addition, rapid responses, including rapid vaccine development, are required to effectively control emerging infectious diseases and pandemics. Thus, there exists a need for the prevention and/or treatment of infectious diseases and cancer.

SUMMARY

[0007] The present disclosure describes RNA molecules that are useful for inducing immune responses. Both self-replicating RNA molecules and messenger RNA (mRNA) molecules are described.

[0008] In some aspects, disclosed herein are RNA molecules comprising: (a) a first polynucleotide encoding one or more viral replication proteins, wherein one or more miRNA binding sites in the first polynucleotide have been modified as compared to a reference polynucleotide; and (b) a second polynucleotide comprising a first transgene encoding a first antigenic protein or a fragment thereof. In other aspects, disclosed herein are DNA molecules encoding the RNA molecules as disclosed herein. In other aspects, disclosed herein are compositions comprising the RNA molecules as disclosed herein and a lipid formulation 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. In other aspects, disclosed herein are uses of the compositions as disclosed herein in the manufacture of a medicament for preventing or ameliorating COVID-19. In other aspects, disclosed herein are uses of the RNA molecules as disclosed herein or the compositions as disclosed herein in the manufacture of a medicament for inducing an immune response in a subject.

[0009] Also described herein, in some examples, are RNA molecules comprising: (i) a first polynucleotide comprising a sequence having at least 80% identity to a sequence of SEQ ID NO:6; and (ii) a second polynucleotide comprising a first transgene encoding a first antigenic protein or a fragment thereof.

[0010] In some examples, modification of the one or more miRNA binding sites reduces or eliminates miRNA binding. In some examples, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, or 15 miRNA binding sites in the first polynucleotide have been modified. In some examples, the one or more miRNA binding sites are selected from regions that bind a miRNA having a sequence of SEQ ID NOs:58, 59, 72, 80, 81, 83, 101, 102, 103, 112, 113, 114, 128, 131, 142, 156, 157, 171, 175, and any combination thereof.

[0011] In some examples, the one or more viral replication proteins of RNA molecules described herein are alphavirus proteins or rubivirus proteins. In some examples, the alphavirus proteins are from Venezuelan Equine Encephalitis Virus (VEEV), Eastern Equine Encephalitis Virus (EEEV), Everglades Virus (EVEV), Mucambo Virus (MUCV), Semliki Forest Virus (SFV), Pixuna Virus (PIXV), Middleburg Virus (MIDV), Chikungunya Virus (CHIKV), O'Nyong-Nyong Virus (ONNV), Ross River Virus (RRV), Barmah Forest Virus (BFV), Getah Virus (GETV), Sagiyama Virus (SAGV), Bebaru Virus (BEBV), Mayaro Virus (MAYV), Una Virus (UNAV), Sindbis Virus (SINV), Aura Virus (AURAV), Whataroa Virus (WHAV), Babanki Virus (BABV), Kyzylagach Virus (KYZV), Western Equine Encephalitis Virus (WEEV), Highland J Virus (HJV), Fort Morgan Virus (FMV), Ndumu Virus (NDUV), Salmonid Alphavirus (SAV), Buggy Creek Virus (BCRV), or any combination thereof.

[0012] In some examples, first polynucleotides of RNA molecules described herein encode a polyprotein comprising an alphavirus nsP1 protein, an alphavirus nsP2 protein, an alphavirus nsP3 protein, an alphavirus nsP4 protein, or any combination thereof. In some examples, first polynucleotides encode a polyprotein comprising an alphavirus nsP1 protein, an alphavirus nsP2 protein, an alphavirus nsP3 protein, or any combination thereof, and an alphavirus nsP4 protein. In some examples, first polynucleotides comprise a sequence having at least 80% identity to a sequence of SEQ ID NO:6. In some examples, first polynucleotides comprise a sequence having at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:6. In some examples, first polynucleotides encode a polyprotein comprising a sequence having at least 80%, at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:187.

[0013] In some examples, RNA molecules described herein include a 5' untranslated region (UTR). In some examples, the 5' UTR comprises a viral 5' UTR, a non-viral 5' UTR, or a combination of viral and non-viral 5' UTR sequences. In some examples, the 5' UTR comprises an alphavirus 5' UTR. In some examples, the alphavirus 5' UTR comprises a Venezuelan Equine Encephalitis Virus (VEEV), Eastern Equine Encephalitis Virus (EEEV), Everglades Virus (EVEV), Mucambo Virus (MUCV), Semliki Forest Virus (SFV), Pixuna Virus (PIXV), Middleburg Virus (MIDV), Chikungunya Virus (CHIKV), O'Nyong-Nyong Virus (ONNV), Ross River Virus (RRV), Barmah Forest Virus (BFV), Getah Virus (GETV), Sagiyama Virus (SAGV), Bebaru Virus (BEBV), Mayaro Virus (MAYV), Una Virus (UNAV), Sindbis Virus (SINV), Aura Virus (AURAV), Whataroa Virus (WHAV), Babanki Virus (BABV), Kyzylagach Virus (KYZV), Western Equine Encephalitis Virus (WEEV), Highland J Virus (HJV), Fort Morgan Virus (FMV), Ndumu Virus (NDUV), Salmonid Alphavirus (SAV), or Buggy Creek Virus (BCRV) 5' UTR sequence. In some examples, the 5' UTR comprises a sequence of SEQ ID NO:5.

