] In some cases, the spacer separating two TEE sequences can include other sequences known in the art that can regulate the translation of the polynucleotide of the disclosure, *e.g.,* miR sequences described herein (*e.g.,* miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences can include a different miR sequence or component of a miR sequence (*e.g.,* miR seed sequence).

[0481] In some cases, a polynucleotide of the disclosure comprises a miR and/or TEE sequence. In some cases, the incorporation of a miR sequence and/or a TEE sequence into a polynucleotide of the disclosure can change the shape of the stem loop region, which can increase and/or decrease translation. See *e.g.,* Kedde et al., Nature Cell Biology 2010 12(10): 1014-20).

14. MicroRNA (miRNA) Binding Sites

[0482] Sensor sequences include, for example, microRNA (miRNA) binding sites, transcription factor binding sites, structured mRNA sequences and/or motifs, artificial binding sites engineered to act as pseudo-receptors for endogenous

nucleic acid binding molecules, and combinations thereof. Non-limiting examples of sensor sequences are described in U.S. Publication 2014/0200261.

[0483] In some cases, a polynucleotide (*e.g.,* a ribonucleic acid (RNA), *e.g.,* a messenger RNA (mRNA)) of the disclosure comprising an open reading frame (ORF) encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides further comprises a sensor sequence. In some cases, the sensor sequence is a miRNA binding site.

[0484] A miRNA is a 19-25 nucleotide long noncoding RNA that binds to a polynucleotide and down-regulates gene expression either by reducing stability or by inhibiting translation of the polynucleotide. A miRNA sequence comprises a "seed" region, *i.e.,* a sequence in the region of positions 2-8 of the mature miRNA. A miRNA seed can comprise positions 2-8 or 2-7 of the mature miRNA. In some cases, a miRNA seed can comprise 7 nucleotides (*e.g.,* nucleotides 2-8 of the mature miRNA), wherein the seed-complementary site in the corresponding miRNA binding site is flanked by an adenosine (A) opposed to miRNA position 1. In some cases, a miRNA seed can comprise 6 nucleotides (*e.g.,* nucleotides 2-7 of the mature miRNA), wherein the seed-complementary site in the corresponding miRNA binding site is flanked by an adenosine (A) opposed to miRNA position 1. See, for example, Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP; Mol Cell. 2007 Jul 6;27(1):91-105. miRNA profiling of the target cells or tissues can be conducted to determine the presence or absence of miRNA in the cells or tissues. In some cases, a polynucleotide (*e.g.,* a ribonucleic acid (RNA), *e.g.,* a messenger RNA (mRNA)) of the disclosure comprises one or more microRNA target sequences, microRNA sequences, or microRNA seeds. Such sequences can correspond to any known microRNA such as those taught in US Publication US2005/0261218 and US Publication US2005/0059005.

[0485] As used herein, the term "microRNA (miRNA or miR) binding site" refers to a sequence within a polynucleotide, *e.g.,* within a DNA or within an RNA transcript, including in the 5'UTR and/or 3'UTR, that has sufficient complementarity to all or a region of a miRNA to interact with, associate with or bind to the miRNA. In some cases, a polynucleotide of the disclosure comprising an ORF encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides further comprises a miRNA binding site. In exemplary cases, a 5'UTR and/or 3'UTR of the polynucleotide (*e.g.,* a ribonucleic acid (RNA), *e.g.,* a messenger RNA (mRNA)) comprises a miRNA binding site.

[0486] A miRNA binding site having sufficient complementarity to a miRNA refers to a degree of complementarity sufficient to facilitate miRNA-mediated regulation of a polynucleotide, *e.g.,* miRNA-mediated translational repression or degradation of the polynucleotide. In exemplary aspects of the disclosure, a miRNA binding site having sufficient complementarity to the miRNA refers to a degree of complementarity sufficient to facilitate miRNA-mediated degradation of the polynucleotide, *e.g.,* miRNA-guided RNA-induced silencing complex (RISC)-mediated cleavage of mRNA. The miRNA binding site can have complementarity to, for example, a 19-25 nucleotide miRNA sequence, to a 19-23 nucleotide miRNA sequence, or to a 22 nucleotide miRNA sequence. A miRNA binding site can be complementary to only a portion of a miRNA, *e.g.,* to a portion less than 1, 2, 3, or 4 nucleotides of the full length of a naturally-occurring miRNA sequence. Full or complete complementarity (*e.g.,* full complementarity or complete complementarity over all or a significant portion of the length of a naturally-occurring miRNA) is preferred when the desired regulation is mRNA degradation.

[0487] In some cases, a miRNA binding site includes a sequence that has complementarity (*e.g.,* partial or complete complementarity) with an miRNA seed sequence. In some cases, the miRNA binding site includes a sequence that has complete complementarity with a miRNA seed sequence. In some cases, a miRNA binding site includes a sequence that has complementarity (*e.g.,* partial or complete complementarity) with an miRNA sequence. In some cases, the miRNA binding site includes a sequence that has complete complementarity with a miRNA sequence. In some cases, a miRNA binding site has complete complementarity with a miRNA sequence but for 1, 2, or 3 nucleotide substitutions, terminal additions, and/or truncations.

[0488] In some cases, the miRNA binding site is the same length as the corresponding miRNA. In other cases, the miRNA binding site is one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve nucleotide(s) shorter than the corresponding miRNA at the 5' terminus, the 3' terminus, or both. In still other cases, the microRNA binding site is two nucleotides shorter than the corresponding microRNA at the 5' terminus, the 3' terminus, or both. The miRNA binding sites that are shorter than the corresponding miRNAs are still capable of degrading the mRNA incorporating one or more of the miRNA binding sites or preventing the mRNA from translation.

[0489] In some cases, the miRNA binding site binds to the corresponding mature miRNA that is part of an active RISC containing Dicer. In another case, binding of the miRNA binding site to the corresponding miRNA in RISC degrades the mRNA containing the miRNA binding site or prevents the mRNA from being translated. In some cases, the miRNA binding site has sufficient complementarity to miRNA so that a RISC complex comprising the miRNA cleaves the polynucleotide comprising the miRNA binding site. In other cases, the miRNA binding site has imperfect complementarity so that a RISC complex comprising the miRNA induces instability in the polynucleotide comprising the miRNA binding site. In another case, the miRNA binding site has imperfect complementarity so that a RISC complex comprising the miRNA represses transcription of the polynucleotide comprising the miRNA binding site.

[0490] In some cases, the miRNA binding site has one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve mismatch(es) from the corresponding miRNA.

[0491] In some cases, the miRNA binding site has at least about ten, at least about eleven, at least about twelve, at least about thirteen, at least about fourteen, at least about fifteen, at least about sixteen, at least about seventeen, at least about eighteen, at least about nineteen, at least about twenty, or at least about twenty-one contiguous nucleotides complementary to at least about ten, at least about eleven, at least about twelve, at least about thirteen, at least about fourteen, at least about fifteen, at least about sixteen, at least about seventeen, at least about eighteen, at least about nineteen, at least about twenty, or at least about twenty-one, respectively, contiguous nucleotides of the corresponding miRNA.

[0492] By engineering one or more miRNA binding sites into a polynucleotide of the disclosure, the polynucleotide can be targeted for degradation or reduced translation, provided the miRNA in question is available. This can reduce off-target effects upon delivery of the polynucleotide. For example, if a polynucleotide of the disclosure is not intended to be delivered to a tissue or cell but ends up there, then a miRNA abundant in the tissue or cell can inhibit the expression of the gene of interest if one or multiple binding sites of the miRNA are engineered into the 5'UTR and/or 3'UTR of the polynucleotide.

[0493] Conversely, miRNA binding sites can be removed from polynucleotide sequences in which they naturally occur in order to increase protein expression in specific tissues. For example, a binding site for a specific miRNA can be removed from a polynucleotide to improve protein expression in tissues or cells containing the miRNA.

[0494] In one case, a polynucleotide of the disclosure can include at least one miRNA-binding site in the 5'UTR and/or 3'UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells. In another case, a polynucleotide of the disclosure can include two, three, four, five, six, seven, eight, nine, ten, or more miRNA-binding sites in the 5'-UTR and/or 3'-UTR in order to direct cytotoxic or cytoprotective mRNA therapeutics to specific cells such as, but not limited to, normal and/or cancerous cells.

[0495] Regulation of expression in multiple tissues can be accomplished through introduction or removal of one or more miRNA binding sites. The decision whether to remove or insert a miRNA binding site can be made based on miRNA expression patterns and/or their profilings in diseases. Identification of miRNAs, miRNA binding sites, and their expression patterns and role in biology have been reported (*e.g.,* Bonauer et al., Curr Drug Targets 2010 11:943-949; Anand and Cheresh Curr Opin Hematol 2011 18:171-176; Contreras and Rao Leukemia 2012 26:404-413 (2011 Dec 20. doi: 10.1038/leu.2011.356); Bartel Cell 2009 136:215-233; Landgraf et al, Cell, 2007 129:1401-1414; Gentner and Naldini, Tissue Antigens. 2012 80:393-403 and all references therein).

[0496] miRNAs and miRNA binding sites can correspond to any known sequence, including non-limiting examples described in U.S. Publication Nos. 2014/0200261, 2005/0261218, and 2005/0059005.

[0497] Examples of tissues where miRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223, miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-1d, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126).

[0498] Some microRNAs, *e.g.*, miR-122, are abundant in normal tissue but are present in much lower levels in cancer or tumor tissue. Thus, engineering microRNA target sequences (*i.e.*, microRNA binding site) into the polynucleotides encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides (*e.g.,* in a 3'UTR like region or other region) can effectively target the molecule for degradation or reduced translation in normal tissue (where the microRNA is abundant) while providing high levels of translation in the cancer or tumor tissue (where the microRNA is present in much lower levels). This provides a tumor-targeting approach for the methods and compositions of the disclosure.

[0499] Further examples of the miRNA binding sites that can be useful for the present disclosure include immune cell specific miRNAs including, but not limited to, hsa-let-7a-2-3p, hsa-let-7a-3p, hsa-7a-5p, hsa-let-7c, hsa-let-7e-3p, hsa-let-7e-5p, hsa-let-7g-3p, hsa-let-7g-5p, hsa-let-7i-3p, hsa-let-7i-5p, miR-10a-3p, miR-10a-5p, miR-1184, hsa-let-7f-1-3p, hsa-let-7f-2--5p, hsa-let-7f-5p, miR-125b-1-3p, miR-125b-2-3p, miR-125b-5p, miR-1279, miR-130a-3p, miR-130a-5p, miR-132-3p, miR-132-5p, miR-142-3p, miR-142-5p, miR-143-3p, miR-143-5p, miR-146a-3p, miR-146a-5p, miR-146b-3p, miR-146b-5p, miR-147a, miR-147b, miR-148a-5p, miR-148a-3p, miR-150-3p, miR-150-5p, miR-151b, miR-155-3p, miR-155-5p, miR-15a-3p, miR-15a-5p, miR-15b-5p, miR-15b-3p, miR-16-1-3p, miR-16-2-3p, miR-16-5p, miR-17-5p, miR-181a-3p, miR-181a-5p, miR-181a-2-3p, miR-182-3p, miR-182-5p, miR-197-3p, miR-197-5p, miR-21-5p, miR-21-3p, miR-214-3p, miR-214-5p, miR-223-3p, miR-223-5p, miR-221-3p, miR-221-5p, miR-23b-3p, miR-23b-5p, miR-24-1-5p,miR-24-2-5p, miR-24-3p, miR-26a-1-3p, miR-26a-2-3p, miR-26a-5p, miR-26b-3p, miR-26b-5p, miR-27a-3p, miR-27a-5p, miR-27b-3p,miR-27b-5p, miR-28-3p, miR-28-5p, miR-2909, miR-29a-3p, miR-29a-5p, miR-29b-1-5p, miR-29b-2-5p, miR-29c-3p, miR-29c-5p,, miR-30e-3p, miR-30e-5p, miR-331-5p, miR-339-3p, miR-339-5p, miR-345-3p, miR-345-5p, miR-346, miR-34a-3p, miR-34a-5p, , miR-363-3p, miR-363-5p, miR-372, miR-377-3p, miR-377-5p, miR-493-3p, miR-493-5p, miR-542, miR-548b-5p, miR548c-5p, miR-548i, miR-548j, miR-548n, miR-574-3p, miR-598, miR-718, miR-935, miR-99a-3p, miR-99a-5p, miR-99b-3p, and miR-99b-5p. Furthermore, novel miRNAs can be identified in immune cell through micro-array hybridization and microtome analysis (*e.g.,* Jima DD et al, Blood, 2010, 116:e118-e127; Vaz C et al., BMC Genomics, 2010, 11,288.)

[0500] MiRNAs that are known to be expressed in the liver include, but are not limited to, miR-107, miR-122-3p, miR-122-5p, miR-1228-3p, miR-1228-5p, miR-1249, miR-129-5p, miR-1303, miR-151a-3p, miR-151a-5p, miR-152, miR-194-3p, miR-194-5p, miR-199a-3p, miR-199a-5p, miR-199b-3p, miR-199b-5p, miR-296-5p, miR-557, miR-581, miR-939-3p, and miR-939-5p. MiRNA binding sites from any liver specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the liver. Liver specific miRNA binding sites can be engineered alone or further in combination with immune cell (*e.g.,* APC) miRNA binding sites in a polynucleotide of the disclosure.

[0501] MiRNAs that are known to be expressed in the lung include, but are not limited to, let-7a-2-3p, let-7a-3p, let-7a-5p, miR-126-3p, miR-126-5p, miR-127-3p, miR-127-5p, miR-130a-3p, miR-130a-5p, miR-130b-3p, miR-130b-5p, miR-133a, miR-133b, miR-134, miR-18a-3p, miR-18a-5p, miR-18b-3p, miR-18b-5p, miR-24-1-5p, miR-24-2-5p, miR-24-3p, miR-296-3p, miR-296-5p, miR-32-3p, miR-337-3p, miR-337-5p, miR-381-3p, and miR-381-5p. MiRNA binding sites from any lung specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the lung. Lung specific miRNA binding sites can be engineered alone or further in combination with immune cell (*e.g.,* APC) miRNA binding sites in a polynucleotide of the disclosure.

[0502] MiRNAs that are known to be expressed in the heart include, but are not limited to, miR-1, miR-133a, miR-133b, miR-149-3p, miR-149-5p, miR-186-3p, miR-186-5p, miR-208a, miR-208b, miR-210, miR-296-3p, miR-320, miR-451a, miR-451b, miR-499a-3p, miR-499a-5p, miR-499b-3p, miR-499b-5p, miR-744-3p, miR-744-5p, miR-92b-3p, and miR-92b-5p. MiRNA binding sites from any heart specific microRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the heart. Heart specific miRNA binding sites can be engineered alone or further in combination with immune cell (*e.g.,* APC) miRNA binding sites in a polynucleotide of the disclosure.

[0503] MiRNAs that are known to be expressed in the nervous system include, but are not limited to, miR-124-5p, miR-125a-3p, miR-125a-5p, miR-125b-1-3p, miR-125b-2-3p, miR-125b-5p,miR-1271-3p, miR-1271-5p, miR-128, miR-132-5p, miR-135a-3p, miR-135a-5p, miR-135b-3p, miR-135b-5p, miR-137, miR-139-5p, miR-139-3p, miR-149-3p, miR-149-5p, miR-153, miR-181c-3p, miR-181c-5p, miR-183-3p, miR-183-5p, miR-190a, miR-190b, miR-212-3p, miR-212-5p, miR-219-1-3p, miR-219-2-3p, miR-23a-3p, miR-23a-5p,miR-30a-5p, miR-30b-3p, miR-30b-5p, miR-30c-1-3p, miR-30c-2-3p, miR-30c-5p, miR-30d-3p, miR-30d-5p, miR-329, miR-342-3p, miR-3665, miR-3666, miR-380-3p, miR-380-5p, miR-383, miR-410, miR-425-3p, miR-425-5p, miR-454-3p, miR-454-5p, miR-483, miR-510, miR-516a-3p, miR-548b-5p, miR-548c-5p, miR-571, miR-7-1-3p, miR-7-2-3p, miR-7-5p, miR-802, miR-922, miR-9-3p, and miR-9-5p. MiRNAs enriched in the nervous system further include those specifically expressed in neurons, including, but not limited to, miR-132-3p, miR-132-3p, miR-148b-3p, miR-148b-5p, miR-151a-3p, miR-151a-5p, miR-212-3p, miR-212-5p, miR-320b, miR-320e, miR-323a-3p, miR-323a-5p, miR-324-5p, miR-325, miR-326, miR-328, miR-922 and those specifically expressed in glial cells, including, but not limited to, miR-1250, miR-219-1-3p, miR-219-2-3p, miR-219-5p, miR-23a-3p, miR-23a-5p, miR-3065-3p, miR-3065-5p, miR-30e-3p, miR-30e-5p, miR-32-5p, miR-338-5p, and miR-657. MiRNA binding sites from any CNS specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the nervous system. Nervous system specific miRNA binding sites can be engineered alone or further in combination with immune cell (*e.g.,* APC) miRNA binding sites in a polynucleotide of the disclosure.

[0504] MiRNAs that are known to be expressed in the pancreas include, but are not limited to, miR-105-3p, miR-105-5p, miR-184, miR-195-3p, miR-195-5p, miR-196a-3p, miR-196a-5p, miR-214-3p, miR-214-5p, miR-216a-3p, miR-216a-5p, miR-30a-3p, miR-33a-3p, miR-33a-5p, miR-375, miR-7-1-3p, miR-7-2-3p, miR-493-3p, miR-493-5p, and miR-944. MiRNA binding sites from any pancreas specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the pancreas. Pancreas specific miRNA binding sites can be engineered alone or further in combination with immune cell (e.g. APC) miRNA binding sites in a polynucleotide of the disclosure.

[0505] MiRNAs that are known to be expressed in the kidney include, but are not limited to, miR-122-3p, miR-145-5p, miR-17-5p, miR-192-3p, miR-192-5p, miR-194-3p, miR-194-5p, miR-20a-3p, miR-20a-5p, miR-204-3p, miR-204-5p, miR-210, miR-216a-3p, miR-216a-5p, miR-296-3p, miR-30a-3p, miR-30a-5p, miR-30b-3p, miR-30b-5p, miR-30c-1-3p, miR-30c-2-3p, miR30c-5p, miR-324-3p, miR-335-3p, miR-335-5p, miR-363-3p, miR-363-5p, and miR-562. MiRNA binding sites from any kidney specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the kidney. Kidney specific miRNA binding sites can be engineered alone or further in combination with immune cell (*e.g.,* APC) miRNA binding sites n a polynucleotide of the disclosure.

[0506] MiRNAs that are known to be expressed in the muscle include, but are not limited to, let-7g-3p, let-7g-5p, miR-1, miR-1286, miR-133a, miR-133b, miR-140-3p, miR-143-3p, miR-143-5p, miR-145-3p, miR-145-5p, miR-188-3p, miR-188-5p, miR-206, miR-208a, miR-208b, miR-25-3p, and miR-25-5p. MiRNA binding sites from any muscle specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the muscle. Muscle specific miRNA binding sites can be engineered alone or further in combination with immune cell (*e.g.,* APC) miRNA binding sites in a polynucleotide of the disclosure .

[0507] MiRNAs are also differentially expressed in different types of cells, such as, but not limited to, endothelial cells, epithelial cells, and adipocytes.

[0508] MiRNAs that are known to be expressed in endothelial cells include, but are not limited to, let-7b-3p, let-7b-5p, miR-100-3p, miR-100-5p, miR-101-3p, miR-101-5p, miR-126-3p, miR-126-5p, miR-1236-3p, miR-1236-5p, miR-130a-3p, miR-130a-5p, miR-17-5p, miR-17-3p, miR-18a-3p, miR-18a-5p, miR-19a-3p, miR-19a-5p, miR-19b-1-5p, miR-19b-2-5p, miR-19b-3p, miR-20a-3p, miR-20a-5p, miR-217, miR-210, miR-21-3p, miR-21-5p, miR-221-3p, miR-221-5p, miR-222-3p, miR-222-5p, miR-23a-3p, miR-23a-5p, miR-296-5p, miR-361-3p, miR-361-5p, miR-421, miR-424-3p, miR-424-5p, miR-513a-5p, miR-92a-1-5p, miR-92a-2-5p, miR-92a-3p, miR-92b-3p, and miR-92b-5p. Many novel miRNAs are discovered in endothelial cells from deep-sequencing analysis (e.g., Voellenkle C et al., RNA, 2012, 18, 472-484). MiRNA binding sites from any endothelial cell specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the endothelial cells.

[0509] MiRNAs that are known to be expressed in epithelial cells include, but are not limited to, let-7b-3p, let-7b-5p, miR-1246, miR-200a-3p, miR-200a-5p, miR-200b-3p, miR-200b-5p, miR-200c-3p, miR-200c-5p, miR-338-3p, miR-429, miR-451a, miR-451b, miR-494, miR-802 and miR-34a, miR-34b-5p, miR-34c-5p, miR-449a, miR-449b-3p, miR-449b-5p specific in respiratory ciliated epithelial cells, let-7 family, miR-133a, miR-133b, miR-126 specific in lung epithelial cells, miR-382-3p, miR-382-5p specific in renal epithelial cells, and miR-762 specific in corneal epithelial cells. MiRNA binding sites from any epithelial cell specific miRNA can be introduced to or removed from a polynucleotide of the disclosure to regulate expression of the polynucleotide in the epithelial cells.

[0510] In addition, a large group of miRNAs are enriched in embryonic stem cells, controlling stem cell self-renewal as well as the development and/or differentiation of various cell lineages, such as neural cells, cardiac, hematopoietic cells, skin cells, osteogenic cells and muscle cells (*e.g.,* Kuppusamy KT et al., Curr. Mol Med, 2013, 13(5), 757-764; Vidigal JA and Ventura A, Semin Cancer Biol. 2012, 22(5-6), 428-436; GoffLA et al., PLoS One, 2009, 4:e7192; Morin RD et al., Genome Res,2008,18, 610-621; Yoo JK et al., Stem Cells Dev. 2012, 21(11), 2049-2057). MiRNAs abundant in embryonic stem cells include, but are not limited to, let-7a-2-3p, let-a-3p, let-7a-5p, let7d-3p, let-7d-5p, miR-103a-2-3p, miR-103a-5p, miR-106b-3p, miR-106b-5p, miR-1246, miR-1275, miR-138-1-3p, miR-138-2-3p, miR-138-5p, miR-154-3p, miR-154-5p, miR-200c-3p, miR-200c-5p, miR-290, miR-301a-3p, miR-301a-5p, miR-302a-3p, miR-302a-5p, miR-302b-3p, miR-302b-5p, miR-302c-3p, miR-302c-5p, miR-302d-3p, miR-302d-5p, miR-302e, miR-367-3p, miR-367-5p, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-373, miR-380-5p, miR-423-3p, miR-423-5p, miR-486-5p, miR-520c-3p, miR-548e, miR-548f, miR-548g-3p, miR-548g-5p, miR-548i, miR-548k, miR-5481, miR-548m, miR-548n, miR-548o-3p, miR-548o-5p, miR-548p, miR-664a-3p, miR-664a-5p, miR-664b-3p, miR-664b-5p, miR-766-3p, miR-766-5p, miR-885-3p, miR-885-5p,miR-93-3p, miR-93-5p, miR-941,miR-96-3p, miR-96-5p, miR-99b-3p and miR-99b-5p. Many predicted novel miRNAs are discovered by deep sequencing in human embryonic stem cells (*e.g.,* Morin RD et al., Genome Res,2008,18, 610-621; GoffLA et al., PLoS One, 2009, 4:e7192; Bar M et al., Stem cells, 2008, 26, 2496-2505).

[0511] In one case, the binding sites of embryonic stem cell specific miRNAs can be included in or removed from the 3'UTR of a polynucleotide of the disclosure to modulate the development and/or differentiation of embryonic stem cells, to inhibit the senescence of stem cells in a degenerative condition (e.g. degenerative diseases), or to stimulate the senescence and apoptosis of stem cells in a disease condition (e.g. cancer stem cells).

[0512] Many miRNA expression studies are conducted to profile the differential expression of miRNAs in various cancer cells/tissues and other diseases. Some miRNAs are abnormally over-expressed in certain cancer cells and others are under-expressed. For example, miRNAs are differentially expressed in cancer cells (WO2008/154098, US2013/0059015, US2013/0042333, WO2011/157294); cancer stem cells (US2012/0053224); pancreatic cancers and diseases (US2009/0131348, US2011/0171646, US2010/0286232, US8389210); asthma and inflammation (US8415096); prostate cancer (US2013/0053264); hepatocellular carcinoma (WO2012/151212, US2012/0329672, WO2008/054828, US8252538); lung cancer cells (WO2011/076143, WO2013/033640, WO2009/070653, US2010/0323357); cutaneous T cell lymphoma (WO2013/011378); colorectal cancer cells (WO2011/0281756, WO2011/076142); cancer positive lymph nodes (WO2009/100430, US2009/0263803); nasopharyngeal carcinoma (EP2112235); chronic obstructive pulmonary disease (US2012/0264626, US2013/0053263); thyroid cancer (WO2013/066678); ovarian cancer cells (US2012/0309645, WO2011/095623); breast cancer cells (WO2008/154098, WO2007/081740, US2012/0214699), leukemia and lymphoma (WO2008/073915, US2009/0092974, US2012/0316081, US2012/0283310, WO2010/018563.)

[0513] As a non-limiting example, miRNA binding sites for miRNAs that are over-expressed in certain cancer and/or tumor cells can be removed from the 3'UTR of a polynucleotide of the disclosure, restoring the expression suppressed by the over-expressed miRNAs in cancer cells, thus ameliorating the corresponsive biological function, for instance, transcription stimulation and/or repression, cell cycle arrest, apoptosis and cell death. Normal cells and tissues, wherein miRNAs expression is not up-regulated, will remain unaffected.

[0514] MiRNA can also regulate complex biological processes such as angiogenesis (*e.g.,* miR-132) (Anand and Cheresh Curr Opin Hematol 2011 18:171-176). In the polynucleotides of the disclosure, miRNA binding sites that are

involved in such processes can be removed or introduced, in order to tailor the expression of the po ynucleotides to biologically relevant cell types or relevant biological processes. In this context, the polynucleotides of the disclosure are defined as auxotrophic polynucleotides.

[0515] In some cases, the microRNA binding site (*e.g.*, miR-122 binding site) is fully complementary to miRNA (*e.g.*, miR-122), thereby degrading the mRNA fused to the miRNA binding site. In other cases, the miRNA binding site is not fully complementary to the corresponding miRNA. In certain cases, the miRNA binding site (*e.g.*, miR-122 binding site) is the same length as the corresponding miRNA (*e.g.*, miR-122). In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is one nucleotide shorter than the corresponding microRNA (*e.g.*, miR-122, which has 22 nts) at the 5' terminus, the 3' terminus, or both. In still other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is two nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus, the 3' terminus, or both. In yet other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is three nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus, the 3' terminus, or both. In some cases, the microRNA binding site (*e.g.*, miR-122 binding site) is four nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus, the 3' terminus, or both. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is five nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus, the 3' terminus, or both. In some cases, the microRNA binding site (*e.g.*, miR-122 binding site) is six nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus, the 3' terminus, or both. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is seven nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus or the 3' terminus. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is eight nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus or the 3' terminus. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is nine nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus or the 3' terminus. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is ten nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus or the 3' terminus. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is eleven nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus or the 3' terminus. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) is twelve nucleotides shorter than the corresponding microRNA (*e.g.*, miR-122) at the 5' terminus or the 3' terminus. The miRNA binding sites that are shorter than the corresponding miRNAs are still capable of degrading the mRNA incorporating one or more of the miRNA binding sites or preventing the mRNA from translation.

[0516] In some cases, the microRNA binding site (*e.g.*, miR-122 binding site) has sufficient complementarity to miRNA (*e.g.*, miR-122) so that a RISC complex comprising the miRNA (*e.g.*, miR-122) cleaves the polynucleotide comprising the microRNA binding site. In other cases, the microRNA binding site (*e.g.*, miR-122 binding site) has imperfect complementarity so that a RISC complex comprising the miRNA (*e.g.*, miR-122) induces instability in the polynucleotide comprising the microRNA binding site. In another case, the microRNA binding site (*e.g.*, miR-122 binding site) has imperfect complementarity so that a RISC complex comprising the miRNA (*e.g.*, miR-122) represses transcription of the polynucleotide comprising the microRNA binding site. In one case, the miRNA binding site (*e.g.*, miR-122 binding site) has one mismatch from the corresponding miRNA(*e.g.*, miR-122). In another case, the miRNA binding site has two mismatches from the corresponding miRNA. In other cases, the miRNA binding site has three mismatches from the corresponding miRNA. In other cases, the miRNA binding site has four mismatches from the corresponding miRNA. In some cases, the miRNA binding site has five mismatches from the corresponding miRNA. In other cases, the miRNA binding site has six mismatches from the corresponding miRNA. In certain cases, the miRNA binding site has seven mismatches from the corresponding miRNA. In other cases, the miRNA binding site has eight mismatches from the corresponding miRNA. In other cases, the miRNA binding site has nine mismatches from the corresponding miRNA. In other cases, the miRNA binding site has ten mismatches from the corresponding miRNA. In other cases, the miRNA binding site has eleven mismatches from the corresponding miRNA. In other cases, the miRNA binding site has twelve mismatches from the corresponding miRNA.

[0517] In certain cases, the miRNA binding site (*e.g.*, miR-122 binding site) has at least about ten contiguous nucleotides complementary to at least about ten contiguous nucleotides of the corresponding miRNA (*e.g.*, miR-122), at least about eleven contiguous nucleotides complementary to at least about eleven contiguous nucleotides of the corresponding miRNA, at least about twelve contiguous nucleotides complementary to at least about twelve contiguous nucleotides of the corresponding miRNA, at least about thirteen contiguous nucleotides complementary to at least about thirteen contiguous nucleotides of the corresponding miRNA, or at least about fourteen contiguous nucleotides complementary to at least about fourteen contiguous nucleotides of the corresponding miRNA. In some cases, the miRNA binding sites have at least about fifteen contiguous nucleotides complementary to at least about fifteen contiguous nucleotides of the corresponding miRNA, at least about sixteen contiguous nucleotides complementary to at least about sixteen contiguous nucleotides of the corresponding miRNA, at least about seventeen contiguous nucleotides complementary to at least about seventeen contiguous nucleotides of the corresponding miRNA, at least about eighteen contiguous nucleotides complementary to at least about eighteen contiguous nucleotides of the corresponding miRNA, at least about nineteen contiguous nucleotides complementary to at least about nineteen contiguous nucleotides of the corresponding miRNA,

at least about twenty contiguous nucleotides complementary to at least about twenty contiguous nucleotides of the corresponding miRNA, or at least about twenty one contiguous nucleotides complementary to at least about twenty one contiguous nucleotides of the corresponding miRNA.

[0518] In some cases, the polynucleotides comprise an mRNA encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides and at least one miR-122 binding site, at least two miR-122 binding sites, at least three miR-122 binding sites, at least four miR-122 binding sites, or at least five miR-122 binding sites. In one aspect, the miRNA binding site binds miR-122 or is complementary to miR-122. In another aspect, the miRNA binding site binds to miR-122-3p or miR-122-5p. In a particular aspect, the miRNA binding site comprises a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO: 52 or 54, wherein the miRNA binding site binds to miR-122. In another particular aspect, the miRNA binding site comprises a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO: 54, wherein the miRNA binding site binds to miR-122. These sequences are shown below in Table 7.

Table 7. miR-122 and miR-122 binding sites

SEQ ID NO.	Description	Sequence
SEQ ID NO: 50	miR-122	CCUUAGCAGAGCUGUGGAGUGUGACAAUGGUGUUUGUG UCUAAACUAUCAAACGCCAUUAUCACACUAAAUAGCUA CUGCUAGGC
SEQ ID NO: 51	miR-122-3p	AACGCCAUUAUCACACUAAAUA
SEQ ID NO: 52	miR-122-3p binding site	UAUUUAGUGUGAUAAUGGCGUU
SEQ ID NO: 53	miR-122-5p	UGGAGUGUGACAAUGGUGUUUG
SEQ ID NO: 54	miR-122-5p binding site	CAAACACCAUUGUCACACUCCA

[0519] In some cases, a miRNA binding site (*e.g.*, miR-122 binding site) is inserted in the polynucleotide of the disclosure in any position of the polynucleotide (*e.g.*, 3' UTR); the insertion site in the polynucleotide can be anywhere in the polynucleotide as long as the insertion of the miRNA binding site in the polynucleotide does not interfere with the translation of the functional IL12B polypeptide, IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides in the absence of the corresponding miRNA (*e.g.*, miR-122); and in the presence of the miRNA (*e.g.*, miR-122), the insertion of the miRNA binding site in the polynucleotide and the binding of the miRNA binding site to the corresponding miRNA are capable of degrading the polynucleotide or preventing the translation of the polynucleotide. In one case, a miRNA binding site is inserted in a 3'UTR of the polynucleotide.

[0520] In certain cases, a miRNA binding site is inserted in at least about 30 nucleotides downstream from the stop codon of the an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides-encoding mRNA. In other cases, a miRNA binding site is inserted in at least about 10 nucleotides, at least about 15 nucleotides, at least about 20 nucleotides, at least about 25 nucleotides, at least about 30 nucleotides, at least about 35 nucleotides, at least about 40 nucleotides, at least about 45 nucleotides, at least about 50 nucleotides, at least about 55 nucleotides, at least about 60 nucleotides, at least about 65 nucleotides, at least about 70 nucleotides, at least about 75 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, or at least about 100 nucleotides downstream from the stop codon of the polynucleotide, *e.g.*, the IL12B polypeptide, IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides encoding mRNA. In other cases, a miRNA binding site is inserted in about 10 nucleotides to about 100 nucleotides, about 20 nucleotides to about 90 nucleotides, about 30 nucleotides to about 80 nucleotides, about 40 nucleotides to about 70 nucleotides, about 50 nucleotides to about 60 nucleotides, about 45 nucleotides to about 65 nucleotides downstream from the stop codon of the polynucleotide, *e.g.*, the IL12B polypeptide, IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides-encoding mRNA. In some cases, the miRNA binding site is inserted downstream of the stop codon in the nucleic acid sequence encoding an IL12 polypeptide as disclosed herein. In some cases, the miRNA binding site is inserted downstream of the stop codon in the nucleic acid sequence encoding membrane domain as disclosed herein. In some cases, the miRNA binding site is inserted downstream of the stop codon in the nucleic acid sequence encoding heterologous polypeptide as disclosed herein.

[0521] In some cases, a miRNA binding site is inserted in the polynucleotide of the disclosure in any position of the polynucleotide (*e.g.*, the 5'UTR and/or 3'UTR). In some cases, the 5'UTR comprises a miRNA binding site. In some cases, the 3'UTR comprises a miRNA binding site. In some cases, the 5'UTR and the 3'UTR comprise a miRNA binding site. The insertion site in the polynucleotide can be anywhere in the polynucleotide as long as the insertion of the miRNA binding site in the polynucleotide does not interfere with the translation of a functional polypeptide in the absence of the corresponding miRNA; and in the presence of the miRNA, the insertion of the miRNA binding site in the polynucleotide and the binding of the miRNA binding site to the corresponding miRNA are capable of degrading the polynucleotide or

preventing the translation of the polynucleotide.

[0522] In some cases, a miRNA binding site is inserted in at least about 30 nucleotides downstream from the stop codon of an ORF in a polynucleotide of the disclosure comprising the ORF. In some cases, a miRNA binding site is inserted in at least about 10 nucleotides, at least about 15 nucleotides, at least about 20 nucleotides, at least about 25 nucleotides, at least about 30 nucleotides, at least about 35 nucleotides, at least about 40 nucleotides, at least about 45 nucleotides, at least about 50 nucleotides, at least about 55 nucleotides, at least about 60 nucleotides, at least about 65 nucleotides, at least about 70 nucleotides, at least about 75 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, or at least about 100 nucleotides downstream from the stop codon of an ORF in a polynucleotide of the disclosure. In some cases, a miRNA binding site is inserted in about 10 nucleotides to about 100 nucleotides, about 20 nucleotides to about 90 nucleotides, about 30 nucleotides to about 80 nucleotides, about 40 nucleotides to about 70 nucleotides, about 50 nucleotides to about 60 nucleotides, about 45 nucleotides to about 65 nucleotides downstream from the stop codon of an ORF in a polynucleotide of the disclosure.

[0523] MiRNA gene regulation can be influenced by the sequence surrounding the miRNA such as, but not limited to, the species of the surrounding sequence, the type of sequence (*e.g.,* heterologous, homologous, exogenous, endogenous, or artificial), regulatory elements in the surrounding sequence and/or structural elements in the surrounding sequence. The miRNA can be influenced by the 5'UTR and/or 3'UTR. As a non-limiting example, a non-human 3'UTR can increase the regulatory effect of the miRNA sequence on the expression of a polypeptide of interest compared to a human 3'UTR of the same sequence type.

[0524] In one case, other regulatory elements and/or structural elements of the 5'UTR can influence miRNA mediated gene regulation. One example of a regulatory element and/or structural element is a structured IRES (Internal Ribosome Entry Site) in the 5'UTR, which is necessary for the binding of translational elongation factors to initiate protein translation. EIF4A2 binding to this secondarily structured element in the 5'-UTR is necessary for miRNA mediated gene expression (Meijer HA et al., Science, 2013, 340, 82-85). The polynucleotides of the disclosure can further include this structured 5'UTR in order to enhance microRNA mediated gene regulation.

[0525] At least one miRNA binding site can be engineered into the 3'UTR of a polynucleotide of the disclosure. In this context, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or more miRNA binding sites can be engineered into a 3'UTR of a polynucleotide of the disclosure. For example, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 2, or 1 miRNA binding sites can be engineered into the 3'UTR of a polynucleotide of the disclosure. In one case, miRNA binding sites incorporated into a polynucleotide of the disclosure can be the same or can be different miRNA sites. A combination of different miRNA binding sites incorporated into a polynucleotide of the disclosure can include combinations in which more than one copy of any of the different miRNA sites are incorporated. In another case, miRNA binding sites incorporated into a polynucleotide of the disclosure can target the same or different tissues in the body. As a non-limiting example, through the introduction of tissue-, cell-type-, or disease-specific miRNA binding sites in the 3'-UTR of a polynucleotide of the disclosure, the degree of expression in specific cell types (*e.g.,* hepatocytes, myeloid cells, endothelial cells, cancer cells, etc.) can be reduced.

[0526] In one case, a miRNA binding site can be engineered near the 5' terminus of the 3'UTR, about halfway between the 5' terminus and 3' terminus of the 3'UTR and/or near the 3' terminus of the 3'UTR in a polynucleotide of the disclosure. As a non-limiting example, a miRNA binding site can be engineered near the 5' terminus of the 3'UTR and about halfway between the 5' terminus and 3' terminus of the 3'UTR. As another non-limiting example, a miRNA binding site can be engineered near the 3' terminus of the 3'UTR and about halfway between the 5' terminus and 3' terminus of the 3'UTR. As yet another non-limiting example, a miRNA binding site can be engineered near the 5' terminus of the 3'UTR and near the 3' terminus of the 3'UTR.

[0527] In another case, a 3'UTR can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 miRNA binding sites. The miRNA binding sites can be complementary to a miRNA, miRNA seed sequence, and/or miRNA sequences flanking the seed sequence.

[0528] In one case, a polynucleotide of the disclosure can be engineered to include more than one miRNA site expressed in different tissues or different cell types of a subject. As a non-limiting example, a polynucleotide of the disclosure can be engineered to include miR-192 and miR-122 to regulate expression of the polynucleotide in the liver and kidneys of a subject. In another case, a polynucleotide of the disclosure can be engineered to include more than one miRNA site for the same tissue.

[0529] In some cases, the therapeutic window and or differential expression associated with the polypeptide encoded by a polynucleotide of the disclosure can be altered with a miRNA binding site. For example, a polynucleotide encoding a polypeptide that provides a death signal can be designed to be more highly expressed in cancer cells by virtue of the miRNA signature of those cells. Where a cancer cell expresses a lower level of a particular miRNA, the polynucleotide encoding the binding site for that miRNA (or miRNAs) would be more highly expressed. Hence, the polypeptide that provides a death signal triggers or induces cell death in the cancer cell. Neighboring noncancer cells, harboring a higher expression of the same miRNA would be less affected by the encoded death signal as the polynucleotide would be expressed at a lower level due to the effects of the miRNA binding to the binding site or "sensor" encoded in the 3'UTR.

Conversely, cell survival or cytoprotective signals can be delivered to tissues containing cancer and non-cancerous cells where a miRNA has a higher expression in the cancer cells-the result being a lower survival signal to the cancer cell and a larger survival signal to the normal cell. Multiple polynucleotides can be designed and administered having different signals based on the use of miRNA binding sites as described herein.

[0530] In some cases, the expression of a polynucleotide of the disclosure can be controlled by incorporating at least one sensor sequence in the polynucleotide and formulating the polynucleotide for administration. As a non-limiting example, a polynucleotide of the disclosure can be targeted to a tissue or cell by incorporating a miRNA binding site and formulating the polynucleotide in a lipid nanoparticle comprising a cationic lipid, including any of the lipids described herein.

[0531] A polynucleotide of the disclosure can be engineered for more targeted expression in specific tissues, cell types, or biological conditions based on the expression patterns of miRNAs in the different tissues, cell types, or biological conditions. Through introduction of tissue-specific miRNA binding sites, a polynucleotide of the disclosure can be designed for optimal protein expression in a tissue or cell, or in the context of a biological condition.

[0532] In some cases, a polynucleotide of the disclosure can be designed to incorporate miRNA binding sites that either have 100% identity to known miRNA seed sequences or have less than 100% identity to miRNA seed sequences. In some cases, a polynucleotide of the disclosure can be designed to incorporate miRNA binding sites that have at least: 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to known miRNA seed sequences. The miRNA seed sequence can be partially mutated to decrease miRNA binding affinity and as such result in reduced downmodulation of the polynucleotide. In essence, the degree of match or mis-match between the miRNA binding site and the miRNA seed can act as a rheostat to more finely tune the ability of the miRNA to modulate protein expression. In addition, mutation in the non-seed region of a miRNA binding site can also impact the ability of a miRNA to modulate protein expression.

[0533] In one case, a miRNA sequence can be incorporated into the loop of a stem loop.

[0534] In another case, a miRNA seed sequence can be incorporated in the loop of a stem loop and a miRNA binding site can be incorporated into the 5' or 3' stem of the stem loop.

[0535] In one case, a translation enhancer element (TEE) can be incorporated on the 5'end of the stem of a stem loop and a miRNA seed can be incorporated into the stem of the stem loop. In another case, a TEE can be incorporated on the 5' end of the stem of a stem loop, a miRNA seed can be incorporated into the stem of the stem loop and a miRNA binding site can be incorporated into the 3' end of the stem or the sequence after the stem loop. The miRNA seed and the miRNA binding site can be for the same and/or different miRNA sequences.

[0536] In one case, the incorporation of a miRNA sequence and/or a TEE sequence changes the shape of the stem loop region which can increase and/or decrease translation. (see *e.g.,* Kedde et al., "A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility." Nature Cell Biology. 2010).

[0537] In one case, the 5'-UTR of a polynucleotide of the disclosure can comprise at least one miRNA sequence. The miRNA sequence can be, but is not limited to, a 19 or 22 nucleotide sequence and/or a miRNA sequence without the seed.

[0538] In one case the miRNA sequence in the 5'UTR can be used to stabilize a polynucleotide of the disclosure described herein.

[0539] In another case, a miRNA sequence in the 5'UTR of a polynucleotide of the disclosure can be used to decrease the accessibility of the site of translation initiation such as, but not limited to a start codon. See, *e.g.*, Matsuda et al., PLoS One. 2010 11(5):e15057, which used antisense locked nucleic acid (LNA) oligonucleotides and exon-junction complexes (EJCs) around a start codon (-4 to +37 where the A of the AUG codons is +1) in order to decrease the accessibility to the first start codon (AUG). Matsuda showed that altering the sequence around the start codon with an LNA or EJC affected the efficiency, length and structural stability of a polynucleotide. A polynucleotide of the disclosure can comprise a miRNA sequence, instead of the LNA or EJC sequence described by Matsuda et al, near the site of translation initiation in order to decrease the accessibility to the site of translation initiation. The site of translation initiation can be prior to, after or within the miRNA sequence. As a non-limiting example, the site of translation initiation can be located within a miRNA sequence such as a seed sequence or binding site. As another non-limiting example, the site of translation initiation may be located within a miR-122 sequence such as the seed sequence or the mir-122 binding site.

[0540] In some cases, a polynucleotide of the disclosure can include at least one miRNA in order to dampen expression of the encoded polypeptide in a tissue or cell of interest. As a non-limiting example, a polynucleotide of the disclosure can include at least one miR-122 binding site in order to dampen expression of an encoded polypeptide of interest in the liver. As another non-limiting example a polynucleotide of the disclosure can include at least one miR-142-3p binding site, miR-142-3p seed sequence, miR-142-3p binding site without the seed, miR-142-5p binding site, miR-142-5p seed sequence, miR-142-5p binding site without the seed, miR-146 binding site, miR-146 seed sequence and/or miR-146 binding site without the seed sequence.

[0541] In some cases, a polynucleotide of the disclosure can comprise at least one miRNA binding site in the 3'UTR in order to selectively degrade mRNA therapeutics in the immune cells to subdue unwanted immunogenic reactions caused by therapeutic delivery. As a non-limiting example, the miRNA binding site can make a polynucleotide of the

disclosure more unstable in antigen presenting cells. Non-limiting examples of these miRNAs include mir-122-5p or mir-122-3p.

[0542] In one case, a polynucleotide of the disclosure comprises at least one miRNA sequence in a region of the polynucleotide that can interact with a RNA binding protein.

[0543] In some cases, the polynucleotide of the disclosure *(e.g.,* a RNA, *e.g.,* an mRNA) comprising (i) a sequence-optimized nucleotide sequence (e.g., an ORF) encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides *(e.g.,* the wild-type sequence, functional fragment, or variant thereof) and (ii) a miRNA binding site *(e.g.,* a miRNA binding site that binds to miR-122).

[0544] In some cases, the polynucleotide of the disclosure comprises a uracil-modified sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides disclosed herein and a miRNA binding site disclosed herein, *e.g.,* a miRNA binding site that binds to miR-122. In some cases, the uracil-modified sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides comprises at least one chemically modified nucleobase, *e.g.*, 5-methoxyuracil. In some cases, at least 95% of a type of nucleobase (e.g., uracil) in a uracil-modified sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides of the disclosure are modified nucleobases. In some cases, at least 95% of uracil in a uracil-modified sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides is 5-methoxyuridine. In some cases, the polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides disclosed herein and a miRNA binding site is formulated with a delivery agent, *e.g.,* a compound having the **Formula (I),** *e.g.,* any of Compounds 1-147 or any of Compounds 1-232.

15. 3' UTR and the AU Rich Elements

[0545] In certain cases, a polynucleotide of the present disclosure (e.g., a polynucleotide comprising a nucleotide sequence (e.g., an ORF) encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides of the disclosure) further comprises a 3' UTR.

[0546] 3'-UTR is the section of mRNA that immediately follows the translation termination codon and often contains regulatory regions that post-transcriptionally influence gene expression. Regulatory regions within the 3'-UTR can influence polyadenylation, translation efficiency, localization, and stability of the mRNA. In one case, the 3'-UTR useful for the disclosure comprises a binding site for regulatory proteins or microRNAs. In some cases, the 3'-UTR has a silencer region, which binds to repressor proteins and inhibits the expression of the mRNA. In other cases, the 3'-UTR comprises an AU-rich element. Proteins bind AREs to affect the stability or decay rate of transcripts in a localized manner or affect translation initiation. In other cases, the 3'-UTR comprises the sequence AAUAAA that directs addition of several hundred adenine residues called the poly(A) tail to the end of the mRNA transcript.

[0547] Natural or wild type 3'UTRs are known to have stretches of Adenosines and Uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo.*

[0548] Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of polynucleotides of the disclosure. When engineering specific polynucleotides, one or more copies of an ARE can be introduced to make polynucleotides of the disclosure less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using polynucleotides of the disclosure and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hour, 12 hour, 24 hour, 48 hour, and 7 days post-transfection.

[0549] In certain cases, the 3' UTR useful for the polynucleotides of the disclosure comprises a 3'UTR selected from those shown in this application.

[0550] In certain cases, the 3'UTR sequence useful for the disclosure comprises a nucleotide sequence at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from the group consisting

of 3'UTR sequences listed herein and any combination thereof.

16. Regions having a 5' Cap

[0551] The disclosure also includes a polynucleotide that comprises both a 5' Cap and a polynucleotide of the present disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides).

[0552] The 5' cap structure of a natural mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5' proximal introns during mRNA splicing.

[0553] Endogenous mRNA molecules can be 5'-end capped generating a 5'-ppp-5'-triphosphate linkage between a terminal guanosine cap residue and the 5'-terminal transcribed sense nucleotide of the mRNA molecule. This 5'-guanylate cap can then be methylated to generate an N7-methyl-guanylate residue. The ribose sugars of the terminal and/or anteterminal transcribed nucleotides of the 5' end of the mRNA can optionally also be 2'-O-methylated. 5'-decapping through hydrolysis and cleavage of the guanylate cap structure can target a nucleic acid molecule, such as an mRNA molecule, for degradation.

[0554] In some cases, the polynucleotides of the present disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) incorporate a cap moiety.

[0555] In some cases, polynucleotides of the present disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) comprise a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5'-ppp-5' phosphorodiester linkages, modified nucleotides can be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, MA) can be used with α-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5'-ppp-5' cap. Additional modified guanosine nucleotides can be used such as α-methylphosphonate and seleno-phosphate nucleotides.

[0556] Additional modifications include, but are not limited to, 2'-O-methylation of the ribose sugars of 5'-terminal and/or 5'-anteterminal nucleotides of the polynucleotide (as mentioned above) on the 2'-hydroxyl group of the sugar ring. Multiple distinct 5'-cap structures can be used to generate the 5'-cap of a nucleic acid molecule, such as a polynucleotide that functions as an mRNA molecule. Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural *(i.e.,* endogenous, wild-type or physiological) 5'-caps in their chemical structure, while retaining cap function. Cap analogs can be chemically *(i.e.,* non-enzymatically) or enzymatically synthesized and/or linked to the polynucleotides of the disclosure.

[0557] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5'-5'-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3'-O-methyl group (*i.e.,* N7,3'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine (m^7G-3'mppp-G; which may equivalently be designated 3' O-Mem7G(5')ppp(5')G). The 3'-O atom of the other, unmodified, guanine becomes linked to the 5'-terminal nucleotide of the capped polynucleotide. The N7- and 3'-O-methlyated guanine provides the terminal moiety of the capped polynucleotide.

[0558] Another exemplary cap is mCAP, which is similar to ARCA but has a 2'-O-methyl group on guanosine (*i.e.,* N7,2'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine, m^7Gm-ppp-G).

[0559] In some cases, the cap is a dinucleotide cap analog. As a non-limiting example, the dinucleotide cap analog can be modified at different phosphate positions with a boranophosphate group or a phophoroselenoate group such as the dinucleotide cap analogs described in U.S. Patent No. US 8,519,110.

[0560] In another case, the cap is a cap analog is a N7-(4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog known in the art and/or described herein. Non-limiting examples of a N7-(4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog include a N7-(4-chlorophenoxyethyl)-G(5')ppp(5')G and a N7-(4-chlorophenoxyethyl)-$m^{3'\text{-}O}$G(5')ppp(5')G cap analog (*See, e.g.,* the various cap analogs and the methods of synthesizing cap analogs described in Kore et al. Bioorganic & Medicinal Chemistry 2013 21:4570-4574). In another case, a cap analog of the present disclosure is a 4-chloro/bromophenoxyethyl analog.

[0561] While cap analogs allow for the concomitant capping of a polynucleotide or a region thereof, in an *in vitro* transcription reaction, up to 20% of transcripts can remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5'-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, can lead to reduced translational competency and reduced cellular stability.

[0562] Polynucleotides of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) can also be capped post-manufacture (whether IVT or chemical synthesis), using enzymes, in order to generate more authentic 5'-cap structures. As used

herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5'cap structures of the present disclosure are those that, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5' endonucleases and/or reduced 5'decapping, as compared to synthetic 5'cap structures known in the art (or to a wild-type, natural or physiological 5'cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of a polynucleotide and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5'-terminal nucleotide of the mRNA contains a 2'-O-methyl. Such a structure is termed the Cap1 structure. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, *e.g.,* to other 5'cap analog structures known in the art. Cap structures include, but are not limited to, 7mG(5')ppp(5')N,pN2p (cap 0), 7mG(5')ppp(5')NlmpNp (cap 1), and 7mG(5')-ppp(5')NlmpN2mp (cap 2).

[0563] As a non-limiting example, capping chimeric polynucleotides post-manufacture can be more efficient as nearly 100% of the chimeric polynucleotides can be capped. This is in contrast to ~80% when a cap analog is linked to a chimeric polynucleotide in the course of an *in vitro* transcription reaction.

[0564] According to the present disclosure, 5' terminal caps can include endogenous caps or cap analogs. According to the present disclosure, a 5' terminal cap can comprise a guanine analog. Useful guanine analogs include, but are not limited to, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0565] In certain cases, the 5' terminal cap structure is a CapO, Cap1, ARCA, inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azidoguanosine, Cap2, Cap4, 5' methylG cap, or an analog thereof.

17. Poly-A Tails

[0566] In some cases, the polynucleotides of the present disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) further comprise a poly-A tail. In further cases, terminal groups on the poly-A tail can be incorporated for stabilization. In other cases, a poly-A tail comprises des-3' hydroxyl tails.

[0567] During RNA processing, a long chain of adenine nucleotides (poly-A tail) can be added to a polynucleotide such as an mRNA molecule in order to increase stability. Immediately after transcription, the 3' end of the transcript can be cleaved to free a 3' hydroxyl. Then poly-A polymerase adds a chain of adenine nucleotides to the RNA. The process, called polyadenylation, adds a poly-A tail that can be between, for example, approximately 80 to approximately 250 residues long, including approximately 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240 or 250 residues long.

[0568] PolyA tails can also be added after the construct is exported from the nucleus.

[0569] According to the present disclosure, terminal groups on the poly A tail can be incorporated for stabilization. Polynucleotides of the present disclosure can include des-3' hydroxyl tails. They can also include structural moieties or 2'-Omethyl modifications as taught by Junjie Li, et al. (Current Biology, Vol. 15, 1501-1507, August 23, 2005).

[0570] The polynucleotides of the present disclosure can be designed to encode transcripts with alternative poly A tail structures including histone mRNA. According to Norbury, "Terminal uridylation has also been detected on human replication-dependent histone mRNAs. The turnover of these mRNAs is thought to be important for the prevention of potentially toxic histone accumulation following the completion or inhibition of chromosomal DNA replication. These mRNAs are distinguished by their lack of a 3' poly(A) tail, the function of which is instead assumed by a stable stem-loop structure and its cognate stem-loop binding protein (SLBP); the latter carries out the same functions as those of PABP on polyadenylated mRNAs" (Norbury, "Cytoplasmic RNA: a case of the tail wagging the dog," Nature Reviews Molecular Cell Biology; AOP, published online 29 August 2013; doi:10.1038/nrm3645).

[0571] Unique poly-A tail lengths provide certain advantages to the polynucleotides of the present disclosure. Generally, the length of a poly-A tail, when present, is greater than 30 nucleotides in length. In another case, the poly-A tail is greater than 35 nucleotides in length *(e.g.,* at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000 nucleotides).

[0572] In some cases, the polynucleotide or region thereof includes from about 30 to about 3,000 nucleotides (*e.g.*, from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 750, from 30 to 1,000, from 30 to 1,500, from 30 to 2,000, from 30 to 2,500, from 50 to 100, from 50 to 250, from 50 to 500, from 50 to 750, from 50 to 1,000, from 50 to 1,500, from 50 to 2,000, from 50 to 2,500, from 50 to 3,000, from 100 to 500, from 100 to 750, from 100 to 1,000,

from 100 to 1,500, from 100 to 2,000, from 100 to 2,500, from 100 to 3,000, from 500 to 750, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 2,500, from 500 to 3,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 2,500, from 1,000 to 3,000, from 1,500 to 2,000, from 1,500 to 2,500, from 1,500 to 3,000, from 2,000 to 3,000, from 2,000 to 2,500, and from 2,500 to 3,000).

[0573] In some cases, the poly-A tail is designed relative to the length of the overall polynucleotide or the length of a particular region of the polynucleotide. This design can be based on the length of a coding region, the length of a particular feature or region or based on the length of the ultimate product expressed from the polynucleotides.

[0574] In this context, the poly-A tail can be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% greater in length than the polynucleotide or feature thereof. The poly-A tail can also be designed as a fraction of the polynucleotides to which it belongs. In this context, the polyA tail can be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the construct, a construct region or the total length of the construct minus the poly-A tail. Further, engineered binding sites and conjugation of polynucleotides for Poly-A binding protein can enhance expression.

[0575] Additionally, multiple distinct polynucleotides can be linked together via the PABP (Poly-A binding protein) through the 3'-end using modified nucleotides at the 3'terminus of the poly-A tail. Transfection experiments can be conducted in relevant cell lines at and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72hr and day 7 post-transfection.

[0576] In some cases, the polynucleotides of the present disclosure are designed to include a polyA-G Quartet region. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In this case, the G-quartet is incorporated at the end of the poly-A tail. The resultant polynucleotide is assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production from an mRNA equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

18. **Start codon region**

[0577] The disclosure also includes a polynucleotide that comprises both a start codon region and the polynucleotide described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides). In some cases, the polynucleotides of the present disclosure can have regions that are analogous to or function like a start codon region.

[0578] In some cases, the translation of a polynucleotide can initiate on a codon that is not the start codon AUG. Translation of the polynucleotide can initiate on an alternative start codon such as, but not limited to, ACG, AGG, AAG, CTG/CUG, GTG/GUG, ATA/AUA, ATT/AUU, TTG/UUG (see Touriol et al. Biology of the Cell 95 (2003) 169-178 and Matsuda and Mauro PLoS ONE, 2010 5:11).

[0579] As a non-limiting example, the translation of a polynucleotide begins on the alternative start codon ACG. As another non-limiting example, polynucleotide translation begins on the alternative start codon CTG or CUG. As yet another non-limiting example, the translation of a polynucleotide begins on the alternative start codon GTG or GUG.

[0580] Nucleotides flanking a codon that initiates translation such as, but not limited to, a start codon or an alternative start codon, are known to affect the translation efficiency, the length and/or the structure of the polynucleotide. *(See, e.g.,* Matsuda and Mauro PLoS ONE, 2010 5:11). Masking any of the nucleotides flanking a codon that initiates translation can be used to alter the position of translation initiation, translation efficiency, length and/or structure of a polynucleotide.

[0581] In some cases, a masking agent can be used near the start codon or alternative start codon in order to mask or hide the codon to reduce the probability of translation initiation at the masked start codon or alternative start codon. Non-limiting examples of masking agents include antisense locked nucleic acids (LNA) polynucleotides and exon-junction complexes (EJCs) (*See, e.g.,* Matsuda and Mauro describing masking agents LNA polynucleotides and EJCs (PLoS ONE, 2010 5:11)).

[0582] In another case, a masking agent can be used to mask a start codon of a polynucleotide in order to increase the likelihood that translation will initiate on an alternative start codon. In some cases, a masking agent can be used to mask a first start codon or alternative start codon in order to increase the chance that translation will initiate on a start codon or alternative start codon downstream to the masked start codon or alternative start codon.

[0583] In some cases, a start codon or alternative start codon can be located within a perfect complement for a miR binding site. The perfect complement of a miR binding site can help control the translation, length and/or structure of the polynucleotide similar to a masking agent. As a non-limiting example, the start codon or alternative start codon can be located in the middle of a perfect complement for a miRNA binding site. The start codon or alternative start codon can be located after the first nucleotide, second nucleotide, third nucleotide, fourth nucleotide, fifth nucleotide, sixth nucleotide, seventh nucleotide, eighth nucleotide, ninth nucleotide, tenth nucleotide, eleventh nucleotide, twelfth nucleotide, thirteenth nucleotide, fourteenth nucleotide, fifteenth nucleotide, sixteenth nucleotide, seventeenth nucleotide, eighteenth nucleotide, nineteenth nucleotide, twentieth nucleotide or twenty-first nucleotide.

[0584] In another case, the start codon of a polynucleotide can be removed from the polynucleotide sequence in order

to have the translation of the polynucleotide begin on a codon that is not the start codon. Translation of the polynucleotide can begin on the codon following the removed start codon or on a downstream start codon or an alternative start codon. In a non-limiting example, the start codon ATG or AUG is removed as the first 3 nucleotides of the polynucleotide sequence in order to have translation initiate on a downstream start codon or alternative start codon. The polynucleotide sequence where the start codon was removed can further comprise at least one masking agent for the downstream start codon and/or alternative start codons in order to control or attempt to control the initiation of translation, the length of the polynucleotide and/or the structure of the polynucleotide.

19. Stop Codon Region

[0585] The disclosure also includes a polynucleotide that comprises both a stop codon region and the polynucleotide described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides). In some cases, the polynucleotides of the present disclosure can include at least two stop codons before the 3' untranslated region (UTR). The stop codon can be selected from TGA, TAA and TAG in the case of DNA, or from UGA, UAA and UAG in the case of RNA. In some cases, the polynucleotides of the present disclosure include the stop codon TGA in the case or DNA, or the stop codon UGA in the case of RNA, and one additional stop codon. In a further case the addition stop codon can be TAA or UAA. In another case, the polynucleotides of the present disclosure include three consecutive stop codons, four stop codons, or more.

20. Insertions and Substitutions

[0586] The disclosure also includes a polynucleotide of the present disclosure that further comprises insertions and/or substitutions.

[0587] In some cases, the 5'UTR of the polynucleotide can be replaced by the insertion of at least one region and/or string of nucleosides of the same base. The region and/or string of nucleotides can include, but is not limited to, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 nucleotides and the nucleotides can be natural and/or unnatural. As a non-limiting example, the group of nucleotides can include 5-8 adenine, cytosine, thymine, a string of any of the other nucleotides disclosed herein and/or combinations thereof.

[0588] In some cases, the 5'UTR of the polynucleotide can be replaced by the insertion of at least two regions and/or strings of nucleotides of two different bases such as, but not limited to, adenine, cytosine, thymine, any of the other nucleotides disclosed herein and/or combinations thereof. For example, the 5'UTR can be replaced by inserting 5-8 adenine bases followed by the insertion of 5-8 cytosine bases. In another example, the 5'UTR can be replaced by inserting 5-8 cytosine bases followed by the insertion of 5-8 adenine bases.

[0589] In some cases, the polynucleotide can include at least one substitution and/or insertion downstream of the transcription start site that can be recognized by an RNA polymerase. As a non-limiting example, at least one substitution and/or insertion can occur downstream of the transcription start site by substituting at least one nucleic acid in the region just downstream of the transcription start site (such as, but not limited to, +1 to +6). Changes to region of nucleotides just downstream of the transcription start site can affect initiation rates, increase apparent nucleotide triphosphate (NTP) reaction constant values, and increase the dissociation of short transcripts from the transcription complex curing initial transcription (Brieba et al, Biochemistry (2002) 41: 5144-5149). The modification, substitution and/or insertion of at least one nucleoside can cause a silent mutation of the sequence or can cause a mutation in the amino acid sequence.

[0590] In some cases, the polynucleotide can include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12 or at least 13 guanine bases downstream of the transcription start site.

[0591] In some cases, the polynucleotide can include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 guanine bases in the region just downstream of the transcription start site. As a non-limiting example, if the nucleotides in the region are GGGAGA, the guanine bases can be substituted by at least 1, at least 2, at least 3 or at least 4 adenine nucleotides (SEQ ID NO: 233). In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases can be substituted by at least 1, at least 2, at least 3 or at least 4 cytosine bases (SEQ ID NO: 234). In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases can be substituted by at least 1, at least 2, at least 3 or at least 4 thymine, and/or any of the nucleotides described herein (SEQ ID NO: 235).

[0592] In some cases, the polynucleotide can include at least one substitution and/or insertion upstream of the start codon. For the purpose of clarity, one of skill in the art would appreciate that the start codon is the first codon of the protein coding region whereas the transcription start site is the site where transcription begins. The polynucleotide can include, but is not limited to, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 substitutions and/or insertions of nucleotide bases. The nucleotide bases can be inserted or substituted at 1, at least 1, at least 2, at least 3, at least 4 or at least 5 locations upstream of the start codon. The nucleotides inserted and/or

substituted can be the same base *(e.g.,* all A or all C or all T or all G), two different bases (*e.g.,* A and C, A and T, or C and T), three different bases (*e.g.,* A, C and T or A, C and T) or at least four different bases.

[0593] As a non-limiting example, the guanine base upstream of the coding region in the polynucleotide can be substituted with adenine, cytosine, thymine, or any of the nucleotides described herein. In another non-limiting example the substitution of guanine bases in the polynucleotide can be designed so as to leave one guanine base in the region downstream of the transcription start site and before the start codon (see Esvelt et al. Nature (2011) 472(7344):499-503). As a non-limiting example, at least 5 nucleotides can be inserted at 1 location downstream of the transcription start site but upstream of the start codon and the at least 5 nucleotides can be the same base type.

21. Polynucleotide Comprising an mRNA Encoding an IL12 Polypeptide

[0594] In certain cases, a polynucleotide of the present disclosure, for example a polynucleotide comprising an mRNA nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides, comprises from 5' to 3' end:

(i) a 5' cap provided above;
(ii) a 5' UTR, such as the sequences provided above;
(iii) an open reading frame encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides, *e.g.*, a sequence optimized nucleic acid sequence encoding IL12 disclosed herein;
(iv) at least one stop codon;
(v) a 3' UTR, such as the sequences provided above; and
(vi) a poly-A tail provided above.

[0595] In some cases, the polynucleotide further comprises a miRNA binding site, *e.g.,* a miRNA binding site that binds to miRNA-142. In some cases, the 5'UTR comprises the miRNA binding site.

[0596] In some cases, a polynucleotide of the present disclosure comprises a nucleotide sequence encoding a polypeptide sequence at least 70%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% , at least 97%, at least 98%, at least 99%, or 100% identical to the protein sequence of a wild type IL12 (e.g., isoform 1, 2, 3, or 4).

22. Methods of Making Polynucleotides

[0597] The present disclosure also provides methods for making a polynucleotide of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) or a complement thereof.

[0598] In some aspects, a polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) disclosed herein, and encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides, can be constructed using *in vitro* transcription. In other aspects, a polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) disclosed herein, and encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides, can be constructed by chemical synthesis using an oligonucleotide synthesizer.

[0599] In other aspects, a polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) disclosed herein, and encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides is made by using a host cell. In certain aspects, a polynucleotide (e.g., a RNA, *e.g.,* an mRNA) disclosed herein, and encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides is made by one or more combination of the IVT, chemical synthesis, host cell expression, or any other methods known in the art. In other cases, a host cell is a eukaryotic cell, *e.g., in vitro* mammalian cells.

[0600] Naturally occurring nucleosides, non-naturally occurring nucleosides, or combinations thereof, can totally or partially naturally replace occurring nucleosides present in the candidate nucleotide sequence and can be incorporated into a sequence-optimized nucleotide sequence *(e.g.,* a RNA, *e.g.,* an mRNA) encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides. The resultant polynucleotides, *e.g.*, mRNAs, can then be examined for their ability to produce protein and/or produce a therapeutic outcome.

a. In Vitro Transcription / Enzymatic Synthesis

[0601] The polynucleotides of the present disclosure disclosed herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) can be transcribed using an *in vitro* transcription (IVT) system. The system typically comprises a transcription buffer, nucleotide

triphosphates (NTPs), an RNase inhibitor and a polymerase. The NTPs can be selected from, but are not limited to, those described herein including natural and unnatural (modified) NTPs. The polymerase can be selected from, but is not limited to, T7 RNA polymerase, T3 RNA polymerase and mutant polymerases such as, but not limited to, polymerases able to incorporate polynucleotides disclosed herein. See U.S. Publ. No. US20130259923.

[0602] Any number of RNA polymerases or variants can be used in the synthesis of the polynucleotides of the present disclosure. RNA polymerases can be modified by inserting or deleting amino acids of the RNA polymerase sequence. As a non-limiting example, the RNA polymerase can be modified to exhibit an increased ability to incorporate a 2'-modified nucleotide triphosphate compared to an unmodified RNA polymerase (see International Publication WO2008078180 and U.S. Patent 8,101,385).

[0603] Variants can be obtained by evolving an RNA polymerase, optimizing the RNA polymerase amino acid and/or nucleic acid sequence and/or by using other methods known in the art. As a non-limiting example, T7 RNA polymerase variants can be evolved using the continuous directed evolution system set out by Esvelt et al. (Nature 472:499-503 (2011)) where clones of T7 RNA polymerase can encode at least one mutation such as, but not limited to, lysine at position 93 substituted for threonine (K93T), I4M, A7T, E63V, V64D, A65E, D66Y, T76N, C125R, S128R, A136T, N165S, G175R, H176L, Y178H, F182L, L196F, G198V, D208Y, E222K, S228A, Q239R, T243N, G259D, M267I, G280C, H300R, D351A, A354S, E356D, L360P, A383V, Y385C, D388Y, S397R, M401T, N410S, K450R, P451T, G452V, E484A, H523L, H524N, G542V, E565K, K577E, K577M, N601S, S684Y, L699I, K713E, N748D, Q754R, E775K, A827V, D851N or L864F. As another non-limiting example, T7 RNA polymerase variants can encode at least mutation as described in U.S. Pub. Nos. 20100120024 and 20070117112. Variants of RNA polymerase can also include, but are not limited to, substitutional variants, conservative amino acid substitution, insertional variants, deletional variants and/or covalent derivatives.

[0604] In one aspect, the polynucleotide can be designed to be recognized by the wild type or variant RNA polymerases. In doing so, the polynucleotide can be modified to contain sites or regions of sequence changes from the wild type or parent chimeric polynucleotide.

[0605] Polynucleotide or nucleic acid synthesis reactions can be carried out by enzymatic methods utilizing polymerases. Polymerases catalyze the creation of phosphodiester bonds between nucleotides in a polynucleotide or nucleic acid chain. Currently known DNA polymerases can be divided into different families based on amino acid sequence comparison and crystal structure analysis. DNA polymerase I (pol I) or A polymerase family, including the Klenow fragments of *E. coli, Bacillus* DNA polymerase I, *Thermus aquaticus* (Taq) DNA polymerases, and the T7 RNA and DNA polymerases, is among the best studied of these families. Another large family is DNA polymerase α (pol α) or B polymerase family, including all eukaryotic replicating DNA polymerases and polymerases from phages T4 and RB69. Although they employ similar catalytic mechanism, these families of polymerases differ in substrate specificity, substrate analog-incorporating efficiency, degree and rate for primer extension, mode of DNA synthesis, exonuclease activity, and sensitivity against inhibitors.

[0606] DNA polymerases are also selected based on the optimum reaction conditions they require, such as reaction temperature, pH, and template and primer concentrations. Sometimes a combination of more than one DNA polymerases is employed to achieve the desired DNA fragment size and synthesis efficiency. For example, Cheng et al. increase pH, add glycerol and dimethyl sulfoxide, decrease denaturation times, increase extension times, and utilize a secondary thermostable DNA polymerase that possesses a 3' to 5' exonuclease activity to effectively amplify long targets from cloned inserts and human genomic DNA. (Cheng et al., PNAS 91:5695-5699 (1994)). RNA polymerases from bacteriophage T3, T7, and SP6 have been widely used to prepare RNAs for biochemical and biophysical studies. RNA polymerases, capping enzymes, and poly-A polymerases are disclosed in the co-pending International Publication No. WO2014028429.

[0607] In one aspect, the RNA polymerase which can be used in the synthesis of the polynucleotides of the present disclosure is a Syn5 RNA polymerase. (see Zhu et al. Nucleic Acids Research 2013, doi:10.1093/nar/gkt1193). The Syn5 RNA polymerase was recently characterized from marine cyanophage Syn5 by Zhu et al. where they also identified the promoter sequence (see Zhu et al. Nucleic Acids Research 2013). Zhu et al. found that Syn5 RNA polymerase catalyzed RNA synthesis over a wider range of temperatures and salinity as compared to T7 RNA polymerase. Additionally, the requirement for the initiating nucleotide at the promoter was found to be less stringent for Syn5 RNA polymerase as compared to the T7 RNA polymerase making Syn5 RNA polymerase promising for RNA synthesis.

[0608] In one aspect, a Syn5 RNA polymerase can be used in the synthesis of the polynucleotides described herein. As a non-limiting example, a Syn5 RNA polymerase can be used in the synthesis of the polynucleotide requiring a precise 3 '-terminus.

[0609] In one aspect, a Syn5 promoter can be used in the synthesis of the polynucleotides. As a non-limiting example, the Syn5 promoter can be 5'-ATTGGGCACCCGTAAGGG-3' (SEQ ID NO: 47) as described by Zhu et al. (Nucleic Acids Research 2013).

[0610] In one aspect, a Syn5 RNA polymerase can be used in the synthesis of polynucleotides comprising at least one chemical modification described herein and/or known in the art (see *e.g.,* the incorporation of pseudo-UTP and 5Me-

CTP described in Zhu et al. Nucleic Acids Research 2013).

[0611] In one aspect, the polynucleotides described herein can be synthesized using a Syn5 RNA polymerase which has been purified using modified and improved purification procedure described by Zhu et al. (Nucleic Acids Research 2013).

[0612] Various tools in genetic engineering are based on the enzymatic amplification of a target gene which acts as a template. For the study of sequences of individual genes or specific regions of interest and other research needs, it is necessary to generate multiple copies of a target gene from a small sample of polynucleotides or nucleic acids. Such methods can be applied in the manufacture of the polynucleotides of the disclosure.

[0613] Polymerase chain reaction (PCR) has wide applications in rapid amplification of a target gene, as well as genome mapping and sequencing. The key components for synthesizing DNA comprise target DNA molecules as a template, primers complementary to the ends of target DNA strands, deoxynucleoside triphosphates (dNTPs) as building blocks, and a DNA polymerase. As PCR progresses through denaturation, annealing and extension steps, the newly produced DNA molecules can act as a template for the next circle of replication, achieving exponentially amplification of the target DNA. PCR requires a cycle of heating and cooling for denaturation and annealing. Variations of the basic PCR include asymmetric PCR (Innis et al., PNAS 85: 9436-9440 (1988)), inverse PCR (Ochman et al., Genetics 120(3): 621-623, (1988)), reverse transcription PCR (RT-PCR) (Freeman et al., BioTechniques 26(1): 112-22, 124-5 (1999)). In RT-PCR, a single stranded RNA is the desired target and is converted to a double stranded DNA first by reverse transcriptase.

[0614] A variety of isothermal *in vitro* nucleic acid amplification techniques have been developed as alternatives or complements of PCR. For example, strand displacement amplification (SDA) is based on the ability of a restriction enzyme to form a nick (Walker et al., PNAS 89: 392-396 (1992))). A restriction enzyme recognition sequence is inserted into an annealed primer sequence. Primers are extended by a DNA polymerase and dNTPs to form a duplex. Only one strand of the duplex is cleaved by the restriction enzyme. Each single strand chain is then available as a template for subsequent synthesis. SDA does not require the complicated temperature control cycle of PCR.

[0615] Nucleic acid sequence-based amplification (NASBA), also called transcription mediated amplification (TMA), is also an isothermal amplification method that utilizes a combination of DNA polymerase, reverse transcriptase, RNAse H, and T7 RNA polymerase. (Compton, Nature 350:91-92 (1991)). A target RNA is used as a template and a reverse transcriptase synthesizes its complementary DNA strand. RNAse H hydrolyzes the RNA template, making space for a DNA polymerase to synthesize a DNA strand complementary to the first DNA strand which is complementary to the RNA target, forming a DNA duplex. T7 RNA polymerase continuously generates complementary RNA strands of this DNA duplex. These RNA strands act as templates for new cycles of DNA synthesis, resulting in amplification of the target gene.

[0616] Rolling-circle amplification (RCA) amplifies a single stranded circular polynucleotide and involves numerous rounds of isothermal enzymatic synthesis where Φ29 DNA polymerase extends a primer by continuously progressing around the polynucleotide circle to replicate its sequence over and over again. Therefore, a linear copy of the circular template is achieved. A primer can then be annealed to this linear copy and its complementary chain can be synthesized. See Lizardi et al., Nature Genetics 19:225-232 (1998). A single stranded circular DNA can also serve as a template for RNA synthesis in the presence of an RNA polymerase. (Daubendiek et al., JACS 117:7818-7819 (1995)). An inverse rapid amplification of cDNA ends (RACE) RCA is described by Polidoros et al. A messenger RNA (mRNA) is reverse transcribed into cDNA, followed by RNAse H treatment to separate the cDNA. The cDNA is then circularized by CircLigase into a circular DNA. The amplification of the resulting circular DNA is achieved with RCA. (Polidoros et al., BioTechniques 41:35-42 (2006)).

[0617] Any of the foregoing methods can be utilized in the manufacture of one or more regions of the polynucleotides of the present disclosure.

[0618] Assembling polynucleotides or nucleic acids by a ligase is also widely used. DNA or RNA ligases promote intermolecular ligation of the 5' and 3' ends of polynucleotide chains through the formation of a phosphodiester bond. Ligase chain reaction (LCR) is a promising diagnosing technique based on the principle that two adjacent polynucleotide probes hybridize to one strand of a target gene and couple to each other by a ligase. If a target gene is not present, or if there is a mismatch at the target gene, such as a single-nucleotide polymorphism (SNP), the probes cannot ligase. (Wiedmann et al., PCR Methods and Application, vol.3 (4), s51-s64 (1994)). LCR can be combined with various amplification techniques to increase sensitivity of detection or to increase the amount of products if it is used in synthesizing polynucleotides and nucleic acids.

[0619] Several library preparation kits for nucleic acids are now commercially available. They include enzymes and buffers to convert a small amount of nucleic acid samples into an indexed library for downstream applications. For example, DNA fragments can be placed in a NEBNEXT® ULTRATM DNA Library Prep Kit by NEWENGLAND BIOLABS® for end preparation, ligation, size selection, clean-up, PCR amplification and final clean-up.

[0620] Continued development is going on to improvement the amplification techniques. For example, U.S. Pat. No. 8,367,328 to Asada et al., teaches utilizing a reaction enhancer to increase the efficiency of DNA synthesis reactions

by DNA polymerases. The reaction enhancer comprises an acidic substance or cationic complexes of an acidic substance. U.S. Pat. No. 7.384,739 to Kitabayashi et al., teaches a carboxylate ion-supplying substance that promotes enzymatic DNA synthesis, wherein the carboxylate ion-supplying substance is selected from oxalic acid, malonic acid, esters of oxalic acid, esters of malonic acid, salts of malonic acid, and esters of maleic acid. US Pat. 7,378,262 to Sobek et al., discloses an enzyme composition to increase fidelity of DNA amplifications. The composition comprises one enzyme with 3' exonuclease activity but no polymerase activity and another enzyme that is a polymerase. Both of the enzymes are thermostable and are reversibly modified to be inactive at lower temperatures.

[0621] U.S. Pat. No. 7,550,264 to Getts et al. teaches multiple round of synthesis of sense RNA molecules are performed by attaching oligodeoxynucleotides tails onto the 3' end of cDNA molecules and initiating RNA transcription using RNA polymerase. U.S. Pat. Pub. No. 2013/0183718 to Rohayem teaches RNA synthesis by RNA-dependent RNA polymerases (RdRp) displaying an RNA polymerase activity on single-stranded DNA templates. Oligonucleotides with non-standard nucleotides can be synthesized with enzymatic polymerization by contacting a template comprising non-standard nucleotides with a mixture of nucleotides that are complementary to the nucleotides of the template as disclosed in U.S. Pat. No. 6,617,106 to Benner.

b. Chemical synthesis

[0622] Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest, such as a polynucleotide of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides). For example, a single DNA or RNA oligomer containing a codon-optimized nucleotide sequence coding for the particular isolated polypeptide can be synthesized. In other aspects, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. In some aspects, the individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[0623] A polynucleotide disclosed herein *(e.g.,* a RNA, *e.g.,* an mRNA) can be chemically synthesized using chemical synthesis methods and potential nucleobase substitutions known in the art. See, for example, International Publication Nos. WO2014093924, WO2013052523; WO2013039857, WO2012135805, WO2013151671; U.S. Publ. No. US20130115272; or U.S. Pat. Nos. US8999380 or US8710200.

c. *Purification of Polynucleotides Encoding IL12*

[0624] Purification of the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) can include, but is not limited to, polynucleotide clean-up, quality assurance and quality control. Clean-up can be performed by methods known in the arts such as, but not limited to, AGENCOURT® beads (Beckman Coulter Genomics, Danvers, MA), poly-T beads, LNA™ oligo-T capture probes (EXIQON® Inc., Vedbaek, Denmark) or HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC).

[0625] The term "purified" when used in relation to a polynucleotide such as a "purified polynucleotide" refers to one that is separated from at least one contaminant. As used herein, a "contaminant" is any substance that makes another unfit, impure or inferior. Thus, a purified polynucleotide (e.g., DNA and RNA) is present in a form or setting different from that in which it is found in nature, or a form or setting different from that which existed prior to subjecting it to a treatment or purification method.

[0626] In some cases, purification of a polynucleotide of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) removes impurities that can reduce or remove an unwanted immune response, *e.g.*, reducing cytokine activity.

[0627] In some cases, the polynucleotide of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) is purified prior to administration using column chromatography (e.g., strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), or (LCMS)).

[0628] In some cases, the polynucleotide of the disclosure (e.g., a polynucleotide comprising a nucleotide sequence an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) purified using column chromatography (*e.g.*, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC, hydrophobic interaction HPLC (HIC-HPLC), or (LCMS)) presents increased expression of the encoded IL12 protein compared to the expression level obtained with the same polynucleotide of the present disclosure purified by a different purification method.

[0629] In some cases, a column chromatography (e.g., strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), hydrophobic interaction HPLC (HIC-HPLC), or (LCMS)) purified polynucleotide com-

prises a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides comprising one or more of the point mutations known in the art.

[0630] In some cases, the use of RP-HPLC purified polynucleotide increases IL12 protein expression levels in cells when introduced into those cells, *e.g.,* by 10-100%, *i.e.,* at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, or at least about 100% with respect to the expression levels of IL12 protein in the cells before the RP-HPLC purified polynucleotide was introduced in the cells, or after a non-RP-HPLC purified polynucleotide was introduced in the cells.

[0631] In some cases, the use of RP-HPLC purified polynucleotide increases functional IL12 protein expression levels in cells when introduced into those cells, *e.g.,* by 10-100%, *i.e.,* at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, or at least about 100% with respect to the functional expression levels of IL12 protein in the cells before the RP-HPLC purified polynucleotide was introduced in the cells, or after a non-RP-HPLC purified polynucleotide was introduced in the cells.

[0632] In some cases, the use of RP-HPLC purified polynucleotide increases detectable IL12 activity in cells when introduced into those cells, *e.g.,* by 10-100%, *i.e.,* at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, or at least about 100% with respect to the activity levels of functional IL12 in the cells before the RP-HPLC purified polynucleotide was introduced in the cells, or after a non-RP-HPLC purified polynucleotide was introduced in the cells.

[0633] In some cases, the purified polynucleotide is at least about 80% pure, at least about 85% pure, at least about 90% pure, at least about 95% pure, at least about 96% pure, at least about 97% pure, at least about 98% pure, at least about 99% pure, or about 100% pure.

[0634] A quality assurance and/or quality control check can be conducted using methods such as, but not limited to, gel electrophoresis, UV absorbance, or analytical HPLC. In another case, the polynucleotide can be sequenced by methods including, but not limited to reverse-transcriptase-PCR.

d. Quantification of Expressed Polynucleotides Encoding IL12

[0635] In some cases, the polynucleotides of the present disclosure (e.g., a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides), their expression products, as well as degradation products and metabolites can be quantified according to methods known in the art.

[0636] In some cases, the polynucleotides of the present disclosure can be quantified in exosomes or when derived from one or more bodily fluid. As used herein "bodily fluids" include peripheral blood, serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, and umbilical cord blood. Alternatively, exosomes can be retrieved from an organ selected from the group consisting of lung, heart, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colon, breast, prostate, brain, esophagus, liver, and placenta.

[0637] In the exosome quantification method, a sample of not more than 2mL is obtained from the subject and the exosomes isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof. In the analysis, the level or concentration of a polynucleotide can be an expression level, presence, absence, truncation or alteration of the administered construct. It is advantageous to correlate the level with one or more clinical phenotypes or with an assay for a human disease biomarker.

[0638] The assay can be performed using construct specific probes, cytometry, qRT-PCR, real-time PCR, PCR, flow cytometry, electrophoresis, mass spectrometry, or combinations thereof while the exosomes can be isolated using immunohistochemical methods such as enzyme linked immunosorbent assay (ELISA) methods. Exosomes may also be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

[0639] These methods afford the investigator the ability to monitor, in real time, the level of polynucleotides remaining or delivered. This is possible because the polynucleotides of the present disclosure differ from the endogenous forms

due to the structural or chemical modifications.

[0640] In some cases, the polynucleotide can be quantified using methods such as, but not limited to, ultraviolet visible spectroscopy (UV/Vis). A non-limiting example of a UV/Vis spectrometer is a NANODROP® spectrometer (ThermoFisher, Waltham, MA). The quantified polynucleotide can be analyzed in order to determine if the polynucleotide can be of proper size, check that no degradation of the polynucleotide has occurred. Degradation of the polynucleotide can be checked by methods such as, but not limited to, agarose gel electrophoresis, HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), liquid chromatography-mass spectrometry (LCMS), capillary electrophoresis (CE) and capillary gel electrophoresis (CGE).

23. Pharmaceutical Compositions and Formulations

[0641] The present disclosure provides pharmaceutical compositions and formulations that comprise any of the polynucleotides described above. In some cases, the composition or formulation further comprises a delivery agent.

[0642] In some cases, the composition or formulation can contain a polynucleotide comprising a sequence optimized nucleic acid sequence disclosed herein which encodes an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides. In some cases, the composition or formulation can contain a polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) comprising a polynucleotide *(e.g.,* an ORF) having significant sequence identity to a sequence optimized nucleic acid sequence disclosed herein which encodes an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides. In some cases, the polynucleotide further comprises a miRNA binding site, *e.g.,* a miRNA binding site that binds miR-122.

[0643] Pharmaceutical compositions or formulation can optionally comprise one or more additional active substances, *e.g.*, therapeutically and/or prophylactically active substances. Pharmaceutical compositions or formulation of the present disclosure can be sterile and/or pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents can be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005. In some cases, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers to polynucleotides to be delivered as described herein.

[0644] Formulations and pharmaceutical compositions described herein can be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0645] A pharmaceutical composition or formulation in accordance with the present disclosure can be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0646] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition can comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition can comprise between 0.1% and 100%, e.g., between 0.5 and 50%, between 1 and 30%, between 5 and 80%, or at least 80% (w/w) active ingredient.

[0647] In some cases, the compositions and formulations described herein can contain at least one polynucleotide of the disclosure. As a non-limiting example, the composition or formulation can contain 1, 2, 3, 4 or 5 polynucleotides of the disclosure. In some cases, the compositions or formulations described herein can comprise more than one type of polynucleotide. In some cases, the composition or formulation can comprise a polynucleotide in linear and circular form. In another case, the composition or formulation can comprise a circular polynucleotide and an IVT polynucleotide. In yet another case, the composition or formulation can comprise an IVT polynucleotide, a chimeric polynucleotide and a circular polynucleotide.

