MRNA THERAPEUTIC COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to U.S. Provisional Application No. 63/291,686, filed December 20, 2021; U.S. Provisional Application No. 63/320,664, filed March 16, 2022; and U.S. Provisional Application No. 63/401,214, filed August 26, 2022; all of which are herein incorporated by reference in their entirety.

BACKGROUND

[0002] Commercial or developing vaccines (e.g., cancer vaccines) are typically based on whole microorganisms, protein antigens, peptides, polysaccharides or deoxyribonucleic acid (DNA) vaccines and their combinations. The use of RNA polynucleotides as therapeutics is a new and emerging field.

[0003] A need therefore exists for developing an enhanced RNA delivery system for a more effective, easily scalable, and stable delivery of RNA therapeutics (such as cancer vaccines).

SUMMARY OF THE INVENTION

[0004] In one aspect, provided herein is a mRNA therapeutic composition, comprising one or more polynucleotides encoding one or more antigenic (e.g., tumor antigenic) or signaling polypeptides. The one or more polynucleotides are formulated within a lipid reconstructed plant messenger packs (LPMPs) comprising natural lipids and an ionizable lipid. The ionizable lipid has two or more of the characteristics listed below:

(i) at least 2 ionizable amines;

(ii) at least 3 lipid tails, wherein each of the lipid tails is at least 6 carbon atoms in length;

(iii) a pKa of about 4.5 to about 7.5;

(iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and

(v) an N:P ratio of at least 10.

[0005] In another aspect, provided herein is a method for making a mRNA therapeutic composition. The method comprises reconstituting a film comprising purified PMP lipids in the presence of an ionizable lipid to produce a lipid reconstructed plant messenger packs (LPMP) comprising the ionizable lipid. The ionizable lipid has two or more of the characteristics listed below:

(i) at least 2 ionizable amines;

(ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length;

(iii) a pKa of about 4.5 to about 7.5;

(iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and

(v) an N:P ratio of at least 10.

[0006] The method further comprises loading into the LPMPs with one or more polynucleotides encoding one or more antigenic (e.g., tumor antigenic) or signaling polypeptides.

[0007] In some embodiments, the polynucleotides are polynucleotide constructs, which encode one

naturally occurring IL-15 molecule, or a variant thereof. In one embodiment, the IL-15 molecule comprises a variant of a naturally occurring IL-15 molecule (e.g., an IL-15 variant, e.g., as described herein), or a fragment thereof. In one embodiment, the polynucleotide is an mRNA which encodes an IL-15 superagonist, IL-15 molecule, IL-15RA molecule, or a combination thereof.

[0023] In some embodiments, the mRNA comprises a 5' untranslated region (UTR) and/or a 3' UTR.

[0024] In some embodiments, the mRNA comprises a 5' UTR. The 5' UTR may comprise a Kozak sequence.

[0025] In some embodiments, the mRNA comprises a 3' UTR. In some embodiments, the 3' UTR comprises one or more sequences derived from an amino-terminal enhancer of split (AES). In some embodiments, the 3' UTR comprises a sequence derived from mitochondrially encoded 12S rRNA (mtRNRl).

[0026] In some embodiments, the mRNA comprises a poly(A) sequence. In one embodiment, the poly(A) sequence is a 110-nucleotide sequence consisting of a sequence of 30 adenosine residues (SEQ ID NO: 44), a 10-nucleotide linker sequence, and a sequence of 70 adenosine residues (SEQ ID NO: 45).

[0027] In some embodiments, the polynucleotide is encapsulated by the lipid reconstructed plant messenger packs (LPMPs). In some embodiments, the polynucleotide is embedded on the surface of the LPMPs. In some embodiments, the polynucleotide is conjugated to the surface of the LPMPs.

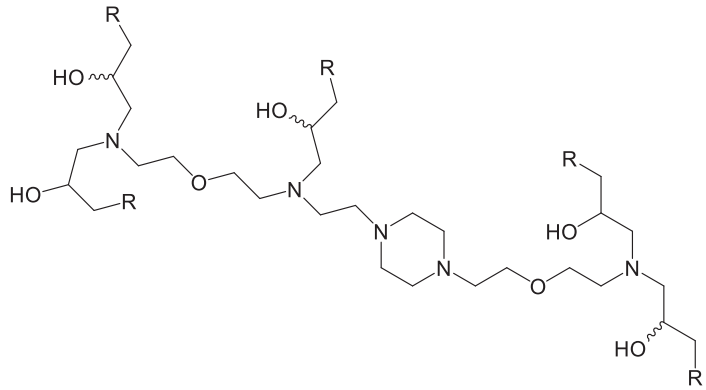
[0028] In some embodiments, the LPMP is produced by a method comprising lipid extrusion. In some embodiments, the LPMP is produced by a method comprising processing a solution comprising a lipid extract of the PMPs in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs. In some embodiments, the aqueous phase comprises the polynucleotides.

[0029] In some embodiments, the natural lipids of the LPMPs are extracted from lemon or algae.

[0030] In some embodiments, the ionizable lipid of the LPMPs is selected from the group consisting of 1,1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5, SM-102 (Lipid H), and ALC-315.

[0031] In one embodiment, the ionizable lipid is C12-200.

[0032] In some embodiments, the ionizable lipid is



(I), wherein R is C8-C14 alkyl group.

[0033] In some embodiments, the reconstitution is performed in the presence of a sterol, thereby producing a LPMP that comprises natural lipids, an ionizable lipid, and a sterol. In some embodiments, the sterol is cholesterol or sitosterol.

development or differentiation state of the organism. In one instance, provided is a composition to alter expression of one or more proteins, e.g., proteins that affect activity, structure, or function. Expression of the one or more proteins may be restricted to a specific location(s) or widespread throughout the organism.

mRNA

[00303] The LPMP / mRNA therapeutic composition may include a mRNA molecule, e.g., a mRNA molecule encoding a polypeptide. The mRNA molecule can be synthetic and modified (e.g., chemically). The mRNA molecule can be chemically synthesized or transcribed in vitro. The mRNA molecule can be disposed on a plasmid, e.g., a viral vector, bacterial vector, or eukaryotic expression vector. In some examples, the mRNA molecule can be delivered to cells by transfection, electroporation, or transduction (e.g., adenoviral or lentiviral transduction).

[00304] In some instances, the modified RNA agent of interest described herein has modified nucleosides or nucleotides. Such modifications are known and are described, e.g., in WO 2012/019168. Additional modifications are described, e.g., in WO 2015/038892; WO 2015/038892; WO 2015/089511; WO 2015/196130; WO 2015/196118 and WO 2015/196128 A2, which are herein incorporated by reference in their entirety.

[00305] In some instances, the modified RNA encoding a polypeptide of interest has one or more terminal modification, e.g., a 5' cap structure and/or a poly-A tail (e.g., of between 100-200 nucleotides in length (SEQ ID NO: 46)). The 5' cap structure may be selected from the group consisting of CapO, Capl, ARCA, inosine, Nl-methyl-guanosine, 2'fluoro- guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido- guanosine. In some cases, the modified RNAs also contain a 5‘ UTR including at least one Kozak sequence, and a 3‘ UTR. Such modifications are known and are described, e.g., in WO 2012/135805 and WO 2013/052523, which are incorporated herein by reference in their entirety. Additional terminal modifications are described, e.g., in WO 2014/164253 and WO 2016/011306, WO 2012/045075, and WO 2014/093924, which are incorporated herein by reference in their entirety. Chimeric enzymes for synthesizing capped RNA molecules (e.g., modified mRNA) which may include at least one chemical modification are described in WO 2014/028429, which is incorporated herein by reference in its entirety.

[00306] In some instances, a modified mRNA (mmRNA) may be cyclized, or concatemerized, to generate a translation competent molecule to assist interactions between poly-A binding proteins and 5 '-end binding proteins. The mechanism of cyclization or concatemerization may occur through at least 3 different routes: 1) chemical, 2) enzymatic, and 3) ribozyme catalyzed. The newly formed 5'-/3'- linkage may be intramolecular or intermolecular. Such modifications are described, e.g., in WO 2013/151736.

[00307] Methods of making and purifying modified RNAs are known and disclosed in the art. For example, modified RNAs are made using only in vitro transcription (IVT) enzymatic synthesis. Methods of making IVT polynucleotides are known in the art and are described in WO 2013/151666, WO 2013/151668, WO 2013/151663, WO 2013/151669, WO 2013/151670, WO 2013/151664, WO 2013/151665, WO 2013/151671, WO 2013/151672, WO 2013/151667 and WO 2013/151736, which are incorporated herein by reference in their entirety. Methods of purification include purifying an

herein), or a fragment thereof.

[00366] In one embodiment, the polynucleotide is an mRNA which encodes an IL-2 molecule comprising an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence of an IL-2 molecule provided in any one of Tables I-III.

[00367] In some embodiments, the polynucleotide is an mRNA which encodes an IL-15 molecule. In one embodiment, the IL-15 molecule comprises a naturally occurring IL-15 molecule, a fragment of a naturally occurring IL-15 molecule, or a variant thereof. In one embodiment, the IL-15 molecule comprises a variant of a naturally occurring IL-15 molecule (e.g., an IL-15 variant, e.g., as described herein), or a fragment thereof.

[00368] In one embodiment, the polynucleotide is an mRNA which encodes an IL-15 molecule comprising an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence of an IL-15 molecule provided in Table IV.

[00369] In some embodiments, the mRNA comprises a 5' untranslated region (UTR) and/or a 3' UTR.

[00370] In some embodiments, the mRNA comprises a 5' UTR. The 5' UTR may comprise a Kozak sequence.