[0014] In some examples, RNA molecules described herein include a 3' untranslated region (UTR). In some examples, the 3' UTR comprises a viral 3' UTR, a non-viral 3' UTR, or a combination of viral and non-viral 3' UTR sequences. In some examples, the 3' UTR comprises an alphavirus 3' UTR. In some examples, the alphavirus 3' UTR comprises a Venezuelan Equine Encephalitis Virus (VEEV), Eastern Equine Encephalitis Virus (EEEV), Everglades Virus

(EVEV), Mucambo Virus (MUCV), Semliki Forest Virus (SFV), Pixuna Virus (PIXV), Middleburg Virus (MIDV), Chikungunya Virus (CHIKV), O'Nyong-Nyong Virus (ONNV), Ross River Virus (RRV), Barmah Forest Virus (BFV), Getah Virus (GETV), Sagiyama Virus (SAGV), Bebaru Virus (BEBV), Mayaro Virus (MAYV), Una Virus (UNAV), Sindbis Virus (SINV), Aura Virus (AURAV), Whataroa Virus (WHAV), Babanki Virus (BABV), Kyzylagach Virus (KYZV), Western Equine Encephalitis Virus (WEEV), Highland J Virus (HJV), Fort Morgan Virus (FMV), Ndumu Virus (NDUV), Salmonid Alphavirus (SAV), or Buggy Creek Virus (BCRV) 3' UTR sequence. In some examples, the 3' UTR comprises a sequence of SEQ ID NO:9. In some examples, the 3' UTR further comprises a poly-A sequence.

[0015] In some examples, the first antigenic protein of RNA molecules described herein is a viral protein, a bacterial protein, a fungal protein, a protozoan protein, or a parasite protein. In some examples, the viral protein is a coronavirus protein, an orthomyxovirus protein, a paramyxovirus protein, a picornavirus protein, a flavivirus protein, a filovirus protein, a rhabdovirus protein, a togavirus protein, an arterivirus protein, a bunyavirus protein, an arenavirus protein, a reovirus protein, a bornavirus protein, a retrovirus protein, an adenovirus protein, a herpesvirus protein, a polyomavirus protein, a papillomavirus protein, a poxvirus protein, or a hepadnavirus protein. In some examples, the first antigenic protein is a SARS-CoV-2 protein, or an influenza virus protein, a respiratory syncytial virus (RSV) protein, a human immunodeficiency virus (HIV) protein, a hepatitis C virus (HCV) protein, a cytomegalovirus (CMV) protein, a Lassa Fever Virus (LFV) protein, an Ebola Virus (EBOV) protein, a *Mycobacterium* protein, a *Bacillus* protein, a *Yersinia* protein, a *Streptococcus* protein, a *Pseudomonas* protein, a *Shigella* protein, a *Campylobacter* protein, a *Salmonella* protein, a *Plasmodium* protein, or a *Toxoplasma* protein. In some examples, the first antigenic protein is a SARS-CoV-2 spike glycoprotein. In some examples, the SARS-CoV-2 spike glycoprotein comprises an amino acid sequence having at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17. In some examples, the second polynucleotide of RNA molecules described herein comprises a sequence having at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12,

or SEQ ID NO:13. In some examples, the first transgene of RNA molecules described herein is expressed from a first subgenomic promoter.

[0016] In some examples, the second polynucleotide of RNA molecules described herein includes at least two transgenes. In some examples, a second transgene of the second polynucleotide encodes a second antigenic protein or a fragment thereof or an immunomodulatory protein. In some examples, the second polynucleotide further comprises a sequence encoding a 2A peptide, an internal ribosomal entry site (IRES), a second subgenomic promoter, or a combination thereof, located between transgenes. In some examples, the immunomodulatory protein is a cytokine, a chemokine, or an interleukin. In some examples, first and second transgenes of second polynucleotides encode viral proteins, bacterial proteins, fungal proteins, protozoan proteins, parasite proteins, immunomodulatory proteins, or any combination thereof.

[0017] In some examples, the first polynucleotide is located 5' of the second polynucleotide. In some examples, RNA molecules described herein further include an intergenic region located between the first polynucleotide and the second polynucleotide. In some examples, the intergenic region comprises a sequence having at least 85% identity to a sequence of SEQ ID NO:7.

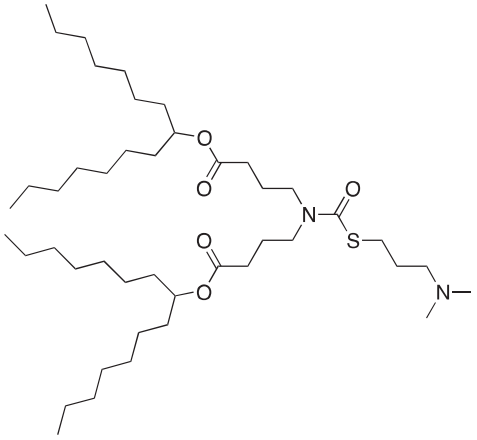
[0018] In some examples, RNA molecules described herein are self-replicating RNA molecules. In some examples, RNA molecules described herein include a sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4. In some examples, RNA molecules described herein are self-replicating RNA molecules. In some examples, RNA molecules described herein include a sequence having at least 80%, at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:40, or SEQ ID NO:48.