[0648] Although the descriptions of pharmaceutical compositions and formulations provided herein are principally directed to pharmaceutical compositions and formulations that are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, *e.g.*, to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions or formulations suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions or formulation is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant

mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[0649] The present disclosure provides pharmaceutical formulations that comprise a polynucleotide described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides). The polynucleotides described herein can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (e.g., from a depot formulation of the polynucleotide); (4) alter the biodistribution (e.g., target the polynucleotide to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo;* and/or (6) alter the release profile of encoded protein *in vivo.* In some cases, the pharmaceutical formulation further comprises a delivery agent, *(e.g.,* a compound having the **Formula (I),** *e.g.,* any of Compounds 1-147 or any of Compounds 1-232).

[0650] A pharmaceutically acceptable excipient, as used herein, includes, but are not limited to, any and all solvents, dispersion media, or other liquid vehicles, dispersion or suspension aids, diluents, granulating and/or dispersing agents, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, binders, lubricants or oil, coloring, sweetening or flavoring agents, stabilizers, antioxidants, antimicrobial or antifungal agents, osmolality adjusting agents, pH adjusting agents, buffers, chelants, cyoprotectants, and/or bulking agents, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006).

[0651] Exemplary diluents include, but are not limited to, calcium or sodium carbonate, calcium phosphate, calcium hydrogen phosphate, sodium phosphate, lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, etc., and/or combinations thereof.

[0652] Exemplary granulating and/or dispersing agents include, but are not limited to, starches, pregelatinized starches, or microcrystalline starch, alginic acid, guar gum, agar, poly(vinyl-pyrrolidone), (providone), cross-linked poly(vinyl-pyrrolidone) (crospovidone), cellulose, methylcellulose, carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), magnesium aluminum silicate (VEEGUM®), sodium lauryl sulfate, etc., and/or combinations thereof.

[0653] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (e.g., acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), sorbitan fatty acid esters (*e.g.*, polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40], glyceryl monooleate, polyoxyethylene esters, polyethylene glycol fatty acid esters (e.g., CREMOPHOR®), polyoxyethylene ethers (e.g., polyoxyethylene lauryl ether [BRIJ®30]), PLUORINC®F 68, POLOXAMER®188, etc. and/or combinations thereof.

[0654] Exemplary binding agents include, but are not limited to, starch, gelatin, sugars (e.g., sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol), amino acids *(e.g.,* glycine), natural and synthetic gums *(e.g.,* acacia, sodium alginate), ethylcellulose, hydroxyethylcellulose, hydroxypropyl methylcellulose, etc., and combinations thereof.

[0655] Oxidation is a potential degradation pathway for mRNA, especially for liquid mRNA formulations. In order to prevent oxidation, antioxidants can be added to the formulations. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, benzyl alcohol, butylated hydroxyanisole, m-cresol, methionine, butylated hydroxytoluene, monothioglycerol, sodium or potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, etc., and combinations thereof.

[0656] Exemplary chelating agents include, but are not limited to, ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, trisodium edetate, etc., and combinations thereof.

[0657] Exemplary antimicrobial or antifungal agents include, but are not limited to, benzalkonium chloride, benzethonium chloride, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, benzoic acid, hydroxybenzoic acid, potassium or sodium benzoate, potassium or sodium sorbate, sodium propionate, sorbic acid, etc., and combinations thereof.

[0658] Exemplary preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, ascorbic acid, butylated hydroxyanisol, ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), etc., and combinations thereof.

[0659] In some cases, the pH of polynucleotide solutions are maintained between pH 5 and pH 8 to improve stability. Exemplary buffers to control pH can include, but are not limited to sodium phosphate, sodium citrate, sodium succinate, histidine (or histidine-HCl), sodium malate, sodium carbonate, etc., and/or combinations thereof.

[0660] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium or magnesium lauryl sulfate, etc., and combinations thereof.

[0661] The pharmaceutical composition or formulation described here may contain a cyroprotectant to stabilize a polynucleotide described herein during freezing. Exemplary cryoprotectants include, but are not limited to mannitol,

sucrose, trehalose, lactose, glycerol, dextrose, etc., and combinations thereof.

[0662] The pharmaceutical composition or formulation described here may contain a bulking agent in lyophilized polynucleotide formulations to yield a "pharmaceutically elegant" cake, stabilize the lyophilized polynucleotides during long term *(e.g.*, 36 month) storage. Exemplary bulking agents of the present disclosure can include, but are not limited to sucrose, trehalose, mannitol, glycine, lactose, raffinose, and combinations thereof.

[0663] In some cases, the pharmaceutical composition or formulation further comprises a delivery agent. The delivery agent of the present disclosure can include, without limitation, liposomes, lipid nanoparticles, lipidoids, polymers, lipoplexes, microvesicles, exosomes, peptides, proteins, cells transfected with polynucleotides, hyaluronidase, nanoparticle mimics, nanotubes, conjugates, and combinations thereof.

24. Delivery Agents

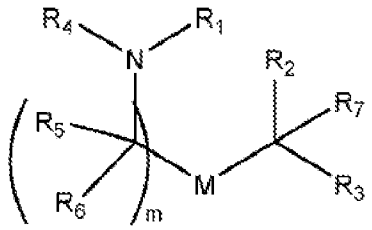
a. Lipid Compound

[0664] The present disclosure provides pharmaceutical compositions with advantageous properties. In particular, the present application provides pharmaceutical compositions comprising:

(a) a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides; and
(b) a delivery agent.

[0665] In some cases, the delivery agent for the present disclosure comprises any one or more compounds disclosed in International Application No. PCT/US2016/052352, filed on September 16, 2016, and published as WO 2017/049245.

[0666] In some cases, the delivery agent comprises a compound having the formula (I)



(I),

wherein

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, $-P(O)(OR')O-$, $-S(O)_2-$, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0667] In some cases, a subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or stereoisomers thereof, wherein alkyl and alkenyl groups may be linear or branched.

[0668] In some cases, a subset of compounds of Formula (I) includes those in which when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

[0669] In another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, and C_{1-3} alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
Rs is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or stereoisomers thereof.

[0670] In another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, and C_{1-3} alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0671] In yet another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)\ C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)\ C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and $-C(=NR_9)N(R)_2$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R_4 is $-(CH_2)_nQ$ in which n is 1 or 2, or (ii) R_4 is $-(CH_2)_nCHQR$ in which n is 1, or (iii) R_4 is -CHQR, and $-CQ(R)_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0672] In yet another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R_4 is $-(CH_2)_nQ$ in which n is 1 or 2, or (ii) R_4 is $-(CH_2)_nCHQR$ in which n is 1, or (iii) R_4 is -CHQR, and $-CQ(R)_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0673] In still another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, -N(OR)C(O)R, $-N(OR)S(O)_2R$, -N(OR)C(O)OR, -N(OR)C (O)N(R)$_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)$ R, -C(O)N(R)OR, and $-C(=NR_9)N(R)_2$, and each n is independently selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0674] In still another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0675] In yet another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{2-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is $-(CH_2)_nQ$ or $-(CH_2)_nCHQR$, where Q is $-N(R)_2$, and n is selected from 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;
each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0676] In yet another cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';
R_2 and R_3 are independently selected from the group consisting of H, C_{2-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is $-(CH_2)_nQ$ or $-(CH_2)_nCHQR$, where Q is $-N(R)_2$, and n is selected from 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0677] In still other cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR'', -YR'', and -R''M'R';
R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR'', -YR'', and -R*OR'', or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, and $-CQ(R)_2$, where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2$-, -S-S-, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR'', -YR'', and H;
each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

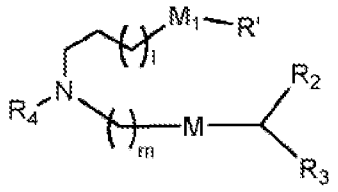
[0678] In still other cases, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R*YR'', -YR'', and -R''M'R';
R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR'', -YR'', and -R*OR'', or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;
R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, and $-CQ(R)_2$, where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;
each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2$-, an aryl group, and a heteroaryl group;
R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR'', -YR'', and H;
each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or stereoisomers thereof.

[0679] In certain cases, a subset of compounds of Formula (I) includes those of Formula (IA):



(IA),

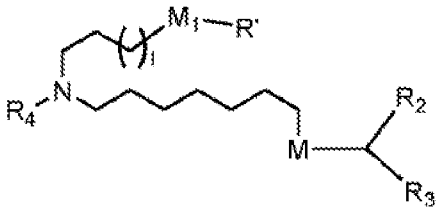
or a salt or stereoisomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl, or heterocycloalkyl; M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-P(O)(OR')O-$, -S-S-, an aryl group, and a heteroaryl group; and

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

[0680] In some cases, a subset of compounds of Formula (I) includes those of Formula (IA), or a salt or stereoisomer thereof,
wherein

l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9;
M_1 is a bond or M';
R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is OH, $-NHC(S)N(R)_2$, or $-NHC(O)N(R)_2$;
M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-P(O)(OR')O-$, an aryl group, and a heteroaryl group; and
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

[0681] In certain cases, a subset of compounds of Formula (I) includes those of Formula (II):



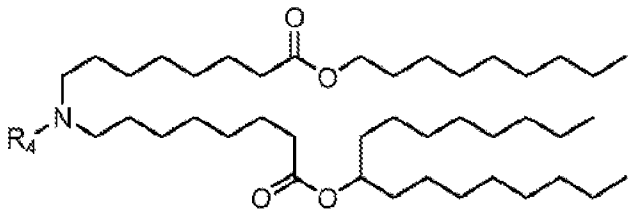
(II)

or a salt or stereoisomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl, or heterocycloalkyl; M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-P(O)(OR')O-$, -S-S-, an aryl group, and a heteroaryl group; and
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

[0682] In some cases, a subset of compounds of Formula (I) includes those of Formula (II), or a salt or stereoisomer thereof, wherein

l is selected from 1, 2, 3, 4, and 5;
M_1 is a bond or M';
R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is OH, $-NHC(S)N(R)_2$, or $-NHC(O)N(R)_2$;
M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-P(O)(OR')O-$, an aryl group, and a heteroaryl group; and
R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

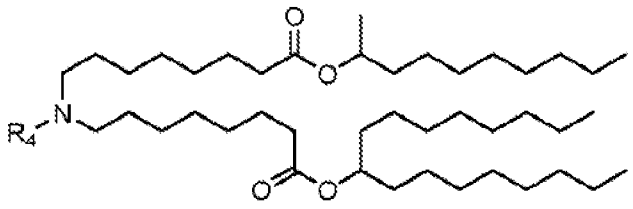
[0683] In some cases, the compound of formula (I) is of the formula (IIa),



(IIa),

or a salt thereof, wherein R_4 is as described above.

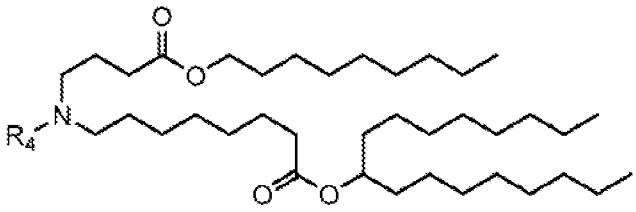
[0684] In some cases, the compound of formula (I) is of the formula (IIb),



(IIb),

or a salt thereof, wherein R_4 is as described above.

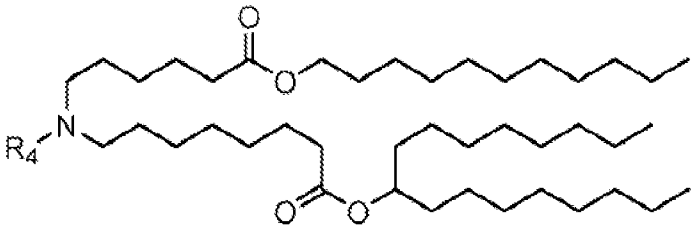
[0685] In some cases, the compound of formula (I) is of the formula (IIc),



(IIc),

or a salt thereof, wherein R_4 is as described above.

[0686] In some cases, the compound of formula (I) is of the formula (IIe):



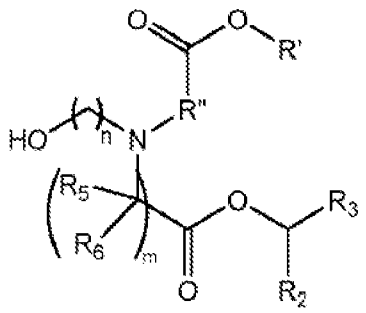
(IIe),

or a salt thereof, wherein R_4 is as described above.

[0687] In some cases, the compound of formula (IIa), (IIb), (IIc), or (IIe) comprises an R_4 which is selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$, wherein Q, R and n are as defined above.

[0688] In some cases, Q is selected from the group consisting of -OR, -OH, $-O(CH_2)_nN(R)_2$, -OC(O)R, $-CX_3$, -CN, -N(R)C(O)R, -N(H)C(O)R, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, -N(H)C(O)N(H)(R), $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, -N(H)C(S)N(H)(R), and a heterocycle, wherein R is as defined above. In some aspects, n is 1 or 2. In some cases, Q is OH, $-NHC(S)N(R)_2$, or $-NHC(O)N(R)_2$.

[0689] In some cases, the compound of formula (I) is of the formula (IId),



(IId),

or a salt thereof, wherein R_2 and R_3 are independently selected from the group consisting of C_{5-14} alkyl and C_{5-14} alkenyl, n is selected from 2, 3, and 4, and R', R", R_5, R_6 and m are as defined above.

[0690] In some aspects of the compound of formula (IId), R_2 is C_8 alkyl. In some aspects of the compound of formula (IId), R_3 is C_5-C_9 alkyl. In some aspects of the compound of formula (IId), m is 5, 7, or 9. In some aspects of the compound of formula (IId), each R_5 is H. In some aspects of the compound of formula (IId), each R_6 is H.

[0691] In another aspect, the present application provides a lipid composition (e.g., a lipid nanoparticle (LNP)) comprising: (1) a compound having the formula (I); (2) optionally a helper lipid (e.g. a phospholipid); (3) optionally a structural lipid (e.g. a sterol); (4) optionally a lipid conjugate (e.g. a PEG-lipid); and (5) optionally a quaternary amine compound. In exemplary cases, the lipid composition (e.g., LNP) further comprises a polynucleotide encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides, *e.g.*, a polynucleotide encapsulated therein.

[0692] As used herein, the term "alkyl" or "alkyl group" means a linear or branched, saturated hydrocarbon including one or more carbon atoms *(e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms).

[0693] The notation "C_{1-14} alkyl" means a linear or branched, saturated hydrocarbon including 1-14 carbon atoms. An alkyl group may be optionally substituted.

[0694] As used herein, the term "alkenyl" or "alkenyl group" means a linear or branched hydrocarbon including two or more carbon atoms (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms) and at least one double bond.

[0695] The notation "C_{2-14} alkenyl" means a linear or branched hydrocarbon including 2-14 carbon atoms and at least one double bond. An alkenyl group may include one, two, three, four, or more double bonds. For example, C_{18} alkenyl may include one or more double bonds. A C_{18} alkenyl group including two double bonds may be a linoleyl group. An alkenyl group may be optionally substituted.

[0696] As used herein, the term "carbocycle" or "carbocyclic group" means a mono- or multi-cyclic system including one or more rings of carbon atoms. Rings may be three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen membered rings.

[0697] The notation "C_{3-6} carbocycle" means a carbocycle including a single ring having 3-6 carbon atoms. Carbocycles may include one or more double bonds and may be aromatic (e.g., aryl groups). Examples of carbocycles include cyclopropyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, and 1,2-dihydronaphthyl groups. Carbocycles may be optionally substituted.

[0698] As used herein, the term "heterocycle" or "heterocyclic group" means a mono- or multi-cyclic system including one or more rings, where at least one ring includes at least one heteroatom. Heteroatoms may be, for example, nitrogen, oxygen, or sulfur atoms. Rings may be three, four, five, six, seven, eight, nine, ten, eleven, or twelve membered rings. Heterocycles may include one or more double bonds and may be aromatic (e.g., heteroaryl groups). Examples of heterocycles include imidazolyl, imidazolidinyl, oxazolyl, oxazolidinyl, thiazolyl, thiazolidinyl, pyrazolidinyl, pyrazolyl, isoxazolidinyl, isoxazolyl, isothiazolidinyl, isothiazolyl, morpholinyl, pyrrolyl, pyrrolidinyl, furyl, tetrahydrofuryl, thiophenyl, pyridinyl, piperidinyl, quinolyl, and isoquinolyl groups. Heterocycles may be optionally substituted.

[0699] As used herein, a "biodegradable group" is a group that may facilitate faster metabolism of a lipid in a subject. A biodegradable group may be, but is not limited to, -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -$S(O)_2$-, an aryl group, and a heteroaryl group.

[0700] As used herein, an "aryl group" is a carbocyclic group including one or more aromatic rings. Examples of aryl groups include phenyl and naphthyl groups.

[0701] As used herein, a "heteroaryl group" is a heterocyclic group including one or more aromatic rings. Examples of heteroaryl groups include pyrrolyl, furyl, thiophenyl, imidazolyl, oxazolyl, and thiazolyl. Both aryl and heteroaryl groups may be optionally substituted. For example, M and M' can be selected from the non-limiting group consisting of optionally substituted phenyl, oxazole, and thiazole. In the formulas herein, M and M' can be independently selected from the list of biodegradable groups above.

[0702] Alkyl, alkenyl, and cyclyl (e.g., carbocyclyl and heterocyclyl) groups may be optionally substituted unless otherwise specified. Optional substituents may be selected from the group consisting of, but are not limited to, a halogen atom *(e.g.,* a chloride, bromide, fluoride, or iodide group), a carboxylic acid *(e.g.,* -C(O)OH), an alcohol *(e.g.,* a hydroxyl, -OH), an ester *(e.g.,* -C(O)OR or -OC(O)R), an aldehyde *(e.g.,* -C(O)H), a carbonyl *(e.g.,* -C(O)R, alternatively represented by C=O), an acyl halide *(e.g.,* -C(O)X, in which X is a halide selected from bromide, fluoride, chloride, and iodide), a carbonate *(e.g.,* -OC(O)OR), an alkoxy *(e.g.,* -OR), an acetal (*e.g.*, -C(OR)$_2$R"", in which each OR are alkoxy groups that can be the same or different and R"" is an alkyl or alkenyl group), a phosphate (*e.g.*, P(O)$_4^{3-}$), a thiol *(e.g.,* -SH), a sulfoxide *(e.g.,* -S(O)R), a sulfinic acid *(e.g.,* -S(O)OH), a sulfonic acid (*e.g.*, -S(O)$_2$OH), a thial *(e.g.,* -C(S)H), a sulfate (*e.g.,* S(O)$_4^{2-}$), a sulfonyl (*e.g.,* -S(O)$_2$-), an amide (*e.g.*, -C(O)NR$_2$, or -N(R)C(O)R), an azido *(e.g.,* -N$_3$), a nitro (*e.g.*, -NO$_2$), a cyano *(e.g.,* -CN), an isocyano *(e.g.,* -NC), an acyloxy *(e.g.,* -OC(O)R), an amino (*e.g.*, -NR$_2$, -NRH, or -NH$_2$), a carbamoyl (*e.g.*, -OC(O)NR$_2$, -OC(O)NRH, or -OC(O)NH$_2$), a sulfonamide (e.g., -S(O)$_2$NR$_2$, -S(O)$_2$NRH, -S(O)$_2$NH$_2$, -N(R)S(O)$_2$R, -N(H)S(O)$_2$R, -N(R)S(O)$_2$H, or -N(H)S(O)$_2$H), an alkyl group, an alkenyl group, and a cyclyl (e.g., carbocyclyl or heterocyclyl) group.

[0703] In any of the preceding, R is an alkyl or alkenyl group, as defined herein. In some cases, the substituent groups themselves may be further substituted with, for example, one, two, three, four, five, or six substituents as defined herein. For example, a C$_{1-6}$ alkyl group may be further substituted with one, two, three, four, five, or six substituents as described herein.

[0704] The compounds of any one of formulae (I), (IA), (II), (IIa), (IIb), (IIc), (IId), and (IIe) include one or more of the following features when applicable.

[0705] In some cases, R$_4$ is selected from the group consisting of a C$_{3-6}$ carbocycle, -(CH$_2$)$_n$Q, -(CH$_2$)$_n$CHQR, -CHQR, and -CQ(R)$_2$, where Q is selected from a C$_{3-6}$ carbocycle, 5- to 14- membered aromatic or non-aromatic heterocycle having one or more heteroatoms selected from N, O, S, and P, -OR, -O(CH$_2$)$_n$N(R)$_2$, -C(O)OR, -OC(O)R, -CX$_3$, -CX$_2$H, -CXH$_2$, -CN, -N(R)$_2$, -C(O)N(R)$_2$, -N(R)C(O)R, -N(R)S(O)$_2$R, -N(R)C(O)N(R)$_2$, -N(R)C(S)N(R)$_2$, and -C(R)N(R)$_2$C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5.

[0706] In another case, R$_4$ is selected from the group consisting of a C$_{3-6}$ carbocycle, -(CH$_2$)$_n$Q, -(CH$_2$)$_n$CHQR, -CHQR, and -CQ(R)$_2$, where Q is selected from a C$_{3-6}$ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH$_2$)$_n$N(R)$_2$, -C(O)OR, -OC(O)R, -CX$_3$, -CX$_2$H, -CXH$_2$, -CN, -C(O)N(R)$_2$, -N(R)C(O)R, -N(R)S(O)$_2$R, -N(R)C(O)N(R)$_2$, -N(R)C(S)N(R)$_2$, -C(R)N(R)$_2$C(O)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, and C$_{1-3}$ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5.

[0707] In another case, R$_4$ is selected from the group consisting of a C$_{3-6}$ carbocycle, -(CH$_2$)$_n$Q, -(CH$_2$)$_n$CHQR, -CHQR, and -CQ(R)$_2$, where Q is selected from a C$_{3-6}$ carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH$_2$)$_n$N(R)$_2$, -C(O)OR, -OC(O)R, -CX$_3$, -CX$_2$H, -CXH$_2$, -CN, -C(O)N(R)$_2$, -N(R)C(O)R, -N(R)S(O)$_2$R, -N(R)C(O)N(R)$_2$, -N(R)C(S)N(R)$_2$, -C(R)N(R)$_2$C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R$_4$ is -(CH$_2$)$_n$Q in which n is 1 or 2, or (ii) R$_4$ is -(CH$_2$)$_n$CHQR in which n is 1, or (iii) R$_4$ is -CHQR, and -CQ(R)$_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl.

[0708] In another case, R$_4$ is selected from the group consisting of a C$_{3-6}$ carbocycle, -(CH$_2$)$_n$Q, -(CH$_2$)$_n$CHQR, -CHQR, and -CQ(R)$_2$, where Q is selected from a C$_{3-6}$ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH$_2$)$_n$N(R)$_2$, -C(O)OR, -OC(O)R, -CX$_3$, -CX$_2$H, -CXH$_2$, -CN, -C(O)N(R)$_2$, -N(R)C(O)R, -N(R)S(O)$_2$R, -N(R)C(O)N(R)$_2$, -N(R)C(S)N(R)$_2$, -C(R)N(R)$_2$C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5.

[0709] In another case, R$_4$ is unsubstituted C$_{1-4}$ alkyl, *e.g.*, unsubstituted methyl.

[0710] In certain cases, the disclosure provides a compound having the Formula (I), wherein R$_4$ is -(CH$_2$)$_n$Q or -(CH$_2$)$_n$CHQR, where Q is -N(R)$_2$, and n is selected from 3, 4, and 5.

[0711] In certain cases, the disclosure provides a compound having the Formula (I), wherein R$_4$ is selected from the group consisting of -(CH$_2$)$_n$Q, -(CH$_2$)$_n$CHQR, -CHQR, and -CQ(R)$_2$, where Q is -N(R)$_2$, and n is selected from 1, 2, 3, 4, and 5.

[0712] In certain cases, the disclosure provides a compound having the Formula (I), wherein R$_2$ and R$_3$ are independently selected from the group consisting of C$_{2-14}$ alkyl, C$_{2-14}$ alkenyl, -R*YR", -YR", and -R*OR", or R$_2$ and R$_3$, together with the atom to which they are attached, form a heterocycle or carbocycle, and R$_4$ is -(CH$_2$)$_n$Q or -(CH$_2$)$_n$CHQR, where Q is -N(R)$_2$, and n is selected from 3, 4, and 5.

[0713] In certain cases, R$_2$ and R$_3$ are independently selected from the group consisting of C$_{2-14}$ alkyl, C$_{2-14}$ alkenyl, -R*YR", -YR", and -R*OR", or R$_2$ and R$_3$, together with the atom to which they are attached, form a heterocycle or carbocycle.

[0714] In some cases, R$_1$ is selected from the group consisting of C$_{5-20}$ alkyl and C$_{5-20}$ alkenyl.

[0715] In other cases, R$_1$ is selected from the group consisting of -R*YR", -YR", and -R"M'R'.

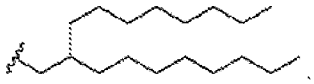
[0716] In certain cases, R$_1$ is selected from -R*YR" and -YR". In some cases, Y is a cyclopropyl group. In some cases,

R" is C_8 alkyl or C_8 alkenyl. In certain cases, R" is C_{3-12} alkyl. For example, R" may be C_3 alkyl. For example, R" may be C_{4-8} alkyl (*e.g.*, C_4, C_5, C_6, C_7, or C_8 alkyl).

[0717] In some cases, R_1 is C_{5-20} alkyl. In some cases, R_1 is C_6 alkyl. In some cases, R_1 is C_8 alkyl. In other cases, R_1 is C_9 alkyl. In certain cases, R_1 is C_{14} alkyl. In other cases, R_1 is C_{18} alkyl.

[0718] In some cases, R_1 is C_{5-20} alkenyl. In certain cases, R_1 is C_{18} alkenyl. In some cases, R_1 is linoleyl.

[0719] In certain cases, R_1 is branched (e.g., decan-2-yl, undecan-3-yl, dodecan-4-yl, tridecan-5-yl, tetradecan-6-yl, 2-methylundecan-3-yl, 2-methyldecan-2-yl, 3-methylundecan-3-yl, 4-methyldodecan-4-yl, or heptadeca-9-yl). In certain cases, R_1 is



[0720] In certain cases, R_1 is unsubstituted C_{5-20} alkyl or C_{5-20} alkenyl. In certain cases, R' is substituted C_{5-20} alkyl or C_{5-20} alkenyl (e.g., substituted with a C_{3-6} carbocycle such as 1-cyclopropylnonyl).

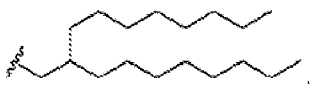
[0721] In other cases, R_1 is -R"M'R'.

[0722] In some cases, R' is selected from -R"YR" and -YR". In some cases, Y is C_{3-8} cycloalkyl. In some cases, Y is C_{6-10} aryl. In some cases, Y is a cyclopropyl group. In some cases, Y is a cyclohexyl group. In certain cases, R" is C_1 alkyl.

[0723] In some cases, R" is selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl. In some cases, R" adjacent to Y is C_1 alkyl. In some cases, R" adjacent to Y is C_{4-9} alkyl (*e.g.*, C_4, C_5, C_6, C_7 or C_8 or C_9 alkyl).

[0724] In some cases, R' is selected from C_4 alkyl and C_4 alkenyl. In certain cases, R' is selected from C_5 alkyl and C_5 alkenyl. In some cases, R' is selected from C_6 alkyl and C_6 alkenyl. In some cases, R' is selected from C_7 alkyl and C_7 alkenyl. In some cases, R' is selected from C_9 alkyl and C_9 alkenyl.

[0725] In other cases, R' is selected from C_{11} alkyl and C_{11} alkenyl. In other cases, R' is selected from C_{12} alkyl, C_{12} alkenyl, C_{13} alkyl, C_{13} alkenyl, C_{14} alkyl, C_{14} alkenyl, C_{15} alkyl, C_{15} alkenyl, C_{16} alkyl, C_{16} alkenyl, C_{17} alkyl, C_{17} alkenyl, C_{18} alkyl, and C_{18} alkenyl. In certain cases, R' is branched (e.g., decan-2-yl, undecan-3-yl, dodecan-4-yl, tridecan-5-yl, tetradecan-6-yl, 2-methylundecan-3-yl, 2-methyldecan-2-yl, 3-methylundecan-3-yl, 4-methyldodecan-4-yl or heptadeca-9-yl). In certain cases, R' is



[0726] In certain cases, R' is unsubstituted C_{1-18} alkyl. In certain cases, R' is substituted C_{1-18} alkyl (*e.g.*, C_{1-15} alkyl substituted with a C_{3-6} carbocycle such as 1-cyclopropylnonyl).

[0727] In some cases, R" is selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl. In some cases, R" is C_3 alkyl, C_4 alkyl, C_5 alkyl, C_6 alkyl, C_7 alkyl, or C_8 alkyl. In some cases, R" is C_9 alkyl, C_{10} alkyl, C_{11} alkyl, C_{12} alkyl, C_{13} alkyl, or C_{14} alkyl.

[0728] In some cases, M' is -C(O)O-. In some cases, M' is -OC(O)-.

[0729] In other cases, M' is an aryl group or heteroaryl group. For example, M' may be selected from the group consisting of phenyl, oxazole, and thiazole.

[0730] In some cases, M is -C(O)O- In some cases, M is -OC(O)-. In some cases, M is -C(O)N(R')-. In some cases, M is -P(O)(OR')O-.

[0731] In other cases, M is an aryl group or heteroaryl group. For example, M may be selected from the group consisting of phenyl, oxazole, and thiazole.

[0732] In some cases, M is the same as M'. In other cases, M is different from M'.

[0733] In some cases, each R_5 is H. In certain such cases, each R_6 is also H.

[0734] In some cases, R_7 is H. In other cases, R_7 is C_{1-3} alkyl (e.g., methyl, ethyl, propyl, or i-propyl).

[0735] In some cases, R_2 and R_3 are independently C_{5-14} alkyl or C_{5-14} alkenyl.

[0736] In some cases, R_2 and R_3 are the same. In some cases, R_2 and R_3 are C_8 alkyl. In certain cases, R_2 and R_3 are C_2 alkyl. In other cases, R_2 and R_3 are C_3 alkyl. In some cases, R_2 and R_3 are C_4 alkyl. In certain cases, R_2 and R_3 are C_5 alkyl. In other cases, R_2 and R_3 are C_6 alkyl. In some cases, R_2 and R_3 are C_7 alkyl.

[0737] In other cases, R_2 and R_3 are different. In certain cases, R_2 is C_8 alkyl. In some cases, R_3 is C_{1-7} (*e.g.*, C_1, C_2, C_3, C_4, C_5, C_6, or C_7 alkyl) or C_9 alkyl.

[0738] In some cases, R_7 and R_8 are H.

[0739] In certain cases, R_2 is H.

[0740] In some cases, m is 5, 7, or 9.

[0741] In some cases, R_4 is selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$

[0742] In some cases, Q is selected from the group consisting of -OR, -OH, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, -CN, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, $-C(R)N(R)_2C(O)OR$, a carbocycle, and a heterocycle.

[0743] In certain cases, Q is -OH.

[0744] In certain cases, Q is a substituted or unsubstituted 5- to 10- membered heteroaryl, *e.g.*, Q is an imidazole, a pyrimidine, a purine, 2-amino-1,9-dihydro-6*H*-purin-6-one-9-yl (or guanin-9-yl), adenin-9-yl, cytosin-1-yl, or uracil-1-yl. In certain cases, Q is a substituted 5- to 14-membered heterocycloalkyl, *e.g.*, substituted with one or more substituents selected from oxo (=O), OH, amino, and C_{1-3} alkyl. For example, Q is 4-methylpiperazinyl, 4-(4-methoxybenzyl)piperazinyl, or isoindolin-2-yl-1,3-dione.

[0745] In certain cases, Q is an unsubstituted or substituted C_{6-10} aryl (such as phenyl) or C_{3-6} cycloalkyl.

[0746] In some cases, n is 1. In other cases, n is 2. In further cases, n is 3. In certain other cases, n is 4. For example, R_4 may be $-(CH_2)_2OH$. For example, R_4 may be $-(CH_2)_3OH$. For example, R_4 may be $-(CH_2)_4OH$. For example, R_4 may be benzyl. For example, R_4 may be 4-methoxybenzyl.

[0747] In some cases, R_4 is a C_{3-6} carbocycle. In some cases, R_4 is a C_{3-6} cycloalkyl. For example, R_4 may be cyclohexyl optionally substituted with *e.g.*, OH, halo, C_{1-6} alkyl, etc. For example, R_4 may be 2-hydroxycyclohexyl.

[0748] In some cases, R is H.

[0749] In some cases, R is unsubstituted C_{1-3} alkyl or unsubstituted C_{2-3} alkenyl. For example, R_4 may be $-CH_2CH(OH)CH_3$ or $-CH_2CH(OH)CH_2CH_3$.

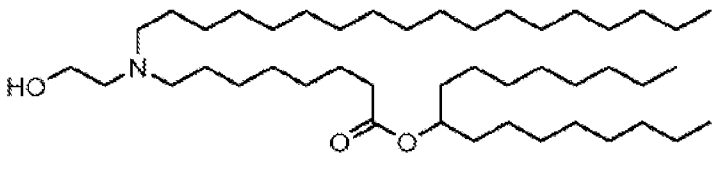
[0750] In some cases, R is substituted C_{1-3} alkyl, *e.g.*, CH_2OH. For example, R_4 may be $-CH_2CH(OH)CH_2OH$.

[0751] In some cases, R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle. In some cases, R_2 and R_3, together with the atom to which they are attached, form a 5- to 14- membered aromatic or non-aromatic heterocycle having one or more heteroatoms selected from N, O, S, and P. In some cases, R_2 and R_3, together with the atom to which they are attached, form an optionally substituted C_{3-20} carbocycle (e.g., C_{3-18} carbocycle, C_{3-15} carbocycle, C_{3-12} carbocycle, or C_{3-10} carbocycle), either aromatic or non-aromatic. In some cases, R_2 and R_3, together with the atom to which they are attached, form a C_{3-6} carbocycle. In other cases, R_2 and R_3, together with the atom to which they are attached, form a C_6 carbocycle, such as a cyclohexyl or phenyl group. In certain cases, the heterocycle or C_{3-6} carbocycle is substituted with one or more alkyl groups (*e.g.*, at the same ring atom or at adjacent or non-adjacent ring atoms). For example, R_2 and R_3, together with the atom to which they are attached, may form a cyclohexyl or phenyl group bearing one or more C_5 alkyl substitutions. In certain cases, the heterocycle or C_{3-6} carbocycle formed by R_2 and R_3, is substituted with a carbocycle groups. For example, R_2 and R_3, together with the atom to which they are attached, may form a cyclohexyl or phenyl group that is substituted with cyclohexyl. In some cases, R_2 and R_3, together with the atom to which they are attached, form a C_{7-15} carbocycle, such as a cycloheptyl, cyclopentadecanyl, or naphthyl group.

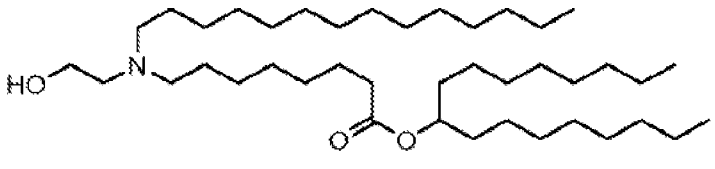
[0752] In some cases, R_4 is selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$. In some cases, Q is selected from the group consisting of -OR, -OH, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, -CN, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, and a heterocycle. In other cases, Q is selected from the group consisting of an imidazole, a pyrimidine, and a purine.

[0753] In some cases, R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle. In some cases, R_2 and R_3, together with the atom to which they are attached, form a C_{3-6} carbocycle, such as a phenyl group. In certain cases, the heterocycle or C_{3-6} carbocycle is substituted with one or more alkyl groups (*e.g.*, at the same ring atom or at adjacent or non-adjacent ring atoms). For example, R_2 and R_3, together with the atom to which they are attached, may form a phenyl group bearing one or more C_5 alkyl substitutions.

[0754] In some cases, the pharmaceutical compositions of the present disclosure, the compound of formula (I) is selected from the group consisting of:



(Compound 1),



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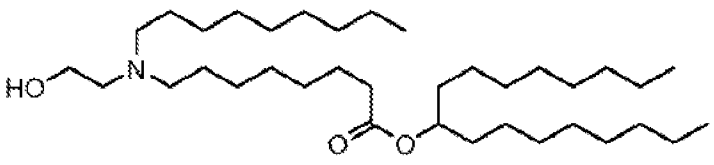

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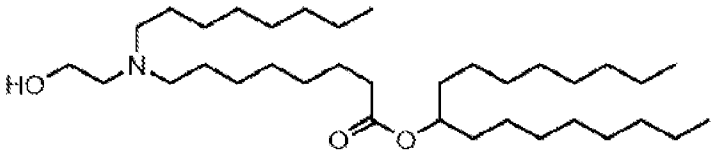

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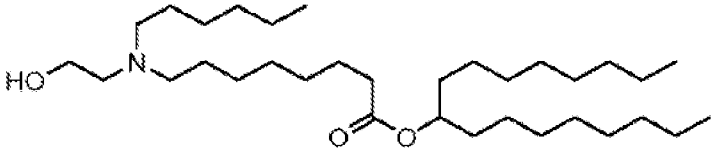

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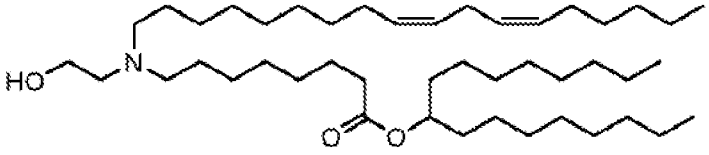
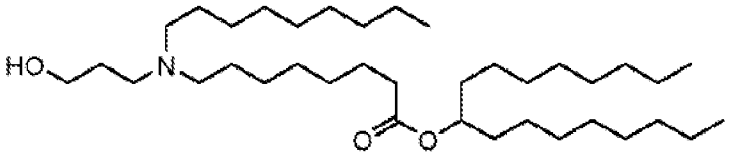
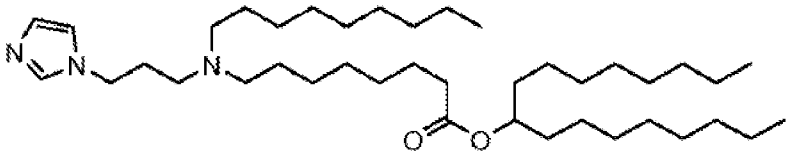


(Compound 6),



(Compound 7),



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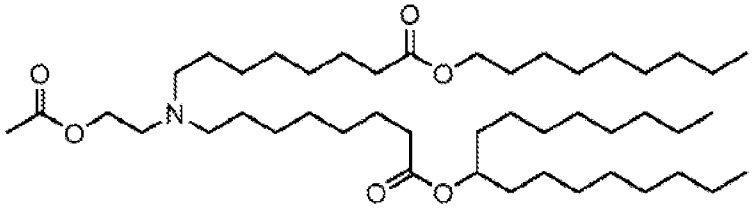

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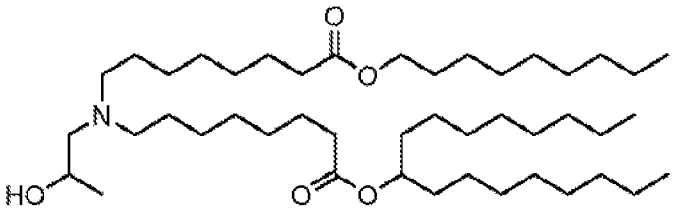

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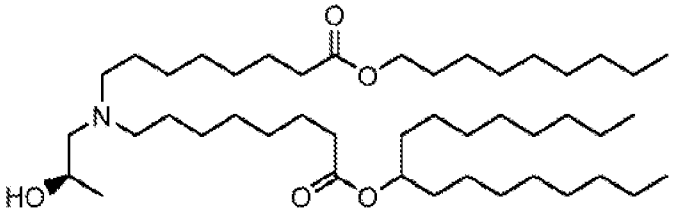

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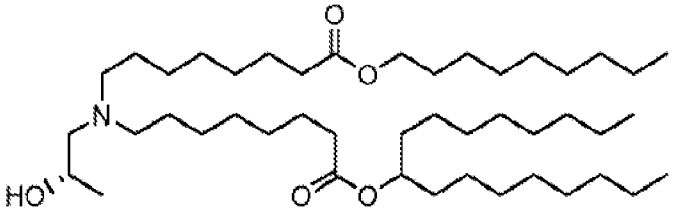

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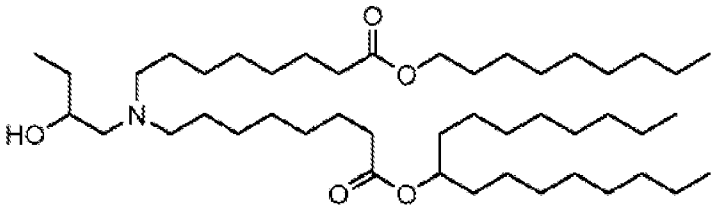

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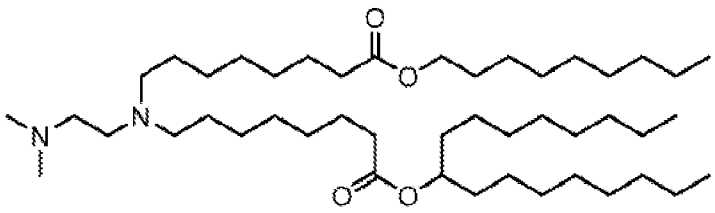

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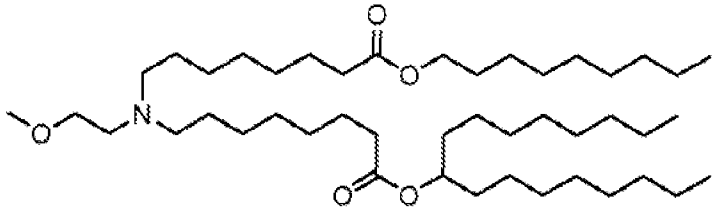

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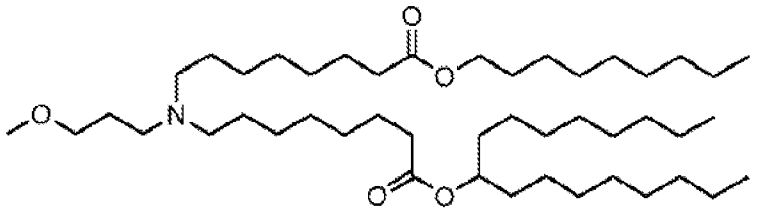

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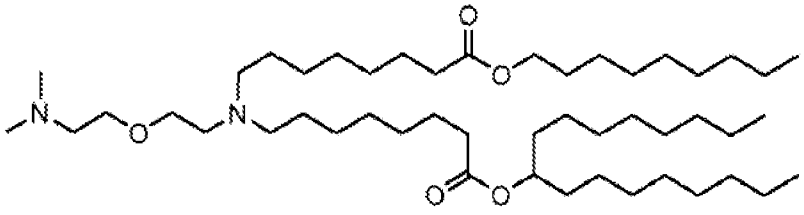

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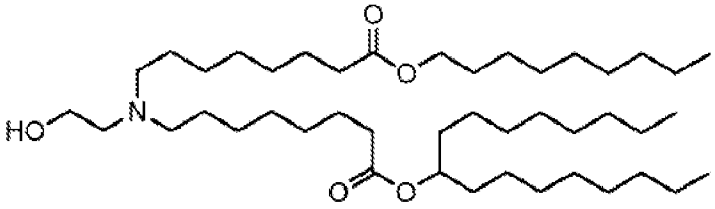

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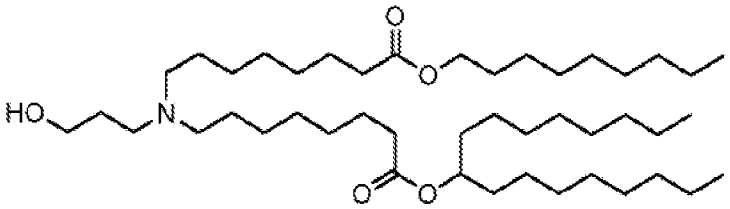

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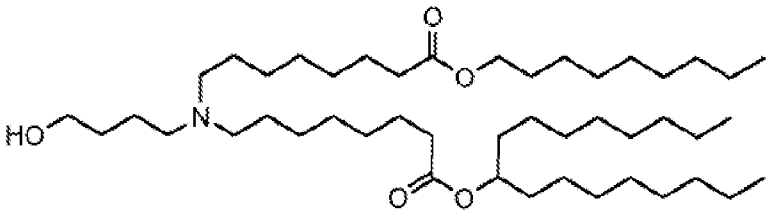

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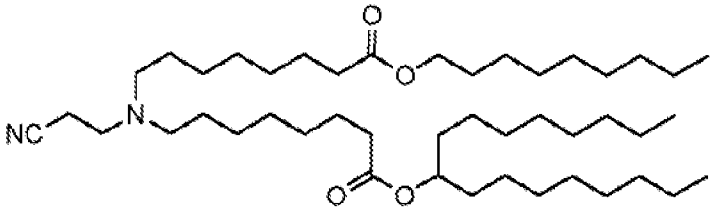

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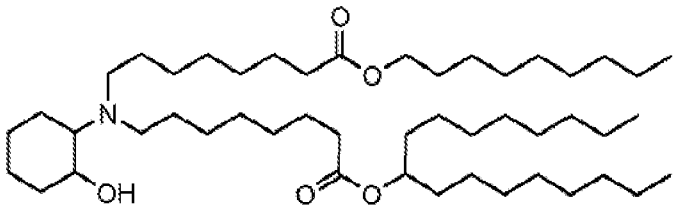

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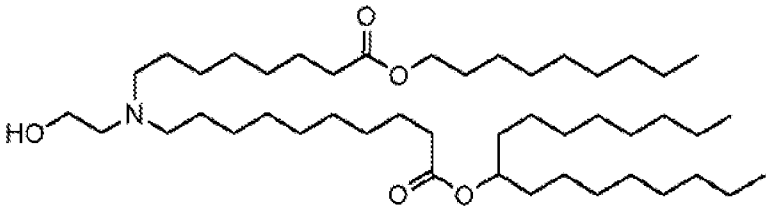

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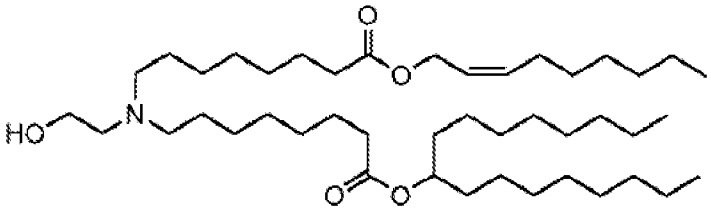

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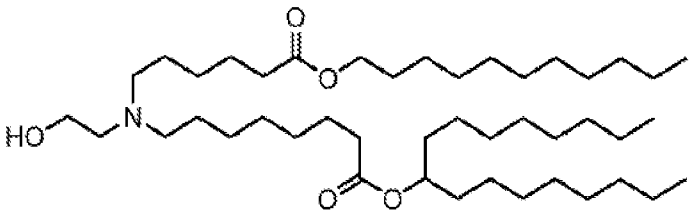

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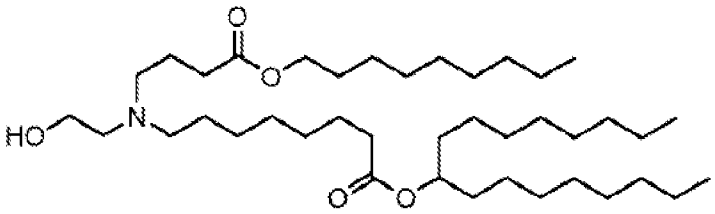

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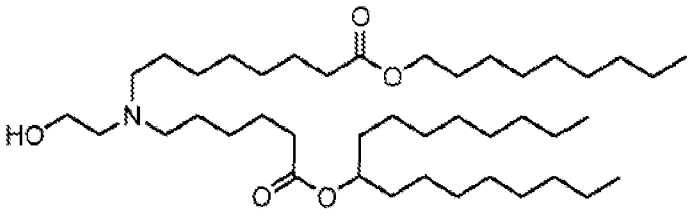

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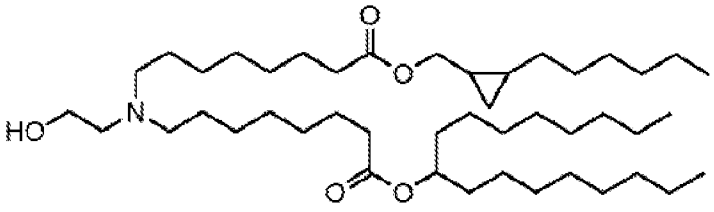

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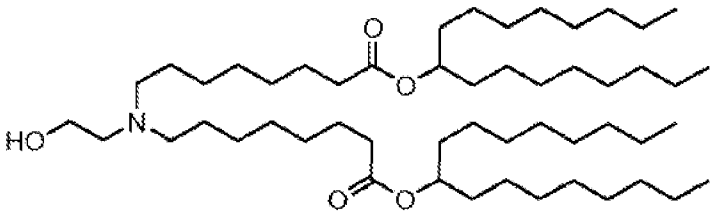

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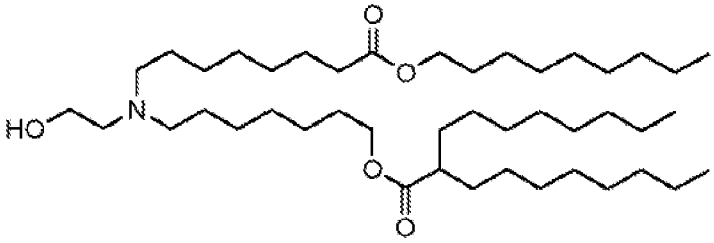

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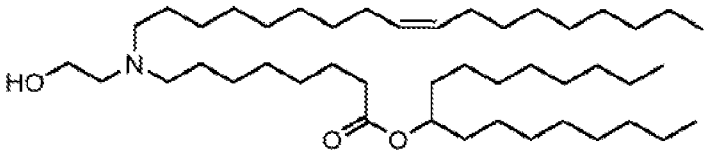

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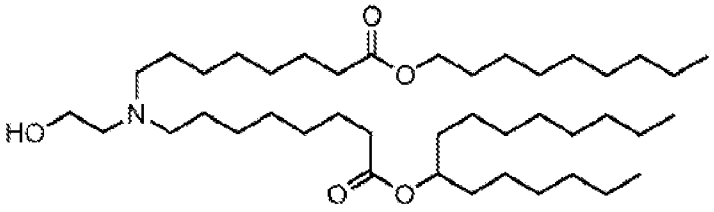

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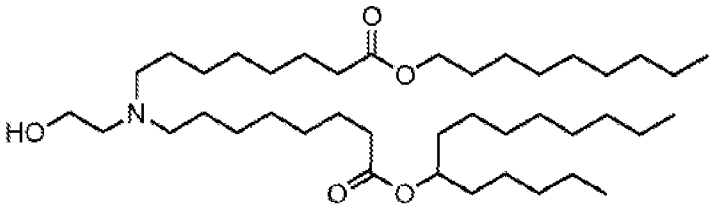

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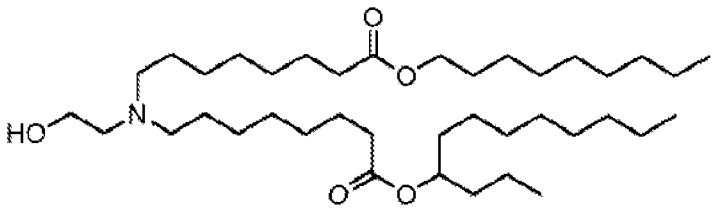

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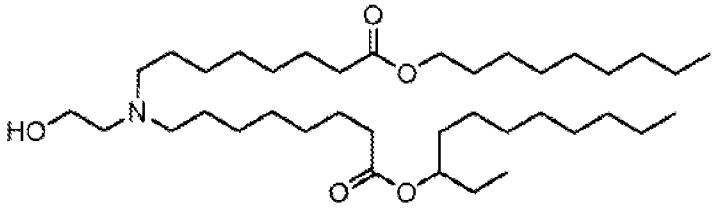

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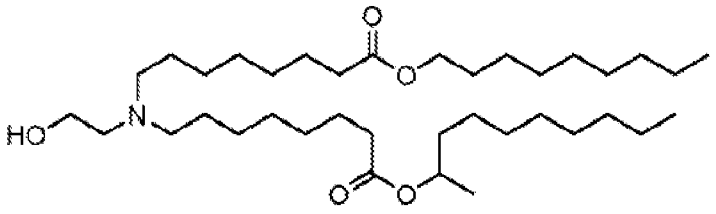

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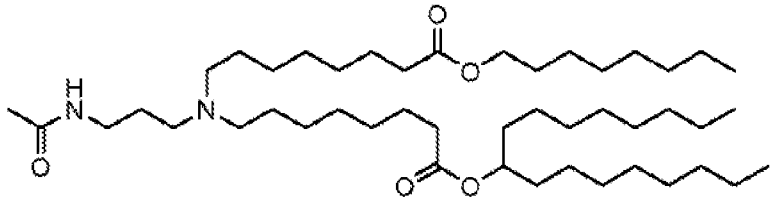

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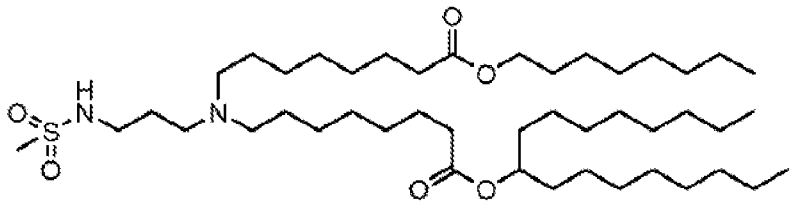

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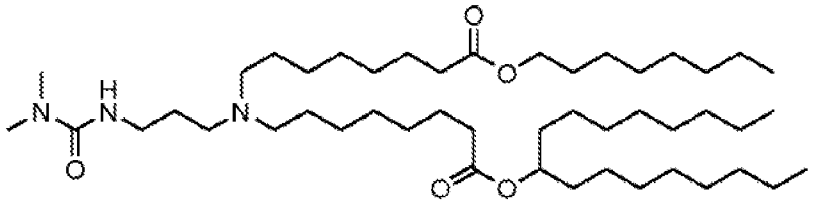

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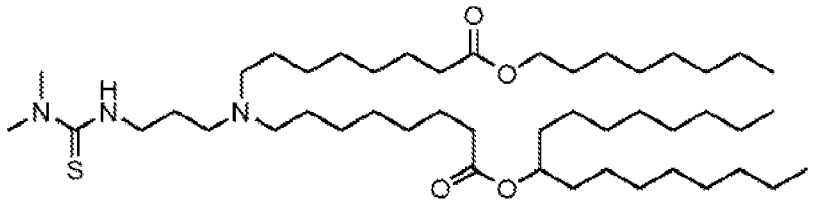

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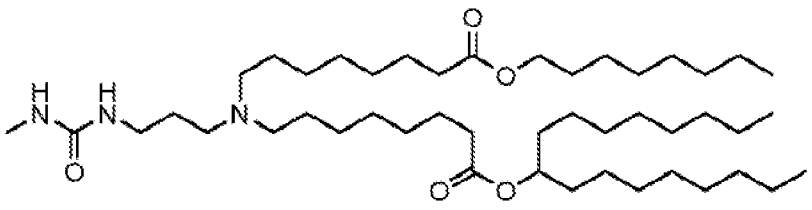

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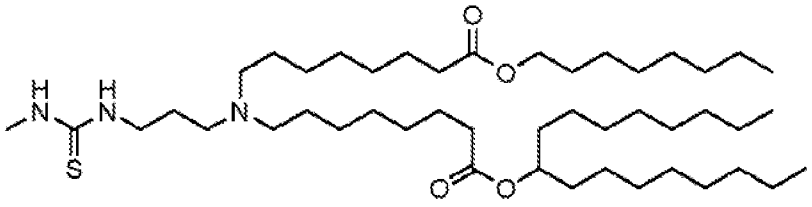

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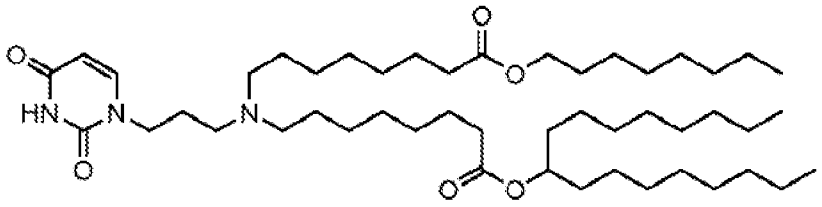

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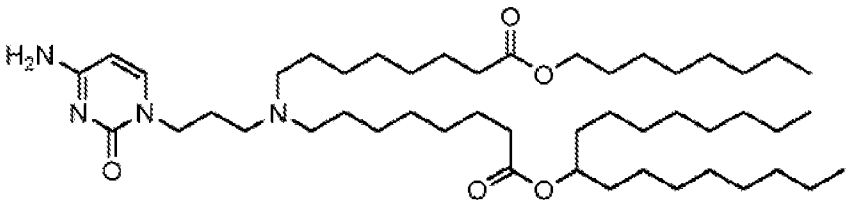

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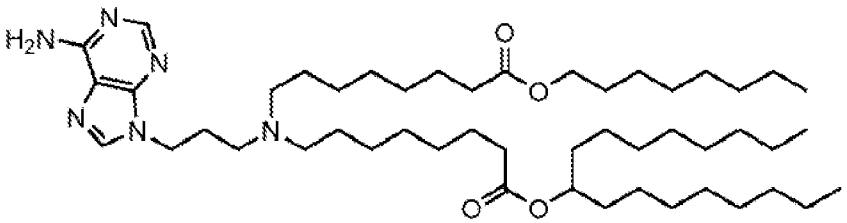

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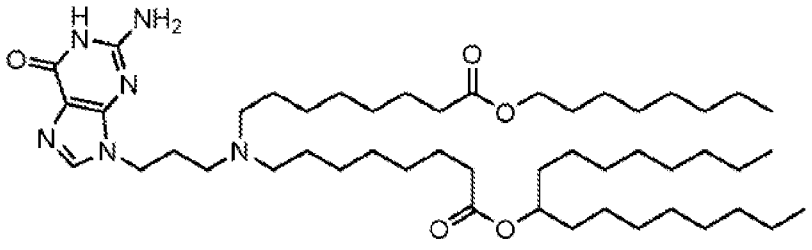

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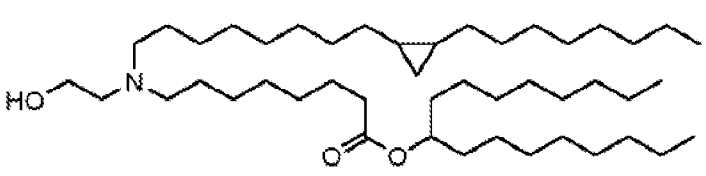

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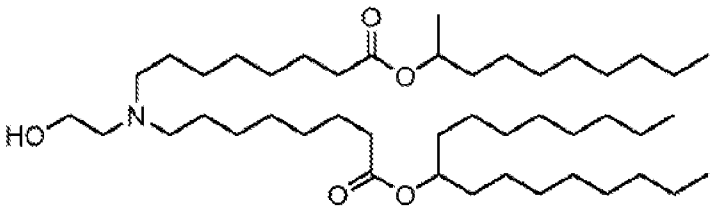

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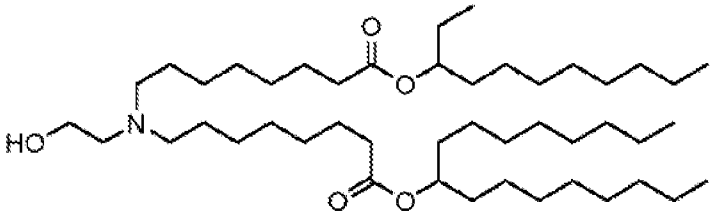

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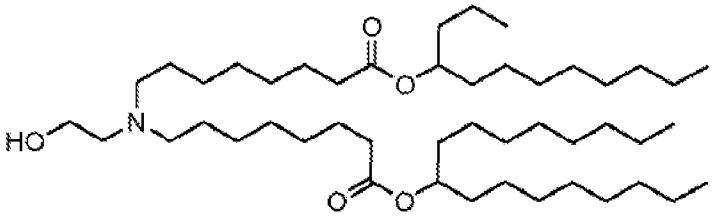

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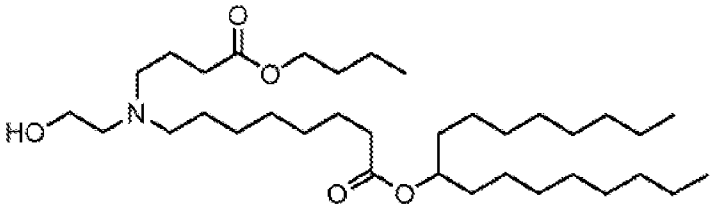

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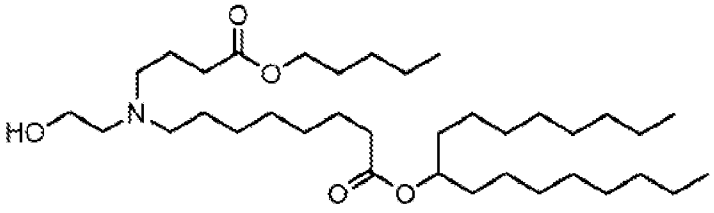

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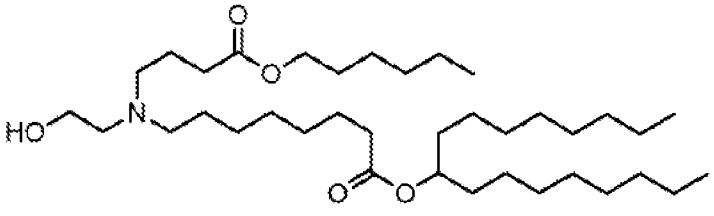

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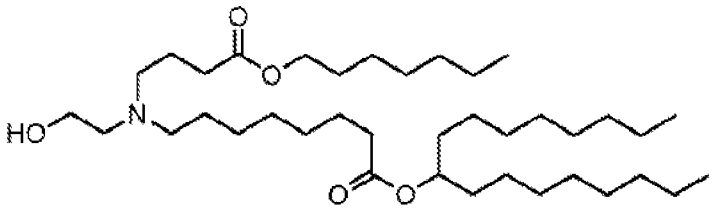

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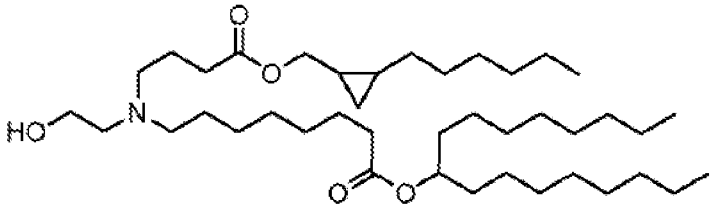

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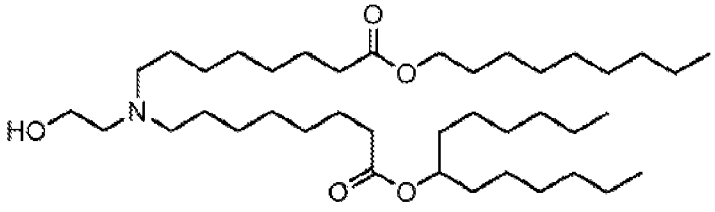

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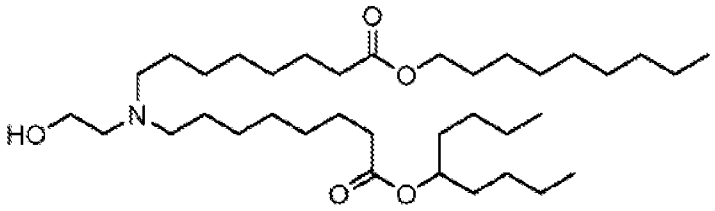

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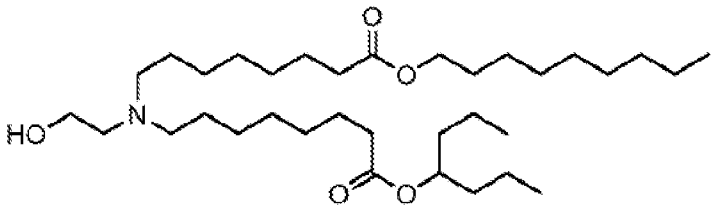

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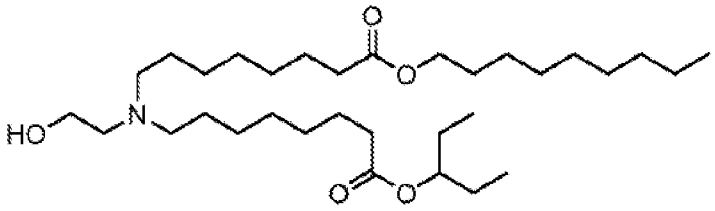

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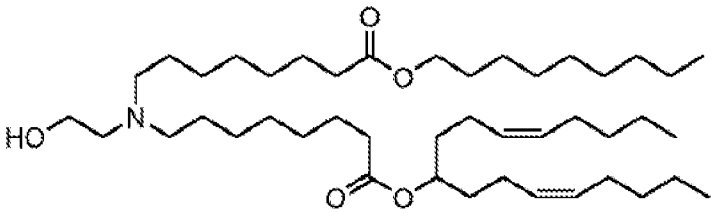

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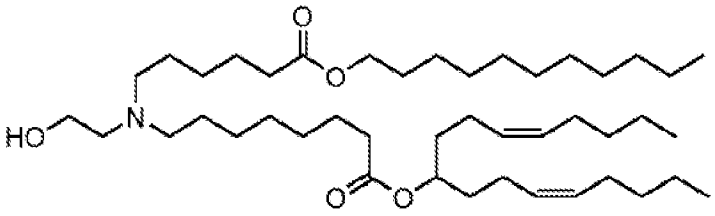

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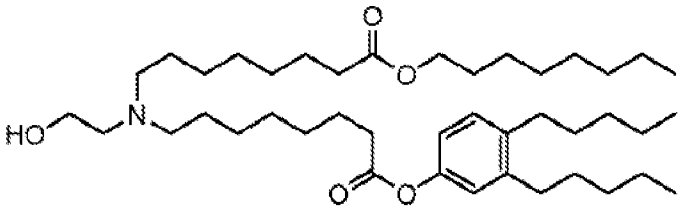

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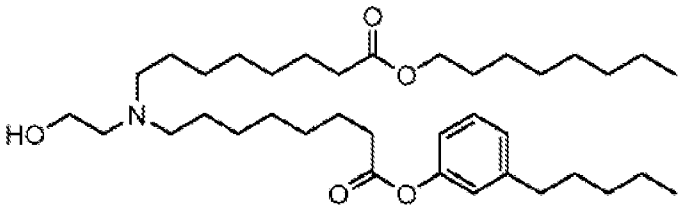

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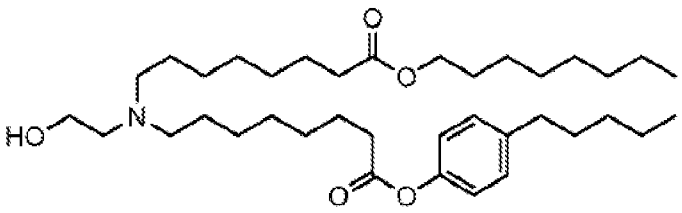

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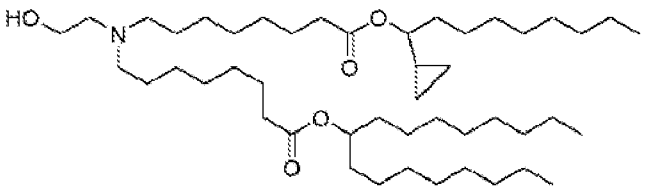

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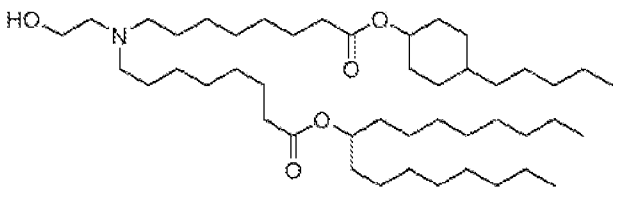

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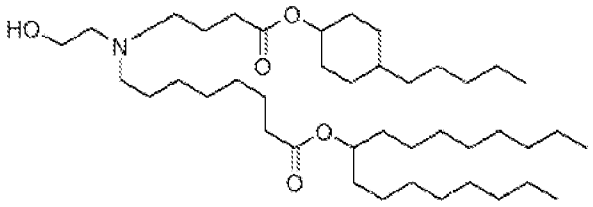

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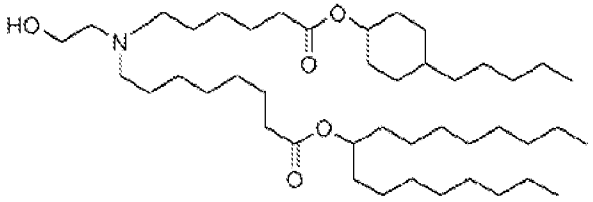

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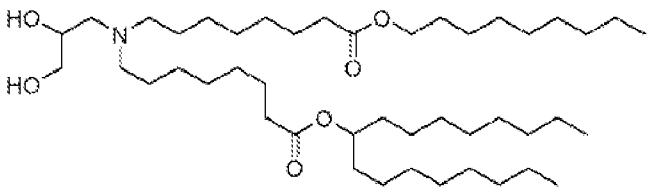

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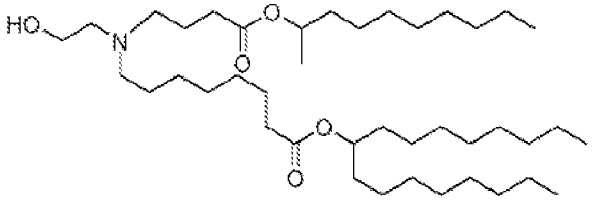

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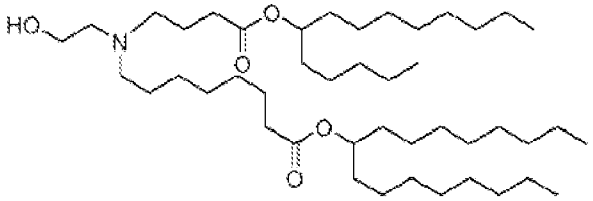

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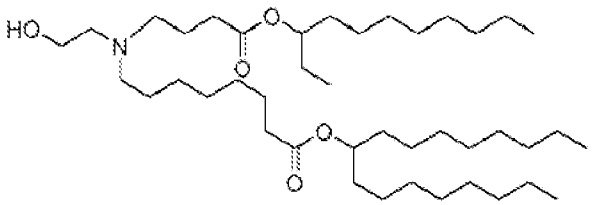

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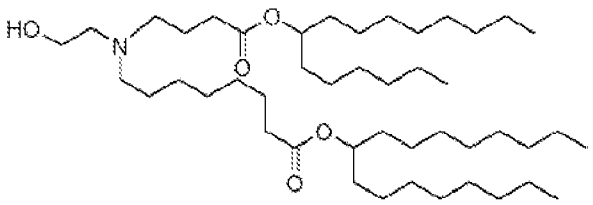

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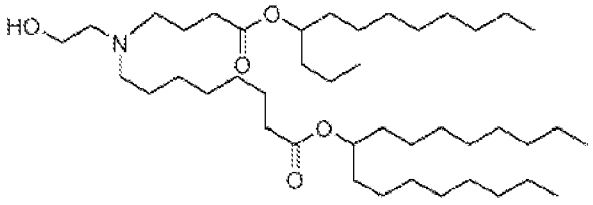

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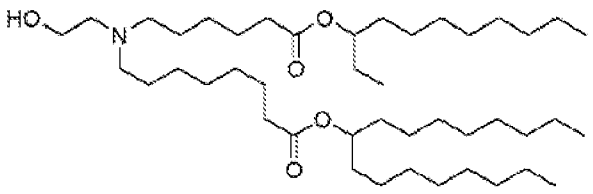

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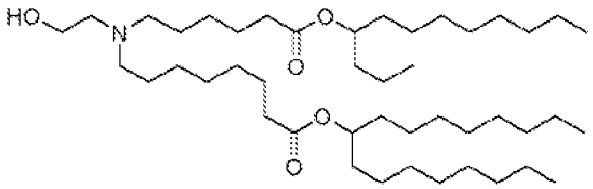

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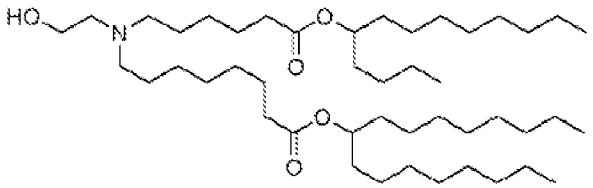

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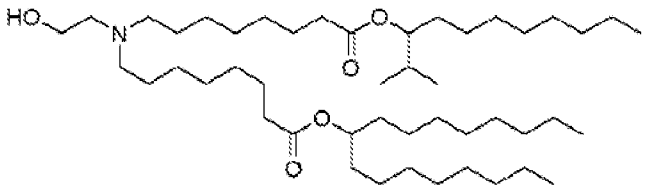

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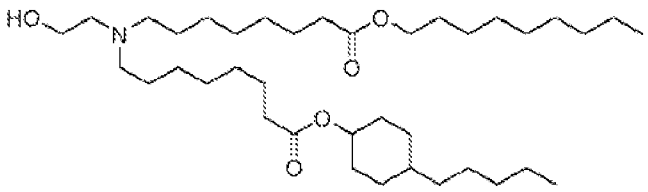

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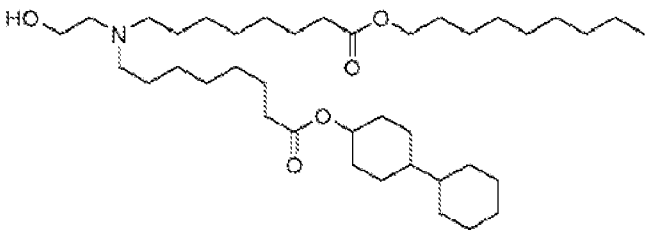

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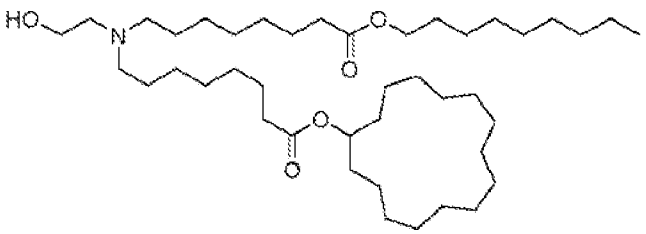

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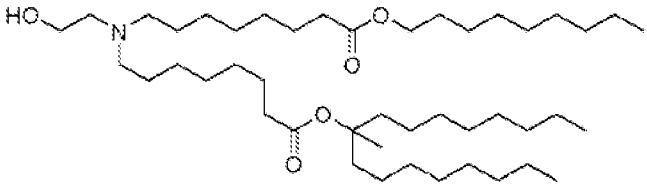

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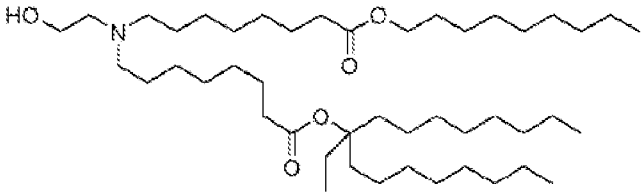

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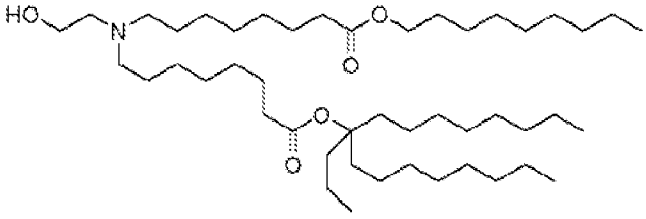

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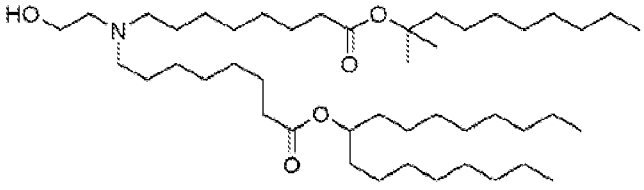

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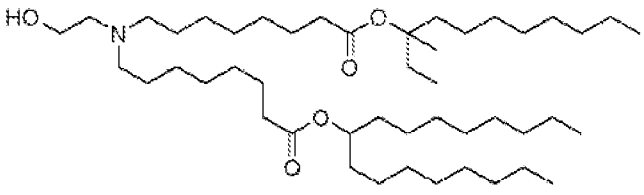

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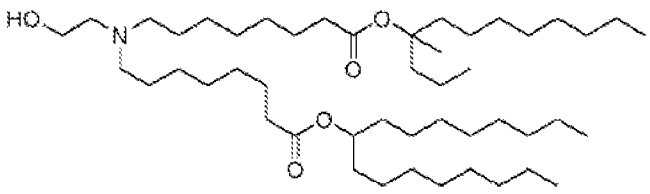

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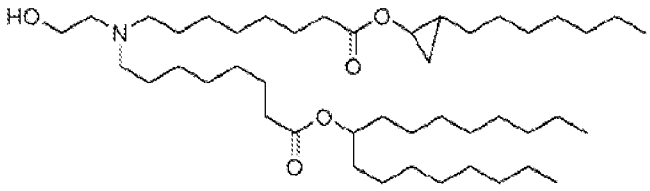

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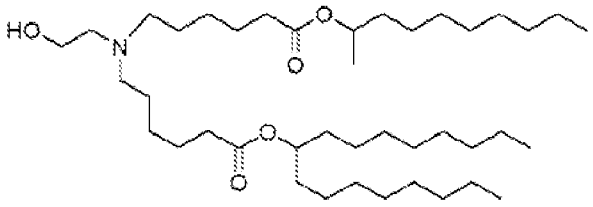

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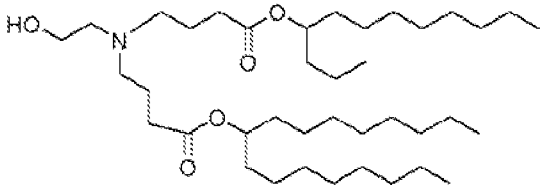

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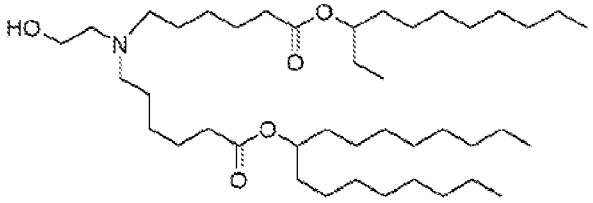

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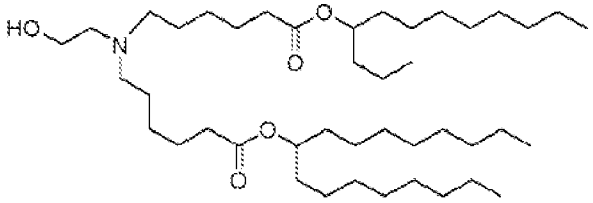

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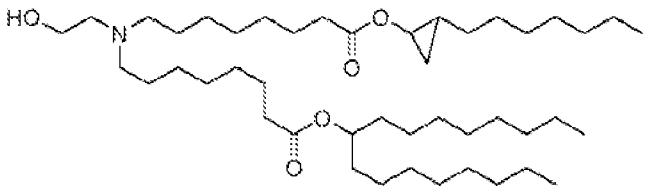

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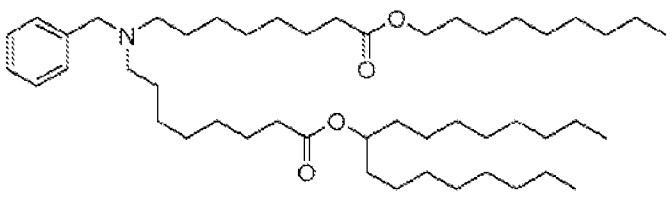

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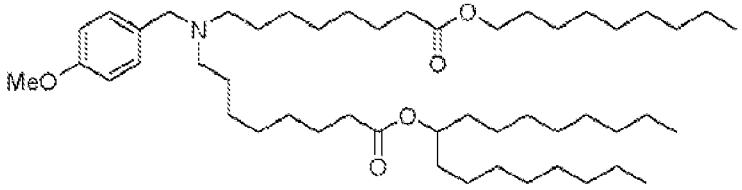

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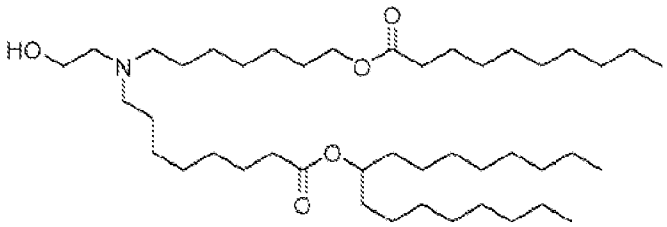

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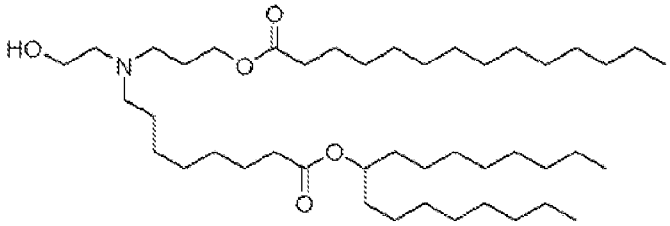

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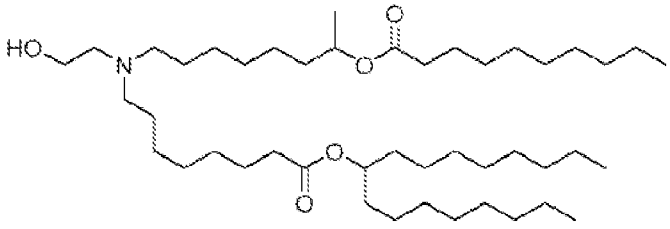

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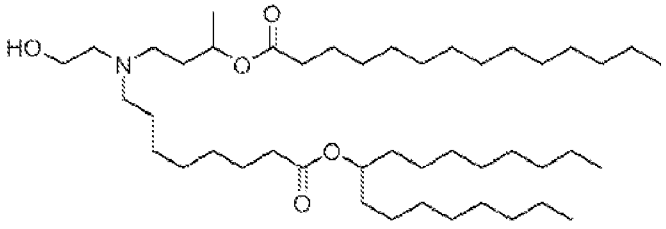

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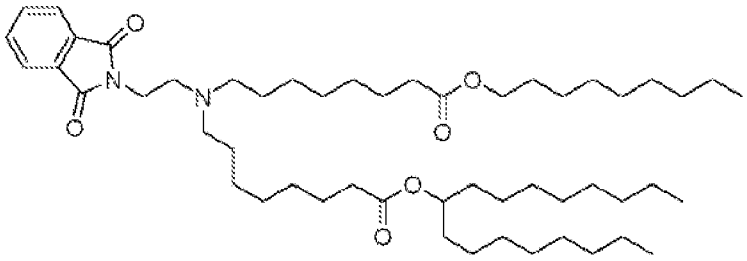

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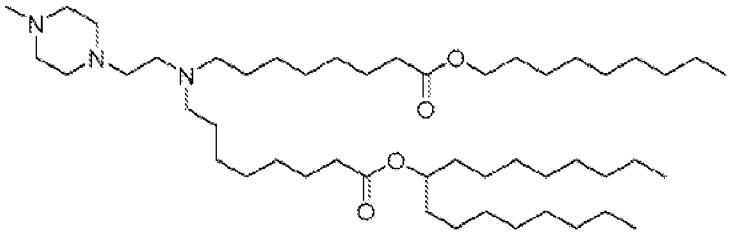

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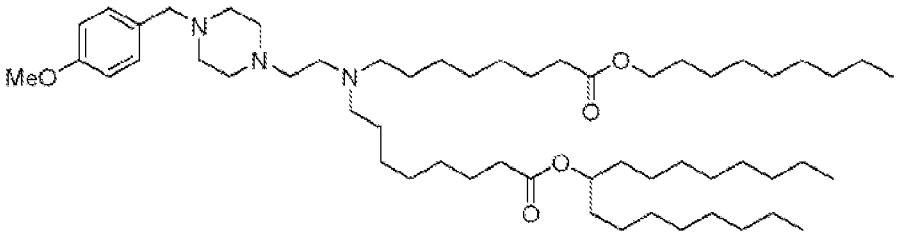

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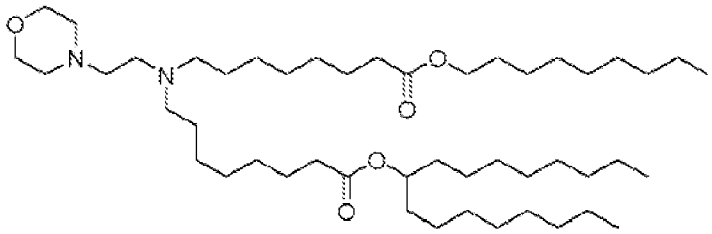

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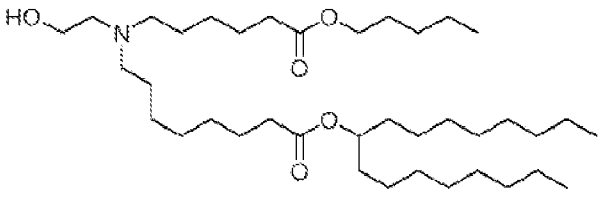

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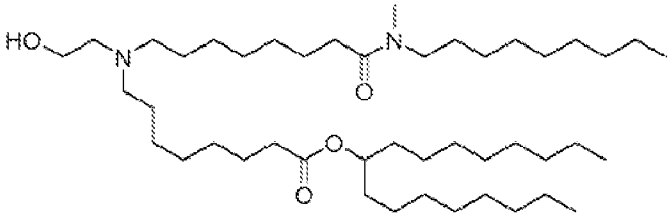

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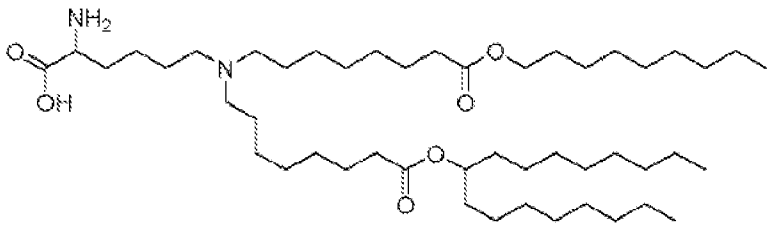

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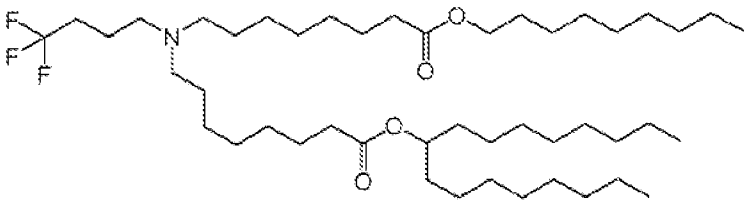

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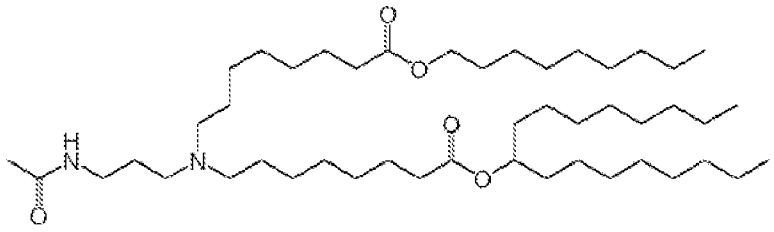