[00371] In some embodiments, the mRNA comprises a 3' UTR. In some embodiments, the 3' UTR comprises one or more sequences derived from an amino-terminal enhancer of split (AES). In some embodiments, the 3' UTR comprises a sequence derived from mitochondrially encoded 12S rRNA (mtRNRl).

In some embodiments, the mRNA comprises a poly(A) sequence. In one embodiment, the poly(A) sequence is a 110-nucleotide sequence consisting of a sequence of 30 adenosine residues (SEQ ID NO: 44), a 10-nucleotide linker sequence, and a sequence of 70 adenosine residues (SEQ ID NO: 45).

	mRNA Name	ORF Sequence (Amino Acid)	ORF Sequence (Nucleotide)	5' UTR Sequence	3' UTR Sequence	Con-struct Sequence
			CCACUGGAAGAGGC CCUGAACCUGGCCC CUAGUAAGAAUUU CCACCUCAGACCUC GCGACCUUAUUAGC GACAUCAACGUGAU CGUGCUCGAAUUAA AGGGCUCCGAGACA ACAUUCAUGUGCGA AUACGCCGACGAGA CAGCCACCAUCGUG GAGUUCCUGAACCG CUGGAUCACCUUCU GCCAGAGCAUCAUC UCUACUCUGACC			

Table IV: Exemplary IL-15 and IL-15RA sequences

Sequence ID No	Sequence ID Info	Sequence
14	IL-15	MRISKPHLRS ISIQCYLCLL LNSHFLTEAG IHVFILGCFS AGLPKTEANW VNVISDLKKI EDLIQSMHID ATLYTESDVH PSCKVTAMKC FLLELQVISL ESGDASIHDT VENLIILANN SLSSNGNVTE SGCKECEELE EKNIKEFLQS FVHIVQMFIN TS
15	IL-15	ATGAGAATTTCGAAACCACATTTGAGAAGTATTTCCATCCAG TGCTACTTGTGTTTACTTCTAAACAGTCATTTTCTAACTGAA GCTGGCATTCATGTCTTCATTTTGGGCTGTTTCAGTGCAGG GCTTCCTAAAACAGAAGCCAACTGGGTGAATGTAATAAGTG ATTTGAAAAAAATTGAAGATCTTATTCAATCTATGCATATTGA TGCTACTTTATATACGGAAAGTGATGTTCACCCCAGTTGCA AAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTA TTTCACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTA GAAAATCTGATCATCCTAGCAAACAACAGTTTGTCTTCTAAT GGGAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAACT GGAGGAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTACA TATTGTCCAAATGTTCATCAACACTTCTTGA
16	IL-15RA	MAPRRARGCRTLGLPALLLLLLLRPPATRGITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHW TTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSH GTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVL LCGLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSSR DEDLENCSHHL
24	IL-15RA	ATGGCCCCGCGGCGGGCGCGCGGCTGCCGGACCCTCGGTCT CCCGGCGCTGCTACTGCTGCTGCTGCTCCGGCCGCCGGCGAC GCGGGGCATCACGTGCCCTCCCCCCATGTCCGTGGAACACG CAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGGGAG CGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACG TCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGT CGCCCACTGGACAACCCCCAGTCTCAAATGCATTAGAGACC CTGCCCTGGTTCACCAAAGGCCAGCGCCACCCTCCACAGTA

type untranslated regions of a nucleic acid sequence are transcribed but not translated. In mRNA, the 5 ' UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the polynucleotides of the present disclosure to, among other things, enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites. A variety of 5' UTR and 3' UTR sequences are known and available in the art.

[00412] A 5' UTR is region of an mRNA that is directly upstream (5') from the start codon (the first codon of an mRNA transcript translated by a ribosome). A 5' UTR does not encode a protein (is non-coding). Natural 5' UTRs have features that play roles in translation initiation. They harbor signatures like Kozak sequences, which 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, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5' UTR also have been known to form secondary structures which are involved in elongation factor binding.

[00413] In some embodiments, the 5' UTR is a heterologous UTR, i.e., is a UTR found in nature associated with a different ORF. In another embodiment, a 5' UTR is a synthetic UTR, i.e., does not occur in nature. Synthetic UTRs include UTRs that have been mutated to improve their properties, e.g., which increase gene expression as well as those which are completely synthetic. Exemplary 5' UTRs include Xenopus or human derived a-globin or b- globin (8278063; 9012219), human cytochrome b-245 a polypeptide, and hydroxysteroid (17b) dehydrogenase, and Tobacco etch virus (U.S.8278063, 9012219, which are incorporated herein by reference in their entirety). CMV immediate-early 1 (IE1) gene (US2014/0206753, WO2013/185069, which are incorporated herein by reference in their entirety), the sequence GGGAUCCUACC (SEQ ID NO: 47) (WO2014/144196) may also be used. In another embodiment, 5' UTR of a TOP gene is a 5' UTR of a TOP gene lacking the 5' TOP motif (the oligopyrimidine tract) (e.g., WO/2015/101414, W02015/101415, WO/2015/062738, WO2015/024667, WO2015/024667; 5' UTR element derived from ribosomal protein Large 32 (L32) gene (WO/2015/101414, W02015/101415, WO/2015/062738), 5' UTR element derived from the 5' UTR of an hydroxysteroid (17-b) dehydrogenase 4 gene (HSD17B4) (WO2015/024667), or a 5' UTR element derived from the 5' UTR of ATP5A1 (WO2015/024667) can be used. In some embodiments, an internal ribosome entry site (IRES) is used instead of a 5' UTR.

[00414] A 3' UTR is region of an mRNA that is directly downstream (3') from the stop codon (the codon of an mRNA transcript that signals a termination of translation). A 3' UTR does not encode a protein (is non-coding). 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.

Embodiment 26. The mRNA therapeutic composition of embodiment 24, wherein the 3' UTR comprises a sequence derived from an amino-terminal enhancer of split (AES).

Embodiment 27. The mRNA therapeutic composition of embodiment 24, wherein the 3' UTR comprises a sequence derived from mitochondrially encoded 12S rRNA (mtRNRl).

Embodiment 28. The mRNA therapeutic composition of embodiment 15, wherein the mRNA comprises a poly(A) sequence.

Embodiment 29. The mRNA therapeutic composition of embodiment 28, wherein the poly(A) sequence is a 110-nucleotide sequence consisting of a sequence of 30 adenosine residues (SEQ ID NO: 44), a 10-nucleotide linker sequence, and a sequence of 70 adenosine residues (SEQ ID NO: 45).

Embodiment 30. The mRNA therapeutic composition of embodiment 1, wherein the LPMP is a lipophilic moiety selected from the group consisting of a lipoplex, a liposome, a lipid nanoparticle, a polymer-based carrier, an exosome, a lamellar body, a micelle, and an emulsion.

Embodiment 31. The mRNA therapeutic composition of embodiment 1, wherein the LPMP is a liposome selected from the group consisting of a cationic liposome, a nanoliposome, a proteoliposome, a unilamellar liposome, a multilamellar liposome, a ceramide-containing nanoliposome, and a multivesicular liposome.

Embodiment 32. The mRNA therapeutic composition of embodiment 1, wherein the LPMP is a lipid nanoparticle.

Embodiment 33. The mRNA therapeutic composition of embodiment 1, wherein the LPMP has a size of less than about 200 nm.

Embodiment 34. The mRNA therapeutic composition of embodiment 33, wherein the LPMP has a size of less than about 150 nm.

Embodiment 35. The mRNA therapeutic composition of embodiment 33, wherein the LPMP has a size of less than about 100 nm.

Embodiment 36. The mRNA therapeutic composition of embodiment 32, wherein the lipid nanoparticle has a size of about 55 nm to about 80 nm.

Embodiment 37. The mRNA therapeutic composition of embodiment 3, wherein the PEG-lipid conjugate is PEG-DMG or PEG-PE.

concentration using Zetasizer Ultra (Malvern Panalytical). The mRNA encapsulation efficiency was characterized by Quant-iT RiboGreen RNA Assay Kit (ThermoFisher Scientific). The particles were diluted to the desired mRNA concentration to get a final 10% sucrose solution in PBS. The formulations were then flash frozen in liquid nitrogen.

[00500] The resulting formulations with mRNA are shown in Table 2.