[0019] In some examples, RNA molecules described herein further include a 5' cap. In some examples, the 5' cap has a Cap 1 structure, a Cap 1 (^{m6}A) structure, a Cap 2 structure, or a Cap 0 structure.

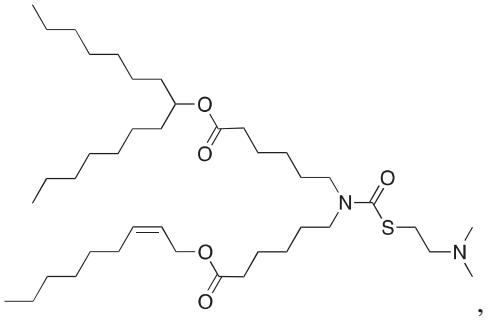
[0020] Described herein, in some examples, are DNA molecules encoding any of the RNA molecules described herein. In some examples, DNA molecules described herein include a

promoter. In some examples, the promoter is located 5' of the 5 'UTR. In some examples, the promoter is a T7 promoter, a T3 promoter, or an SP6 promoter.

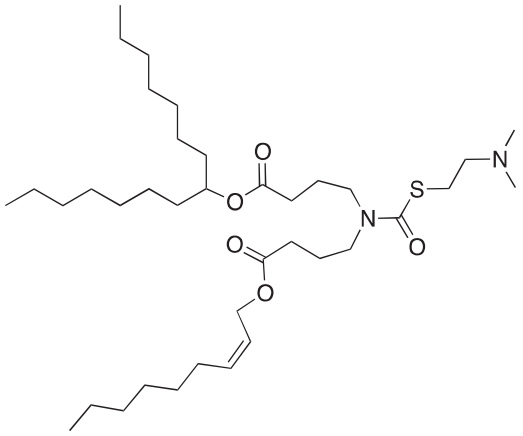
[0021] Described herein, in some examples, are compositions comprising any RNA molecule described herein and a lipid. In some examples, the lipid comprises an ionizable cationic lipid. In some examples, the ionizable cationic lipid has a structure of



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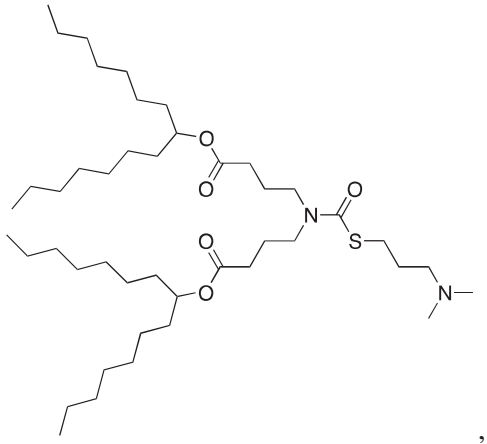
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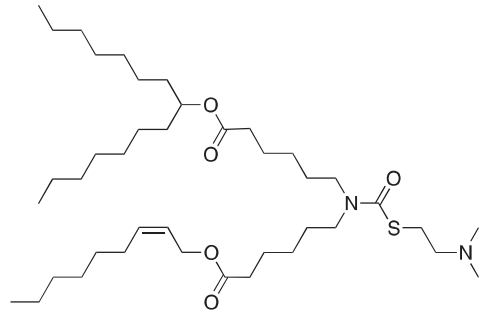
, or a pharmaceutically acceptable salt thereof.

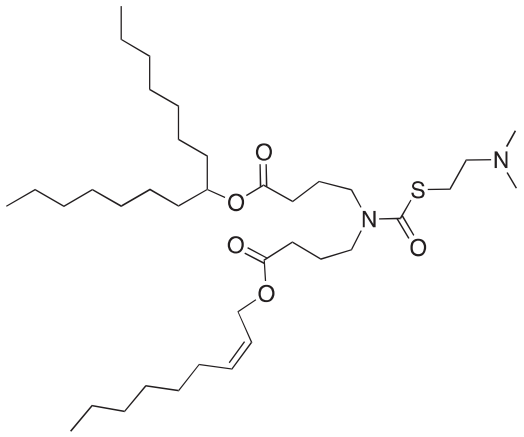
[0022] Described herein, in some examples, are compositions comprising any RNA molecule described herein and a lipid formulation.