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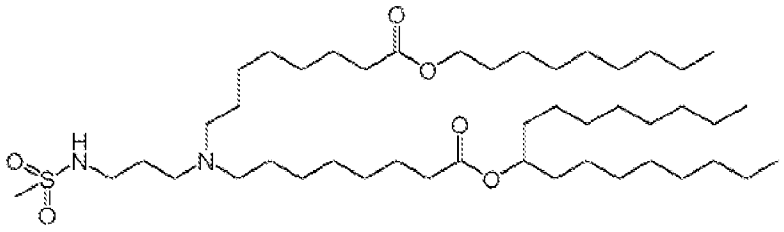

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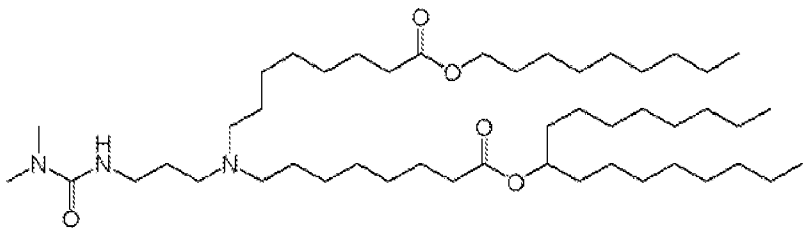

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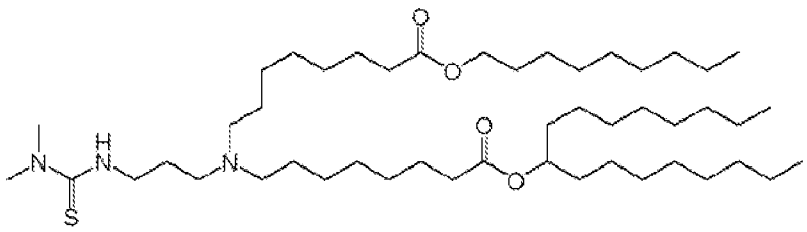

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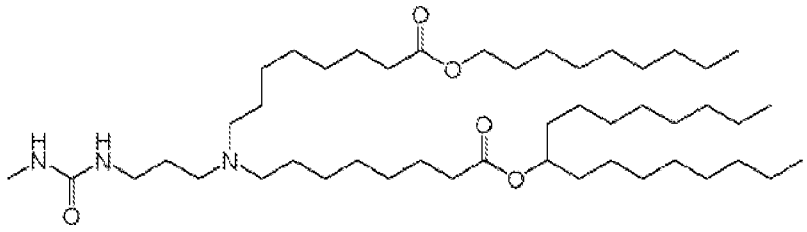

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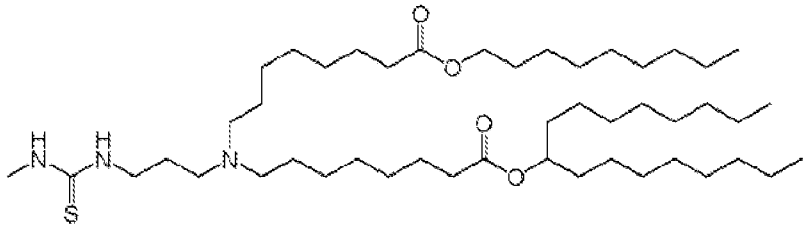

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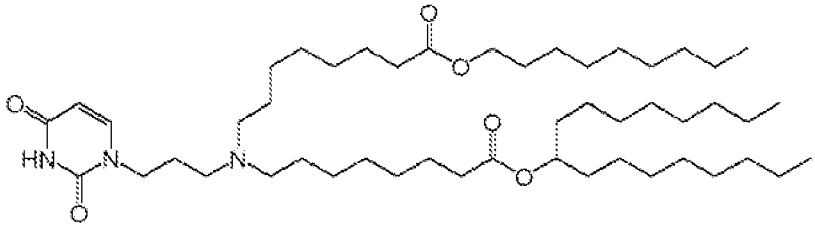

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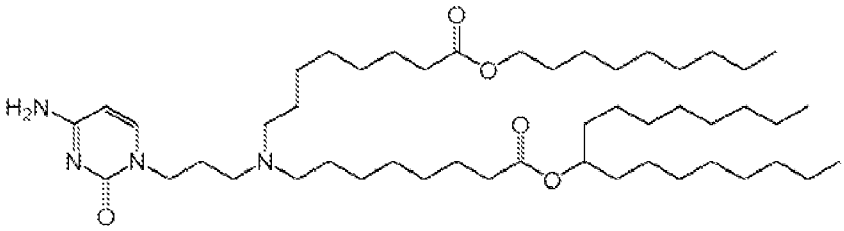

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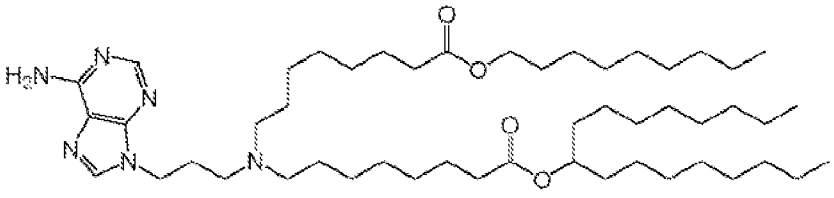

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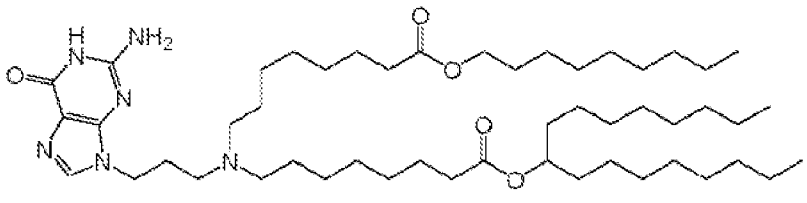

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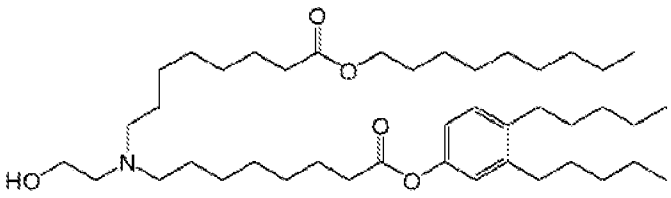

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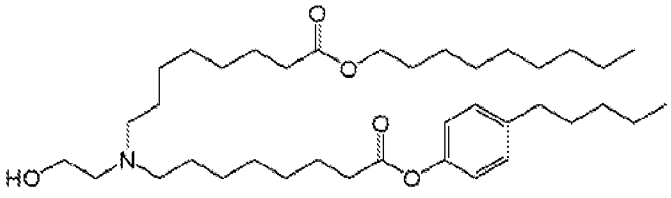

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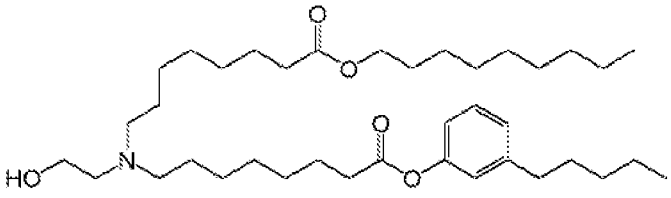

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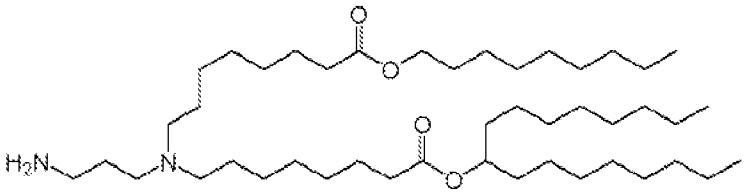

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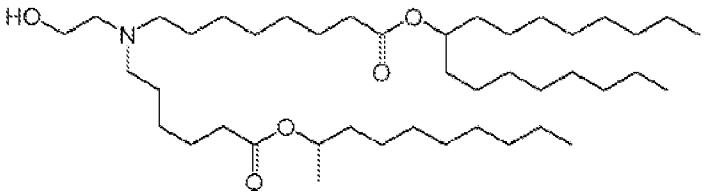

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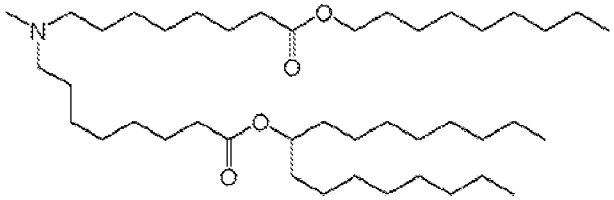

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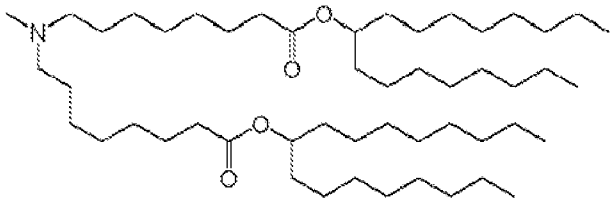

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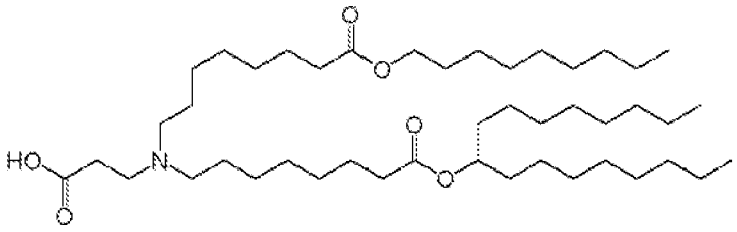

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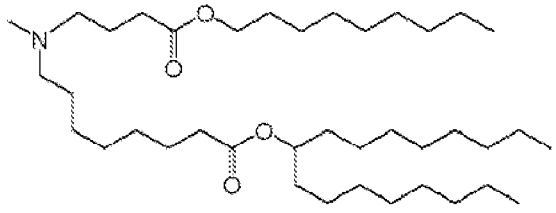

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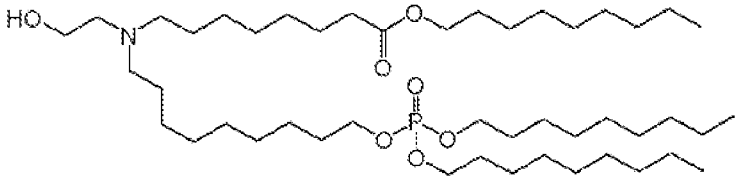

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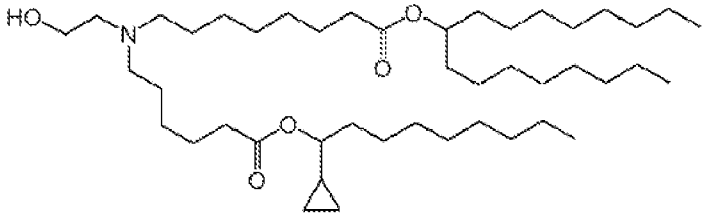

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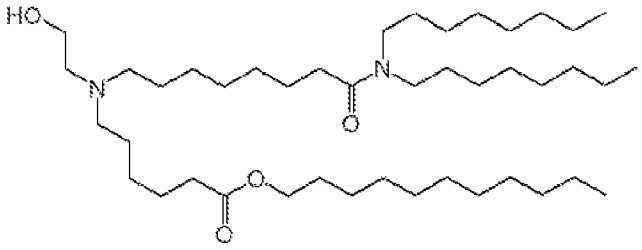

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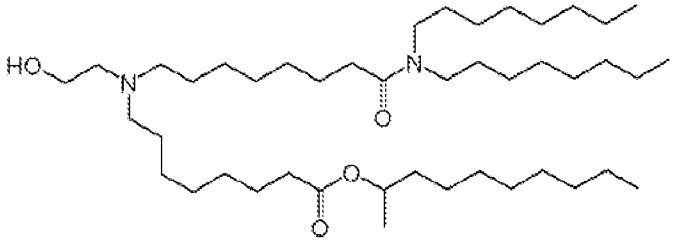

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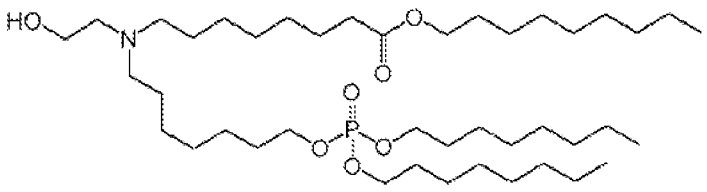

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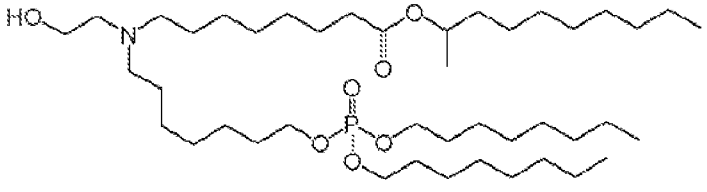

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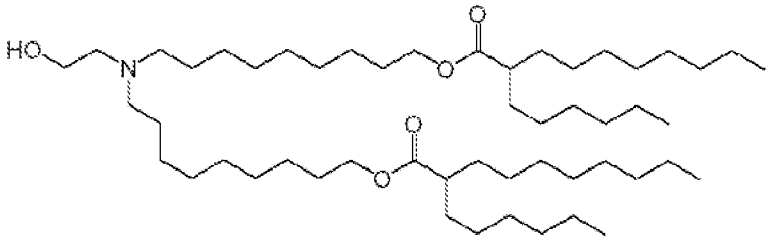

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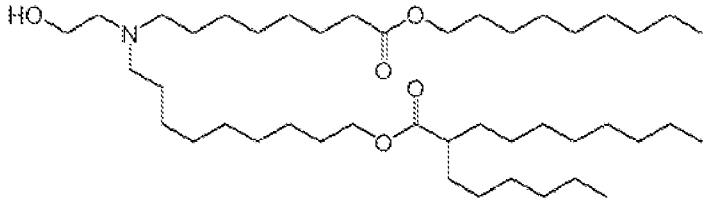

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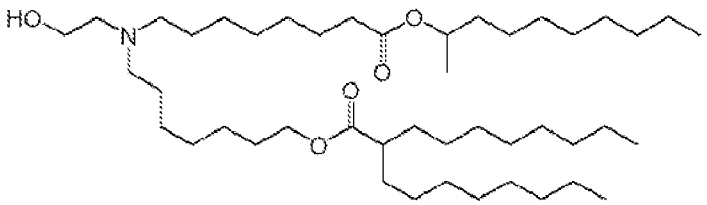

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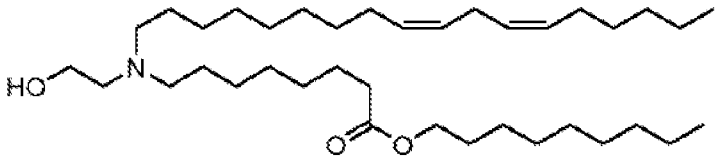

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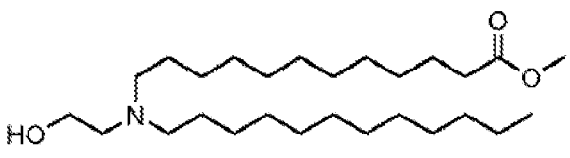

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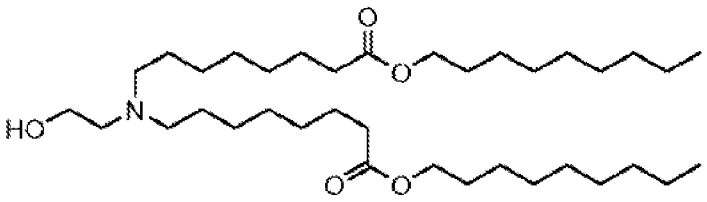

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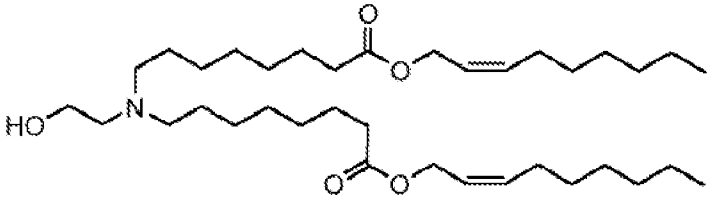

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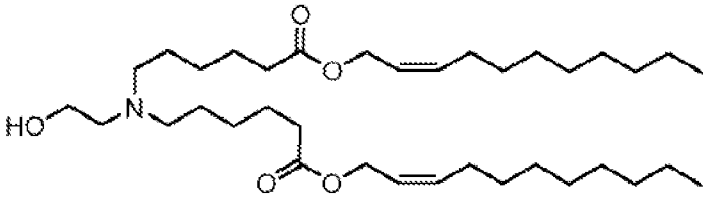

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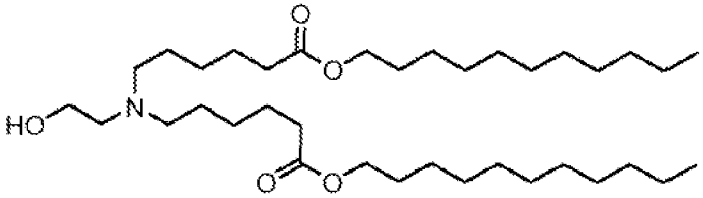

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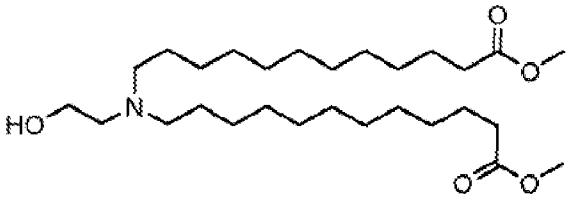

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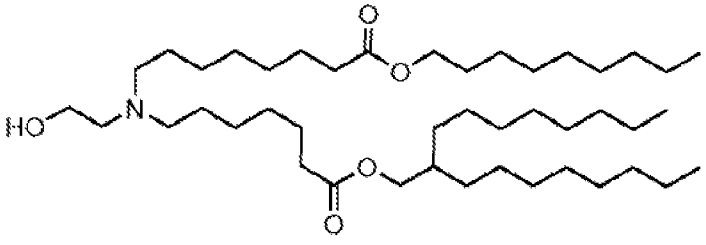

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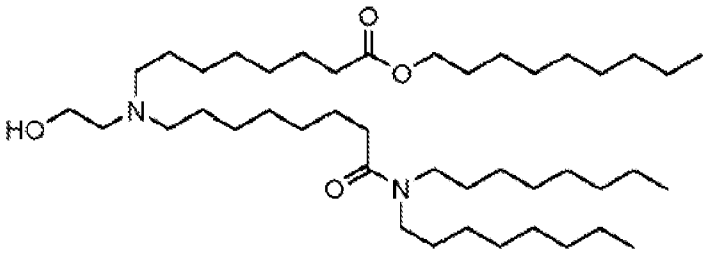

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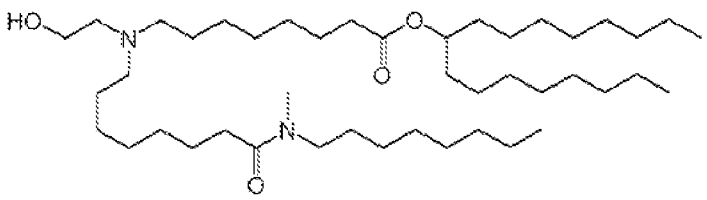

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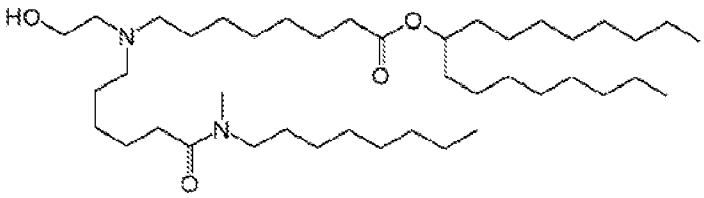

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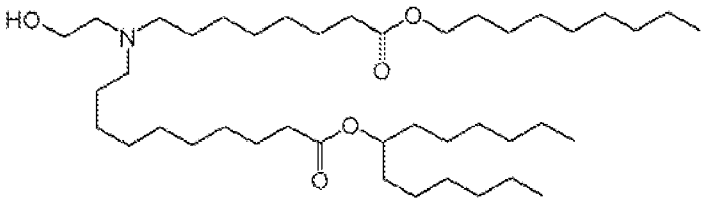

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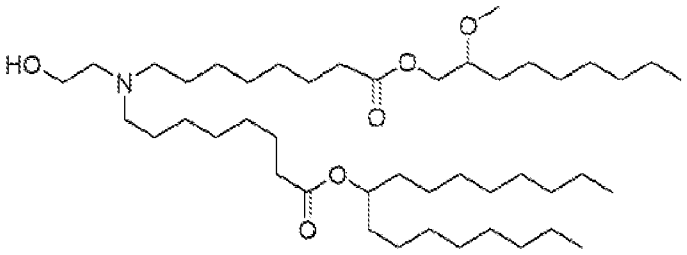

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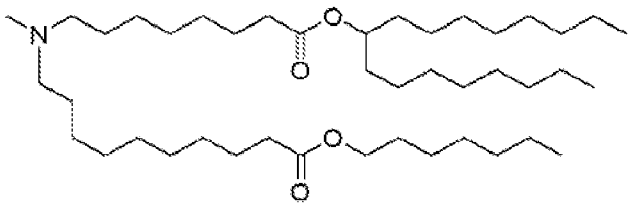

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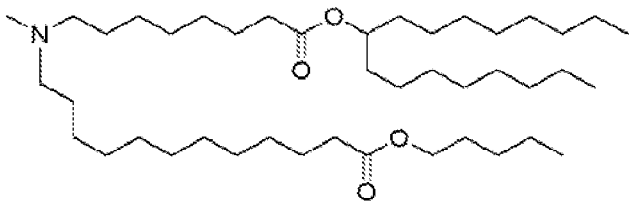

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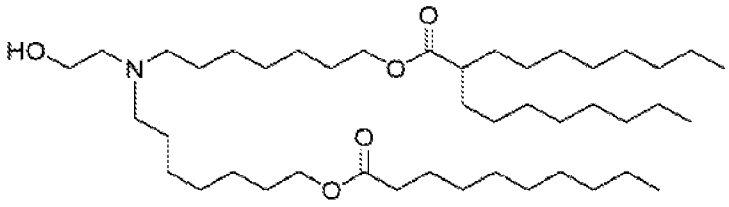

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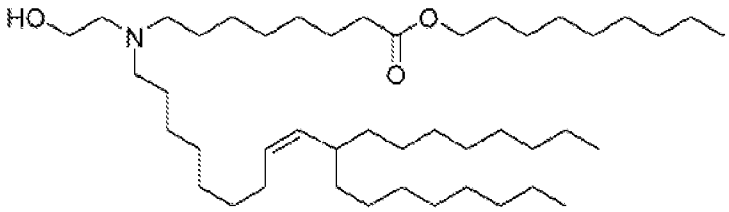

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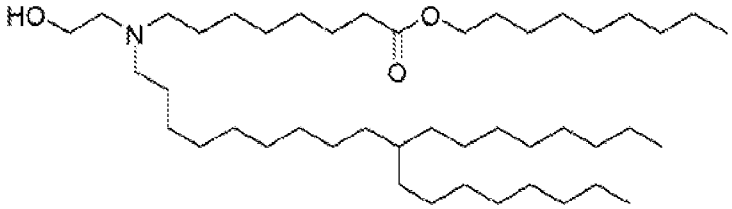

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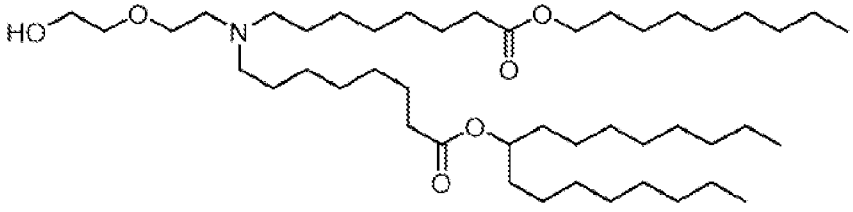

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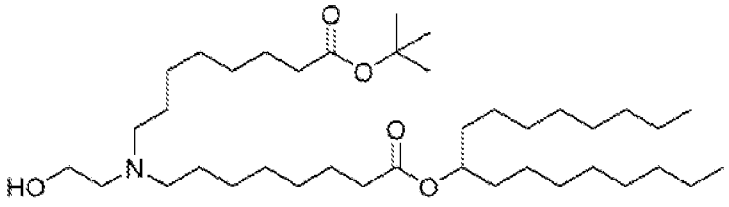

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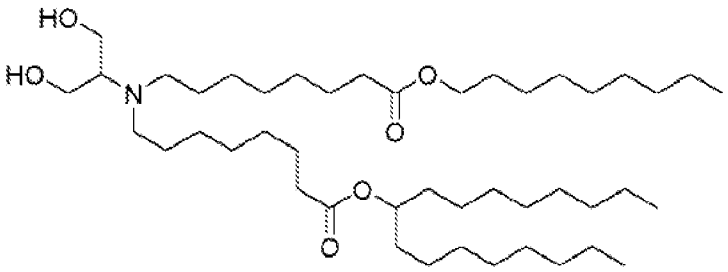

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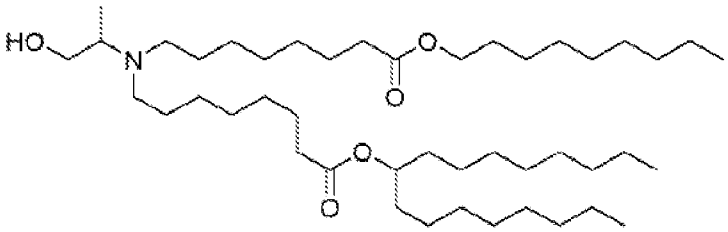

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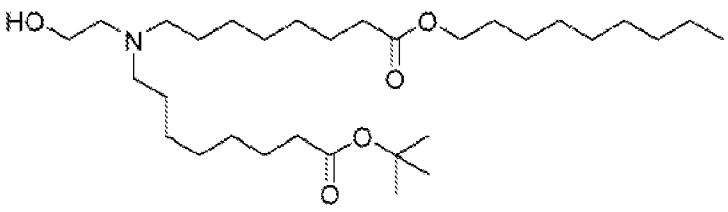

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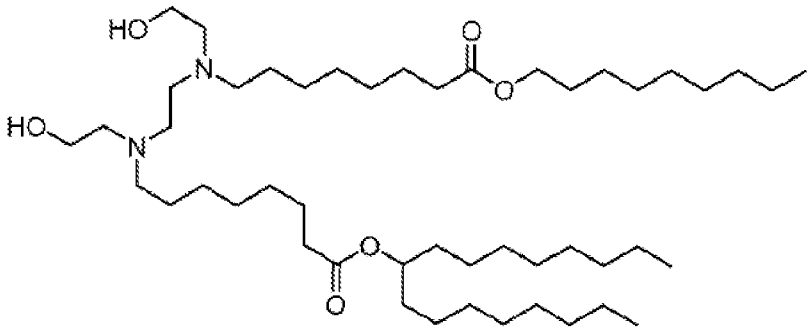

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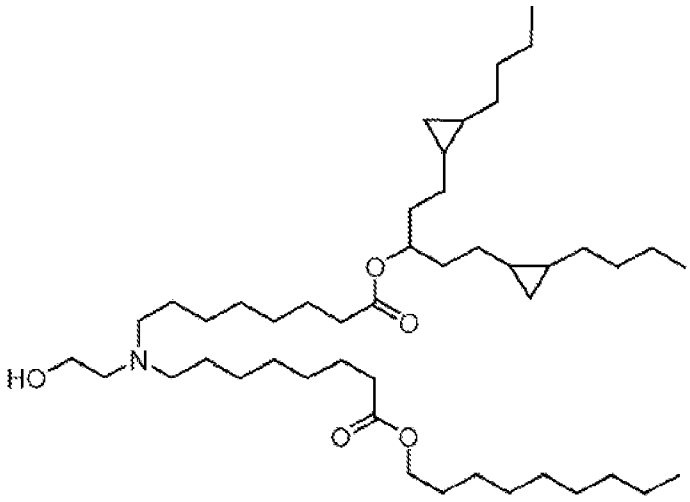

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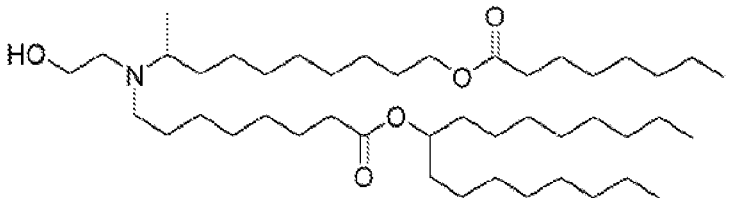

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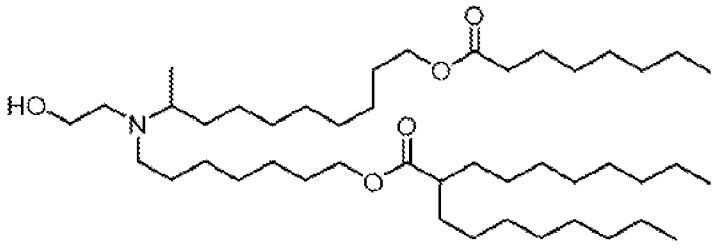

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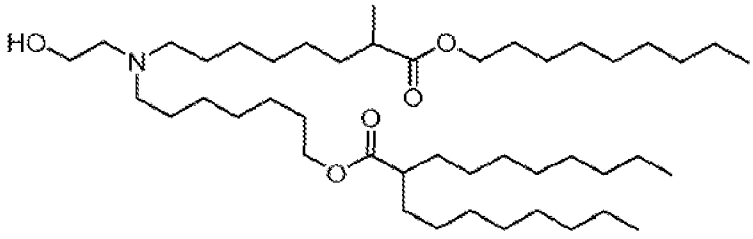

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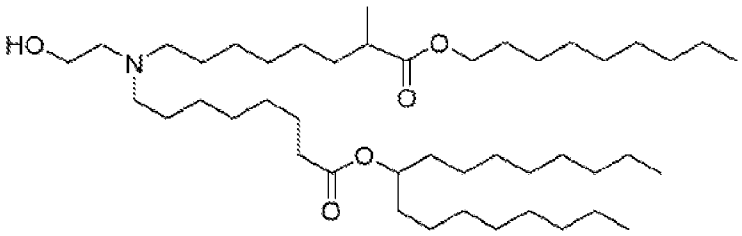

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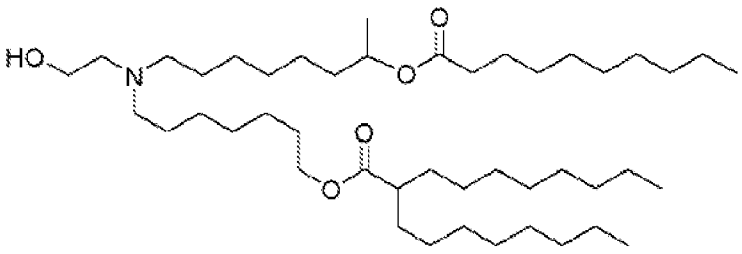

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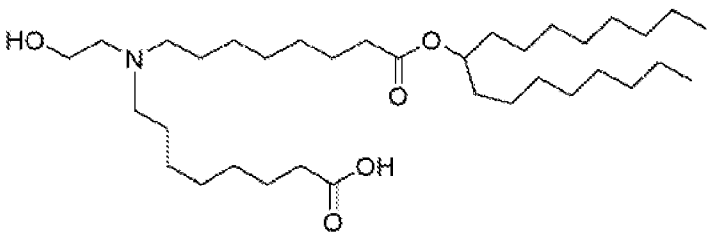

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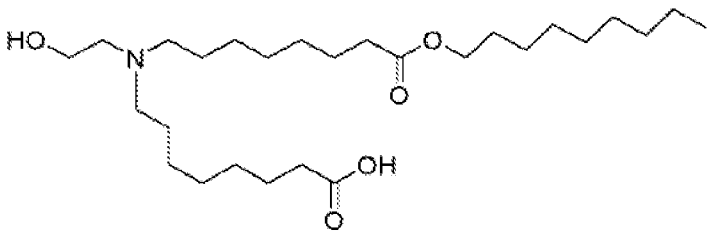

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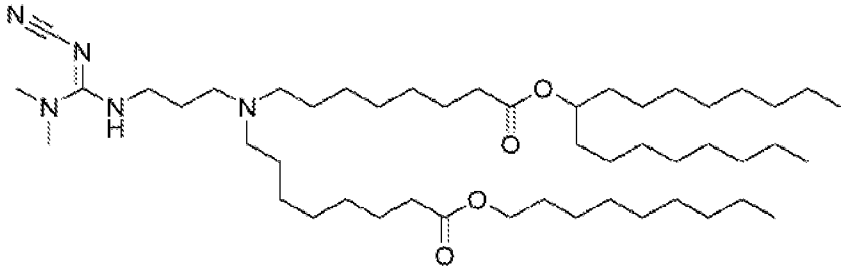

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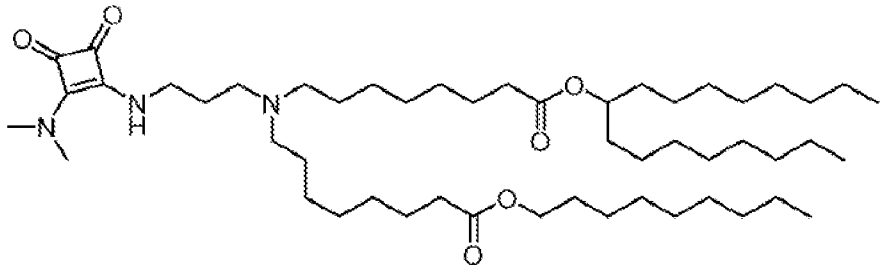

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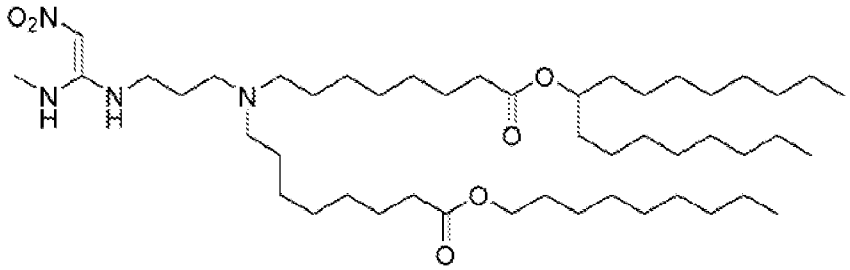

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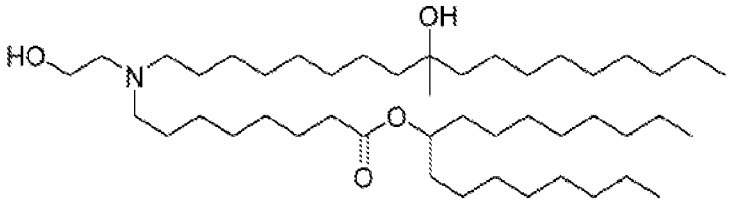

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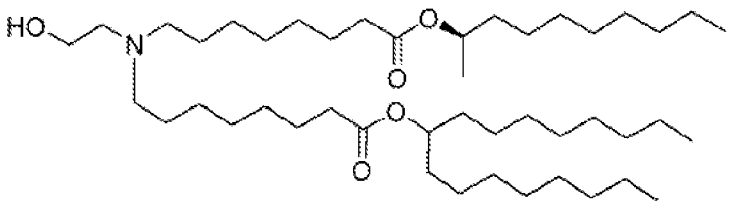

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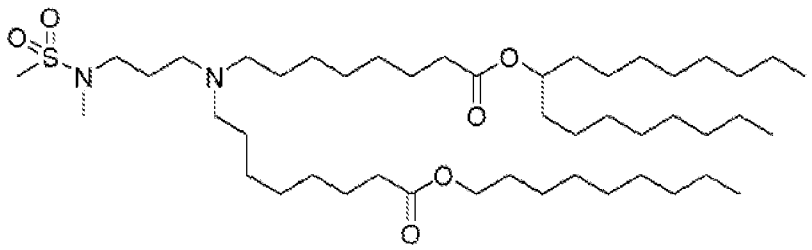

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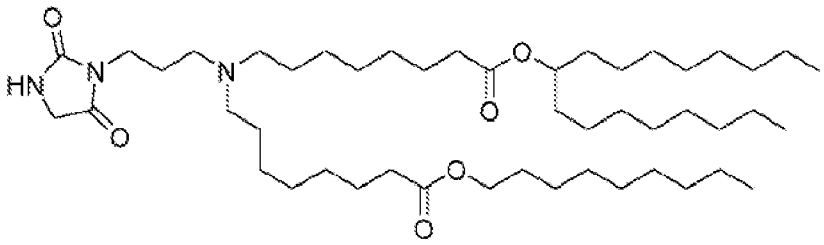

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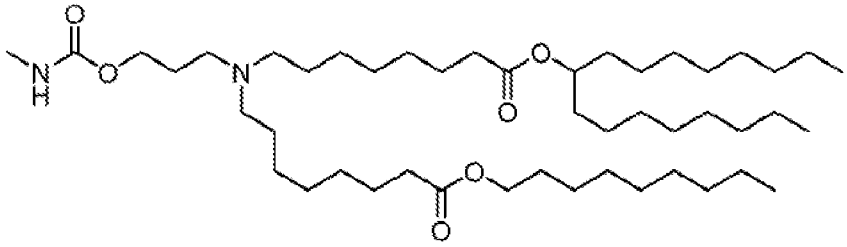

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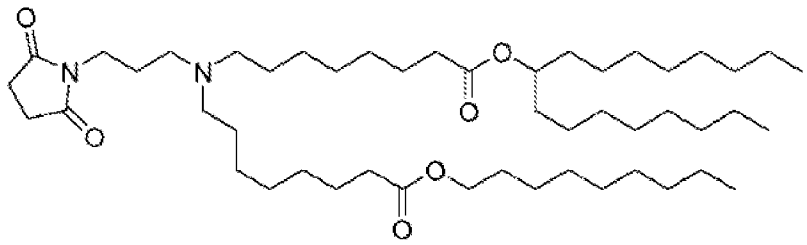

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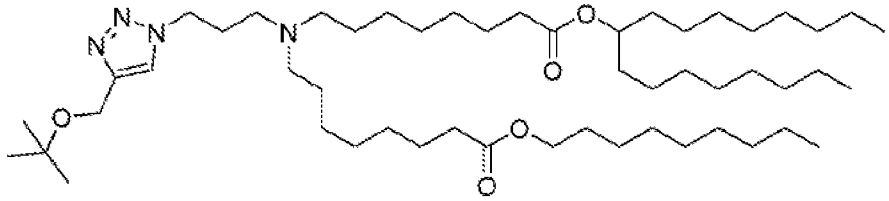

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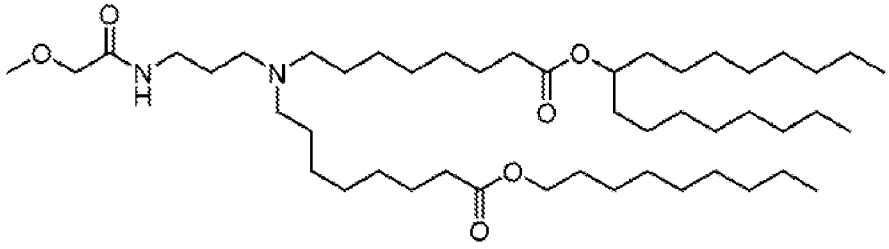

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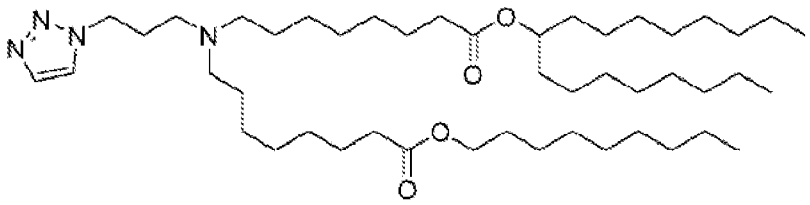

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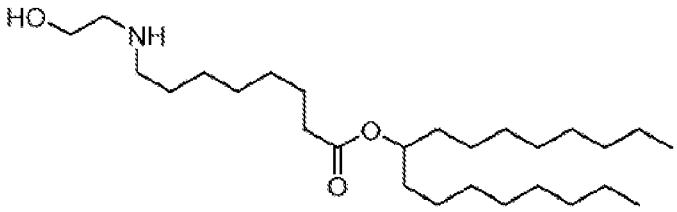

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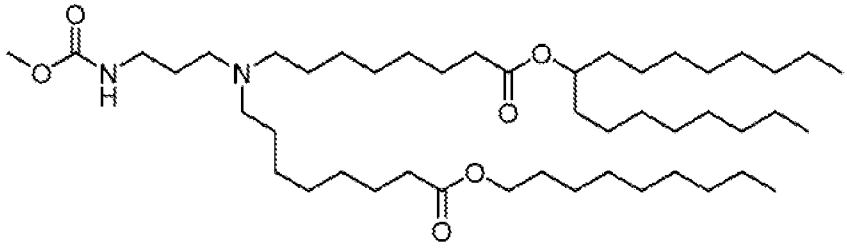

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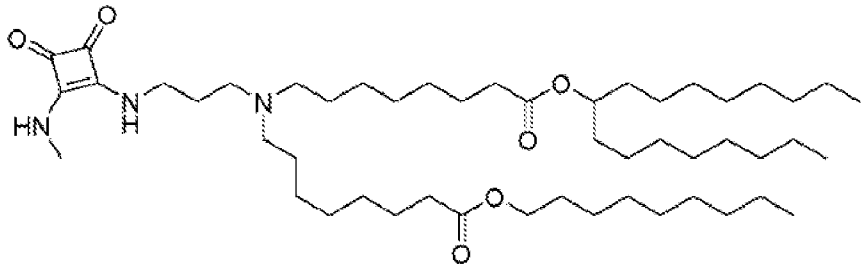

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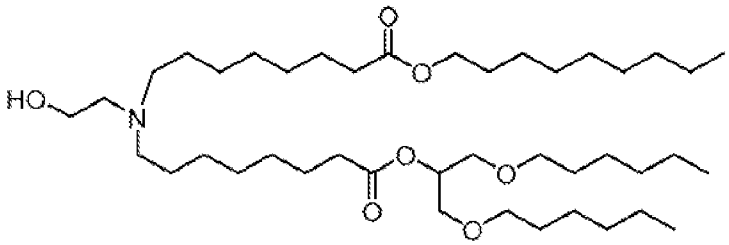

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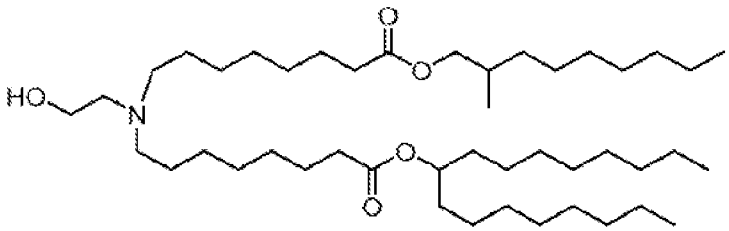

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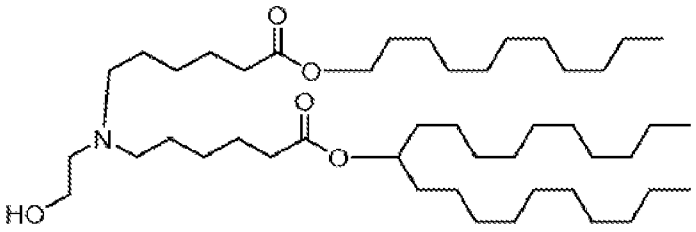

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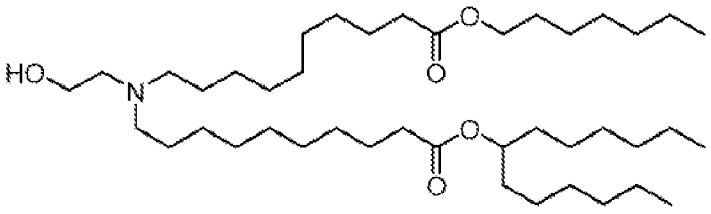

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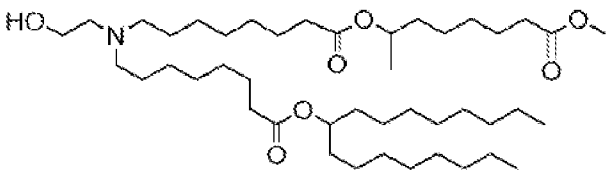

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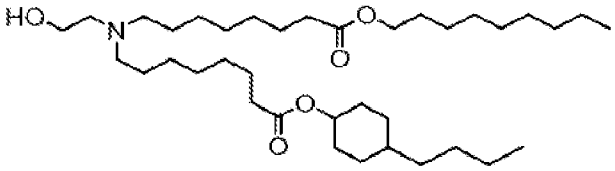

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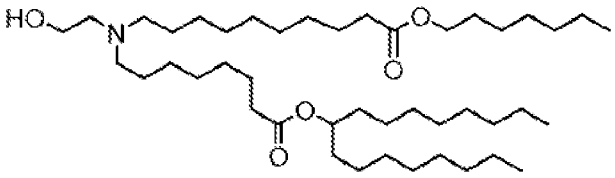

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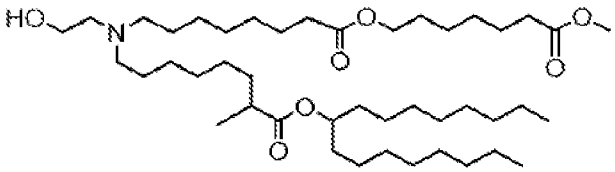

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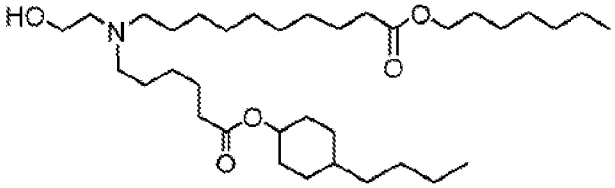

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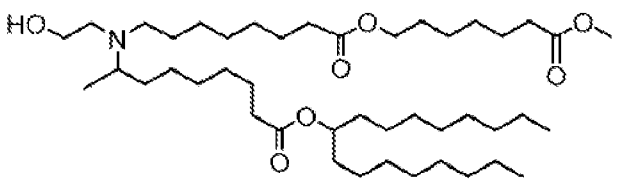

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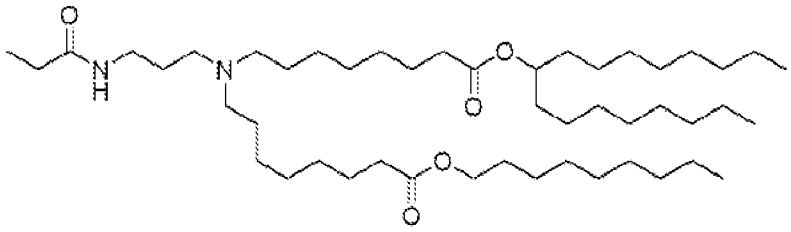

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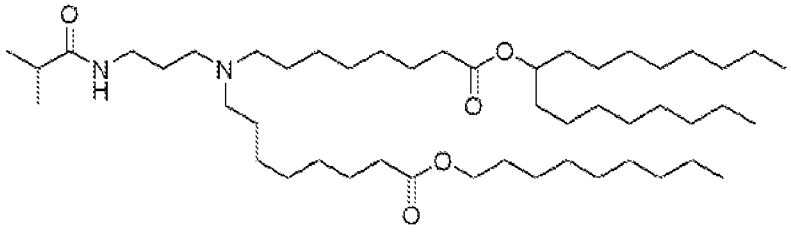

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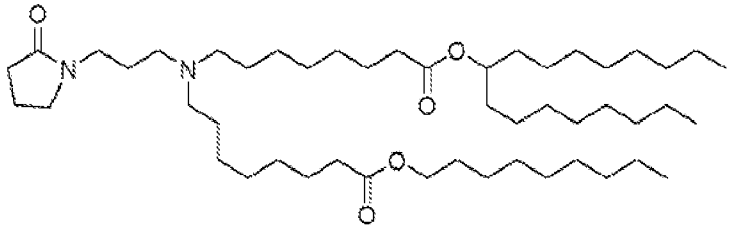

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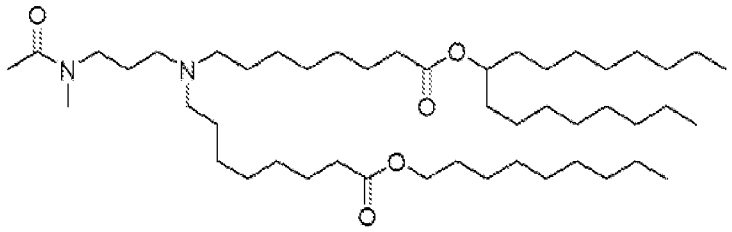

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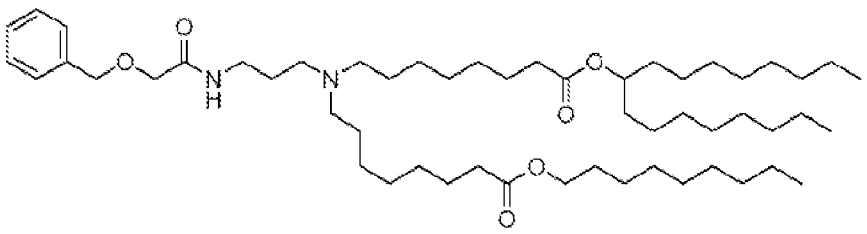

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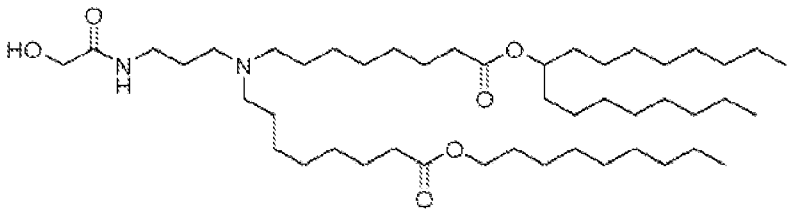

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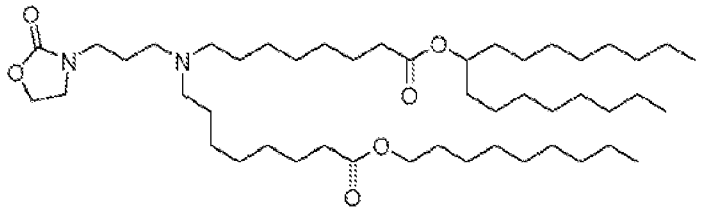

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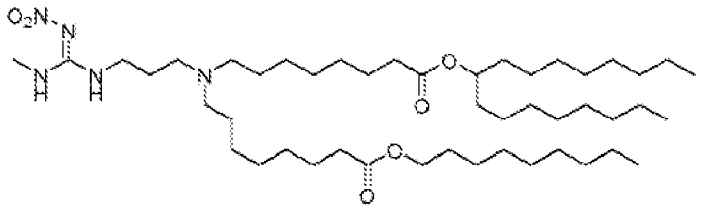

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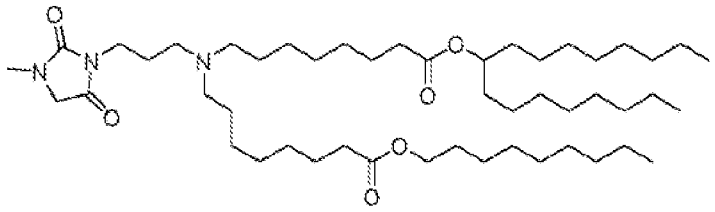

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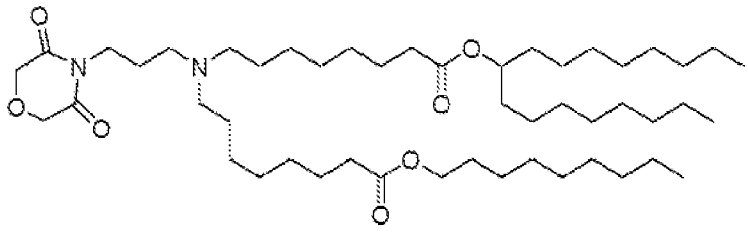

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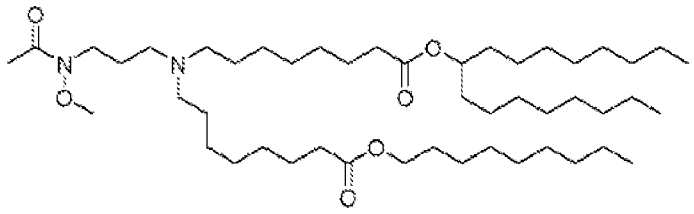

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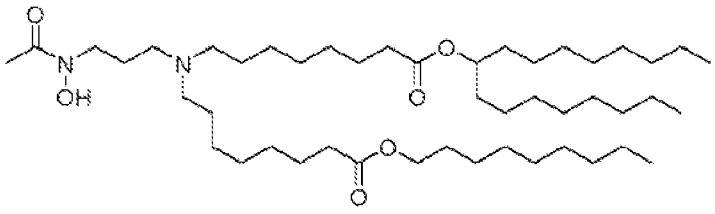

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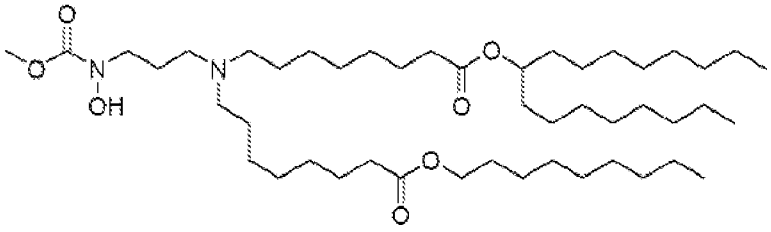

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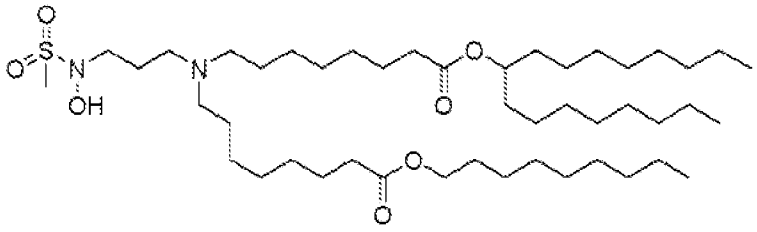

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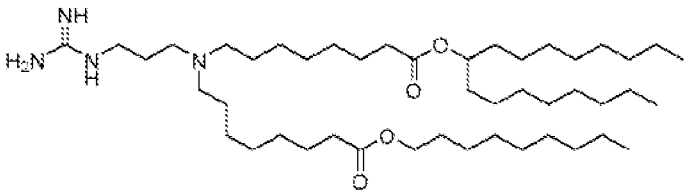

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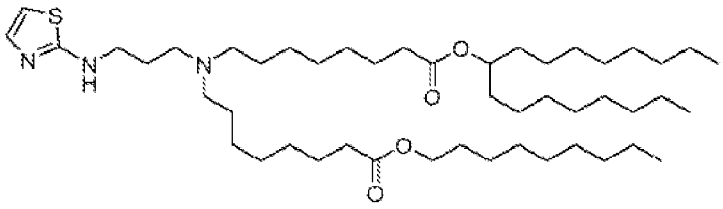

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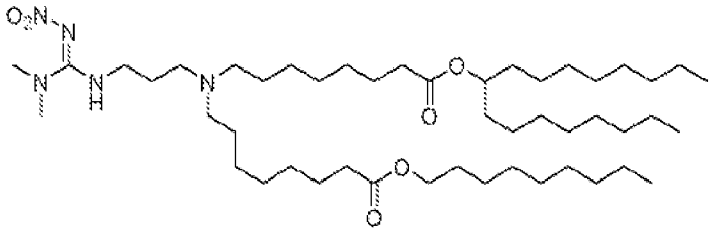

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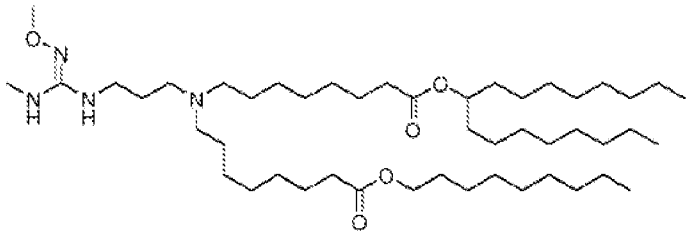

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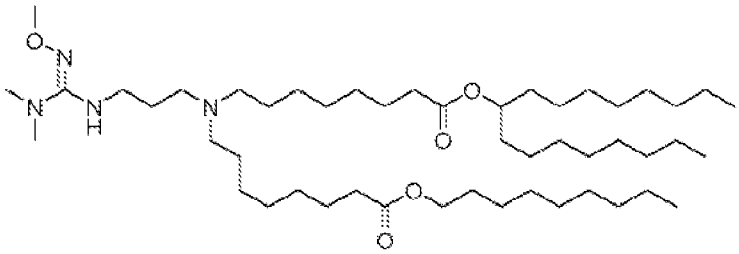

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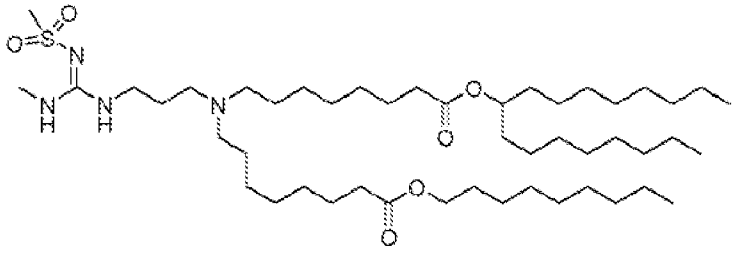

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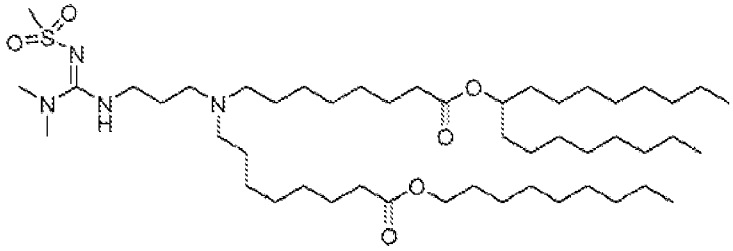

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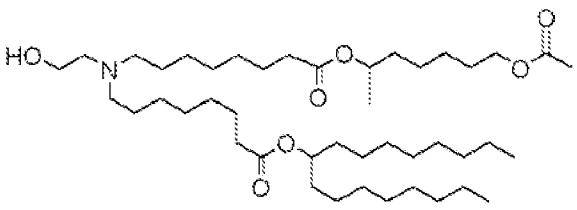

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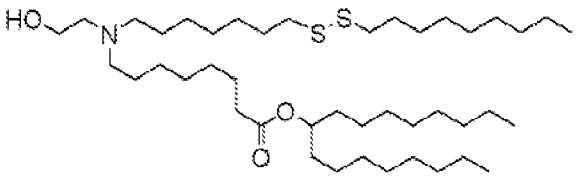

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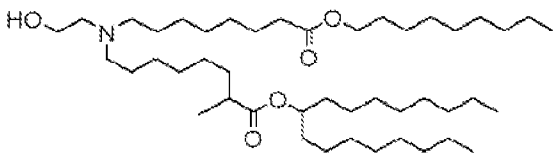

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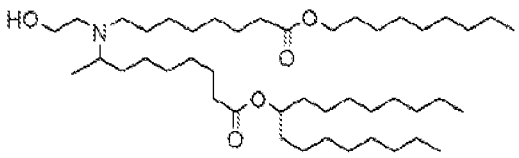

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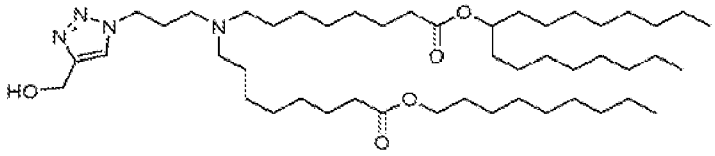

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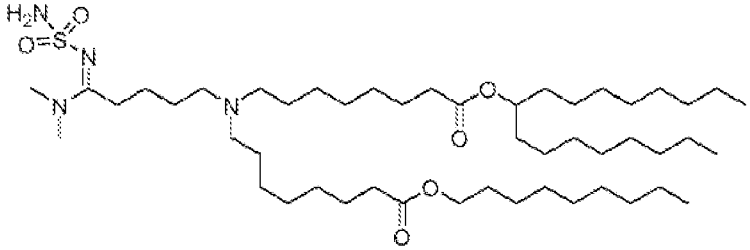

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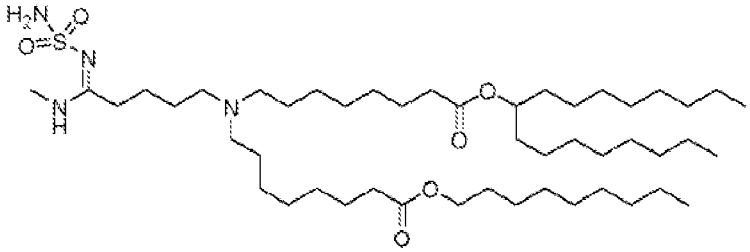

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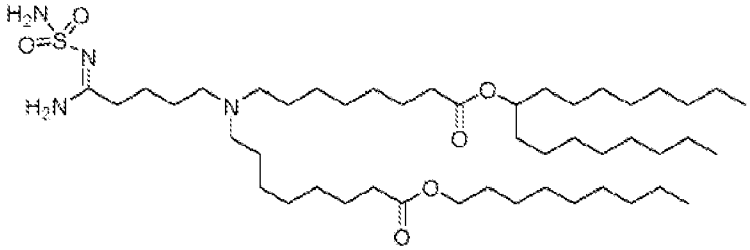

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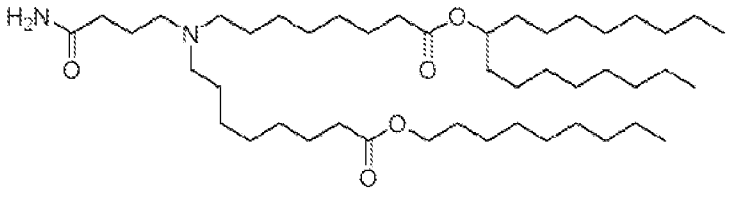

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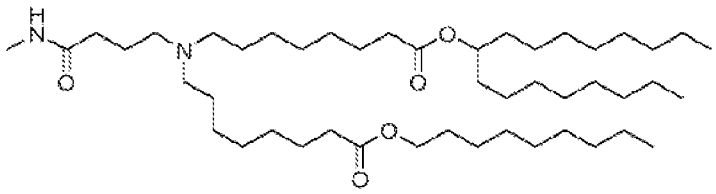

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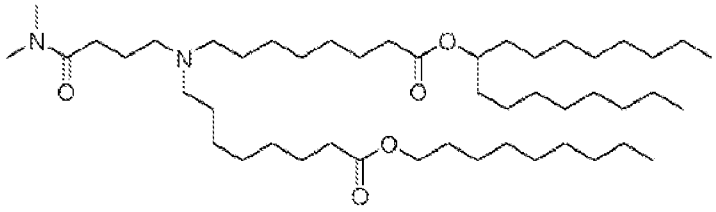

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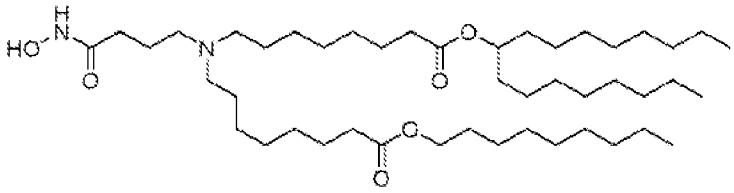

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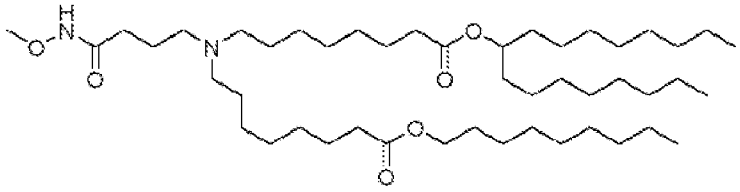

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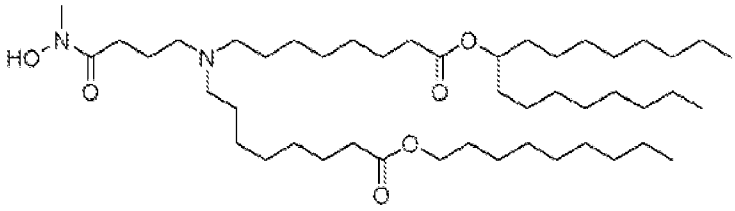

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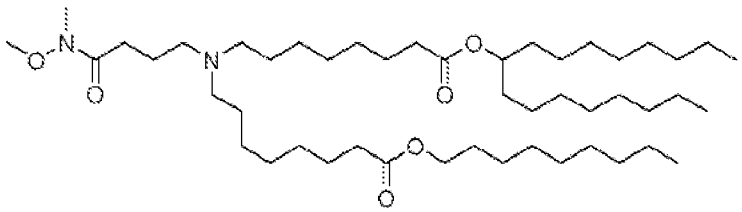

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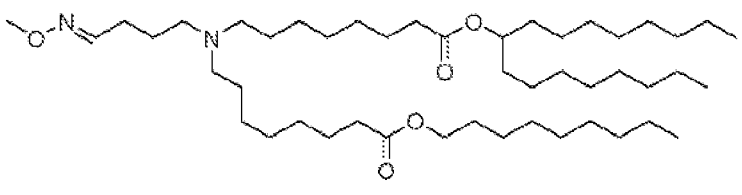

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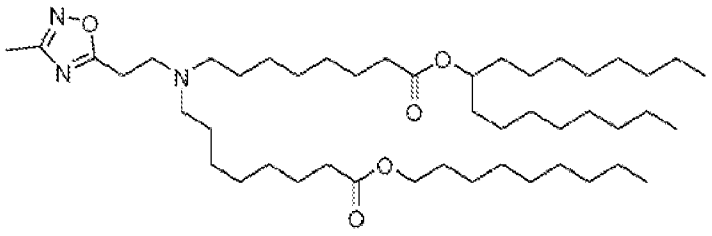

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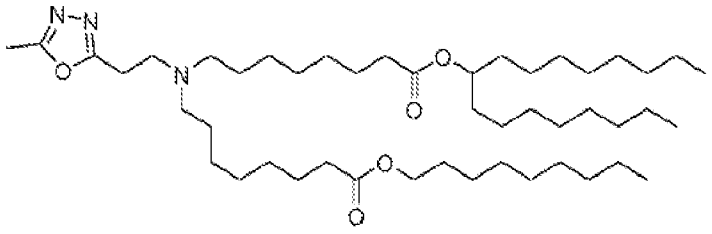
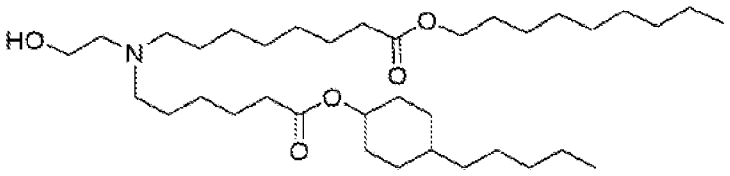


(Compound 231),



(Compound 232),

and salts or stereoisomers thereof.

[0755] In other cases, the compound of Formula (I) is selected from the group consisting of Compound 1-Compound 147, or salt or stereoisomers thereof.

[0756] Amine moieties of the lipid compounds disclosed herein can be protonated under certain conditions. For example, the central amine moiety of a lipid according to formula (I) is typically protonated (*i.e.*, positively charged) at a pH below the pKa of the amino moiety and is substantially not charged at a pH above the pKa. Such lipids may be referred to ionizable amino lipids.

[0757] In one specific case, the ionizable amino lipidis Compound 18.

[0758] In some cases, the amount the ionizable amino lipid, *e.g.*, compound of formula (I), ranges from about 1 mol % to 99 mol % in the lipid composition.

[0759] In one case, the amount of the ionizable amino lipid, *e.g.*, compound of formula (I), is at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 mol % in the lipid composition.

[0760] In one case, the amount of the ionizable amino lipid, *e.g.*, the compound of formula (I), ranges from about 30 mol % to about 70 mol %, from about 35 mol % to about 65 mol %, from about 40 mol % to about 60 mol %, and from about 45 mol % to about 55 mol % in the lipid composition.

[0761] In one specific case, the amount of the ionizable amino lipid, *e.g.*, compound of formula (I), is about 50 mol % in the lipid composition.

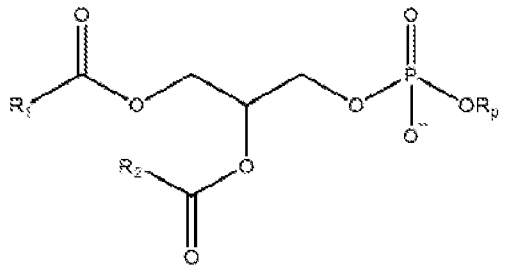
[0762] In addition to the ionizable amino lipid disclosed herein, *e.g.*, compound of formula (I), the lipid composition of the pharmaceutical compositions disclosed herein can comprise additional components such as phospholipids, structural lipids, quaternary amine compounds, PEG-lipids, and any combination thereof.

[0763] In other aspects, the lipid nanoparticle comprises a molar ratio of about 20-60% ionizable amino lipid: 5-25% phospholipid lipid: 25-55% sterol; and 0.5-15% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 20-60% Compound 18: 5-25% phospholipid: 25-55% cholesterol; and 0.5-15% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 50% ionizable amino lipid: about 10% phospholipid: about 38.5% cholesterol; and about 1.5% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 50% Compound 18: about 10% phospholipid: about 38.5% cholesterol; and about 1.5% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 49.83% ionizable amino lipid: about 9.83% phospholipid: about 30.33% cholesterol; and about 2.0% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 49.83% Compound 18: about 9.83% phospholipid: about 30.33% cholesterol; and about 2.0% PEG-modified lipid.

b. Additional Components in the Lipid Composition

(i) Phospholipids

[0764] The lipid composition of the pharmaceutical composition disclosed herein can comprise one or more phospholipids, for example, one or more saturated or (poly)unsaturated phospholipids or a combination thereof. In general, phospholipids comprise a phospholipid moiety and one or more fatty acid moieties. For example, a phospholipid can be a lipid according to formula (VII):



(VII)

in which R_p represents a phospholipid moiety and R_1 and R_2 represent fatty acid moieties with or without unsaturation that may be the same or different.

[0765] A phospholipid moiety may be selected, for example, from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and a sphingomyelin.

[0766] A fatty acid moiety may be selected, for example, from the non-limiting group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanoic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid.

[0767] Particular phospholipids may facilitate fusion to a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (e.g., a cellular or intracellular membrane). Fusion of a phospholipid to a membrane may allow one or more elements *(e.g.,* a therapeutic agent) of a lipid-containing composition *(e.g.,* LNPs) to pass through the membrane permitting, e.g., delivery of the one or more elements to a target tissue (e.g., tumoral tissue).

[0768] Non-natural phospholipid species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated. For example, a phospholipid may be functionalized with or cross-linked to one or more alkynes (e.g., an alkenyl group in which one or more double bonds is replaced with a triple bond). Under appropriate reaction conditions, an alkyne group may undergo a copper-catalyzed cycloaddition upon exposure to an azide. Such reactions may be useful in functionalizing a lipid bilayer of a nanoparticle composition to facilitate membrane permeation or cellular recognition or in conjugating a nanoparticle composition to a useful component such as a targeting or imaging moiety *(e.g.,* a dye).

[0769] Phospholipids include, but are not limited to, glycerophospholipids such as phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols, phosphatidy glycerols, and phosphatidic acids. Phospholipids also include phosphosphingolipid, such as sphingomyelin. In some cases, a pharmaceutical composition for intratumoral delivery disclosed herein can comprise more than one phospholipid. When more than one phospholipid is used, such phospholipids can belong to the same phospholipid class *(e.g.,* MSPC and DSPC) or different classes *(e.g.,* MSPC and MSPE).

[0770] Phospholipids may be of a symmetric or an asymmetric type. As used herein, the term "symmetric phospholipid" includes glycerophospholipids having matching fatty acid moieties and sphingolipids in which the variable fatty acid moiety and the hydrocarbon chain of the sphingosine backbone include a comparable number of carbon atoms. As used herein, the term "asymmetric phospholipid" includes lysolipids, glycerophospholipids having different fatty acid moieties (e.g., fatty acid moieties with different numbers of carbon atoms and/or unsaturations (e.g., double bonds)), and sphingolipids in which the variable fatty acid moiety and the hydrocarbon chain of the sphingosine backbone include a dissimilar number of carbon atoms *(e.g.,* the variable fatty acid moiety include at least two more carbon atoms than the hydrocarbon chain or at least two fewer carbon atoms than the hydrocarbon chain).

[0771] In some cases, the lipid composition of a pharmaceutical composition disclosed herein comprises at least one symmetric phospholipid. Symmetric phospholipids may be selected from the non-limiting group consisting of

1,2-dipropionyl-*sn*-glycero-3-phosphocholine (03:0 PC),

1,2-dibutyryl-*sn*-glycero-3-phosphocholine (04:0 PC),
1,2-dipentanoyl-*sn*-glycero-3-phosphocholine (05:0 PC),
1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (06:0 PC),
1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (07:0 PC),
1,2-dioctanoyl-*sn*-glycero-3-phosphocholine (08:0 PC),
1,2-dinonanoyl-*sn*-glycero-3-phosphocholine (09:0 PC),
1,2-didecanoyl-*sn*-glycero-3-phosphocholine (10:0 PC),
1,2-diundecanoyl-*sn*-glycero-3-phosphocholine (11:0 PC, DUPC),
1,2-dilauroyl-*sn*-glycero-3-phosphocholine (12:0 PC),
1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine (13:0 PC),
1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (14:0 PC, DMPC),
1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine (15:0 PC),
1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (16:0 PC, DPPC),
1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (4ME 16:0 PC),
1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine (17:0 PC),
1,2-distearoyl-*sn*-glycero-3-phosphocholine (18:0 PC, DSPC),
1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine (19:0 PC),
1,2-diarachidoyl-*sn*-glycero-3-phosphocholine (20:0 PC),
1,2-dihenarachidoyl-*sn*-glycero-3-phosphocholine (21:0 PC),
1,2-dibehenoyl-*sn*-glycero-3-phosphocholine (22:0 PC),
1,2-ditricosanoyl-*sn*-glycero-3-phosphocholine (23:0 PC),
1,2-dilignoceroyl-*sn*-glycero-3-phosphocholine (24:0 PC),
1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (14:1 (Δ9-Cis) PC),
1,2-dimyristelaidoyl-*sn*-glycero-3-phosphocholine (14:1 (Δ9-Trans) PC),
1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (16:1 (Δ9-Cis) PC),
1,2-dipalmitelaidoyl-*sn*-glycero-3-phosphocholine (16:1 (Δ9-Trans) PC),
1,2-dipetroselenoyl-*sn*-glycero-3-phosphocholine (18:1 (Δ6-Cis) PC),
1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18:1 (Δ9-Cis) PC, DOPC),
1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (18:1 (Δ9-Trans) PC),
1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (18:2 (Cis) PC, DLPC),
1,2-dilinolenoyl-*sn*-glycero-3-phosphocholine (18:3 (Cis) PC, DLnPC),
1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine (20:1 (Cis) PC),
1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine (20:4 (Cis) PC, DAPC),
1,2-dierucoyl-*sn*-glycero-3-phosphocholine (22:1 (Cis) PC),
1,2-didocosahexaenoyl-*sn*-glycero-3-phosphocholine (22:6 (Cis) PC, DHAPC),
1,2-dinervonoyl-*sn*-glycero-3-phosphocholine (24:1 (Cis) PC),
1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine (06:0 PE),
1,2-dioctanoyl-*sn*-glycero-3-phosphoethanolamine (08:0 PE),
1,2-didecanoyl-*sn*-glycero-3-phosphoethanolamine (10:0 PE),
1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (12:0 PE),
1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (14:0 PE),
1,2-dipentadecanoyl-*sn*-glycero-3-phosphoethanolamine (15:0 PE),
1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (16:0 PE),
1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (4ME 16:0 PE),
1,2-diheptadecanoyl-*sn*-glycero-3-phosphoethanolamine (17:0 PE),
1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (18:0 PE, DSPE),
1,2-dipalmitoleoyl-*sn*-glycero-3-phosphoethanolamine (16:1 PE),
1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (18:1 (Δ9-Cis) PE, DOPE),
1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine (18:1 (Δ9-Trans) PE),
1,2-dilinoleoyl-*sn*-glycero-3-phosphoethanolamine (18:2 PE, DLPE),
1,2-dilinolenoyl-*sn*-glycero-3-phosphoethanolamine (18:3 PE, DLnPE),
1,2-diarachidonoyl-*sn*-glycero-3-phosphoethanolamine (20:4 PE, DAPE),
1,2-didocosahexaenoyl-*sn*-glycero-3-phosphoethanolamine (22:6 PE, DHAPE),
1,2-di-O-octadecenyl-*sn*-glycero-3-phosphocholine (18:0 Diether PC),
1,2-dioleoyl-*sn*-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and
any combination thereof.

[0772] In some cases, the lipid composition of a pharmaceutical composition disclosed herein comprises at least one

symmetric phospholipid selected from the non-limiting group consisting of DLPC, DMPC, DOPC, DPPC, DSPC, DUPC, 18:0 Diether PC, DLnPC, DAPC, DHAPC, DOPE, 4ME 16:0 PE, DSPE, DLPE,DLnPE, DAPE, DHAPE, DOPG, and any combination thereof.

[0773] In some cases, the lipid composition of a pharmaceutical composition disclosed herein comprises at least one asymmetric phospholipid. Asymmetric phospholipids may be selected from the non-limiting group consisting of

1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (14:0-16:0 PC, MPPC),
1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (14:0-18:0 PC, MSPC),
1-palmitoyl-2-acetyl-*sn*-glycero-3-phosphocholine (16:0-02:0 PC),
1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (16:0-14:0 PC, PMPC),
1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (16:0-18:0 PC, PSPC),
1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16:0-18:1 PC, POPC),
1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (16:0-18:2 PC, PLPC),
1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (16:0-20:4 PC),
1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (14:0-22:6 PC),
1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (18:0-14:0 PC, SMPC),
1-stearoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (18:0-16:0 PC, SPPC),
1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (18:0-18:1 PC, SOPC),
1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (18:0-18:2 PC),
1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (18:0-20:4 PC),
1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (18:0-22:6 PC),
1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (18:1-14:0 PC, OMPC),
1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (18:1-16:0 PC, OPPC),
1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (18:1-18:0 PC, OSPC),
1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (16:0-18:1 PE, POPE),
1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoethanolamine (16:0-18:2 PE),
1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine (16:0-20:4 PE),
1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine (16:0-22:6 PE),
1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (18:0-18:1 PE),
1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphoethanolamine (18:0-18:2 PE),
1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine (18:0-20:4 PE),
1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine (18:0-22:6 PE),
1-oleoyl-2-cholesterylhemisuccinoyl-*sn*-glycero-3-phosphocholine (OChemsPC), and
any combination thereof.

[0774] Asymmetric lipids useful in the lipid composition may also be lysolipids. Lysolipids may be selected from the non-limiting group consisting of

1-hexanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (06:0 Lyso PC),
1-heptanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (07:0 Lyso PC),
1-octanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (08:0 Lyso PC),
1-nonanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (09:0 Lyso PC),
1-decanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (10:0 Lyso PC),
1-undecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (11:0 Lyso PC),
1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine (12:0 Lyso PC),
1-tridecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (13:0 Lyso PC),
1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (14:0 Lyso PC),
1-pentadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (15:0 Lyso PC),
1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (16:0 Lyso PC),
1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (17:0 Lyso PC),
1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (18:0 Lyso PC),
1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (18:1 Lyso PC),
1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (19:0 Lyso PC),
1-arachidoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (20:0 Lyso PC),
1-behenoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (22:0 Lyso PC),
1-lignoceroyl-2-hydroxy-*sn*-glycero-3-phosphocholine (24:0 Lyso PC),
1-hexacosanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (26:0 Lyso PC),
1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (14:0 Lyso PE),

1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (16:0 Lyso PE),
1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (18:0 Lyso PE),
1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (18:1 Lyso PE),
1-hexadecyl-*sn*-glycero-3-phosphocholine (C16 Lyso PC), and
any combination thereof.

[0775] In some case, the lipid composition of a pharmaceutical composition disclosed herein comprises at least one asymmetric phospholipid selected from the group consisting of MPPC, MSPC, PMPC, PSPC, SMPC, SPPC, and any combination thereof. In some cases, the asymmetric phospholipid is 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine (MSPC). In a particular case, the asymmetric phospholipid is one or more phospholipid disclosed in International Application No. PCT/US17/27492, filed on April 13, 2017.

[0776] In some cases, the lipid compositions disclosed herein may contain one or more symmetric phospholipids, one or more asymmetric phospholipids, or a combination thereof. When multiple phospholipids are present, they can be present in equimolar ratios, or non-equimolar ratios.

[0777] In one case, the lipid composition of a pharmaceutical composition disclosed herein comprises a total amount of phospholipid (e.g., MSPC) which ranges from about 1 mol % to about 20 mol %, from about 5 mol %to about 20 mol %, from about 10 mol % to about 20 mol %, from about 15 mol % to about 20 mol %, from about 1 mol % to about 15 mol %, from about 5 mol % to about 15 mol %, from about 10 mol % to about 15 mol %, from about 5 mol % to about 10 mol % in the lipid composition. In one case, the amount of the phospholipid is from about 8 mol % to about 15 mol % in the lipid composition. In one case, the amount of the phospholipid (e.g., MSPC) is about 10 mol % in the lipid composition.

[0778] In some aspects, the amount of a specific phospholipid (e.g., MSPC) is at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mol % in the lipid composition.

(ii) Quaternary Amine Compounds

[0779] The lipid composition of a pharmaceutical composition disclosed herein can comprise one or more quaternary amine compounds (e.g., DOTAP). The term "quaternary amine compound" is used to include those compounds having one or more quaternary amine groups (e.g., trialkylamino groups) and permanently carrying a positive charge and existing in a form of a salt. For example, the one or more quaternary amine groups can be present in a lipid or a polymer *(e.g.,* PEG). In some cases, the quaternary amine compound comprises (1) a quaternary amine group and (2) at least one hydrophobic tail group comprising (i) a hydrocarbon chain, linear or branched, and saturated or unsaturated, and (ii) optionally an ether, ester, carbonyl, or ketal linkage between the quaternary amine group and the hydrocarbon chain. In some cases, the quaternary amine group can be a trimethylammonium group. In some cases, the quaternary amine compound comprises two identical hydrocarbon chains. In some cases, the quaternary amine compound comprises two different hydrocarbon chains.

[0780] In some cases, the lipid composition of a pharmaceutical composition disclosed herein comprises at least one quaternary amine compound. Quaternary amine compound may be selected from the non-limiting group consisting of

1,2-dioleoyl-3-trimethylammonium-propane (DOTAP),
N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA),
1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM),
2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA),
N,N-distearyl-N,N-dimethylammonium bromide (DDAB),
N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE),
N-(1,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DORIE),
N,N-dioleyl-N,N-dimethylammonium chloride (DODAC),
1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC),
1,2-distearoyl-3-trimethylammonium-propane (DSTAP),
1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP),
1,2-dilinoleoyl-3-trimethylammonium-propane (DLTAP),
1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP)
1,2-distearoyl -sn-glycero-3-ethylphosphocholine (DSePC)
1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC),
1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC),
1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOePC),
1,2-di-(9Z-tetradecenoyl)-sn-glycero-3-ethylphosphocholine (14:1 EPC),
1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18:1 EPC),

and any combination thereof.

[0781] In one case, the quaternary amine compound is 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP).

[0782] Quaternary amine compounds are known in the art, such as those described in US 2013/0245107 A1, US 2014/0363493 A1, US 8,158,601, WO 2015/123264 A1, and WO 2015/148247 A1.

[0783] In one case, the amount of the quaternary amine compound (e.g., DOTAP) in the lipid composition disclosed herein ranges from about 0.01 mol % to about 20 mol %.

[0784] In one case, the amount of the quaternary amine compound (e.g., DOTAP) in the lipid composition disclosed herein ranges from about 0.5 mol % to about 20 mol %, from about 0.5 mol % to about 15 mol %, from about 0.5 mol % to about 10 mol %, from about 1 mol % to about 20 mol %, from about 1 mol % to about 15 mol %, from about 1 mol % to about 10 mol %, from about 2 mol % to about 20 mol %, from about 2 mol % to about 15 mol %, from about 2 mol % to about 10 mol %, from about 3 mol % to about 20 mol %, from about 3 mol % to about 15 mol %, from about 3 mol % to about 10 mol %, from about 4 mol % to about 20 mol %, from about 4 mol % to about 15 mol %, from about 4 mol % to about 10 mol %, from about 5 mol % to about 20 mol %, from about 5 mol % to about 15 mol %, from about 5 mol % to about 10 mol %, from about 6 mol % to about 20 mol %, from about 6 mol % to about 15 mol %, from about 6 mol % to about 10 mol %, from about 7 mol % to about 20 mol %, from about 7 mol % to about 15 mol %, from about 7 mol % to about 10 mol %, from about 8 mol % to about 20 mol %, from about 8 mol % to about 15 mol %, from about 8 mol % to about 10 mol %, from about 9 mol % to about 20 mol %, from about 9 mol % to about 15 mol %, from about 9 mol % to about 10 mol %.

[0785] In one case, the amount of the quaternary amine compound (e.g., DOTAP) in the lipid composition disclosed herein ranges from about 5 mol % to about 10 mol %.

[0786] In one case, the amount of the quaternary amine compound (e.g., DOTAP) in the lipid composition disclosed herein is about 5 mol %. In one case, the amount of the quaternary amine compound (e.g., DOTAP) in the lipid composition disclosed herein is about 10 mol %.

[0787] In some cases, the amount of the quaternary amine compound (e.g., DOTAP) is at least about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5 or 20 mol % in the lipid composition disclosed herein.

[0788] In some cases, the lipid composition of the pharmaceutical compositions disclosed herein comprises a compound of formula (I). In one case, the mole ratio of the compound of formula (I) (e.g., Compounds 18, 25, 26 or 48) to the quaternary amine compound *(e.g.,* DOTA) is about 100:1 to about 2.5:1. In one case, the mole ratio of the compound of formula (I) (e.g., Compounds 18, 25, 26 or 48) to the quaternary amine compound *(e.g.,* DOTAP) is about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, or about 2.5:1. In one case, the mole ratio of the compound of formula (I) (*e.g.,* Compounds 18, 25, 26 or 48) to the quaternary amine compound (e.g., DOTAP) in the lipid composition disclosed herein is about 10:1.

[0789] In some cases, the lipid composition of the pharmaceutical compositions disclosed herein comprises the lipid composition disclosed in International Application No. PCT/US2017/027492, filed April 13, 2017.

[0790] In some aspects, the lipid composition of the pharmaceutical compositions disclosed herein does not comprise a quaternary amine compound. In some aspects, the lipid composition of the pharmaceutical compositions disclosed herein does not comprise DOTAP.

(iii) Structural Lipids

[0791] The lipid composition of a pharmaceutical composition disclosed herein can comprise one or more structural lipids. As used herein, the term "structural lipid" refers to sterols and also to lipids containing sterol moieties. In some cases, the structural lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and mixtures thereof. In some cases, the structural lipid is cholesterol.

[0792] In one case, the amount of the structural lipid *(e.g.,* an sterol such as cholesterol) in the lipid composition of a pharmaceutical composition disclosed herein ranges from about 20 mol % to about 60 mol %, from about 25 mol % to about 55 mol %, from about 30 mol % to about 50 mol %, or from about 35 mol % to about 45 mol %.