Table 2. The characterizations of a typical LPMP formulation with mRNA

Formulation	Ionizable: Structural: Sterol: PEG-lipid Molar ratio	Ionizable	Structural	Sterol	PEG-lipid	Cargo	N : P	Size (nm)	PDI	Encapsulation efficiency (%)
LNP	*35:16:46.5:2.5*	C12-200	DOPE	Chol	DMPE-PEG2k	an mRNA	15: 1	78.47	0.126	94.4
LPMP / mRNA A (recPMP1)	*35:50:12.5:2.5*	C12-200	Naturally derived lemon lipid	Chol	DMPE-PEG2k	an mRNA		96.49	0.300	94.8
LPMP / mRNA B (recPMP2)	*35:20:42.5:2.5*	C12-200	Naturally derived algae lipid	Chol	DMPE-PEG2k	an mRNA		93.96	0.206	96.3

Example 3. Administration of LPMP/mRNA formulation in mice

[00501] If not specified, the LPMP / mRNA formulation were prepared according to those described in Example 2. The coding portion of the EPO mRNA used in this example is as follows:

AUGGGCGUGCACGAGUGCCCCGCCUGGCUGUGGCUGCUGCUGAGCCUGCUGAGCCU
GCCCCUGGGCCUGCCCGUGCUGGGCGCCCCCCCCCGGCUGAUCUGCGACAGCCGGG
UGCUGGAGCGGUACCUGCUGGAGGCCAAGGAGGCCGAGAACAUCACCACCGGCUGC
GCCGAGCACUGCAGCCUGAACGAGAACAUCACCGUGCCCGACACCAAGGUGAACUUC
UACGCCUGGAAGCGGAUGGAGGUGGGCCAGCAGGCCGUGGAGGUGUGGCAGGGCCU
GGCCCUGCUGAGCGAGGCCGUGCUGCGGGGCCAGGCCCUGCUGGUGAACAGCAGCC
AGCCCUGGGAGCCCCUGCAGCUGCACGUGGACAAGGCCGUGAGCGGCCUGCGGAGC
CUGACCACCCUGCUGCGGGCCCUGGGCGCCCAGAAGGAGGCCAUCAGCCCCCCCGAC
GCCGCCAGCGCCGCCCCCCUGCGGACCAUCACCGCCGACACCUUCCGGAAGCUGUUC
CGGGUGUACAGCAACUUCCUGCGGGGCAAGCUGAAGCUGUACACCGGCGAGGCCUG
CCGGACCGGCGACCGGUGA (SEQ ID NO: 48)

[00502] Figure 1 shows the expression level of erythropoietin (EPO) in pg/mL in serum from Ai9 (tomato red loxP) mice treated orally with reconstructed LPMPs (recPMPs) derived from lemon and comprising Cre recombinase mRNA. Ai9 mice comprise a *loxP*-flanked STOP cassette, which prevents transcription of the red fluorescent protein tdTomato. These mice are used as a Cre reporter strain: tdTomato fluorescence is visible following Cre-mediated recombination. Fluorescence thus indicates that the Cre mRNA has been delivered.

[00503] Figure 2 shows the percentage of *in vivo* immune cells (CD-4 cells, CD-8 cells, or B cells) transfected from the spleens of Ai9 (tomato red loxP) mice treated with recLPMPs derived from lemon and comprising Cre recombinase mRNA. The control: a lipid nanoparticle (LNP) as control; or a phosphate-buffered saline (PBS).

[00504] Figure 9 shows the expression of EPO in serum following oral (intra-jejunal) delivery of

reconstructed lemon LPMPs comprising EPO mRNA in NHPs, wherein the reconstructed lemon LPMPs were administered intramuscularly (IM) at doses of 0.1 mg/kg on Day 1; 0.05 mg/kg on Day 8; and 0.05 mg/kg on Day 15 (Figure 5) or 0.1 mg/kg on Day 1; 0.05 mg/kg on Day 8; and 0.01 mg/kg on Day 15 (Figure 6).

[00522] Figure 7 shows the expression of EPO following repeat doses of reconstructed lemon LPMPs comprising EPO mRNA, wherein the reconstructed lemon LPMPs were administered subcutaneously (SubQ) at doses of 0.1 mg/kg on Day 1, 0.05 mg/kg on Day 8, and 0.01 mg/kg on Day 15. The concentration of hEPO in serum was shown pre-dose and at 6, 12, and 24 hours post-dose.

[00523] Figure 8 shows the results of a NHP study in which the expression of EPO mRNA was measured following repeat doses of reconstructed lemon LPMPs comprising EPO, wherein the reconstructed lemon LPMPs were administered SubQ at doses of 0.1 mg/kg on Day 1, 0.05 mg/kg on Day 8, and 0.05 mg/kg on Day 15. The concentration of hEPO in serum was shown pre-dose and at 6, 12, and 24 hours post-dose.

Example 5. Intramuscular and subcutaneous delivery of recLemon LPMP / GFP mRNA formulation transfected *in vivo* immune cells

[00524] Figure 11 shows the percentage of GFP-positive immune cells (CD4 T cells, CD8 T cells, and B cells) in cynomolgus macaques treated with intramuscularly administered (IM; top panel) or subcutaneously administered (SubQ; bottom panel) reconstructed lemon LPMPs comprising GFP mRNA.

Example 6. IL-2 induction of anti-tumor responses against MC-38 tumors with recLemon LPMP / mRNA formulation

[00525] This example describes a recLemon LPMP / mRNA formulation comprising a lemon lipid reconstructed with ionizable lipids (C12-200), sterols (cholesterol), and PEG lipids (DMPE-PEG2k), to encapsulate mRNA (e.g., mRNA for IL-2). The recLemon LPMP / mRNA formulation tested in this example was prepared according to Example 2. The coding portion of the IL-2 mRNA used in this example is as follows:

MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO: 49)

Treatment schedule

[00526] The anti-tumor response of the recLemon LPMP / mRNA formulation was evaluated in colorectal adenocarcinoma murine model (MC38). The design of subcutaneous delivery of the recLemon LPMP / mRNA formulation and evaluation is shown in Scheme 1.

Table 9

Payload	SEQ ID NO:	Sequence
tdTomato Cytosolic	50	ATGGTGAGCAAGGGCGAGGAGGTCATCAAAGAGTTCATGCGCTTCAAGGTGCGCAT GGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCG CCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTG CCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGT GAAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGGCTTCA AGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGTGACCCAGGA CTCCTCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCACCAACT TCCCCCCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCAC CGAGCGCCTGTACCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGGCCCTG AAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGACCATCTACATGGCCAA GAAGCCCGTGCAACTGCCCGGCTACTACTACGTGGACACCAAGCTGGACATCACCT CCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCTCCGAGGGCCGCCA CCACCTGTTCCTGGGGCATGGCACCGGCAGCACCGGCAGCGGCAGCTCCGGCACC GCCTCCTCCGAGGACAACAACATGGCCGTCATCAAAGAGTTCATGCGCTTCAAGGT GCGCATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAG GGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGC CCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGC GTACGTGAAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGG GCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGTGAC CCAGGACTCCTCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGC ACCAACTTCCCCCCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGG CCTCCACCGAGCGCCTGTACCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCA GGCCCTGAAGCTGAAGGACGGCGGCCGCTACCTGGTGGAGTTCAAGACCATCTACA TGGCCAAGAAGCCCGTGCAACTGCCCGGCTACTACTACGTGGACACCAAGCTGGAC ATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCTCCGAGGG CCGCCACCACCTGTTCCTGTACGGCATGGACGAGCTGTACAAGTAA
tdTomato Secreted	51	ATGTGGTGGCGCCTGTGGTGGCTGCTGCTGCTGCTGCTGCTGCTGTGGCCCATGGT GTGGGCCGTGAGCAAGGGCGAGGAGGTCATCAAAGAGTTCATGCGCTTCAAGGTG CGCATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGG GCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCC CCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGT ACGTGAAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGGC TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGTGACCC AGGACTCCTCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCAC CAACTTCCCCCCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCC TCCACCGAGCGCCTGTACCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGG CCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGACCATCTACATG GCCAAGAAGCCCGTGCAACTGCCCGGCTACTACTACGTGGACACCAAGCTGGACAT CACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCTCCGAGGGCC GCCACCACCTGTTCCTGGGGCATGGCACCGGCAGCACCGGCAGCGGCAGCTCCGG CACCGCCTCCTCCGAGGACAACAACATGGCCGTCATCAAAGAGTTCATGCGCTTCA AGGTGCGCATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGG CGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGC GGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAA GGCGTACGTGAAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCG AGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGT GACCCAGGACTCCTCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGC GGCACCAACTTCCCCCCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGG AGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGCGTGCTGAAGGGCGAGATCCA CCAGGCCCTGAAGCTGAAGGACGGCGGCCGCTACCTGGTGGAGTTCAAGACCATC TACATGGCCAAGAAGCCCGTGCAACTGCCCGGCTACTACTACGTGGACACCAAGCT GGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCTCCG AGGGCCGCCACCACCTGTTCCTGTACGGCATGGACGAGCTGTACAAGTAA
tdTomato Membrane	52	ATGGGCTGCGTGTGCAGCAGCAACCCCGAGAACAACAACAACATGGTGAGCAAGG GCGAGGAGGTCATCAAAGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCATG AACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGC ACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGG ACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTGAAGCACCCCGCC GACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGT GATGAACTTCGAGGACGGCGGTCTGGTGACCGTGACCCAGGACTCCTCCCTGCAG GACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCACCAACTTCCCCCCCGACG GCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTA

		CCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGGCCCTGAAGCTGAAGGAC GGCGGCCACTACCTGGTGGAGTTCAAGACCATCTACATGGCCAAGAAGCCCGTGCA ACTGCCCGGCTACTACTACGTGGACACCAAGCTGGACATCACCTCCCACAACGAGG ACTACACCATCGTGGAACAGTACGAGCGCTCCGAGGGCCGCCACCACCTGTTCCTG GGGCATGGCACCGGCAGCACCGGCAGCGGCAGCTCCGGCACCGCCTCCTCCGAG GACAACAACATGGCCGTCATCAAAGAGTTCATGCGCTTCAAGGTGCGCATGGAGGG CTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTAC GAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCG CCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTGAAGCAC CCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGA GCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGTGACCCAGGACTCCTCC CTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCACCAACTTCCCCCC CGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGC CTGTACCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGGCCCTGAAGCTGA AGGACGGCGGCCGCTACCTGGTGGAGTTCAAGACCATCTACATGGCCAAGAAGCCC GTGCAACTGCCCGGCTACTACTACGTGGACACCAAGCTGGACATCACCTCCCACAA CGAGGACTACACCATCGTGGAACAGTACGAGCGCTCCGAGGGCCGCCACCACCTG TTCCTGTACGGCATGGACGAGCTGTACAAGTAA
eGFP	53	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGC TGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGA TGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCG TGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGC TACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCG AGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGA CTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCC ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGA TCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAA CACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGA GTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
IL-2	54	ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACA GTGCACCCACCAGCAGCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCACCTGCT GCTGGACCTGCAGATGATCCTGAACGGCATCAACAACTACAAGAACCCCAAGCTGA CCAGAATGCTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCAC CTGCAGTGCCTGGAGGAGGAGCTGAAGCCCCTGGAGGAGGTGCTGAACCTGGCCC AGAGCAAGAACTTCCACCTGAGACCCAGAGACCTGATCAGCAACATCAACGTGATC GTGCTGGAGCTGAAGGGCAGCGAGACCACCTTCATGTGCGAGTACGCCGACGAGA CCGCCACCATCGTGGAGTTCCTGAACAGATGGATCACCTTCAGCCAGAGCATCATC AGCACCCTGACCTGA
sumIL-2	55	ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACA GTGCACCCACCAGCAGCAGCACCAAGAAGACCCAGCTGCAGCTGGAGCACCTGCT GCTGGACCTGCAGATGATCCTGAACGGCATCAACAACTACAAGAACCCCAAGCTGA CCAGAATGCTGACCGCCAAGTTCTACATGCCCAAGAAGGCCACCGAGCTGAAGCAC CTGCAGTGCCTGGAGGAGGAGCTGAAGCCCCTGGAGGAGGTGCTGAACCTGGCCC AGAGCAAGAACTTCCACTTCGACCCCAGAGACGTGGTGAGCAACATCAACGTGTTC GTGCTGGAGCTGAAGGGCAGCGAGACCACCTTCATGTGCGAGTACGCCGACGAGA CCGCCACCATCGTGGAGTTCCTGAACAGATGGATCACCTTCAGCCAGAGCATCATC AGCACCCTGACCTGA
IL-2 + IL-2-Ra	56	ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACA GTAGCAAGAACTTCCACCTGAGACCCAGAGACCTGATCAGCAACATCAACGTGATC GTGCTGGAGCTGAAGGGCAGCGAGACCACCTTCATGTGCGAGTACGCCGACGAGA CCGCCACCATCGTGGAGTTCCTGAACAGATGGATCACCTTCAGCCAGAGCATCATC AGCACCCTGACCGGCGGCAGCAGCAGCACCAAGAAGACCCAGCTGCAGCTGGAGC ACCTGCTGCTGGACCTGCAGATGATCCTGAACGGCATCAACAACTACAAGAACCCC AAGCTGACCAGAATGCTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGAGCT GAAGCACCTGCAGTGCCTGGAGGAGGAGCTGAAGCCCCTGGAGGAGGTGCTGAAC CTGGCCCAGGGCAGCGGCGGCGGCAGCGAGCTGTGCGACGACGACCCCCCCGAG ATCCCCCACGCCACCTTCAAGGCCATGGCCTACAAGGAGGGCACCATGCTGAACTG CGAGTGCAAGAGAGGCTTCAGAAGAATCAAGAGCGGCAGCCTGTACATGCTGTGCA CCGGCAACAGCAGCCACAGCAGCTGGGACAACCAGTGCCAGTGCACCAGCAGCGC CACCAGAAACACCACCAAGCAGGTGACCCCCCAGCCCGAGGAGCAGAAGGAGAGA AAGACCACCGAGATGCAGAGCCCCATGCAGCCCGTGGACCAGGCCAGCCTGCCCG GCCACTGCAGAGAGCCCCCCCCCTGGGAGAACGAGGCCACCGAGAGAATCTACCA CTTCGTGGTGGGCCAGATGGTGTACTACCAGTGCGTGCAGGGCTACAGAGCCCTGC

		ACAGAGGCCCCGCCGAGAGCGTGTGCAAGATGACCCACGGCAAGACCAGATGGAC CCAGCCCCAGCTGATCTGCACCGGCTGA
Anti-CD19 & CD20 CAR-T	57	ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCCTTCCT TTTGATTCCAGAGGTGCAATTACAGCAAAGCGGTGCAGAACTCGTGAAGCCAGGCG CTTCAGTAAAAATGTCCTGCAAAGCTTCCGGCTACACATTCACTAGCTACAACATGCA TTGGGTTAAACAAACACCTGGCCAGGGATTGGAGTGGATCGGGGCTATATATCCGG GTAACGGTGACACTAGTTACAATCAGAAATTCAAGGGAAAGGCCACGCTTACAGCAG ACAAGAGTAGCAGTACCGCCTATATGCAACTCAGCAGCTTGACCAGCGAAGACAGC GCAGATTATTACTGTGCCAGATCCAACTATTACGGCTCAAGCTACTGGTTCTTTGAC GTGTGGGGCGCCGGAACCACTGTTACTGTCAGCAGCGGAGGCGGTGGGAGTGGCG GCGGTGGTTCCGGCGGGGGCGGGTCAGATATTGTGTTGACGCAATCCCCCGCAAT CCTGTCCGCGTCCCCAGGCGAAAAAGTGACAATGACATGCCGGGCCAGCTCAAGC GTGAATTACATGGATTGGTATCAGAAGAAACCCGGGAGCAGCCCCAAGCCGTGGAT CTATGCGACGAGTAACCTGGCTTCCGGCGTCCCCGCCCGATTCAGCGGGTCCGGC AGCGGCACTAGTTACAGTTTAACCATCAGCAGGGTGGAGGCTGAAGACGCGGCAAC CTATTATTGCCAGCAGTGGTCATTTAATCCTCCCACGTTTGGCGGCGGCACCAAGCT GGAGATAAAGGGCGGGGGGGGCAGTGGAGGCGGAGGTAGCGGCGGAGGAGGATC AGGAGGGGGGGGTAGCGGAGGAGGGGGTTCAGACATACAGATGACACAGACAACC TCGAGCCTGAGCGCATCCCTGGGGGACCGAGTCACCATCTCATGTAGAGCATCGCA GGACATCAGCAAATATCTCAACTGGTACCAGCAAAAACCCGATGGTACAGTGAAACT CTTAATTTACCACACATCCCGGTTACATAGCGGAGTCCCAAGCCGCTTTAGCGGATC CGGAAGCGGGACCGACTACTCCCTCACGATCAGCAACCTCGAACAGGAGGATATAG CCACTTATTTCTGCCAGCAGGGTAACACCCTGCCGTATACATTTGGCGGGGGCACA AAACTAGAAATCACAGGCAGTACAAGTGGTAGTGGGAAGCCCGGTAGCGGAGAAGG TAGCACGAAAGGCGAGGTTAAACTTCAGGAATCAGGCCCAGGCCTAGTTGCCCCTT CACAAAGTCTAAGCGTCACGTGTACCGTGTCCGGCGTAAGCCTGCCAGATTATGGA GTCAGCTGGATTAGGCAGCCTCCACGGAAGGGACTGGAGTGGCTGGGCGTGATTT GGGGGTCGGAGACCACCTATTATAACAGCGCGCTGAAATCGCGGCTGACTATTATT AAGGATAATAGCAAGTCACAGGTGTTTCTTAAGATGAATAGCCTGCAGACTGATGAC ACTGCGATATACTACTGTGCCAAGCACTACTACTACGGAGGGTCATATGCTATGGAT TACTGGGGGCAGGGAACCTCCGTGACTGTAAGTTCCGCCGCCGCTACTACAACCCC TGCCCCACGTCCCCCGACCCCAGCACCTACTATCGCATCCCAGCCCCTTTCCCTTC GCCCTGAGGCCTGCAGGCCTGCAGCTGGAGGAGCCGTTCACACCAGGGGCCTCGA CTTTGCATGCGATATTTACATCTGGGCCCCACTGGCTGGCACCTGCGGCGTCTTGCT GCTGAGCCTCGTGATCACCCTCTATTGTAAGAGGGGGCGAAAAAAGCTCCTGTACAT TTTCAAACAGCCTTTCATGAGACCTGTGCAGACAACACAGGAAGAGGACGGTTGCTC CTGTAGGTTTCCGGAGGAAGAAGAGGGAGGGTGTGAATTGCGTGTAAAGTTCTCCA GATCAGCTGATGCCCCAGCCTATCAACAAGGACAGAATCAGCTGTACAACGAGCTTA ATTTGGGGCGGAGAGAGGAATACGATGTCTTGGACAAGAGACGGGGGCGCGACCC CGAGATGGGCGGGAAGCCTAGAAGGAAGAACCCTCAGGAGGGGCTGTATAATGAG CTCCAAAAGGATAAGATGGCCGAAGCTTACAGCGAGATCGGGATGAAAGGAGAGCG CCGCCGCGGAAAAGGCCATGATGGGTTATACCAGGGCCTTAGCACCGCCACTAAGG ACACTTACGACGCTCTACACATGCAGGCTCTCCCACCCCGATGA
Anti-HER2 Heavy Chain	58	ATGAGGGCTTGGATCTTCTTTCTGCTCTGCCTGGCCGGGCGCGCCTTGGCCGAAGT TCAACTGGTAGAGAGTGGAGGTGGGCTTGTGCAACCAGGCGGATCCTTGCGACTGT CCTGCGCCGCTTCAGGCTTCAACATCAAGGACACCTACATCCATTGGGTCCGCCAG GCACCAGGAAAAGGTCTTGAATGGGTGGCCAGAATCTACCCTACTAACGGTTACAC CAGATATGCAGACTCCGTTAAGGGGCGATTTACCATTTCAGCAGACACCTCTAAGAA CACCGCTTACCTGCAGATGAACTCACTTCGAGCTGAGGACACCGCCGTTTACTATTG CAGCAGATGGGGCGGTGACGGCTTCTACGCTATGGATTACTGGGGACAGGGGACTT TGGTAACTGTGAGTAGTGCATCTACAAAGGGGCCAAGCGTGTTCCCACTTGCCCCAT CTTCTAAAAGCACCTCAGGAGGGACTGCAGCCTTGGGTTGCTTGGTTAAAGATTATT TTCCAGAGCCTGTAACTGTATCCTGGAATAGTGGGGCCCTCACAAGCGGAGTACATA CTTTCCCTGCAGTATTGCAGTCTAGTGGACTCTACTCTCTCAGCAGTGTAGTGACCG TACCTTCCAGTTCACTTGGAACACAGACCTATATTTGCAATGTGAATCATAAGCCATC TAATACTAAAGTGGATAAGAAAGTGGAGCCTAAATCTTGTGACAAGACTCATACATGC CCTCCCTGCCCTGCCCCTGAACTGTTGGGAGGGCCCTCTGTATTTCTCTTCCCCCCT AAACCAAAGGACACCCTGATGATCAGTCGAACTCCTGAGGTGACTTGTGTGGTTGTT GACGTGTCACATGAGGATCCCGAAGTGAAATTCAACTGGTACGTCGATGGAGTAGA GGTACACAATGCAAAGACAAAACCTAGGGAGGAACAGTATAATTCTACCTATAGAGT GGTGTCTGTTCTCACAGTTCTCCATCAAGACTGGTTGAACGGTAAAGAATATAAATG CAAAGTCTCCAATAAGGCTTTGCCCGCTCCCATTGAAAAAACAATCAGTAAAGCCAA AGGCCAGCCACGCGAACCACAGGTCTACACCCTTCCACCATCTCGCGAAGAAATGA CTAAGAATCAAGTGTCACTCACATGCCTCGTCAAGGGCTTTTACCCTTCTGATATTGC AGTTGAATGGGAGTCTAACGGGCAGCCCGAGAATAATTACAAGACTACCCCCCCCG TCCTTGACTCTGATGGCAGCTTTTTCCTGTACTCAAAATTGACTGTGGACAAGTCTCG