[0023] In some examples, the lipid formulation comprises an ionizable cationic lipid. In some examples, the ionizable cationic lipid has a structure of



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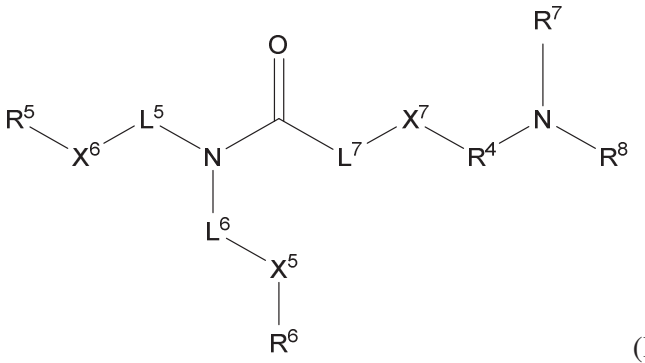




, or a pharmaceutically acceptable salt thereof.

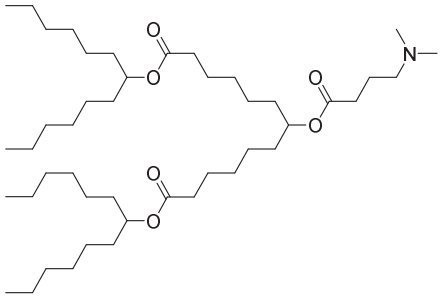
[0024] In some examples, the lipid formulation is selected from a lipoplex, a liposome, a lipid nanoparticle, a polymer-based carrier, an exosome, a lamellar body, a micelle, and an emulsion. In some examples, the lipid formulation is a liposome selected from a cationic liposome, a nanoliposome, a proteoliposome, a unilamellar liposome, a multilamellar liposome, a ceramide-containing nanoliposome, and a multivesicular liposome. In some examples, the lipid formulation is a lipid nanoparticle. In some examples, the lipid nanoparticle has a size of less than about 200 nm. In some examples, the lipid nanoparticle has a size of less than about 150 nm. In some examples, the lipid nanoparticle has a size of less than about 100 nm. In some examples, the lipid nanoparticle has a size of about 55 nm to about 90 nm. In some examples, the lipid formulation comprises one or more cationic lipids. In some examples, the one or more cationic lipids is selected from 5-carboxyspermylglycinedioctadecylamide (DOGS), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium (DOSPA), 1,2-Dioleoyl-3-Dimethylammonium-Propane (DODAP), 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP), 1,2-distearyloxy-N,N-dimethyl-3-aminopropane

(DSDMA), 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane (DLenDMA), N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-oc-tadecadienoxy)propane (CLinDMA), 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethy 1-1-(cis,cis-9′,1-2′-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (DLinDAP), 1,2-N,N′-Dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP), 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane (DLinCDAP), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), and 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane or (DLin-K-XTC2-DMA). In some examples, the lipid formulation comprises an ionizable cationic lipid. In some examples, the ionizable cationic lipid has a structure of Formula I:

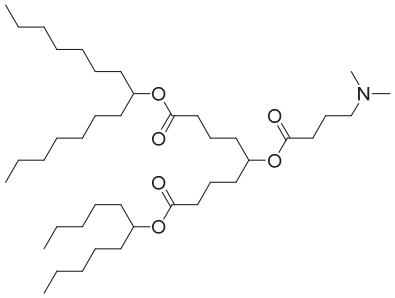


(I)

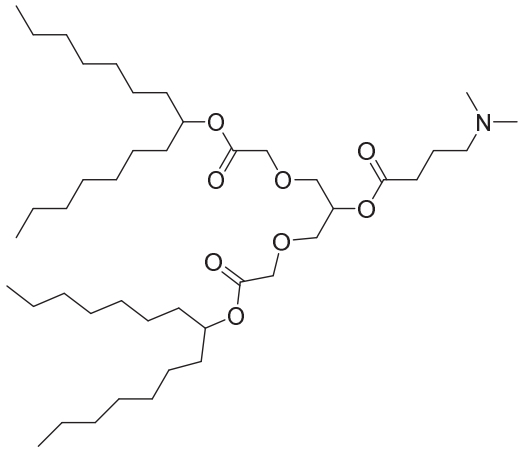
or a pharmaceutically acceptable salt or solvate thereof, wherein R^5 and R^6 are each independently selected from the group consisting of a linear or branched C_1-C_{31} alkyl, C_2-C_{31} alkenyl or C_2-C_{31} alkynyl and cholesteryl; L^5 and L^6 are each independently selected from the group consisting of a linear C_1-C_{20} alkyl and C_2-C_{20} alkenyl; X^5 is -C(O)O-, whereby -C(O)O-R^6 is formed or -OC(O)- whereby -OC(O)-R^6 is formed; X^6 is -C(O)O- whereby -C(O)O-R^5 is formed or -OC(O)- whereby -OC(O)-R^5 is formed; X^7 is S or O; L^7 is absent or lower alkyl; R^4 is a linear or branched C_1-C_6 alkyl; and R^7 and R^8 are each independently selected from the group consisting of a hydrogen and a linear or branched C_1-C_6 alkyl. In some examples, the ionizable cationic lipid is selected from