[0793] In one case, the amount of the structural lipid *(e.g.,* an sterol such as cholesterol) in the lipid composition disclosed herein ranges from about 25 mol % to about 30 mol %, from about 30 mol % to about 35 mol %, or from about 35 mol % to about 40 mol %.

[0794] In one case, the amount of the structural lipid *(e.g.,* a sterol such as cholesterol) in the lipid composition disclosed herein is about 23.5 mol %, about 28.5 mol %, about 33.5 mol %, or about 38.5 mol %.

[0795] In some cases, the amount of the structural lipid *(e.g.,* an sterol such as cholesterol) in the lipid composition

disclosed herein is at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 mol %.

[0796] In some aspects, the lipid composition component of the pharmaceutical compositions for intratumoral delivery disclosed does not comprise cholesterol.

(iv) Polyethylene Glycol (PEG)-Lipids

[0797] The lipid composition of a pharmaceutical composition disclosed herein can comprise one or more a polyethylene glycol (PEG) lipid.

[0798] As used herein, the term "PEG-lipid" refers to polyethylene glycol (PEG)-modified lipids. Non-limiting examples of PEG-lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (e.g., PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. Such lipids are also referred to as PEGylated lipids. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid.

[0799] In some cases, the PEG-lipid includes, but not limited to 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-distearyl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA).

[0800] In one case, the PEG-lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, and mixtures thereof.

[0801] In some cases, the lipid moiety of the PEG-lipids includes those having lengths of from about C_{14} to about C_{22}, preferably from about C_{14} to about C_{16}. In some cases, a PEG moiety, for example an mPEG-NH_2, has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 daltons. In one case, the PEG-lipid is PEG_{2k}-DMG.

[0802] In one case, the lipid nanoparticles described herein may comprise a PEG lipid which is a non-diffusible PEG. Non-limiting examples of non-diffusible PEGs include PEG-DSG and PEG-DSPE.

[0803] PEG-lipids are known in the art, such as those described in U.S. Patent No. 8158601 and International Publ. No. WO 2015/130584 A2.

[0804] In one case, the amount of PEG-lipid in the lipid composition of a pharmaceutical composition disclosed herein ranges from about 0.1 mol % to about 5 mol %, from about 0.5 mol % to about 5 mol %, from about 1 mol % to about 5 mol %, from about 1.5 mol % to about 5 mol %, from about 2 mol % to about 5 mol % mol %, from about 0.1 mol % to about 4 mol %, from about 0.5 mol % to about 4 mol %, from about 1 mol % to about 4 mol %, from about 1.5 mol % to about 4 mol %, from about 2 mol % to about 4 mol %, from about 0.1 mol % to about 3 mol %, from about 0.5 mol % to about 3 mol %, from about 1 mol % to about 3 mol %, from about 1.5 mol % to about 3 mol %, from about 2 mol % to about 3 mol %, from about 0.1 mol % to about 2 mol %, from about 0.5 mol % to about 2 mol %, from about 1 mol % to about 2 mol %, from about 1.5 mol % to about 2 mol %, from about 0.1 mol % to about 1.5 mol %, from about 0.5 mol % to about 1.5 mol %, or from about 1 mol % to about 1.5 mol %.

[0805] In one case, the amount of PEG-lipid in the lipid composition disclosed herein is about 1.5 mol %.

[0806] In one case, the amount of PEG-lipid in the lipid composition disclosed herein is at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5 mol %.

[0807] In some aspects, the lipid composition of the pharmaceutical compositions disclosed herein does not comprise a PEG-lipid.

[0808] In some cases, the lipid composition disclosed herein comprises an ionizable amino lipid, *e.g.,* a compound of formula (I), and an asymmetric phospholipid. In some cases, the lipid composition comprises compound 18 and MSPC.

[0809] In some cases, the lipid composition disclosed herein comprises an ionizable amino lipid, *e.g.,* a compound of formula (I), and a quaternary amine compound. In some cases, the lipid composition comprises compound 18 and DOTAP.

[0810] In some cases, the lipid composition disclosed herein comprises an ionizable amino lipid, *e.g.,* a compound of formula (I), an asymmetric phospholipid, and a quaternary amine compound. In some cases, the lipid composition comprises compound 18, MSPC and DOTAP.

[0811] In one case, the lipid composition comprises about 50 mol % of a compound of formula (I) (e.g. Compounds 18, 25, 26 or 48), about 10 mol % of DSPC or MSPC, about 33.5 mol % of cholesterol, about 1.5 mol % of PEG-DMG, and about 5 mol % of DOTAP. In one case, the lipid composition comprises about 50 mol % of a compound of formula (I) (e.g. Compounds 18, 25, 26 or 48), about 10 mol % of DSPC or MSPC, about 28.5 mol % of cholesterol, about 1.5 mol % of PEG-DMG, and about 10 mol % of DOTAP

[0812] In some cases, the lipid nanoparticle comprises a molar ratio of about 20-60% ionizable amino lipid: 5-25% phospholipid: 25-55% sterol; and 0.5-15% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 20-60% Compound 18: 5-25% phospholipid: 25-55% cholesterol; and 0.5-15% PEG-modified

lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 50% ionizable amino lipid: about 10% phospholipid: about 38.5% cholesterol; and about 1.5% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 50% Compound 18: about 10% phospholipid: about 38.5% cholesterol; and about 1.5% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 49.83% ionizable amino lipid: about 9.83% phospholipid: about 30.33% cholesterol; and about 2.0% PEG-modified lipid. In other aspects, the lipid nanoparticle carrier comprises a molar ratio of about 49.83% Compound 18: about 9.83% phospholipid: about 30.33% cholesterol; and about 2.0% PEG-modified lipid.

[0813] The components of the lipid nanoparticle may be tailored for optimal delivery of the polynucleotides based on the desired outcome. As a non-limiting example, the lipid nanoparticle may comprise 40-60 mol % an ionizable amino lipid *(e.g.,* a compound of formula (I)), 8-16 mol % phospholipid, 30-45 mol % cholesterol, 1-5 mol % PEG lipid, and optionally 1-15 mol % quaternary amine compound.

[0814] In some cases, the lipid nanoparticle may comprise 45-65 mol % of an ionizable amino lipid *(e.g.,* a compound of formula (I)), 5-10 mol % phospholipid, 25-40 mol % cholesterol, 0.5-5 mol % PEG lipid, and optionally 1-15 mol % quaternary amine compound.

[0815] Non-limiting examples of nucleic acid lipid particles are disclosed in U.S. Patent Publication No. 20140121263.

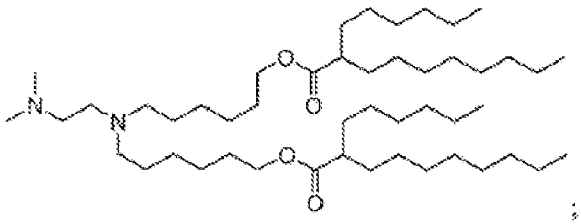
(v) Other Ionizable Amino Lipids

[0816] The lipid composition of the pharmaceutical composition disclosed herein can comprise one or more ionizable lipids in addition to a lipid according to formula (I). As used herein, the term "ionizable lipid" has its ordinary meaning in the art and may refer to a lipid comprising one or more charged moieties. In some cases, an ionizable lipid may be positively charged or negatively charged. An ionizable lipid may be positively charged, in which case it can be referred to as 'cationic lipid." For example, an ionizable molecule may comprise an amine group, referred to as ionizable amino lipids.

[0817] Ionizable lipids may be selected from the non-limiting group consisting of

3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10),
N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25),
1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA),
2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA),
2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA),
1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), (13Z,16Z)-N,N-dimethyl-3-nonydocosa-13-16-dien-1-amine (L608),
2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-di en-1-yloxy]propan-1-amine (Octyl-CLinDMA),
(2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9, 12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and
(2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9, 12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)). In addition to these, an ionizable amino lipid may also be a lipid including a cyclic amine group.

[0818] Ionizable lipids can also be the compounds disclosed in International Publication No. WO 2015/199952 A1. For example, the ionizable amino lipids include, but not limited to:



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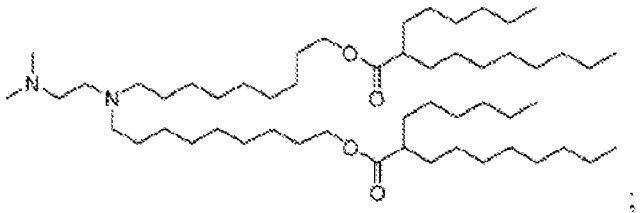
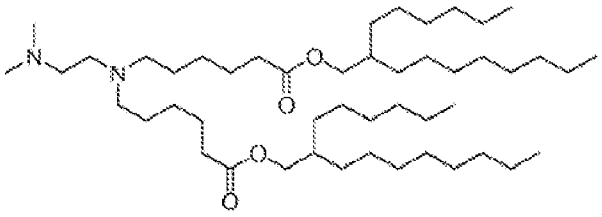
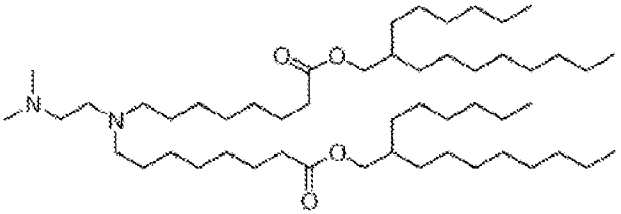
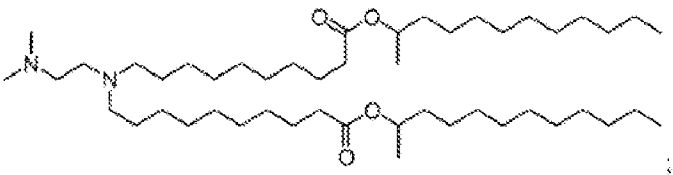
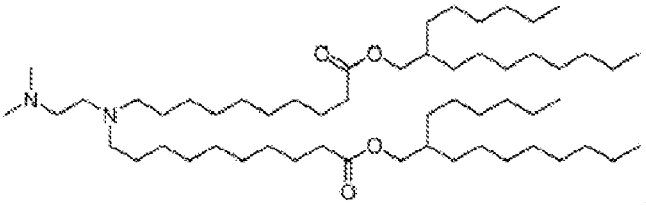
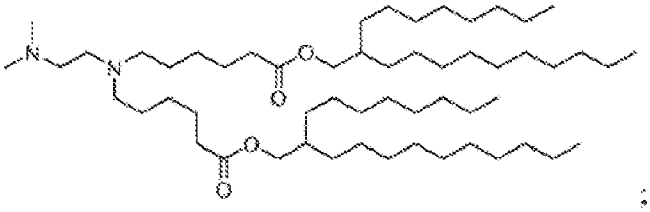


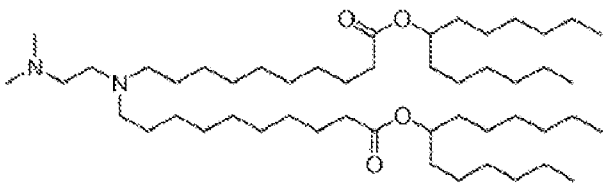


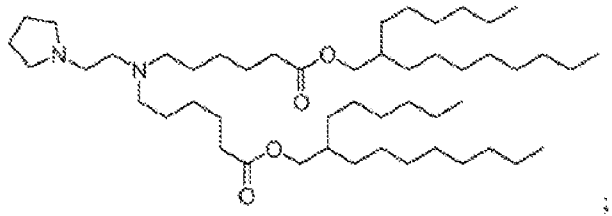


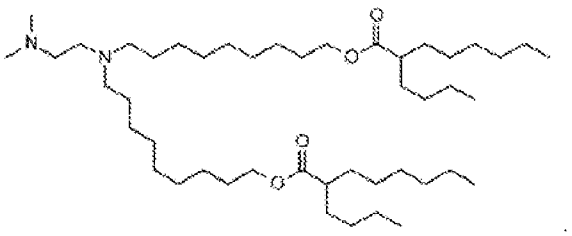












and any combination thereof.

(vi) Other Lipid Composition Components

[0819] The lipid composition of a pharmaceutical composition disclosed herein may include one or more components in addition to those described above. For example, the lipid composition may include one or more permeability enhancer molecules, carbohydrates, polymers, surface altering agents (e.g., surfactants), or other components. For example, a permeability enhancer molecule may be a molecule described by U.S. Patent Application Publication No. 2005/0222064. Carbohydrates may include simple sugars *(e.g.,* glucose) and polysaccharides *(e.g.,* glycogen and derivatives and analogs thereof). The lipid composition may include a buffer such as, but not limited to, citrate or phosphate at a pH of 7, salt and/or sugar. Salt and/or sugar may be included in the formulations described herein for isotonicity.

[0820] A polymer may be included in and/or used to encapsulate or partially encapsulate a pharmaceutical composition disclosed herein *(e.g.,* a pharmaceutical composition in lipid nanoparticle form). A polymer may be biodegradable and/or biocompatible. A polymer may be selected from, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates.

[0821] The ratio between the lipid composition and the polynucleotide range can be from about 10:1 to about 60:1 (wt/wt).

[0822] In some cases, the ratio between the lipid composition and the polynucleotide can be about 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 21:1, 22:1, 23:1, 24:1, 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 36:1, 37:1, 38:1, 39:1, 40:1, 41:1, 42:1, 43:1, 44:1, 45:1, 46:1, 47:1, 48:1, 49:1, 50:1, 51:1, 52:1, 53:1, 54:1, 55:1, 56:1, 57:1, 58:1, 59:1 or 60:1 (wt/wt). In some cases, the wt/wt ratio of the lipid composition to the polynucleotide encoding a therapeutic agent is about 20:1 or about 15:1.

[0823] In some cases, the pharmaceutical composition disclosed herein can contain more than one polypeptides. For example, a pharmaceutical composition disclosed herein can contain two or more polynucleotides *(e.g.,* RNA, *e.g.,* mRNA).

[0824] In one case, the lipid nanoparticles described herein may comprise polynucleotides (e.g., mRNA) in a lipid:polynucleotide weight ratio of 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1 or 70:1, or a range or any of these ratios such as, but not limited to, 5:1 to about 10:1, from about 5:1 to about 15:1, from about 5:1 to about 20:1, from about 5:1 to about 25:1, from about 5:1 to about 30:1, from about 5:1 to about 35:1, from about 5:1 to about 40:1, from about 5:1 to about 45:1, from about 5:1 to about 50:1, from about 5:1 to about 55:1, from about 5:1 to about 60:1, from about 5:1 to about 70:1, from about 10:1 to about 15:1, from about 10:1 to about 20:1, from about 10:1 to about 25:1, from about 10:1 to about 30:1, from about 10:1 to about 35:1, from about 10:1 to about 40:1, from about 10:1 to about 45:1, from about 10:1 to about 50:1, from about 10:1 to about 55:1, from about 10:1 to about 60:1, from about 10:1 to about 70:1, from about 15:1 to about 20:1, from about 15:1 to about 25:1,from about 15:1 to about 30:1, from about 15:1 to about 35:1, from about 15:1 to about 40:1, from about 15:1 to about 45:1, from about 15:1 to about 50:1, from about 15:1 to about 55:1, from about 15:1 to about 60:1 or from about 15:1 to about 70:1.

[0825] In one case, the lipid nanoparticles described herein may comprise the polynucleotide in a concentration from approximately 0.1 mg/ml to 2 mg/ml such as, but not limited to, 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml,

0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1.0 mg/ml, 1.1 mg/ml, 1.2 mg/ml, 1.3 mg/ml, 1.4 mg/ml, 1.5 mg/ml, 1.6 mg/ml, 1.7 mg/ml, 1.8 mg/ml, 1.9 mg/ml, 2.0 mg/ml or greater than 2.0 mg/ml.

[0826] In one case, formulations comprising the polynucleotides and lipid nanoparticles described herein may comprise 0.15 mg/ml to 2 mg/ml of the polynucleotide described herein (e.g., mRNA). In some cases, the formulation may further comprise 10 mM of citrate buffer and the formulation may additionally comprise up to 10% w/w of sucrose *(e.g.,* at least 1% w/w, at least 2% w/w/, at least 3% w/w, at least 4% w/w, at least 5% w/w, at least 6% w/w, at least 7% w/w, at least 8% w/w, at least 9% w/w or 10% w/w).

(vii) Nanoparticle Compositions

[0827] In some cases, the pharmaceutical compositions disclosed herein are formulated as lipid nanoparticles (LNP). Accordingly, the present disclosure also provides nanoparticle compositions comprising (i) a lipid composition comprising a delivery agent such as a compound of formula (I) as described herein, and (ii) a polynucleotide encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides. In such nanoparticle composition, the lipid composition disclosed herein can encapsulate the polynucleotide encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides.

[0828] Nanoparticle compositions are typically sized on the order of micrometers or smaller and may include a lipid bilayer. Nanoparticle compositions encompass lipid nanoparticles (LNPs), liposomes (e.g., lipid vesicles), and lipoplexes. For example, a nanoparticle composition may be a liposome having a lipid bilayer with a diameter of 500 nm or less.

[0829] Nanoparticle compositions include, for example, lipid nanoparticles (LNPs), liposomes, and lipoplexes. In some cases, nanoparticle compositions are vesicles including one or more lipid bilayers. In certain cases, a nanoparticle composition includes two or more concentric bilayers separated by aqueous compartments. Lipid bilayers may be functionalized and/or crosslinked to one another. Lipid bilayers may include one or more ligands, proteins, or channels.

[0830] Nanoparticle compositions of the present disclosure comprise at least one compound according to formula (I). For example, the nanoparticle composition can include one or more of Compounds 1-147, or one or more of Compounds 1-342. Nanoparticle compositions can also include a variety of other components. For example, the nanoparticle composition may include one or more other lipids in addition to a lipid according to formula (I), such as (i) at least one phospholipid, (ii) at least one quaternary amine compound, (iii) at least one structural lipid, (iv) at least one PEG-lipid, or (v) any combination thereof.

[0831] In some cases, the nanoparticle composition comprises a compound of formula (I), (e.g., Compounds 18, 25, 26 or 48). In some cases, the nanoparticle composition comprises a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48) and a phospholipid (e.g., DSPC or MSPC). In some cases, the nanoparticle composition comprises a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48), a phospholipid *(e.g.,* DSPC or MSPC), and a quaternary amine compound *(e.g.,* DOTAP). In some cases, the nanoparticle composition comprises a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48), and a quaternary amine compound (e.g., DOTAP).

[0832] In some cases, the nanoparticle composition comprises a lipid composition consisting or consisting essentially of compound of formula (I) (e.g., Compounds 18, 25, 26 or 48). In some cases, the nanoparticle composition comprises a lipid composition consisting or consisting essentially of a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48) and a phospholipid (e.g., DSPC or MSPC). In some cases, the nanoparticle composition comprises a lipid composition consisting or consisting essentially of a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48), a phospholipid (e.g., DSPC or MSPC), and a quaternary amine compound (e.g., DOTAP). In some cases, the nanoparticle composition comprises a lipid composition consisting or consisting essentially of a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48), and a quaternary amine compound (e.g., DOTAP).

[0833] In one case, the nanoparticle composition comprises (1) a lipid composition comprising about 50 mole % of a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48); about 10 mole % of DSPC or MSPC; about 33.5 mole % of cholesterol; about 1.5 mole % of PEG-DMG (e.g., PEG_{2k}-DMG); about 5 mole % of DOTAP; and (2) a polynucleotide.

[0834] In one case, the nanoparticle composition comprises (1) a lipid composition comprising about 50 mole % of a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48); about 10 mole % of DSPC or MSPC; about 28.5 mole % of cholesterol; about 1.5 mole % of PEG-DMG (e.g., PEG_{2k}-DMG); about 10 mole % of DOTAP; and (2) a polynucleotide.

[0835] In one case, the nanoparticle composition comprises (1) a lipid composition comprising about 50 mole % of a compound of formula (I) (e.g., Compounds 18, 25, 26 or 48); about 10 mole % of DSPC or MSPC; about 23.5 mole % of cholesterol; about 1.5 mole % of PEG-DMG (e.g., PEG_{2k}-DMG); about 15 mole % of DOTAP; and (2) a polynucleotide.

[0836] Nanoparticle compositions may be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy or scanning electron microscopy) may be used to examine the morphology and size distribution of a nanoparticle composition. Dynamic light scattering or potentiometry (e.g., potentiometric titrations) may be used to measure zeta potentials. Dynamic light scattering may also be utilized to determine particle sizes. Instruments such as the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) may also be used to measure multiple characteristics of a nanoparticle composition, such as particle size, polydispersity index, and zeta potential.

[0837] As generally defined herein, the term "lipid" refers to a small molecule that has hydrophobic or amphiphilic properties. Lipids may be naturally occurring or synthetic. Examples of classes of lipids include, but are not limited to, fats, waxes, sterol-containing metabolites, vitamins, fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides, and prenol lipids. In some instances, the amphiphilic properties of some lipids leads them to form liposomes, vesicles, or membranes in aqueous media.

[0838] In some cases, a lipid nanoparticle (LNP) may comprise an ionizable lipid. As used herein, the term "ionizable lipid" has its ordinary meaning in the art and may refer to a lipid comprising one or more charged moieties. In some cases, an ionizable lipid may be positively charged or negatively charged. An ionizable lipid may be positively charged, in which case it can be referred to as "cationic lipid". For example, an ionizable molecule may comprise an amine group, referred to as ionizable amino lipids. As used herein, a "charged moiety" is a chemical moiety that carries a formal electronic charge, e.g., monovalent (+1, or -1), divalent (+2, or -2), trivalent (+3, or -3), etc. The charged moiety may be anionic (i.e., negatively charged) or cationic (i.e., positively charged). Examples of positively-charged moieties include amine groups (e.g., primary, secondary, and/or tertiary amines), ammonium groups, pyridinium group, guanidine groups, and imidizolium groups. In a particular case, the charged moieties comprise amine groups. Examples of negatively-charged groups or precursors thereof, include carboxylate groups, sulfonate groups, sulfate groups, phosphonate groups, phosphate groups, hydroxyl groups, and the like. The charge of the charged moiety may vary, in some cases, with the environmental conditions, for example, changes in pH may alter the charge of the moiety, and/or cause the moiety to become charged or uncharged. In general, the charge density of the molecule may be selected as desired.

[0839] It should be understood that the terms "charged" or "charged moiety" does not refer to a "partial negative charge" or "partial positive charge" on a molecule. The terms "partial negative charge" and "partial positive charge" are given its ordinary meaning in the art. A "partial negative charge" may result when a functional group comprises a bond that becomes polarized such that electron density is pulled toward one atom of the bond, creating a partial negative charge on the atom. Those of ordinary skill in the art will, in general, recognize bonds that can become polarized in this way.

[0840] In some cases, the ionizable lipid is an ionizable amino lipid, sometimes referred to in the art as an "ionizable cationic lipid". In one case, the ionizable amino lipid may have a positively charged hydrophilic head and a hydrophobic tail that are connected via a linker structure.

[0841] The size of the nanoparticles can help counter biological reactions such as, but not limited to, inflammation, or can increase the biological effect of the polynucleotide.

[0842] As used herein, "size" or "mean size" in the context of nanoparticle compositions refers to the mean diameter of a nanoparticle composition.

[0843] In one case, the polynucleotide encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides are formulated in lipid nanoparticles having a diameter from about 10 to about 100 nm such as, but not limited to, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about 20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm, about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm.

[0844] In one case, the nanoparticles have a diameter from about 10 to 500 nm. In one case, the nanoparticle has a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750 nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

[0845] In some cases, the largest dimension of a nanoparticle composition is 1 μm or shorter (e.g., 1 μm, 900 nm, 800 nm, 700 nm, 600 nm, 500 nm, 400 nm, 300 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, 50 nm, or shorter).

[0846] A nanoparticle composition may be relatively homogenous. A polydispersity index may be used to indicate the homogeneity of a nanoparticle composition, *e.g.*, the particle size distribution of the nanoparticle composition. A small *(e.g.,* less than 0.3) polydispersity index generally indicates a narrow particle size distribution. A nanoparticle composition may have a polydispersity index from about 0 to about 0.25, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25. In some cases, the polydispersity index of a nanoparticle composition disclosed herein may be from about 0.10 to about 0.20.

[0847] The zeta potential of a nanoparticle composition may be used to indicate the electrokinetic potential of the composition. For example, the zeta potential may describe the surface charge of a nanoparticle composition. Nanoparticle compositions with relatively low charges, positive or negative, are generally desirable, as more highly charged species

may interact undesirably with cells, tissues, and other elements in the body. In some cases, the zeta potential of a nanoparticle composition disclosed herein may be from about -10 mV to about +20 mV, from about -10 mV to about +15 mV, from about 10 mV to about +10 mV, from about -10 mV to about +5 mV, from about -10 mV to about 0 mV, from about -10 mV to about -5 mV, from about -5 mV to about +20 mV, from about -5 mV to about +15 mV, from about -5 mV to about +10 mV, from about -5 mV to about +5 mV, from about -5 mV to about 0 mV, from about 0 mV to about +20 mV, from about 0 mV to about +15 mV, from about 0 mV to about +10 mV, from about 0 mV to about +5 mV, from about +5 mV to about +20 mV, from about +5 mV to about +15 mV, or from about +5 mV to about +10 mV.

[0848] In some cases, the zeta potential of the lipid nanoparticles may be from about 0 mV to about 100 mV, from about 0 mV to about 90 mV, from about 0 mV to about 80 mV, from about 0 mV to about 70 mV, from about 0 mV to about 60 mV, from about 0 mV to about 50 mV, from about 0 mV to about 40 mV, from about 0 mV to about 30 mV, from about 0 mV to about 20 mV, from about 0 mV to about 10 mV, from about 10 mV to about 100 mV, from about 10 mV to about 90 mV, from about 10 mV to about 80 mV, from about 10 mV to about 70 mV, from about 10 mV to about 60 mV, from about 10 mV to about 50 mV, from about 10 mV to about 40 mV, from about 10 mV to about 30 mV, from about 10 mV to about 20 mV, from about 20 mV to about 100 mV, from about 20 mV to about 90 mV, from about 20 mV to about 80 mV, from about 20 mV to about 70 mV, from about 20 mV to about 60 mV, from about 20 mV to about 50 mV, from about 20 mV to about 40 mV, from about 20 mV to about 30 mV, from about 30 mV to about 100 mV, from about 30 mV to about 90 mV, from about 30 mV to about 80 mV, from about 30 mV to about 70 mV, from about 30 mV to about 60 mV, from about 30 mV to about 50 mV, from about 30 mV to about 40 mV, from about 40 mV to about 100 mV, from about 40 mV to about 90 mV, from about 40 mV to about 80 mV, from about 40 mV to about 70 mV, from about 40 mV to about 60 mV, and from about 40 mV to about 50 mV. In some cases, the zeta potential of the lipid nanoparticles may be from about 10 mV to about 50 mV, from about 15 mV to about 45 mV, from about 20 mV to about 40 mV, and from about 25 mV to about 35 mV. In some cases, the zeta potential of the lipid nanoparticles may be about 10 mV, about 20 mV, about 30 mV, about 40 mV, about 50 mV, about 60 mV, about 70 mV, about 80 mV, about 90 mV, and about 100 mV.

[0849] The term "encapsulation efficiency" of a polynucleotide describes the amount of the polynucleotide that is encapsulated by or otherwise associated with a nanoparticle composition after preparation, relative to the initial amount provided. As used herein, "encapsulation" may refer to complete, substantial, or partial enclosure, confinement, surrounding, or encasement.

[0850] Encapsulation efficiency is desirably high *(e.g.,* close to 100%). The encapsulation efficiency may be measured, for example, by comparing the amount of the polynucleotide in a solution containing the nanoparticle composition before and after breaking up the nanoparticle composition with one or more organic solvents or detergents.

[0851] Fluorescence may be used to measure the amount of free polynucleotide in a solution. For the nanoparticle compositions described herein, the encapsulation efficiency of a polynucleotide may be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some cases, the encapsulation efficiency may be at least 80%. In certain cases, the encapsulation efficiency may be at least 90%.

[0852] The amount of a polynucleotide present in a pharmaceutical composition disclosed herein can depend on multiple factors such as the size of the polynucleotide, desired target and/or application, or other properties of the nanoparticle composition as well as on the properties of the polynucleotide.

[0853] For example, the amount of an mRNA useful in a nanoparticle composition may depend on the size (expressed as length, or molecular mass), sequence, and other characteristics of the mRNA. The relative amounts of a polynucleotide in a nanoparticle composition may also vary.

[0854] The relative amounts of the lipid composition and the polynucleotide present in a lipid nanoparticle composition of the present disclosure can be optimized according to considerations of efficacy and tolerability. For compositions including an mRNA as a polynucleotide, the N:P ratio can serve as a useful metric.

[0855] As the N:P ratio of a nanoparticle composition controls both expression and tolerability, nanoparticle compositions with low N:P ratios and strong expression are desirable. N:P ratios vary according to the ratio of lipids to RNA in a nanoparticle composition.

[0856] In general, a lower N:P ratio is preferred. The one or more RNA, lipids, and amounts thereof may be selected to provide an N:P ratio from about 2:1 to about 30:1, such as 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 12:1, 14:1, 16:1, 18:1, 20:1, 22:1, 24:1, 26:1, 28:1, or 30:1. In certain cases, the N:P ratio may be from about 2:1 to about 8:1. In other cases, the N:P ratio is from about 5:1 to about 8:1. In certain cases, the N:P ratio is between 5:1 and 6:1. In one specific aspect, the N:P ratio is about is about 5.67:1.

[0857] In addition to providing nanoparticle compositions, the present disclosure also provides methods of producing lipid nanoparticles comprising encapsulating a polynucleotide. Such method comprises using any of the pharmaceutical compositions disclosed herein and producing lipid nanoparticles in accordance with methods of production of lipid nanoparticles known in the art. *See, e.g.,* Wang et al. (2015) "Delivery of oligonucleotides with lipid nanoparticles" Adv. Drug Deliv. Rev. 87:68-80; Silva et al. (2015) "Delivery Systems for Biopharmaceuticals. Part I: Nanoparticles and

Microparticles" Curr. Pharm. Technol. 16: 940-954; Naseri et al. (2015) "Solid Lipid Nanoparticles and Nanostructured Lipid Carriers: Structure, Preparation and Application" Adv. Pharm. Bull. 5:305-13; Silva et al. (2015) "Lipid nanoparticles for the delivery of biopharmaceuticals" Curr. Pharm. Biotechnol. 16:291-302, and references cited therein.

25. Other Delivery Agents

a. Liposomes, Lipoplexes, and Lipid Nanoparticles

[0858] In some cases, the compositions or formulations of the present disclosure comprise a delivery agent, *e.g.,* a liposome, a lioplexes, a lipid nanoparticle, or any combination thereof. The polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) can be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. Liposomes, lipoplexes, or lipid nanoparticles can be used to improve the efficacy of the polynucleotides directed protein production as these formulations can increase cell transfection by the polynucleotide; and/or increase the translation of encoded protein. The liposomes, lipoplexes, or lipid nanoparticles can also be used to increase the stability of the polynucleotides.

[0859] Liposomes are artificially-prepared vesicles that may primarily be composed of a lipid bilayer and may be used as a delivery vehicle for the administration of pharmaceutical formulations. Liposomes can be of different sizes. A multilamellar vesicle (MLV) may be hundreds of nanometers in diameter, and may contain a series of concentric bilayers separated by narrow aqueous compartments. A small unicellular vesicle (SUV) may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes may contain a low or a high pH value in order to improve the delivery of the pharmaceutical formulations.

[0860] The formation of liposomes may depend on the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimal size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and scale up production of safe and efficient liposomal products, etc.

[0861] As a non-limiting example, liposomes such as synthetic membrane vesicles may be prepared by the methods, apparatus and devices described in U.S. Pub. Nos. US20130177638, US20130177637, US20130177636, US20130177635, US20130177634, US20130177633, US20130183375, US20130183373, and US20130183372. In some cases, the polynucleotides described herein may be encapsulated by the liposome and/or it may be contained in an aqueous core that may then be encapsulated by the liposome as described in, *e.g.,* Intl. Pub. Nos. WO2012031046, WO2012031043, WO2012030901, WO2012006378, and WO2013086526; and U.S. Pub.Nos. US20130189351, US20130195969 and US20130202684.

[0862] In some cases, the polynucleotides described herein can be formulated in a cationic oil-in-water emulsion where the emulsion particle comprises an oil core and a cationic lipid that can interact with the polynucleotide anchoring the molecule to the emulsion particle. In some cases, the polynucleotides described herein can be formulated in a water-in-oil emulsion comprising a continuous hydrophobic phase in which the hydrophilic phase is dispersed. Exemplary emulsions can be made by the methods described in Intl. Pub. Nos. WO2012006380 and WO201087791.

[0863] In some cases, the polynucleotides described herein can be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex can be accomplished by methods as described in, *e.g.,* U.S. Pub. No. US20120178702. As a non-limiting example, the polycation can include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine and the cationic peptides described in Intl. Pub. No. WO2012013326 or U.S. Pub. No. US20130142818.

[0864] In some cases, the polynucleotides described herein can be formulated in a lipid nanoparticle (LNP) such as those described in Intl. Pub. Nos. WO2013123523, WO2012170930, WO2011127255 and WO2008103276; and U.S. Pub. No. US20130171646.

[0865] Lipid nanoparticle formulations typically comprise one or more lipids. In some cases, the lipid is an ionizable lipid *(e.g.,* an ionizable amino lipid). In some cases, the lipid is a cationic lipid. In some cases, lipid nanoparticle formulations further comprise other components, including a phospholipid, a structural lipid, a quaternary amine compound, and a molecule capable of reducing particle aggregation, for example a PEG or PEG-modified lipid.

[0866] Cationic and ionizable lipids may include those as described in, *e.g.,* Intl. Pub. Nos. WO2015199952, WO 2015130584, WO 2015011633, and WO2012040184 WO2013126803, WO2011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365, WO2012044638, WO2010080724, WO201021865, WO2008103276, and WO2013086373; U.S. Patent Nos. 7,893,302, 7,404,969, 8,283,333, and 8,466,122; and U.S. Pub. Nos. US20110224447, US20120295832, US20150315112, US20100036115,

US20120202871, US20130064894, US20130129785, US20130150625, US20130178541, US20130123338 and US20130225836. In some cases, the amount of the cationic and ionizable lipids in the lipid composition ranges from about 0.01 mol% to about 99 mol%.

[0867] Exemplary ionizable lipids include, but not limited to, any one of Compounds 1-147 disclosed herein, DLin-MC3-DMA (MC3), DLin-DMA, DLenDMA, DLin-D-DMA, DLin-K-DMA, DLin-M-C2-DMA, DLin-K-DMA, DLin-KC2-DMA, DLin-KC3-DMA, DLin-KC4-DMA, DLin-C2K-DMA, DLin-MP-DMA, DODMA, 98N12-5, C12-200, DLin-C-DAP, DLin-DAC, DLinDAP, DLinAP, DLin-EG-DMA, DLin-2-DMAP, KL10, KL22, KL25, Octyl-CLinDMA, Octyl-CLinDMA (2R), Octyl-CLinDMA (2S), and any combination thereof. Other exemplary ionizable lipids include, (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine (L608), (20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine, (17Z,20Z)-N,N-dimemyl-hexacosa-17,20-dien-9-amine, (16Z,19Z)-N5N-dimethylpentacosa-16,19-dien-8-amine, (13Z,16Z)-N,N-dimethyldocosa-13,16-dien-5-amine, (12Z,15Z)-N,N-dimethylhenicosa-12,15-dien-4-amine, (14Z,17Z)-N,N-dimethyltricosa-14,17-dien-6-amine, (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-7-amine, (18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-10-amine, (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-5-amine, (14Z,17Z)-N,N-dimethyltricosa-14,17-dien-4-amine, (19Z,22Z)-N,N-dimeihyloctacosa-19,22-dien-9-amine, (18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-8-amine, (17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-7-amine, (16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-6-amine, (22Z,25Z)-N,N-dimethylhentriaconta-22,25-dien-10-amine, (21Z,24Z)-N,N-dimethyltriaconta-21,24-dien-9-amine, (18Z)-N,N-dimetylheptacos-18-en-10-amine, (17Z)-N,N-dimethylhexacos-17-en-9-amine, (19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-7-amine, N,N-dimethylheptacosan-10-amine, (20Z,23Z)-N-ethyl-N-methylnonacosa-20,23-dien-10-amine, 1-[(11Z,14Z)-1-nonylicosa-11,14-dien-1-yl]pyrrolidine, (20Z)-N,N-dimethylheptacos-20-en-10-amine, (15Z)-N,N-dimethyl eptacos-15-en-10-amine, (14Z)-N,N-dimethylnonacos-14-en-10-amine, (17Z)-N,N-dimethylnonacos-17-en-10-amine, (24Z)-N,N-dimethyltritriacont-24-en-10-amine, (20Z)-N,N-dimethylnonacos-20-en-10-amine, (22Z)-N,N-dimethylhentriacont-22-en-10-amine, (16Z)-N,N-dimethylpentacos-16-en-8-amine, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl] eptadecan-8-amine, 1-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]nonadecan-10-amine, N,N-dimethyl-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine, N,N-dimethyl-1-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]nonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine, N,N-dimethyl-[(1R,2S)-2-undecylcyclopropyl]tetradecan-5-amine, N,N-dimethyl-3-{7-[(1S,2R)-2-octylcyclopropyl]heptyl}dodecan-1-amine, 1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine, 1-[(1S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine, R-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, S-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy)methyl]ethyl}pyrrolidine, (2S)-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-[(5Z)-oct-5-en-1-yloxy]propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy)methyl]ethyl}azetidine, (2S)-1-(hexyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, (2S)-1-(heptyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-(nonyloxy)-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-[(9Z)-octadec-9-en-1-yloxy]-3-(octyloxy)propan-2-amine; (2S)-N,N-dimethyl-1-[(6Z,9Z,12Z)-octadeca-6,9,12-trien-1-yloxy]-3-(octyloxy)propan-2-amine, (2S)-1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(pentyloxy)propan-2-amine, (2S)-1-(hexyloxy)-3-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethylpropan-2-amine, 1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3 -(octyloxy)propan-2-amine, 1-[(13Z,16Z)-docosa-13,16-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2S)-1-[(13Z,16Z)-docosa-13,16-dien-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, (2S)-1-[(13Z)-docos-13-en-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, 1-[(13Z)-docos-13-en-1-yloxy]-N,N-dimethyl-3 -(octyloxy)propan-2-amine, 1-[(9Z)-hexadec-9-en-1-yloxy]-N,N-dimethyl-3 - (octyloxy)propan-2-amine, (2R)-N,N-dimethyl-H(1-metoyloctyl)oxy]-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, (2R)-1-[(3,7-dimethyloctyl)oxy]-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-(octyloxy)-3-({8-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]octyl} oxy)propan-2-amine, N,N-dimethyl-1-{[8-(2-oclylcyclopropyl)octyl]oxy}-3-(octyloxy)propan-2-amine, and (11E,20Z,23Z)-N,N-dimethylnonacosa-11,20,2-trien-10-amine, and any combination thereof.

[0868] Phospholipids include, but are not limited to, glycerophospholipids such as phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols, phosphatidy glycerols, and phosphatidic acids. Phospholipids also include phosphosphingolipid, such as sphingomyelin. In some cases, the phospholipids are DLPC, DMPC, DOPC, DPPC, DSPC, DUPC, 18:0 Diether PC, DLnPC, DAPC, DHAPC, DOPE, 4ME 16:0 PE, DSPE, DLPE,DLnPE, DAPE, DHAPE, DOPG, and any combination thereof. In some cases, the phospholipids are MPPC, MSPC, PMPC, PSPC, SMPC, SPPC, DHAPE, DOPG, and any combination thereof. In some cases, the amount of phospholipids (e.g., DSPC and/or MSPC) in the lipid composition ranges from about 1 mol% to about 20 mol%.

[0869] The structural lipids include sterols and lipids containing sterol moieties. In some cases, the structural lipids include cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and mixtures thereof. In some cases, the structural lipid is cholesterol. In some cases, the amount of the structural lipids (e.g., cholesterol) in the lipid composition ranges from about 20 mol% to about 60 mol%.

[0870] The quaternary amine compound as described herein include 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N-(1,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), 1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), 1,2-distearoyl-3-trimethylammonium-propane (DSTAP), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), 1,2-dilinoleoyl-3-trimethylammonium-propane (DLTAP), 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3-ethylphosphocholine (DSePC), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOePC), 1,2-di-(9Z-tetradecenoyl)-sn-glycero-3-ethylphosphocholine (14:1 EPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18:1 EPC), and any combination thereof. In some cases, the amount of the quaternary amine compounds (*e.g.*, DOTAP) in the lipid composition ranges from about 0.01 mol% to about 20 mol%.

[0871] The PEG-modified lipids include PEG-modified phosphatidylethanolamine and phosphatidic acid, PEG-ceramide conjugates (*e.g.*, PEG-CerC14 or PEG-CerC20), PEG-modified dialkylamines and PEG-modified 1,2-diacyloxypropan-3-amines. Such lipids are also referred to as PEGylated lipids. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG DMPE, PEG-DPPC, or a PEG-DSPE lipid. In some cases, the PEG-lipid are 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA). In some cases, the PEG moiety has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 daltons. In some cases, the amount of PEG-lipid in the lipid composition ranges from about 0.1 mol% to about 5 mol%.

[0872] In some cases, the LNP formulations described herein can additionally comprise a permeability enhancer molecule. Non-limiting permeability enhancer molecules are described in U.S. Pub. No. US20050222064.

[0873] The LNP formulations can further contain a phosphate conjugate. The phosphate conjugate can increase *in vivo* circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates can be made by the methods described in, *e.g.,* Intl. Pub. No. WO2013033438 or U.S. Pub. No. US20130196948. The LNP formulation may also contain a polymer conjugate *(e.g.,* a water soluble conjugate) as described in, *e.g.,* U.S. Pub. Nos. US20130059360, US20130196948, and US20130072709.

[0874] The LNP formulations can comprise a conjugate to enhance the delivery of nanoparticles of the present disclosure in a subject. Further, the conjugate can inhibit phagocytic clearance of the nanoparticles in a subject. In some cases, the conjugate may be a "self" peptide designed from the human membrane protein CD47 *(e.g.,* the "self" particles described by Rodriguez et al, Science 2013 339, 971-975). As shown by Rodriguez et al. the self peptides delayed macrophage-mediated clearance of nanoparticles which enhanced delivery of the nanoparticles.

[0875] The LNP formulations can comprise a carbohydrate carrier. As a non-limiting example, the carbohydrate carrier can include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified phytoglycogen beta-dextrin (*e.g.*, Intl. Pub. No. WO2012109121).

[0876] The LNP formulations can be coated with a surfactant or polymer to improve the delivery of the particle. In some cases, the LNP may be coated with a hydrophilic coating such as, but not limited to, PEG coatings and/or coatings that have a neutral surface charge as described in U.S. Pub. No. US20130183244.

[0877] The LNP formulations can be engineered to alter the surface properties of particles so that the lipid nanoparticles may penetrate the mucosal barrier as described in U.S. Pat. No. 8,241,670 or Intl. Pub. No. WO2013110028.

[0878] The LNP engineered to penetrate mucus can comprise a polymeric material *(i.e.,* a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material can include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyeneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates.

[0879] LNP engineered to penetrate mucus can also include surface altering agents such as, but not limited to, polynucleotides, anionic proteins (*e.g.*, bovine serum albumin), surfactants (*e.g.*, cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (*e.g.*, cyclodextrin), nucleic acids, polymers *(e.g.,* heparin, polyethylene glycol and poloxamer), mucolytic agents *(e.g.,* N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase.

[0880] In some cases, the mucus penetrating LNP can be a hypotonic formulation comprising a mucosal penetration enhancing coating. The formulation can be hypotonic for the epithelium to which it is being delivered. Non-limiting

examples of hypotonic formulations may be found in, *e.g.,* Intl. Pub. No. WO2013110028.

[0881] In some cases, the polynucleotide described herein is formulated as a lipoplex, such as, without limitation, the ATUPLEXTM system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECTTM from STEMGENT® (Cambridge, MA), and polyethylenimine (PEI) or protamine-based targeted and non-targeted delivery of nucleic acids (Aleku et al. Cancer Res. 2008 68:9788-9798; Strumberg et al. Int J Clin Pharmacol Ther 2012 50:76-78; Santel et al., Gene Ther 2006 13:1222-1234; Santel et al., Gene Ther 2006 13:1360-1370; Gutbier et al., Pulm Pharmacol. Ther. 2010 23:334-344; Kaufmann et al. Microvasc Res 2010 80:286-293Weide et al. J Immunother. 2009 32:498-507; Weide et al. J Immunother. 2008 31:180-188; Pascolo Expert Opin. Biol. Ther. 4:1285-1294; Fotin-Mleczek et al., 2011 J. Immunother. 34:1-15; Song et al., Nature Biotechnol. 2005, 23:709-717; Peer et al., Proc Natl Acad Sci U S A. 2007 6;104:4095-4100; deFougerolles Hum Gene Ther. 2008 19:125-132).

[0882] In some cases, the polynucleotides described herein are formulated as a solid lipid nanoparticle (SLN), which may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. Exemplary SLN can be those as described in Intl. Pub. No. WO2013105101.

[0883] In some cases, the polynucleotides described herein can be formulated for controlled release and/or targeted delivery. As used herein, "controlled release" refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In one case, the polynucleotides can be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the compounds of the disclosure, encapsulation may be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.9 or greater than 99.999% of the pharmaceutical composition or compound of the disclosure may be enclosed, surrounded or encased within the delivery agent. "Partially encapsulation" means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical composition or compound of the disclosure may be enclosed, surrounded or encased within the delivery agent.

[0884] Advantageously, encapsulation can be determined by measuring the escape or the activity of the pharmaceutical composition or compound of the disclosure using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the pharmaceutical composition or compound of the disclosure are encapsulated in the delivery agent.

[0885] In some cases, the polynucleotide controlled release formulation can include at least one controlled release coating (*e.g.*, OPADRY®, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®)). In some cases, the polynucleotide controlled release formulation can comprise a polymer system as described in U.S. Pub. No. US20130130348, or a PEG and/or PEG related polymer derivative as described in U.S. Pat. No. 8,404,222.

[0886] In some cases, the polynucleotides described herein can be encapsulated in a therapeutic nanoparticle, referred to herein as "therapeutic nanoparticle polynucleotides." Therapeutic nanoparticles may be formulated by methods described in, *e.g.,* Intl. Pub. Nos. WO2010005740, WO2010030763, WO2010005721, WO2010005723, and WO2012054923; and U.S. Pub. Nos. US20110262491, US20100104645, US20100087337, US20100068285, US20110274759, US20100068286, US20120288541, US20120140790, US20130123351 and US20130230567; and U.S. Pat. Nos. 8,206,747, 8,293,276, 8,318,208 and 8,318,211.

[0887] In some cases, the therapeutic nanoparticle polynucleotide can be formulated for sustained release. As used herein, "sustained release" refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle of the polynucleotides described herein can be formulated as disclosed in Intl. Pub. No. WO2010075072 and U.S. Pub. Nos. US20100216804, US20110217377, US20120201859 and US20130150295.

[0888] In some cases, the therapeutic nanoparticle polynucleotide can be formulated to be target specific, such as those described in Intl. Pub. Nos. WO2008121949, WO2010005726, WO2010005725, WO2011084521 and WO2011084518; and U.S. Pub. Nos. US20100069426, US20120004293 and US20100104655.

[0889] The LNPs can be prepared using microfluidic mixers or micromixers. Exemplary microfluidic mixers can include, but are not limited to, a slit interdigitial micromixer including, but not limited to those manufactured by Microinnova (Allerheiligen bei Wildon, Austria) and/or a staggered herringbone micromixer (SHM) (see Zhigaltsevet al., "Bottom-up design and synthesis of limit size lipid nanoparticle systems with aqueous and triglyceride cores using millisecond microfluidic mixing," Langmuir 28:3633-40 (2012); Belliveau et al., "Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA," Molecular Therapy-Nucleic Acids. 1:e37 (2012); Chen et al., "Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation," J. Am. Chem. Soc. 134(16):6948-51 (2012)). Exemplary micromixers include Slit Interdigital Microstructured Mixer (SIMM-V2) or a Standard Slit Interdigital Micro Mixer (SSIMM) or Caterpillar (CPMM) or Impinging-jet (IJMM,) from the Institut für Mikrotechnik

Mainz GmbH, Mainz Germany. In some cases, methods of making LNP using SHM further comprise mixing at least two input streams wherein mixing occurs by microstructure-induced chaotic advection (MICA). According to this method, fluid streams flow through channels present in a herringbone pattern causing rotational flow and folding the fluids around each other. This method can also comprise a surface for fluid mixing wherein the surface changes orientations during fluid cycling. Methods of generating LNPs using SHM include those disclosed in U.S. Pub. Nos. US20040262223 and US20120276209.

[0890] In some cases, the polynucleotides described herein can be formulated in lipid nanoparticles using microfluidic technology (see Whitesides, George M., "The Origins and the Future of Microfluidics," Nature 442: 368-373 (2006); and Abraham et al., "Chaotic Mixer for Microchannels," Science 295: 647-651 (2002)). In some cases, the polynucleotides can be formulated in lipid nanoparticles using a micromixer chip such as, but not limited to, those from Harvard Apparatus (Holliston, MA) or Dolomite Microfluidics (Royston, UK). A micromixer chip can be used for rapid mixing of two or more fluid streams with a split and recombine mechanism.

[0891] In some cases, the polynucleotides described herein can be formulated in lipid nanoparticles having a diameter from about 1 nm to about 100 nm such as, but not limited to, about 1 nm to about 20 nm, from about 1 nm to about 30 nm, from about 1 nm to about 40 nm, from about 1 nm to about 50 nm, from about 1 nm to about 60 nm, from about 1 nm to about 70 nm, from about 1 nm to about 80 nm, from about 1 nm to about 90 nm, from about 5 nm to about from 100 nm, from about 5 nm to about 10 nm, about 5 nm to about 20 nm, from about 5 nm to about 30 nm, from about 5 nm to about 40 nm, from about 5 nm to about 50 nm, from about 5 nm to about 60 nm, from about 5 nm to about 70 nm, from about 5 nm to about 80 nm, from about 5 nm to about 90 nm, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about 20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm.

[0892] In some cases, the lipid nanoparticles can have a diameter from about 10 to 500 nm. In one case, the lipid nanoparticle may have a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750 nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

[0893] In some cases, the polynucleotides can be delivered using smaller LNPs. Such particles may comprise a diameter from below 0.1 μm up to 100 nm such as, but not limited to, less than 0.1 μm, less than 1.0 μm, less than μm, less than 10 μm, less than 15 um, less than 20 um, less than 25 um, less than 30 um, less than 35 um, less than 40 um, less than 50 um, less than 55 um, less than 60 um, less than 65 um, less than 70 um, less than 75 um, less than 80 um, less than 85 um, less than 90 um, less than 95 um, less than 100 um, less than 125 um, less than 150 um, less than 175 um, less than 200 um, less than 225 um, less than 250 um, less than 275 um, less than 300 um, less than 325 um, less than 350 um, less than 375 um, less than 400 um, less than 425 um, less than 450 um, less than 475 um, less than 500 um, less than 525 um, less than 550 um, less than 575 um, less than 600 um, less than 625 um, less than 650 um, less than 675 um, less than 700 um, less than 725 um, less than 750 um, less than 775 um, less than 800 um, less than 825 um, less than 850 um, less than 875 um, less than 900 um, less than 925 um, less than 950 um, or less than 975 um.

[0894] The nanoparticles and microparticles described herein can be geometrically engineered to modulate macrophage and/or the immune response. The geometrically engineered particles can have varied shapes, sizes and/or surface charges to incorporate the polynucleotides described herein for targeted delivery such as, but not limited to, pulmonary delivery (see, *e.g.,* Intl. Pub. No. WO2013082111). Other physical features the geometrically engineering particles can include, but are not limited to, fenestrations, angled arms, asymmetry and surface roughness, charge that can alter the interactions with cells and tissues.

[0895] In some case, the nanoparticles described herein are stealth nanoparticles or target-specific stealth nanoparticles such as, but not limited to, those described in U.S. Pub. No. US20130172406. The stealth or target-specific stealth nanoparticles can comprise a polymeric matrix, which may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polyesters, polyanhydrides, polyethers, polyurethanes, polymethacrylates, polyacrylates, polycyanoacrylates, or combina-

tions thereof.

b. Lipidoids

[0896] In some cases, the compositions or formulations of the present disclosure comprise a delivery agent, *e.g.,* a lipidoid. The polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) can be formulated with lipidoids. Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and therefore to achieve an effective delivery of the polynucleotide, as judged by the production of an encoded protein, following the injection of a lipidoid formulation via localized and/or systemic routes of administration. Lipidoid complexes of polynucleotides can be administered by various means including, but not limited to, intravenous, intramuscular, or subcutaneous routes.

[0897] The synthesis of lipidoids is described in literature (see Mahon et al., Bioconjug. Chem. 2010 21:1448-1454; Schroeder et al., J Intern Med. 2010 267:9-21; Akinc et al., Nat Biotechnol. 2008 26:561-569; Love et al., Proc Natl Acad Sci U S A. 2010 107:1864-1869; Siegwart et al., Proc Natl Acad Sci U S A. 2011 108:12996-3001).

[0898] Formulations with the different lipidoids, including, but not limited to penta[3-(1-laurylaminopropionyl)]-triethylenetetramine hydrochloride (TETA-5LAP; also known as 98N12-5, see Murugaiah et al., Analytical Biochemistry, 401:61 (2010)), C12-200 (including derivatives and variants), and MD1, can be tested for *in vivo* activity. The lipidoid "98N12-5" is disclosed by Akinc et al., Mol Ther. 2009 17:872-879. The lipidoid "C12-200" is disclosed by Love et al., Proc Natl Acad Sci U S A. 2010 107:1864-1869 and Liu and Huang, Molecular Therapy. 2010 669-670.

[0899] In one case, the polynucleotides described herein can be formulated in an aminoalcohol lipidoid. Aminoalcohol lipidoids may be prepared by the methods described in U.S. Patent No. 8,450,298.

[0900] The lipidoid formulations can include particles comprising either 3 or 4 or more components in addition to polynucleotides. Lipidoids and polynucleotide formulations comprising lipidoids are described in Intl. Pub. No. WO 2015051214.

c. *Hyaluronidase*

[0901] In some cases, the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) and hyaluronidase for injection (*e.g.*, intramuscular or subcutaneous injection). Hyaluronidase catalyzes the hydrolysis of hyaluronan, which is a constituent of the interstitial barrier. Hyaluronidase lowers the viscosity of hyaluronan, thereby increases tissue permeability (Frost, Expert Opin. Drug Deliv. (2007) 4:427-440). Alternatively, the hyaluronidase can be used to increase the number of cells exposed to the polynucleotides administered intramuscularly, intratumorally, or subcutaneously.

d. Nanoparticle Mimics

[0902] In some cases, the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) is encapsulated within and/or absorbed to a nanoparticle mimic. A nanoparticle mimic can mimic the delivery function organisms or particles such as, but not limited to, pathogens, viruses, bacteria, fungus, parasites, prions and cells. As a non-limiting example, the polynucleotides described herein can be encapsulated in a non-viron particle that can mimic the delivery function of a virus (see *e.g.,* Intl. Pub. No. WO2012006376 and U.S. Pub. Nos. US20130171241 and US20130195968.

e. Nanotubes

[0903] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) attached or otherwise bound to *(e.g.,* through steric, ionic, covalent and/or other forces) at least one nanotube, such as, but not limited to, rosette nanotubes, rosette nanotubes having twin bases with a linker, carbon nanotubes and/or single-walled carbon nanotubes. Nanotubes and nanotube formulations comprising a polynucleotide are described in, *e.g.,* Intl. Pub. No. WO2014152211.

f. Self-Assembled Nanoparticles, or Self-Assembled Macromolecules

[0904] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in self-assembled nanoparticles, or amphiphilic macromolecules (AMs) for delivery. AMs comprise biocompatible amphiphilic polymers that have an alkylated sugar backbone

covalently linked to poly(ethylene glycol). In aqueous solution, the AMs self-assemble to form micelles. Nucleic acid self-assembled nanoparticles are described in Intl. Appl. No. PCT/US2014/027077, and AMs and methods of forming AMs are described in U.S. Pub. No. US20130217753.

g. Inorganic Nanoparticles, Semi-Conductive and Metallic Nanoparticles

[0905] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in inorganic nanoparticles, or water-dispersible nanoparticles comprising a semiconductive or metallic material. The inorganic nanoparticles can include, but are not limited to, clay substances that are water swellable. The water-dispersible nanoparticles can be hydrophobic or hydrophilic nanoparticles. As a non-limiting example, the inorganic, semi-conductive and metallic nanoparticles are described in, *e.g.,* U.S. Pat. Nos. 5,585,108 and 8,257,745; and U.S. Pub. Nos. US20120228565, US 20120265001 and US 20120283503.

h. Surgical Sealants: Gels and Hydrogels

[0906] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in a surgical sealant. Surgical sealants such as gels and hydrogels are described in Intl. Appl. No. PCT/US2014/027077.

i. Suspension formulations

[0907] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in suspensions. In some cases, suspensions comprise a polynucleotide, water immiscible oil depots, surfactants and/or co-surfactants and/or co-solvents. Suspensions can be formed by first preparing an aqueous solution of a polynucleotide and an oil-based phase comprising one or more surfactants, and then mixing the two phases (aqueous and oil-based).

[0908] Exemplary oils for suspension formulations can include, but are not limited to, sesame oil and Miglyol (comprising esters of saturated coconut and palmkernel oil-derived caprylic and capric fatty acids and glycerin or propylene glycol), corn oil, soybean oil, peanut oil, beeswax and/or palm seed oil. Exemplary surfactants can include, but are not limited to Cremophor, polysorbate 20, polysorbate 80, polyethylene glycol, transcutol, Capmul®, labrasol, isopropyl myristate, and/or Span 80. In some cases, suspensions can comprise co-solvents including, but not limited to ethanol, glycerol and/or propylene glycol.

[0909] In some cases, suspensions can provide modulation of the release of the polynucleotides into the surrounding environment by diffusion from a water immiscible depot followed by resolubilization into a surrounding environment (*e.g.*, an aqueous environment).

[0910] In some cases, the polynucleotides can be formulated such that upon injection, an emulsion forms spontaneously (*e.g.*, when delivered to an aqueous phase), which may provide a high surface area to volume ratio for release of polynucleotides from an oil phase to an aqueous phase. In some cases, the polynucleotide is formulated in a nanoemulsion, which can comprise a liquid hydrophobic core surrounded by or coated with a lipid or surfactant layer. Exemplary nanoemulsions and their preparations are described in, *e.g.,* U.S. Pat. No. 8,496,945.

j. Cations and Anions

[0911] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) and a cation or anion, such as Zn2+, Ca2+, Cu2+, Mg2+ and combinations thereof. Exemplary formulations can include polymers and a polynucleotide complexed with a metal cation as described in, *e.g.,* U.S. Pat. Nos. 6,265,389 and 6,555,525. In some cases, cationic nanoparticles can contain a combination of divalent and monovalent cations. The delivery of polynucleotides in cationic nanoparticles or in one or more depot comprising cationic nanoparticles may improve polynucleotide bioavailability by acting as a long-acting depot and/or reducing the rate of degradation by nucleases.

k. Molded Nanoparticles and Microparticles

[0912] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides de-

scribed herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in molded nanoparticles in various sizes, shapes and chemistry. For example, the nanoparticles and/or microparticles can be made using the PRINT® technology by LIQUIDA TECHNOLOGIES® (Morrisville, NC) (*e.g.,* International Pub. No. WO2007024323.

[0913] In some cases, the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) is formulated in microparticles. The microparticles may contain a core of the polynucleotide and a cortex of a biocompatible and/or biodegradable polymer, including but not limited to, poly(α-hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester and a polyanhydride. The microparticle may have adsorbent surfaces to adsorb polynucleotides. The microparticles may have a diameter of from at least 1 micron to at least 100 microns (*e.g.*, at least 1 micron, at least 10 micron, at least 20 micron, at least 30 micron, at least 50 micron, at least 75 micron, at least 95 micron, and at least 100 micron). In some case, the compositions or formulations of the present disclosure are microemulsions comprising microparticles and polynucleotides. Exemplary microparticles, microemulsions and their preparations are described in, *e.g.,* U.S. Pat. Nos. 8,460,709, 8,309,139 and 8,206,749; U.S. Pub. Nos. US20130129830, US2013195923 and US20130195898; and Intl. Pub. No. WO2013075068.

l. NanoJackets and NanoLiposomes

[0914] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in NanoJackets and NanoLiposomes by Keystone Nano (State College, PA). NanoJackets are made of materials that are naturally found in the body including calcium, phosphate and may also include a small amount of silicates. Nanojackets may have a size ranging from 5 to 50 nm.

[0915] NanoLiposomes are made of lipids such as, but not limited to, lipids that naturally occur in the body. NanoLiposomes may have a size ranging from 60-80 nm. In some cases, the polynucleotides disclosed herein are formulated in a NanoLiposome such as, but not limited to, Ceramide NanoLiposomes.

m. Cells or Minicells

[0916] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) that is transfected ex vivo into cells, which are subsequently transplanted into a subject. Cell-based formulations of the polynucleotide disclosed herein can be used to ensure cell transfection (*e.g.*, in the cellular carrier), alter the biodistribution of the polynucleotide (*e.g.*, by targeting the cell carrier to specific tissues or cell types), and/or increase the translation of encoded protein.

[0917] Exemplary cells include, but are not limited to, red blood cells, virosomes, and electroporated cells (see *e.g.,* Godfrin et al., Expert Opin Biol Ther. 2012 12:127-133; Fang et al., Expert Opin Biol Ther. 2012 12:385-389; Hu et al., Proc Natl Acad Sci U S A. 2011 108:10980-10985; Lund et al., Pharm Res. 2010 27:400-420; Huckriede et al., J Liposome Res. 2007;17:39-47; Cusi, Hum Vaccin. 2006 2:1-7; de Jonge et al., Gene Ther. 2006 13:400-411).

[0918] A variety of methods are known in the art and are suitable for introduction of nucleic acid into a cell, including viral and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion.

[0919] In some cases, the polynucleotides described herein can be delivered in synthetic virus-like particles (VLPs) synthesized by the methods as described in Intl. Pub Nos. WO2011085231 and WO2013116656; and U.S. Pub. No. 20110171248.

[0920] The technique of sonoporation, or cellular sonication, is the use of sound *(e.g.,* ultrasonic frequencies) for modifying the permeability of the cell plasma membrane. Sonoporation methods are known to deliver nucleic acids *in vivo* (Yoon and Park, Expert Opin Drug Deliv. 2010 7:321-330; Postema and Gilja, Curr Pharm Biotechnol. 2007 8:355-361; Newman and Bettinger, Gene Ther. 2007 14:465-475; U.S. Pub. Nos. US20100196983 and US20100009424).

[0921] In some cases, the polynucleotides described herein can be delivered by electroporation. Electroporation techniques are known to deliver nucleic acids *in vivo* and clinically (Andre et al., Curr Gene Ther. 2010 10:267-280; Chiarella et al., Curr Gene Ther. 2010 10:281-286; Hojman, Curr Gene Ther. 2010 10:128-138). Electroporation devices are sold by many companies worldwide including, but not limited to BTX® Instruments (Holliston, MA) *(e.g.,* the AgilePulse *In vivo* System) and Inovio (Blue Bell, PA) *(e.g.,* Inovio SP-5P intramuscular delivery device or the CELLECTRA® 3000 intradermal delivery device).

[0922] In some cases, the cells are selected from the group consisting of mammalian cells, bacterial cells, plant, microbial, algal and fungal cells. In some cases, the cells are mammalian cells, such as, but not limited to, human, mouse, rat, goat, horse, rabbit, hamster or cow cells. In a further case, the cells can be from an established cell line, including, but not limited to, HeLa, NS0, SP2/0, KEK 293T, Vero, Caco, Caco-2, MDCK, COS-1, COS-7, K562, Jurkat, CHO-K1, DG44, CHOK1SV, CHO-S, Huvec, CV-1, Huh-7, NIH3T3, HEK293, 293, A549, HepG2, IMR-90, MCF-7, U-20S, Per.C6, SF9, SF21 or Chinese Hamster Ovary (CHO) cells.

[0923] In certain cases, the cells are fungal cells, such as, but not limited to, Chrysosporium cells, Aspergillus cells, Trichoderma cells, Dictyostelium cells, Candida cells, Saccharomyces cells, Schizosaccharomyces cells, and Penicillium cells.

[0924] In certain cases, the cells are bacterial cells such as, but not limited to, E. coli, B. subtilis, or BL21 cells. Primary and secondary cells to be transfected by the methods of the disclosure can be obtained from a variety of tissues and include, but are not limited to, all cell types that can be maintained in culture. The primary and secondary cells include, but are not limited to, fibroblasts, keratinocytes, epithelial cells (*e.g.*, mammary epithelial cells, intestinal epithelial cells), endothelial cells, glial cells, neural cells, formed elements of the blood (*e.g.*, lymphocytes, bone marrow cells), muscle cells and precursors of these somatic cell types. Primary cells may also be obtained from a donor of the same species or from another species (*e.g.*, mouse, rat, rabbit, cat, dog, pig, cow, bird, sheep, goat, horse).

[0925] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein in bacterial minicells. As a non-limiting example, bacterial minicells can be those described in Intl. Pub. No. WO2013088250 or U.S. Pub. No. US20130177499.

n. Semi-solid Compositions

[0926] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in a hydrophobic matrix to form a semi-solid or paste-like composition. As a non-limiting example, the semi-solid or paste-like composition can be made by the methods described in Intl. Pub. No. WO201307604.

o. Exosomes

[0927] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in exosomes, which can be loaded with at least one polynucleotide and delivered to cells, tissues and/or organisms. As a non-limiting example, the polynucleotides can be loaded in the exosomes as described in Intl. Pub. No. WO2013084000.

p. Silk-Based Delivery

[0928] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) that is formulated for silk-based delivery. The silk-based delivery system can be formed by contacting a silk fibroin solution with a polynucleotide described herein. As a non-limiting example, a sustained release silk-based delivery system and methods of making such system are described in U.S. Pub. No. US20130177611.

q. Amino Acid Lipids

[0929] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) that is formulation with an amino acid lipid. Amino acid lipids are lipophilic compounds comprising an amino acid residue and one or more lipophilic tails. Non-limiting examples of amino acid lipids and methods of making amino acid lipids are described in U.S. Pat. No. 8,501,824. The amino acid lipid formulations may deliver a polynucleotide in releasable form that comprises an amino acid lipid that binds and releases the polynucleotides. As a non-limiting example, the release of the polynucleotides described herein can be provided by an acid-labile linker as described in, *e.g.,* U.S. Pat. Nos. 7,098,032, 6,897,196, 6,426,086, 7,138,382, 5,563,250, and 5,505,931.

r. Microvesicles

[0930] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in a microvesicle formulation. Exemplary microvesicles include those described in U.S. Pub. No. US20130209544. In some cases, the microvesicle is an ARRDC1-mediated microvesicles (ARMMs) as described in Intl. Pub. No. WO2013119602.

s. Interpolyelectrolyte Complexes

[0931] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in an interpolyelectrolyte complex. Interpolyelectrolyte complexes are formed when charge-dynamic polymers are complexed with one or more anionic molecules. Non-limiting examples of charge-dynamic polymers and interpolyelectrolyte complexes and methods of making interpolyelectrolyte complexes are described in U.S. Pat. No. 8,524,368.

t. Crystalline Polymeric Systems

[0932] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in crystalline polymeric systems. Crystalline polymeric systems are polymers with crystalline moieties and/or terminal units comprising crystalline moieties. Exemplary polymers are described in U.S. Pat. No. 8,524,259.

u. Polymers, Biodegradable Nanoparticles, and Core-Shell Nanoparticles

[0933] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) and a natural and/or synthetic polymer. The polymers include, but not limited to, polyethenes, polyethylene glycol (PEG), poly(l-lysine)(PLL), PEG grafted to PLL, cationic lipopolymer, biodegradable cationic lipopolymer, polyethyleneimine (PEI), cross-linked branched poly(alkylene imines), a polyamine derivative, a modified poloxamer, elastic biodegradable polymer, biodegradable copolymer, biodegradable polyester copolymer, biodegradable polyester copolymer, multiblock copolymers, poly[a-(α-aminobutyl)-L-glycolic acid) (PAGA), biodegradable cross-linked cationic multi-block copolymers, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), amine-containing polymers, dextran polymers, dextran polymer derivatives or combinations thereof.

[0934] Exemplary polymers include, DYNAMIC POLYCONJUGATE® (Arrowhead Research Corp., Pasadena, CA) formulations from MIRUS® Bio (Madison, WI) and Roche Madison (Madison, WI), PHASERXTM polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY™ (PHASERX®, Seattle, WA), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, CA), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, CA), dendrimers and poly(lactic-co-glycolic acid) (PLGA) polymers. RONDELTM (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, CA) and pH responsive co-block polymers such as PHASERX® (Seattle, WA).

[0935] The polymer formulations allow a sustained or delayed release of the polynucleotide (*e.g.*, following intramuscular or subcutaneous injection). The altered release profile for the polynucleotide can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation can also be used to increase the stability of the polynucleotide. Sustained release formulations can include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA), TISSELL® (Baxter International, Inc. Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc. Deerfield, IL).

[0936] As a non-limiting example modified mRNA can be formulated in PLGA microspheres by preparing the PLGA microspheres with tunable release rates (*e.g.*, days and weeks) and encapsulating the modified mRNA in the PLGA microspheres while maintaining the integrity of the modified mRNA during the encapsulation process. EVAc are non-biodegradable, biocompatible polymers that are used extensively in pre-clinical sustained release implant applications (*e.g.*, extended release products Ocusert a pilocarpine ophthalmic insert for glaucoma or progestasert a sustained

release progesterone intrauterine device; transdermal delivery systems Testoderm, Duragesic and Selegiline; catheters). Poloxamer F-407 NF is a hydrophilic, non-ionic surfactant triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene having a low viscosity at temperatures less than 5°C and forms a solid gel at temperatures greater than 15°C.

[0937] As a non-limiting example, the polynucleotides described herein can be formulated with the polymeric compound of PEG grafted with PLL as described in U.S. Pat. No. 6,177,274. As another non-limiting example, the polynucleotides described herein can be formulated with a block copolymer such as a PLGA-PEG block copolymer (see *e.g.,* U.S. Pub. No. US20120004293 and U.S. Pat. Nos. 8,236,330 and 8,246,968), or a PLGA-PEG-PLGA block copolymer (see *e.g.,* U.S. Pat. No. 6,004,573).

[0938] In some cases, the polynucleotides described herein can be formulated with at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers, poly(amine-co-esters) or combinations thereof. Exemplary polyamine polymers and their use as delivery agents are described in, *e.g.,* U.S. Pat. Nos. 8,460,696, 8,236,280.

[0939] In some cases, the polynucleotides described herein can be formulated in a biodegradable cationic lipopolymer, a biodegradable polymer, or a biodegradable copolymer, a biodegradable polyester copolymer, a biodegradable polyester polymer, a linear biodegradable copolymer, PAGA, a biodegradable cross-linked cationic multi-block copolymer or combinations thereof as described in, *e.g.,* U.S. Pat. Nos. 6,696,038, 6,517,869, 6,267,987, 6,217,912, 6,652,886, 8,057,821, and 8,444,992; U.S. Pub. Nos. US20030073619, US20040142474, US20100004315, US2012009145 and US20130195920; and Intl Pub. Nos. WO2006063249 and WO2013086322.

[0940] In some cases, the polynucleotides described herein can be formulated in or with at least one cyclodextrin polymer as described in U.S. Pub. No. US20130184453. In some cases, the polynucleotides described herein can be formulated in or with at least one crosslinked cation-binding polymers as described in Intl. Pub. Nos. WO2013106072, WO2013106073 and WO2013106086. In some cases, the polynucleotides described herein can be formulated in or with at least PEGylated albumin polymer as described in U.S. Pub. No. US20130231287.

[0941] In some cases, the polynucleotides disclosed herein can be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components can be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle for delivery (Wang et al., Nat Mater. 2006 5:791-796; Fuller et al., Biomaterials. 2008 29:1526-1532; DeKoker et al., Adv Drug Deliv Rev. 2011 63:748-761; Endres et al., Biomaterials. 2011 32:7721-7731; Su et al., Mol Pharm. 2011 Jun 6;8(3):774-87). As a non-limiting example, the nanoparticle can comprise a plurality of polymers such as, but not limited to hydrophilic-hydrophobic polymers (*e.g.*, PEG-PLGA), hydrophobic polymers (*e.g.*, PEG) and/or hydrophilic polymers (Intl. Pub. No. WO20120225129).

[0942] The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et al., Proc Natl Acad Sci U S A. 2011 108:12996-13001). The complexation, delivery, and internalization of the polymeric nanoparticles can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle. For example, the core-shell nanoparticles may efficiently deliver siRNA to mouse hepatocytes after they covalently attach cholesterol to the nanoparticle.

[0943] In some cases, a hollow lipid core comprising a middle PLGA layer and an outer neutral lipid layer containing PEG can be used to delivery of the polynucleotides as described herein. In some cases, the lipid nanoparticles can comprise a core of the polynucleotides disclosed herein and a polymer shell, which is used to protect the polynucleotides in the core. The polymer shell can be any of the polymers described herein and are known in the art., the polymer shell can be used to protect the polynucleotides in the core.

[0944] Core-shell nanoparticles for use with the polynucleotides described herein are described in U.S. Pat. No. 8,313,777 or Intl. Pub. No. WO2013124867.

v. Peptides and Proteins

[0945] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) that is formulated with peptides and/or proteins to increase transfection of cells by the polynucleotide, and/or to alter the biodistribution of the polynucleotide (*e.g.*, by targeting specific tissues or cell types), and/or increase the translation of encoded protein (*e.g.,* Intl. Pub. Nos. WO2012110636 and WO2013123298. In some cases, the peptides can be those described in U.S. Pub. Nos. US20130129726, US20130137644 and US20130164219.

w. Conjugates

[0946] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides de-

scribed herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) that is covalently linked to a carrier or targeting group, or including two encoding regions that together produce a fusion protein *(e.g.,* bearing a targeting group and therapeutic protein or peptide) as a conjugate. The conjugate can be a peptide that selectively directs the nanoparticle to neurons in a tissue or organism, or assists in crossing the blood-brain barrier.

[0947] The conjugates include a naturally occurring substance, such as a protein *(e.g.,* human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); an carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.,* a synthetic polyamino acid, an oligonucleotide (*e.g.*, an aptamer). Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), poly-urethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

[0948] In some cases, the conjugate can function as a carrier for the polynucleotide disclosed herein. The conjugate can comprise a cationic polymer such as, but not limited to, polyamine, polylysine, polyalkylenimine, and polyethylenimine that can be grafted to with poly(ethylene glycol). Exemplary conjugates and their preparations are described in U.S. Pat. No. 6,586,524 and U.S. Pub. No. US20130211249.

[0949] The conjugates can also include targeting groups, *e.g.,* a cell or tissue targeting agent, *e.g.,* a lectin, glycoprotein, lipid or protein, *e.g.,* an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

[0950] Targeting groups can be proteins, *e.g.,* glycoproteins, or peptides, *e.g.,* molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell. Targeting groups may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fructose, or aptamers. The ligand can be, for example, a lipopolysaccharide, or an activator of p38 MAP kinase.

[0951] The targeting group can be any ligand that is capable of targeting a specific receptor. Examples include, without limitation, folate, GalNAc, galactose, mannose, mannose-6P, apatamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL, and HDL ligands. In particular cases, the targeting group is an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein. As a non-limiting example, the targeting group can be a glutathione receptor (GR)-binding conjugate for targeted delivery across the blood-central nervous system barrier as described in, *e.g.,* U.S. Pub. No. US2013021661012.

[0952] In some cases, the conjugate can be a synergistic biomolecule-polymer conjugate, which comprises a long-acting continuous-release system to provide a greater therapeutic efficacy. The synergistic biomolecule-polymer conjugate can be those described in U.S. Pub. No. US20130195799. In some cases, the conjugate can be an aptamer conjugate as described in Intl. Pat. Pub. No. WO2012040524. In some cases, the conjugate can be an amine containing polymer conjugate as described in U.S. Pat. No. 8,507,653. In some cases, the polynucleotides can be conjugated to SMARTT POLYMER TECHNOLOGY® (PHASERX®, Inc. Seattle, WA).

[0953] In some cases, the polynucleotides described herein are covalently conjugated to a cell penetrating polypeptide, which can also include a signal sequence or a targeting sequence. The conjugates can be designed to have increased stability, and/or increased cell transfection; and/or altered the biodistribution (*e.g.*, targeted to specific tissues or cell types).

[0954] In some cases, the polynucleotides described herein can be conjugated to an agent to enhance delivery. In some cases, the agent can be a monomer or polymer such as a targeting monomer or a polymer having targeting blocks as described in Intl. Pub. No. WO2011062965. In some cases, the agent can be a transport agent covalently coupled to a polynucleotide as described in, *e.g.,* U.S. Pat. Nos. 6,835.393 and 7,374,778. In some cases, the agent can be a membrane barrier transport enhancing agent such as those described in U.S. Pat. Nos. 7,737,108 and 8,003,129.

x. Micro-Organs

[0955] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A

polypeptide, and/or IL12B and IL12A fusion polypeptides) in a micro-organ that can then express an encoded polypeptide of interest in a long-lasting therapeutic formulation. Exemplary micro-organs and formulations are described in Intl. Pub. No. WO2014152211.

y. Pseudovirions

[0956] In some cases, the compositions or formulations of the present disclosure comprise the polynucleotides described herein *(e.g.,* a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides) in pseudovirions (*e.g.*, pseudovirions developed by Aura Biosciences,Cambridge, MA).

[0957] In some cases, the pseudovirion used for delivering the polynucleotides can be derived from viruses such as, but not limited to, herpes and papillomaviruses as described in, *e.g.,* U.S. Pub. Nos. US20130012450, US20130012566, US21030012426 and US20120207840; and Intl. Pub. No. WO2013009717.

[0958] The pseudovirion can be a virus-like particle (VLP) prepared by the methods described in U.S. Pub. Nos. US20120015899 and US20130177587, and Intl. Pub. Nos. WO2010047839, WO2013116656, WO2013106525 and WO2013122262. In one aspect, the VLP can be bacteriophages MS, Qβ, R17, fr, GA, Sp, MI, I, MXI, NL95, AP205, f2, PP7, and the plant viruses Turnip crinkle virus (TCV), Tomato bushy stunt virus (TBSV), Southern bean mosaic virus (SBMV) and members of the genus Bromovirus including Broad bean mottle virus, Brome mosaic virus, Cassia yellow blotch virus, Cowpea chlorotic mottle virus (CCMV), Melandrium yellow fleck virus, and Spring beauty latent virus. In another aspect, the VLP can be derived from the influenza virus as described in U.S. Pub. No. US20130177587 and U.S. Pat. No. 8,506,967. In one aspect, the VLP can comprise a B7-1 and/or B7-2 molecule anchored to a lipid membrane or the exterior of the particle such as described in Intl. Pub. No. WO2013116656. In one aspect, the VLP can be derived from norovirus, rotavirus recombinant VP6 protein or double layered VP2/VP6 such as the VLP as described in Intl. Pub. No. WO2012049366.

[0959] In some cases, the pseudovirion can be a human papilloma virus-like particle as described in Intl. Pub. No. WO2010120266 and U.S. Pub. No. US20120171290. In some cases, the virus-like particle (VLP) can be a self-assembled particle. In one aspect, the pseudovirions can be virion derived nanoparticles as described in U.S. Pub. Nos. US20130116408 and US20130115247; and Intl. Pub. No. WO2013119877.

[0960] Non-limiting examples of formulations and methods for formulating the polynucleotides described herein are also provided in Intl. Pub. No WO2013090648.

26. Compositions and Formulations for Use

[0961] Certain aspects of the disclosure are directed to compositions or formulations comprising any of the polynucleotides disclosed above.

[0962] In some cases, the composition or formulation comprises:

(i) a polynucleotide *(e.g.,* a RNA, *e.g.,* an mRNA) comprising a sequence-optimized nucleotide sequence (*e.g.*, an ORF) encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides (*e.g.*, the wild-type sequence, functional fragment, or variant thereof), wherein the polynucleotide comprises at least one chemically modified nucleobase, *e.g.,* 5-methoxyuracil *(e.g.,* wherein at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 99%, or 100% of the uracils are 5-methoxyuracils), and wherein the polynucleotide further comprises a miRNA binding site, *e.g.,* a miRNA binding site that binds to miR-122 *(e.g.,* a miR-122-3p or miR-122-5p binding site); and

(ii) a delivery agent comprising a compound having Formula (I), *e.g.,* any of Compounds 1-147 *(e.g.,* Compound 18, 25, 26 or 48) or any of Compounds 1-232.

[0963] In some cases, the uracil or thymine content of the ORF relative to the theoretical minimum uracil or thymine content of a nucleotide sequence encoding the IL12B polypeptide, the IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides ($\%U_{TM}$ or $\%T_{TM}$), is between about 100% and about 150%.

[0964] In some cases, the polynucleotides, compositions or formulations above are used to treat and/or prevent an IL12-related diseases, disorders or conditions, *e.g.*, cancer.

27. Forms of Administration

[0965] The polynucleotides, pharmaceutical compositions and formulations of the disclosure described above can be administered by any route that results in a therapeutically effective outcome. These include, but are not limited to enteral

(into the intestine), gastroenteral, epidural (into the dura matter), oral (by way of the mouth), transdermal, peridural, intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intravenous bolus, intravenous drip, intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection (into a pathologic cavity) intracavitary (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), transvaginal, insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), in ear drops, auricular (in or by way of the ear), buccal (directed toward the cheek), conjunctival, cutaneous, dental (to a tooth or teeth), electro-osmosis, endocervical, endosinusial, endotracheal, extracorporeal, hemodialysis, infiltration, interstitial, intra-abdominal, intra-amniotic, intra-articular, intrabiliary, intrabronchial, intrabursal, intracartilaginous (within a cartilage), intracaudal (within the cauda equine), intracisternal (within the cisterna magna cerebellomedularis), intracorneal (within the cornea), dental intracornal, intracoronary (within the coronary arteries), intracorporus cavernosum (within the dilatable spaces of the corporus cavernosa of the penis), intradiscal (within a disc), intraductal (within a duct of a gland), intraduodenal (within the duodenum), intradural (within or beneath the dura), intraepidermal (to the epidermis), intraesophageal (to the esophagus), intragastric (within the stomach), intragingival (within the gingivae), intraileal (within the distal portion of the small intestine), intralesional (within or introduced directly to a localized lesion), intraluminal (within a lumen of a tube), intralymphatic (within the lymph), intramedullary (within the marrow cavity of a bone), intrameningeal (within the meninges), intraocular (within the eye), intraovarian (within the ovary), intrapericardial (within the pericardium), intrapleural (within the pleura), intraprostatic (within the prostate gland), intrapulmonary (within the lungs or its bronchi), intrasinal (within the nasal or periorbital sinuses), intraspinal (within the vertebral column), intrasynovial (within the synovial cavity of a joint), intratendinous (within a tendon), intratesticular (within the testicle), intrathecal (within the cerebrospinal fluid at any level of the cerebrospinal axis), intrathoracic (within the thorax), intratubular (within the tubules of an organ), intratumor (within a tumor), intratympanic (within the aurus media), intravascular (within a vessel or vessels), intraventricular (within a ventricle), iontophoresis (by means of electric current where ions of soluble salts migrate into the tissues of the body), irrigation (to bathe or flush open wounds or body cavities), laryngeal (directly upon the larynx), nasogastric (through the nose and into the stomach), occlusive dressing technique (topical route administration that is then covered by a dressing that occludes the area), ophthalmic (to the external eye), oropharyngeal (directly to the mouth and pharynx), parenteral, percutaneous, periarticular, peridural, perineural, periodontal, rectal, respiratory (within the respiratory tract by inhaling orally or nasally for local or systemic effect), retrobulbar (behind the pons or behind the eyeball), intramyocardial (entering the myocardium), soft tissue, subarachnoid, subconjunctival, submucosal, topical, transplacental (through or across the placenta), transtracheal (through the wall of the trachea), transtympanic (across or through the tympanic cavity), ureteral (to the ureter), urethral (to the urethra), vaginal, caudal block, diagnostic, nerve block, biliary perfusion, cardiac perfusion, photopheresis or spinal. In specific cases, compositions can be administered in a way that allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier. In some cases, a formulation for a route of administration can include at least one inactive ingredient.

[0966] The polynucleotides of the present disclosure (*e.g.*, a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides or a functional fragment or variant thereof) can be delivered to a cell naked. As used herein in, "naked" refers to delivering polynucleotides free from agents that promote transfection. For example, the polynucleotides delivered to the cell may contain no modifications. The naked polynucleotides can be delivered to the cell using routes of administration known in the art and described herein.

[0967] The polynucleotides of the present disclosure (*e.g.*, a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, I an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides or a functional fragment or variant thereof) can be formulated, using the methods described herein. The formulations can contain polynucleotides that can be modified and/or unmodified. The formulations may further include, but are not limited to, cell penetration agents, a pharmaceutically acceptable carrier, a delivery agent, a bioerodible or biocompatible polymer, a solvent, and a sustained-release delivery depot. The formulated polynucleotides can be delivered to the cell using routes of administration known in the art and described herein.

[0968] The compositions can also be formulated for direct delivery to an organ or tissue in any of several ways in the art including, but not limited to, direct soaking or bathing, via a catheter, by gels, powder, ointments, creams, gels, lotions, and/or drops, by using substrates such as fabric or biodegradable materials coated or impregnated with the compositions, and the like.

[0969] The present disclosure encompasses the delivery of polynucleotides of the disclosure (*e.g.*, a polynucleotide comprising a nucleotide sequence encoding an IL12B polypeptide, an IL12A polypeptide, and/or IL12B and IL12A fusion polypeptides or a functional fragment or variant thereof) in forms suitable for parenteral and injectable administration. Liquid dosage forms for parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms can

comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain cases for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

[0970] A pharmaceutical composition for parenteral administration can comprise at least one inactive ingredient. Any or none of the inactive ingredients used may have been approved by the US Food and Drug Administration (FDA). A non-exhaustive list of inactive ingredients for use in pharmaceutical compositions for parenteral administration includes hydrochloric acid, mannitol, nitrogen, sodium acetate, sodium chloride and sodium hydroxide.

[0971] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations can be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables. The sterile formulation may also comprise adjuvants such as local anesthetics, preservatives and buffering agents.

[0972] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0973] Injectable formulations can be for direct injection into a region of a tissue, organ and/or subject. As a non-limiting example, a tissue, organ and/or subject can be directly injected a formulation by intramyocardial injection into the ischemic region. (*See, e.g.,* Zangi et al. Nature Biotechnology 2013).

[0974] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissues.

28. Kits and Devices

a. Kits

[0975] The disclosure provides a variety of kits for conveniently and/or effectively using the claimed nucleotides of the present disclosure. Typically kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[0976] In one aspect, the present disclosure provides kits comprising the molecules (polynucleotides) of the disclosure.

[0977] Said kits can be for protein production, comprising a first polynucleotides comprising a translatable region. The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent can comprise a saline, a buffered solution, a lipidoid or any delivery agent disclosed herein.

[0978] In some cases, the buffer solution can include sodium chloride, calcium chloride, phosphate and/or EDTA. In another case, the buffer solution can include, but is not limited to, saline, saline with 2mM calcium, 5% sucrose, 5% sucrose with 2mM calcium, 5% Mannitol, 5% Mannitol with 2mM calcium, Ringer's lactate, sodium chloride, sodium chloride with 2mM calcium and mannose (*See, e.g.,* U.S. Pub. No. 20120258046). In a further case, the buffer solutions can be precipitated or it can be lyophilized. The amount of each component can be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of modified RNA in the buffer solution over a period of time and/or under a variety of conditions. In one aspect, the present disclosure provides kits for protein production, comprising: a polynucleotide comprising a translatable region, provided in an amount effective to produce a desired amount of a protein encoded by the translatable region when introduced into a target cell; a second polynucleotide comprising an inhibitory nucleic acid, provided in an amount

effective to substantially inhibit the innate immune response of the cell; and packaging and instructions.