		ATGGCAACAGGGTAATGTTTTCTCCTGTAGCGTAATGCACGAGGCCCTTCATAACCA TTATACCCAAAAATCTCTTTCATTGTCCCCTGGAAAATGA
Anti-HER2 Light Chain	59	ATGAGGGCTTGGATCTTCTTTCTGCTCTGCCTGGCCGGGCGCGCCTTGGCCGATAT TCAGATGACTCAGAGCCCCAGCAGCCTGTCTGCTAGCGTTGGGGATAGAGTCACTA TAACATGTCGGGCTTCCCAGGATGTGAATACTGCTGTCGCTTGGTATCAACAGAAGC CCGGCAAGGCACCAAAACTGCTGATATATAGTGCCTCTTTCCTTTACTCCGGGGTTC CCAGTCGATTCTCTGGAAGCCGCAGCGGCACTGATTTCACACTTACTATAAGTAGTC TGCAACCTGAGGACTTTGCTACATACTACTGCCAGCAGCACTATACAACACCCCCAA CTTTCGGGCAAGGCACTAAGGTAGAAATTAAAAGGACCGTTGCTGCTCCATCCGTCT TTATTTTTCCACCATCTGATGAACAGTTGAAGAGTGGAACAGCAAGCGTCGTTTGTCT CCTGAACAATTTTTACCCACGAGAGGCAAAAGTTCAATGGAAGGTAGACAATGCCCT TCAGAGCGGCAATTCCCAGGAGAGCGTAACCGAGCAGGATAGCAAAGACTCTACAT ACTCTTTGAGTTCAACCCTTACCCTGAGCAAGGCTGATTACGAAAAACACAAGGTGT ACGCTTGCGAGGTAACCCATCAGGGATTGTCATCCCCAGTCACAAAATCATTCAATA GGGGCGAGTGCTGA
Keytruda	60	AUGGAAUUUGGACUGUCUUGGGUUUUUCUGGUAGCUCUGUUUCGUGGAGUGCAA UGCCAGGUUCAGUUGGUCCAGUCGGGUGUGGAGGUUAAAAAGCCCGGCGCCUCU GUGAAGGUUAGCUGCAAGGCUAGCGGAUAUACUUUCACCAAUUACUACAUGUAUU GGGUGAGGCAGGCUCCAGGGCAGGGACUCGAGUGGAUGGGAGGCAUCAACCCGU CCAACGGCGGCACCAACUUCAAUGAGAAGUUCAAAAAUAGAGUGACUCUCACCAC UGAUAGUUCCACGACAACCGCAUACAUGGAACUGAAAAGUCUGCAAUUCGAUGAC ACCGCCGUGUAUUACUGCGCCCGCAGAGACUACCGGUUUGACAUGGGAUUUGAU UACUGGGGUCAGGGCACCACAGUGACAGUCUCAUCCGCCUCUACCAAAGGACCCU CGGUUUUUCCACUCGCACCUUGCUCCCGAAGCACCUCAGAGUCCACCGCGGCCC UUGGGUGUCUGGUGAAAGAUUAUUUCCCCGAACCAGUGACCGUGUCAUGGAAUU CAGGGGCACUGACAAGUGGGGUCCACACGUUCCCAGCGGUGCUGCAGUCCUCCG GGCUGUAUAGCCUUAGCUCUGUAGUCACUGUCCCGUCCAGUUCCCUGGGGACUA AGACUUACACAUGUAACGUAGAUCACAAACCUAGUAACACCAAGGUGGACAAGCG CGUGGAGUCAAAGUACGGACCUCCAUGUCCCCCAUGUCCCGCCCCCGAAUUUUU GGGAGGACCUUCAGUGUUCCUGUUCCCUCCAAAACCUAAAGACACACUGAUGAUA AGCAGGACACCCGAGGUCACCUGCGUCGUGGUCGACGUAAGCCAGGAAGAUCCA GAGGUACAGUUCAACUGGUACGUCGACGGUGUAGAGGUCCAUAAUGCCAAGACAA AGCCCAGGGAGGAGCAGUUUAACUCAACAUAUCGUGUUGUGUCUGUGUUGACGG UUCUACAUCAAGACUGGCUAAACGGCAAGGAGUAUAAAUGCAAGGUUAGUAAUAA GGGCCUCCCCAGCUCUAUUGAGAAAACUAUCAGCAAAGCUAAGGGCCAGCCUCGG GAACCACAAGUUUACACUUUACCGCCCAGCCAGGAGGAAAUGACGAAGAAUCAGG UGAGCCUGACAUGUCUUGUCAAGGGGUUUUACCCCUCUGAUAUCGCUGUAGAAU GGGAAUCCAAUGGUCAACCUGAAAACAACUACAAGACCACCCCGCCUGUGCUCGA CUCUGACGGCAGCUUUUUCCUGUAUUCGAGACUUACAGUGGAUAAAAGCCGGUG GCAGGAGGGCAAUGUGUUUUCCUGCAGUGUGAUGCACGAAGCAUUACACAACCAU UAUACUCAAAAAUCACUCAGUUUGUCUCUGGGGAAG
Epogen	61	AUGGGGGUCCACGAAUGCCCGGCAUGGCUGUGGUUACUGCUAUCGCUGCUCAGC CUGCCACUGGGCCUGCCCGUACUUGGCGCCCCUCCACGCCUCAUCUGUGACUCC CGGGUCCUGGAGCGGUACCUUUUGGAGGCCAAAGAAGCAGAGAAUAUCACUACA GGCUGCGCUGAGCACUGUAGUUUGAACGAAAAUAUAACAGUACCUGACACAAAGG UGAAUUUUUACGCCUGGAAAAGAAUGGAGGUGGGCCAACAGGCAGUGGAAGUGU GGCAGGGUCUCGCUUUACUUAGCGAGGCCGUGCUGCGAGGUCAGGCACUCCUAG UUAACUCCAGCCAGCCCUGGGAGCCACUCCAGUUGCAUGUGGAUAAGGCCGUCU CUGGACUAAGAUCCCUCACCACCCUGCUGAGGGCAUUGGGAGCGCAAAAGGAGG CUAUUUCACCUCCCGAUGCCGCUAGUGCUGCGCCCCUUCGCACUAUCACCGCCG ACACGUUCCGUAAGCUGUUCAGGGUUUAUUCAAACUUUCUCCGGGGGAAGCUGA AACUGUAUACUGGGGAAGCCUGCAGAACCGGAGAUAGG
Enbrel	62	AUGAAAUGGGUUACGUUCAUCUCCUUGCUGUUCCUGUUCUCUUCCGCUUACUCC CUGCCAGCCCAGGUCGCUUUCACGCCAUACGCUCCUGAACCAGGUAGCACUUGU CGUCUACGGGAAUACUAUGACCAGACUGCACAGAUGUGCUGUUCCAAGUGCUCUC CUGGCCAACAUGCCAAAGUGUUUUGCACUAAGACUAGCGAUACAGUGUGCGAUAG CUGCGAGGAUUCUACAUAUACCCAGCUCUGGAACUGGGUUCCCGAGUGUCUCAG CUGUGGCAGCCGGUGUAGUAGCGACCAGGUGGAGACCCAGGCCUGCACACGAGA GCAGAACCGGAUCUGCACAUGUAGACCCGGGUGGUACUGUGCGUUGAGUAAACA GGAGGGAUGUAGACUCUGCGCCCCUCUUAGGAAGUGCCGCCCAGGGUUUGGAGU CGCACGCCCAGGCACAGAAACCAGUGACGUCGUCUGCAAGCCGUGUGCCCCGGG CACAUUCUCCAACACUACCUCAAGUACAGAUAUUUGUAGACCUCAUCAGAUCUGC AAUGUCGUGGCAAUUCCCGGUAACGCCUCGAUGGAUGCCGUGUGCACCUCUACG UCGCCCACUAGGUCCAUGGCCCCAGGAGCAGUACACUUACCUCAGCCUGUUAGCA CACGCUCCCAGCACACGCAACCCACCCCUGAGCCUUCCACUGCACCGUCCACCAG