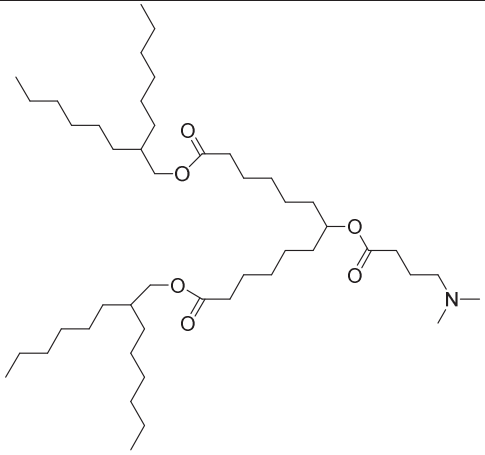
ATX-209



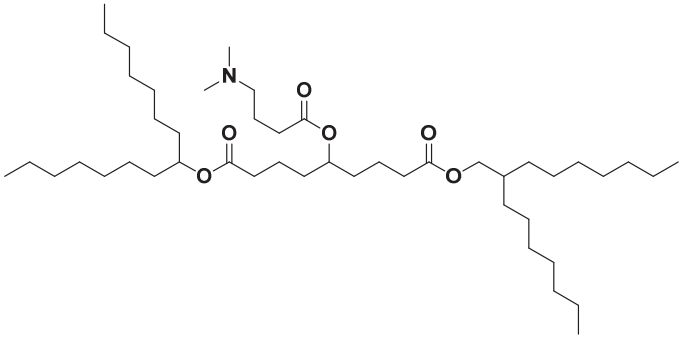
ATX-210



ATX-230



ATX-231

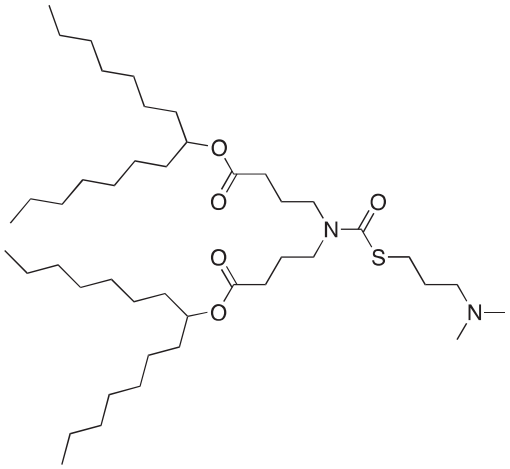


and

ATX-232

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[0025] In some examples, the ionizable cationic lipid is ATX-126:



ATX-126.

[0026] In some examples, the lipid formulation of compositions described herein encapsulates the nucleic acid molecule. In some examples, the lipid formulation is complexed to the nucleic acid molecule.

[0027] In some examples, the lipid formulation further comprises a helper lipid. In some examples, the helper lipid is a phospholipid. In some examples, the helper lipid is selected from dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidyl choline (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), and phosphatidylcholine (PC). In some examples, the helper lipid is distearoylphosphatidylcholine (DSPC).

[0028] In some examples, the lipid formulation of compositions described herein further comprises cholesterol. In some examples, the lipid formulation further comprises a polyethylene glycol (PEG)-lipid conjugate. In some examples, the PEG-lipid conjugate is PEG-DMG. In some examples, the PEG-DMG is PEG2000-DMG.

[0029] In some examples, the lipid portion of the lipid formulation comprises about 40 mol% to about 60 mol% of the ionizable cationic lipid, about 4 mol% to about 16 mol% DSPC, about 30 mol% to about 47 mol% cholesterol, and about 0.5 mol% to about 3 mol% PEG2000-DMG. In some examples, the lipid portion of the lipid formulation comprises about 42 mol% to about 58 mol% of the ionizable cationic lipid, about 6 mol% to about 14 mol% DSPC, about 32 mol% to about 44 mol% cholesterol, and about 1 mol% to about 2 mol% PEG2000-DMG. In some examples, the lipid portion of the lipid formulation comprises about 45 mol% to about 55 mol% of the ionizable cationic lipid, about 8 mol% to about 12 mol% DSPC, about 35 mol% to about 42 mol% cholesterol, and about 1.25 mol% to about 1.75 mol% PEG2000-DMG.

[0030] In some examples, the composition has a total lipid:nucleic acid molecule weight ratio of about 50:1 to about 10:1. In some examples, the composition has a total lipid:nucleic acid molecule weight ratio of about 44:1 to about 24:1. In some examples, the composition has a total lipid: nucleic acid molecule weight ratio of about 40:1 to about 28:1. In some examples, the composition has a total lipid: nucleic acid molecule weight ratio of about 38:1 to about 30:1. In some examples, the composition has a total lipid: nucleic acid molecule weight ratio of about 37:1 to about 33:1.

[0031] In some examples, the composition comprises a HEPES or TRIS buffer at a pH of about 7.0 to about 8.5. In some examples, the HEPES or TRIS buffer is at a concentration of about 7 mg/mL to about 15 mg/mL.

[0032] In some examples, the composition further comprises about 2.0 mg/mL to about 4.0 mg/mL of NaCl. In some examples, the composition further comprises one or more cryoprotectants. In some examples, the one or more cryoprotectants are selected from sucrose, glycerol, or a combination of sucrose and glycerol. In some examples, the composition comprises a combination of sucrose at a concentration of about 70 mg/mL to about 110 mg/mL and glycerol at a concentration of about 50 mg/mL to about 70 mg/mL.