[0979] In one aspect, the present disclosure provides kits for protein production, comprising a polynucleotide comprising a translatable region, wherein the polynucleotide exhibits reduced degradation by a cellular nuclease, and packaging and instructions.

[0980] In one aspect, the present disclosure provides kits for protein production, comprising a polynucleotide comprising a translatable region, wherein the polynucleotide exhibits reduced degradation by a cellular nuclease, and a mammalian cell suitable for translation of the translatable region of the first nucleic acid.

b. Devices

[0981] The present disclosure provides for devices that may incorporate polynucleotides that encode polypeptides of interest. These devices contain in a stable formulation the reagents to synthesize a polynucleotide in a formulation available to be immediately delivered to a subject in need thereof, such as a human patient

[0982] Devices for administration can be employed to deliver the polynucleotides of the present disclosure according to single, multi- or split-dosing regimens taught herein. Such devices are taught in, for example, International Application Publ. No. WO2013151666.

[0983] Method and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as cases of the present disclosure. These include, for example, those methods and devices having multiple needles, hybrid devices employing for example lumens or catheters as well as devices utilizing heat, electric current or radiation driven mechanisms.

[0984] According to the present disclosure, these multi-administration devices can be utilized to deliver the single, multi- or split doses contemplated herein. Such devices are taught for example in, International Application Publ. No. WO2013151666.

[0985] In some cases, the polynucleotide is administered subcutaneously or intramuscularly via at least 3 needles to three different, optionally adjacent, sites simultaneously, or within a 60 minutes period (*e.g.*, administration to 4, 5, 6, 7, 8, 9, or 10 sites simultaneously or within a 60 minute period).

c. *Methods and Devices utilizing catheters and/or lumens*

[0986] Methods and devices using catheters and lumens can be employed to administer the polynucleotides of the present disclosure on a single, multi- or split dosing schedule. Such methods and devices are described in International Application Publication No. WO2013151666.

d. Methods and Devices utilizing electrical current

[0987] Methods and devices utilizing electric current can be employed to deliver the polynucleotides of the present disclosure according to the single, multi- or split dosing regimens taught herein. Such methods and devices are described in International Application Publication No. WO2013151666.

29. Definitions

[0988] In order that the present disclosure can be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0989] The disclosure includes cases in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes cases in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[0990] In this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein. In certain aspects, the term "a" or "an" means "single." In other aspects, the term "a" or "an" includes "two or more" or "multiple."

[0991] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0992] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of

Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0993] Wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0994] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Where a range of values is recited, it is to be understood that each intervening integer value, and each fraction thereof, between the recited upper and lower limits of that range is also specifically disclosed, along with each subrange between such values. The upper and lower limits of any range can independently be included in or excluded from the range, and each range where either, neither or both limits are included is also encompassed within the disclosure. Where a value is explicitly recited, it is to be understood that values which are about the same quantity or amount as the recited value are also within the scope of the disclosure. Where a combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is within the scope of the disclosure. Conversely, where different elements or groups of elements are individually disclosed, combinations thereof are also disclosed. Where any element of an disclosure is disclosed as having a plurality of alternatives, examples of that disclosure in which each alternative is excluded singly or in any combination with the other alternatives are also hereby disclosed; more than one element of an disclosure can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

[0995] Nucleotides are referred to by their commonly accepted single-letter codes. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation. Nucleobases are referred to herein by their commonly known one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Accordingly, A represents adenine, C represents cytosine, G represents guanine, T represents thymine, U represents uracil.

[0996] Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation.

[0997] *About:* The term "about" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is $\pm$ 10 %.

[0998] Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different cases of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0999] *Administered in combination:* As used herein, the term "administered in combination" or "combined administration" means that two or more agents are administered to a subject at the same time or within an interval such that there can be an overlap of an effect of each agent on the patient. In some cases, they are administered within about 60, 30, 15, 10, 5, or 1 minute of one another. In some cases, the administrations of the agents are spaced sufficiently closely together such that a combinatorial (*e.g.*, a synergistic) effect is achieved.

[1000] *Amino acid substitution:* The term "amino acid substitution" refers to replacing an amino acid residue present in a parent or reference sequence *(e.g.,* a wild type IL12 sequence) with another amino acid residue. An amino acid can be substituted in a parent or reference sequence *(e.g.,* a wild type IL12 polypeptide sequence), for example, via chemical peptide synthesis or through recombinant methods known in the art. Accordingly, a reference to a "substitution at position X" refers to the substitution of an amino acid present at position X with an alternative amino acid residue. In some aspects, substitution patterns can be described according to the schema AnY, wherein A is the single letter code corresponding to the amino acid naturally or originally present at position n, and Y is the substituting amino acid residue. In other aspects, substitution patterns can be described according to the schema An(YZ), wherein A is the single letter code corresponding to the amino acid residue substituting the amino acid naturally or originally present at position X, and Y and Z are alternative substituting amino acid residue, *i.e.,*

[1001] In the context of the present disclosure, substitutions (even when they referred to as amino acid substitution) are conducted at the nucleic acid level, *i.e.,* substituting an amino acid residue with an alternative amino acid residue is conducted by substituting the codon encoding the first amino acid with a codon encoding the second amino acid.

[1002] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some cases, "animal" refers to humans at any stage of development. In some cases, "animal" refers to non-human animals at any stage of development. In certain cases, 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, or a pig). In some cases, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some cases, the animal is a transgenic animal, genetically-engineered animal, or a clone.

[1003] *Approximately:* As used herein, the term "approximately," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain cases, the term "approximately" 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).

[1004] *Associated with:* As used herein with respect to a disease, the term "associated with" means that the symptom, measurement, characteristic, or status in question is linked to the diagnosis, development, presence, or progression of that disease. As association may, but need not, be causatively linked to the disease. For example, symptoms, sequelae, or any effects causing a decrease in the quality of life of a patient of cancer are considered associated with cancer and in some cases of the present disclosure can be treated, ameliorated, or prevented by administering the polynucleotides of the present disclosure to a subject in need thereof.

[1005] When used with respect to two or more moieties, the terms "associated with," "conjugated," "linked," "attached," and "tethered," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, *e.g.*, physiological conditions. An "association" need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization based connectivity sufficiently stable such that the "associated" entities remain physically associated.

[1006] *Bifunctional:* As used herein, the term "bifunctional" refers to any substance, molecule or moiety that is capable of or maintains at least two functions. The functions may affect the same outcome or a different outcome. The structure that produces the function can be the same or different. For example, bifunctional modified RNAs of the present disclosure may encode an IL12 peptide (a first function) while those nucleosides that comprise the encoding RNA are, in and of themselves, capable of extending the half-life of the RNA (second function). In this example, delivery of the bifunctional modified RNA to a subject suffering from a protein deficiency would produce not only a peptide or protein molecule that may ameliorate or treat a disease or conditions, but would also maintain a population modified RNA present in the subject for a prolonged period of time. In other aspects, a bifunctionally modified mRNA can be a chimeric molecule comprising, for example, an RNA encoding an IL12 peptide (a first function) and a second protein either fused to first protein or co-expressed with the first protein.

[1007] *Biocompatible:* As used herein, the term "biocompatible" means compatible with living cells, tissues, organs or systems posing little to no risk of injury, toxicity or rejection by the immune system.

[1008] *Biodegradable:* As used herein, the term "biodegradable" means capable of being broken down into innocuous products by the action of living things.

[1009] *Biologically active:* As used herein, the phrase "biologically active" refers to a characteristic of any substance that has activity in a biological system and/or organism. For instance, a substance that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular cases, a polynucleotide of the present disclosure can be considered biologically active if even a portion of the polynucleotide is biologically active or mimics an activity considered biologically relevant.

[1010] *Chimera:* As used herein, "chimera" is an entity having two or more incongruous or heterogeneous parts or regions. For example, a chimeric molecule can comprise a first part comprising an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides, and a second part (*e.g.*, genetically fused to the first part) comprising a second therapeutic protein *(e.g.,* a protein with a distinct enzymatic activity, an antigen binding moiety, or a moiety capable of extending the plasma half-life of IL12, for example, an Fc region of an antibody).

[1011] *Sequence Optimization:* The term "sequence optimization" refers to a process or series of processes by which nucleobases in a reference nucleic acid sequence are replaced with alternative nucleobases, resulting in a nucleic acid sequence with improved properties, *e.g.*, improved protein expression or decreased immunogenicity.

[1012] In general, the goal in sequence optimization is to produce a synonymous nucleotide sequence than encodes the same polypeptide sequence encoded by the reference nucleotide sequence. Thus, there are no amino acid substitutions (as a result of codon optimization) in the polypeptide encoded by the codon optimized nucleotide sequence with respect to the polypeptide encoded by the reference nucleotide sequence.

[1013] *Codon substitution:* The terms "codon substitution" or "codon replacement" in the context of sequence optimization refer to replacing a codon present in a reference nucleic acid sequence with another codon. A codon can be substituted in a reference nucleic acid sequence, for example, via chemical peptide synthesis or through recombinant methods known in the art. Accordingly, references to a "substitution" or "replacement" at a certain location in a nucleic acid sequence (*e.g.*, an mRNA) or within a certain region or subsequence of a nucleic acid sequence (*e.g.*, an mRNA) refer to the substitution of a codon at such location or region with an alternative codon.

[1014] As used herein, the terms "coding region" and "region encoding" and grammatical variants thereof, refer to an Open Reading Frame (ORF) in a polynucleotide that upon expression yields a polypeptide or protein.

[1015] *Compound:* As used herein, the term "compound," is meant to include all stereoisomers and isotopes of the structure depicted. As used herein, the term "stereoisomer" means any geometric isomer (*e.g.*, cis- and trans- isomer), enantiomer, or diastereomer of a compound. The present disclosure encompasses any and all stereoisomers of the

compounds described herein, including stereomerically pure forms (*e.g.*, geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, *e.g.*, racemates. Enantiomeric and stereomeric mixtures of compounds and means of resolving them into their component enantiomers or stereoisomers are well-known. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium. Further, a compound, salt, or complex of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[1016] *Contacting:* As used herein, the term "contacting" means establishing a physical connection between two or more entities. For example, contacting a mammalian cell with a nanoparticle composition means that the mammalian cell and a nanoparticle are made to share a physical connection. Methods of contacting cells with external entities both *in vivo* and ex vivo are well known in the biological arts. For example, contacting a nanoparticle composition and a mammalian cell disposed within a mammal may be performed by varied routes of administration (*e.g.*, intravenous, intramuscular, intradermal, and subcutaneous) and may involve varied amounts of nanoparticle compositions. Moreover, more than one mammalian cell may be contacted by a nanoparticle composition.

[1017] *Conservative amino acid substitution:* A "conservative amino acid substitution" is one in which the amino acid residue in a protein sequence is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, or histidine), acidic side chains (*e.g.*, aspartic acid or glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, or cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, or tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, or histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the amino acid substitution is considered to be conservative. In another aspect, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[1018] *Non-conservative amino acid substitution:* Non-conservative amino acid substitutions include those in which (i) a residue having an electropositive side chain *(e.g.,* Arg, His or Lys) is substituted for, or by, an electronegative residue *(e.g.,* Glu or Asp), (ii) a hydrophilic residue *(e.g.,* Ser or Thr) is substituted for, or by, a hydrophobic residue (*e.g.*, Ala, Leu, Ile, Phe or Val), (iii) a cysteine or proline is substituted for, or by, any other residue, or (iv) a residue having a bulky hydrophobic or aromatic side chain *(e.g.,* Val, His, Ile or Trp) is substituted for, or by, one having a smaller side chain *(e.g.,* Ala or Ser) or no side chain *(e.g.,* Gly).

[1019] Other amino acid substitutions can be readily identified by workers of ordinary skill. For example, for the amino acid alanine, a substitution can be taken from any one of D-alanine, glycine, beta-alanine, L-cysteine and D-cysteine. For lysine, a replacement can be any one of D-lysine, arginine, D-arginine, homo-arginine, methionine, D-methionine, ornithine, or D- ornithine. Generally, substitutions in functionally important regions that can be expected to induce changes in the properties of isolated polypeptides are those in which (i) a polar residue, *e.g.*, serine or threonine, is substituted for (or by) a hydrophobic residue, *e.g.*, leucine, isoleucine, phenylalanine, or alanine; (ii) a cysteine residue is substituted for (or by) any other residue; (iii) a residue having an electropositive side chain, *e.g.*, lysine, arginine or histidine, is substituted for (or by) a residue having an electronegative side chain, *e.g.*, glutamic acid or aspartic acid; or (iv) a residue having a bulky side chain, *e.g.*, phenylalanine, is substituted for (or by) one not having such a side chain, *e.g.*, glycine. The likelihood that one of the foregoing non-conservative substitutions can alter functional properties of the protein is also correlated to the position of the substitution with respect to functionally important regions of the protein: some non-conservative substitutions can accordingly have little or no effect on biological properties.

[1020] *Conserved:* As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, that are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[1021] In some cases, two or more sequences are said to be "completely conserved" if they are 100% identical to one another. In some cases, two or more sequences are said to be "highly conserved" if they are at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some cases, two or more sequences are said to be "highly conserved" if they are about 70% identical, about 80% identical, about 90% identical, about 95%, about 98%, or about 99% identical to one another. In some cases, two or more sequences are said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some cases, two or more sequences are said to be "conserved" if they are about 30% identical, about 40% identical, about 50% identical, about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. Conservation of sequence may apply to the entire length of an polynucleotide or polypeptide or may apply to a portion, region or feature thereof.

[1022] *Controlled Release:* As used herein, the term "controlled release" refers to a pharmaceutical composition or

compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome.

[1023] *Covalent Derivative:* The term "covalent derivative" when referring to polypeptides include modifications of a native or starting protein with an organic proteinaceous or non-proteinaceous derivatizing agent, and/or post-translational modifications. Covalent modifications are traditionally introduced by reacting targeted amino acid residues of the protein with an organic derivatizing agent that is capable of reacting with selected side-chains or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. The resultant covalent derivatives are useful in programs directed at identifying residues important for biological activity, for immunoassays, or for the preparation of anti-protein antibodies for immunoaffinity purification of the recombinant glycoprotein. Such modifications are within the ordinary skill in the art and are performed without undue experimentation.

[1024] *Cyclic or Cyclized:* As used herein, the term "cyclic" refers to the presence of a continuous loop. Cyclic molecules need not be circular, only joined to form an unbroken chain of subunits. Cyclic molecules such as the engineered RNA or mRNA of the present disclosure can be single units or multimers or comprise one or more components of a complex or higher order structure.

[1025] *Cytotoxic:* As used herein, "cytotoxic" refers to killing or causing injurious, toxic, or deadly effect on a cell *(e.g.,* a mammalian cell *(e.g.,* a human cell)), bacterium, virus, fungus, protozoan, parasite, prion, or a combination thereof.

[1026] *Delivering:* As used herein, the term "delivering" means providing an entity to a destination. For example, delivering a polynucleotide to a subject may involve administering a nanoparticle composition including the polynucleotide to the subject *(e.g.,* by an intravenous, intramuscular, intradermal, or subcutaneous route). Administration of a nanoparticle composition to a mammal or mammalian cell may involve contacting one or more cells with the nanoparticle composition.

[1027] *Delivery Agent:* As used herein, "delivery agent" refers to any substance that facilitates, at least in part, the *in vivo, in vitro, or ex vivo* delivery of a polynucleotide to targeted cells.

[1028] *Destabilized:* As used herein, the term "destable," "destabilize," or "destabilizing region" means a region or molecule that is less stable than a starting, wild-type or native form of the same region or molecule.

[1029] *Detectable label:* As used herein, "detectable label" refers to one or more markers, signals, or moieties that are attached, incorporated or associated with another entity that is readily detected by methods known in the art including radiography, fluorescence, chemiluminescence, enzymatic activity, absorbance and the like. Detectable labels include radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, ligands such as biotin, avidin, streptavidin and haptens, quantum dots, and the like. Detectable labels can be located at any position in the peptides or proteins disclosed herein. They can be within the amino acids, the peptides, or proteins, or located at the Nor C- termini.

[1030] *Diastereomer:* As used herein, the term "diastereomer," means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

[1031] *Digest:* As used herein, the term "digest" means to break apart into smaller pieces or components. When referring to polypeptides or proteins, digestion results in the production of peptides.

[1032] *Distal:* As used herein, the term "distal" means situated away from the center or away from a point or region of interest.

[1033] *Domain:* As used herein, when referring to polypeptides, the term "domain" refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (*e.g.*, binding capacity, serving as a site for protein-protein interactions).

[1034] *Dosing regimen:* As used herein, a "dosing regimen" or a "dosing regimen" is a schedule of administration or physician determined regimen of treatment, prophylaxis, or palliative care.

[1035] *Effective Amount:* As used herein, the term "effective amount" of an agent is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats a protein deficiency (*e.g.*, an IL12 deficiency), an effective amount of an agent is, for example, an amount of mRNA expressing sufficient PBGF to ameliorate, reduce, eliminate, or prevent the symptoms associated with the IL12 deficiency, as compared to the severity of the symptom observed without administration of the agent. The term "effective amount" can be used interchangeably with "effective dose," "therapeutically effective amount," or "therapeutically effective dose."

[1036] *Enantiomer:* As used herein, the term "enantiomer" means each individual optically active form of a compound of the disclosure, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (*i.e.,* at least 90% of one enantiomer and at most 10% of the other enantiomer), at least 90%, or at least 98%.

[1037] *Encapsulate:* As used herein, the term "encapsulate" means to enclose, surround or encase.

[1038] *Encapsulation Efficiency:* As used herein, "encapsulation efficiency" refers to the amount of a polynucleotide that becomes part of a nanoparticle composition, relative to the initial total amount of polynucleotide used in the preparation of a nanoparticle composition. For example, if 97 mg of polynucleotide are encapsulated in a nanoparticle composition out of a total 100 mg of polynucleotide initially provided to the composition, the encapsulation efficiency may be given as 97%. As used herein, "encapsulation" may refer to complete, substantial, or partial enclosure, confinement, surrounding, or encasement.

[1039] *Encoded protein cleavage signal:* As used herein, "encoded protein cleavage signal" refers to the nucleotide sequence that encodes a protein cleavage signal.

[1040] *Engineered:* As used herein, cases of the disclosure are "engineered" when they are designed to have a feature or property, whether structural or chemical, that varies from a starting point, wild type or native molecule.

[1041] *Enhanced Delivery:* As used herein, the term "enhanced delivery" means delivery of more *(e.g.,* at least 1.5 fold more, at least 2-fold more, at least 3-fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more) of a polynucleotide by a nanoparticle to a target tissue of interest (*e.g.*, mammalian liver) compared to the level of delivery of a polynucleotide by a control nanoparticle to a target tissue of interest (*e.g.*, MC3, KC2, or DLinDMA). The level of delivery of a nanoparticle to a particular tissue may be measured by comparing the amount of protein produced in a tissue to the weight of said tissue, comparing the amount of polynucleotide in a tissue to the weight of said tissue, comparing the amount of protein produced in a tissue to the amount of total protein in said tissue, or comparing the amount of polynucleotide in a tissue to the amount of total polynucleotide in said tissue. It will be understood that the enhanced delivery of a nanoparticle to a target tissue need not be determined in a subject being treated, it may be determined in a surrogate such as an animal model *(e.g.,* a rat model).

[1042] *Exosome:* As used herein, "exosome" is a vesicle secreted by mammalian cells or a complex involved in RNA degradation.

[1043] *Expression:* As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an mRNA template from a DNA sequence *(e.g.,* by transcription); (2) processing of an mRNA transcript *(e.g.,* by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an mRNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[1044] *Ex Vivo:* As used herein, the term "ex vivo" refers to events that occur outside of an organism (*e.g.*, animal, plant, or microbe or cell or tissue thereof). Ex vivo events may take place in an environment minimally altered from a natural (*e.g., in vivo)* environment.

[1045] *Feature:* As used herein, a "feature" refers to a characteristic, a property, or a distinctive element. When referring to polypeptides, "features" are defined as distinct amino acid sequence-based components of a molecule. Features of the polypeptides encoded by the polynucleotides of the present disclosure include surface manifestations, local conformational shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

[1046] *Formulation:* As used herein, a "formulation" includes at least a polynucleotide and one or more of a carrier, an excipient, and a delivery agent.

[1047] *Fragment:* A "fragment," as used herein, refers to a portion. For example, fragments of proteins can comprise polypeptides obtained by digesting full-length protein isolated from cultured cells. In some cases, a fragment is a subsequences of a full length protein (*e.g.*, IL12) wherein N-terminal, and/or C-terminal, and/or internal subsequences have been deleted. In some preferred aspects of the present disclosure, the fragments of a protein of the present disclosure are functional fragments.

[1048] *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. Thus, a functional fragment of a polynucleotide of the present disclosure is a polynucleotide capable of expressing a functional IL12 fragment. As used herein, a functional fragment of IL12 refers to a fragment of wild type IL12 (*i.e.,* a fragment of any of its naturally occurring isoforms), or a mutant or variant thereof, wherein the fragment retains a least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the biological activity of the corresponding full length protein.

[1049] *Helper Lipid:* As used herein, the term "helper lipid" refers to a compound or molecule that includes a lipidic moiety (for insertion into a lipid layer, *e.g.,* lipid bilayer) and a polar moiety (for interaction with physiologic solution at the surface of the lipid layer). Typically the helper lipid is a phospholipid. A function of the helper lipid is to "complement" the amino lipid and increase the fusogenicity of the bilayer and/or to help facilitate endosomal escape, *e.g.,* of nucleic acid delivered to cells. Helper lipids are also believed to be a key structural component to the surface of the LNP.

[1050] *Homology:* As used herein, the term "homology" 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. Generally, the term "homology" implies an evolutionary relationship between two molecules. Thus, two molecules that are homologous will have a common evolutionary ancestor. In the context of the present disclosure, the term homology encompasses both to identity and similarity.

[1051] In some cases, polymeric molecules are considered to be "homologous" to one another if at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the monomers in the molecule are identical (exactly the same monomer) or are similar (conservative substitutions). The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences).

[1052] *Identity:* As used herein, the term "identity" refers to the overall monomer conservation between polymeric molecules, *e.g.,* between polynucleotide molecules *(e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide 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 cases, 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 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. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent.

[1053] Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is bl2seq, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.,* Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at www.ebi.ac.uk/Tools/psa.

[1054] Sequence alignments can be conducted using methods known in the art such as MAFFT, Clustal (ClustalW, Clustal X or Clustal Omega), MUSCLE, etc.

[1055] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[1056] In certain aspects, the percentage identity "%ID" of a first amino acid sequence (or nucleic acid sequence) to a second amino acid sequence (or nucleic acid sequence) is calculated as %ID = 100 x (Y/Z), where Y is the number of amino acid residues (or nucleobases) scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[1057] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (*e.g.*, crystallographic protein structures), functional data (*e.g.*, location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.*, from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

[1058] *Immune response:* The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues. In some cases, the administration of a nanoparticle comprising a lipid component and an encapsulated therapeutic agent can trigger an immune response, which can be caused by (i) the encapsulated therapeutic agent *(e.g.,* an mRNA), (ii) the expression product of such encapsulated therapeutic agent *(e.g.,* a polypeptide encoded by the mRNA), (iii) the lipid component of the nanoparticle, or (iv) a combination thereof.

[1059] *Inflammatory response:* "Inflammatory response" refers to immune responses involving specific and non-specific defense systems. A specific defense system reaction is a specific immune system reaction to an antigen. Examples of specific defense system reactions include antibody responses. A non-specific defense system reaction is an inflammatory response mediated by leukocytes generally incapable of immunological memory, *e.g.*, macrophages, eosinophils and neutrophils. In some aspects, an immune response includes the secretion of inflammatory cytokines, resulting in elevated inflammatory cytokine levels.

[1060] *Inflammatory cytokines:* The term "inflammatory cytokine" refers to cytokines that are elevated in an inflamma-

tory response. Examples of inflammatory cytokines include interleukin-6 (IL-6), CXCL1 (chemokine (C-X-C motif) ligand 1; also known as GROα, interferon-y (IFNγ), tumor necrosis factor α (TNFα), interferon γ-induced protein 10 (IP-10), or granulocyte-colony stimulating factor (G-CSF). The term inflammatory cytokines includes also other cytokines associated with inflammatory responses known in the art, *e.g.*, interleukin-1 (IL-1), interleukin-8 (IL-8), interleukin-12 (IL12), interleukin-13 (11-13), interferon α (IFN-α), etc.

[1061] *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, in a Petri dish, etc., rather than within an organism (*e.g.*, animal, plant, or microbe).

[1062] *In Vivo:* As used herein, the term *"in vivo"* refers to events that occur within an organism (*e.g.*, animal, plant, or microbe or cell or tissue thereof).

[1063] *Insertional and deletional variants:* "Insertional variants" when referring to polypeptides are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native or starting sequence. "Immediately adjacent" to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid. "Deletional variants" when referring to polypeptides are those with one or more amino acids in the native or starting amino acid sequence removed. Ordinarily, deletional variants will have one or more amino acids deleted in a particular region of the molecule.

[1064] *Intact:* As used herein, in the context of a polypeptide, the term "intact" means retaining an amino acid corresponding to the wild type protein, *e.g.,* not mutating or substituting the wild type amino acid. Conversely, in the context of a nucleic acid, the term "intact" means retaining a nucleobase corresponding to the wild type nucleic acid, *e.g.,* not mutating or substituting the wild type nucleobase.

[1065] *Ionizable amino lipid:* The term "ionizable amino lipid" includes those lipids having one, two, three, or more fatty acid or fatty alkyl chains and a pH-titratable amino head group *(e.g.,* an alkylamino or dialkylamino head group). An ionizable amino lipid is typically protonated *(i.e.,* positively charged) at a pH below the pKa of the amino head group and is substantially not charged at a pH above the pKa. Such ionizable amino lipids include, but are not limited to DLin-MC3-DMA (MC3) and (13Z,165Z)-N,N-dimethyl-3-nonydocosa-13-16-dien-1-amine (L608).

[1066] *Isolated*: As used herein, the term "isolated" refers to a substance or entity that has been separated from at least some of the components with which it was associated (whether in nature or in an experimental setting). Isolated substances (*e.g.,* polynucleotides or polypeptides) can have varying levels of purity in reference to the substances from which they have been isolated. Isolated substances and/or entities can be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some cases, isolated substances are more than 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.

[1067] *Substantially isolated*: By "substantially isolated" is meant that the compound is substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compound of the present disclosure. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compound of the present disclosure, or salt thereof.

[1068] A polynucleotide, vector, polypeptide, cell, or any composition disclosed herein which is "isolated" is a polynucleotide, vector, polypeptide, cell, or composition which is in a form not found in nature. Isolated polynucleotides, vectors, polypeptides, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some aspects, a polynucleotide, vector, polypeptide, or composition which is isolated is substantially pure.

[1069] *Isomer:* As used herein, the term "isomer" means any tautomer, stereoisomer, enantiomer, or diastereomer of any compound of the disclosure. It is recognized that the compounds of the disclosure can have one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers *(i.e.,* geometric E/Z isomers) or diastereomers *(e.g.,* enantiomers *(i.e.,* (+) or (-)) or cis/trans isomers). According to the disclosure, the chemical structures depicted herein, and therefore the compounds of the disclosure, encompass all of the corresponding stereoisomers, that is, both the stereomerically pure form (*e.g.*, geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, *e.g.*, racemates. Enantiomeric and stereoisomeric mixtures of compounds of the disclosure can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[1070] *Linker:* As used herein, a "linker" (including a subunit linker and a heterologous polypeptide linker as referred to herein) refers to a group of atoms, *e.g.*, 10-1,000 atoms, and can be comprised of the atoms or groups such as, but not limited to, carbon, amino, alkylamino, oxygen, sulfur, sulfoxide, sulfonyl, carbonyl, and imine. The linker can be attached to a modified nucleoside or nucleotide on the nucleobase or sugar moiety at a first end, and to a payload, *e.g.,*

a detectable or therapeutic agent, at a second end. The linker can be of sufficient length as to not interfere with incorporation into a nucleic acid sequence. The linker can be used for any useful purpose, such as to form polynucleotide multimers (*e.g.*, through linkage of two or more chimeric polynucleotides molecules or IVT polynucleotides) or polynucleotides conjugates, as well as to administer a payload, as described herein. Examples of chemical groups that can be incorporated into the linker include, but are not limited to, alkyl, alkenyl, alkynyl, amido, amino, ether, thioether, ester, alkylene, heteroalkylene, aryl, or heterocyclyl, each of which can be optionally substituted, as described herein. Examples of linkers include, but are not limited to, unsaturated alkanes, polyethylene glycols (*e.g.*, ethylene or propylene glycol monomeric units, *e.g.*, diethylene glycol, dipropylene glycol, triethylene glycol, tripropylene glycol, tetraethylene glycol, or tetraethylene glycol), and dextran polymers and derivatives thereof., Other examples include, but are not limited to, cleavable moieties within the linker, such as, for example, a disulfide bond (-S-S-) or an azo bond (-N=N-), which can be cleaved using a reducing agent or photolysis. Non-limiting examples of a selectively cleavable bond include an amido bond can be cleaved for example by the use of tris(2-carboxyethyl)phosphine (TCEP), or other reducing agents, and/or photolysis, as well as an ester bond can be cleaved for example by acidic or basic hydrolysis.

[1071] *Methods of Administration:* As used herein, "methods of administration" may include intravenous, intramuscular, intradermal, subcutaneous, or other methods of delivering a composition to a subject. A method of administration may be selected to target delivery *(e.g.,* to specifically deliver) to a specific region or system of a body.

[1072] *Modified:* As used herein "modified" refers to a changed state or structure of a molecule of the disclosure. Molecules can be modified in many ways including chemically, structurally, and functionally. In some cases, the mRNA molecules of the present disclosure are modified by the introduction of non-natural nucleosides and/or nucleotides, *e.g.*, as it relates to the natural ribonucleotides A, U, G, and C. Noncanonical nucleotides such as the cap structures are not considered "modified" although they differ from the chemical structure of the A, C, G, U ribonucleotides.

[1073] *Mucus:* As used herein, "mucus" refers to the natural substance that is viscous and comprises mucin glycoproteins.

[1074] *Nanoparticle Composition:* As used herein, a "nanoparticle composition" is a composition comprising one or more lipids. Nanoparticle compositions are typically sized on the order of micrometers or smaller and may include a lipid bilayer. Nanoparticle compositions encompass lipid nanoparticles (LNPs), liposomes (*e.g.*, lipid vesicles), and lipoplexes. For example, a nanoparticle composition may be a liposome having a lipid bilayer with a diameter of 500 nm or less.

[1075] *Naturally occurring:* As used herein, "naturally occurring" means existing in nature without artificial aid.

[1076] *Non-human vertebrate:* As used herein, a "non-human vertebrate" includes all vertebrates except *Homo sapiens,* including wild and domesticated species. Examples of non-human vertebrates include, but are not limited to, mammals, such as alpaca, banteng, bison, camel, cat, cattle, deer, dog, donkey, gayal, goat, guinea pig, horse, llama, mule, pig, rabbit, reindeer, sheep water buffalo, and yak.

[1077] *Nucleic acid sequence:* The terms "nucleic acid sequence," "nucleotide sequence," or "polynucleotide sequence" are used interchangeably and refer to a contiguous nucleic acid sequence. The sequence can be either single stranded or double stranded DNA or RNA, *e.g.,* an mRNA.

[1078] The term "nucleic acid," in its broadest sense, includes any compound and/or substance that comprises a polymer of nucleotides. These polymers are often referred to as polynucleotides. Exemplary nucleic acids or polynucleotides of the disclosure include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β- D-ribo configuration, α-LNA having an α-L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α-LNA having a 2'-amino functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) or hybrids or combinations thereof.

[1079] The phrase "nucleotide sequence encoding" refers to the nucleic acid *(e.g.,* an mRNA or DNA molecule) coding sequence which encodes a polypeptide. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to which the nucleic acid is administered. The coding sequence can further include sequences that encode signal peptides.

[1080] *Off-target:* As used herein, "off target" refers to any unintended effect on any one or more target, gene, or cellular transcript.

[1081] *Open reading frame:* As used herein, "open reading frame" or "ORF" refers to a sequence which does not contain a stop codon in a given reading frame.

[1082] *Operably linked:* As used herein, the phrase "operably linked" refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties or the like.

[1083] *Optionally substituted:* Herein a phrase of the form "optionally substituted X" (*e.g.*, optionally substituted alkyl) is intended to be equivalent to "X, wherein X is optionally substituted" (*e.g.*, "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" *(e.g.,* alkyl) *per se* is optional.

[1084] *Part:* As used herein, a "part" or "region" of a polynucleotide is defined as any portion of the polynucleotide that is less than the entire length of the polynucleotide.

[1085] *Patient:* As used herein, "patient" refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition.

[1086] *Pharmaceutically acceptable:* The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, 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.

[1087] *Pharmaceutically acceptable excipients:* The phrase "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients can include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

[1088] *Pharmaceutically acceptable salts:* The present disclosure also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form *(e.g.,* by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, acetic acid, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzene sulfonic acid, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound that contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, Pharmaceutical Salts: Properties, Selection, and Use, P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., Journal of Pharmaceutical Science, 66, 1-19 (1977).

[1089] *Pharmaceutically acceptable solvate:* The term "pharmaceutically acceptable solvate," as used herein, means a compound of the disclosure wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. For example, solvates can be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N,N'*-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate."

[1090] *Pharmacokinetic:* As used herein, "pharmacokinetic" refers to any one or more properties of a molecule or compound as it relates to the determination of the fate of substances administered to a living organism. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as ADME where: (A) Absorption is the process of a substance entering the blood circulation; (D) Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body; (M) Metabolism

(or Biotransformation) is the irreversible transformation of parent compounds into daughter metabolites; and (E) Excretion (or Elimination) refers to the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

[1091] *Physicochemical:* As used herein, "physicochemical" means of or relating to a physical and/or chemical property.

[1092] *Polynucleotide:* The term "polynucleotide" as used herein refers to polymers of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof. This term refers to the primary structure of the molecule. Thus, the term includes triple-, double- and single-stranded deoxyribonucleic acid ("DNA"), as well as triple-, double- and single-stranded ribonucleic acid ("RNA"). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the polynucleotide. More particularly, the term "polynucleotide" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, hRNA, siRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (e.g., peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA. In particular aspects, the polynucleotide comprises an mRNA. In other aspect, the mRNA is a synthetic mRNA. In some aspects, the synthetic mRNA comprises at least one unnatural nucleobase. In some aspects, all nucleobases of a certain class have been replaced with unnatural nucleobases (*e.g.,* all uridines in a polynucleotide disclosed herein can be replaced with an unnatural nucleobase, *e.g.,* 5-methoxyuridine). In some aspects, the polynucleotide (*e.g.,* a synthetic RNA or a synthetic DNA) comprises only natural nucleobases, *i.e.,* A (adenosine), G (guanosine), C (cytidine), and T (thymidine) in the case of a synthetic DNA, or A, C, G, and U (uridine) in the case of a synthetic RNA.

[1093] The skilled artisan will appreciate that the T bases in the codon maps disclosed herein are present in DNA, whereas the T bases would be replaced by U bases in corresponding RNAs. For example, a codon-nucleotide sequence disclosed herein in DNA form, *e.g.,* a vector or an in-vitro translation (IVT) template, would have its T bases transcribed as U based in its corresponding transcribed mRNA. In this respect, both codon-optimized DNA sequences (comprising T) and their corresponding mRNA sequences (comprising U) are considered codon-optimized nucleotide sequence of the present disclosure. A skilled artisan would also understand that equivalent codon-maps can be generated by replaced one or more bases with non-natural bases. Thus, *e.g.,* a TTC codon (DNA map) would correspond to a UUC codon (RNA map), which in turn would correspond to a ΨΨC codon (RNA map in which U has been replaced with pseudouridine).

[1094] Standard A-T and G-C base pairs form under conditions which allow the formation of hydrogen bonds between the N3-H and C4-oxy of thymidine and the N1 and C6-NH2, respectively, of adenosine and between the C2-oxy, N3 and C4-NH2, of cytidine and the C2-NH2, N'-H and C6-oxy, respectively, of guanosine. Thus, for example, guanosine (2-amino-6-oxy-9-β-D-ribofuranosyl-purine) can be modified to form isoguanosine (2-oxy-6-amino-9-β-D-ribofuranosyl-purine). Such modification results in a nucleoside base which will no longer effectively form a standard base pair with cytosine. However, modification of cytosine (1-β-D-ribofuranosyl-2-oxy-4-amino-pyrimidine) to form isocytosine (1-β-D-ribofuranosyl-2-amino-4-oxy-pyrimidine-) results in a modified nucleotide which will not effectively base pair with guanosine but will form a base pair with isoguanosine (U.S. Pat. No. 5,681,702 to Collins et al.). Isocytosine is available from Sigma Chemical Co. (St. Louis, Mo.); isocytidine can be prepared by the method described by Switzer et al. (1993) Biochemistry 32:10489-10496 and references cited therein; 2'-deoxy-5-methyl-isocytidine can be prepared by the method of Tor et al., 1993, J. Am. Chem. Soc. 115:4461-4467 and references cited therein; and isoguanine nucleotides can be prepared using the method described by Switzer et al., 1993, supra, and Mantsch et al., 1993, Biochem. 14:5593-5601, or by the method described in U.S. Pat. No. 5,780,610 to Collins et al. Other nonnatural base pairs can be synthesized by the method described in Piccirilli et al., 1990, Nature 343:33-37, for the synthesis of 2,6-diaminopyrimidine and its complement (1-methylpyrazolo-[4,3]pyrimidine-5,7-(4H,6H)-dione. Other such modified nucleotide units which form unique base pairs are known, such as those described in Leach et al. (1992) J. Am. Chem. Soc. 114:3675-3683 and Switzer et al., supra.

[1095] *Polypeptide:* The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can comprise modified amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids such as homocysteine, ornithine, p-acetylphenylalanine, D-amino acids, and creatine), as well as other modifications known in the art.

[1096] The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. Polypeptides include encoded polynucleotide products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide can be a monomer or can be a multi-molecular complex such as a dimer, trimer or tetramer. They can also comprise single chain or multichain polypeptides. Most commonly disulfide linkages are found in multichain polypeptides. The term

polypeptide can also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid. In some cases, a "peptide" can be less than or equal to 50 amino acids long, *e.g.,* about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[1097] *Polypeptide variant:* As used herein, the term "polypeptide variant" refers to molecules that differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants can possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants will possess at least about 50% identity, at least about 60% identity, at least about 70% identity, at least about 80% identity, at least about 90% identity, at least about 95% identity, at least about 99% identity to a native or reference sequence. In some cases, they will be at least about 80%, or at least about 90% identical to a native or reference sequence.

[1098] *Polypeptide per unit drug (PUD):* As used herein, a PUD or product per unit drug, is defined as a subdivided portion of total daily dose, usually 1 mg, pg, kg, etc., of a product (such as a polypeptide) as measured in body fluid or tissue, usually defined in concentration such as pmol/mL, mmol/mL, etc. divided by the measure in the body fluid.

[1099] *Preventing:* As used herein, the term "preventing" refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[1100] *Prodrug:* The present disclosure also includes prodrugs of the compounds described herein. As used herein, "prodrugs" refer to any substance, molecule or entity that is in a form predicate for that substance, molecule or entity to act as a therapeutic upon chemical or physical alteration. Prodrugs may by covalently bonded or sequestered in some way and that release or are converted into the active drug moiety prior to, upon or after administered to a mammalian subject. Prodrugs can be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or *in vivo,* to the parent compounds. Prodrugs include compounds wherein hydroxyl, amino, sulfhydryl, or carboxyl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, sulfhydryl, or carboxyl group respectively. Preparation and use of prodrugs is discussed in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987.

[1101] *Proliferate:* As used herein, the term "proliferate" means to grow, expand or increase or cause to grow, expand or increase rapidly. "Proliferative" means having the ability to proliferate. "Anti-proliferative" means having properties counter to or inapposite to proliferative properties.

[1102] *Prophylactic:* As used herein, "prophylactic" refers to a therapeutic or course of action used to prevent the spread of disease.

[1103] *Prophylaxis:* As used herein, a "prophylaxis" refers to a measure taken to maintain health and prevent the spread of disease. An "immune prophylaxis" refers to a measure to produce active or passive immunity to prevent the spread of disease.

[1104] *Protein cleavage site:* As used herein, "protein cleavage site" refers to a site where controlled cleavage of the amino acid chain can be accomplished by chemical, enzymatic or photochemical means.

[1105] *Protein cleavage signal:* As used herein "protein cleavage signal" refers to at least one amino acid that flags or marks a polypeptide for cleavage.

[1106] *Protein of interest:* As used herein, the terms "proteins of interest" or "desired proteins" include those provided herein and fragments, mutants, variants, and alterations thereof.

[1107] *Proximal:* As used herein, the term "proximal" means situated nearer to the center or to a point or region of interest.

[1108] *Pseudouridine:* As used herein, pseudouridine (ψ) refers to the C-glycoside isomer of the nucleoside uridine. A "pseudouridine analog" is any modification, variant, isoform or derivative of pseudouridine. For example, pseudouridine analogs include but are not limited to 1-carboxymethyl-pseudouridine, 1-propynyl-pseudouridine, 1-taurinomethyl-pseudouridine, 1-taurinomethyl-4-thio-pseudouridine, 1-methylpseudouridine ($m^1\psi$), 1-methyl-4-thio-pseudouridine ($m^1s^4\psi$), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine ($m^3\psi$), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydropseudouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($acp^3\ \psi$), and 2'-O-methyl-pseudouridine (ψm).

[1109] *Purified:* As used herein, "purify," "purified," "purification" means to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection.

[1110] *Reference Nucleic Acid Sequence:* The term "reference nucleic acid sequence" or "reference nucleic acid" or "reference nucleotide sequence" or "reference sequence" refers to a starting nucleic acid sequence *(e.g.,* a RNA, *e.g.,*

an mRNA sequence) that can be sequence optimized. In some cases, the reference nucleic acid sequence is a wild type nucleic acid sequence, a fragment or a variant thereof. In some cases, the reference nucleic acid sequence is a previously sequence optimized nucleic acid sequence.

[1111] *Repeated transfection:* As used herein, the term "repeated transfection" refers to transfection of the same cell culture with a polynucleotide a plurality of times. The cell culture can be transfected at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, at least 10 times, at least 11 times, at least 12 times, at least 13 times, at least 14 times, at least 15 times, at least 16 times, at least 17 times at least 18 times, at least 19 times, at least 20 times, at least 25 times, at least 30 times, at least 35 times, at least 40 times, at least 45 times, at least 50 times or more.

[1112] *Salts:* In some aspects, the pharmaceutical composition for intratumoral delivery disclosed herein and comprises salts of some of their lipid constituents. The term "salt" includes any anionic and cationic complex. Non-limiting examples of anions include inorganic and organic anions, *e.g.,* fluoride, chloride, bromide, iodide, oxalate (*e.g.,* hemioxalate), phosphate, phosphonate, hydrogen phosphate, dihydrogen phosphate, oxide, carbonate, bicarbonate, nitrate, nitrite, nitride, bisulfite, sulfide, sulfite, bisulfate, sulfate, thiosulfate, hydrogen sulfate, borate, formate, acetate, benzoate, citrate, tartrate, lactate, acrylate, polyacrylate, fumarate, maleate, itaconate, glycolate, gluconate, malate, mandelate, tiglate, ascorbate, salicylate, polymethacrylate, perchlorate, chlorate, chlorite, hypochlorite, bromate, hypobromite, iodate, an alkylsulfonate, an arylsulfonate, arsenate, arsenite, chromate, dichromate, cyanide, cyanate, thiocyanate, hydroxide, peroxide, permanganate, and mixtures thereof.

[1113] *Sample:* As used herein, the term "sample" or "biological sample" refers to a subset of its tissues, cells or component parts (e.g., body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). A sample further can include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule.

[1114] *Signal Sequence:* As used herein, the phrases "signal sequence," "signal peptide," and "transit peptide" are used interchangeably and refer to a sequence that can direct the transport or localization of a protein to a certain organelle, cell compartment, or extracellular export. The term encompasses both the signal sequence polypeptide and the nucleic acid sequence encoding the signal sequence. Thus, references to a signal sequence in the context of a nucleic acid refer in fact to the nucleic acid sequence encoding the signal sequence polypeptide.

[1115] *Signal transduction pathway:* A "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell.

[1116] *Similarity:* As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[1117] *Single unit dose:* As used herein, a "single unit dose" is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, *i.e.,* single administration event.

[1118] *Split dose:* As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses.

[1119] *Specific delivery:* As used herein, the term "specific delivery," "specifically deliver," or "specifically delivering" means delivery of more *(e.g.,* at least 1.5 fold more, at least 2-fold more, at least 3-fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more) of a polynucleotide by a nanoparticle to a target tissue of interest (*e.g.,* mammalian liver) compared to an off-target tissue (*e.g.,* mammalian spleen). The level of delivery of a nanoparticle to a particular tissue may be measured by comparing the amount of protein produced in a tissue to the weight of said tissue, comparing the amount of polynucleotide in a tissue to the weight of said tissue, comparing the amount of protein produced in a tissue to the amount of total protein in said tissue, or comparing the amount of polynucleotide in a tissue to the amount of total polynucleotide in said tissue. For example, for renovascular targeting, a polynucleotide is specifically provided to a mammalian kidney as compared to the liver and spleen if 1.5, 2-fold, 3-fold, 5-fold, 10-fold, 15 fold, or 20 fold more polynucleotide per 1 g of tissue is delivered to a kidney compared to that delivered to the liver or spleen following systemic administration of the polynucleotide. It will be understood that the ability of a nanoparticle to specifically deliver to a target tissue need not be determined in a subject being treated, it may be determined in a surrogate such as an animal model (*e.g.,* a rat

model).

[1120] *Stable:* As used herein "stable" refers to a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and in some cases capable of formulation into an efficacious therapeutic agent.

[1121] *Stabilized:* As used herein, the term "stabilize," "stabilized," "stabilized region" means to make or become stable.

[1122] *Stereoisomer:* As used herein, the term "stereoisomer" refers to all possible different isomeric as well as conformational forms that a compound may possess (*e.g.,* a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure. Some compounds of the present disclosure may exist in different tautomeric forms, all of the latter being included within the scope of the present disclosure.

[1123] *Subject:* By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, but are not limited to, humans, domestic animals, farm animals, zoo animals, sport animals, pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows; primates such as apes, monkeys, orangutans, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; bears, food animals such as cows, pigs, and sheep; ungulates such as deer and giraffes; rodents such as mice, rats, hamsters and guinea pigs; and so on. In certain cases, the mammal is a human subject. In other cases, a subject is a human patient. In a particular case, a subject is a human patient in need of treatment.

[1124] *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 characteristics 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 characteristics.

[1125] *Substantially equal:* As used herein as it relates to time differences between doses, the term means plus/minus 2%.

[1126] *Substantially simultaneous:* As used herein and as it relates to plurality of doses, the term means within 2 seconds.

[1127] *Sufferingfrom:* An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of the disease, disorder, and/or condition.

[1128] *Susceptible to:* An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some cases, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) can be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some cases, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some cases, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[1129] *Sustained release:* As used herein, the term "sustained release" refers to a pharmaceutical composition or compound release profile that conforms to a release rate over a specific period of time.

[1130] *Synthetic:* The term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or other molecules of the present disclosure can be chemical or enzymatic.

[1131] *Targeted Cells:* As used herein, "targeted cells" refers to any one or more cells of interest. The cells can be found *in vitro, in vivo, in situ* or in the tissue or organ of an organism. The organism can be an animal, for example a mammal, a human, a subject or a patient.

[1132] *Target tissue:* As used herein "target tissue" refers to any one or more tissue types of interest in which the delivery of a polynucleotide would result in a desired biological and/or pharmacological effect. Examples of target tissues of interest include specific tissues, organs, and systems or groups thereof. In particular applications, a target tissue may be a kidney, a lung, a spleen, vascular endothelium in vessels (*e.g.,* intra-coronary or intra-femoral), or tumor tissue (*e.g.,* via intratumoral injection). An "off-target tissue" refers to any one or more tissue types in which the expression of the encoded protein does not result in a desired biological and/or pharmacological effect. In particular applications, off-target tissues may include the liver and the spleen.

[1133] The presence of a therapeutic agent in an off-target issue can be the result of: (i) leakage of a polynucleotide from the administration site to peripheral tissue or distant off-target tissue *(e.g.,* liver) via diffusion or through the bloodstream *(e.g.,* a polynucleotide intended to express a polypeptide in a certain tissue would reach the liver and the polypeptide would be expressed in the liver); or (ii) leakage of an polypeptide after administration of a polynucleotide encoding

such polypeptide to peripheral tissue or distant off-target tissue *(e.g.,* liver) via diffusion or through the bloodstream *(e.g.,* a polynucleotide would expressed a polypeptide in the target tissue, and the polypeptide would diffuse to peripheral tissue).

[1134] *Targeting sequence:* As used herein, the phrase "targeting sequence" refers to a sequence that can direct the transport or localization of a protein or polypeptide.

[1135] *Terminus:* As used herein the terms "termini" or "terminus," when referring to polypeptides, refers to an extremity of a peptide or polypeptide. Such extremity is not limited only to the first or final site of the peptide or polypeptide but can include additional amino acids in the terminal regions. The polypeptide based molecules of the disclosure can be characterized as having both an N-terminus (terminated by an amino acid with a free amino group (NH_2)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins of the disclosure are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These sorts of proteins will have multiple N- and C-termini. Alternatively, the termini of the polypeptides can be modified such that they begin or end, as the case can be, with a non-polypeptide based moiety such as an organic conjugate.

[1136] *Therapeutic Agent:* The term "therapeutic agent" refers to an agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect. For example, in some cases, an mRNA encoding an IL12B polypeptide, IL12A polypeptide, or both IL12B and IL12A polypeptides can be a therapeutic agent.

[1137] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (e.g., nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, etc.) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[1138] *Therapeutically effective outcome:* As used herein, the term "therapeutically effective outcome" means an outcome that is sufficient in a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[1139] *Total daily dose:* As used herein, a "total daily dose" is an amount given or prescribed in 24 hr. period. The total daily dose can be administered as a single unit dose or a split dose.

[1140] *Transcription factor:* As used herein, the term "transcription factor" refers to a DNA-binding protein that regulates transcription of DNA into RNA, for example, by activation or repression of transcription. Some transcription factors effect regulation of transcription alone, while others act in concert with other proteins. Some transcription factor can both activate and repress transcription under certain conditions. In general, transcription factors bind a specific target sequence or sequences highly similar to a specific consensus sequence in a regulatory region of a target gene. Transcription factors may regulate transcription of a target gene alone or in a complex with other molecules.

[1141] *Transcription:* As used herein, the term "transcription" refers to methods to introduce exogenous nucleic acids into a cell. Methods of transfection include, but are not limited to, chemical methods, physical treatments and cationic lipids or mixtures.

[1142] *Transfection:* As used herein, "transfection" refers to the introduction of a polynucleotide into a cell wherein a polypeptide encoded by the polynucleotide is expressed *(e.g.,* mRNA) or the polypeptide modulates a cellular function *(e.g.,* siRNA, miRNA). As used herein, "expression" of a nucleic acid sequence refers to translation of a polynucleotide (*e.g.,* an mRNA) into a polypeptide or protein and/or post-translational modification of a polypeptide or protein.

[1143] *Treating, treatment, therapy:* As used herein, the term "treating" or "treatment" or "therapy" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severit of, and/or reducing incidence of one or more symptoms or features of a disease, e.g., acute intermittent porphyria. For example, "treating" acute intermittent porphyria can refer to diminishing symptoms associate with the disease, prolong the lifespan (increase the survival rate) of patients, reducing the severity of the disease, preventing or delaying the onset of the disease, etc. Treatment can be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[1144] *Unmodified:* As used herein, "unmodified" refers to any substance, compound or molecule prior to being changed in some way. Unmodified can, but does not always, refer to the wild type or native form of a biomolecule. Molecules can undergo a series of modifications whereby each modified molecule can serve as the "unmodified" starting molecule for a subsequent modification.

[1145] *Uracil:* Uracil is one of the four nucleobases in the nucleic acid of RNA, and it is represented by the letter U. Uracil can be attached to a ribose ring, or more specifically, a ribofuranose via a β-N_1-glycosidic bond to yield the nucleoside uridine. The nucleoside uridine is also commonly abbreviated according to the one letter code of its nucleobase, *i.e.,* U. Thus, in the context of the present disclosure, when a monomer in a polynucleotide sequence is U, such

U is designated interchangeably as a "uracil" or a "uridine."

[1146] *Uridine Content:* The terms "uridine content" or "uracil content" are interchangeable and refer to the amount of uracil or uridine present in a certain nucleic acid sequence. Uridine content or uracil content can be expressed as an absolute value (total number of uridine or uracil in the sequence) or relative (uridine or uracil percentage respect to the total number of nucleobases in the nucleic acid sequence).

[1147] *Uridine-Modified Sequence:* The terms "uridine-modified sequence" refers to a sequence optimized nucleic acid *(e.g.,* a synthetic mRNA sequence) with a different overall or local uridine content (higher or lower uridine content) or with different uridine patterns (*e.g.,* gradient distribution or clustering) with respect to the uridine content and/or uridine patterns of a candidate nucleic acid sequence. In the content of the present disclosure, the terms "uridine-modified sequence" and "uracil-modified sequence" are considered equivalent and interchangeable.

[1148] A "high uridine codon" is defined as a codon comprising two or three uridines, a "low uridine codon" is defined as a codon comprising one uridine, and a "no uridine codon" is a codon without any uridines. In some cases, a uridine-modified sequence comprises substitutions of high uridine codons with low uridine codons, substitutions of high uridine codons with no uridine codons, substitutions of low uridine codons with high uridine codons, substitutions of low uridine codons with no uridine codons, substitution of no uridine codons with low uridine codons, substitutions of no uridine codons with high uridine codons, and combinations thereof. In some cases, a high uridine codon can be replaced with another high uridine codon. In some cases, a low uridine codon can be replaced with another low uridine codon. In some cases, a no uridine codon can be replaced with another no uridine codon. A uridine-modified sequence can be uridine enriched or uridine rarefied.

[1149] *Uridine Enriched:* As used herein, the terms "uridine enriched" and grammatical variants refer to the increase in uridine content (expressed in absolute value or as a percentage value) in an sequence optimized nucleic acid *(e.g.,* a synthetic mRNA sequence) with respect to the uridine content of the corresponding candidate nucleic acid sequence. Uridine enrichment can be implemented by substituting codons in the candidate nucleic acid sequence with synonymous codons containing less uridine nucleobases. Uridine enrichment can be global *(i.e.,* relative to the entire length of a candidate nucleic acid sequence) or local *(i.e.,* relative to a subsequence or region of a candidate nucleic acid sequence).

[1150] *Uridine Rarefied:* As used herein, the terms "uridine rarefied" and grammatical variants refer to a decrease in uridine content (expressed in absolute value or as a percentage value) in an sequence optimized nucleic acid *(e.g.,* a synthetic mRNA sequence) with respect to the uridine content of the corresponding candidate nucleic acid sequence. Uridine rarefication can be implemented by substituting codons in the candidate nucleic acid sequence with synonymous codons containing less uridine nucleobases. Uridine rarefication can be global *(i.e.,* relative to the entire length of a candidate nucleic acid sequence) or local *(i.e.,* relative to a subsequence or region of a candidate nucleic acid sequence).

[1151] *Variant:* The term variant as used in present disclosure refers to both natural variants (*e.g.,* polymorphisms, isoforms, etc) and artificial variants in which at least one amino acid residue in a native or starting sequence *(e.g.,* a wild type sequence) has been removed and a different amino acid inserted in its place at the same position. These variants can be described as "substitutional variants." The substitutions can be single, where only one amino acid in the molecule has been substituted, or they can be multiple, where two or more amino acids have been substituted in the same molecule. If amino acids are inserted or deleted, the resulting variant would be an "insertional variant" or a "deletional variant" respectively.

[1152] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes cases in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes cases in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[1153] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

[1154] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different aspects of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

Examples

Example 1: *In Vivo* Anti-Tumor Efficacy of IL12 Modified mRNA in a Colon Adenocarcinoma (MC38) Syngeneic Model *(intravenous* administration)

[1155] The *in vivo* anti-tumor efficacy of IL12 mRNA, administered as a single intravenous (IV) dose in mice bearing M38 adenocarcinoma tumors, was assessed.

A. Preparation of IL12 modified mRNA and control

[1156] A polynucleotide (mRNA) comprising a codon-optimized nucleotide sequence encoding a wild-type murine IL12 polypeptide (murine IL12) and a miRNA binding site (miR-122) in its 3' UTR were prepared (mIL12_miR122) (sequence set forth below).

GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUGUCCUCAGAAGCUAACCAUC
UCCUGGUUUGCCAUCGUUUUGCUGGUGUCUCCACUCAUGGCCAUGUGGGAGCUGGAGAAAGACGUUUAUG
UUGUAGAGGUGGACUGGACUCCCGAUGCCCCUGGAGAAACAGUGAACCUCACCUGUGACACGCCUGAAGAA
GAUGACAUCACCUGGACCUCAGACCAGAGACAUGGAGUCAUAGGCUCUGGAAAGACCCUGACCAUCACUGUC
AAAGAGUUCCUAGAUGCUGGCCAGUACACCUGCCACAAAGGAGGCGAGACUCUGAGCCACUCACAUCUGCUG
CUCCACAAGAAGGAAAAUGGAAUUUGGUCCACUGAAAUUUUAAAAAAUUUCAAAAACAAGACUUUCCUGAA
GUGUGAAGCACCAAAUUACUCCGGACGGUUCACGUGCUCAUGGCUGGUGCAAAGAAACAUGGACUUGAAGU
UCAACAUCAAGAGCAGUAGCAGUUCCCCUGACUCUCGGGCAGUGACAUGUGGAAUGGCGUCUCUGUCUGCA
GAGAAGGUCACACUGGACCAAAGGGACUAUGAGAAGUAUUCAGUGUCCUGCCAGGAGGAUGUCACCUGCCC
AACUGCCGAGGAGACCCUGCCCAUUGAACUGGCGUUGGAAGCACGGCAGCAGAAUAAAUAUGAGAACUACAG
CACCAGCUUCUUCAUCAGGGACAUCAUCAAACCAGACCCGCCCAAGAACUUGCAGAUGAAGCCUUUGAAGAA
CUCACAGGUGGAGGUCAGCUGGGAGUACCCUGACUCCUGGAGCACUCCCCAUUCCUACUUCUCCCUCAAGUU
CUUUGUUCGAAUCCAGCGCAAGAAAGAAAAGAUGAAGGAGACAGAGGAGGGGUGUAACCAGAAAGGUGCGU
UCCUCGUAGAGAAGACAUCUACCGAAGUCCAAUGCAAAGGCGGGAAUGUCUGCGUGCAAGCUCAGGAUCGC
UAUUACAAUUCCUCAUGCAGCAAGUGGGCAUGUGUUCCCUGCAGGGUCCGAUCCGGAGGCGGAGGGAGCGG
AGGCGGAGGGAGCGGAGGCGGAGGGAGCAGGGUCAUUCCAGUCUCUGGACCUGCCAGGUGUCUUAGCCAG
UCCCGAAACCUGCUGAAGACCACAGAUGACAUGGUGAAGACGGCCAGAGAAAAACUGAAACAUUAUUCCUGC
ACUGCUGAAGACAUCGAUCAUGAAGACAUCACACGGGACCAAACCAGCACAUUGAAGACCUGUUUACCACUG
GAACUACACAAGAACGAGAGUUGCCUGGCUACUAGAGAGACUUCUUCCACAACAAGAGGGAGCUGCCUGCCC
CCACAGAAGACGUCUUUGAUGAUGACCCUGUGCCUUGGUAGCAUCUAUGAGGACUUGAAGAUGUACCAGAC
AGAGUUCCAGGCCAUCAACGCAGCACUUCAGAAUCACAACCAUCAGCAGAUCAUUUUAGACAAGGGCAUGCU
GGUGGCCAUCGAUGAGCUGAUGCAGUCUCUGAAUCAUAAUGGCGAGACUCUGCGCCAGAAACCUCCUGUGG
GAGAAGCAGACCCUUACAGAGUGAAAAUGAAGCUCUGCAUCCUGCUUCACGCCUUCAGCACCCGCGUCGUGA
CCAUCAACAGGGUGAUGGGCUAUCUGAGCUCCGCC**UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCU**
UGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGUACCCCCCAAACACCAUUGUCACACUCC
AGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 241)

[1157] The miR-122 binding element was incorporated to decrease protein production from the liver. A negative control mRNA was also prepared (non-translatable version of mRNAs), e.g., NST-FIX. The mRNAs were fully modified with N1-methylpseudouridine. Both modified mRNAs were formulated in MC3 lipid nanoparticles (LNP).

B. MC-38 Colon Adenocarcinoma Mouse Model

[1158] MC-38 colon adenocarcinoma tumors were implanted subcutaneously in mice as described in Rosenberg et al., Science 233:1318-1321 (1986)).

[1159] Eleven days after tumor implantation, two groups of animals were administered a single intravenous dose of LNP-formulated IL12 modified mRNA (at a dose of either 0.1 mg/kg (Group 4) or 0.05 mg/kg (Group 5). Two groups of control animals were treated with equivalent doses of negative control mRNA (NST-FIX LNP) (Group 7 and Group 8), PBS (Group 1), or recombinant murine IL12 protein, 1 μg (Group 2).

[1160] Tumor volume was measured using manual calipers. Mean tumor volume to day 24 **(Fig. 5)** was recorded in

cubic millimeters (mm^3).

C. Results

[1161] Intravenous administration of murine IL12 mRNA resulted in dose dependent plasma IL12 **(FIG. 2A)** and IPNγ **(FIG. 2B)** levels and increased IL12 and IPNγ AUC **(Table 8),** each of which were higher than those levels following administration of a comparable amount of recombinant IL12 protein by intraperitoneal injection **(FIGs. 2A-2B).**

Table 8: AUC levels for IL12 and IPNγ plasma levels.

Treatment	IL12 AUC (ng/mL)*hr 0.5-196	IFNγ AUC (ng/mL)*hr 0.5-196
0.1 mpk IL12 mRNA	18805 (↑ *10.4x)*	5290 (↑ *21.3x)*
0.05 mpk IL12 mRNA	7500 *(↑4.1x)*	1856 *(↑ 7.5x)*
~0.05 mpk IL12 protein	1801	248

The numbers in parentheses indicate the x-fold increase for mRNA over protein.

[1162] **FIG. 3** depicts the robust efficacy of a single intravenous (IV) dose of murine IL12 mRNA in lipid nanoparticle (LNP), at doses of 0.1 mg/kg (Group 4) and 0.05 mg/kg (Group 5) (as indicated by lines with the inverted triangles), compared to Groups 1 (PBS), 2 (IL12 protein), 7 and 8 (controls NST-FIX, 0.1 mg/kg and 0.05 mg/kg, respectively).

[1163] **FIGs. 4A-4F** depict the mean tumor volume and the number of complete responses (CR) seen following administration of a single intravenous (IV) dose of: murine IL12 mRNA in lipid nanoparticle (LNP), at doses of 0.1 mg/kg (Group 4)(**FIG. 4F)** and 0.05 mg/kg (Group 5)**(FIG. 4E),** PBS (Group 1)(**FIG. 4A), IL12** protein (Group 2)(**FIG. 4D),** controls NST-FIX, 0.1 mg/kg and 0.05 mg/kg (Groups 7 and 8, respectively) **(FIGs. 4C** and **4B,** respectively). Complete responses (CRs) are shown in **FIGs. 4E** and **4F** only. **FIG. 4E** shows that 6 of 8 CRs (i.e., 75% CR) were seen in Group 5 (IL12 mRNA in lipid nanoparticle (LNP), at a dose of 0.05 mg/kg). **FIG. 4F** shows that 5 of 9 CRs (i.e., 56% CR) were seen in Group 4 (IL12 mRNA in lipid nanoparticle (LNP), at a dose of 0.1 mg/kg). Aside from the IL12 mRNA groups, no other group observed any CRs.

[1164] **FIG. 5** depicts the survival benefit at day 47 post tumor-implantation from a single intravenous (IV) dose of murine IL12 mRNA in lipid nanoparticle (LNP) at a dose of 0.05 mg/kg (Group 5) and at a dose of 0.1 mg/kg (Group 4).

[1165] Notably, **FIGs. 3-5** demonstrate the advantage of administering intravenous murine **IL12** mRNA over protein in terms of improved pharmacokinetics (PK), pharmacodynamics (PD), and therapeutic efficacy, with a single IV dose.

[1166] **Table 9** depicts the *tolerability* advantage of local (intratumoral) administration of IL12 mRNA over systemic (intravenous) administration. Nine (9) of 10 mice intratumorally administered IL12 mRNA were viable at day 20 compared to 3 of 12 mice intravenously administered IL12 mRNA.

Table 9: Intravenous and intratumoral tolerability of murine IL12 administration.

Route	IL12 mRNA in LNP (mg/kg)	Treatment tolerability outcome (to Day 20)	Plasma levels of IL12 24 hr post dose (ng/ml)
Intravenous	0.2	3/12 viable	1592
Intratumoral	~0.2 (4 μg fixed)	9/10 viable	89

Example 2: *In Vivo* Anti-Tumor Efficacy of Murine IL12 Modified mRNA in a Colon Adenocarcinoma (MC38) Model *(intratumoral* administration)

[1167] The *in vivo* anti-tumor efficacy of murine IL12 mRNA, administered intratumorally in mice bearing M38 adenocarcinoma tumors, was assessed.

A. Preparation of IL12 modified mRNA and control

[1168] The mIL12_miR122 polynucleotide as described in Example 1 was prepared. A negative control mRNA, NST-FIX mRNA, was also prepared. Both modified mRNAs were formulated in MC3 lipid nanoparticles (LNP) as described in Example 1.

B. MC-38 Colon Adenocarcinoma Mouse Model

[1169] MC-38 colon adenocarcinoma tumors were implanted subcutaneously in mice as described in Rosenberg et al., Science 233:1318-1321 (1986)).

[1170] Eleven days after tumor implantation, animals were administered a single intratumoral dose of MC3 LNP-formulated murine IL12 modified mRNA (4 μg mRNA per dose). Two groups of control animals were treated with an equivalent dose and regimen of negative control mRNA (NST-FIX LNP) or PBS.

[1171] Tumor volume was measured at the indicated time points in **FIG. 6B** using manual calipers. Tumor volume mean to day 24 **(FIG. 6A)** and individual tumor volume to day 47 **(FIG.6B)** were recorded in cubic millimeters (mm^3). Endpoints in the study were either death of the animal or a tumor volume reaching 1500 mm^3.

C. Results

[1172] **FIG. 6A** shows that mean tumor volume was reduced when MC3 LNP-formulated murine IL12 modified mRNA was administered. In contrast, administering a control modified mRNA (NST-FIX) or PBS to the mice had little effect on reducing the tumor volume mean when assessed up to day 24.

[1173] **FIG. 6B** shows that administering about 4 μg MC3 LNP-formulated murine **IL12** modified mRNA per animal to the mice decreased individual tumor volumes in some animals compared to animals administered control modified mRNA (NST-FIX) or PBS. Complete responses (CRs) were seen in 3 of 7 animals (44%), with 3 animals removed due to ulceration. These data show that mIL12_miR122 polynucleotides have anti-tumor efficacy when administered intratumorally *in vivo*.

Example 3: *In Vivo* Anti-Tumor Efficacy of IL12 Modified mRNA in a B-Cell Lymphoma (A20) Syngeneic Model

[1174] The *in vivo* anti-tumor efficacy of murine IL12 mRNA, administered intratumorally in mice bearing A20 B-cell lymphoma tumors, was assessed.

A. Preparation **of IL12** *modified mRNAs and controls*

[1175] A polynucleotide comprising a codon-optimized nucleotide sequence encoding an IL12 polypeptide (murine IL12) *without* a miRNA binding site (miRless) was prepared (IL12 miRless) (sequence set forth below).

GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUG
UCCUCAGAAGCUAACCAUCUCCUGGUUUGCCAUCGUUUUGCUGGUGUCUCCAC
UCAUGGCCAUGUGGGAGCUGGAGAAAGACGUUUAUGUUGUAGAGGUGGACUG
GACUCCCGAUGCCCCUGGAGAAACAGUGAACCUCACCUGUGACACGCCUGAAG
AAGAUGACAUCACCUGGACCUCAGACCAGAGACAUGGAGUCAUAGGCUCUGGA
AAGACCCUGACCAUCACUGUCAAAGAGUUCCUAGAUGCUGGCCAGUACACCUG
CCACAAAGGAGGCGAGACUCUGAGCCACUCACAUCUGCUGCUCCACAAGAAGG
AAAAUGGAAUUUGGUCCACUGAAAUUUUAAAAAAUUUCAAAAACAAGACUUU
CCUGAAGUGUGAAGCACCAAAUUACUCCGGACGGUUCACGUGCUCAUGGCUGG
UGCAAAGAAACAUGGACUUGAAGUUCAACAUCAAGAGCAGUAGCAGUUCCCCU
GACUCUCGGGCAGUGACAUGUGGAAUGGCGUCUCUGUCUGCAGAGAAGGUCAC
ACUGGACCAAAGGGACUAUGAGAAGUAUUCAGUGUCCUGCCAGGAGGAUGUC
ACCUGCCCAACUGCCGAGGAGACCCUGCCCAUUGAACUGGCGUUGGAAGCACG
GCAGCAGAAUAAAUAUGAGAACUACAGCACCAGCUUCUUCAUCAGGGACAUCA
UCAAACCAGACCCGCCCAAGAACUUGCAGAUGAAGCCUUUGAAGAACUCACAG
GUGGAGGUCAGCUGGGAGUACCCUGACUCCUGGAGCACUCCCCAUUCCUACUU
CUCCCUCAAGUUCUUUGUUCGAAUCCAGCGCAAGAAAGAAAAGAUGAAGGAG
ACAGAGGAGGGGUGUAACCAGAAAGGUGCGUUCCUCGUAGAGAAGACAUCUA
CCGAAGUCCAAUGCAAAGGCGGGAAUGUCUGCGUGCAAGCUCAGGAUCGCUAU
UACAAUUCCUCAUGCAGCAAGUGGGCAUGUGUUCCCUGCAGGGUCCGAUCCGG
AGGCGGAGGGAGCGGAGGCGGAGGGAGCGGAGGCGGAGGGAGCAGGGUCAUU
CCAGUCUCUGGACCUGCCAGGUGUCUUAGCCAGUCCCGAAACCUGCUGAAGAC
CACAGAUGACAUGGUGAAGACGGCCAGAGAAAAACUGAAACAUUAUUCCUGC

ACUGCUGAAGACAUCGAUCAUGAAGACAUCACACGGGACCAAACCAGCACAUU
GAAGACCUGUUUACCACUGGAACUACACAAGAACGAGAGUUGCCUGGCUACUA
GAGAGACUUCUUCCACAACAAGAGGGAGCUGCCUGCCCCCACAGAAGACGUCU
UUGAUGAUGACCCUGUGCCUUGGUAGCAUCUAUGAGGACUUGAAGAUGUACC
AGACAGAGUUCCAGGCCAUCAACGCAGCACUUCAGAAUCACAACCAUCAGCAG
AUCAUUUUAGACAAGGGCAUGCUGGUGGCCAUCGAUGAGCUGAUGCAGUCUC
UGAAUCAUAAUGGCGAGACUCUGCGCCAGAAACCUCCUGUGGGAGAAGCAGAC
CCUUACAGAGUGAAAAUGAAGCUCUGCAUCCUGCUUCACGCCUUCAGCACCCG
CGUCGUGACCAUCAACAGGGUGAUGGGCUAUCUGAGCUCCGCC**UGAUAAUAG**
GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCCAGCCC
CUCCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAG
UGGGCGGC (SEQ ID NO: 242)

[1176] The mIL12_miR122 polynucleotide as described in Example 1 was prepared. A negative control mRNA (NST) was also prepared (non-translatable version of the same mRNA) (NST IL12_miRless). All modified mRNAs were formulated in MC3 lipid nanoparticles (LNP) as described in Example 1.

B. A20 B-Cell Lymphoma Tumor Model

[1177] Mouse models of B-cell lymphoma using the A20 cell line are useful for analyzing tumors. (Kim et al., Journal of Immunology 122:549-554 (1979); Donnou et al., Advances in Hematology 2012:701704 (2012)). A20 cells are derived from a B cell lymphoma from a BALB/c mouse and are typically grown in syngeneic mice as a subcutaneous implant. 500,000 A20 cells were implanted subcutaneously in BALB/c mice to generate subcutaneous tumors. Tumors were monitored for size and palpability.

[1178] Once the tumors reached an average size of 100 mm^3, the mice were cohorted into three groups. One test group was administered intratumorally mIL12_miRless in MC3-based lipid nanoparticles (LNP) at a dose of 5 μg mRNA per animal. The second test group was administered intratumorally mIL12_miR122 in MC3-based lipid nanoparticles (LNP) at a dose of 5 μg mRNA per animal. The control group was administered an equivalent dose of non-translated control mRNA (NST). Animals were dosed on day 10 post implantation. Results are shown in **FIGs. 7A-7C** .

[1179] The study was carried out through day 50 post implantation. Endpoints in the study were either death of the animal or a pre-determined endpoint of 2000 mm^3 tumor volume.

C. Results

[1180] **FIG. 7A** shows that tumor volume (measured in mm^3) increased over time in all 12 animals treated with 5 μg control NST mRNA. **FIG. 7B** shows that tumor volume decreased over time in some animals treated with mIL12_miRless compared to animals administered control mRNA (NST). Complete responses (CRs) were seen in 5 of 12 animals (42%). Therefore, the intratumoral administration of murine IL12 mRNAs is efficacious in the A20 tumor model.

[1181] **FIG. 7C** shows changes in tumor volume in animals treated with 5 μg mIL12-miR122 **(FIG. 7C).** Complete responses (CR) were achieved in 6 out of 12 mice in the IL12-miR122 group **(FIG. 7C).**

[1182] The data in **FIGs. 7A-7C** show that mIL12_miRless and mIL12-miR122 polynucleotides have comparable anti-tumor efficacy when administered intratumorally *in vivo*.

Example 4: Single and Multidose *In Vivo* Anti-tumor Efficacy of a Modified IL12 mRNA

[1183] The *in vivo* anti-tumor efficacy of a modified murine IL12 mRNA, administered as a single 0.5 μg dose and a multidose (0.5 μg for 7 days x 6), was studied in BALB/C mice bearing A20 B-cell lymphoma tumors.

A. Modified **IL12** *mRNA*

[1184] The mIL12_miR122 polynucleotide and mIL12_miRless polynucleotide as described in Examples 1 and 3, respectively, were prepared. One group of mIL12_miR122 mRNA was formulated in MC3-based lipid nanoparticles (LNP) as described in Example 1. Another group of mIL12_miR122 mRNA was formulated in Compound 18-based lipid nanoparticles (LNP).

B. Dosing

[1185] On day 10 post implantation, two groups of mice bearing A20 tumors (n=12 in each group) were administered a *single* 0.5 μg dose of murine IL12 mRNA in the form of IL12 miRless- or IL12 miR122-mRNA in MC3-based LNP.

[1186] Also on day 10 post implantation, another group of mice bearing A20 tumors was intratumorally administered *weekly* dosing of 0.5 μg of IL12 miR122 mRNA in *MC3*-based LNP for 7 days x 6.

[1187] Also on day 10 post implantation, another group of mice bearing A20 tumors was intratumorally administered *weekly* dosing of 0.5 μg of IL12 miR122 mRNA in Compound 18-based LNP for 7 days x 6.

[1188] Finally, 10 days post implantation, another group of mice bearing A20 tumors (n=12 per group) was administered *weekly* dosing of 0.5 μg non-translated negative control mRNA (NST) in either MC3-based LNP or Compound 18-based LNP for 7 days x 6.

C. Results

[1189] As shown in **FIGs. 8A-8B,** *in vivo* anti-tumor efficacy in a B-cell lymphoma tumor model (A20) was achieved after mice bearing A20 tumors were administered a single dose of 0.5 μg murine **IL12** mRNA in MC3-based lipid nanoparticle (LNP). **FIG. 8A** depicts decreased tumor volume in some mice administered IL12 miRless (0.5 μg), and that four (4) complete responses (CR) were achieved. **FIG. 8B** depicts decreased tumor volume in some mice administered IL12 miR122 (0.5 μg), and that three (3) complete responses (CR) were achieved.

[1190] As shown in **FIG. 8C,** *in vivo* anti-tumor efficacy was enhanced with a weekly dosing regimen **of IL12** miR122 mRNA in MC3-based LNP (0.5 μg mRNA for 7 days x 6), compared to single dosing (see FIG. 8B). **FIG. 8C** shows that five (5) CRs were achieved in the 12 A20-bearing mice administered *weekly dosing* of 0.5 μg IL12 miR122 mRNA for seven (7) days x 6.

[1191] *In vivo* anti-tumor efficacy of IL12 mRNA in Compound-18 LNPs was enhanced as compared to the in vivo tumor efficacy of IL12 mRNA in MC3 LNPs, as can be seen by delay in tumor growth **(FIG. 8D). FIG. 8D** shows the individual tumor volumes for 12 mice administered 0.5 μg of murine IL12 mRNA in compound 18-based LNP for 7 days x 6. Complete responses (CR) were also achieved in 5 out of 12 animals.

[1192] As shown in **FIGs. 8E-8F,** tumor growth was observed in mice bearing A20 tumors administered weekly dosing (7 days x 6) of 0.5 μg non-translated negative control mRNA (NST) in MC3-based lipid nanoparticle (LNP) **(FIG. 8E)** and 0.5 μg NST in Compound 18-based LNP **(FIG. 8F).**

[1193] This data shows that polynucleotides comprising modified murine IL12 mRNA (both miR122 and miRless) show anti-tumor efficacy at low doses (0.5 μg). It also shows that *in vivo* anti-tumor efficacy can potentially be enhanced with a multiple dosing regimen. Finally, the data shows *in vivo* anti-tumor efficacy when 0.5 μg IL12 miR122 mRNA in MC3-based *and* Compound 18-based LNP is administered intratumorally weekly (for 7 days x 6). In contrast, tumor growth was observed in mice bearing A20 tumors administered weekly dosing (7 days x 6) of 0.5 μg non-translated negative control mRNA (NST) in MC3-based lipid nanoparticle (LNP) and in Compound 18-based LNP.

Example 5: Analysis of Levels of IL12 p70, IFNγ, IP10, IL6, GCSF, and GROα in Plasma and Tumors of A20-bearing Mice Following Administration of Murine IL12 mRNA

[1194] On day 10 post implantation, groups of mice bearing A20 tumors (n=12 in each group) were administered a dose of miR122 IL12 mRNA (at 5μg, 2.5 μg, or 0.5 μg) in MC3-based LNP or Compound 18-based LNP, NST or IL12 protein at the same dosages, or PBS.

[1195] The levels of tumor and plasma IL12 p70, as well as the level of other cytokines, were determined at 6 and 24 hours after administration of the dosages. The levels of IL12 (p70) were determined using a sandwich ELISA commercial kit (R&D Systems, Minneapolis, MN, USA). The levels of IFNy, IP10, IL6, G-CSF, and GROα were also determined.

[1196] *Results:* **FIGs. 9A-9B** show dose-dependent levels of IL12 in plasma **(FIG. 9A)** and tumor **(FIG. 9B)** at 6 hours and 24 hours following administration of the indicated doses of murine IL12 mRNA in an MC3-based LNP to mice bearing A20 tumors.

[1197] **FIGs. 9C-9D** show elevated levels of IL12 in plasma and tumor following administration of the indicated doses of murine IL12 mRNA in Compound 18-based LNPs compared to murine IL12 mRNA in MC3-based LNPs. **FIG. 9C**

shows plasma IL12 levels at 6 hours and 24 hours; **FIG. 9D** shows tumor IL12 levels at 6 hours and 24 hours. **Table 10** shows the fold increase of IL12 in plasma and tumor after the murine IL12 mRNA administration (6 hrs and 24 hrs).

Table 10: Fold increase of IL12 from Compound 18 compared to MC3.

	mRNA dose	6 hr	24 hr
Plasma	0.5 μg	9.3	2.8
	2.5 μg	2.7	7.2
Tumor	0.5 μg	1.8	2.9
	2.5 μg	2.3	2.1

[1198] **FIGs. 9E-9F** show increased levels of IFNy at 6 hours and 24 hours in plasma **(FIG. 9E)** and in tumor **(FIG. 9F)** following administration of murine IL12 mRNA to mice bearing A20 tumors.

[1199] **FIGs. 9G-9H** show increased levels of IP10 at 6 hours and 24 hours in plasma **(FIG. 9G)** and in tumor **(FIG. 9H)** following administration of murine IL12 mRNA to mice bearing A20 tumors.

[1200] **FIGs. 9I-9J** show decreased levels of IL6 at 6 hours and 24 hours in plasma **(FIG. 91)** and in tumor **(FIG.** 9J) following administration of murine IL12 mRNA in Compound 18-based LNP compared to murine IL12 mRNA in MC3-based LNP.

[1201] **FIGs. 9K-9L** show decreased levels of G-CSF at 6 hours and 24 hours in plasma **(FIG. 9K)** and in tumor **(FIG.9L)** following administration of murine IL12 mRNA in Compound 18-based LNP compared to murine IL12 mRNA in MC3-based LNP.

[1202] **FIGs. 9M-9N** show decreased levels of GROa at 6 hours and at 24 hours in plasma **(FIG. 9M)** and tumor **(FIG. 9N)** following administration of murine IL12 mRNA in Compound 18-based LNP compared to murine **IL12** mRNA in MC3-based LNP.

[1203] The data in this example show dose dependent levels **of IL12** in plasma and tumor with intratumoral administration of murine IL12 mRNA **(FIGs. 9A and 9B).** The data also show increased IL12 levels in plasma and tumor from Compound 18-based LNPs compared to MC3-based LNP **(FIGs. 9C and 9D),** as well as increased IPNγ and IP10 levels, attributable to administration of murine IL12 mRNA **(FIGs. 9E-9H).** Finally, this example shows decreased levels of IL6, G-CSF, and GROα in plasma and tumor with Compound 18-formulated murine IL12 mRNA compared to MC3-formulated murine IL12 mRNA **(FIGs. 9I-9N).**

Example 6: *In Vivo* Anti-tumor Efficacy of Intravenous (IV) Administration of IL12_miR122 mRNA in a subcutaneous A20 model

A. Study Design

[1204] 500,000 A20 cells were implanted subcutaneously in BALB/c mice. A20 tumor-bearing mice were cohorted into 2 treatment groups (N=10) when tumor averages reached approximately ~100 mm^3. Mice were IV dosed with 0.5 mg/kg murine mRNA in a Compound 18 PEG containing LNP every 7 days (Q7D).

[1205] The control group were treated with negative control mRNA formulated in LNP, whereas the treatment group were dosed with LNPs containing murine IL12 mRNA with a miR122 binding element with in the 3' UTR. Use of the Compound 18-containing LNP and incorporation of the miR122 binding element were meant to mitigate potential protein production from the liver.

[1206] Tumor volumes and body weights were measured twice a week, and general clinical observations made in accordance with an accepted IACUC protocol until a pre-determined endpoint of 2000 mm^3 tumor volume was reached.

B. Efficacy Data

[1207] The individual tumor volumes from mice treated with weekly dosing of murine IL12 mRNA plus a miR122 binding element formulated in a Compound 18 PEG-containing LNP are shown in **FIG. 10B** compared to appropriate negative controls in **FIG. 10A.** Dosing days are indicated by vertical red hashed lines, and the pre-determined endpoint of 2000 mm^3 tumor volume indicated by horizontal black hashed line.

C. Assessment of Side Effects/Toxicity

[1208] The individual body weights from mice treated with weekly dosing of murine IL12 mRNA plus a miR122 binding

element formulated in a Compound 18 PEG-containing LNP are shown in **FIG. 10D** compared to appropriate negative controls in

FIG. 10C.

D. Conclusion

[1209] The intravenous administration of murine IL12 mRNA delayed the growth of A20 tumors as compared to an appropriate negative control *(i.e.,* IV treatment of identically formulated mRNA with a miR122 binding element that had no start "NST" codons). The efficacious dosing of 0.5 mg/kg Q7D was well-tolerated as determined by general clinical observations and more quantitatively by body weight measurements.

Example 7: *In Vivo* Anti-Tumor Efficacy of Intraperitoneal (IP) Administration of Murine IL12 Modified mRNA in an IP MC38 Model

A. Study Design

[1210] The luciferase reporter gene was integrated within the MC-38 colon cell line to enable measurement of bioluminescence from these cells if grown in a context that tumor burden could not be assessed by caliper in live animals. The light output from these cells has been correlated with tumor burden.

[1211] 500,000 MC-38 luciferase-enabled (MC38-luc) cells were inoculated in the peritoneal cavity as a model of colon cancer metastasis to this space. Mice were assigned to various treatment groups based on bioluminescent signal, and treatment started 7 days post disease induction.

[1212] Mice were treated with a single intraperitoneal (IP) dose of murine mRNA formulated in a LNP (MC3:cholesterol:DSPC:PEG-DMGat mol %s of 50:38.5:10:1.5) and compared to a single IP dose of 1 μg recombinant mouse IL12 protein. A single dose of 2 fixed dose levels of mRNA (2 μg and 4 μg) were administered at day 7 post disease induction, and murine IL12 mRNAs with and without a miR122 binding element were assessed in different treatment groups compared to appropriate negative controls (non-start NST mRNA with a miR122 binding element encapsulated in an identically formulated LNP).

[1213] Tumor volumes and body weights were frequently measured, and general clinical observations made in accordance with appropriate IACUC protocols.

B. Efficacy Data

[1214] Bioluminescence (BL) was used as a surrogate for tumor burden and measured on several days including day 22 post disease induction as depicted in **FIG. 11A.** Treatment groups and dose levels are indicated in **FIGs. 11B.** Mice were administered no treatment, 2 μg mIL12_miRless, 2 μg mIL12_miR122, 2 μg NST_OX40L_122, 4 μg mIL12_miRless, 4 μg mIL12_miR122, 4μg NST_OX40L_122, and 1 μg rm IL12. Mice treated with murine IL12 mRNA in all arms exhibited lower BL signal than negative controls.

C. Assessment of Side Effects/Toxicity:

[1215] The treatments were generally well tolerated, and no treatment groups exhibited a body weight loss average of over 10% **(FIG. 11B).**

D. Conclusion

[1216] As shown in **FIGs. 11A and 11B,** mice treated out to 150 days post implantation with both fixed doses of murine IL12 mRNA exhibited lower levels of BL signal as a measure of tumor burden compared to negative controls, and this inhibited BL level was associated with an apparent survival benefit of intraperitoneally-dosed murine IL12 mRNA. Murine IL12 mRNA that contained a miR122 binding domain within the 3' UTR exhibited similar efficacy to mRNA without this regulatory element (miR-less). The effective doses employed were considered generally well-tolerated.

[1217] Mice treated with fixed doses of murine IL12 mRNA exhibited lower levels of BL signal as a measure of tumor burden compared to negative controls, and this inhibited BL level was associated with an apparent survival benefit of intraperitoneally-dosed murine IL12 mRNA.

Example 8: Analysis of Levels of IL12 and IPNγ in Mice Following Administration of Murine IL12 mRNA

[1218] Mice bearing MC38 tumors were administered one or more doses of murine miR122 IL12 mRNA in standard Compound 18-based LNP intratumorally (iTu). In particular, mice were given a single intratumoral (iTu) dose of murine miR122 IL12 mRNA (at 0.05 μg, 0.5 μg, or 5.0 μg) in LNP, multiple iTu doses (4x) of murine miR122 IL12 mRNA at the same levels, or 5.0 μg NST as a control.