		CUUCCUGCUGCCCAUGGGCCCAAGUCCUCCAGCGGAGGGCAGCACAGGGGACGA GCCAAAGUCCUGCGACAAAACCCAUACUUGUCCCCCUUGCCCCGCACCCGAACUC CUUGGGGGACCAUCAGUAUUCUUGUUUCCCCCCAAGCCAAAGGACACAUUAAUGA UUUCUAGGACUCCGGAGGUGACUUGCGUCGUUGUUGACGUGAGCCACGAAGACC CAGAGGUGAAAUUCAACUGGUAUGUGGAUGGAGUCGAAGUUCACAAUGCGAAAAC CAAACCUCGAGAGGAACAGUACAACUCAACCUAUAGGGUAGUGAGUGUUCUGACC GUGCUGCACCAGGACUGGCUCAAUGGGAAGGAAUACAAGUGUAAGGUAUCCAAUA AAGCCCUGCCUGCCCCUAUCGAAAAGACAAUAAGCAAGGCUAAGGGGCAACCUCG CGAACCCCAGGUGUACACGCUGCCACCAAGCAGAGAGGAGAUGACAAAAAACCAA GUGUCACUGACCUGCCUGGUGAAAGGCUUUUACCCGAGCGAUAUCGCUGUGGAG UGGGAGUCAAACGGACAACCCGAGAAUAACUAUAAAACCACGCCCCCCGUGCUGG AUUCAGACGGUUCUUUCUUCCUUUAUUCCAAGUUGACCGUGGACAAGUCUCGGU GGCAGCAGGGCAAUGUCUUUUCCUGUAGUGUCAUGCACGAAGCUCUACACAAUCA UUACACCCAAAAAAGCCUGUCACUCUCUCCCGGUAAA
Filgrastim	63	AUGGCAGGCCCAGCUACGCAGAGCCCAAUGAAACUGAUGGCACUGCAGCUCCUG UUAUGGCACAGCGCCCUGUGGACCGUCCAGGAAGCAACACCCCUCGGGCCUGCG UCCAGUCUCCCCCAGAGCUUUCUACUUAAAUGUCUAGAGCAGGUGCGGAAGAUCC AAGGAGACGGGGCCGCACUGCAGGAGAAGCUCUGCGCUACAUACAAGCUUUGUC ACCCAGAGGAACUGGUGCUGUUAGGCCAUUCACUGGGAAUCCCUUGGGCCCCUU UGUCUAGUUGCCCGUCCCAGGCUUUACAGUUGGCCGGCUGCCUGAGCCAGCUGC ACUCCGGACUAUUUCUGUAUCAGGGACUUUUGCAGGCCCUGGAGGGUAUUUCCC CUGAGCUCGGGCCGACUCUGGAUACACUGCAGCUUGAUGUCGCCGACUUUGCUA CUACCAUAUGGCAACAAAUGGAGGAACUCGGUAUGGCCCCAGCGCUGCAACCCAC CCAGGGGGCCAUGCCCGCUUUCGCGUCUGCCUUCCAGCGCAGAGCAGGCGGCGU GUUGGUGGCCUCACAUCUCCAAUCGUUCCUGGAAGUUUCUUACCGAGUACUCAG GCACUUGGCACAGCCC
Humira	38	AUGGAAUUUGGACUGUCUUGGGUUUUUCUGGUAGCUCUGUUUCGUGGAGUGCAA UGCGAGGUUCAGCUUGUCGAGAGCGGCGGCGGUCUCGUGCAACCCGGACGGAG UCUGAGACUCAGCUGUGCUGCUUCAGGAUUUACCUUCGACGAUUAUGCGAUGCA UUGGGUGCGGCAGGCUCCUGGCAAAGGAUUAGAAUGGGUGAGCGCAAUUACAUG GAACUCUGGCCACAUCGACUACGCCGAUUCAGUGGAGGGCAGGUUCACCAUUAG CAGAGAUAAUGCUAAGAAUAGCUUAUACUUGCAGAUGAACAGCCUGCGCGCCGAG GAUACUGCGGUGUACUAUUGCGCCAAGGUCAGUUAUCUGUCCACCGCCAGCUCC CUAGAUUAUUGGGGGCAGGGAACCUUGGUGACAGUCAGUUCCGCAAGCACGAAA GGGCCCAGCGUUUUCCCUCUCGCACCCUCUUCAAAAUCCACUUCAGGGGGGACA GCUGCACUGGGAUGUCUUGUCAAGGAUUACUUCCCCGAACCUGUGACCGUCUCA UGGAAUUCCGGUGCCUUGACCUCAGGGGUGCAUACCUUCCCCGCAGUCCUUCAG AGUUCUGGACUCUACUCCCUGAGUUCUGUGGUAACAGUCCCUUCUAGCUCCCUC GGGACACAGACCUACAUUUGCAAUGUGAACCAUAAACCGAGUAACACUAAAGUGG ACAAAAAAGUAGAACCCAAAAGCUGCGAUAAGACCCACACGUGCCCCCCGUGUCC GGCCCCCGAGCUUCUGGGCGGCCCUUCGGUGUUCCUGUUUCCACCUAAGCCCAA AGACACACUGAUGAUCUCUCGCACUCCUGAAGUAACAUGCGUGGUGGUCGACGU GAGCCACGAAGAUCCAGAAGUUAAGUUCAAUUGGUAUGUUGACGGGGUGGAGGU GCACAACGCCAAGACUAAGCCAAGGGAGGAGCAAUACAAUUCCACAUACCGAGUU GUCAGCGUACUUACGGUUCUGCACCAAGACUGGCUAAAUGGUAAAGAGUACAAGU GUAAGGUGUCUAACAAAGCCCUGCCAGCGCCGAUAGAAAAGACUAUCUCAAAAGC AAAGGGCCAGCCCAGAGAACCUCAGGUGUACACCUUGCCACCAUCAAGGGACGAG CUCACCAAGAAUCAGGUUAGUCUCACCUGUCUGGUGAAGGGGUUCUAUCCAUCC GACAUCGCCGUGGAGUGGGAGAGUAACGGCCAGCCCGAAAACAAUUACAAGACGA CACCACCAGUCUUGGAUAGCGACGGUUCUUUCUUUCUAUAUUCGAAGCUCACUGU CGACAAGUCGCGGUGGCAGCAGGGCAACGUAUUUAGCUGCUCCGUCAUGCAUGA GGCCCUGCACAACCACUAUACUCAAAAAUCCCUGUCCUUAUCUCCUGGCAAG
Rituximab	39	AUGAAGUAUCUACUGCCUACCGCUGCAGCUGGGCUGUUGCUUCUGGCUGCUCAA CCAGCCAUGGCUCAGAUCGUGCUCUCCCAGUCCCCCGCGAUUUUGAGCGCAUCC CCAGGCGAGAAGGUAACAAUGACGUGCCGCGCGAGCAGUUCAGUGUCUUACAUC CACUGGUUCCAGCAAAAGCCAGGCUCUAGCCCCAAGCCCUGGAUUUACGCAACCA GCAAUCUUGCCAGUGGCGUCCCGGUGAGGUUCUCGGGGUCUGGAAGCGGUACAU CUUAUAGUCUCACCAUAAGCAGGGUAGAGGCCGAGGACGCCGCUACUUACUAUUG CCAGCAGUGGACCUCUAACCCACCCACCUUCGGCGGUGGAACCAAGCUGGAAAUC AAACGAACAGUCGCCGCCCCGUCCGUGUUUAUCUUCCCUCCUUCUGACGAGCAG CUGAAAUCCGGGACCGCAUCCGUCGUUUGUCUACUGAACAACUUUUACCCCCGG GAAGCAAAAGUCCAGUGGAAGGUUGAUAACGCCCUCCAAAGUGGAAAUAGCCAGG AAUCAGUGACUGAGCAAGAUUCCAAAGAUAGUACUUACAGCUUAUCAAGCACGCU CACUCUGUCCAAAGCCGACUAUGAAAAACACAAGGUGUACGCCUGUGAGGUGACA CAUCAGGGGCUGUCAUCACCUGUUACAAAGUCGUUUAAUAGAGGCGAAUGC