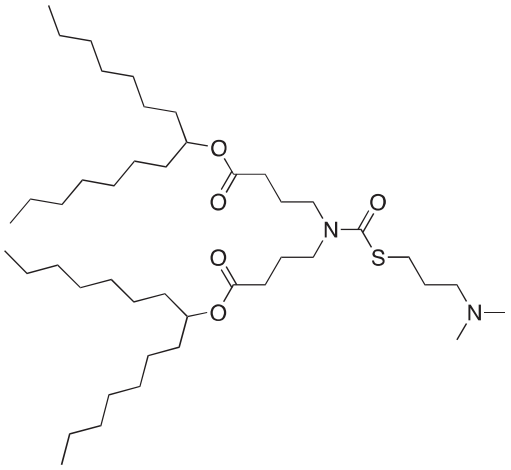
[0033] In some examples, the composition is a lyophilized composition. In some examples, the lyophilized composition comprises one or more lyoprotectants. In some examples, the lyophilized composition comprises a poloxamer, potassium sorbate, sucrose, or any combination thereof. In some examples, the poloxamer is poloxamer 188.

[0034] In some examples, the lyophilized composition comprises about 0.01 to about 1.0 % w/w of the RNA molecule. In some examples, the lyophilized composition comprises about 1.0 to about 5.0 % w/w lipids. In some examples, the lyophilized composition comprises about 0.5 to about 2.5 % w/w of TRIS buffer. In some examples, the lyophilized composition comprises about 0.75 to about 2.75 % w/w of NaCl. In some examples, the lyophilized composition comprises about 85 to about 95 % w/w of a sugar. In some examples, the sugar is sucrose. In some examples, the lyophilized composition comprises about 0.01 to about 1.0 % w/w of a poloxamer. In some examples, the poloxamer is poloxamer 188. In some examples, the lyophilized composition comprises about 1.0 to about 5.0 % w/w of potassium sorbate.

[0035] In some examples, compositions described herein include an RNA molecule comprising (A) a sequence of SEQ ID NO:1; (B) a sequence of SEQ ID NO:2; (C) a sequence of SEQ ID NO:3; or (D) a sequence of SEQ ID NO:4. In some examples, compositions described herein include an RNA molecule comprising a sequence of SEQ ID NO:29. In some examples, compositions described herein include an RNA molecule comprising a sequence of SEQ ID

NO:32. In some examples, compositions described herein include an RNA molecule comprising a sequence of SEQ ID NO:48. In some examples, compositions described herein include an RNA molecule comprising a sequence of SEQ ID NO:40.

[0036] Described herein, in some examples, are lipid nanoparticle compositions comprising a. a lipid formulation comprising i. about 45 mol% to about 55 mol% of an ionizable cationic lipid having the structure of ATX-126:



ATX-126;

ii. about 8 mol% to about 12 mol% DSPC; iii. about 35 mol% to about 42 mol% cholesterol; and iv. about 1.25 mol% to about 1.75 mol% PEG2000-DMG; and b. an RNA molecule having at least 80% identity to a sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4; wherein the lipid formulation encapsulates the RNA molecule and the lipid nanoparticle has a size of about 60 to about 90 nm. In some examples, the RNA molecule included in lipid nanoparticle compositions described herein has at least 80% identity to a sequence of SEQ ID NO:29. In some examples, the RNA molecule included in lipid nanoparticle compositions described herein has at least 80% identity to a sequence of SEQ ID NO:32. In some examples, the RNA molecule included in lipid nanoparticle compositions described herein has at least 80% identity to a sequence of SEQ ID NO:40. In some examples, the RNA molecule included in lipid nanoparticle compositions described herein has at least 80% identity to a sequence of SEQ ID NO:48. In some examples, the RNA molecule included in lipid nanoparticle compositions described herein has at least 80% identity to a sequence of SEQ ID NO:29. In some examples, the RNA molecule included in lipid nanoparticle compositions described herein has at least 80% identity to a sequence of SEQ ID NO:32.

[0037] Described herein, in some examples, are methods for administering compositions described herein to a subject in need thereof. In some examples, compositions described herein

are administered intramuscularly, subcutaneously, intradermally, transdermally, intranasally, orally, sublingually, intravenously, intraperitoneally, topically, by aerosol, or by a pulmonary route. In some examples, compositions described herein are administered intramuscularly.

[0038] Described herein, in some examples, are methods of administering a composition described herein to a subject in need thereof, wherein the composition is lyophilized and is reconstituted prior to administration.

[0039] Described herein, in some examples, are methods of preventing or ameliorating COVID-19, comprising administering a composition described herein to a subject in need thereof. In some examples, the composition is administered one time. In some examples, the composition is administered two times.

[0040] Described herein, in some examples, are methods of administering a booster dose to a vaccinated subject, comprising administering a composition described herein to a subject who was previously vaccinated against coronavirus.

[0041] In some examples, a composition described herein is administered at a dosage of about 0.01 μg to about 1,000 μg of nucleic acid in the methods described herein. In some examples, a composition described herein is administered at a dosage of about 1, 2, 5, 7.5, or 10 μg of nucleic acid.