[1219] The levels of plasma IL12 and IPNγ were determined at 24 hours after administration of the dosages. The levels of IL12 and IPNγ were determined using a Luminex-based multiplex panel (ProcartaPlex Mouse Cytokine & Chemokine Panel 1A 36 plex, Affymetrix eBioscience).

[1220] **FIG. 17A** shows dose-dependent levels of **IL12** in plasma at 24 hours following administration of the indicated doses of murine IL12 mRNA to mice bearing tumors. The plasma concentration of IL12 (ng/ml) is shown below in **Table 11.**

Table 11. IL12 protein levels in plasma (ng/ml).

Dose	1st	2nd	3rd	4th
5ug NST	0.007 ± 0.0008	0.001 ± 0.0012	0.006 ± n/a	---
0.05ug IL12_122	0.320 ± 0.117	0.165 ± 0.095	0.559 ± 0.725	0.143 ± 0.054
0.5ug IL12_122	1.59 ± 1.65	1.02 ± 1.49	5.30 ± 9.57	1.21 ± 1.21
5ug IL12_122	195.8 ± 123.3	176.8 ± 221.4	86.61 ± 40.53	69.87 ± 62.50

[1221] **FIG. 17B** shows increased levels of IFNγ at 24 hours in plasma following administration of murine IL12 mRNA to mice bearing tumors. This figure therefore shows that administration of murine IL12 mRNA induces IPNγ expression over multiple doses.

[1222] Alternatively, mice bearing MC38 colon adenocarcinoma cells within the peritoneal cavity as a model of carcinomatosis were given a single fixed intraperitoneal (IP) dose of murine miR122 IL12 mRNA (at 2 μg or 4 μg), murine miRless IL12 mRNA (at 2 μg or 4 μg), or recombinant IL12 protein (rmIL12) (at 1 μg), on day 7 after MC38 cells were seeded. The levels of plasma IL12 and IPNγ were determined after administration of the dosage using standard techniques.

[1223] **FIGs. 18A-18B** show increased levels of IL12 in plasma **(FIG. 18A)** and IPNγ in plasma **(FIG. 18B)** following intraperitoneal (IP) administration of murine IL12 mRNA to mice bearing MC38 tumors.

[1224] These data show that both intratumoral and intraperitoneal administration of IL-12 induces increased plasma levels of IFNγ. Additionally, multiple dosing of IL-12 in an MC38 tumor model induces repeated levels of both plasma IL-12 and IPNγ.

Example 9: *In Vivo* Anti-tumor Efficacy of Intratumoral (iTu) Administration of IL12_miR122 mRNA in an A20 Model

[1225] A20 tumor cells were implanted subcutaneously in mice. A20 tumor-bearing mice were cohorted into 2 treatment groups (N=12). Mice were iTu dosed with 0.5 μg mRNA in Compound 18-LNP, every 7 days (Q7D).

[1226] The control group was treated with negative control mRNA formulated in the same LNP, whereas the treatment group was dosed with LNPs containing murine IL12 mRNA with a miR122 binding site in the 3' UTR (mIL12-miR122).

[1227] Tumor volumes were measured over the course of 90 days after implantation with A20 cells, and general clinical observations made in accordance with an accepted IACUC protocol until a pre-determined endpoint of 2000 mm^3 tumor volume was reached.

[1228] The individual tumor volumes from mice treated with iTu dosing of mIL12-miR122 formulated in Compound 18- LNP are shown in **FIG. 19B** compared to the negative control in **FIG. 19A. FIG. 19B** shows that four mice achieved complete response by Day 90.

[1229] Apparent complete responders (4/12) from **FIG. 19B** were re-challenged with A20 tumor cells. Individual tumor volumes from re-challenged mice are shown in **FIG. 19D** compared to tumor growth in naive mice challenged with A20

in **FIG. 19C. FIG. 19C** shows that 9 out of 10 control mice bearing newly implanted tumors reached its end point (2000 mm^3 tumor volume) by Day 45. However, **FIG. 19D** shows that all four complete responders from **FIG. 19B** rechalleged with A20 tumor did not show any tumor growth through Day 60. This indicates that the mIL12-miR122 mRNA administration provided the mice an immune memory response that is sufficient to prevent tumor growth upon rechallenge.

Example 10: *In Vivo* Anti-tumor Efficacy of Intratumoral (iTu) Administration of IL12_miR122 mRNA in an MC38-S Model

[1230] MC38-S cells were implanted into mice. Generally, MC38-S, also described as MC38-2, is a more sensitive cell line to therapy. Without being bound by theory, one explanation for this observation is the increased levels of CD45+ T cells, CD8+ Treg cells, Tumor associated macrophages (TAMs), and DCs observed as well as observations that MC38-S tumors are slower growing and more sensitive to tested IMTs. All of these characteristics of MC38-S tumors are by comparison to MC38-R tumors (also described as MC38-1 or MC38-M) and both are useful models.

[1231] MC38-S tumor-bearing mice were cohorted into 4 treatment groups (N=15). Mice were iTu dosed with 0.05 μg, 0.5 μg, or 5 μg murine IL12 mRNA in Compound -18 LNP. As a negative control, mice were iTu dosed with NST-FIX mRNA or untreated. In single and multiple dose studies, mice in the experimental and control groups received the same dosing regimen.

[1232] Tumor volumes and survival were measured in each group over the course of 80 days after implantation with MC38-S cells and general clinical observations made in accordance with an accepted protocol until a pre-determined endpoint of 1500 mm^3 tumor volume was reached.

[1233] The individual tumor volumes from mice treated with a single iTu dose of 0.05 μg murine IL12 mRNA are shown in **FIG. 20A** and **FIG. 21A,** mice treated with a single iTu dose of 0.5 μg murine IL12 mRNA are shown in **FIG. 20B** and **FIG. 21B,** mice treated with a single iTu dose of 5 μg murine IL12 mRNA formulated are shown in **FIG. 20C** and **FIG. 21C,** compared to appropriate negative controls in **FIG. 20D.** The pre-determined endpoint of 1500 mm^3 tumor volume indicated by horizontal black hashed line. **FIGs. 20A** and **21A** show that six out of 15 mice achieved complete response. **FIGs. 20B, 21B, 20C,** and **21C** show that 13 out of 15 mice achieved complete response, indicating that a low dose of 0.5 μg may be sufficient to exert a maximally effective response given the similar response seen with 5 μg.

[1234] The individual tumor volumes from mice treated with two iTu doses of 0.05 μg murine IL12 mRNA are shown in **FIG. 21D,** mice treated with two iTu doses of 0.5 μg murine IL12 mRNA are shown in **FIG. 21E,** and mice treated with two iTu doses of 5 μg murine IL12 mRNA are shown in **FIG. 21F.** The pre-determined endpoint of 1500 mm^3 tumor volume indicated by horizontal black hashed line. **FIG. 21D** shows that 9 out of 15 mice achieved complete response. **FIGs. 21E** and **21F** show that 14 out of 15 mice achieved a complete response. This data shows that in some tumor types, a single administration of murine IL12 mRNA can achieve an anti-tumor effect that is sufficient to reduce or eliminate the tumor.

[1235] A dose response trend was clear between 0.05 and 0.5 μg dose levels. A threshold appeared to be achieved ≤ 0.5 μg murine IL12 mRNA. Two doses of IL12 mRNA were also beneficial above a single dose. All mice administered any dose of murine IL12 mRNA displayed a survival benefit **(FIG. 22).** Therefore, the intratumoral administration of murine IL12 mRNA delayed or reduced the growth or size of MC38-S tumors as compared to an appropriate negative control and provided a survival benefit.

Example 11: *In Vivo* Anti-tumor Efficacy of Intratumoral (iTu) Administration of IL12_miR122 mRNA in an MC38-R Model

[1236] MC38-R cells were implanted into mice. Generally, MC38-R, also referred to herein as MC38-1 or MC38-M, is a more resistant cell line to therapy. Without being bound by theory, one explanation for this observation is the reduced levels of CD45+ T cells, CD8+ Treg cells, and DCs, and more cells that bear markers of "myeloid derived suppressor cells" were observed, as well as observations that MC38-R tumors are faster growing and generally less sensitive to tested immune-mediated therapies. All of these characteristics of MC38-R tumors are by comparison to MC38-S tumors and both are useful models.

[1237] MC38-R tumor-bearing mice were cohorted into 4 treatment groups (N=13). Mice were iTu dosed with 0.05 μg, 0.5 μg, or 5 μg murine IL12 mRNA in Compound-18 LNP. As a negative control, mice were iTu dosed with NST-FIX mRNA or untreated. In single and multiple dose studies, mice in the experimental and control groups received the same dosing regimen.

[1238] Tumor volumes and survival were measured in each group over the course of 75 days after implantation with MC38-R cells.

[1239] The individual tumor volumes from mice treated with a single iTu dose of 0.05 μg murine IL12 mRNA are shown in **FIG. 23A** and **FIG. 23E,** mice treated with a single iTu dose of 0.5 μg murine IL12 mRNA formulated in an LNP are shown in **FIG. 23B** and **FIG. 23F,** mice treated with a single iTu dose of 5 μg murine IL12 mRNA formulated in an LNP

are shown in **FIG. 23C** and **FIG. 23G,** compared to appropriate negative controls in **FIG. 23D.** The pre-determined endpoint of 2000 mm^3 tumor volume indicated by horizontal black hashed line. **FIGs. 23A** and **23E** show that no mice bearing MC38-R tumor after administration of 0.05 μg of murine **IL12** mRNA achieved complete response. **FIGs. 23B** and **23F** show that two mice bearing MC38-R tumor after administration of 0.5 μg of murine **IL12** mRNA achieved complete response. **FIGs. 23C** and **23G** show that four mice bearing MC38-R tumor after administration of 5 μg of murine IL12 mRNA achieved complete response.

[1240] The individual tumor volumes from mice treated with multiple iTu doses of 0.05 μg murine IL12 mRNA are shown in **FIG. 23H,** mice treated with two iTu doses of 0.5 μg murine IL12 mRNA formulated in an LNP are shown in **FIG. 231,** and mice treated with two iTu doses of 5 μg murine IL12 mRNA formulated in an LNP are shown in **FIG. 23J.** The pre-determined endpoint of 2000 mm^3 tumor volume indicated by horizontal black hashed line. **FIGs. 23H-23J** show that multiple administrations have similar efficacy as single administration.

[1241] These results indicate that despite the demonstrated resistance of MC38-R tumors to other immune mediated therapies, such as anti-PDI and anti-PD-LI antagonist antibodies, mice administered murine IL12 mRNA displayed a survival benefit **(FIG. 24).** Survival events included tumor burden endpoints and animals off study due to ulceration/eschar which occurred in treated and non-treated mice. The intratumoral administration of murine IL12 mRNA delayed the growth of MC38-R tumors as compared to an appropriate negative control and provided a survival benefit and was generally well tolerated.

[1242] The levels of tumor and plasma IL12 p70, as well as the level of other cytokines, were determined at 24 hours after administration of the dosages. The results show dose dependent levels of IL12 in plasma with intratumoral administration of murine IL12 mRNA as shown previously. The results also show sustained dose-dependent expression of TNFα, IL-10, IL-13, IL-15/15R, IL-27, MIP-1β, MIP-1α, MCP-1, MCP-3, M-CSF, IL-4, and IL-5 after multiple murine IL12 dosing (data not shown). GM-CSF, IL-18, IL-3, RANTES and IL-6 had elevated expression levels only following 5 μg murine IL12 dosing (data not shown).

Example 12: CD8+ T Cells Involved in IL12 mRNA Efficacy

[1243] To evaluate the role of CD8+ T cells in mediating IL12 mRNA induced efficacy, MC38-R cells were implanted into mice, the CD8+ T cell-depleting clone 24.3 was used to deplete CD8+ T cells and CD8+ T cell levels were monitored in blood to confirm depletion over the course of the efficacy study. The 24.3 clone or a control antibody was given to mice 2 days prior to administration of murine IL12 mRNA. The depleting or control antibody was given at 0.5 mg daily for 3 consecutive days, 3 days off, then another five antibody doses at 0.2 mg given every 4 days. Flow cytometry was performed throughout the experiment to confirm CD8+ T cell depletion.

[1244] Using the 24.3 clone, CD8+ T cells were depleted over the course of 28 days, whereas an antibody control did not deplete CD8+ T cells **(FIG. 25).** CD8+ T cell numbers in blood were significantly decreased at all time points examined by flow cytometry, thus confirming effective depletion.

[1245] Mice given 0.5 μg murine IL12 mRNA after CD8+ T cell depletion via clone 24.3 displayed increased tumor volume **(FIG. 26D)** compared to mice given 0.5 μg murine IL12 mRNA after mock CD8+ T cell depletion **(FIG. 26B).** Likewise, mice given 0.5 μg murine IL12 mRNA after CD8+ T cell depletion displayed poor survival compared to mice given 0.5 μg murine IL12 mRNA after mock CD8+ T cell depletion **(FIG. 26E). FIG. 26A** shows tumor volume in mice treated with negative control mRNA after mock CD8+ T cell depletion, and **FIG. 26C** shows tumor volume in mice treated with negative control mRNA after CD8+ T cell depletion. Although murine IL12 mRNA treatment appeared to result in some delay in tumor growth even with CD8+ T cell depletion, there were no complete responders in the absence of cytotoxic T cells in contrast to the clear survival benefit observed with murine IL12 mRNA treatment and mock depletion. The results indicate that CD8+ T cells play an essential role in IL12 mRNA mediated efficacy. Example 13: Significant changes in the immune infiltrate in MC38-R and B16F10-AP3 tumors following intratumoral administration of IL12 mRNA

[1246] Significant changes occurred in the immune cell infiltrate in both MC38-R and B16F10-AP3 tumors following treatment with IL12 mRNA. MC38-R tumors were treated intratumorally with 0.05 μg or 0.5 μg murine IL12 mRNA and B16F10-AP3 tumors were treated intratumorally with 0.1 μg or 0.5 μg murine IL12 mRNA.

[1247] In both the MC38-R and B16F10-AP3 models the percentage of CD11b+ myeloid cells within the tumor staining positive for PDL1 expression increased significantly at most time points, following IL12 mRNA treatment **(FIG. 27A** and **FIG. 27B).**

[1248] IL12 mRNA treatment further correlated with an increase in CD8+ T cell within tumors in both MC38-R and B16F10-AP3 mouse models at late time points. In particular, the percent of T cells, as evidenced by the proportion of CD8+ T cells out of the immune infiltrate (CD45+ cells) and the number of CD8+ positive cells per mg of tumor tissue, was statistically higher 7 days after administration of 0.5 μg murine IL12 mRNA in both mouse models **(FIGs. 28A** and **28B).** Furthermore, a significantly higher number of T cells were observed in tumors in B16F10-AP3 mice treated with 0.1 μg murine IL12 mRNA, as evidenced by the proportion of CD8+ T cells out of the immune infiltrate (CD45+ cells) **(FIG. 28B).** Both the percent of CD45 positive cells and the cells per mg are informative. At day 7, tumors in treatment

groups are smaller than controls, particularly for the 0.1 μg and 0.5 μg groups.

[1249] An increased ratio of CD8+ cells to T regulatory (Treg) cells (CD8:Treg) was seen in both MC38-R **(FIG. 29A)** and B16F10-AP3 **(FIG. 29B)** mouse models, demonstrating an improved effector to suppressor ratio. In particular, MC38-R mice treated with 0.05 μg or 0.5 μg murine IL12 mRNA exhibited statistically significant higher ratios of CD8+ T cells to Treg cells as compared to negative controls, resulting from both an increase in CD8+ cells and a decrease in Treg cells in tumor tissue at day 7 **(FIG. 29A).** Similarly, B16F10-AP3 mice treated with 0.1 μg or 0.5 μg murine IL12 mRNA exhibited statistically higher ratios of CD8+ T cells to Treg cells as compared to negative controls, resulting from an increase in CD8+ cells **(FIG. 29B).**

[1250] Expression of CD69, which is indicative of activation of T lymphocytes and NK cells, was increased on CD8+ T cells in both MC38-R and B16F10-AP3 tumors **(FIGs. 30A-30B).**

[1251] Activation of natural killer (NK) cells was also seen in both MC38-R and B16F10-AP3 tumors treated with IL-12 mRNA **(FIGs. 31A** and **31B).**

[1252] An increase in cross-presenting dendritic cells was observed in both MC38-R and B16F10-AP3 tumor models. CD103+ cDC increases were observed in both MC38-R **(FIG. 32A)** and B16F10-AP3 tumors **(FIG. 32B)** at day 7. In addition, a greater percent of activated CD8+ cDCs were found in the draining lymph node following treatment with murine **IL12** mRNA at each of 24hrs, 72hrs, and day 7 as compared to controls in B16F10-AP3 tumors **(FIG. 32C).**

Example 14: *In Vivo* Anti-Tumor Efficacy of IL12 Modified mRNA in Combination with Anti-PD-LI Antibody in an MC38-R Model

[1253] Given the increased expression of PD-L1 (see Example 11 above) after administration of murine IL12 mRNA, the efficacy of a combination therapy of murine IL12 mRNA and an anti-PD-LI antibody was studied. To evaluate the efficacy of anti-PD-L1 antibody monotherapy, mice bearing MC38-R tumor were administered a murine anti-PD-LI antibody obtained from clone 80. **FIG. 33A** shows tumor volume changes in mice after administration of an antibody control. **FIG. 33B** shows tumor volume changes after administration of the anti-PD-LI antibody. All mice reached the end point (i.e., 2000 mm^3 tumor) demonstrating insensitivity of this model to anti-PD-LI treatment.

[1254] Mice bearing MC38-R tumor were administered iTu either (i) murine IL12 mRNA alone, or (ii) murine IL12 mRNA and a murine anti-PD-LI antibody in combination. Three dosages of mRNA were tested, i.e., 0.05 μg (data not shown), 0.5 μg, and 5 μg. Tumor volumes and survival were measured in each group (n=15) over the course of 90 days after implantation with MC38-R cells and general clinical observations made in accordance with an accepted protocol until a pre-determined endpoint of 2000 mm^3 tumor volume was reached.

[1255] **FIGs. 33C** and **33E** show that the combination therapy with 0.5 μg murine IL12 mRNA resulted in complete response in 6 out of 15 mice while only 1 out of 15 mice achieved complete response after administration of murine IL12 mRNA alone. **FIGs. 33D** and **33F** show that 11 out of 15 mice achieved complete response in the combination therapy with 5 μg murine IL12 mRNA while only 5 out of 15 mice achieved complete response after administration of murine **IL12** mRNA alone. **FIG. 33G** shows data that confirm a murine anti-PD-LI antibody alone did not have any anti-tumor efficacy in mice bearing MC38-R in this study. The results show that the combination therapy of murine IL12 mRNA and a checkpoint inhibitor, e.g., an anti-PD-LI antibody, can display synergistic efficacy in tumors otherwise resistant to checkpoint inhibitor therapy, e.g., MC38-R.

Example 15: *In Vivo* Anti-Tumor Efficacy of Intratumoral Administration of IL12 Modified mRNA in Combination with an Anti-PD-LI Antibody

[1256] MC-38 colon adenocarcinoma tumors were implanted subcutaneously in mice as described in Rosenberg et al., Science 233:1318-1321 (1986)).

[1257] MC38-R or B16F10-AP3 mice tumor bearing-mice were administered a single intratumoral dose of LNP-formulated murine IL12 modified mRNA (IL12 mRNA with miR122 formulated with Compound 18-based LNP) at a dose of either 0.05 μg, 0.5 μg, or 5 μg, alone or in combination with an intraperitoneal dose of anti-PD-LI antibody (clone 80). Additional doses were administered as indicated below.

A. *Results*

[1258] To test the effect of combined treatment with a murine IL12 mRNA and an anti-PD-L1 antibody (αPD-L1), MC38-R mice were treated eleven days post implant with a murine anti-PD-LI antibody alone **(FIG. 34A),** 0.5 μg murine IL12 mRNA alone **(FIG. 34B),** or both a murine anti-PD-LI antibody and 0.5 μg murine IL12 mRNA **(FIG. 34C).** Treatment using an anti-PD-LI antibody alone had little effect on tumor growth **(FIG. 34A),** and treatment with a single dose of 0.5 μg murine IL12 mRNA resulted in one CR **(FIGs. 34B).** When murine IL12 mRNA intratumoral treatment was combined with anti-PD-L1 administration (administered in six doses), there was a large increase in the number of CRs observed.

Eight out of fifteen mice (53%) exhibited a complete response following a single dose of 0.5 μg murine IL12 mRNA in combination with an anti-PD-LI antibody **(FIG. 34C),**

[1259] The synergistic effect of combination treatment using murine IL12 mRNA and an anti-PD-LI antibody was further observed in B16F10-AP3 mice, which are thought to be immunologically barren and are also resistant to checkpoint blockade therapies such as anti-PDL1. While untreated B16F10-AP3 mice **(FIG. 35A)** and B16F10-AP3 treated with an anti-PD-LI antibody alone **(FIG. 35B)** or a single dose of 0.5 μg murine IL12 mRNA alone **(FIG. 35C)** yielded no CRs, mice treated with a single dose of 0.5 μg murine **IL12** mRNA in combination with an anti-PD-LI antibody (administered in six doses on days 11, 14, 18, 21, 25, and 28) resulted in two CRs out of fifteen mice **(FIG. 35D).**

Example 16: Abscopal Effect Following Murine IL12 mRNA Administration within a Distal Tumor

[1260] To investigate potential abscopal effects of murine IL12 mRNA intratumoral administration, MC38 colon adenocarcinoma tumors were implanted bilaterally in mice (MC38-S; **FIG. 36A).** In an ongoing study, 17/20 MC38-S tumors treated with 0.5 μg murine IL12 mRNA **(FIG. 36D)** and 19/20 MC38-S tumors treated with 5 μg murine IL12 mRNA **(FIG. 36F)** have thus far exhibited a complete response after about 50 months. In addition, 3/20 distal tumors in the 0.5 μg murine IL12 mRNA-treated mice **(FIG. 36E)** and 16/20 distal tumors in the 5 μg murine IL12 mRNA-treated mice have thus far exhibited a complete response after about 50 months **(FIG. 36G).** The results with negative control are shown in **FIGs. 36B** and **36C.**

[1261] These abscopal effects are amplified when murine IL12 mRNA treatment is combined with anti-PD-LI treatment, in an ongoing study. Mice implanted with bilateral MC38 tumors were administered intratumorally into one tumor either 0.5 μg murine IL12 mRNA **(FIG. 37C-37D)** or 5 μg murine IL12 mRNA **(FIG. 37E-37F)** either as a monotherapy **(FIGs. 37C and 37E)** or combined with an anti-PD-LI antibody (administered by IP injection of 20 mg/kg twice per week for two weeks; **FIGs. 37D and 37F).** Thus far, 3/20 CRs and 16/20 CRs were observed bilaterally in the 0.5 μg **(FIG. 37C)** and 5 μg **(FIG. 37E)** murine **IL12** mRNA-treated mice, and 8/20 CRs and 20/20 CRs were observed bilaterally in the mice treated with a combination of anti-PD-LI and 0.5 μg **(FIG. 37D)** or 5 μg **(FIG. 37F)** murine IL12 mRNA. The results with negative control are shown in **FIGs. 37A and 37B.**

Example 17: Evaluation of Codon-Optimized mRNAs Encoding Human IL12

[1262] In addition to the mIL12_miR122 construct generated in Example 1, 20 codon-optimized mRNAs encoding human IL12 polypeptide (hIL12AB 001-020) were prepared. These mRNAs also had a miR-122 binding site in the 3'UTR.

[1263] The mRNAs were fully modified with N1-methylpseudouridine. The modified mRNAs were formulated in MC3 lipid nanoparticles (LNP) for screening. HeLa cells were transfected using a standard transfection protocol and expression was determined -22 hours post-transfection. Expression was normalized to that from the wild-type mulL12 construct. Most constructs tested had expression equal to or up to ~2-fold over the mIL12_miR122 construct.

[1264] Constructs having high expression in HeLa cells were also tested for expression in MC38 cells, Heb3B cells and A20 cells to confirm expression in various tumor cell lines.

[1265] Variants ML12AB_002, hIL12AB_006 and hIL12AB_021 were further selected for testing *in vivo.* C57B16 mice were implanted subcutaneously with A20 cells (A20 tumor model). Tumors were allowed to grow and tumors subsequently injected intratumorally with LNP-encapsulated mRNAs. Plasma, tumor, liver and spleen were collected 6 and 24 hours post-administration of a single intratumoral dose of mRNA. (n=6 per group.) Variants ML12AB_002 and hIL12AB_006 , in particular, showed good expression as compared to mIL12_miR122 mRNA, e.g., in plasma and tumor.

[1266] Based on the collective screening in vitro and in vivo, ML12AB_002 was selected as a preferred mRNA encoding hIL12AB **(FIG. 38).**

Patentkrav

1. Sammensætning til anvendelse i en fremgangsmåde til reduktion af størrelsen af en tumor eller hæmning vækst af en tumor hos et individ med behov derfor, hvor

(a) sammensætningen omfatter et mRNA, der koder for et IL-12-polypeptid, hvor mRNA'et omfatter en åben læseramme ("ORF"), der koder for et interleukin-12-p40-underenheds-("IL12B")-polypeptid og et interleukin-12-p35-underenheds-("IL12A")-polypeptid,

(b) mRNA'et er formuleret som en lipidnanopartikel, der omfatter et kationisk lipid, et phospholipid, et sterol og et PEG-modificeret lipid, og

(c) fremgangsmåden omfatter indgivelse til individet af en virksom mængde af sammensætningen.

2. Sammensætning til anvendelse ifølge krav 1 og endvidere omfattende indgivelse til individet af en virksom mængde af en sammensætning omfattende et mRNA omfattende en ORF, der koder for et checkpoint-hæmmer-polypeptid eller en virksom mængde af en sammensætning omfattende et checkpoint-hæmmer-polypeptid, hvor eventuelt

(a) checkpoint-hæmmer-polypeptidet hæmmer PD1, PD-L1, CTLA-4 eller en kombination deraf; og/eller

(b) checkpoint-hæmmer-polypeptidet omfatter et antistof,

hvor antistoffet endvidere eventuelt er et anti-CTLA-4-antistof eller antigenbindende fragment deraf, der specifikt binder CTLA-4, et anti-PD1-antistof eller antigenbindende fragment deraf, der specifikt binder PD1, et anti-PD-L1-antistof eller antigenbindende fragment deraf, der specifikt binder PD-L1, eller en kombination deraf;

hvor anti-PD-L1-antistoffet endvidere eventuelt er atezolizumab, avelumab eller durvalumab; anti-CTLA-4-antistoffet er tremelimumab eller ipilimumab; anti-PD-1-antistoffet er nivolumab eller pembrolizumab;

og/eller

(c) hvor sammensætningen ifølge krav 1 alene eller i kombination med sammensætningen omfatter et mRNA omfattende en ORF, der koder for et checkpoint-hæmmer-polypeptid, eller sammensætningen, der omfatter et checkpoint-hæmmer-polypeptid, aktiverer T-celler i individet.

3. Sammensætning til anvendelse i en fremgangsmåde til (i) aktivering af T-celler hos et individ med behov derfor, (ii) forøgelse af et forhold mellem effektor- og suppressor-T-celler i en tumor hos et individ med behov derfor, (iii) forøgelse af antallet af aktiverede naturlige dræberceller (NK) hos et individ med behov herfor, og/eller (vi) forøgelse af krydspræsenterende, dendritiske celler i en tumor hos et individ med behov derfor, hvor

(a) sammensætningen omfatter et mRNA, der koder for et IL-12-polypeptid, hvori mRNA'et omfatter en ORF, der koder for et IL12B-polypeptid og et IL12A-polypeptid,

(b) mRNA'et er formuleret som en lipidnanopartikel, der omfatter et kationisk lipid, et phospholipid, et sterol og et PEG-modificeret lipid, og

(c) fremgangsmåden omfatter intratumoral indgivelse til individet af en virksom mængde af sammensætningen.

4. Sammensætning til anvendelse ifølge krav 2(c) eller 3, hvor T-celleaktiveringen omfatter induktion af T-celleproliferation; og/eller hvor T-celleaktiveringen omfatter induktion af T-celleinfiltration i tumoren eller forøgelse af antallet af tumorinfiltrerende T-celler; og/eller hvor T-celleaktiveringen omfatter induktion af et hukommelses-T-celle-respons; og/eller hvor de aktiverede T-celler omfatter $CD4^+$-celler, $CD8^+$-T-celler eller begge dele.

5. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 4, hvor

(a) indgivelse af sammensætningen alene eller i kombination med en sammensætning omfattende et mRNA omfattende en ORF, der koder for et checkpoint-hæmmer-polypeptid, eller en sammensætning, der omfatter et checkpoint-hæmmer-polypeptid, øger et forhold mellem effektor- og suppressor-T-celler i tumoren, hvor eventuelt forholdet mellem effektor- og suppressor-T-celler er et forhold mellem $CD8^+$-T-celler og regulatoriske T-celler (Treg), hvor endvidere eventuelt forholdet mellem $CD8^+$ og Treg er mindst 10, mindst 20, mindst 30, mindst 40, mindst 50, mindst 60, mindst 70, mindst 80, mindst 90, mindst 100, mindst 110, mindst 120 mindst 130, mindst 140 eller mindst 150; og/eller

(b) indgivelse af sammensætningen øger antallet af aktiverede NK-celler hos individet yderligere, hvor antallet af aktiverede NK-celler eventuelt øges mindst ca. to gange, mindst ca. tre gange, mindst ca. fire gange, mindst ca. fem gange, mindst ca. seks gange, mindst ca. syv gange, mindst ca. otte gange, mindst ca. ni gange eller mindst ca. ti gange; og/eller de øgede aktiverede NK-celler opretholdes i mindst ca. en dag, mindst ca. to dage, mindst ca. tre dage, mindst ca. fire dage, mindst ca. fem dage, mindst ca. seks dage, mindst ca. syv dage, mindst ca. otte dage, mindst ca. ni dage, mindst ca. ti dage, mindst ca. 11 dage, mindst ca. 12 dage, mindst ca. 13 dage eller mindst ca. 14 dage; og/eller

(c) indgivelse af sammensætningen øger krydspræsenterende, dendritiske celler i individets tumor, hvor de krydspræsenterende, dendritiske celler eventuelt er $CD103^+$-celler.

6. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor indgivelse af sammensætningen reducerer størrelsen af en distal tumor eller hæmmer vækst af en distal tumor hos individet.

7. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor IL12B-polypeptidet og IL12A-polypeptidet er fusioneret direkte eller via en nukleinsyre, der koder for en linker, hvor eventuelt

(i) IL12B-polypeptidet er placeret ved 5'-terminalen af IL12A-polypeptidet eller linkeren, eller

(ii) IL12A-polypeptidet er placeret ved 5'-terminalen af IL12B-polypeptidet eller linkeren; og/eller

(iii) IL12B-polypeptidet omfatter en aminosyresekvens, der er mindst 80 %, mindst 90 %, mindst 95 % eller mindst 98 % identisk med aminosyrerne 23 til 328 i SEQ ID NO: 48, hvor aminosyresekvensen har IL12B-aktivitet; og/eller

(iv) IL12A-polypeptidet omfatter en aminosyresekvens, der er mindst 80 %, mindst 90 %, mindst 95 % eller mindst 98 % identisk med aminosyrerne 336 til 532 i SEQ ID NO: 48, hvor aminosyresekvensen har IL12A-aktivitet; og/eller

(v) mRNA'et omfatter en nukleotidsekvens, der koder for et signalpeptid, hvor signalpeptidet eventuelt er et IL12B-signalpeptid; og/eller signalpeptidet omfatter en aminosyresekvens, der er mindst ca. 80 %, mindst 90 %, mindst

95 %, eller mindst 98 % identisk med aminosyrerne 1 til 22 i SEQ ID NO: 48; og/eller

(vi) ORF'en koder for et IL12B-polypeptid, der er operativt forbundet via en linker med et IL12A-polypeptid; og/eller

(vii) ORF'en koder for et IL12B-signalpeptid, et IL12B-polypeptid, en linker og et IL12A-polypeptid; og/eller

(viii) linkeren omfatter en Gly/Ser-linker, hvor eventuelt Gly/Ser-linkeren omfatter $(G_nS)m$, hvor n er 1, 2 3, 4, 5, 6, 7, 8, 9, 10, 15 eller 20, og m er 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 eller 20, hvor endvidere eventuelt Gly/Ser-linkeren omfatter $(G_nS)_m$, og hvor n er 6 og m er 1.

8. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor

(a) IL12-polypeptidet omfatter en aminosyresekvens, der er mindst 80 %, mindst 90 %, mindst 95 % eller mindst 98 % identisk med SEQ ID NO: 48;

(b) mRNA'et omfatter en nukleotidsekvens, der er mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 98 % eller mindst 99 % identisk med en nukleotidsekvens valgt fra gruppen bestående af SEQ ID NO: 5 til 44, 236 og 237;

(c) mRNA'et omfatter en nukleotidsekvens, der er mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 98 % eller mindst 99 % identisk med SEQ ID NO: 236 eller 237; og/eller

(d) mRNA'et omfatter en ORF omfattende mindst ét kemisk modificeret nukleosid, hvor eventuelt

(i) det mindst ene kemisk modificerede nukleosid er valgt fra gruppen bestående af pseudouridin, N1-methylpseudouridin, 5-methylcytosin, 5-methoxyuridin og en kombination deraf;

(ii) nukleosiderne i ORF'en er mindst 10 %, mindst 20 %, mindst 30 %, mindst 40 %, mindst 50 %, mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 99 % eller 100 % kemisk modificeret; og/eller

(iii) de kemisk modificerede nukleosider i ORF'en er valgt fra gruppen bestående af uridin, adenin, cytosin, guanin og en hvilken som helst kombination deraf.

9. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 8, hvor

(a) uridinnukleosiderne i ORF'en er mindst 10 %, mindst 20 %, mindst 30 %, mindst 40 %, mindst 50 %, mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 99 % eller 100 % kemisk modificeret;

(b) adenosinnukleosiderne i ORF'en er mindst 10 %, mindst 20 %, mindst 30 %, mindst 40 %, mindst 50 %, mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 99 % eller 100 % kemisk modificeret;

(c) cytidinnukleosiderne i ORF'en er mindst 10 %, mindst 20 %, mindst 30 %, mindst 40 %, mindst 50 %, mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 99 % eller 100 % kemisk modificeret;

(d) guanosinnukleosiderne i ORF'en er mindst 10 %, mindst 20 %, mindst 30 %, mindst 40 %, mindst 50 %, mindst 60 %, mindst 70 %, mindst 80 %, mindst 90 %, mindst 95 %, mindst 99 % eller 100 % kemisk modificeret; og/eller

(e) mRNA'et omfatter et miRNA-bindingssted, hvor eventuelt

(i) miRNA-bindingsstedet er et miR-122-bindingssted; og/eller

(ii) miRNA-bindingsstedet er et miR-122-3p- eller miR-122-5p-bindingssted, eller

(iii) miRNA-bindingsstedet omfatter en nukleotidsekvens, der er mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 % eller 100 % identisk med aacgccauua ucacacuaaa ua (SEQ ID NO: 51), hvor miRNA-bindingsstedet binder til miR-122; eller

(iv) miRNA-bindingsstedet omfatter en nukleotidsekvens, der er mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 % eller 100 % identisk med uggaguguga caaugguguu ug (SEQ ID NO: 53), hvor miRNA-bindingsstedet binder til miR-122; eller

(v) miRNA-bindingsstedet omfatter en nukleotidsekvens, der er mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 % eller 100 % identisk med caaacaccau ugucacacuc ca (SEQ ID NO: 54), hvor miRNA-bindingsstedet binder til miR-122.

10. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor mRNA'et

(a) omfatter et 5'-utranslateret område (UTR), hvor 5'-UTR'et eventuelt omfatter en nukleinsyresekvens, der er mindst 90 %, 95 %, 96 %, 97 %, 98 %, 99 % eller 100 % identisk med en sekvens anført i tabel 3; og/eller

(b) omfatter et 3'-utranslateret område (UTR), hvor 3'-UTR'et eventuelt omfatter en nukleinsyresekvens, der er mindst 90 %, 95 %, 96 %, 97 %, 98 %, 99 % eller 100 % identisk med en sekvens anført i tabel 4A eller 4B, og/eller mRNA'et omfatter et miRNA-bindingssted i 3'-UTR'et, hvor polynukleotidet endvidere eventuelt omfatter en nukleotidspacer-sekvens, der er kondenseret til miRNA-bindingsstedet, hvor endvidere eventuelt nukleotidspacer-sekvensen omfatter mindst 10 nukleotider, mindst 20 nukleotider, mindst 30 nukleotider, mindst 40 nukleotider, mindst 50 nukleotider, mindst 60 nukleotider, mindst 70 nukleotider, mindst 80 nukleotider, mindst 90 nukleotider eller mindst 100 nukleotider; og/eller

(c) omfatter en 5'-terminalkappestruktur; hvor den 5'-terminale kappestruktur eventuelt er en Cap0, Cap1, ARCA, inosin, N1-methyl-guanosin, 2'fluor-guanosin, 7-deaza-guanosin, 8-oxo-guanosin, 2-amino-guanosin, LNA-guanosin, 2-azidoguanosin, Cap2, Cap4, 5' methylG cap eller en analog deraf, og/eller

(d) omfatter en 3'-polyA-hale; og/eller

(e) omfatter mindst to, mindst tre, mindst fire, mindst fem, mindst seks, mindst syv, mindst otte, mindst ni eller mindst ti miRNA-bindingssteder; og/eller

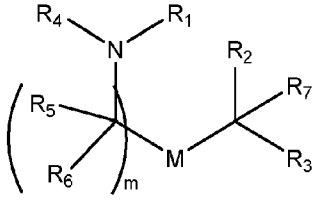
(f) omfatter en kodonoptimeret ORF; og/eller

(g) er et *in vitro*-transskriberet (IVT) polynukleotid og/eller er cirkulært.

11. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor lipidnanopartiklerne omfatter

(a) lipidet valgt fra gruppen bestående af DLin-DMA, DLin-K-DMA, 98N12-5, C12-200, DLin-MC3-DMA, DLin-KC2-DMA, DODMA, PLGA, PEG, PEG-DMG , PEGylerede lipider, aminoalkohollipider, KL22 og kombinationer deraf; eller

(b) en forbindelse med formlen (I)



(I),

eller et salt eller en stereoisomer deraf, hvori

R_1 er valgt fra gruppen bestående af C_{5-20}-alkyl, C_{5-20}-alkenyl, -R*YR'', -YR'' og -R''M'R';

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl, C_{2-14}-alkenyl, -R*YR'', -YR'' og -R*OR'' eller R_2 og R_3, sammen med det atom, hvortil de er bundet, danner en heterocyklus eller carbocyklus;

R_4 er valgt fra gruppen bestående af en C_{3-6}-carbocyklus, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$ og usubstitueret C_{1-6}-alkyl, hvor Q er valgt blandt en carbocyklus, heterocyklus, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$ og $-C(R)N(R)_2C(O)OR$, og hvert n er uafhængigt valgt blandt 1, 2, 3, 4 og 5;

hvert R_5 er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R_6 er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

M og M' er uafhængigt valgt blandt -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2$-, en arylgruppe og en heteroarylgruppe;

R_7 er valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R' er uafhængigt valgt fra gruppen bestående af C_{1-18}-alkyl, C_{2-18}-alkenyl, -R*YR'', -YR'' og H;

hvert R'' er uafhængigt valgt fra gruppen bestående af C_{3-14}-alkyl og C_{3-14}-alkenyl;

hvert R* er uafhængigt valgt fra gruppen bestående af C_{1-12}-alkyl og C_{2-12}-alkenyl;

hvert Y er uafhængigt en C_{3-6}-carbocyklus;

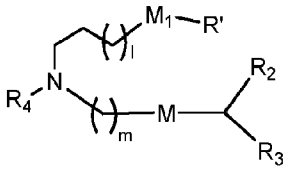
hvert X er uafhængigt udvalgt fra gruppen bestående af F, Cl, Br og I; og

m er valgt blandt 5, 6, 7, 8, 9, 10, 11, 12 og 13; og

forudsat, når R_4 er $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR eller $-CQ(R)_2$, (i) Q ikke er $-N(R)_2$, når n er 1, 2, 3, 4 eller 5, eller (ii) Q så ikke er 5-, 6- eller 7-leddet heterocycloalkyl, når n er 1 eller 2;

hvor eventuelt

(i) forbindelsen har formlen (IA):



(IA),

eller et salt eller en stereoisomer deraf, hvor

l er valgt blandt 1, 2, 3, 4 og 5;

m er valgt blandt 5, 6, 7, 8 og 9;

M_1 er en binding eller M';

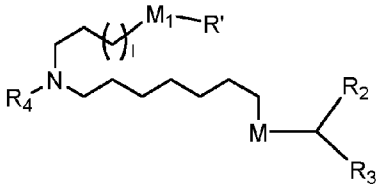
R_4 er usubstitueret C_{1-3}-alkyl eller $-(CH_2)_nQ$, hvori n er 1, 2, 3, 4 eller 5, og Q er OH, $-NHC(S)N(R)_2$ eller $-NHC(O)N(R)_2$;

M og M' er uafhængigt valgt blandt -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, en arylgruppe og en heteroarylgruppe; og

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl og C_{2-14}-alkenyl,

hvor m fortrinsvis er 5, 7 eller 9; eller

(ii) forbindelsen har formlen (II):



(II),

eller et salt eller en stereoisomer deraf, hvori

l er valgt blandt 1, 2, 3, 4 og 5;

M_1 er en binding eller M';

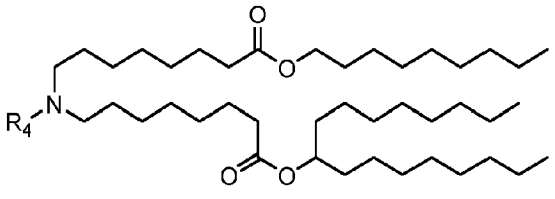
R_4 er usubstitueret C_{1-3}-alkyl eller $-(CH_2)_nQ$, hvori n er 2, 3 eller 4, og Q er OH, $-NHC(S)N(R)_2$ eller $-NHC(O)N(R)_2$;

M og M' er uafhængigt valgt blandt -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, en arylgruppe og en heteroarylgruppe; og

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl og C_{2-14}-alkenyl; eller

(iii) forbindelsen er valgt blandt forbindelse 1 til forbindelse 147 og salte og stereoisomerer deraf; eller

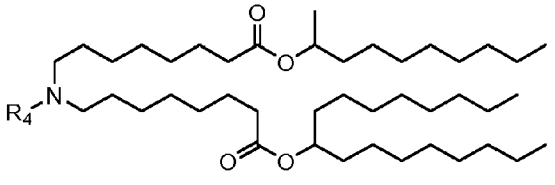
(iv) forbindelsen har formlen (IIa)



(IIa),

eller et salt eller en stereoisomer deraf; eller

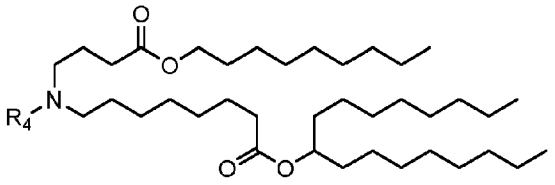
(v) forbindelsen har formlen (IIb)



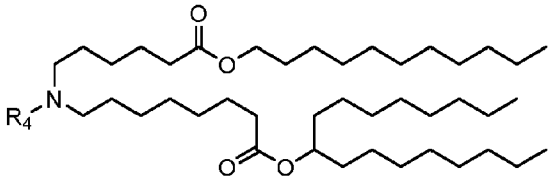
(IIb),

eller et salt eller en stereoisomer deraf; eller

(vi) forbindelsen har formlen (IIc) eller (IIe),



(IIc),

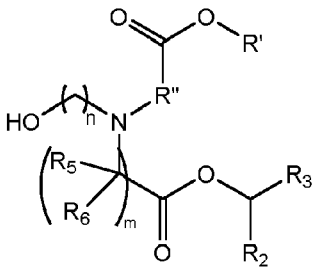


(IIe),

eller et salt eller en stereoisomer deraf; eller

(vii) R_4 i formel I er valgt blandt $-(CH_2)_nQ$ og $-(CH_2)_nCHQR$, hvor Q, R og n er som defineret ovenfor i (b) eller (b)(i); eller

(viii) forbindelsen har formlen (IId)



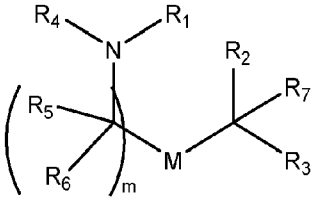
(IId),

eller et salt eller en stereoisomer thereof,

hvor R_2 og R_3 er uafhængigt valgt fra gruppen bestående af C_{5-14}-alkyl og C_{5-14}-alkenyl, n er valgt blandt 2, 3 og 4, og R', R'', R_5, R_6, og m er som defineret ovenfor i (b) eller (b)(i), hvor eventuelt R_2 er C_8-alkyl, og/eller R_3 er C_5-alkyl, C_6-

alkyl, C_7-alkyl, C_8-alkyl eller C_9-alkyl, og/eller m er 5, 7 eller 9, og/eller hvert R_5 er H, hvor eventuelt endvidere hvert R_6 er H.

12. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor lipidnanopartiklerne omfatter en forbindelse med formlen (I)



(I),

eller et salt eller en stereoisomer deraf, hvor

R_1 er valgt fra gruppen bestående af C_{5-30}-alkyl, C_{5-20}-alkenyl, -R*YR'', -YR'' og -R''M'R';

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl, C_{2-14}-alkenyl, -R*YR'', YR'' og R* OR'', eller R_2 og R_3, sammen med det atom, hvortil de er bundet, danner en heterocyklus eller carbocyklus;

R_4 er valgt fra gruppen bestående af en C_{3-6}-carbocyklus, $(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $CQ(R)_2$, og usubstitueret C_{1-6}-alkyl, hvor Q er valgt balndt en carbocyklus, heterocyklus, OR, $O(CH_2)_nN(R)_2$, C(O)OR, OC(O)R, CX_3, CX_2H, CXH_2, CN, $-N(R)_2$, $C(O)N(R)_2$, N(R)C(O)R, $N(R)S(O)_2R$, $N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, -N(OR)C(O)R, $-N(OR)S(O)_2R$, -N(OR)C(O)OR, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR og $C(R)N(R)_2C(O)OR$, og hvert n er uafhængigt valgt blandt 1, 2, 3, 4 og 5;

hvert R_5 er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R_6 er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

M og M' er uafhængigt valgt blandt -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, - (O)-, -C(S)-, -(S)S-, -(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S- en arylgruppe og en heteroarylgruppe;

R_7 er valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

R_8 er valgt fra gruppen bestående af C_{3-6}-carbocyklus og heterocyklus;

R_9 er valgt fra gruppen bestående af H, CN, NO_2, C_{1-6}-alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6}-alkenyl, C_{3-6}-carbocyklus og heterocyklus;

hvert R er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R' er uafhængigt valgt fra gruppen bestående af C_{1-18}-alkyl, C_{2-18}-alkenyl, $-R^*YR''$, -'' og H;

hvert R'' er uafhængigt valgt fra gruppen bestående af C_{3-14}-alkyl og C_{3-14}-alkenyl;

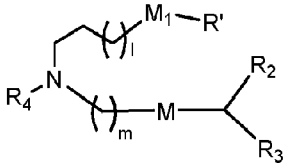
hvert R^* er uafhængigt valgt fra gruppen bestående af C_{1-12}-alkyl og C_{2-12}-alkenyl;

hvert Y er uafhængigt en C_{3-6}-carbocyklus;

hvert X er uafhængigt udvalgt fra gruppen bestående af F, Cl, Br og I; og m er valgt blandt 5, 6, 7, 8, 9, 10, 11, 12 og 13; og

forudsat, når R_4 er $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR eller $-CQ(R)_2$, (i) Q så ikke er $-N(R)_2$, når n er 1, 2, 3, 4 eller 5, eller (ii) Q ikke er 5-, 6- eller 7-leddet heterocycloalkyl, når n er 1 eller 2; hvor eventuelt

(a) indgivelsesmidlet omfatter forbindelsen med formlen (IA):



(IA),

eller et salt eller en stereoisomer deraf, hvor

l er valgt blandt 1, 2, 3, 4 og 5;

m er valgt blandt 5, 6, 7, 8 og 9;

M_1 er en binding eller M';

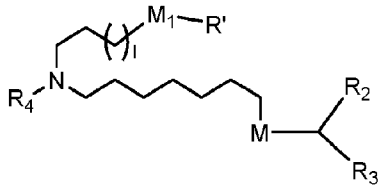
R_4 er usubstitueretC_{1-3}-alkyl eller $(CH_2)_nQ$, hvor Q er OH, $NHC(S)N(R)_2$eller $-NHC(O)N(R)_2$,$-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl eller heterocycloalkyl;

M og M' er uafhængigt valgt blandt -(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, en arylgruppe og en heteroarylgruppe; og

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl og C_{2-14}-alkenyl;

hvor endvidere m eventuelt er 5, 7 eller 9; og/eller

(b) forbindelsen har formlen (II)



(II)

eller et salt eller en stereoisomer deraf, hvor

l er valgt fra 1, 2, 3, 4 og 5;

M_1 er en binding eller M';

R_4 er usubstitueret C_{1-3}-alkyl, eller $(CH_2)_nQ$, hvori n er 2, 3 eller 4, og Q er OH, - $NHC(S)N(R)_2$ eller $NHC(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)R_8$, - $NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, heteroaryl eller heterocycloalkyl;

M and M' er uafhængigt valgt blandt - (O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S- en arylgruppe og en heteroarylgruppe; og

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl og C_{2-14}-alkenyl; og/eller

(c) M_1 er M', hvor M og M' eventuelt uafhængigt er -C(O)O- eller -OC(O)-; og/eller

(d) hvor 1 er 1, 3 eller 5; og/eller

(e) forbindelsen er valgt fra gruppen bestående af forbindelse 1 til forbindelse 232, salte og stereoisomerer deraf og en hvilken som helst kombination deraf.

13. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor

(a) phospholipidet er valgt fra gruppen bestående af

1,2-dilinoleoyl-sn-glycero-3-phosphocholin (DLPC),

1,2-dimyristoyl-sn-glycero-phosphocholin (DMPC),

1,2-dioleoyl-sn-glycero-3-phosphocholin (DOPC),

1,2-dipalmitoyl-sn-glycero-3-phosphocholin (DPPC),

1,2-distearoyl-sn-glycero-3-phosphocholin (DSPC),

1,2-diundecanoyl-sn-glycero-phosphocholin (DUPC),

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholin (POPC),

1,2-di-O-octadecenyl-*sn*-glycero-3-phosphocholin (18:0 Diether PC),

1-oleoyl-2-cholesterylhemisuccinoyl-*sn*-glycero-3-phosphocholin (OChemsPC),

1-hexadecyl-sn-glycero-3-phosphocholin (C16 Lyso PC),

1,2-dilinolenoyl-sn-glycero-3-phosphocholin,

1,2-diarachidonoyl-sn-glycero-3-phosphocholin,

1,2-didocosahexaenoyl-sn-glycero-3-phosphocholin,

1,2-dioleoyl-sn-glycero-3-phosphoethanolamin (DOPE),

1,2-diphytanoyl-sn-glycero-3-phosphoethanolamin (ME 16:0 PE),

1,2-distearoyl-sn-glycero-3-phosphoethanolamin,

1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamin,

1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamin,

1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamin,

1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamin,

1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) natriumsalt (DOPG),

sphingomyelin

og blandinger deraf;

eller hvor phospholipidet er valgt fra gruppen bestående af

1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholin (14:0-16:0 PC, MPPC),

1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholin (14:0-18:0 PC, MSPC),

1-palmitoyl-2-acetyl-sn-glycero-3-phosphocholin (16:0-02:0 PC),

1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholin (16:0-14:0 PC, PMPC),

1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholin (16:0-18:0 PC, PSPC),

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholin (16:0-18:1 PC, POPC),

1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholin (16:0-18:2 PC, PLPC),

1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholin (16:0-20:4 PC),

1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholin (14:0-22:6 PC),

1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholin (18:0-14:0 PC, SMPC),

1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC),

1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholin (18:0-18:1 PC, SOPC),

1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholin (18:0-18:2 PC),

1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholin (18:0-20:4 PC),

1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholin (18:0-22:6 PC),

1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholin (18:1-14:0 PC, OMPC),
1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholin (18:1-16:0 PC, OPPC),
1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholin (18:1-18:0 PC, OSPC),
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamin (16:0-18:1 PE, POPE),
1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamin (16:0-18:2 PE),
1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamin (16:0-20:4 PE),
1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamin (16:0-22:6 PE),
1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamin (18:0-18:1 PE),
1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamin (18:0-18:2 PE),
1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamin (18:0-20:4 PE),
1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamin (18:0-22:6 PE),
1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholin (OChemsPC) og
en hvilken som helst kombination heraf; og/eller
(b) sterolet er valgt fra gruppen bestående af cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidin, ursolsyre, alfa-tocopherol og blandinger deraf; og/eller
(c) PEG-lipidet er valgt fra gruppen bestående af en PEG-modificeret phosphatidylethanolamin, en PEG-modificeret phosphatidinsyre, et PEG-modificeret ceramid, en PEG-modificeret dialkylamin, en PEG-modificeret diacylglycerol, en PEG-modificeret dialkylglycero og blandinger deraf; og/eller
(d) lipidnanopartiklerne omfatter et kationisk lipid valgt fra gruppen bestående af 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazinethanamin (KL10), N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazindiethanamin (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontan (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropan (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolan (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoat (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolan (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropan (DODMA),

2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-die n-1-yloxy]propan-1-amin (Octyl-CLinDMA),
(2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,1 2-dien-1-yloxy]propan-1-amin (Octyl-CLinDMA (2R)) og
(2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)) og/eller
(e) lipidnanopartiklerne omfatter en kvaternær aminforbindelse, hvor den kvaternære aminforbindelse eventuelt er valgt fra gruppen bestående af
1,2-dioleoyl-3-trimethylammonium-propan (DOTAP),
N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniumchlorid (DOTMA),
1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazoliniumchlorid (DOTIM),
2,3-dioleyloxy-N-[2(spermincarboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoracetat (DOSPA),
N,N-distearyl-N,N-dimethylammoniumbromid (DDAB),
N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethylammoniumbromid (DMRIE),
N-(1,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethylammoniumbromid (DORIE),
N,N-dioleyl-N,N-dimethylammoniumchlorid (DODAC),
1,2-dilauroyl-sn-glycero-3-ethylphosphocholin (DLePC),
1,2-distearoyl-3-trimethylammonium-propan (DSTAP),
1,2-dipalmitoyl-3-trimethylammonium-propan (DPTAP),
1,2-dilinoleoyl-3-trimethylammonium-propan (DLTAP),
1,2-dimyristoyl-3-trimethylammonium-propan (DMTAP),
1,2-distearoyl-sn-glycero-3-ethylphosphocholin (DSePC),
1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholin (DPePC),
1,2-dimyristoyl-sn-glycero-3-ethylphosphocholin (DMePC),
1,2-dioleoyl-sn-glycero-3-ethylphosphocholin (DOePC),
1,2-di-(9Z-tetradecenoyl)-sn-glycero-3-ethylphosphocholin (14:1 EPC),
1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholin (16:0-18:1 EPC),
og en hvilken som helst kombination heraf.

14. Sammensætning til anvendelse ifølge et hvilket som helst foregående krav, hvor
(a) der med administrationen behandles en cancer,

hvor canceren eventuelt er udvalgt fra gruppen bestående af binyrebarkcancer, fremskreden cancer, analcancer, aplastisk anæmi, galdevejscancer, blærecancer, knoglecancer, knoglemetastase, hjernetumorer, hjernecancer, brystcancer, børnecancer, cancer af ukendt primær oprindelse, Castlemans sygdom, cervixcancer, colon/rektal cancer, endometriecancer, cancer i spiserøret, tumorer fra Ewing-familien, øjencancer, galdeblærecancer, gastrointestinale carcinoide tumorer, gastrointestinale stromale tumorer, gestationstrofoblastisk sygdom, Hodgkins sygdom, Kaposis sarkom, nyrecellekarcinom, larynx- og hypopharyngeal cancer, akut lymfatisk leukæmi, akut myeloid leukæmi, kronisk lymfatisk leukæmi, kronisk myeloid leukæmi, kronisk myelomonocytisk leukæmi, levercancer, hepatocellulært karcinom (HCC), ikke-småcellet lungecancer, småcellet lungecancer, lungekarcinoid tumor, hudlymfom, malignt mesotheliom, multipelt myelom, myelodysplastisk syndrom, næsehule- og paranasal sinuscancer, nasopharyngeal cancer, neuroblastom, ikke-Hodgkins lymfom, mundhule- og oropharyngeal cancer, osteosarkom, ovariecancer, bugspytkirtelcancer, peniscancer, hypofysetumorer, prostatacancer, retinoblastom, rhabdomyosarkom, spytkirtelcancer, sarkom i blødt væv hos voksne, basal- og pladecellehudcancer, melanom, tyndtarmscancer, mavecancer, testikelcancer, svælgcancer, thymuscancer, skjoldbruskkirtelcancer, livmodersarkom, vaginal cancer, vulvarcancer, Waldenströms makroglobulinæmi, Wilms tumor, sekundære cancere forårsaget af cancerbehandling og en hvilken som helst kombination heraf; og/eller

(b) sammensætningen administreres ved hjælp af en anordning omfattende en pumpe, plaster, lægemiddelreservoir, anordning med kort nål, anordning med enkelt nål, anordning med flere nåle, mikronåleanordning, stråleinjektionsanordning, ballistisk pulver/partikelindgivelsesanordning, kateter, lumen, kryosonde, kanyle, mikrokanyler eller anordninger, der anvender varme, RF-energi, elektrisk strøm eller en hvilken som helst kombination deraf; og/eller

(c) den virksomme mængde er mellem ca. 0,10 mg/kg til omkring 1.000 mg/kg; og/eller

(d) individet er et menneske.

15. Lipidnanopartikel omfattende et mRNA, der koder for et humant IL12-polypeptid, hvor mRNA'et omfatter en ORF, der koder for et humant IL12B-polypeptid, som er operativt forbundet med et humant IL12A-polypeptid, og lipidnanopartiklerne omfatter et kationisk lipid, et phospholipid og et PEG-modificeret lipid.

16. Lipidnanopartikel ifølge krav 15, hvor

(a) IL12B-polypeptidet er operativt forbundet med IL12A-polypeptidet ved hjælp af en peptidlinker; og/eller

(b) IL12B-polypeptidet er placeret ved 5'-terminalen af IL12A-polypeptidet eller peptidlinkeren; eller

(c) IL12A-polypeptidet er placeret ved 5'-terminalen af IL12B-polypeptidet eller peptidlinkeren; og/eller

(d) peptidlinkeren omfatter en Gly/Ser-linker, hvor eventuelt Gly/Ser-linkeren omfatter $(G_nS)m$, hvor n er 1, 2 3, 4, 5, 6, 7, 8, 9, 10, 15 eller 20, og m er 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 eller 20, hvor endvidere Gly/Ser-linkeren eventuelt omfatter $(G_nS)_m$, og hvor n er 6, og m er 1; og/eller

(e) ORF'en koder for et signalpeptid, hvor signalpeptidet eventuelt er et humant IL12B-signalpeptid; og/eller

(f) det humane IL12B-polypeptid omfatter en aminosyresekvens angivet i aminosyrerne 23 til 328 ifølge SEQ ID NO: 48; og/eller

(g) det humane IL12A-polypeptid omfatter en aminosyresekvens angivet i aminosyrerne 336 til 532 ifølge SEQ ID NO: 48; og/eller

(h) humane IL12B-signalpeptid omfatter en aminosyresekvens angivet i aminosyrerne 1 til 22 ifølge SEQ ID NO: 48; og/eller

(i) det humane IL12-polypeptid omfatter en aminosyresekvens angivet i SEQ ID NO: 48; og/eller

(j) mRNA'et omfatter et miRNA-bindingssted, eventuelt et miR-122-bindingssted, endvidere eventuelt et miR-122-3p- eller miR-122-5p-bindingssted; endvidere eventuelt et miR-122-5p-bindingssted omfattende sekvensen angivet i SEQ ID NO: 54; og/eller

(k) mRNA'et omfatter en 3' UTR, hvor 3' UTR'en eventuelt omfatter en sekvens angivet i SEQ ID NO: 240 og/eller eventuelt er et miRNA-bindingssted placeret i 3' UTR'en; og/eller

(l) mRNA'et omfatter en 5' UTR, hvor 5' UTR'en eventuelt omfatter en sekvens angivet i SEQ ID NO:39; og/eller

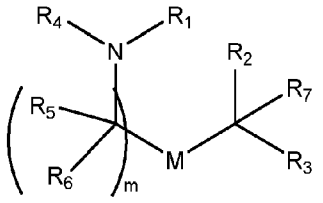
(m) mRNA'et omfatter en 5'-terminal kappestruktur; hvor eventuelt 5'-terminal-kappestrukturen er en Cap0, Cap1, ARCA, inosin, N1-methyl-guanosin, 2'flu eller o-guanosin, 7-deaza-guanosin, 8-oxo-guanosin, 2-amino-guanosin, LNA-guanosin, 2-azidoguanosin, Cap2, Cap4, 5' methylG-cap eller en analog deraf; og/eller

(n) mRNA'et omfatter en 3'-polyA-hale; og/eller

(o) mRNA'et omfatter sekvensen angivet i SEQ ID NO: 6 eller SEQ ID NO: 236 eller 237; og/eller

(p) mRNA'et omfatter en ORF, der omfatter mindst ét modificeret nukleosid, hvor det mindst ene kemisk modificerede nukleosid eventuelt er valgt fra gruppen bestående af pseudouridin, N1-methylpseudouridin, 5-methylcytosin, 5-methoxyuridin og en kombination deraf, hvor det mindst ene kemisk modificerede nukleosid fortrinsvis er N1-methylpseudouridin; og/eller

(q) hvor lipidnanopartiklerne omfatter fra 30 mol-% til 70 mol-%, fra 35 mol-% til 65 mol-%, fra 40 mol-% til 60 mol-% eller fra 45 mol-% til 55 mol-% af en forbindelse med formlen (I):



(I),

eller et salt eller en stereoisomer deraf, hvor

R_1 er valgt fra gruppen bestående af C_{5-20}-alkyl, C_{5-20}-alkenyl, -R*YR'', -YR'' og -R''M'R';

R_2 og R_3 er uafhængigt valgt fra gruppen bestående af H, C_{1-14}-alkyl, C_{2-14}-alkenyl, -R*YR'', -YR'' og -R*OR'', eller R_2 og R_3, sammen med det atom, hvortil de er bundet, danner en heterocyklus eller carbocyklus;

R_4 er valgt fra gruppen bestående af en C_{3-6}-carbocyklus, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$ og usubstitueret C_{1-6}-alkyl, hvor Q er valgt blandt en carbocyklus, heterocyklus, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, -

$N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$ og $-C(R)N(R)_2C(O)OR$, og hvert n er uafhængigt valgt blandt 1, 2, 3, 4 og 5;

hvert R_5 er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R_6 er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

M og M' er uafhængigt valgt blandt -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2$-, en arylgruppe og en heteroarylgruppe;

R_7 er valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R er uafhængigt valgt fra gruppen bestående af C_{1-3}-alkyl, C_{2-3}-alkenyl og H;

hvert R' er uafhængigt valgt fra gruppen bestående af C_{1-18}-alkyl, C_{2-18}-alkenyl, $-R^*YR''$, -YR'' og H;

hvert R'' er uafhængigt valgt fra gruppen bestående af C_{3-14}-alkyl og C_{3-14}-alkenyl;

hvert R^* er uafhængigt valgt fra gruppen bestående af C_{1-12}-alkyl og C_{2-12}-alkenyl;

hvert Y er uafhængigt en C_{3-6}-carboklus;

hvert X er uafhængigt udvalgt fra gruppen bestående af F, Cl, Br og I; og

m er valgt blandt 5, 6, 7, 8, 9, 10, 11, 12 og 13; og

forudsat, når R_4 er $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR eller $-CQ(R)_2$, (i) Q så ikke er $-N(R)_2$-, når n er 1, 2, 3, 4 eller 5, eller (ii) Q ikke er 5-, 6- eller 7-leddet heterocycloalkyl, når n er 1 eller 2; og/eller

(r) det kationiske lipid er forbindelse 18.

17. Lipidnanopartikel som defineret i krav 15 eller 16 til anvendelse i en fremgangsmåde til behandling eller forsinkelse af cancerprogression hos et individ, hvor medikamentet omfatter lipidnanopartiklerne og en eventuelt farmaceutisk acceptabel bærer, og hvor behandlingen omfatter administration af medikamentet i kombination med en sammensætning omfattende et checkpoint-hæmmer-polypeptid og en eventuel farmaceutisk acceptabel bærer.

18. Kit omfattende et medikament, der omfatter en lipidnanopartikel ifølge krav 15 eller 16 og en valgfri farmaceutisk acceptabel bærer, og en indlægsseddel, der omfatter instruktioner til administration af medikamentet alene eller i kombination med en sammensætning, der omfatter et checkpoint-hæmmer-polypeptid og en eventuelt farmaceutisk acceptabel bærer til behandling eller forsinkelse af cancerprogression hos et individ, hvor kittet eventuelt endvidere omfatter en indlægsseddel, der omfatter instruktioner til administration af det første medikament og det andet medikament til behandling eller forsinkelse af cancerprogression hos et individ.

19. Sammensætning omfattende lipidnanopartiklerne ifølge krav 15 eller 16 og eventuelt en farmaceutisk acceptabel bærer til anvendelse ved behandling eller forsinkelse af cancerprogression hos et individ, hvor behandlingen omfatter intratumoral administration af lipidnanopartiklen i kombination med en anden sammensætning, hvor den anden sammensætning omfatter et checkpoint-hæmmer-polypeptid og en eventuel farmaceutisk acceptabel bærer.

20. Farmaceutisk sammensætning omfattende lipidnanopartiklerne ifølge krav 15 eller 16 og en farmaceutisk acceptabel bærer eller excipiens, hvor lipidnanopartiklerne eventuelt er formuleret til intratumoral administration.

21. Kit omfattende en beholder omfattende den farmaceutiske sammensætning ifølge krav 20 og en indlægsseddel omfattende instruktioner til administration af den farmaceutiske sammensætning til behandling eller forsinkelse af cancerprogression hos et individ, hvor indlægssedlen eventuelt endvidere omfatter instruktioner til administration af den farmaceutiske sammensætning i kombination med en sammensætning omfattende et checkpoint-hæmmer-polypeptid og en valgfri farmaceutisk acceptabel bærer til behandling eller forsinkelse af cancerprogression hos et individ.

DRAWINGS

FIG. 1.

(1) Wild Type IL12B without signal (IL12B) Amino Acids (SEQ ID NO: 1):
IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEV
LSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTC
GAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK
PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSS
WSEWASVPCS

(2) Wild Type IL12B without signal (IL12B) Nucleic Acids (SEQ ID NO: 2):
ATATGGCAACTGAAGAAAGATGTTTATGTCGTAGAATTGGATTGGTATCCGGATGCCCCTGGAGAAATGGTGGT
CCTCACCTGTGACACCCCTGAAGAAGATGGTATCACCTGGACCTTGGACCAGAGCAGTGAGGTCTTAGGCTCTG
GCAAAACCCTGACCATCCAAGTCAAAGAGTTTGGAGATGCTGGCCAGTACACCTGTCACAAAGGAGGCGAGGTT
CTAAGCCATTCGCTCCTGCTGCTTCACAAAAAGGAAGATGGAATTTGGTCCACTGATATTTTAAAGGACCAGAA
AGAACCCAAAAATAAGACCTTTCTAAGATGCGAGGCCAAGAATTATTCTGGACGTTTCACCTGCTGGTGGCTGA
CGACAATCAGTACTGATTTGACATTCAGTGTCAAAAGCAGCAGAGGCTCTTCTGACCCCCAAGGGGTGACGTGC
GGAGCTGCTACACTCTCTGCAGAGAGAGTCAGAGGGGACAACAAGGAGTATGAGTACTCAGTGGAGTGCCAGGA
GGACAGTGCCTGCCCAGCTGCTGAGGAGAGTCTGCCCATTGAGGTCATGGTGGATGCCGTTCACAAGCTCAAGT
ATGAAAACTACACCAGCAGCTTCTTCATCAGGGACATCATCAAACCTGACCCACCCAAGAACTTGCAGCTGAAG
CCATTAAAGAATTCTCGGCAGGTGGAGGTCAGCTGGGAGTACCCTGACACCTGGAGTACTCCACATTCCTACTT
CTCCCTGACATTCTGCGTTCAGGTCCAGGGCAAGAGCAAGAGAGAAAAGAAAGATAGAGTCTTCACGGACAAGA
CCTCAGCCACGGTCATCTGCCGCAAAAATGCCAGCATTAGCGTGCGGGCCCAGGACCGCTACTATAGCTCATCT
TGGAGCGAATGGGCATCTGTGCCCTGCAGT

(3) Wild Type IL12A without signal peptide Amino acids (SEQ ID NO: 3):
RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESC
LNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQAL
NFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(4) Wild Type IL12A without signal peptide Nucleic acids (SEQ ID NO: 4):
AGAAACCTCCCCGTGGCCACTCCAGACCCAGGAATGTTCCCATGCCTTCACCACTCCCAAAACCTGCTGAGGCC
GTCAGCAACATGCTCCAGAAGGCCAGACAAACTCTAGAATTTTACCCTTGCACTTCTGAAGAGATTGATCATGAA
GATATCACAAAAGATAAAACCAGCACAGTGGAGGCCTGTTTACCATTGGAATTAACCAAGAATGAGAGTTGCCTA
AATTCCAGAGAGACCTCTTTCATAACTAATGGGAGTTGCCTGGCCTCCAGAAAGACCTCTTTTATGATGGCCCTG
TGCCTTAGTAGTATTTATGAAGACTTGAAGATGTACCAGGTGGAGTTCAAGACCATGAATGCAAAGCTTCTGATG
GATCCTAAGAGGCAGATCTTTCTAGATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTC
AACAGTGAGACTGTGCCACAAAAATCCTCCCTTGAAGAACCGGATTTTTATAAAACTAAAATCAAGCTCTGCATA
CTTCTTCATGCTTTCAGAATTCGGGCAGTGACTATTGATAGAGTGATGAGCTATCTGAATGCTTCC

(5) Wild Type IL12B signal peptide Amino acids: (SEQ ID NO: 45)
MCHQQLVISWFSLVFLASPLVA

(6) Wild Type IL12B signal peptide Nucleic acids: (SEQ ID NO: 46)
ATGTGTCACCAGCAGTTGGTCATCTCTTGGTTTTCCCTGGTTTTTCTGGCATCTCCCCTCGTGGCC

FIG. 2A. IL12 Levels

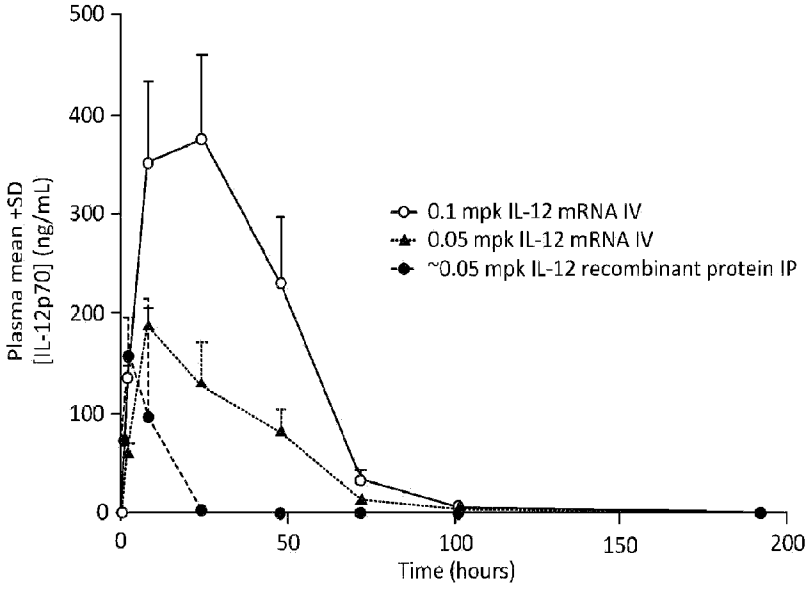


FIG. 2B. IFNγ Levels

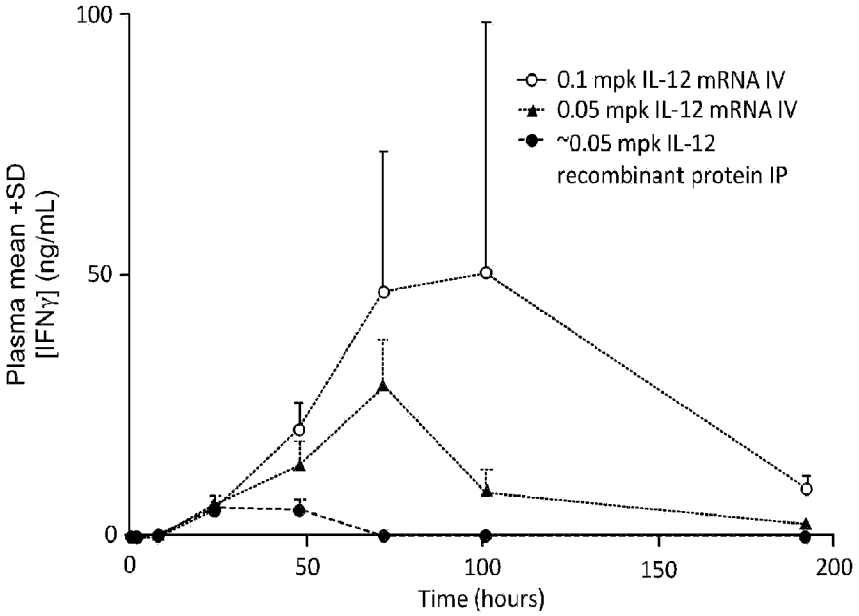


FIG. 3. A single IV dose of IL12 mRNA in LNP has robust efficacy

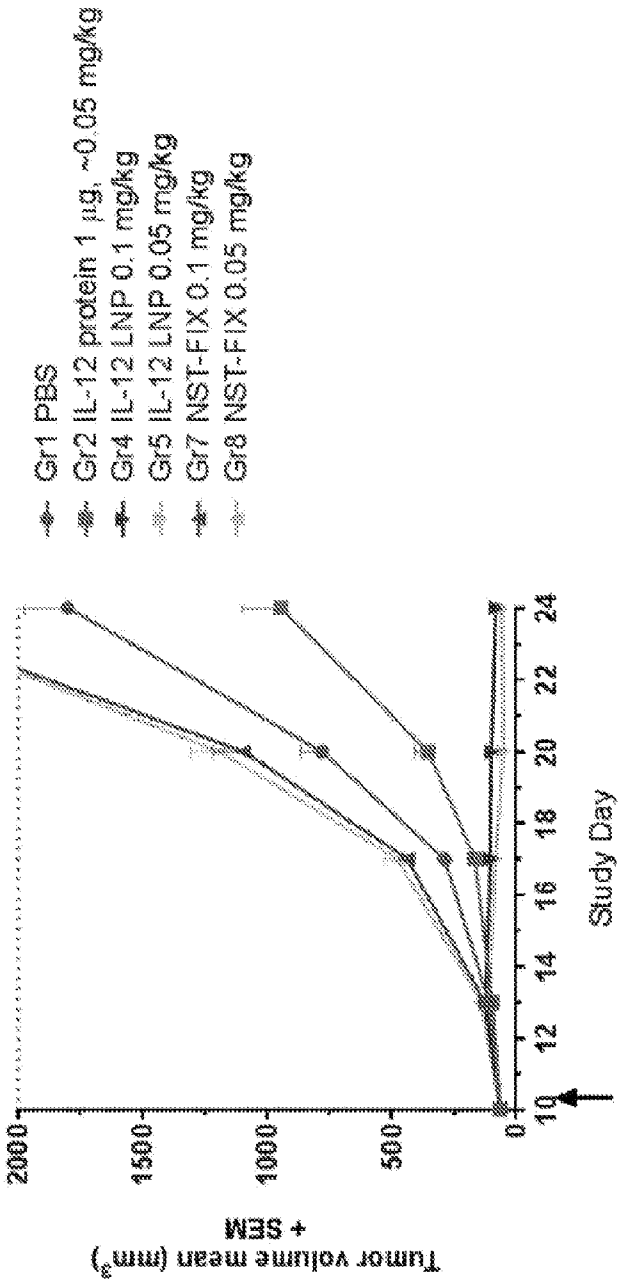


FIG. 4B

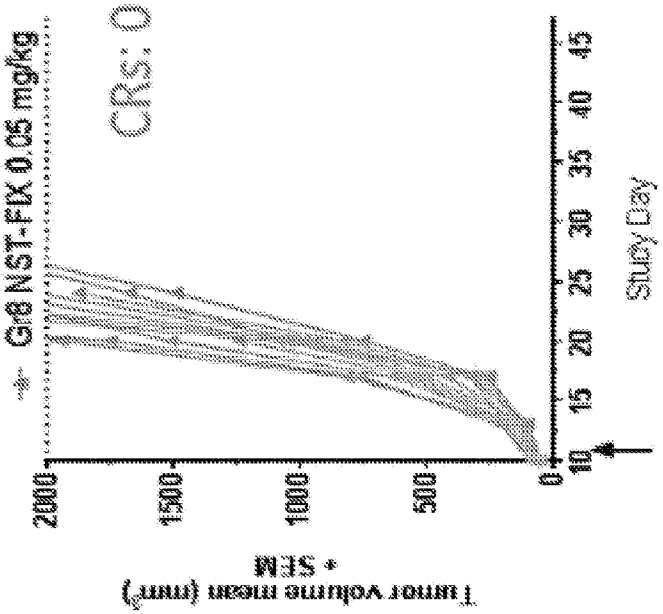

Gr8 NST-FIX 0.05 mg/kg
CRs: 0
Tumor volume mean (mm³) + SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day

FIG. 4A

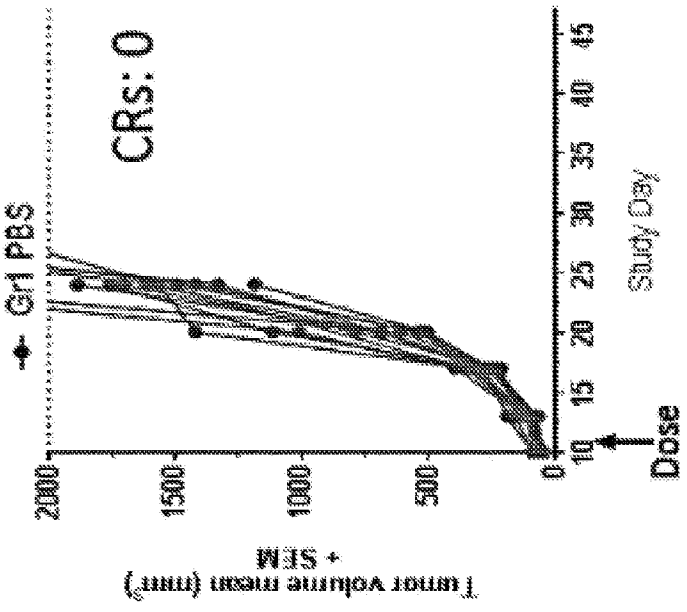

Gr1 PBS
CRs: 0
Tumor volume mean (mm³) + SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day
Dose

FIG. 4D

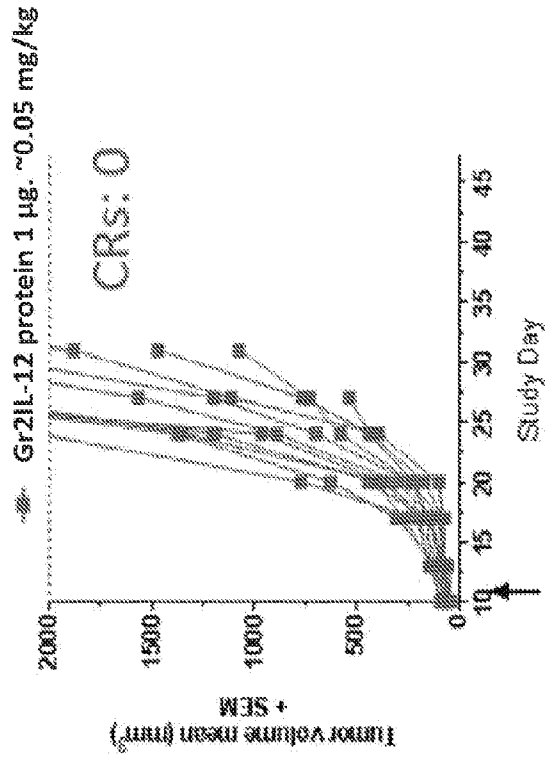

Gr2IL-12 protein 1 µg. ~0.05 mg/kg
CRs: 0
Tumor volume mean (mm³) + SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day

FIG. 4C

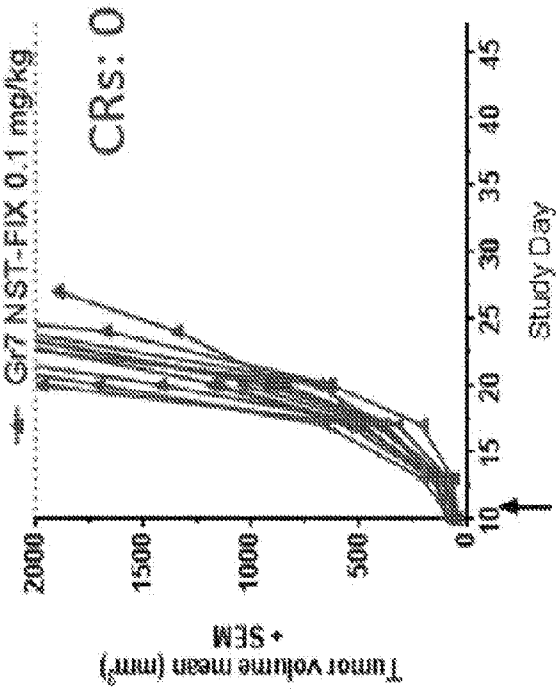

Gr7 NST-FIX 0.1 mg/kg
CRs: 0
Tumor volume mean (mm³) + SEM
2000
1500
1000
500
0
10
15
20
25
30
35
40
45
Study Day

FIG. 4F

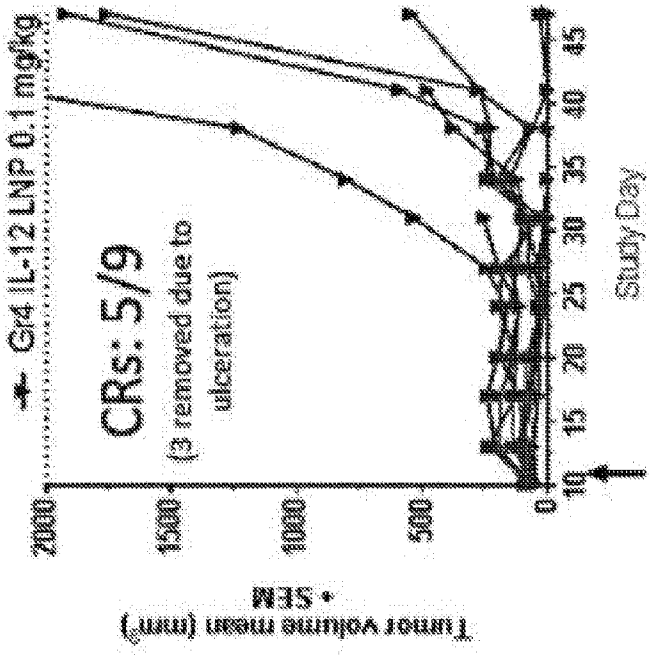

Gr4 IL-12 LNP 0.1 mg/kg
CRs: 5/9
(3 removed due to ulceration)
Tumor volume mean (mm³) + SEM
Study Day
2000
1500
1000
500
0
10
15
20
25
30
35
40
45

FIG. 4E

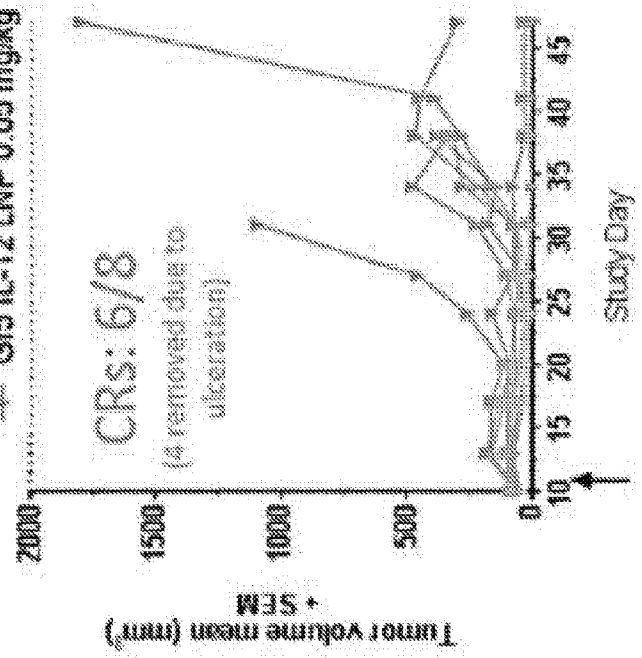

Gr5 IL-12 LNP 0.05 mg/kg
CRs: 6/8
(4 removed due to ulceration)
Tumor volume mean (mm³) + SEM
Study Day
2000
1500
1000
500
0
10
15
20
25
30
35
40
45

FIG. 5.