Romiplostim	40	AUGAAAUGGGUUACGUUCAUCUCCUUGCUGUUCCUGUUCUCUUCCGCUUACUCC GAUAAAACACAUACCUGUCCCCCAUGUCCCGCCCCCGAGCUCCUCGGCGGUCCCA GUGUGUUUCUGUUUCCACCAAAACCCAAGGACACUCUCAUGAUAAGCCGCACGCC CGAGGUUACAUGCGUCGUCGUCGACGUCUCCCACGAGGAUCCGGAGGUGAAAUU UAACUGGUAUGUAGAUGGCGUGGAAGUGCACAACGCAAAAACCAAGCCCCGUGAA GAACAAUACAACUCCACAUACAGGGUCGUGAGCGUGCUGACUGUACUCCAUCAGG AUUGGUUAAAUGGCAAGGAGUAUAAGUGUAAGGUGAGUAAUAAGGCCCUACCUGC ACCUAUUGAAAAGACAAUUAGCAAAGCCAAGGGCCAGCCGAGGGAGCCACAGGUG UAUACACUGCCACCCAGCCGGGAUGAGUUAACAAAGAAUCAGGUUUCACUCACCU GCCUUGUAAAAGGUUUUUACCCAUCAGACAUCGCUGUGGAAUGGGAGUCUAACG GACAGCCUGAAAACAACUAUAAAACCACUCCUCCUGUGCUGGACUCUGACGGAUC GUUCUUCUUGUACUCAAAACUAACCGUGGACAAGAGUAGAUGGCAGCAGGGAAAU GUUUUCUCAUGCAGCGUCAUGCACGAGGCCCUGCAUAAUCACUACACCCAGAAGA GUUUGUCUCUGAGCCCGGGUAAGGGAGGCGGCGGGGGCAUCGAAGGGCCUACU CUGAGACAAUGGCUGGCAGCCCGGGCUGGCGGGGGUGGAGGGGGGGGAGGCAU CGAGGGGCCAACCCUUCGCCAAUGGCUUGCGGCGCGAGCC
Sargramostim	41	AUGUGGCUGCAAUCCCUGCUGCUGCUUGGCACAGUGGCAUGCUCCAUCAGCGCU CCUGCCCGAUCUCCCUCACCCAGCACACAACCCUGGGAGCAUGUCAAUGCGAUAC AGGAGGCCCUCCGGCUCUUAAAUCUCUCGCGCGAUACUGCAGCUGAGAUGAACG AAACCGUGGAAGUGAUCAGUGAAAUGUUCGAUCUGCAGGAACCCACCUGUCUGCA GACCAGGCUAGAGUUGUACAAACAGGGCUUGAGAGGAUCACUAACUAAACUUAAG GGGCCUCUGACGAUGAUGGCCUCCCACUAUAAGCAGCACUGUCCACCAACUCCAG AAACAAGUUGCGCCACACAGAUCAUUACCUUUGAGAGCUUUAAGGAGAACUUAAA AGACUUCCUCCUGGUUAUUCCUUUCGACUGCUGGGAGCCGGUACAGGAG
Anti-CD19	42	AUGGGUGUCCCUACCCAGCUCCUGGGACUGCUCCUGCUGUGGAUCACCGACGCC AUCUGCGACAUCCAGAUGACCCAGAGCCCUGCCAGCCUGUCUACCAGCCUGGGC GAGACAGUGACCAUCCAGUGUCAGGCCAGCGAGGACAUCUACUCUGGCCUGGCU UGGUAUCAGCAGAAGCCCGGCAAGAGCCCUCAGCUGCUGAUCUACGGCGCCAGC GACCUGCAGGACGGCGUGCCUAGCAGAUUCAGCGGCAGCGGCUCCGGAACCCAG UACAGCCUGAAGAUCACCAGCAUGCAGACCGAGGACGAGGGCGUGUACUUCUGC CAGCAAGGCCUGACCUACCCUAGAACCUUCGGAGGAGGCACCAAGCUGGAACUGA AGGGCGGAGGCGGAAGUGGAGGCGGAGGAUCUGGCGGCGGAGGCUCUGAAGUG CAGCUGCAGCAGUCUGGCGCUGAACUGGUCCGGCCUGGCACUAGCGUGAAGCUG UCCUGCAAGGUGUCCGGCGACACCAUCACCUUCUACUACAUGCACUUCGUGAAGC AGAGGCCAGGACAGGGCCUGGAAUGGAUCGGCAGAAUCGACCCUGAGGACGAGA GCACCAAGUACAGCGAGAAGUUCAAGAACAAGGCCACCCUGACCGCCGACACCAG CAGCAACACCGCCUACCUGAAGCUGUCUAGCCUGACCUCCGAGGACACCGCCACC UACUUUUGCAUCUACGGCGGCUACUACUUCGACUACUGGGGCCAGGGCGUGAUG GUCACCGUGUCCAGCAUCGAGUUCAUGUACCCCCCUCCCUACCUGGACAACGAGA GAAGCAACGGCACCAUCAUCCACAUCAAAGAAAAGCACCUGUGCCACACCCAGAG CAGCCCCAAGCUGUUCUGGGCCCUGGUGGUGGUGGCCGGCGUGCUGUUCUGUU ACGGCCUGCUGGUCACAGUGGCCCUGUGCGUGAUCUGGACCAACAGCAGAAGAA ACAGAGGCGGCCAGAGCGACUACAUGAACAUGACCCCCAGAAGGCCAGGCCUGAC CAGAAAGCCCUACCAGCCCUACGCCCCUGCCAGAGACUUCGCCGCCUACAGACCC AGAGCCAAGUUCAGCAGAUCCGCCGAGACAGCCGCCAACCUGCAGGAUCCCAACC AGCUGUUCAACGAGCUGAACCUGGGCAGACGGGAGGAAUUCGACGUGCUGGAAA AGAAGAGAGCCAGGGACCCCGAGAUGGGCGGCAAGCAGCAGAGAAGAAGAAACCC UCAGGAAGGCGUCUACAACGCCCUGCAGAAAGACAAGAUGGCCGAGGCCUACAGC GAGAUCGGCACCAAGGGCGAGAGAAGAAGGGGCAAGGGCCACGAUGGCCUGUUC CAGGGCCUGUCCACCGCCACCAAGGACACCUUCGACGCCCUGCACAUGCAGACCC UGGCCCCCAGA
IL-15	43	MRISKPHLRS ISIQCYLCLL LNSHFLTEAG IHVFILGCFS AGLPKTEANW VNVISDLKKI EDLIQSMHID ATLYTESDVH PSCKVTAMKC FLLELQVISL ESGDASIHDT VENLIILANN SLSSNGNVTE SGCKECEELE EKNIKEFLQS FVHIVQMFIN TS
IL-15	15	ATGAGAATTTCGAAACCACATTTGAGAAGTATTTCCATCCAGTGCTACTTGTGTTTAC TTCTAAACAGTCATTTTCTAACTGAAGCTGGCATTCATGTCTTCATTTTGGGCTGTTTC AGTGCAGGGCTTCCTAAAACAGAAGCCAACTGGGTGAATGTAATAAGTGATTTGAAA AAAATTGAAGATCTTATTCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGA TGTTCACCCCAGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTT ATTTCACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCC TAGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATG TGAGGAACTGGAGGAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTACATATTGTC CAAATGTTCATCAACACTTCTTGA
IL-	16	MAPRRARGCRTLGLPALLLLLLLRPPATRGITCPPPMSVEHADIWVKSYSLYSRERYICN SGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQP

15RA		ESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTT AKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPLASV EMEAMEALPVTWGTSSRDEDLENCSHHL
IL-15RA	24	ATGGCCCCGCGGCGGGCGCGCGGCTGCCGGACCCTCGGTCTCCCGGCGCTGCTA CTGCTGCTGCTGCTCCGGCCGCCGGCGACGCGGGGCATCACGTGCCCTCCCCCCA TGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGGGAG CGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGACGGA GTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAACCCCCAGTCTCAAAT GCATTAGAGACCCTGCCCTGGTTCACCAAAGGCCAGCGCCACCCTCCACAGTAACG ACGGCAGGGGTGACCCCACAGCCAGAGAGCCTCTCCCCTTCTGGAAAAGAGCCCG CAGCTTCATCTCCCAGCTCAAACAACACAGCGGCCACAACAGCAGCTATTGTCCCG GGCTCCCAGCTGATGCCTTCAAAATCACCTTCCACAGGAACCACAGAGATAAGCAGT CATGAGTCCTCCCACGGCACCCCCTCTCAGACAACAGCCAAGAACTGGGAACTCAC AGCATCCGCCTCCCACCAGCCGCCAGGTGTGTATCCACAGGGCCACAGCGACACC ACTGTGGCTATCTCCACGTCCACTGTCCTGCTGTGTGGGCTGAGCGCTGTGTCTCT CCTGGCATGCTACCTCAAGTCAAGGCAAACTCCCCCGCTGGCCAGCGTTGAAATGG AAGCCATGGAGGCTCTGCCGGTGACTTGGGGGACCAGCAGCAGAGATGAAGACTT GGAAAACTGCTCTCACCACCTATGA

Example 9. IL-15 expression in naïve mice with LPMP / mRNA formulation

[00542] This example describes a recLemon LPMP / mRNA formulation comprising a lemon lipid reconstructed with ionizable lipids (C12-200), sterols (cholesterol), and PEG lipids (DMPE-PEG2k), to encapsulate mRNA (e.g., mRNA for IL-15). The recLemon LPMP / mRNA formulation tested in this example was prepared according to Example 2. The coding portion of the IL-15 mRNA used in this example is as follows:

MRISKPHLRS ISIQCYLCLL LNSHFLTEAG IHVFILGCFS AGLPKTEANW VNVISDLKKI EDLIQSMHID ATLYTESDVH PSCKVTAMKC FLLELQVISL ESGDASIHDT VENLIILANN SLSSNGNVTE SGCKECEELE EKNIKEFLQS FVHIVQMFIN TS (SEQ ID NO: 43)

or

ATGAGAATTTCGAAACCACATTTGAGAAGTATTTCCATCCAGTGCTACTTGTGTTTACTTC TAAACAGTCATTTTCTAACTGAAGCTGGCATTCATGTCTTCATTTTGGGCTGTTTCAGTGC AGGGCTTCCTAAAACAGAAGCCAACTGGGTGAATGTAATAAGTGATTTGAAAAAAATTGA AGATCTTATTCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTCACCCC AGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCACTTGAGT CCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCCTAGCAAACAACAGTT TGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAACTGGAGGAAA AAAATATTAAAGAATTTTTGCAGAGTTTTGTACATATTGTCCAAATGTTCATCAACACTTCT TGA (SEQ ID NO: 15)