[0042] Described herein, in some examples, are methods of inducing an immune response in a subject comprising: administering to the subject an effective amount of an RNA molecule described herein. In some examples, the RNA molecule is administered intramuscularly, subcutaneously, intradermally, transdermally, intranasally, orally, sublingually, intravenously, intraperitoneally, topically, by aerosol, or by a pulmonary route.

[0043] Described herein, in some examples, are methods of inducing an immune response in a subject comprising: administering to the subject an effective amount of a composition described herein. In some examples, the composition is administered intramuscularly, subcutaneously, intradermally, transdermally, intranasally, orally, sublingually, intravenously, intraperitoneally, topically, by aerosol, or by a pulmonary route.

[0044] Described herein, in some examples, are RNA molecules for use in inducing an immune response to the first antigenic protein or fragment thereof.

[0045] Also described herein, in some examples, is the use of an RNA molecule described herein in the manufacture of a medicament for inducing an immune response to the first antigenic protein or fragment thereof.

[0046] In another example, the present disclosure describes an RNA molecule for expressing an antigen comprising an open reading frame having at least 80% identity to a sequence of SEQ ID NO:33 or SEQ ID NO:30, wherein T is substituted with U.

[0047] In some examples the RNA molecule further comprises a 5' UTR having a sequence selected from SEQ ID NO:35, SEQ ID NOs:189-218, or SEQ ID NOs:233-279.

[0048] In some examples the RNA molecule further comprises a 3' UTR having a sequence selected from SEQ ID NO:37, SEQ ID NOs:219-225, or SEQ ID NOs:280-317.

[0049] In some examples the RNA molecule further comprises a 5' cap. In some examples the 5' cap has a Cap 1 structure, a Cap 1 (m6A) structure, a Cap 2 structure, or a Cap 0 structure.

[0050] In some examples the RNA molecule further comprises a poly-A tail.

[0051] In another example, the present disclosure describes an RNA molecule for expressing an antigen comprising an open reading frame having at least 80% identity to a sequence of SEQ ID NO:33, a 5' UTR comprising a sequence of SEQ ID NO:35, and a 3' UTR comprising a sequence of SEQ ID NO:37; or an open reading frame having at least 80% identity to a sequence of SEQ ID NO:30, a 5' UTR comprising a sequence of SEQ ID NO:35, and a 3' UTR comprising a sequence of SEQ ID NO:37, wherein T is substituted with U.

[0052] In some examples the RNA molecule further comprises a 5' cap. In some examples the 5' cap has a Cap 1 structure, a Cap 1 (m6A) structure, a Cap 2 structure, or a Cap 0 structure.

[0053] In some examples the RNA molecule further comprises a poly-A tail.

[0054] In another example, the present disclosure describes a DNA molecule encoding any one of the RNA molecules described herein.

[0055] In some examples the DNA molecule comprises a promoter. In some examples the promoter is a T7 promoter, a T3 promoter, or an SP6 promoter.

[0056] In another example, the present disclosure describes a composition comprising any of the RNA molecules described herein, and a lipid formulation.

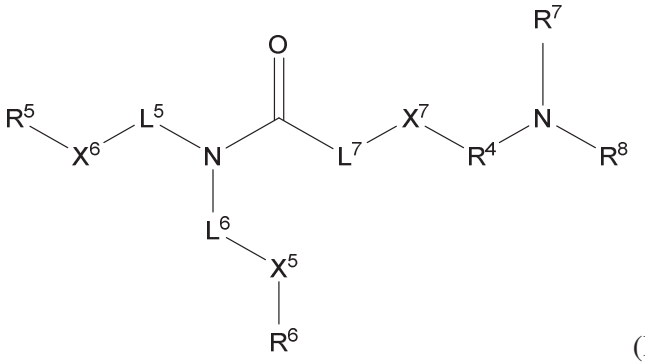
[0057] In some examples the lipid formulation is selected from a lipoplex, a liposome, a lipid nanoparticle, a polymer-based carrier, an exosome, a lamellar body, a micelle, and an emulsion.

[0058] In some examples the lipid formulation is a liposome selected from a cationic liposome, a nanoliposome, a proteoliposome, a unilamellar liposome, a multilamellar liposome, a ceramide-containing nanoliposome, and a multivesicular liposome.

[0059] In some examples the lipid formulation is a lipid nanoparticle.

[0060] In some examples the lipid formulation comprises one or more cationic lipids. In some examples the one or more cationic lipids is selected from 5-carboxyspermylglycinedioctadecylamide (DOGS), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium (DOSPA), 1,2-Dioleoyl-3-Dimethylammonium-Propane (DODAP), 1,2-Dioleoyl-3-Trimethylammonium-Propane (DOTAP), 1,2-distearyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane (DLenDMA), N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-oc-tadecadienoxy)propane (CLinDMA), 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethy 1-1-(cis,cis-9′,1-2′-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (DLinDAP), 1,2-N,N′-Dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP), 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane (DLinCDAP), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), and 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane or (DLin-K-XTC2-DMA).