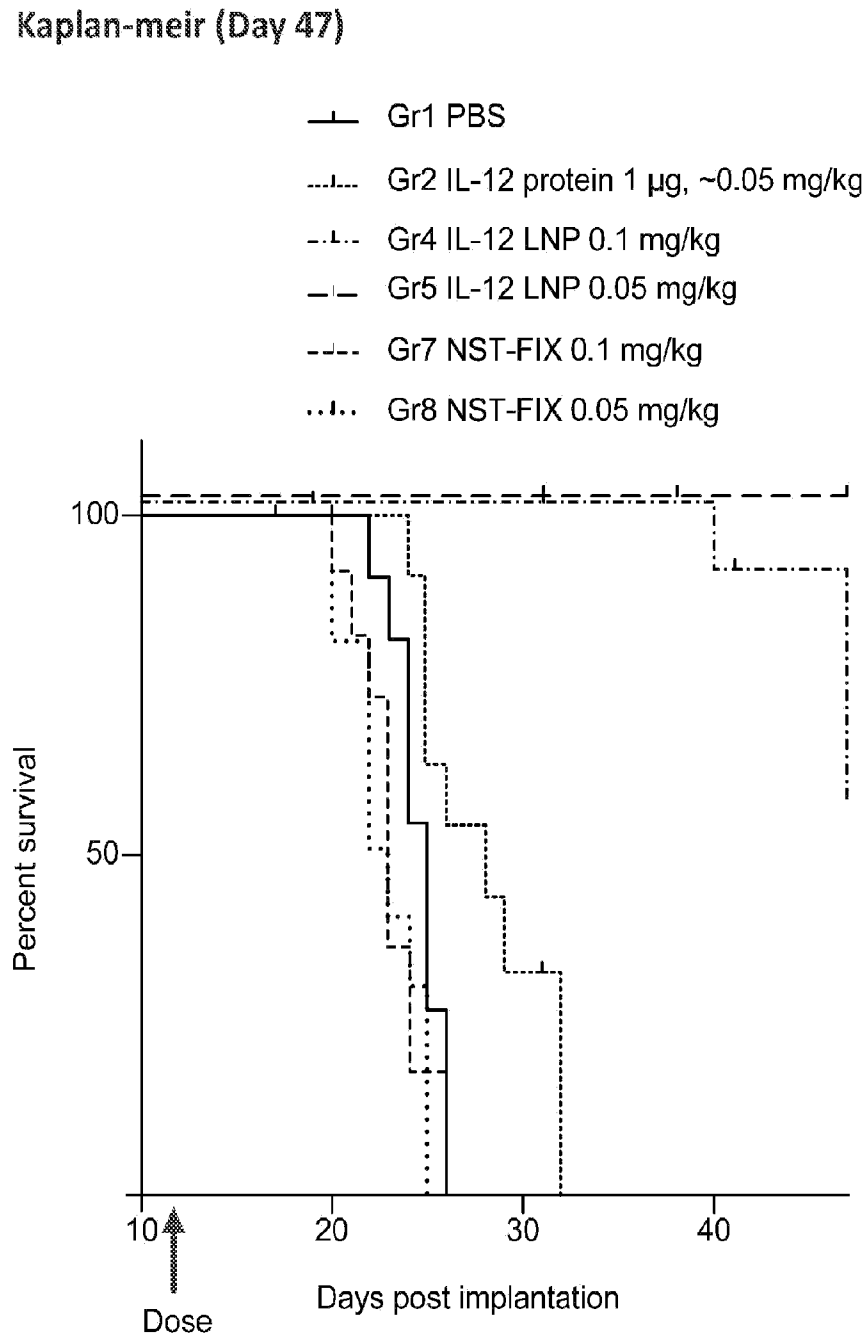

Kaplan-meir (Day 47)
Gr1 PBS
Gr2 IL-12 protein 1 µg, ~0.05 mg/kg
Gr4 IL-12 LNP 0.1 mg/kg
Gr5 IL-12 LNP 0.05 mg/kg
Gr7 NST-FIX 0.1 mg/kg
Gr8 NST-FIX 0.05 mg/kg
Percent survival
100
50
10
20
30
40
Dose
Days post implantation

FIG. 6A

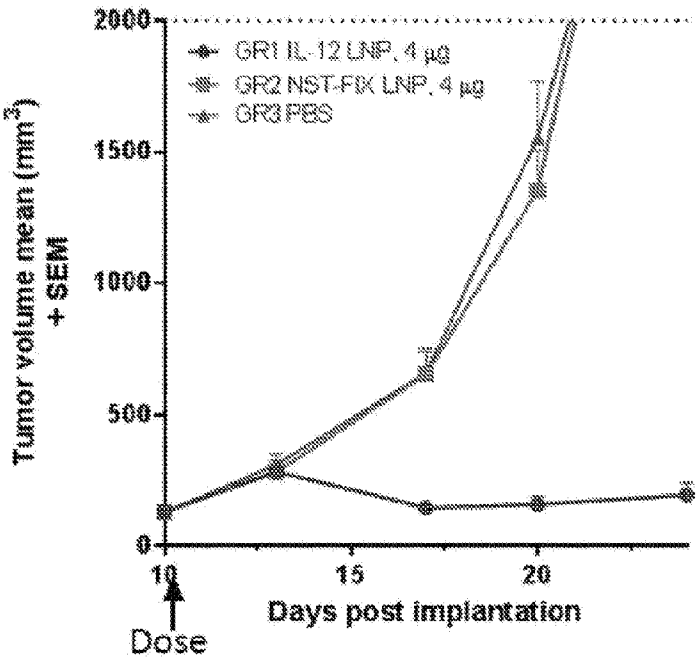

2000
1500
1000
500
0
GR1 IL-12 LNP, 4 µg
GR2 NST-FIK LNP, 4 µg
GR3 PBS
Tumor volume mean (mm3) + SEM
10
15
20
Days post implantation
Dose

FIG. 6B

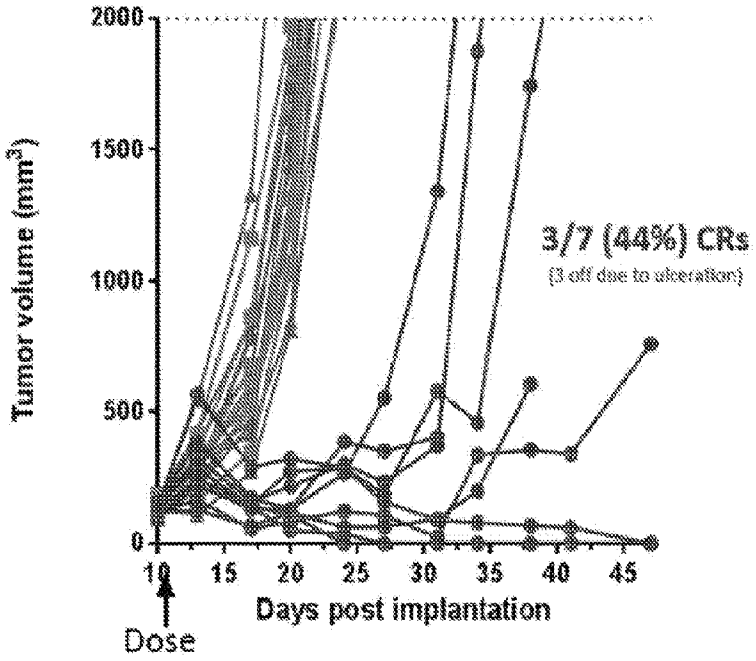

2000
1500
1000
500
0
Tumor volume (mm3)
3/7 (44%) CRs
(3 off due to ulceration)
10
15
20
25
30
35
40
45
Days post implantation
Dose

FIG. 7A

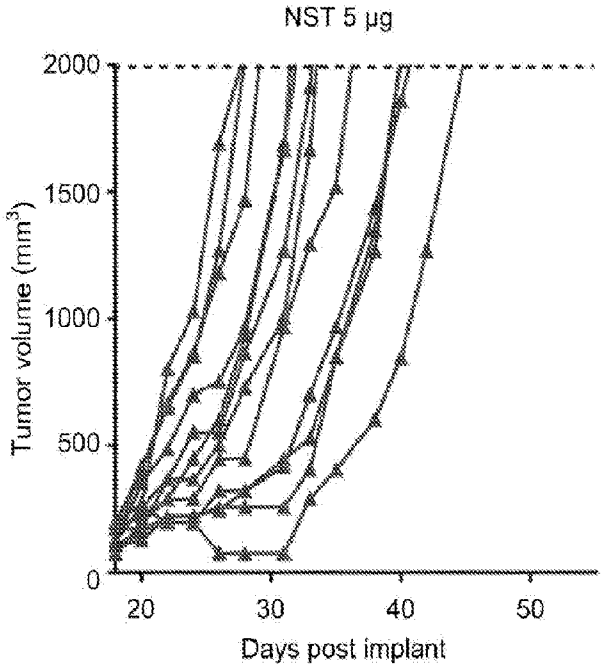

NST 5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant

FIG. 7B

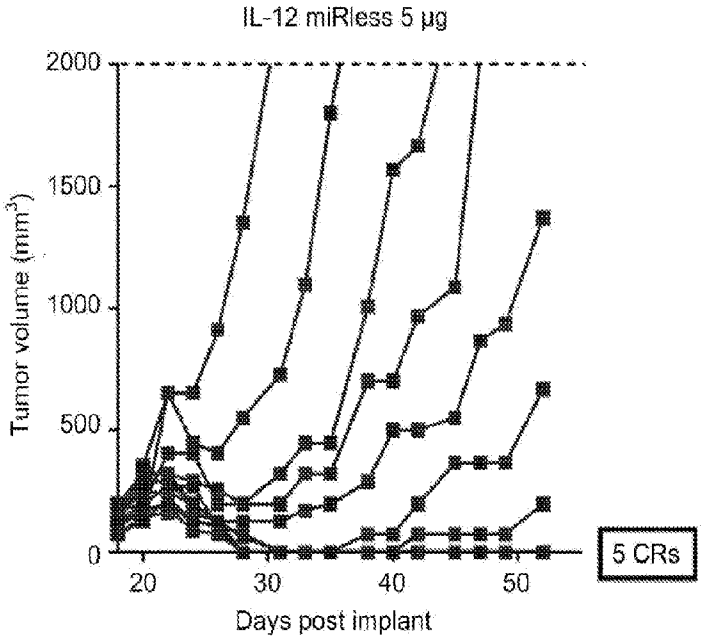

IL-12 miRless 5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
5 CRs

FIG. 7C

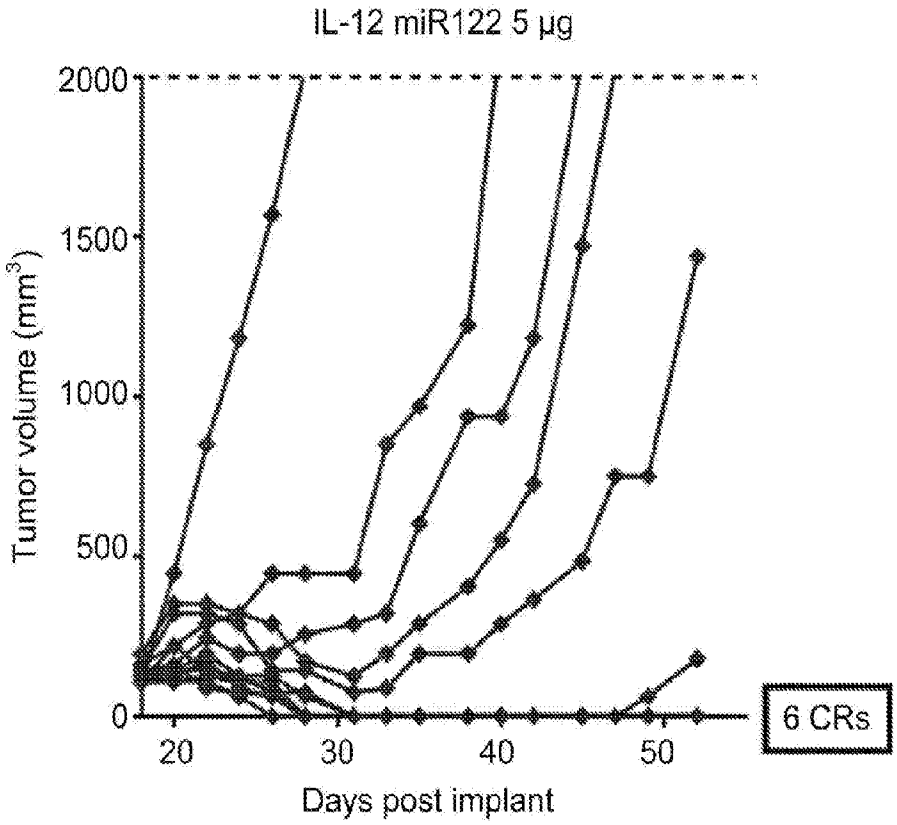

IL-12 miR122 5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
6 CRs

FIG. 8A

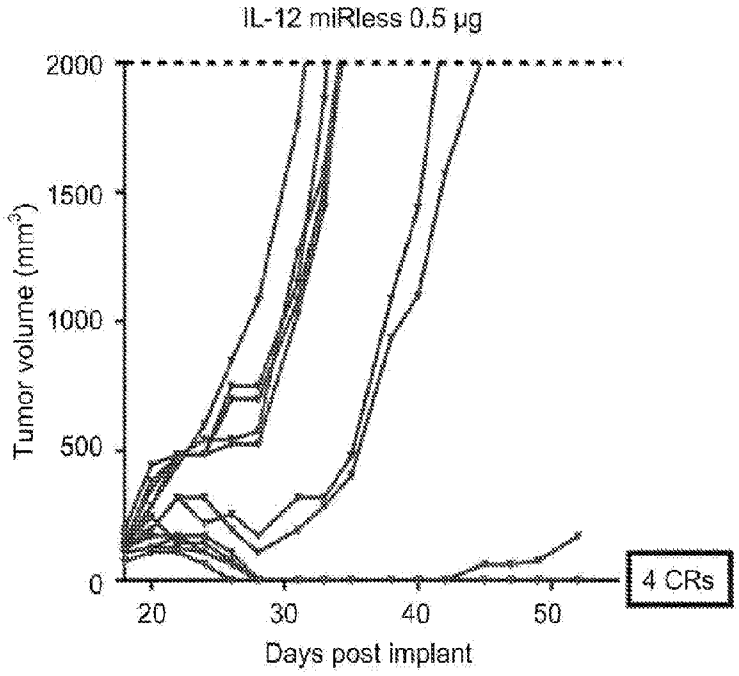

IL-12 miRless 0.5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
4 CRs

FIG. 8B

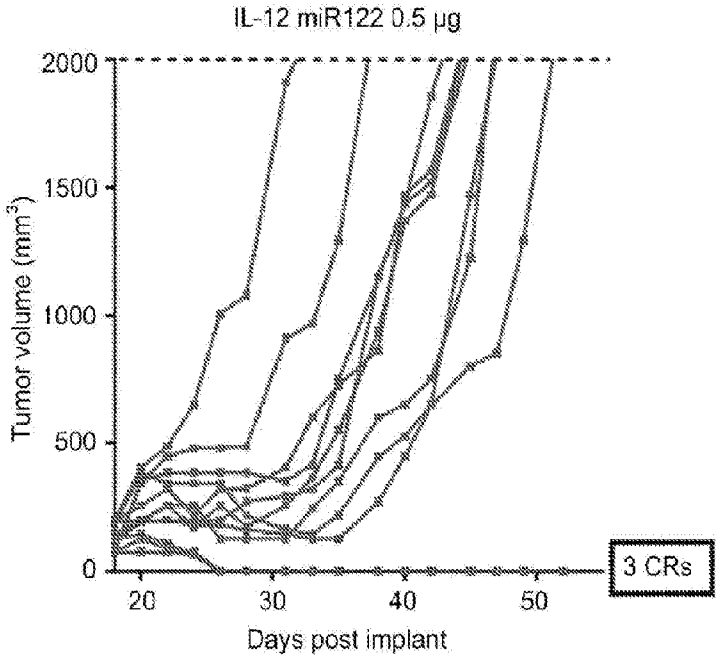

IL-12 miR122 0.5 µg
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant
3 CRs

FIG. 8C

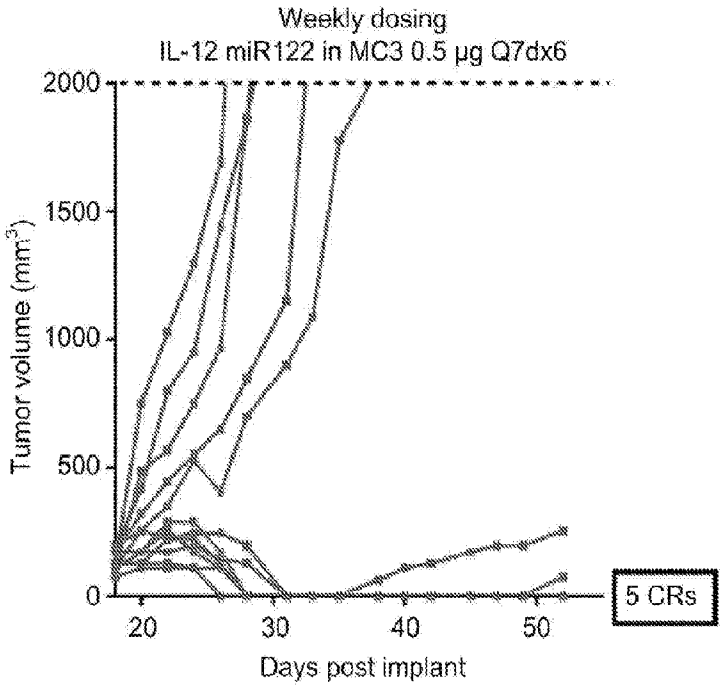

Weekly dosing
IL-12 miR122 in MC3 0.5 µg Q7dx6
Tumor volume (mm3)
2000
1500
1000
500
0
20
30
40
50
Days post implant
5 CRs

FIG. 8D

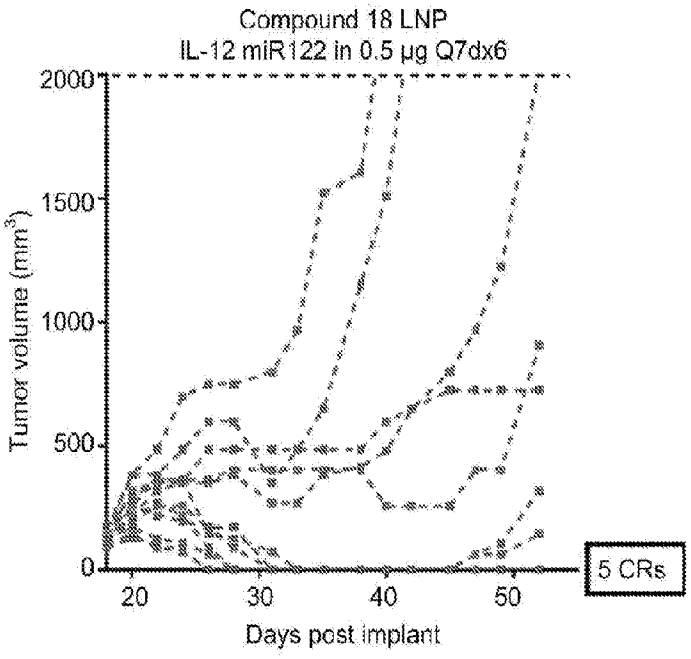

Compound 18 LNP
IL-12 miR122 in 0.5 µg Q7dx6
Tumor volume (mm3)
2000
1500
1000
500
0
20
30
40
50
Days post implant
5 CRs

FIG. 8E

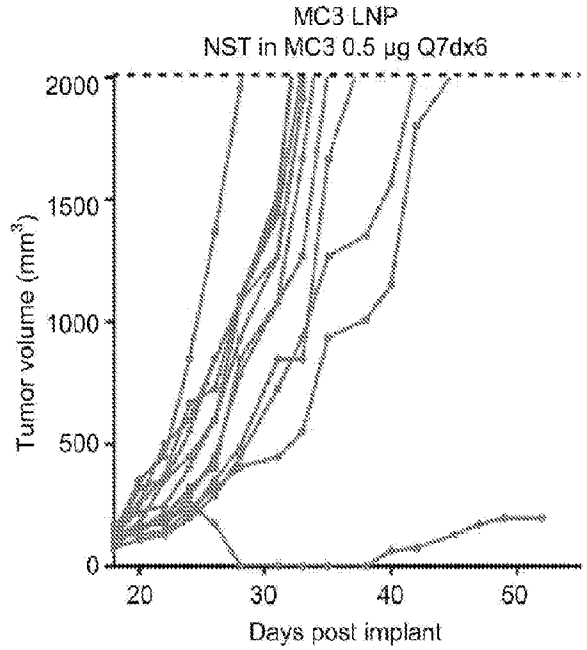

MC3 LNP
NST in MC3 0.5 µg Q7dx6
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant

FIG. 8F

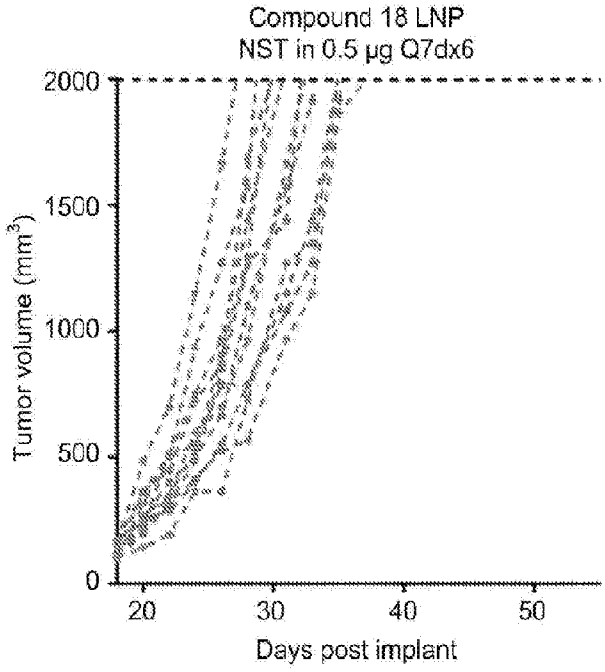

Compound 18 LNP
NST in 0.5 µg Q7dx6
2000
1500
1000
500
0
Tumor volume (mm3)
20
30
40
50
Days post implant

FIG. 9A

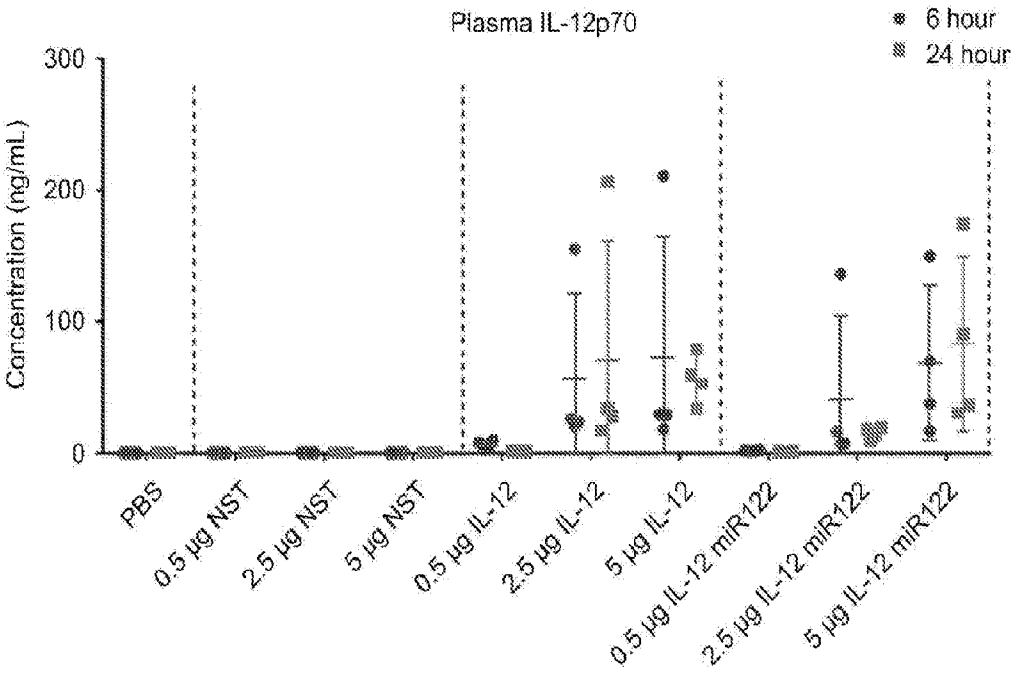

Plasma IL-12p70
6 hour
24 hour
Concentration (ng/mL)
300
200
100
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122

FIG. 9B

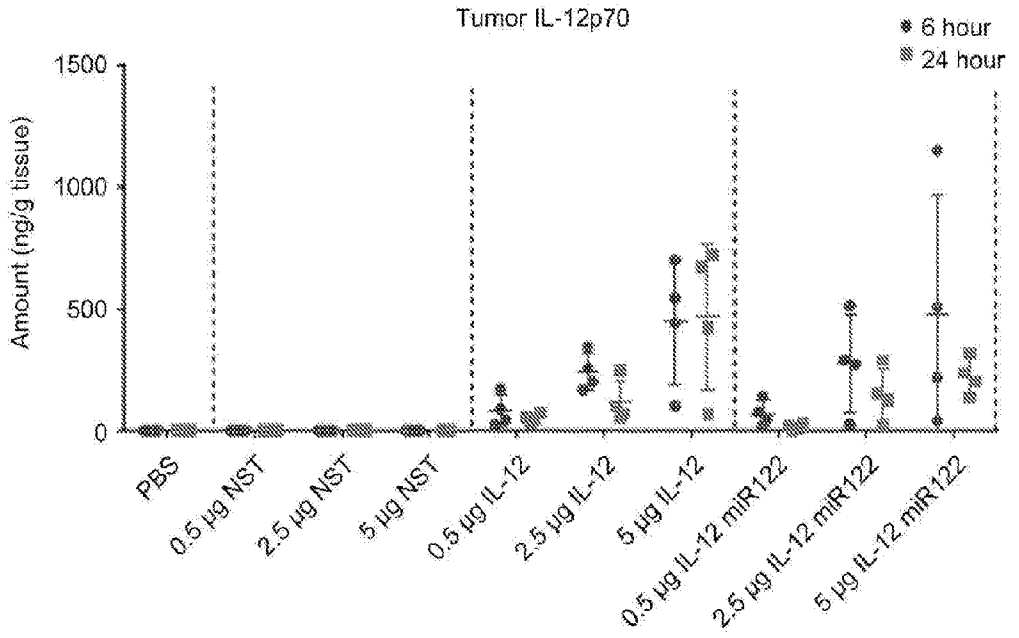

Tumor IL-12p70
6 hour
24 hour
Amount (ng/g tissue)
1500
1000
500
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122

FIG. 9C

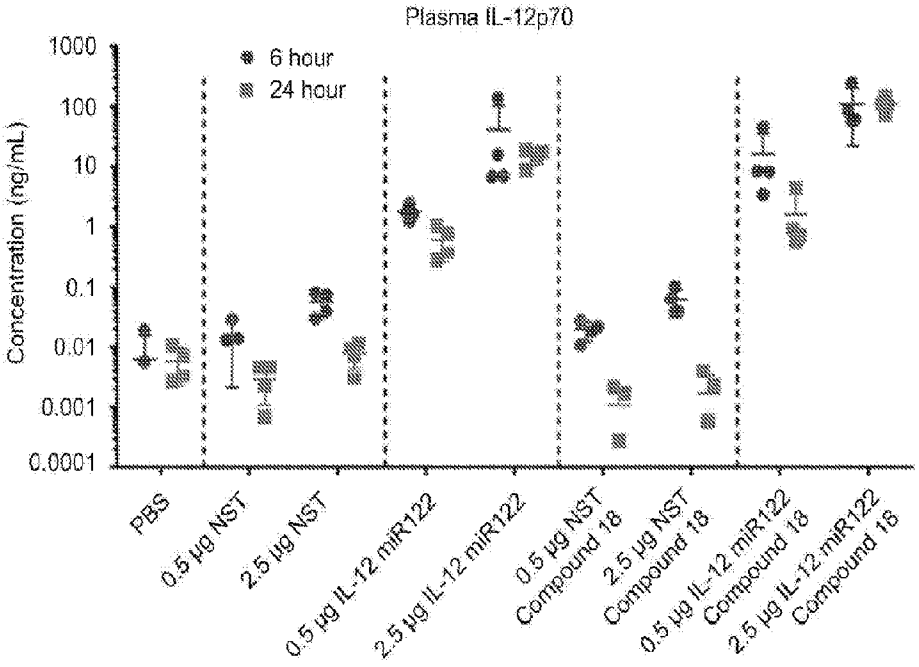

Plasma IL-12p70
6 hour
24 hour
Concentration (ng/mL)
1000
100
10
1
0.1
0.01
0.001
0.0001
PBS
0.5 µg NST
2.5 µg NST
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
0.5 µg NST Compound 18
2.5 µg NST Compound 18
0.5 µg IL-12 miR122 Compound 18
2.5 µg IL-12 miR122 Compound 18

FIG. 9D

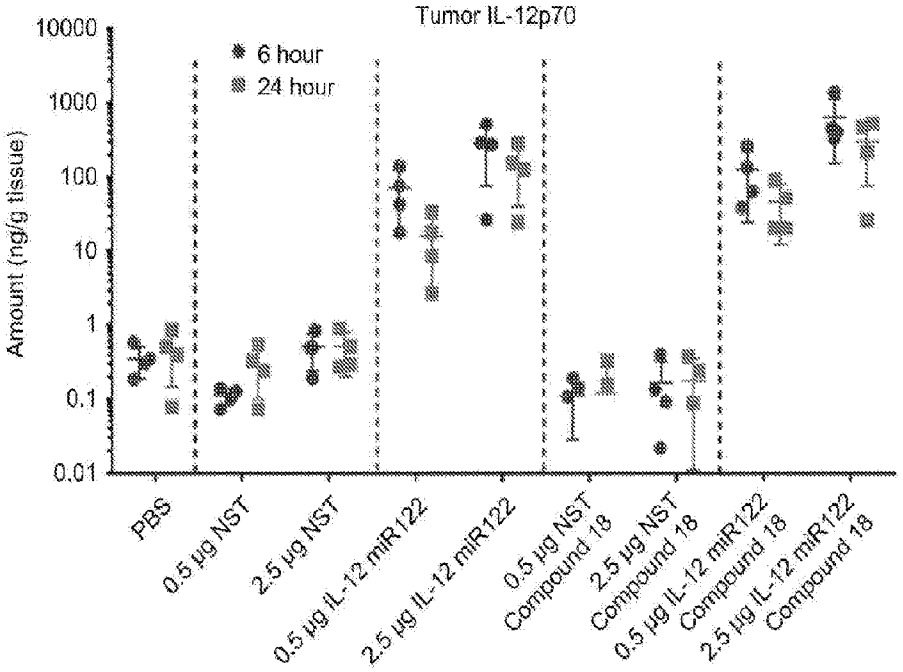

Tumor IL-12p70
6 hour
24 hour
Amount (ng/g tissue)
10000
1000
100
10
1
0.1
0.01
PBS
0.5 µg NST
2.5 µg NST
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
0.5 µg NST Compound 18
2.5 µg NST Compound 18
0.5 µg IL-12 miR122 Compound 18
2.5 µg IL-12 miR122 Compound 18

FIG. 9E

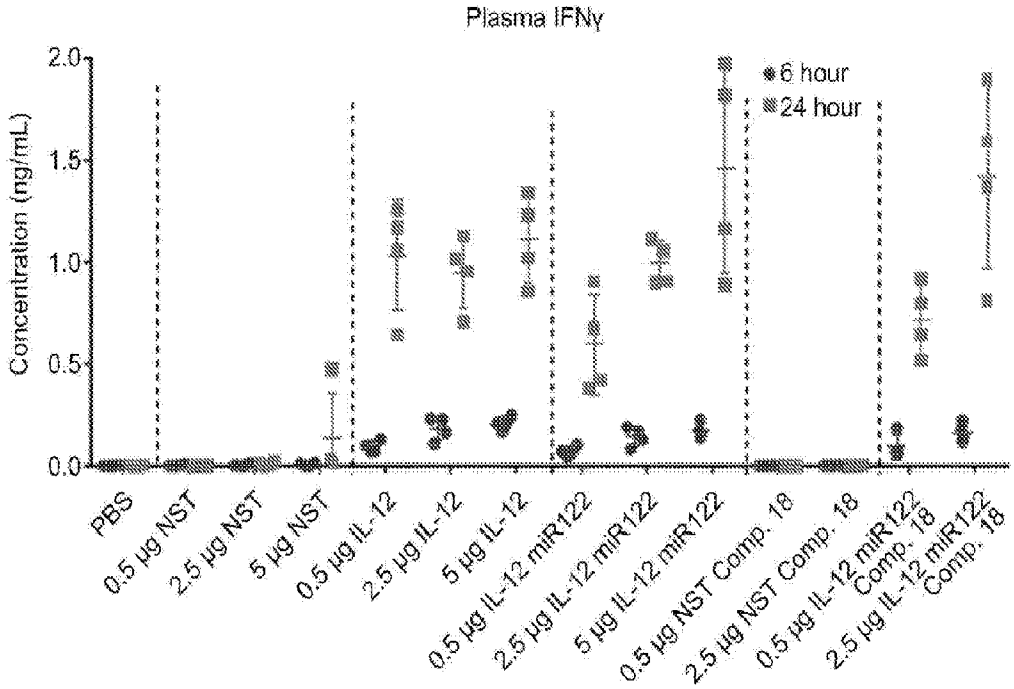

Plasma IFNγ
Concentration (ng/mL)
2.0
1.5
1.0
0.5
0.0
6 hour
24 hour
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9F

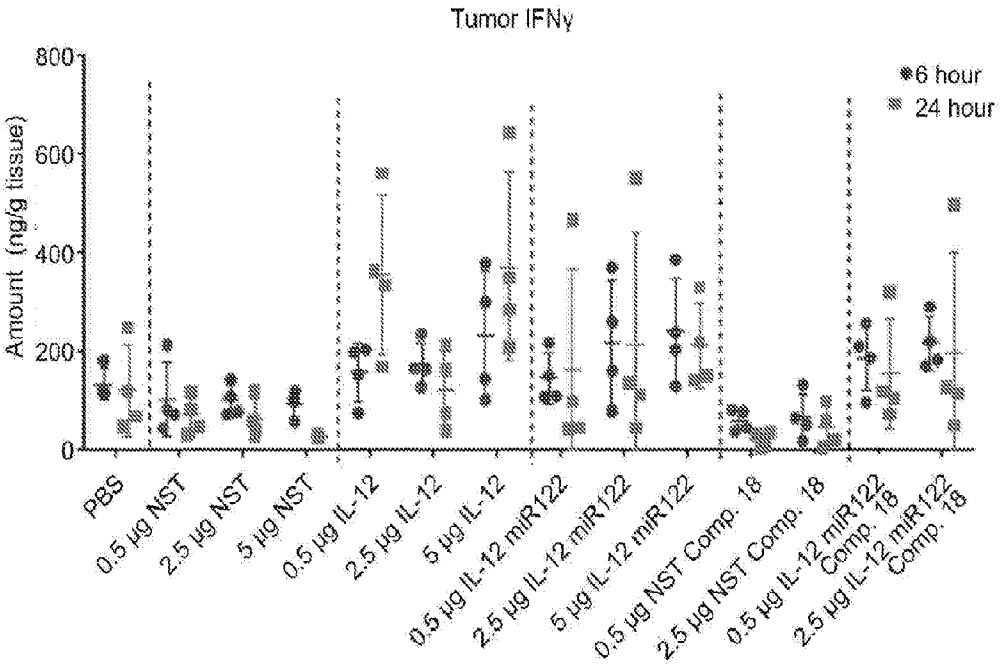

Tumor IFNγ
Amount (ng/g tissue)
800
600
400
200
0
6 hour
24 hour
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9G

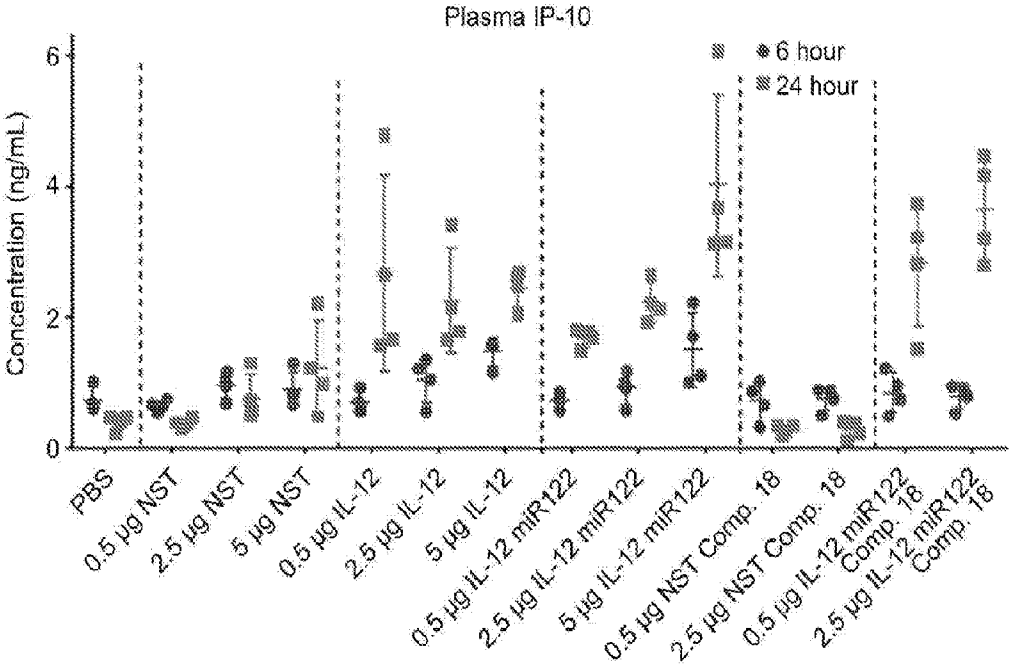

Plasma IP-10
6 hour
24 hour
Concentration (ng/mL)
0
2
4
6
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9H

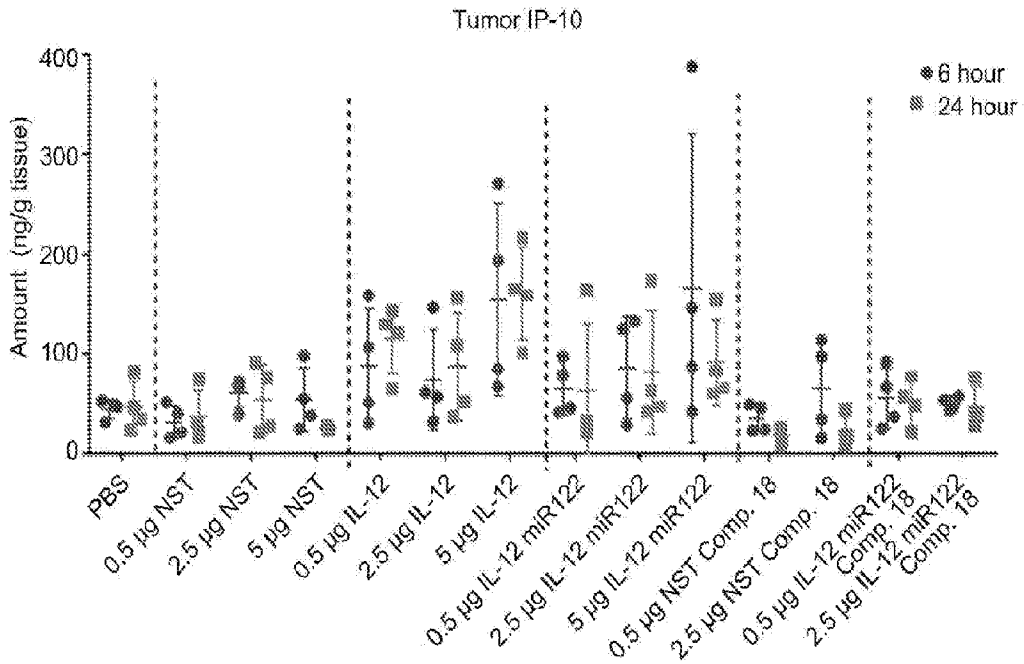

Tumor IP-10
6 hour
24 hour
Amount (ng/g tissue)
0
100
200
300
400
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9I

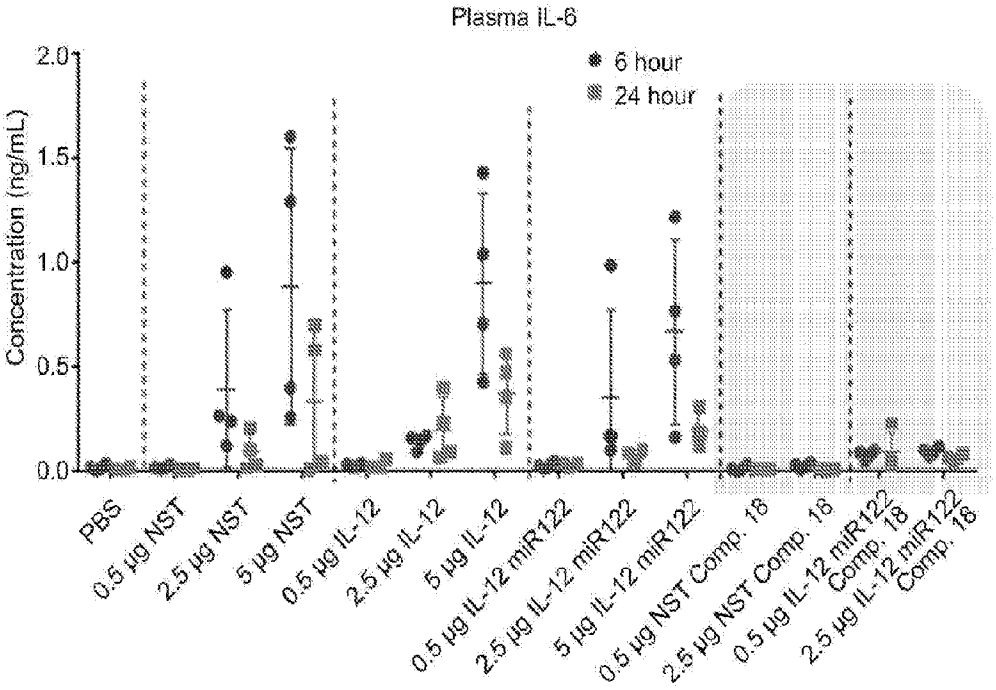

Plasma IL-6
6 hour
24 hour
Concentration (ng/mL)
2.0
1.5
1.0
0.5
0.0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 16
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9J

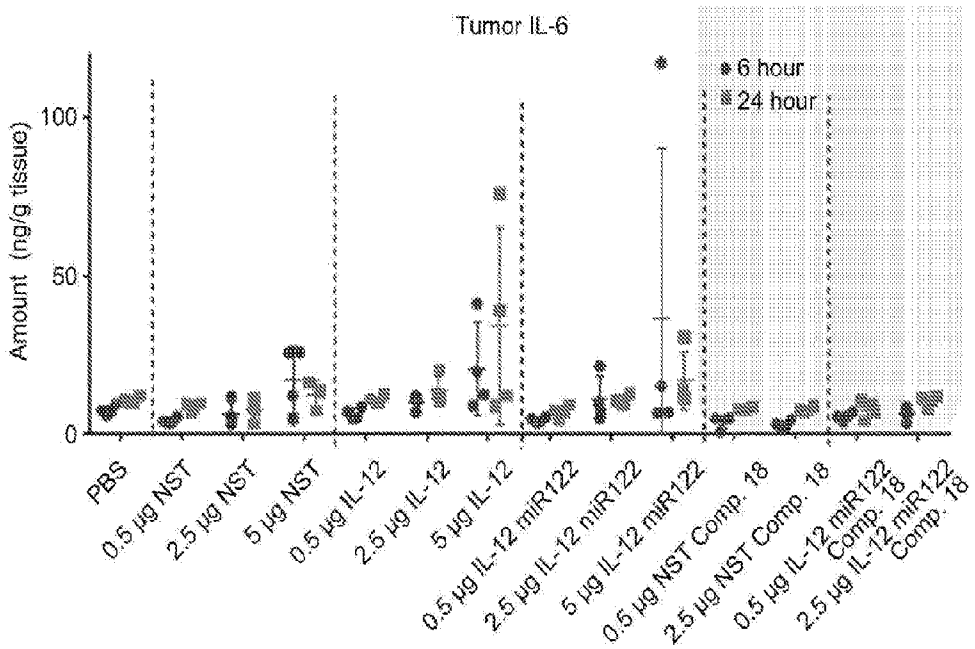

Tumor IL-6
6 hour
24 hour
Amount (ng/g tissue)
100
50
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9K

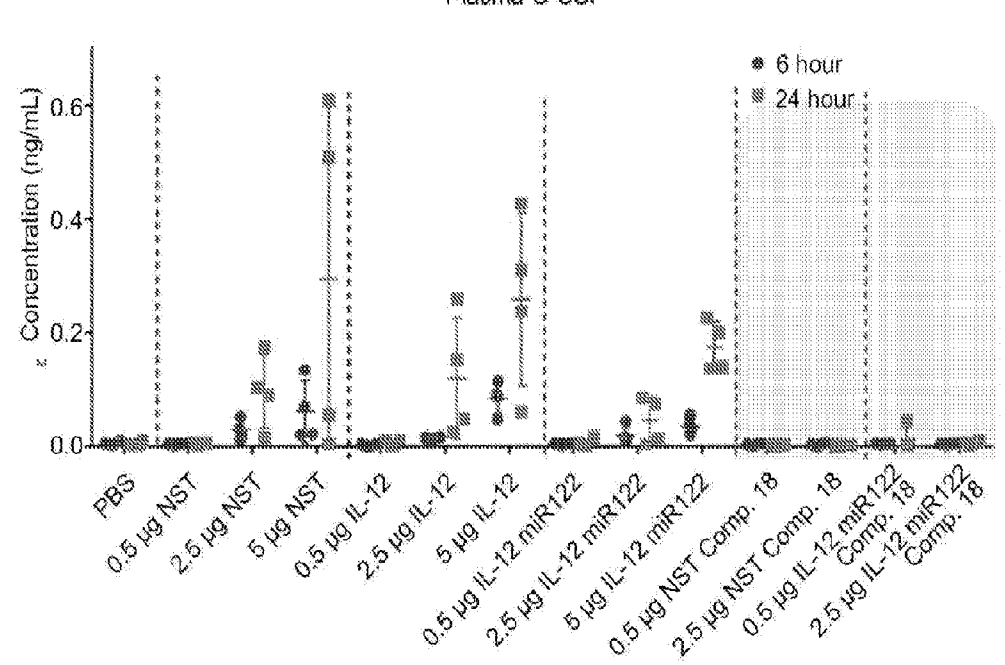

Plasma G-CSF
Concentration (ng/mL)
0.0
0.2
0.4
0.6
6 hour
24 hour
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9L

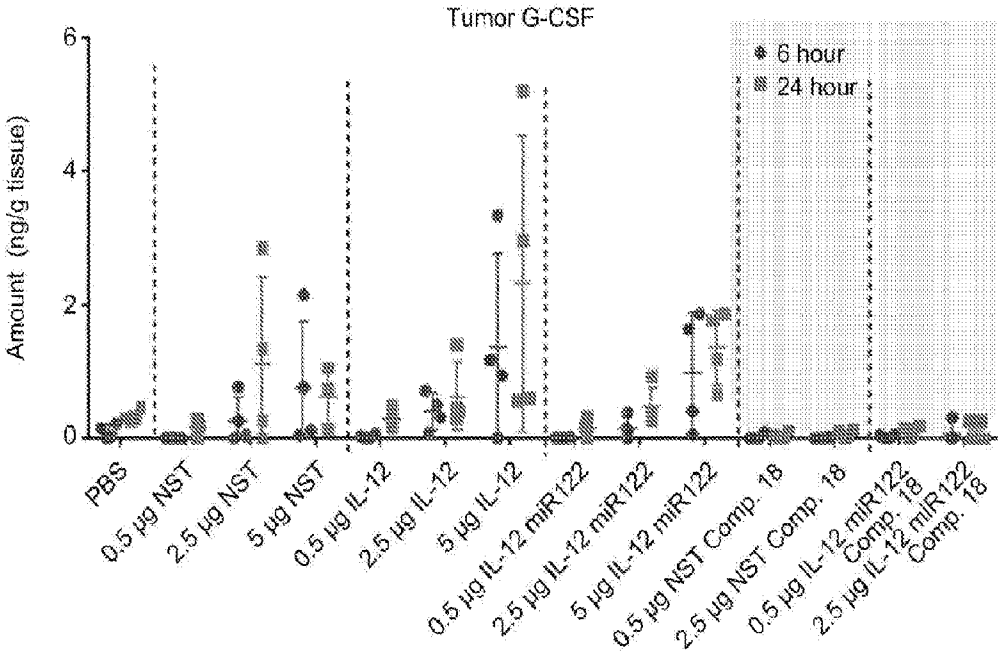

Tumor G-CSF
Amount (ng/g tissue)
0
2
4
6
6 hour
24 hour
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9M

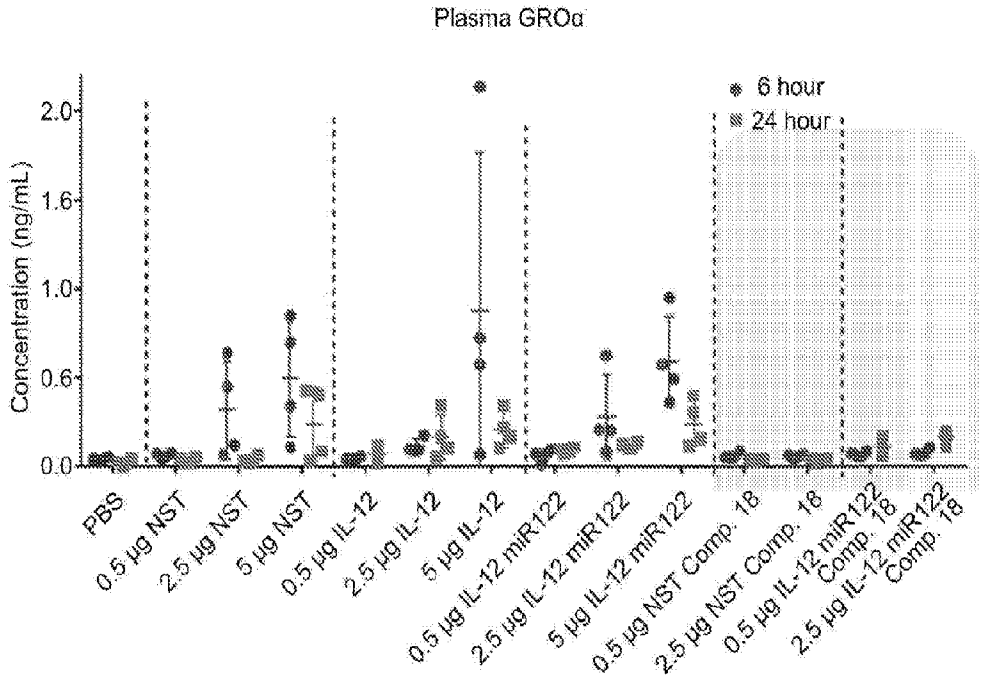

Plasma GROα
6 hour
24 hour
Concentration (ng/mL)
2.0
1.6
1.0
0.6
0.0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 9N

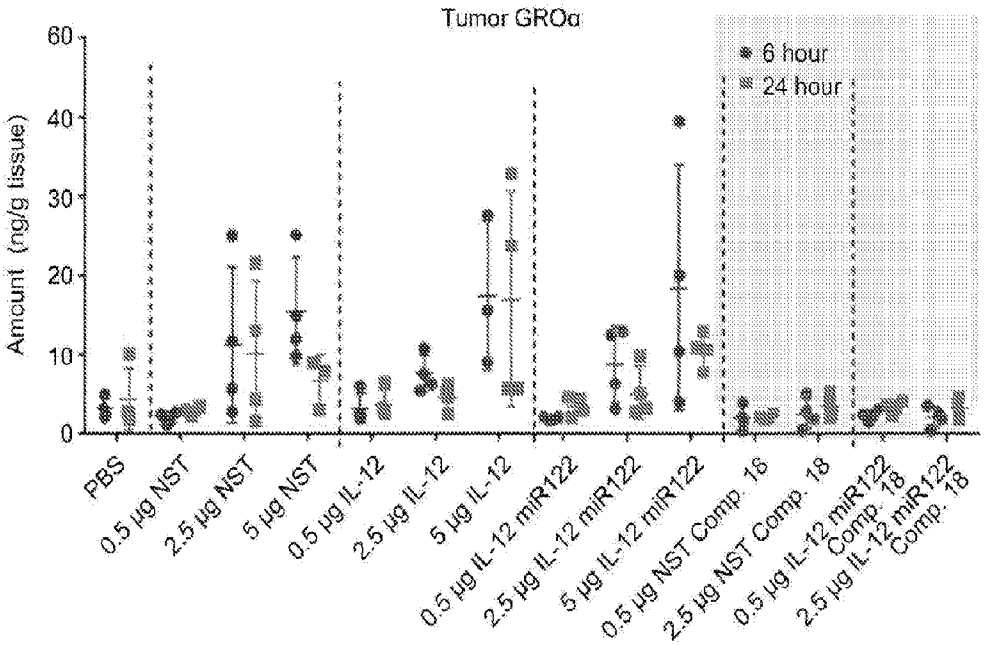

Tumor GROα
6 hour
24 hour
Amount (ng/g tissue)
60
40
30
20
10
0
PBS
0.5 µg NST
2.5 µg NST
5 µg NST
0.5 µg IL-12
2.5 µg IL-12
5 µg IL-12
0.5 µg IL-12 miR122
2.5 µg IL-12 miR122
5 µg IL-12 miR122
0.5 µg NST Comp. 18
2.5 µg NST Comp. 18
0.5 µg IL-12 miR122 Comp. 18
2.5 µg IL-12 miR122 Comp. 18

FIG. 10B

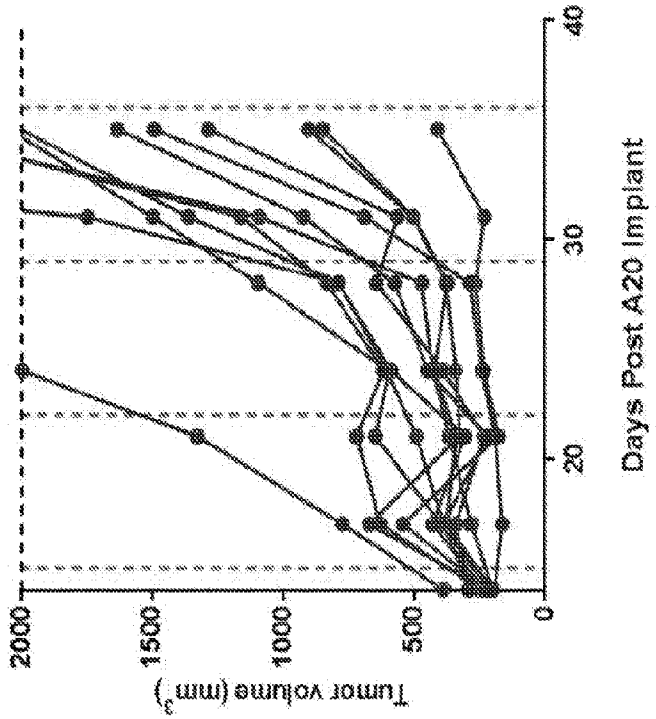


FIG.10A

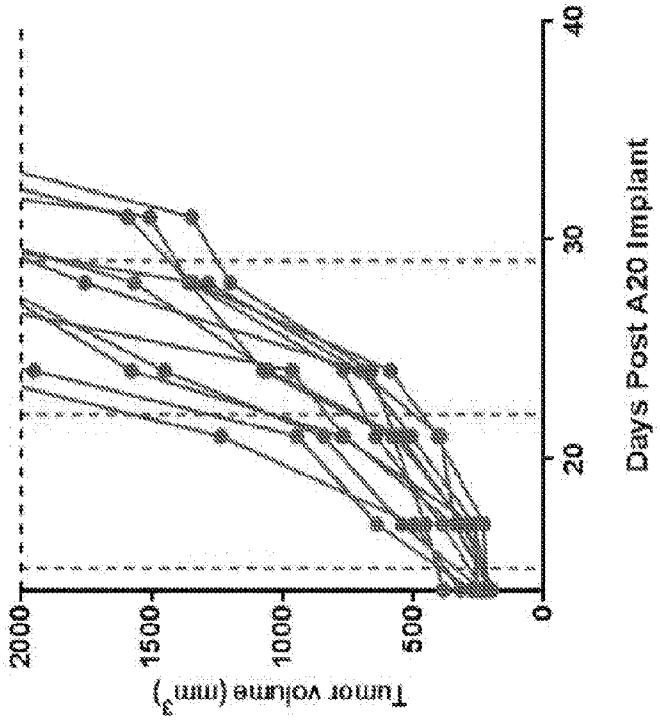


FIG.10C

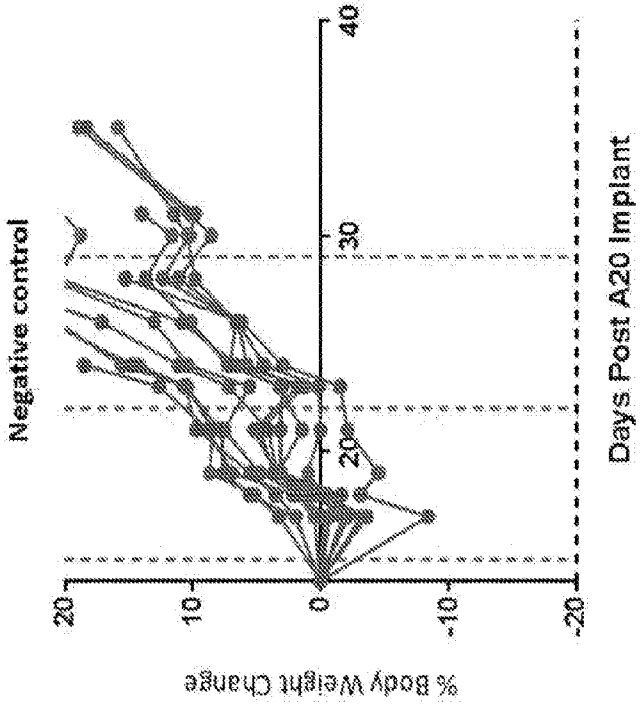


FIG.10D

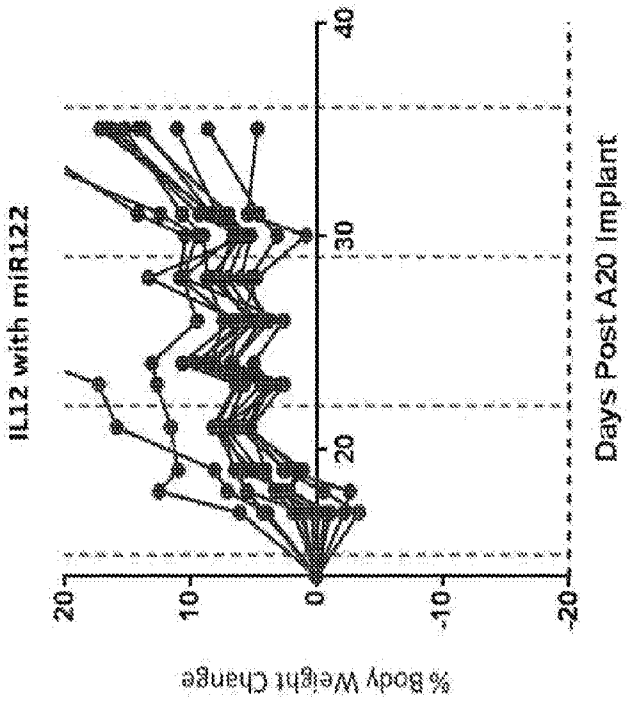


FIG. 11A

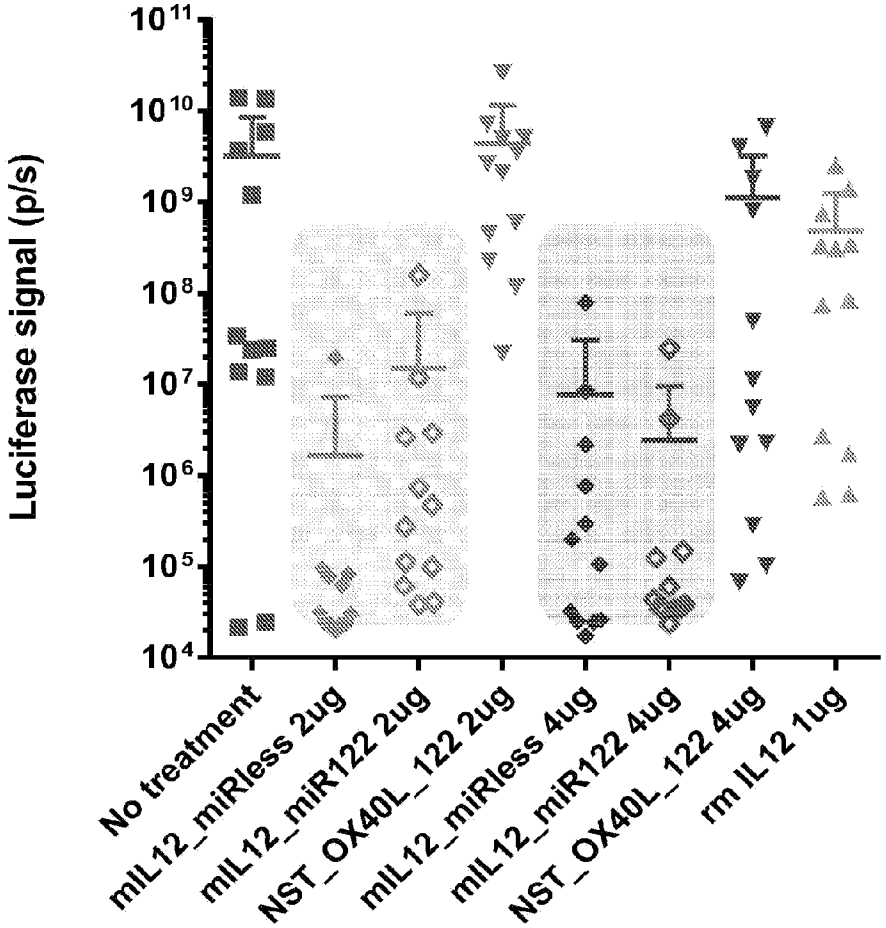
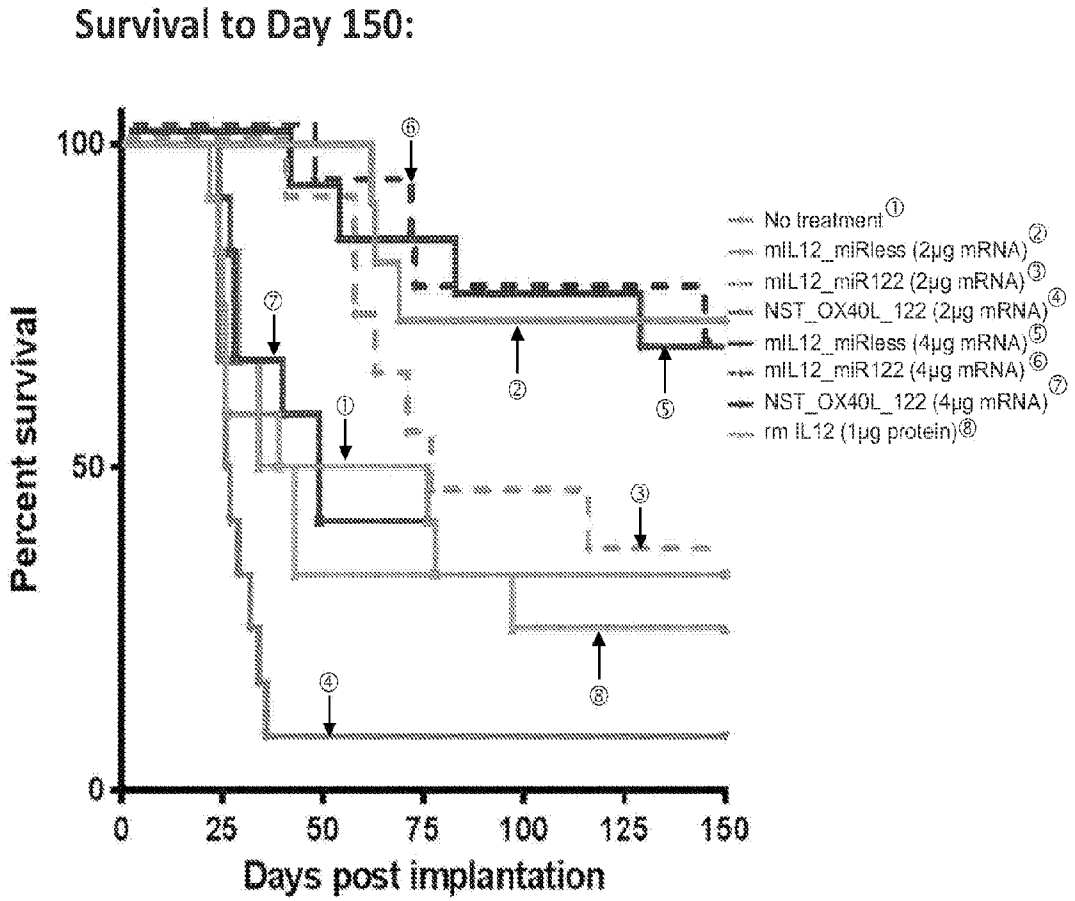

Luciferase signal (p/s)
10^11
10^10
10^9
10^8
10^7
10^6
10^5
10^4
No treatment
mIL12_miRless 2ug
mIL12_miR122 2ug
NST_OX40L_122 2ug
mIL12_miRless 4ug
mIL12_miR122 4ug
NST_OX40L_122 4ug
rm IL12 1ug

FIG. 11B


Survival to Day 150:
Percent survival
100
50
0
0
25
50
75
100
125
150
Days post implantation
No treatment ①
mIL12_miRless (2µg mRNA) ②
mIL12_miR122 (2µg mRNA) ③
NST_OX40L_122 (2µg mRNA) ④
mIL12_miRless (4µg mRNA) ⑤
mIL12_miR122 (4µg mRNA) ⑥
NST_OX40L_122 (4µg mRNA) ⑦
rm IL12 (1µg protein) ⑧

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12B WT	306	10.78%	99		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Minimum (%)
IL12B WT	918	195	21.24%	100.00%	196.97%
	MAX	275	29.96%	141.03%	277.78%
	MIN	109	11.87%	55.90%	110.10%
	MEAN	173.25	18.87%	88.85%	175.00%
	MEDIAN	176	19.17%	90.26%	177.78%
	STD DEV	44.19	4.81%	22.66%	44.63%

FIG. 12A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12B WT	306	40.74%	374		
Nucleic Acid	Length	G Content(abs)	G Content(%)	G Content v WT(%)	G Content v Theoretical Maximum (%)
IL12B WT	918	243	26.47%	100.00%	64.97%
	MAX	374	40.74%	153.91%	100.00%
	MIN	219	23.86%	90.12%	58.56%
	MEAN	260.55	28.38%	107.22%	69.67%
	MEDIAN	249.5	27.18%	102.67%	66.71%
	STD DEV	35.31	3.85%	14.53%	9.44%

FIG. 12B

Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12B WT	306	41.61%	382		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12B WT	918	223	24.29%	100.00%	58.38%
	MAX	279	30.39%	125.11%	73.04%
	MIN	213	23.20%	95.52%	55.76%
	MEAN	238.05	25.93%	106.75%	62.32%
	MEDIAN	238.5	25.98%	106.96%	62.43%
	STD DEV	18.41	2.01%	8.26%	4.82%

FIG. 12C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12B WT	306	65.25%	599		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Minimum (%)
IL12B WT	918	466	50.76%	100.00%	77.80%
	MAX	587	63.94%	125.97%	98.00%
	MIN	449	48.91%	96.35%	74.96%
	MEAN	498.6	54.31%	107.00%	83.24%
	MEDIAN	489.5	53.32%	105.04%	81.72%
	STD DEV	43.23	4.71%	9.28%	7.22%

FIG. 12D

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12B WT	306	10.78%	99		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Minimum (%)
IL12B WT	918	195	21.24%	100.00%	196.97%
	MAX	149	16.23%	76.41%	150.51%
	MIN	131	14.27%	67.18%	132.32%
	MEAN	141.1	15.37%	72.36%	142.53%
	MEDIAN	141	15.36%	72.31%	142.42%
	STD DEV	5.37	0.58%	2.75%	5.42%

FIG. 13A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12B WT	306	40.74%	374		
Nucleic Acid	Length	G Content (abs)	G Content (%)	G Content v WT (%)	G Content v Theoretical Minimum (%)
IL12B WT	918	243	26.47%	100.00%	64.97%
	MAX	294	32.03%	120.99%	78.61%
	MIN	279	30.39%	114.81%	74.60%
	MEAN	287.35	31.30%	118.25%	76.83%
	MEDIAN	288	31.37%	118.52%	77.01%
	STD DEV	4.63	0.50%	1.90%	1.24%

FIG. 13B

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Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12B WT	306	41.61%	382		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12B WT	918	223	24.29%	100.00%	58.38%
	MAX	279	30.39%	125.11%	73.04%
	MIN	262	28.54%	117.49%	68.59%
	MEAN	270.15	29.43%	121.14%	70.72%
	MEDIAN	270	29.41%	121.08%	70.68%
	STD DEV	4.76	0.52%	2.13%	1.25%

FIG. 13C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12B WT	306	65.25%	599		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Maximum (%)
IL12B WT	918	466	50.76%	100.00%	77.80%
	MAX	565	61.55%	121.24%	94.32%
	MIN	547	59.59%	117.38%	91.32%
	MEAN	557.5	60.73%	119.64%	93.07%
	MEDIAN	557	60.68%	119.53%	92.99%
	STD DEV	4.67	0.51%	1.00%	0.78%

FIG. 13D

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12A WT	197	12.86%	76		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Minimum (%)
IL12A WT	591	151	25.55%	100.00%	198.68%
	MAX	189	31.98%	125.17%	248.68%
	MIN	83	14.04%	54.97%	109.21%
	MEAN	126.3	21.37%	83.64%	166.18%
	MEDIAN	125	21.15%	82.78%	164.47%
	STD DEV	28.03	4.74%	18.56%	36.88%

FIG. 14A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12A WT	197	34.69%	205		
Nucleic Acid	Length	G Content (abs)	G Content (%)	G Content v WT (%)	G Content v Theoretical Maximum (%)
IL12A WT	591	123	20.81%	100.00%	60.00%
	MAX	205	34.69%	166.67%	100.00%
	MIN	108	18.27%	87.80%	52.68%
	MEAN	134.7	22.79%	109.51%	65.71%
	MEDIAN	131	22.17%	106.50%	63.90%
	STD DEV	22.88	3.87%	18.60%	11.16%

FIG. 14B

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Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12A WT	197	43.82%	259		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12A WT	591	138	23.35%	100.00%	53.28%
	MAX	191	32.32%	138.41%	73.75%
	MIN	140	23.69%	101.45%	54.05%
	MEAN	164.8	27.88%	119.42%	63.63%
	MEDIAN	164	27.75%	118.84%	63.32%
	STD DEV	12.22	2.07%	8.85%	4.72%

FIG. 14C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12A WT	197	62.27%	368		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Maximum (%)
IL12A WT	591	261	44.16%	100.00%	70.92%
	MAX	359	60.74%	137.55%	97.55%
	MIN	265	44.84%	101.53%	72.01%
	MEAN	299.5	50.68%	114.75%	81.39%
	MEDIAN	293	49.58%	112.26%	79.62%
	STD DEV	29.23	4.95%	11.20%	7.94%

FIG. 14D

Protein	Length	Theoretical Minimum U (%)	Theoretical Minimum U (abs)		
IL12A WT	197	12.86%	76		
Nucleic Acid	Length	U Content (abs)	U Content (%)	U Content v WT (%)	U Content v Theoretical Maximum (%)
IL12A WT	591	151	25.55%	100.00%	198.68%
	MAX	109	18.44%	72.19%	143.42%
	MIN	95	16.07%	62.91%	125.00%
	MEAN	101.9	17.24%	67.48%	134.08%
	MEDIAN	102	17.26%	67.55%	134.21%
	STD DEV	4.27	0.72%	2.83%	5.61%

FIG. 15A

Protein	Length	Theoretical Maximum G (%)	Theoretical Maximum G (abs)		
IL12A WT	197	34.69%	205		
Nucleic Acid	Length	G Content (abs)	G Content (%)	G Content v WT (%)	G Content v Theoretical Maximum (%)
IL12A WT	591	123	20.81%	100.00%	60.00%
	MAX	160	27.07%	130.08%	78.05%
	MIN	147	24.87%	119.51%	71.71%
	MEAN	153.45	25.96%	124.76%	74.85%
	MEDIAN	153.5	25.97%	124.80%	74.88%
	STD DEV	3.38	0.57%	2.75%	1.65%

FIG. 15B

DK/EP 3458083 T5

Protein	Length	Theoretical Maximum C (%)	Theoretical Maximum C (abs)		
IL12A WT	197	43.82%	259		
Nucleic Acid	Length	C Content (abs)	C Content (%)	C Content v WT (%)	C Content v Theoretical Maximum (%)
IL12A WT	591	138	23.35%	100.00%	53.28%
	MAX	195	32.99%	141.30%	75.29%
	MIN	179	30.29%	129.71%	69.11%
	MEAN	187.8	31.78%	136.09%	72.51%
	MEDIAN	187.5	31.73%	135.87%	72.39%
	STD DEV	4.05	0.68%	2.93%	1.56%

FIG. 15C

Protein	Length	Theoretical Maximum GC (%)	Theoretical Maximum GC (abs)		
IL12A WT	197	62.27%	368		
Nucleic Acid	Length	GC Content (abs)	GC Content (%)	GC Content v WT (%)	GC Content v Theoretical Maximum (%)
IL12A WT	591	261	44.16%	100.00%	70.92%
	MAX	350	59.22%	134.10%	95.11%
	MIN	337	57.02%	129.12%	91.58%
	MEAN	341.25	57.74%	130.75%	92.73%
	MEDIAN	340	57.53%	130.27%	92.39%
	STD DEV	4.08	0.69%	1.56%	1.11%

FIG. 15D

IL12B (w/o SP) G5 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12B_WT	**50.76**	**48.04**	**43.14**	**61.11**
MAX	63.94	50.65	43.14	100
MIN	48.91	48.04	43.14	54.25
MEAN	54.31	49.46	43.14	70.34
MEDIAN	53.325	49.35	43.14	66.34
STD DEV	4.71	0.80	0.00	14.42

FIG. 16A

IL12B (w/o SP) G6 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12B_WT	**50.76**	**48.04**	**43.14**	**61.11**
MAX	61.55	51.63	43.14	91.83
MIN	59.59	49.35	43.14	85.29
MEAN	60.73	50.33	43.14	88.73
MEDIAN	60.68	50.33	43.14	88.725
STD DEV	0.51	0.61	0.00	1.60

FIG. 16B

IL12A (w/o SP) G5 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12A_WT	**44.16**	**45.18**	**35.53**	**51.78**
MAX	60.74	48.73	35.53	100
MIN	44.84	43.65	35.53	52.28
MEAN	50.68	46.35	35.53	70.15
MEDIAN	49.58	46.7	35.53	67.765
STD DEV	4.94	1.44	0.00	14.35

FIG. 16C

IL12B (w/o SP) G6 SEQUENCES				
Sequence	GC	GC 1st	GC 2nd	GC 3rd
IL12A_WT	**44.16**	**45.18**	**35.53**	**51.78**
MAX	59.22	51.27	35.53	93.91
MIN	57.02	46.7	35.53	85.79
MEAN	57.74	48.45	35.53	89.24
MEDIAN	57.53	48.22	35.53	88.83
STD DEV	0.69	1.02	0.00	1.80

FIG. 16D

FIG. 17A

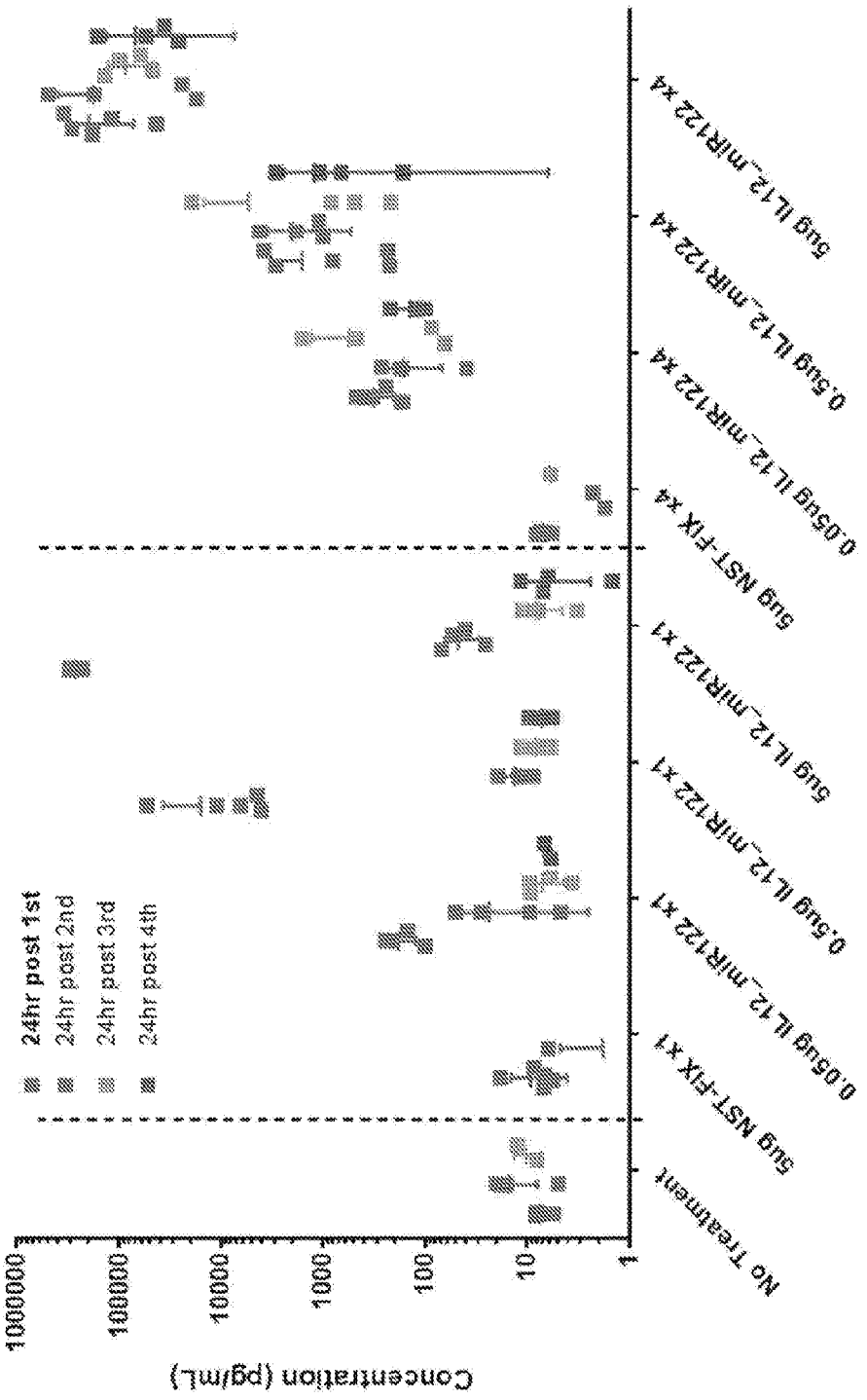

24hr post 1st
24hr post 2nd
24hr post 3rd
24hr post 4th
Concentration (pg/mL)
1000000
100000
10000
1000
100
10
1
No Treatment
5ug NST-FIX x1
0.05ug IL-12_mIR122 x1
0.5ug IL-12_mIR122 x1
5ug IL-12_mIR122 x1
5ug NST-FIX x4
0.05ug IL-12_mIR122 x4
0.5ug IL-12_mIR122 x4
5ug IL-12_mIR122 x4

FIG. 17B

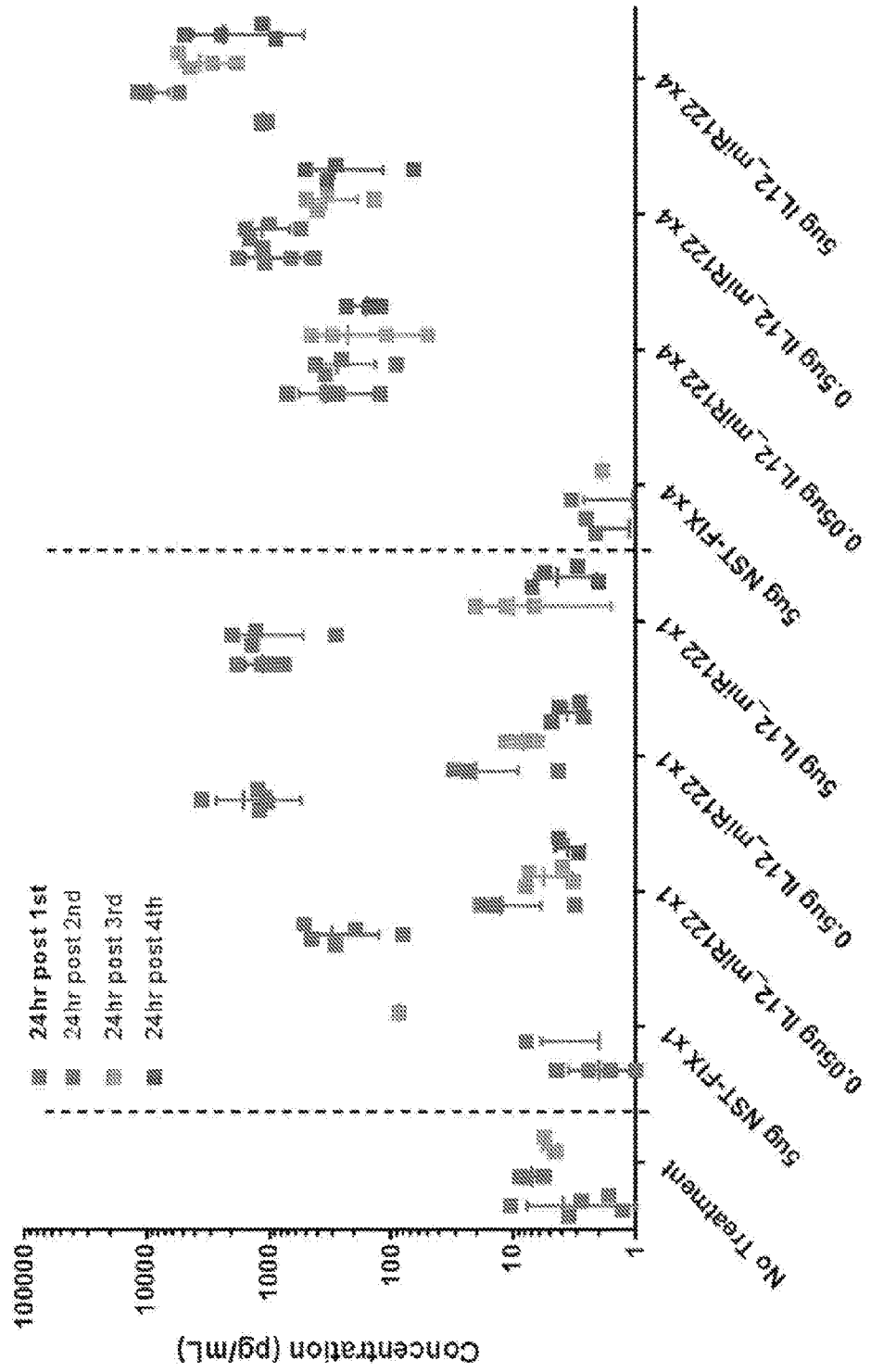

24hr post 1st
24hr post 2nd
24hr post 3rd
24hr post 4th
Concentration (pg/mL)
100000
10000
1000
100
10
1
No Treatment
5ug NST-FIX X1
0.05ug IL12_mIR122 X1
0.5ug IL12_mIR122 X1
5ug IL12_mIR122 X1
5ug NST-FIX X4
0.05ug IL12_mIR122 X4
0.5ug IL12_mIR122 X4
5ug IL12_mIR122 X4

FIG. 18A

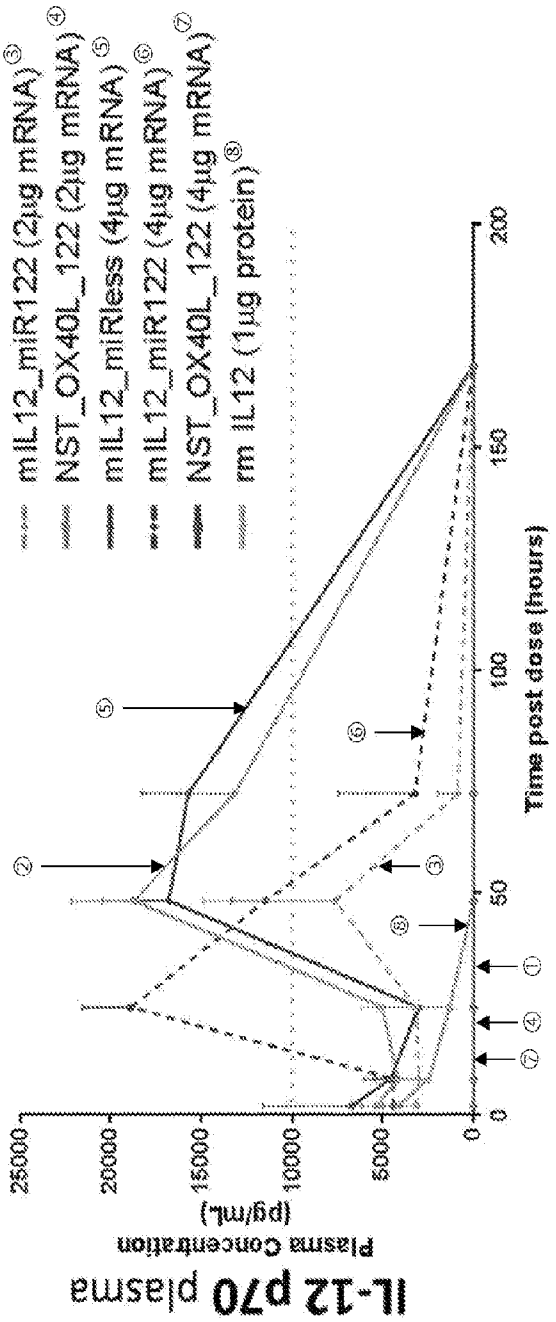

IL-12 p70 plasma
Plasma Concentration
(pg/mL)
25000
20000
15000
10000
5000
0
0
50
100
150
200
Time post dose (hours)
① No treatment
② mIL12_miRless (2μg mRNA)
③ mIL12_miR122 (2μg mRNA)
④ NST_OX40L_122 (2μg mRNA)
⑤ mIL12_miRless (4μg mRNA)
⑥ mIL12_miR122 (4μg mRNA)
⑦ NST_OX40L_122 (4μg mRNA)
⑧ rm IL12 (1μg protein)

FIG. 18B

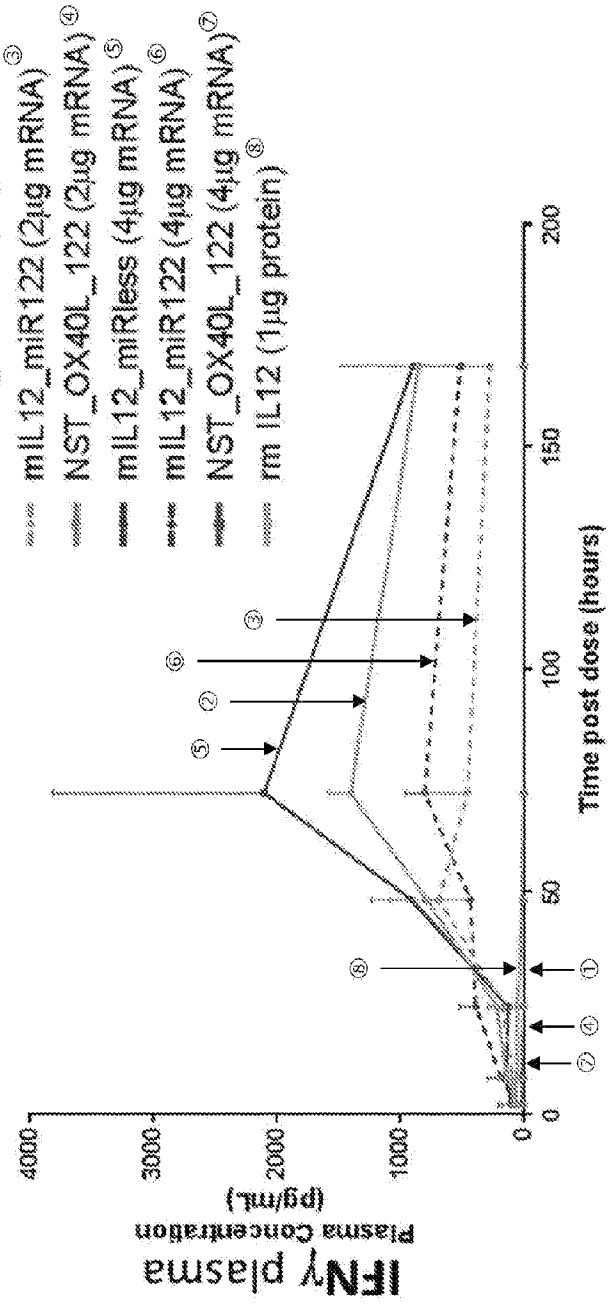

IFNγ plasma
Plasma Concentration
(pg/mL)
4000
3000
2000
1000
0
0
50
100
150
200
Time post dose (hours)
① No treatment
② mIL12_miRless (2μg mRNA)
③ mIL12_miR122 (2μg mRNA)
④ NST_OX40L_122 (2μg mRNA)
⑤ mIL12_miRless (4μg mRNA)
⑥ mIL12_miR122 (4μg mRNA)
⑦ NST_OX40L_122 (4μg mRNA)
⑧ rm IL12 (1μg protein)