[00543] In this example, *in vivo* level of IL-15 and its effect on systemic cytokines upon intramuscular dosing of recLemon LPMP / mRNA formulation (10 µg of IL-15 mRNA) were measured; the effects of intramuscular delivery of recLemon LPMP / mRNA formulation (10 µg IL-15 mRNA) on T cell and NK cell *in vivo* proliferation and intracellular cytokine production (# CD8 T cells, CD4 T cells, and NK cells at 6 and 10 days post intramuscular dose) were assessed. The studies were done in BotaMouse_212. The treatment schedule is shown in the chart below and illustrated in Scheme 3.

this example is as follows:

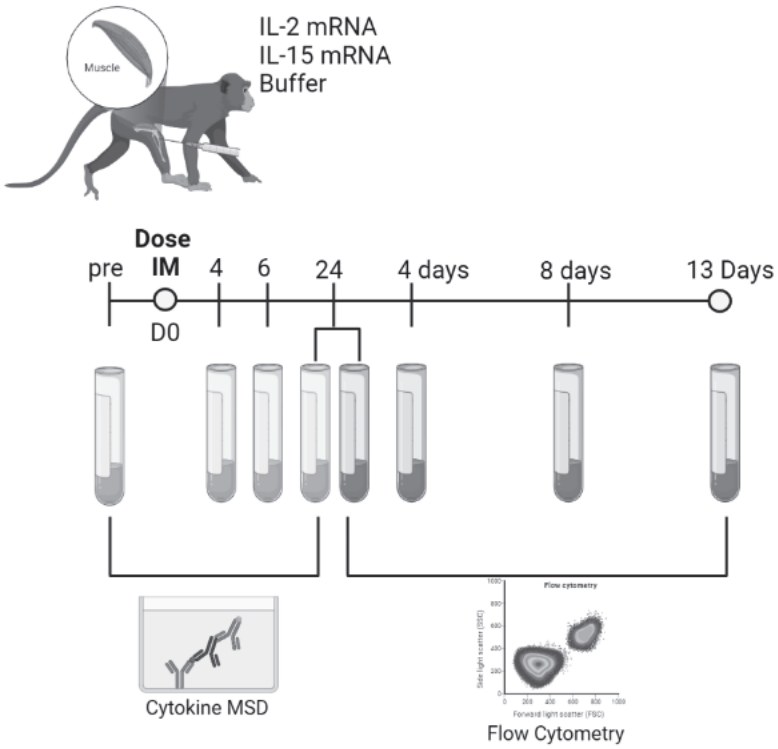
MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO: 49)

[00547] The coding portion of the IL-15 mRNA used in this example is as follows:

MRISKPHLRS ISIQCYLCLL LNSHFLTEAG IHVFILGCFS AGLPKTEANW VNVISDLKKI EDLIQSMHID ATLYTESDVH PSCKVTAMKC FLLELQVISL ESGDASIHDT VENLIILANN SLSSNGNVTE SGCKECEELE EKNIKEFLQS FVHIVQMFIN TS (SEQ ID NO: 43)

[00548] In this example, the effects of intramuscular dosing of recLemon LPMP / mRNA formulation (IL-15 mRNA or IL-2 mRNA) were measured. The studies were done in non-human primates (NHPs). The treatment schedule is shown in the chart below and illustrated in Scheme 4.

Group	Treatment	Particle	Dose and RoA
1 (N=3)	IL-15 mRNA	Lm; C12-200	IM; 100ug (0.02 mg/kg)
2 (N=3)	IL-15 mRNA	Lm; C12-200	IM; 30ug (0.006 mg/kg)
3 (N=3)	IL-2 mRNA	Lm; C12-200	IM; 30ug (0.006 mg/kg)



Scheme 4

What is claimed is:

1. An mRNA therapeutic composition, comprising:

one or more polynucleotides encoding one or more tumor antigenic, or signaling polypeptides, formulated within a lipid reconstructed plant messenger packs (LPMPs) comprising natural lipids and an ionizable lipid, wherein the ionizable lipid has two or more of the characteristics listed below:

(i) at least 2 ionizable amines;

(ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length;

(iii) a pKa of about 4.5 to about 7.5;

(iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and

(v) an N:P ratio of at least 10.

2. The mRNA therapeutic composition of claim 1, wherein the natural lipids are extracted from lemon or algae.

3. The mRNA therapeutic composition of claim 1, wherein the LPMPs further comprise a sterol and a polyethylene glycol (PEG)-lipid conjugate.

4. The mRNA therapeutic composition of claim 3, wherein the LPMPs comprise ionizable lipid:natural lipids:sterol:PEG-lipid at a molar ratio of about 35:50:12.5:2.5 or about 35:20:42.5:2.5.

5. The mRNA therapeutic composition of claim 1, wherein the ionizable lipid is selected from the group consisting of 1,1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5, SM-102 (Lipid H), and ALC-315.

6. The mRNA therapeutic composition of claim 1, wherein the polypeptide is IL-2 peptide, IL-2-Ra, IL-15 peptide, IL-15 Ra, Anti-CD19, CD20, CAR-T, Anti-HER2, Etanercept (Enbrel), Humira, erythropoietin, Epogen, Filgrastim, Keytruda, Rituximab, Romiplostim, Sargramostim, or a fragment or subunit thereof.

7. The mRNA therapeutic composition of claim 1, wherein the polynucleotide is an mRNA which encodes an IL-2 molecule comprising an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence of an IL-2 molecule provided in any one of Tables I-III.

8. The mRNA therapeutic composition of claim 1, wherein the LPMP has a size of less than about 200 nm.

9. The mRNA therapeutic composition of any one of the preceding claims, wherein the LPMP comprises:

about 20 mol% to about 50 mol% of the ionizable lipid,
about 20 mol% to about 60 mol% of the natural lipids,
about 7 mol% to about 20 mol% of the sterol, and
about 0.5 mol% to about 3 mol% of the polyethylene glycol (PEG)-lipid conjugate.

10. The mRNA therapeutic composition of any one of the preceding claims, wherein the mRNA therapeutic composition has a total lipid:polynucleotide weight ratio of about 50:1 to about 10:1.

11. The mRNA therapeutic composition of claim 1, further comprising a HEPES or TRIS buffer at a pH of about 7.0 to about 8.5.

12. The mRNA therapeutic composition of claim 1, further comprising one or more cryoprotectants.

13. The mRNA therapeutic composition of claim 1, wherein the mRNA therapeutic is a lyophilized composition comprising one or more lyoprotectants.

14. Use of the mRNA therapeutic composition of any one of claims 1 to 13 in the manufacture of a medicament for delivering an mRNA therapeutic in a subject.

15. Use of the mRNA therapeutic composition of any one of claims 1 to 13 in the manufacture of a medicament for inducing an immune response in a subject.

16. Use of the mRNA therapeutic composition of any one of claims 1 to 13 in the manufacture of a medicament for treating or preventing a cancer in a subject.

17. The use of any one of claims 14 to 16, wherein the mRNA therapeutic composition is to be administered by oral, intravenous, intradermal, intramuscular, intranasal, intraocular, rectal, intrajejunal, intratumoral, and/or subcutaneous administration.

18. The use of any one of claims 14 to 16, wherein the mRNA therapeutic composition is to be administered at a dosage level sufficient to deliver about 0.006 mg/kg to about 0.5 mg/kg of the polynucleotides to the subject.

19. The use of claim 15 or 16, wherein the mRNA therapeutic composition is to be administered to the

subject one time, two times, three times, four times, or more.

20. The use of any one of claims 14 to 16, wherein an additional therapeutic agent is to be administered to the subject, wherein the additional therapeutic agent is an anti-cancer therapeutic agent or a therapeutic agent that treats and/or prevents a chronic pain.

21. The use of any one of claims 14 to 16, wherein the mRNA therapeutic composition is to be administered at a dosage level sufficient to deliver about 0.0003 mg/kg to about 0.002 mg/kg of the polynucleotides to the subject.

22. The mRNA therapeutic composition of claim 1, wherein the polynucleotide is an mRNA which encodes an IL-15 and/or IL-15Ra molecule comprising an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence of an IL-15 molecule provided in any one of Table 9 or Examples 1-9.

23. The use of any one of claims 14 to 16, wherein the mRNA therapeutic composition modulates proliferation or activation of T cells and/or NK cells.

24. The use of claim 23, wherein the mRNA therapeutic composition modulates proliferation or activation of CD4 Tcells and/or CD8 T cells.

25. The use of any one of claims 14 to 16, wherein the mRNA therapeutic composition modulates activity or expression of Granzyme B/perforin, CD25 (IL-2Ra), TNF-alpha, IL-15, IL-6, IL-8, IL-12, GM-CSF, G-CSF, IL-1, IL-2, IL-4, IL-5, IL-8, IL-9, IL-10, IL-13, IL-17, IL-33, PD-L1, CCL2, CCL3, CCL4, CXCL1, CXCL2, CXCL10 (IP-10), CCL20, CD40, TNF-β, LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-a, IFN-β, IFN-γ or any combination thereof.

26. The use of claim 23 or 25, wherein the mRNA therapeutic composition provides modulations in the blood, in the lymph nodes, in the spleen or in any combination thereof.