FIG. 19A

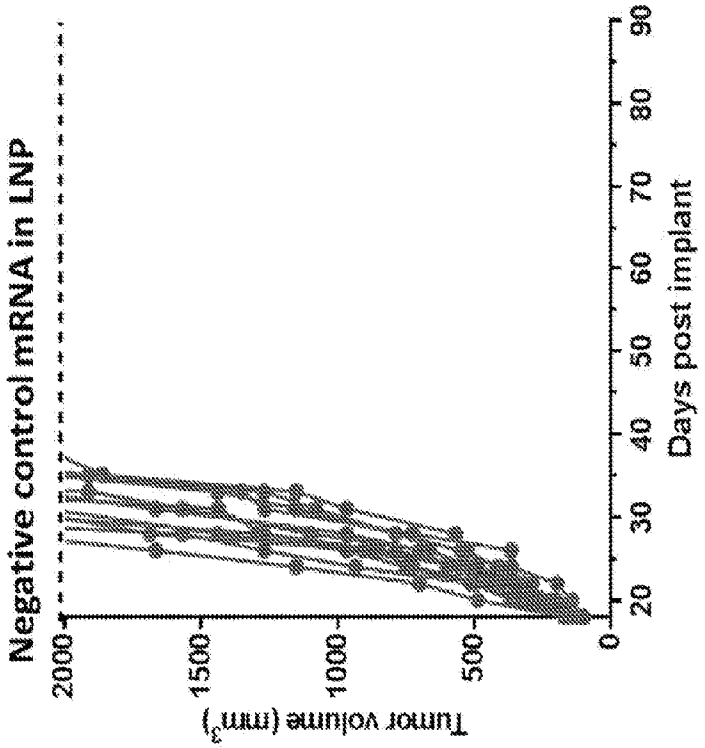


FIG. 19B

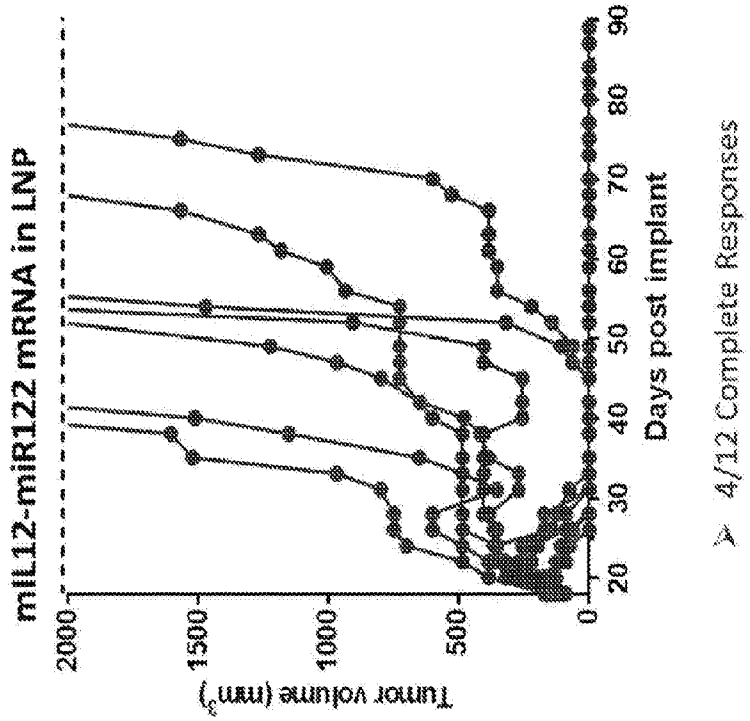
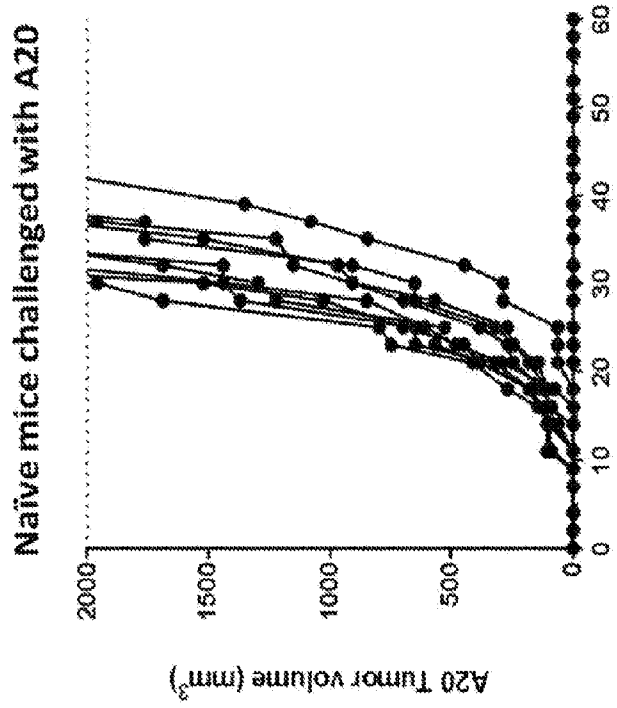
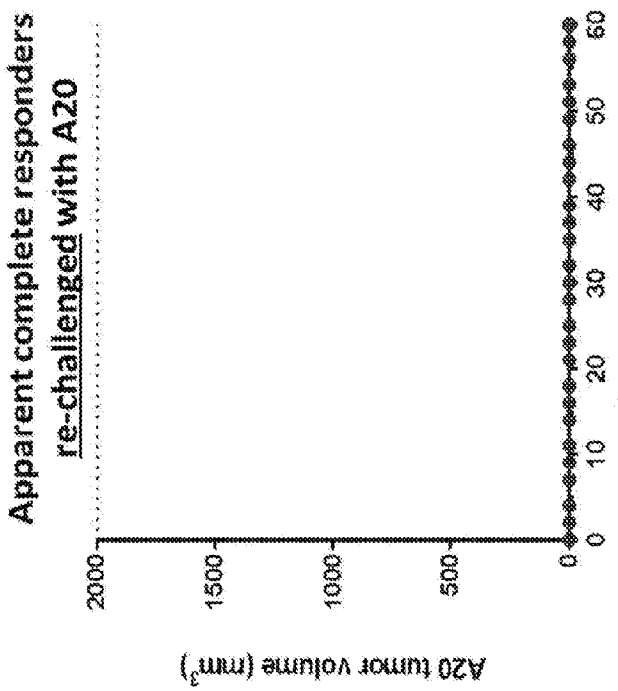


FIG. 19C



9/10 newly implanted tumors hit end point by Day 45

FIG. 19D



➢ 0/4 newly implanted tumors grew

FIG. 20A
0.05 µg IL12

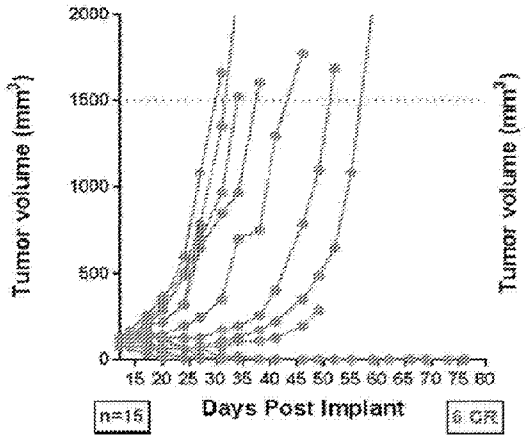


FIG. 20B
0.5 µg IL12

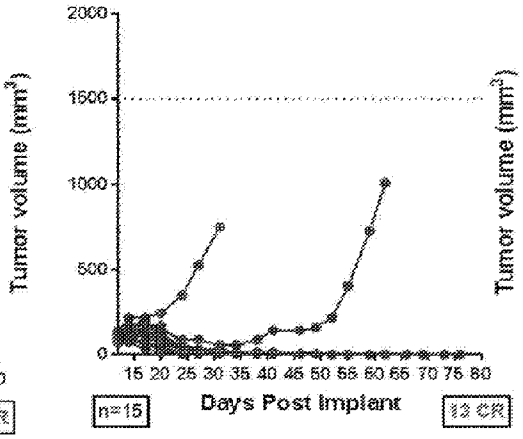
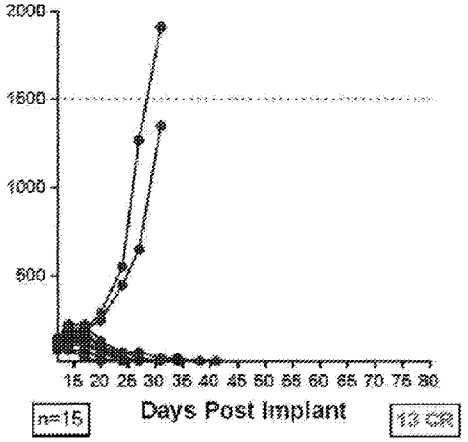
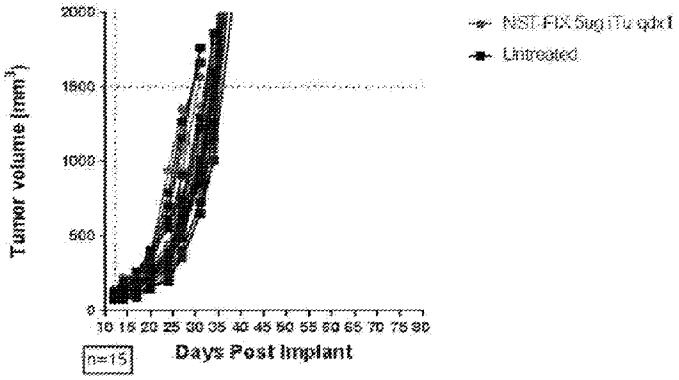


FIG. 20C
5 µg IL12



Negative controls:

FIG. 20D



DK/EP 3458083 T5

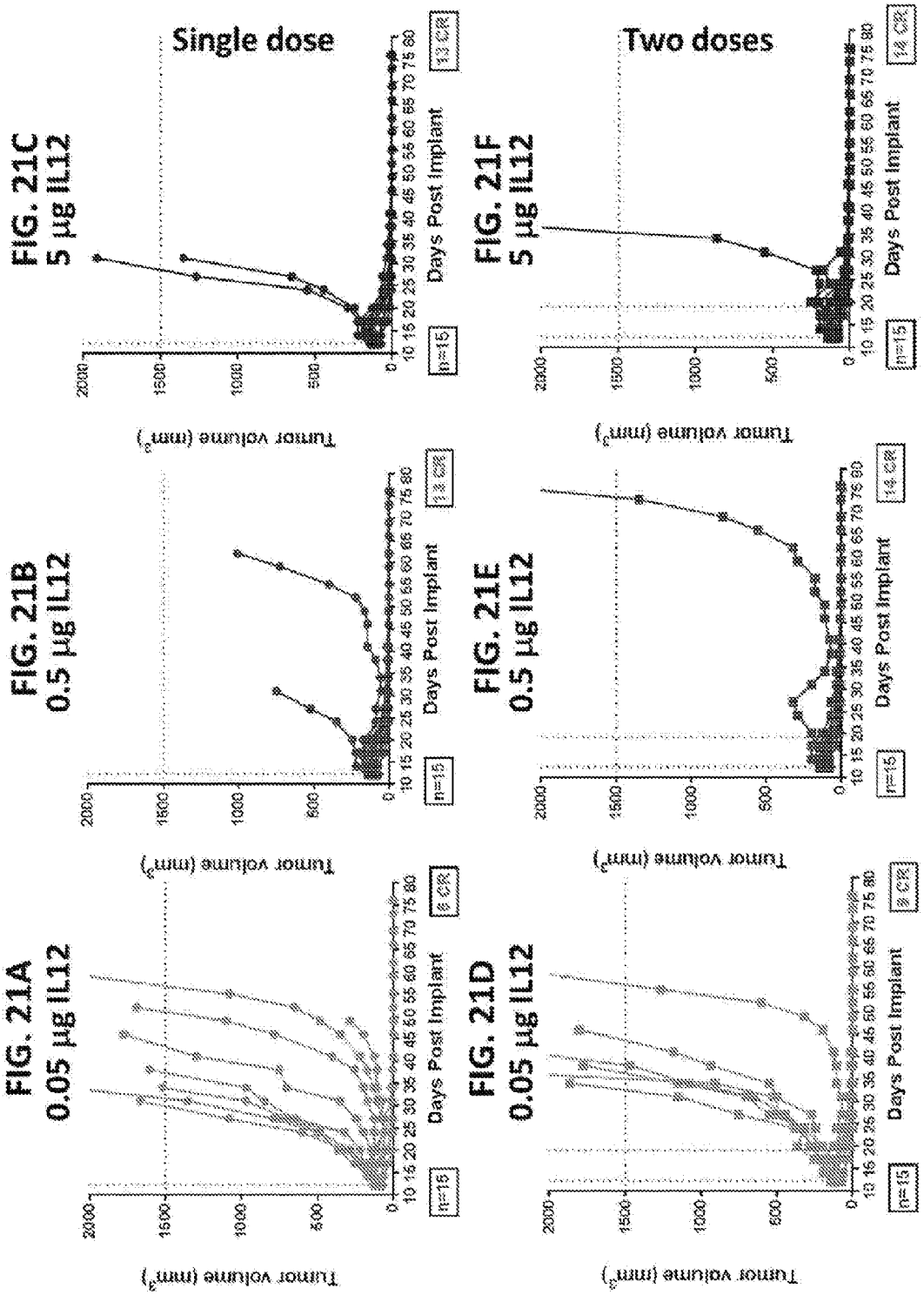
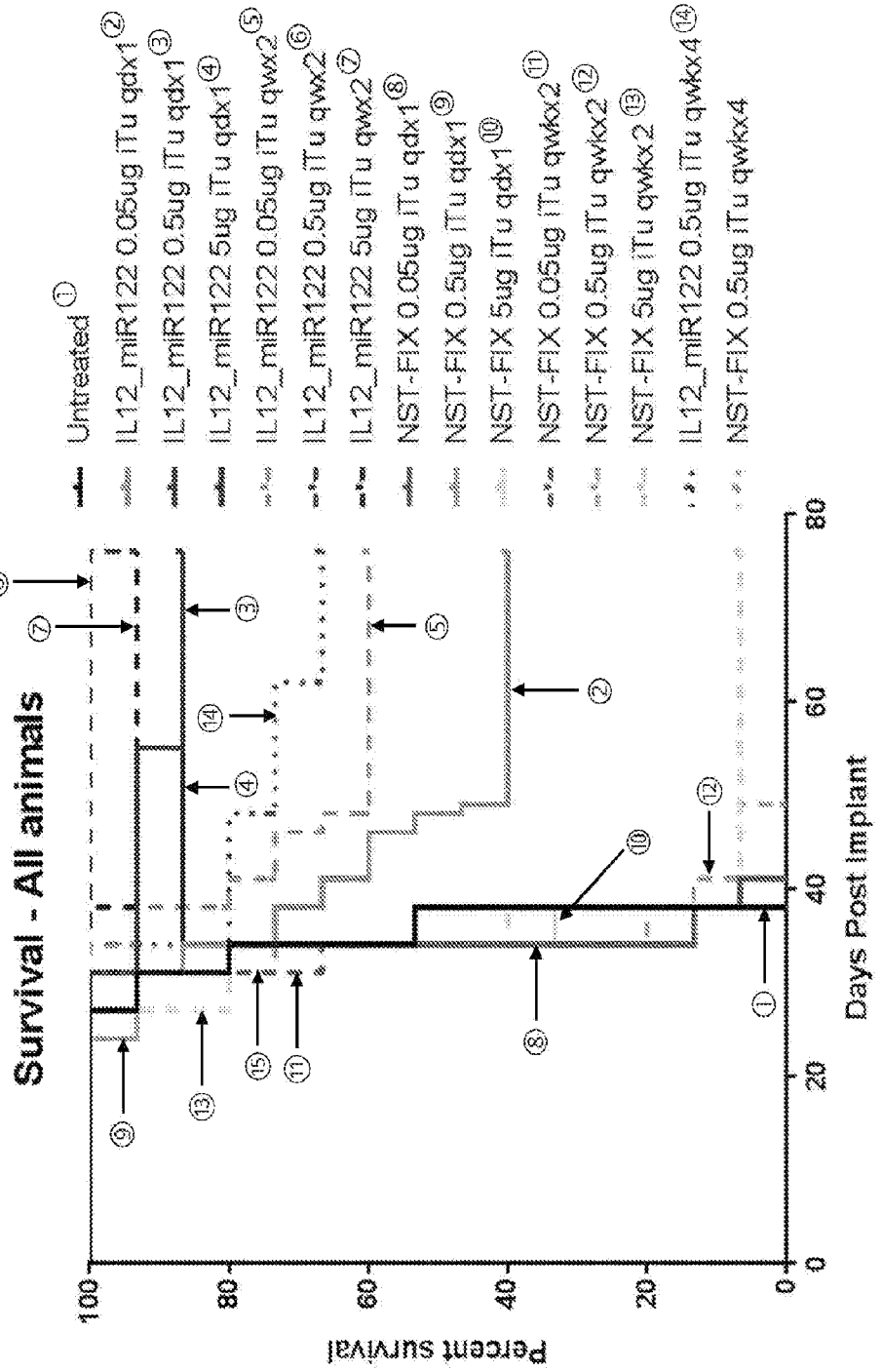


FIG. 21A
0.05 µg IL12
FIG. 21B
0.5 µg IL12
FIG. 21C
5 µg IL12
Single dose
FIG. 21D
0.05 µg IL12
FIG. 21E
0.5 µg IL12
FIG. 21F
5 µg IL12
Two doses
Tumor volume (mm³)
Days Post Implant
n=15
6 CR
8 CR
13 CR
14 CR
18 CR

FIG. 22


Survival - All animals
Percent survival
Days Post Implant
0
20
40
60
80
100
0
20
40
60
80
Untreated ①
IL12_miR122 0.05ug iTu qdx1 ②
IL12_miR122 0.5ug iTu qdx1 ③
IL12_miR122 5ug iTu qdx1 ④
IL12_miR122 0.05ug iTu qwx2 ⑤
IL12_miR122 0.5ug iTu qwx2 ⑥
IL12_miR122 5ug iTu qwx2 ⑦
NST-FIX 0.05ug iTu qdx1 ⑧
NST-FIX 0.5ug iTu qdx1 ⑨
NST-FIX 5ug iTu qdx1 ⑩
NST-FIX 0.05ug iTu qwkx2 ⑪
NST-FIX 0.5ug iTu qwkx2 ⑫
NST-FIX 5ug iTu qwkx2 ⑬
IL12_miR122 0.5ug iTu qwkx4 ⑭
NST-FIX 0.5ug iTu qwkx4

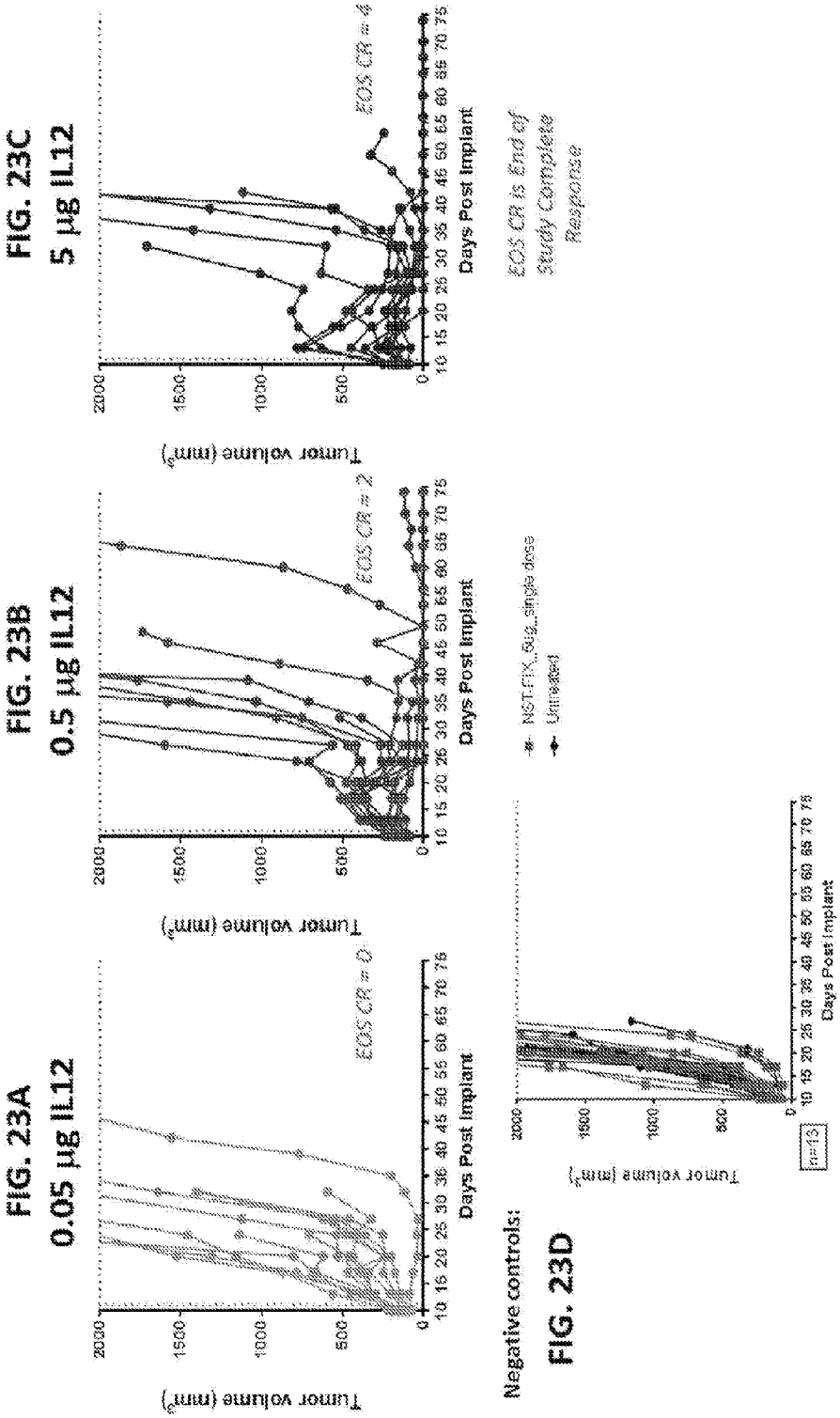


FIG. 23A
0.05 µg IL12
FIG. 23B
0.5 µg IL12
FIG. 23C
5 µg IL12
Tumor volume (mm³)
Days Post Implant
EOS CR = 0
EOS CR = 2
EOS CR = 4
EOS CR is End of Study Complete Response
Negative controls:
NST-FIX_6ug_single dose
Untreated
n=13

FIG. 23D

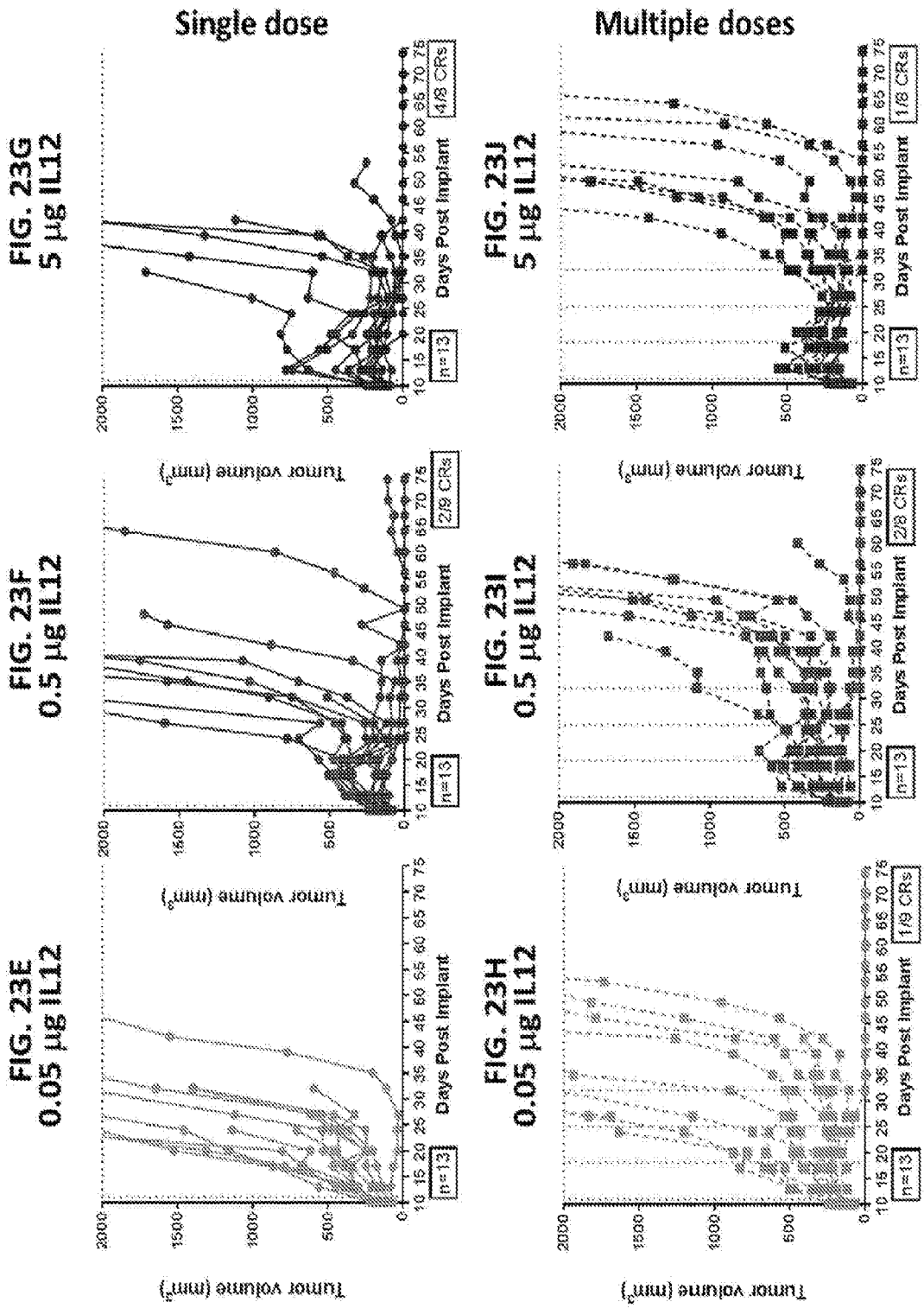

Single dose
Multiple doses
FIG. 23E
0.05 µg IL12
FIG. 23F
0.5 µg IL12
FIG. 23G
5 µg IL12
FIG. 23H
0.05 µg IL12
FIG. 23I
0.5 µg IL12
FIG. 23J
5 µg IL12
Tumor volume (mm³)
Days Post Implant
n=13
1/9 CRs
2/9 CRs
4/8 CRs
1/8 CRs

FIG. 24

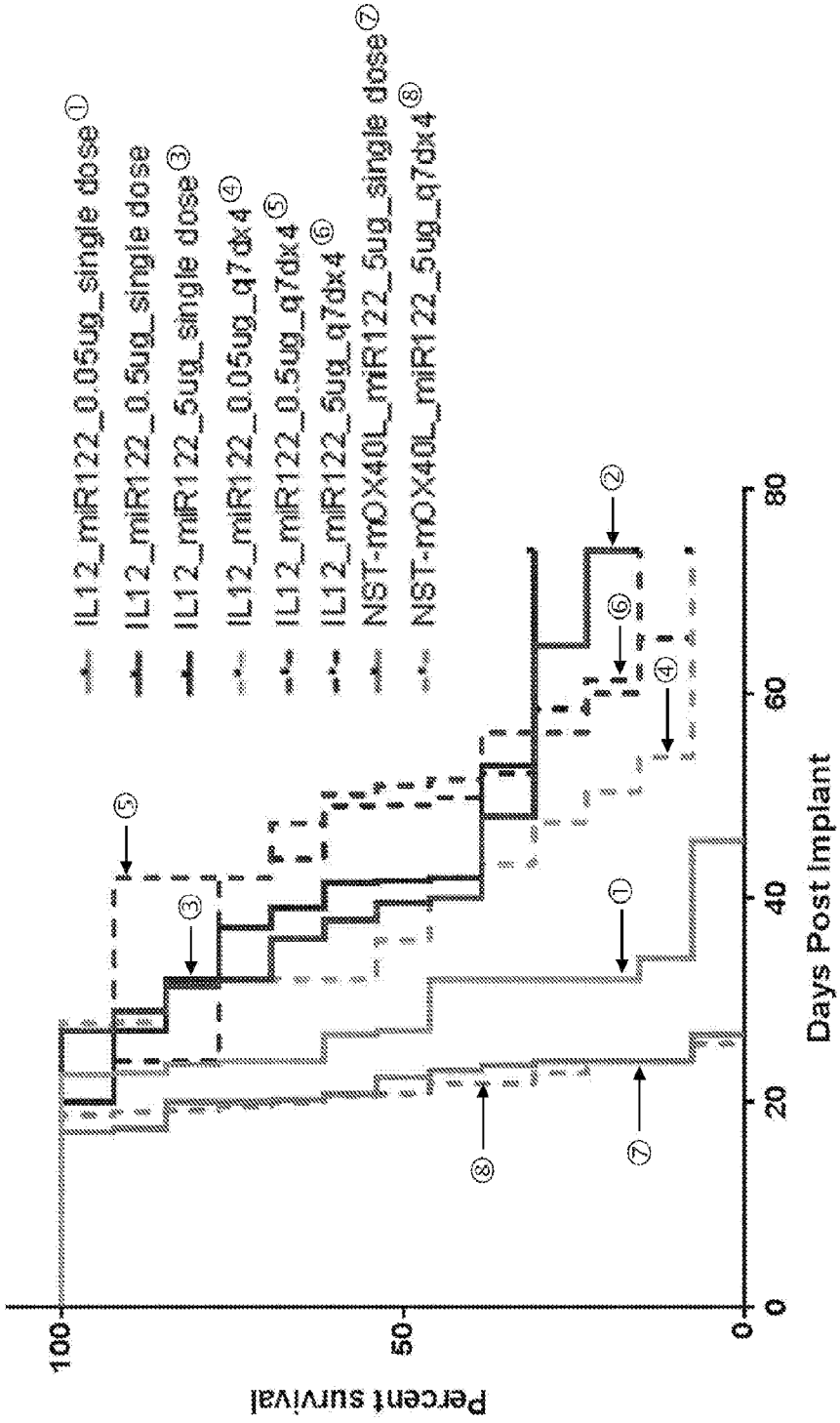

IL12_miR122_0.05ug_single dose①
IL12_miR122_0.5ug_single dose②
IL12_miR122_5ug_single dose③
IL12_miR122_0.05ug_q7dx4④
IL12_miR122_0.5ug_q7dx4⑤
IL12_miR122_5ug_q7dx4⑥
NST-mOX40L_miR122_5ug_single dose⑦
NST-mOX40L_miR122_5ug_q7dx4⑧
Percent survival
100
50
0
0
20
40
60
80
Days Post Implant

FIG. 25

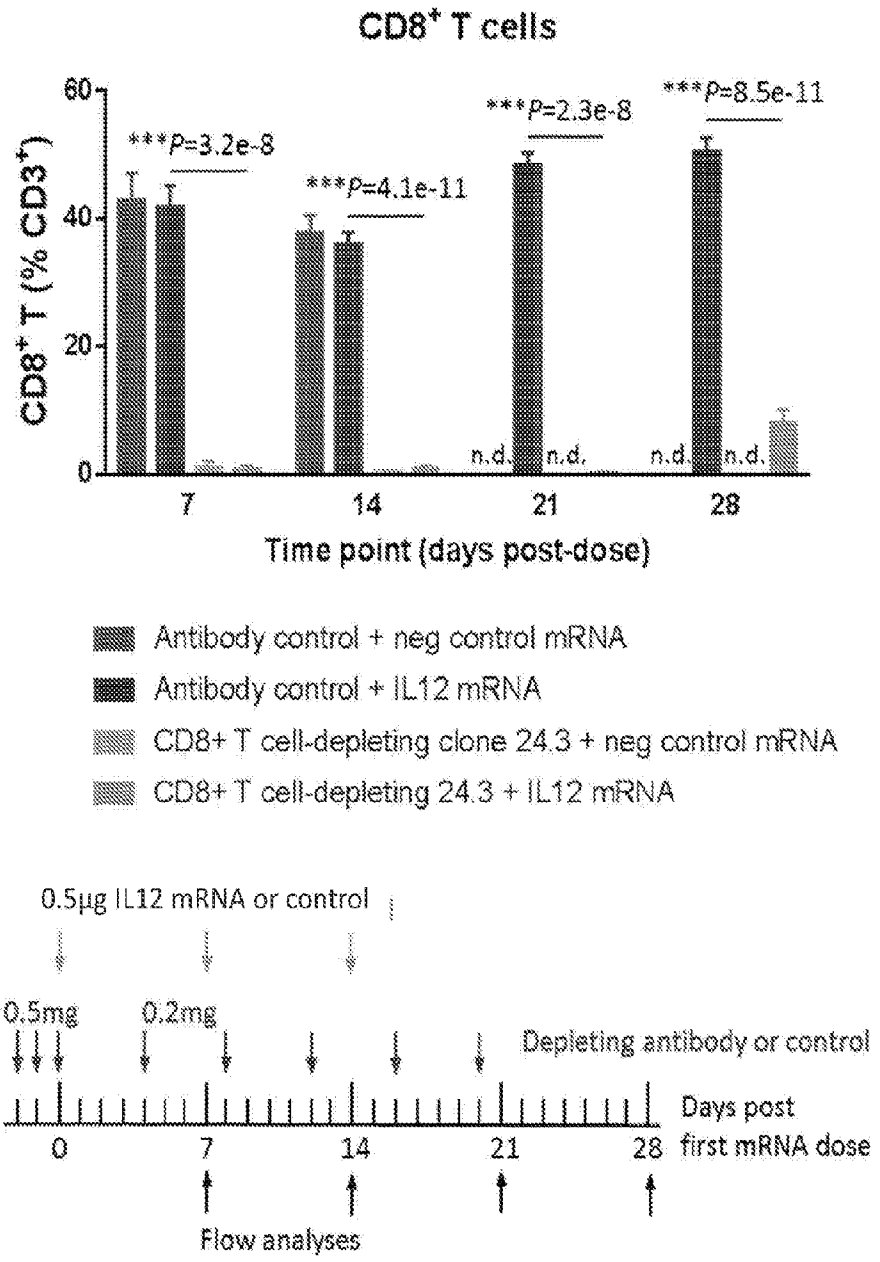

CD8+ T cells
CD8+ T (% CD3+)
60
40
20
0
***P=3.2e-8
***P=4.1e-11
***P=2.3e-8
***P=8.5e-11
n.d.
n.d.
n.d.
n.d.
7
14
21
28
Time point (days post-dose)
Antibody control + neg control mRNA
Antibody control + IL12 mRNA
CD8+ T cell-depleting clone 24.3 + neg control mRNA
CD8+ T cell-depleting 24.3 + IL12 mRNA
0.5µg IL12 mRNA or control
0.5mg
0.2mg
Depleting antibody or control
Days post first mRNA dose
0
7
14
21
28
Flow analyses

FIG. 26A

FIG. 26B

FIG. 26C

FIG. 26D

FIG. 26E

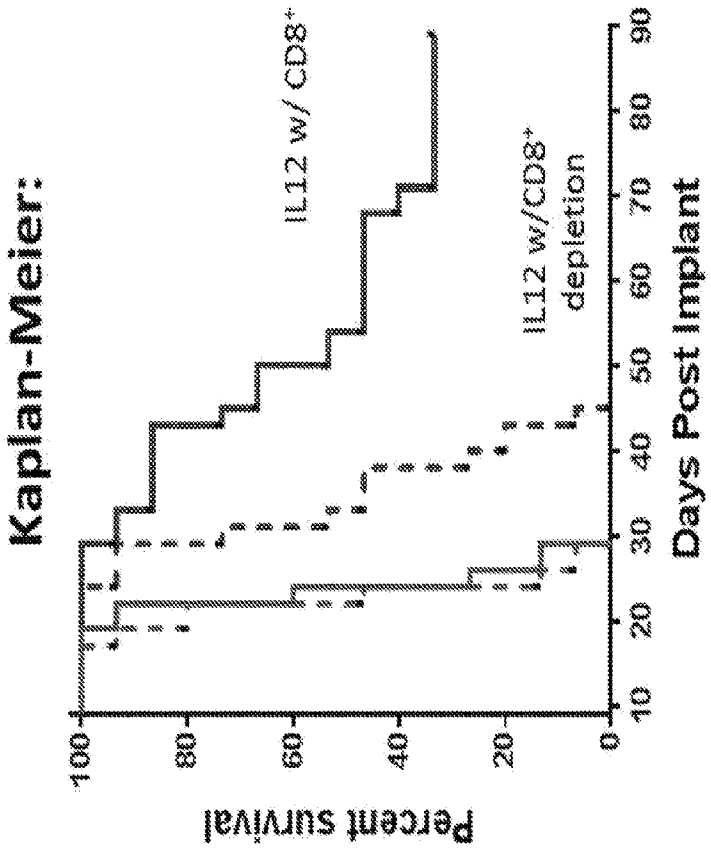

Kaplan-Meier:
Percent survival
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
Days Post Implant
IL12 w/ CD8+
IL12 w/CD8+ depletion

FIG. 27A

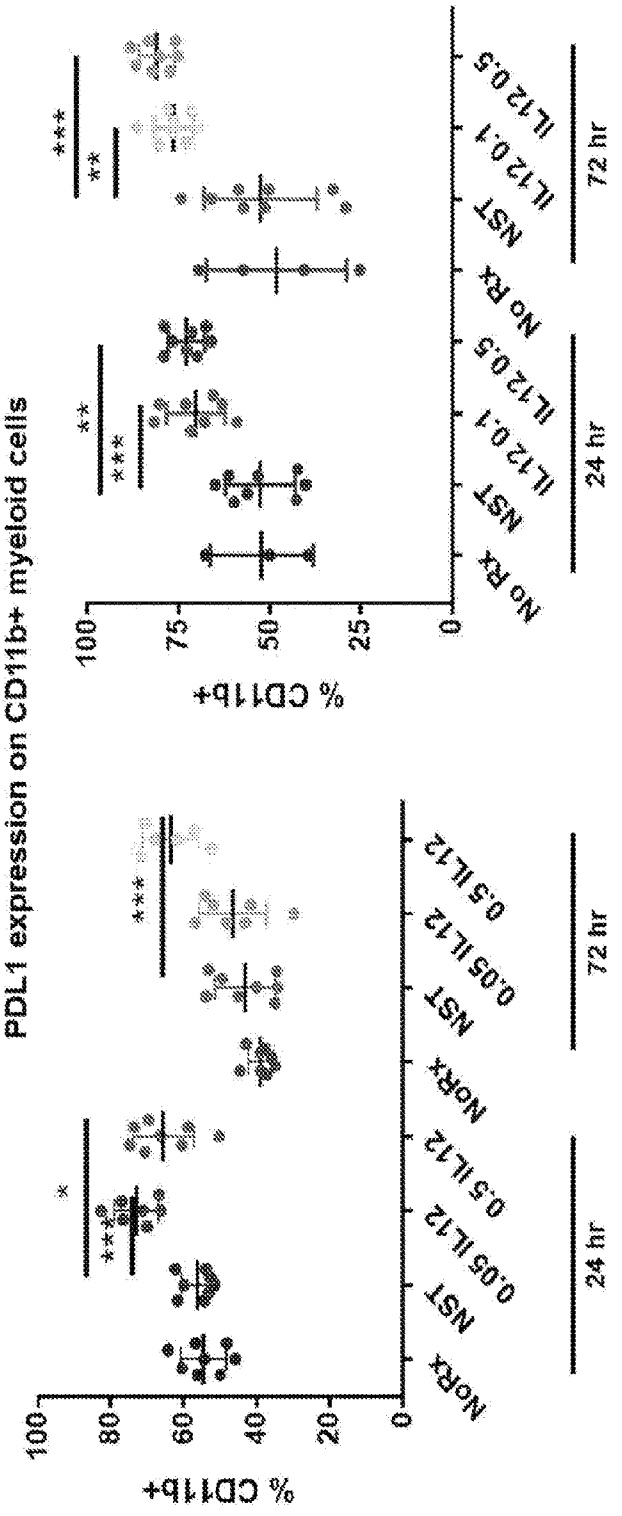


FIG. 27B

FIG. 28A

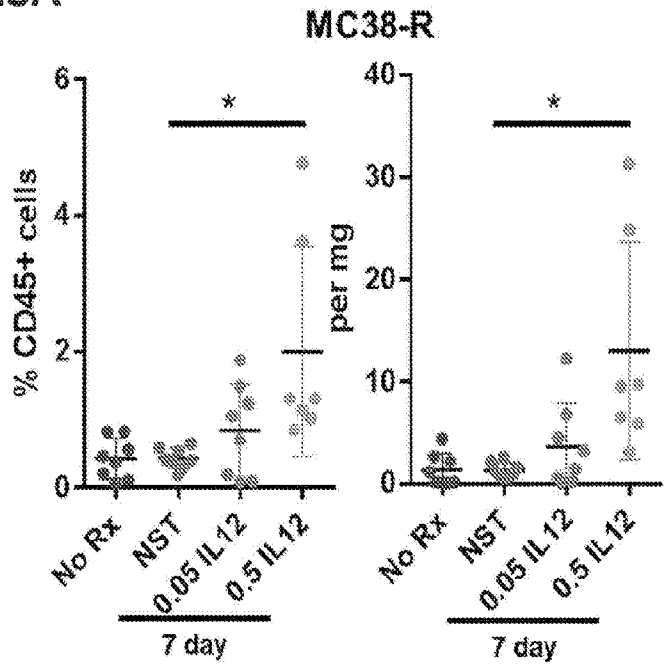


FIG. 28B

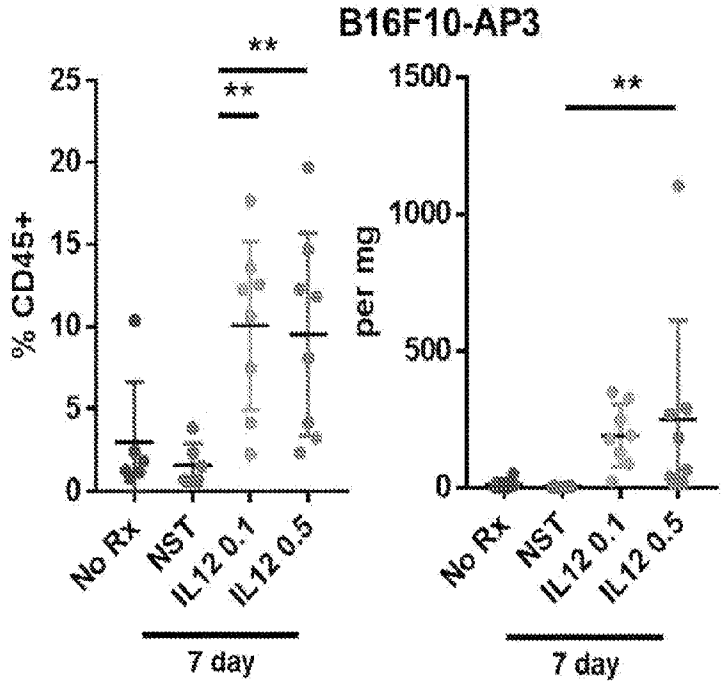


FIG. 29A

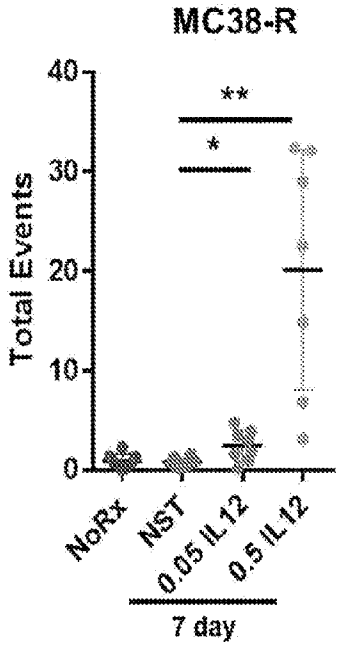


FIG. 29B

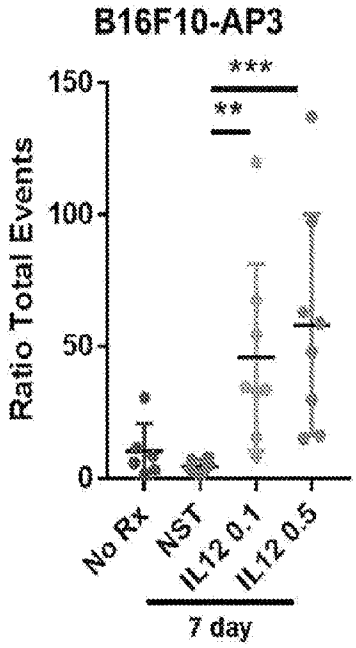


FIG. 30A

MC38-R

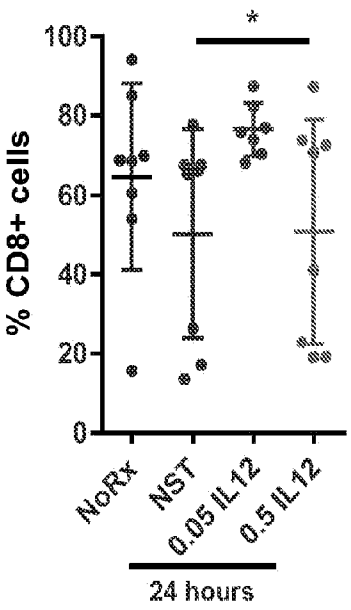


FIG. 30B

B16F10-AP3

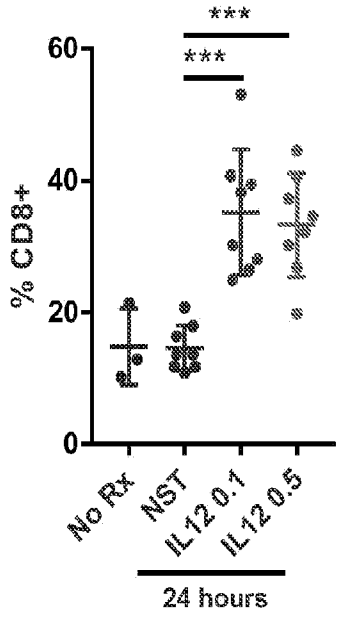


FIG. 31A

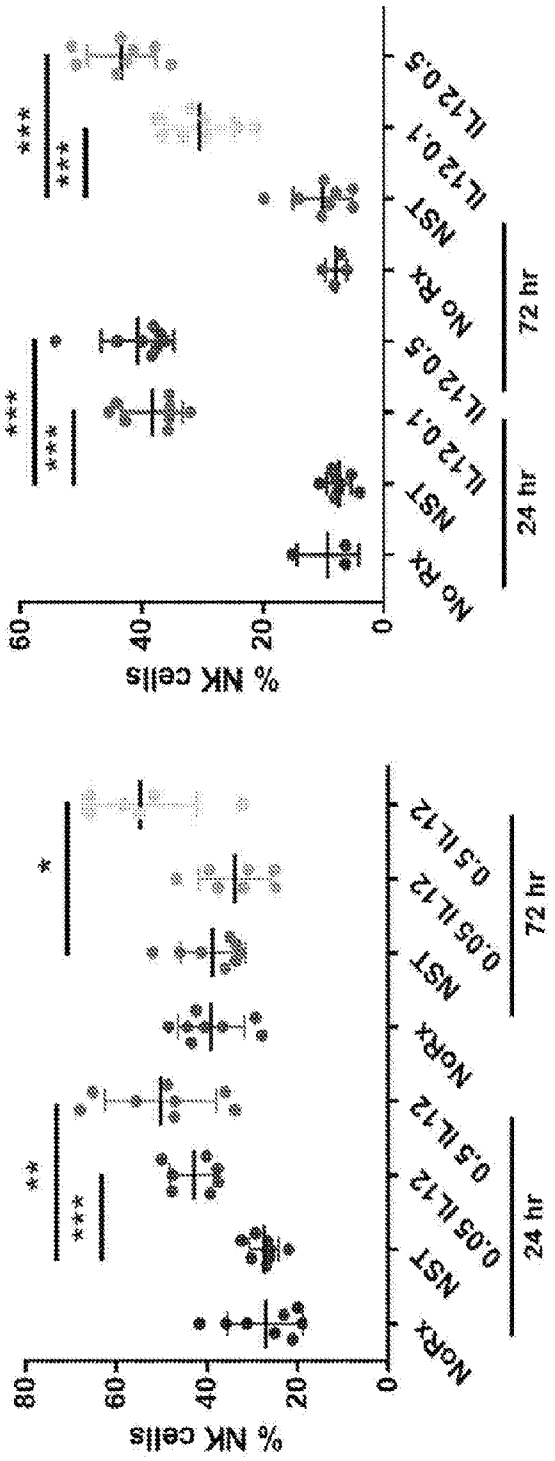


FIG. 31B

FIG. 32A

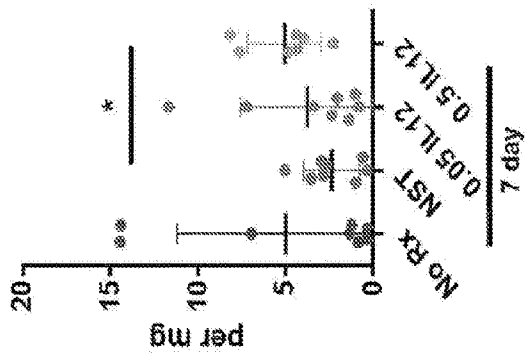


FIG. 32B

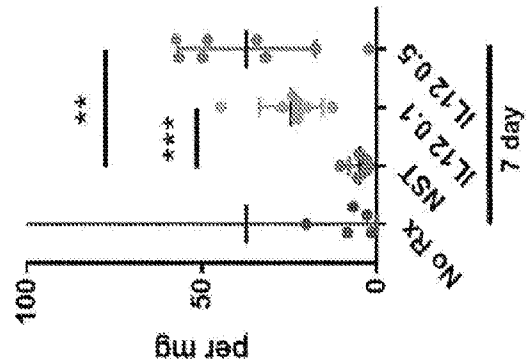


FIG. 32C

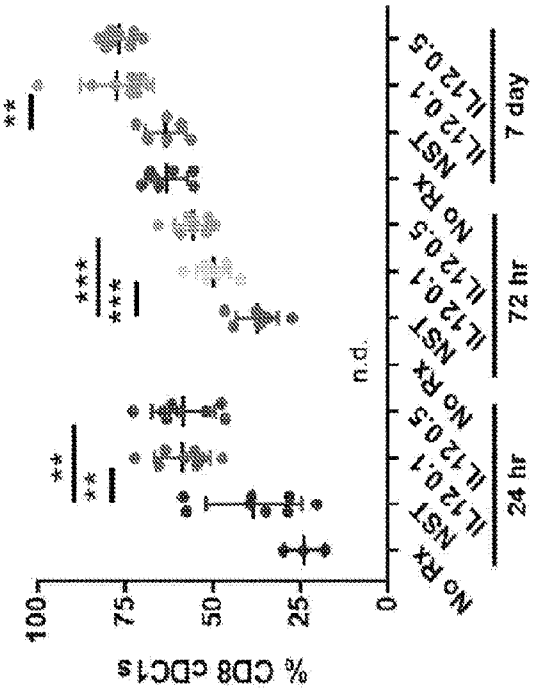


FIG. 33A

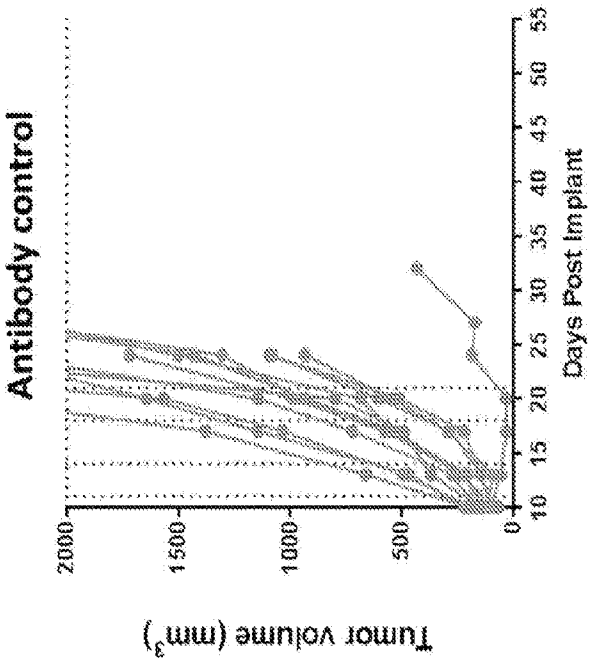


FIG. 33B

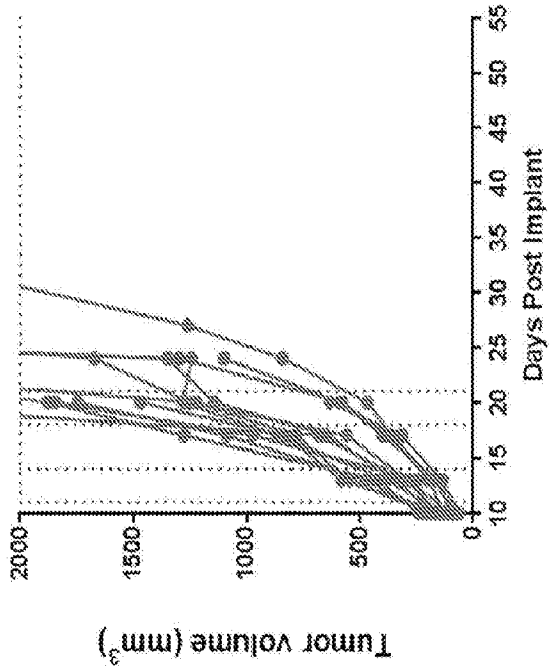


FIG. 33C

FIG. 33D

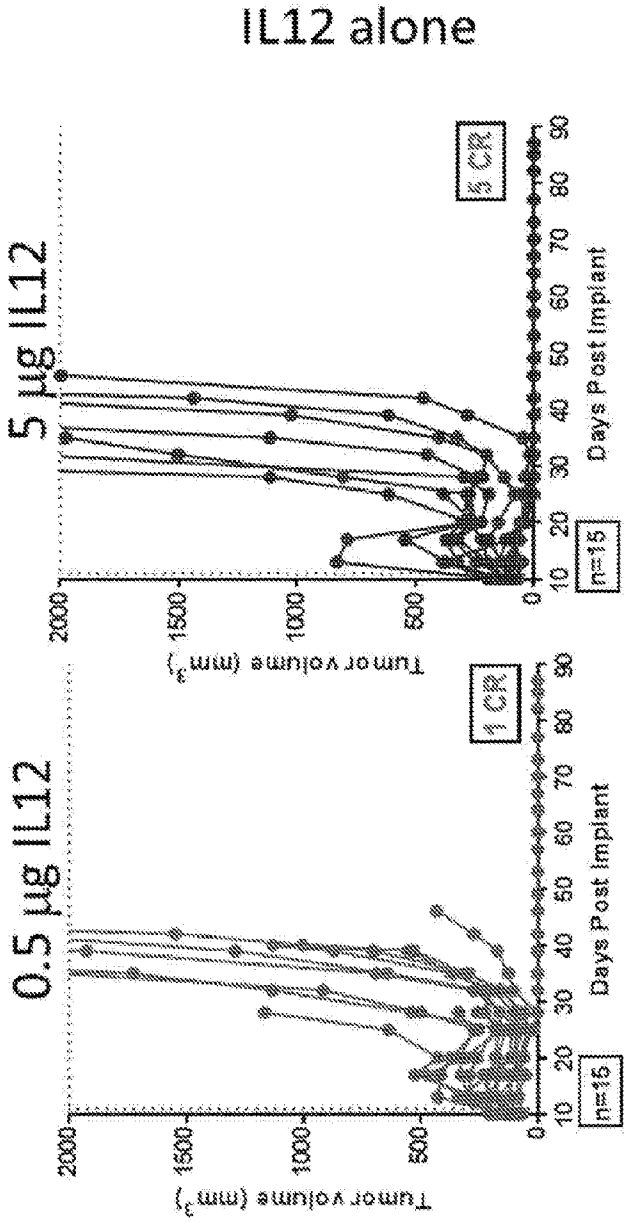


FIG. 33E

FIG. 33F

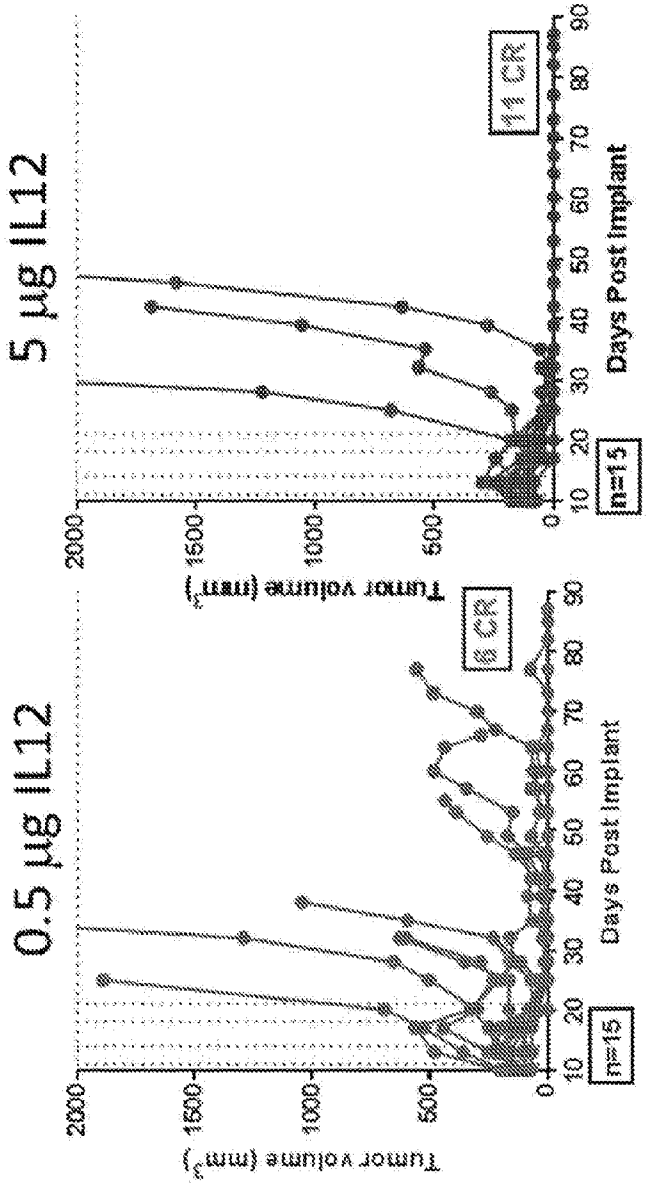


FIG. 33G

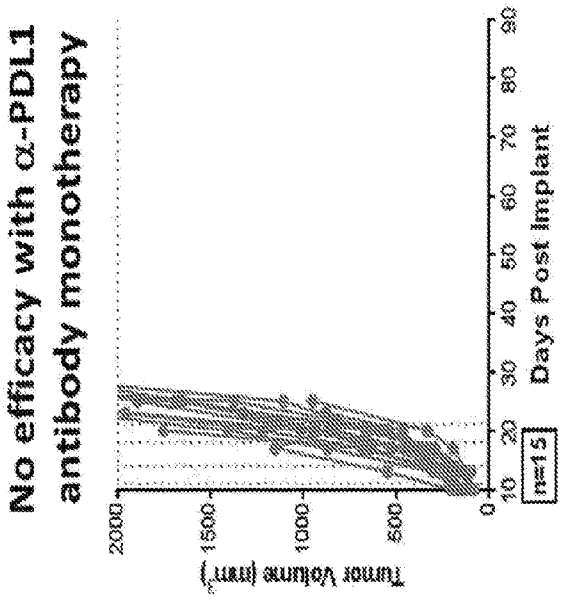

No efficacy with α-PDL1 antibody monotherapy
Tumor Volume (mm³)
2000
1500
1000
500
0
10
20
30
40
50
60
70
80
90
Days Post Implant
n=15

FIG. 34A

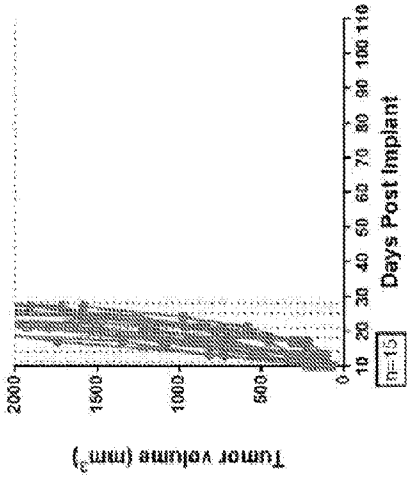


FIG. 34B

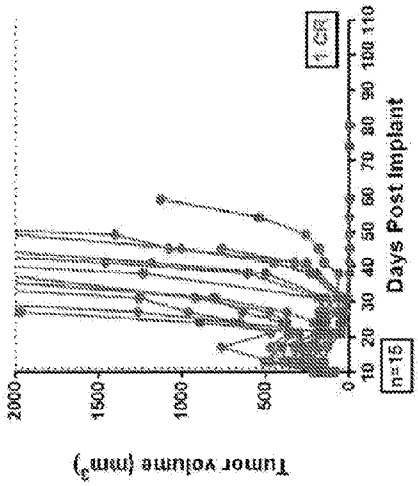


FIG. 34C

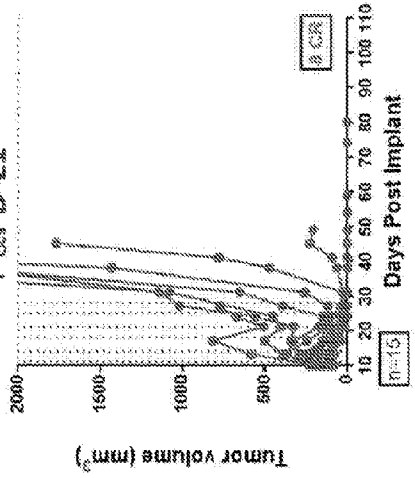


FIG. 35A

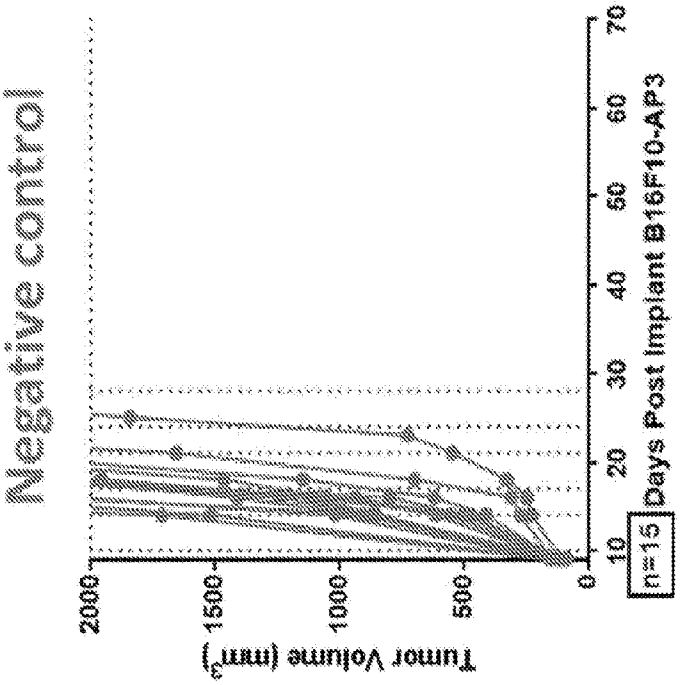


FIG. 35B

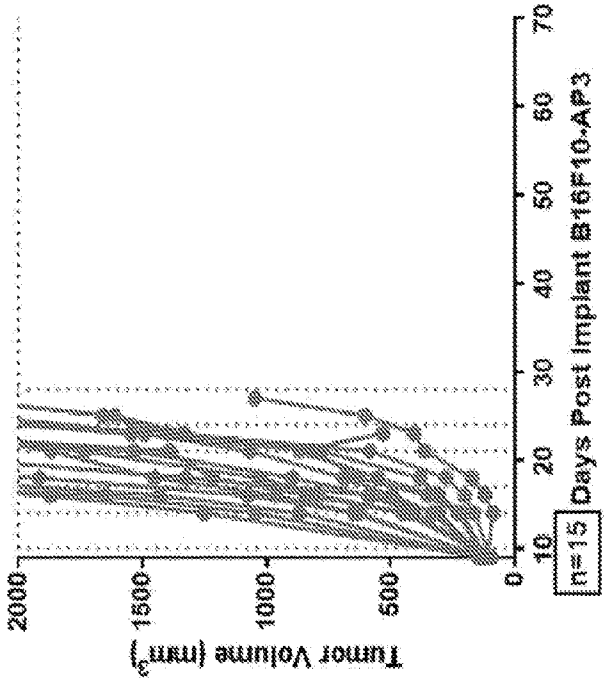


FIG. 35C

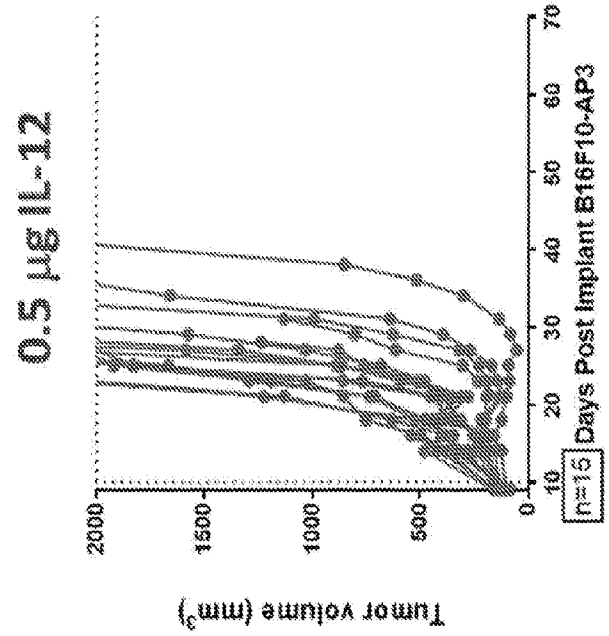


FIG. 35D

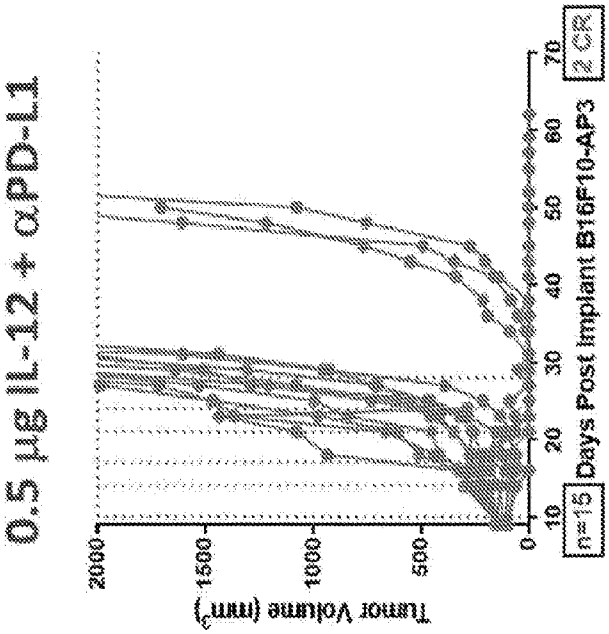


FIG. 36A

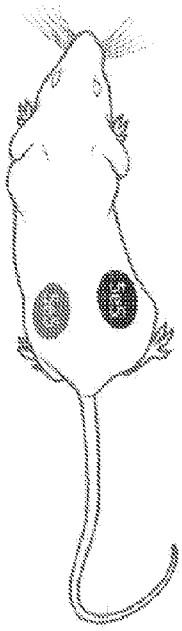


FIG. 36B

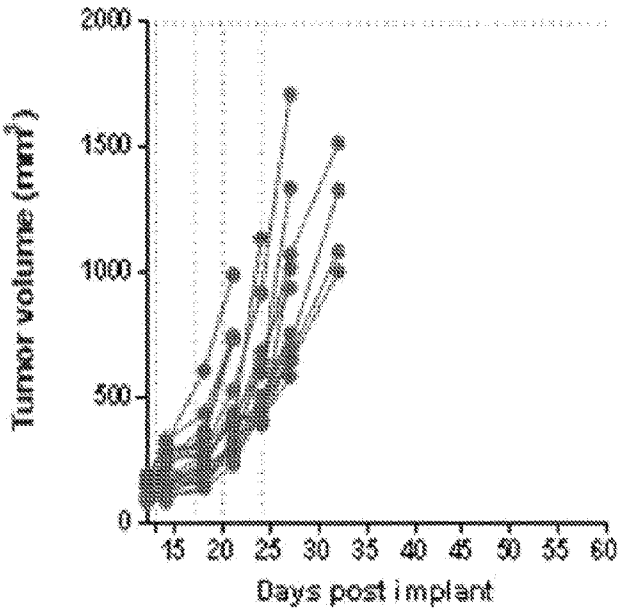


FIG. 36C

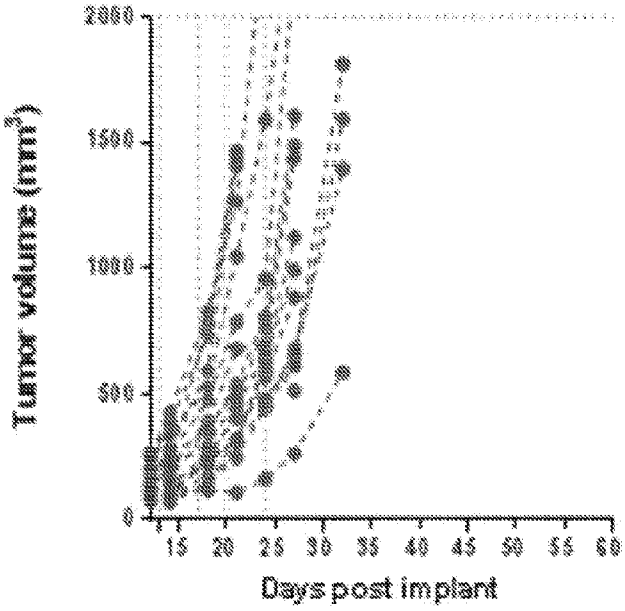
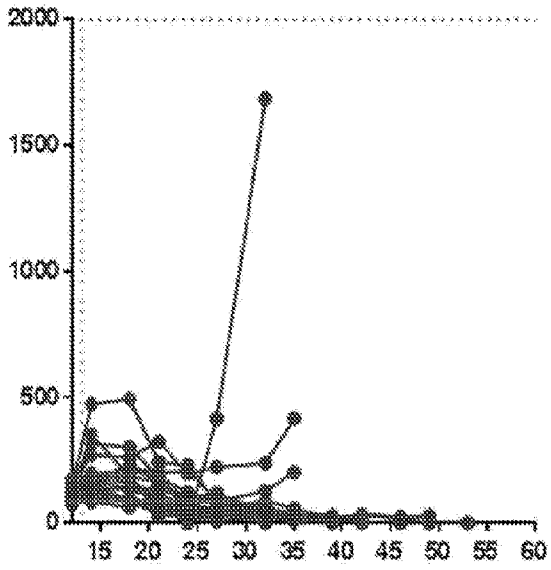


FIG. 36D



~3/20 CRs

FIG. 36E

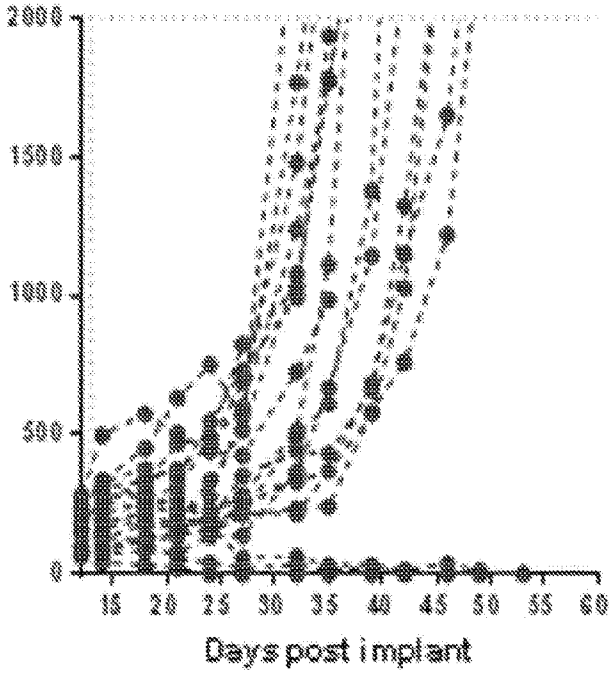
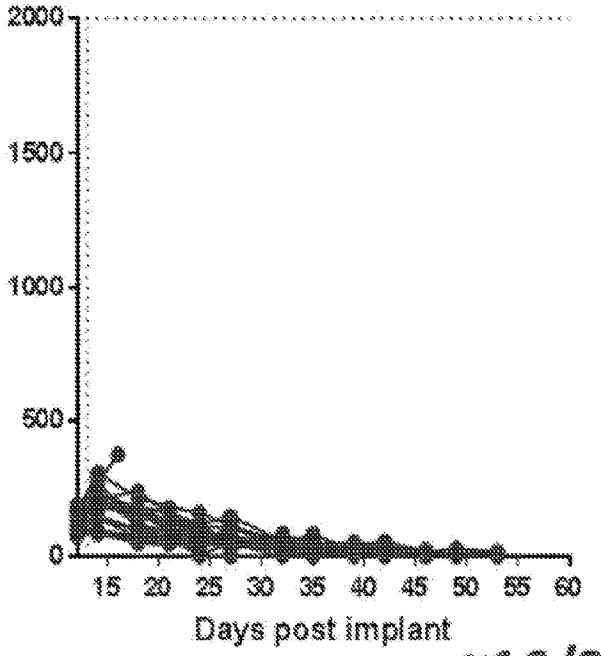


FIG. 36F



~16/20 CRs

FIG. 36G

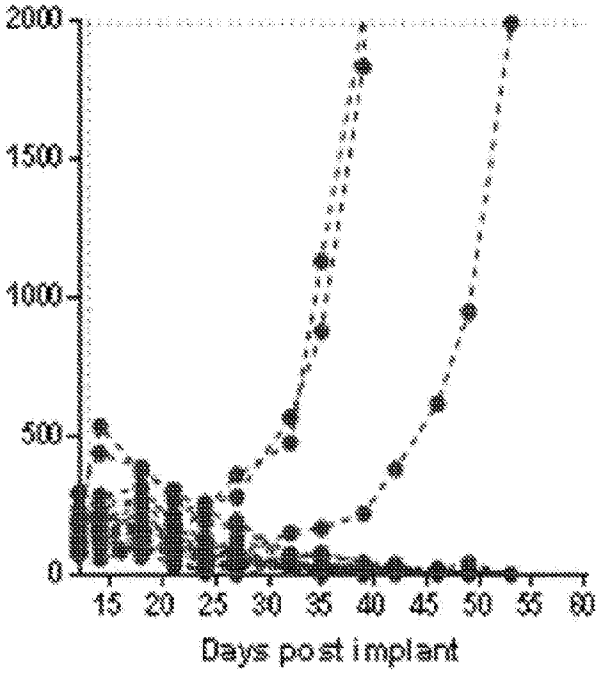


FIG. 37A

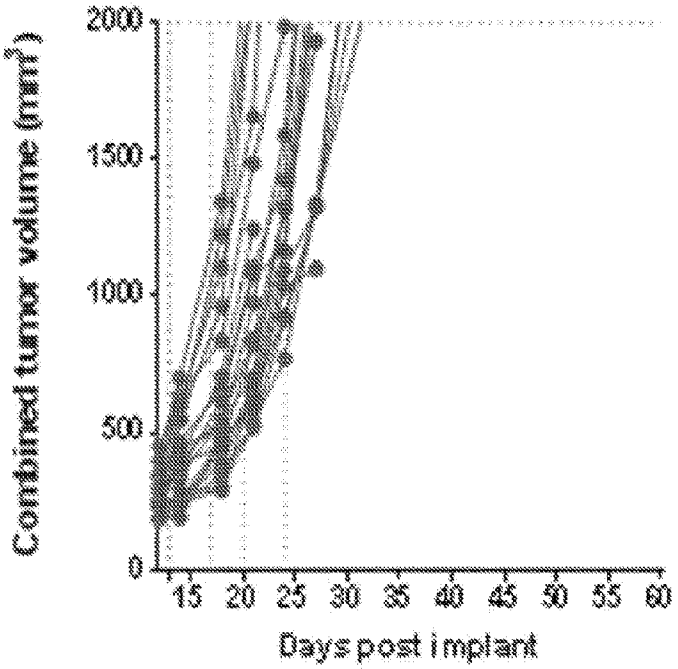


FIG. 37B

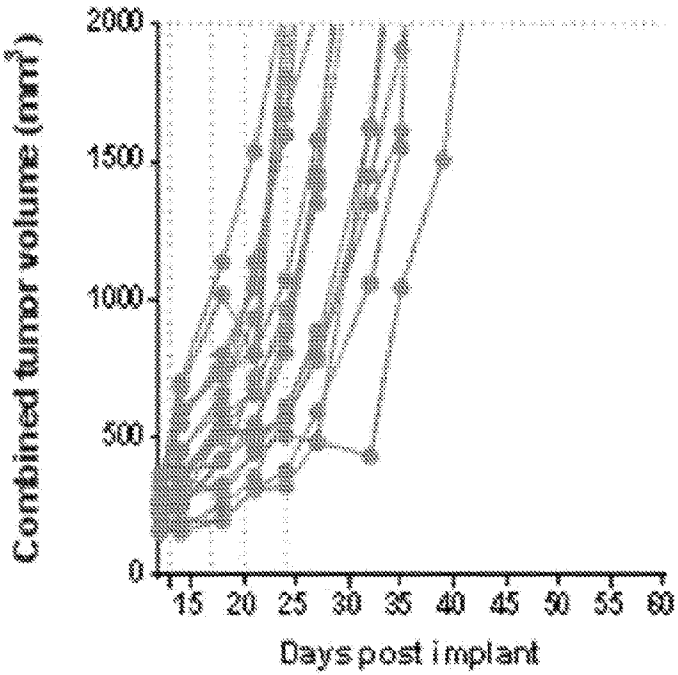


FIG. 37C

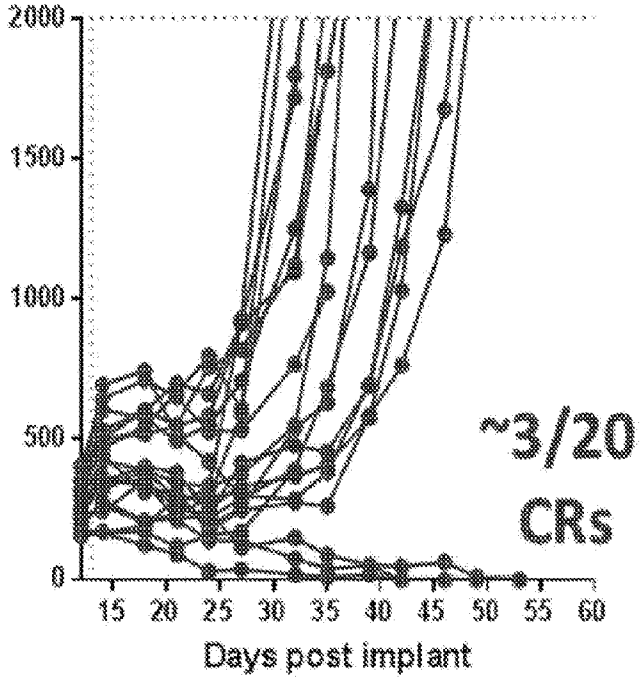


FIG. 37D

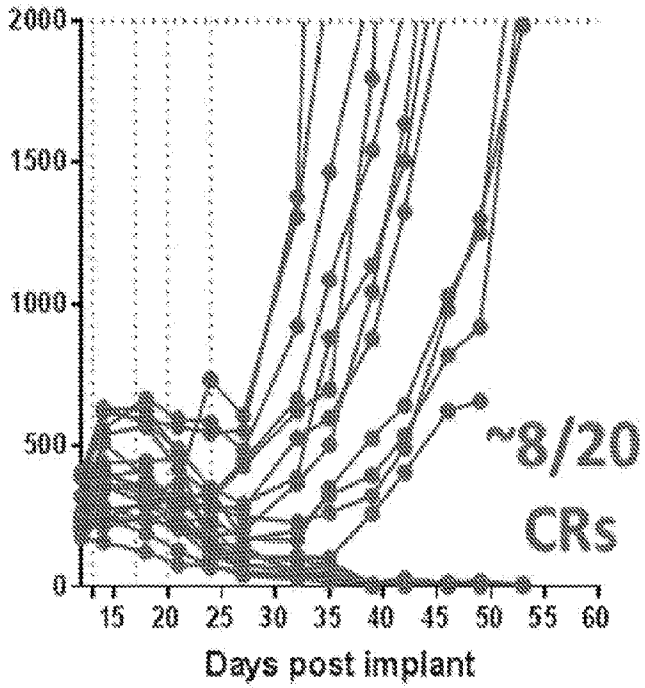


FIG. 37E

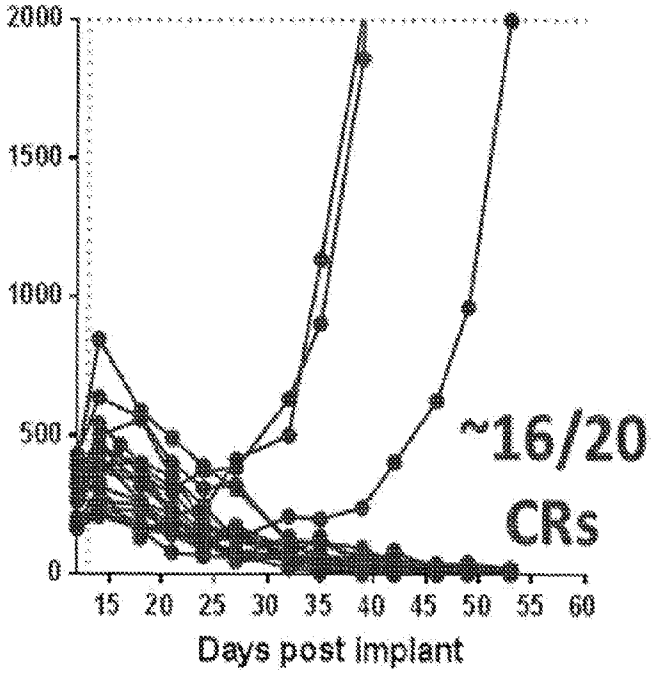


FIG. 37F

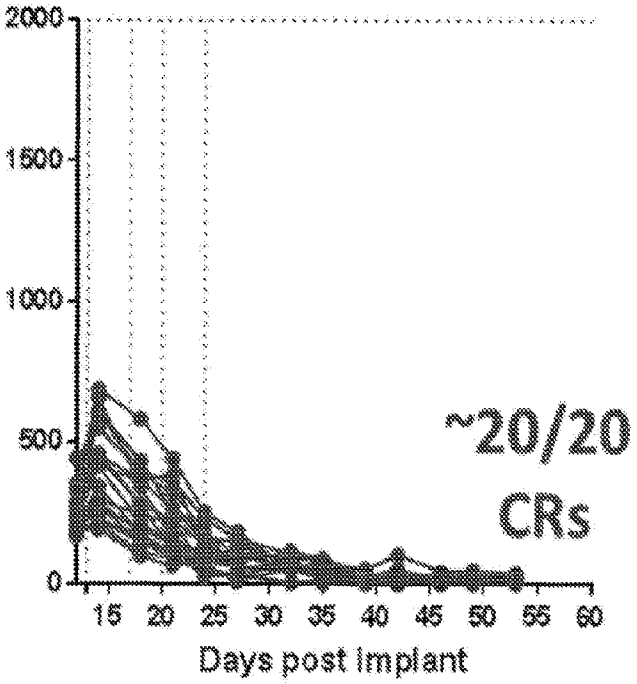
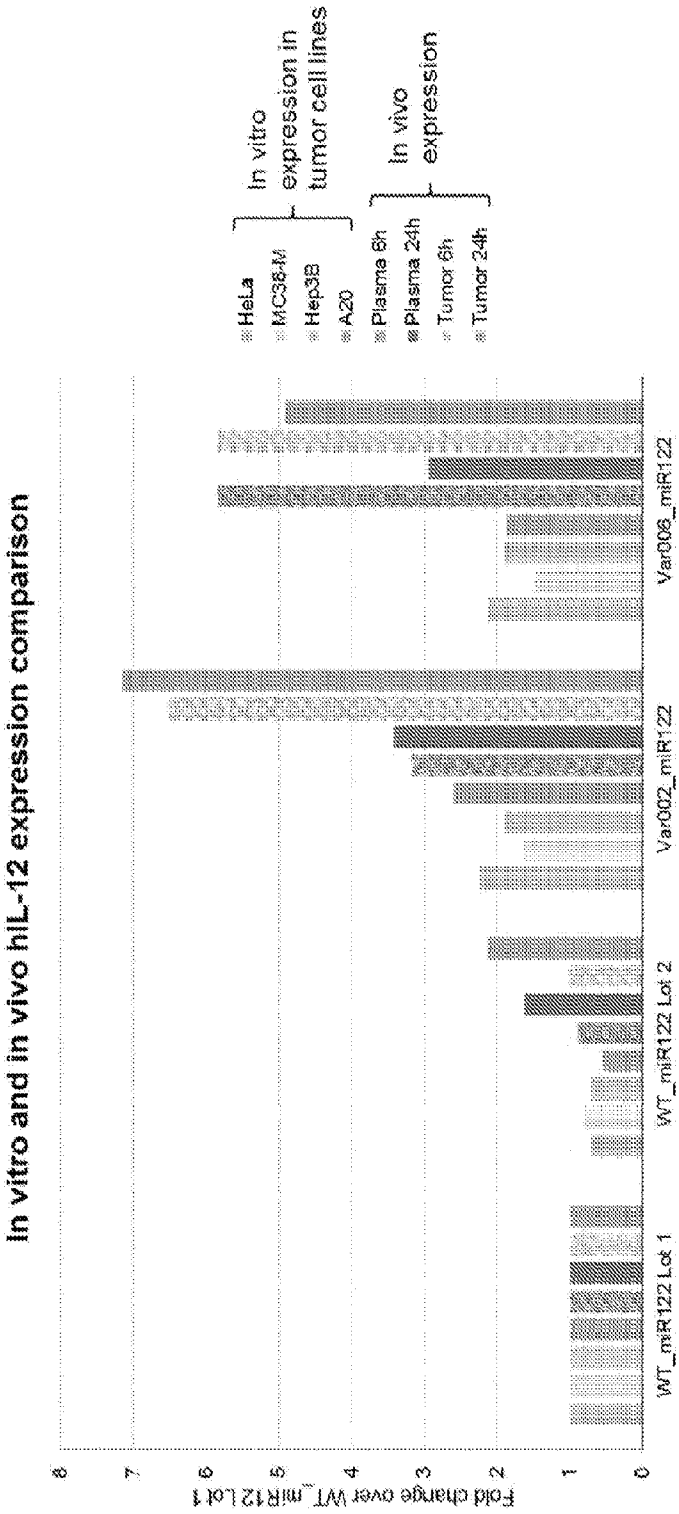


FIG. 38


In vitro and in vivo hIL-12 expression comparison
Fold change over WT_miR122 Lot 1
0
1
2
3
4
5
6
7
8
WT_miR122 Lot 1
WT_miR122 Lot 2
Var002_miR122
Var008_miR122
HeLa
MC38-M
Hep3B
A20
In vitro expression in tumor cell lines
Plasma 6h
Plasma 24h
Tumor 6h
Tumor 24h
In vivo expression

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.