[0061] In some examples the lipid formulation comprises an ionizable cationic lipid. In some examples the ionizable cationic lipid has a structure of Formula I:

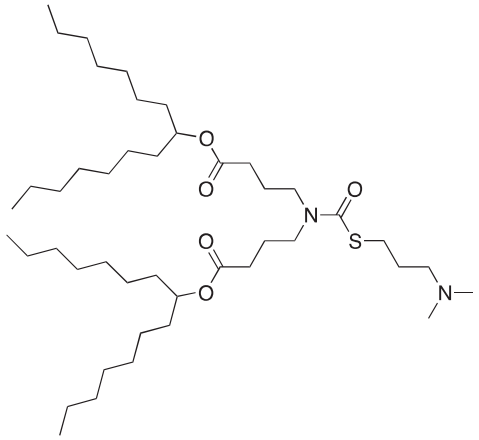


(I)

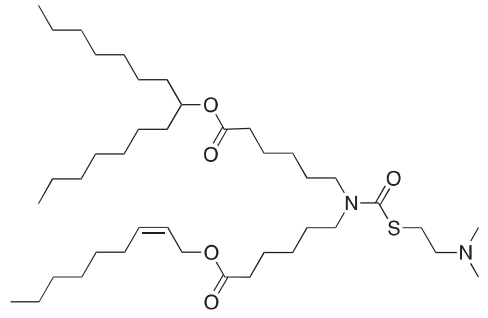
or a pharmaceutically acceptable salt or solvate thereof, wherein R5 and R6 are each independently selected from the group consisting of a linear or branched C1-C31 alkyl, C2-C31 alkenyl or C2-C31 alkynyl and cholesteryl; L5 and L6 are each independently selected

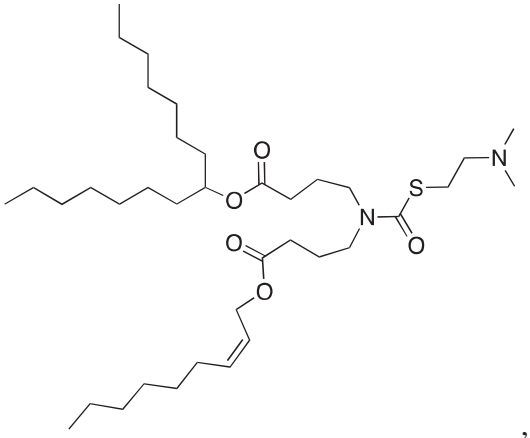
from the group consisting of a linear C1-C20 alkyl and C2-C20 alkenyl; X5 is -C(O)O-, whereby -C(O)O-R6 is formed or -OC(O)- whereby -OC(O)-R6 is formed; X6 is -C(O)O- whereby -C(O)O-R5 is formed or -OC(O)- whereby -OC(O)-R5 is formed; X7 is S or O; L7 is absent or lower alkyl; R4 is a linear or branched C1-C6 alkyl; and R7 and R8 are each independently selected from the group consisting of a hydrogen and a linear or branched C1-C6 alkyl.

[0062] In some examples the ionizable cationic lipid is selected from



,





, or a pharmaceutically acceptable salt thereof.

[0063] In some examples the lipid formulation comprises a helper lipid. In some examples the helper lipid is a phospholipid.

[0064] In some examples the helper lipid is selected from dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidyl choline (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), and phosphatidylcholine (PC).

[0065] In some examples the lipid formulation comprises cholesterol.

[0066] In some examples the lipid formulation comprises a polyethylene glycol (PEG)-lipid conjugate.

[0067] In another example, the present disclosure describes a method of inducing an immune response in a subject comprising administering to the subject an effective amount of any of the RNA molecules or compositions described herein.

[0068] In some examples the method comprises administering the RNA molecule or the composition intramuscularly, subcutaneously, intradermally, transdermally, intranasally, orally, sublingually, intravenously, intraperitoneally, topically, or by a pulmonary route.

[0069] In another example, the present disclosure describes a method of administering administering a booster dose to a vaccinated subject, comprising administering any of the RNA molecules or compositions described herein to a subject who was previously vaccinated against coronavirus.

[0070] In some examples the method comprises administering the RNA molecule or the composition intramuscularly, subcutaneously, intradermally, transdermally, intranasally, orally, sublingually, intravenously, intraperitoneally, topically, or by a pulmonary route.

[0071] In some examples the RNA molecules or compositions described herein are used for inducing an immune response to the antigen.

[0072] In some examples the RNA molecules or compositions described herein are used in the manufacture of a medicament for inducing an immune response to the antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] **FIG. 1A** shows a schematic of an exemplary self-replicating RNA, including nsP1-nsP4 replicase and coronavirus spike transgene regions.

[0074] **FIG. 1B** shows exemplary miRNA binding sites based on predictions by miRanda (Enright, A.J., John, B., Gaul, U. et al. MicroRNA targets in Drosophila. Genome Biol 5, R1 (2003). doi.org/10.1186/gb-2003-5-1-r1). The Venezuelan equine encephalitis virus (VEEV) non-structural protein coding region is shown, with 15 predicted binding sites shown by grey rectangles.

CLAIMS

What is claimed is:

1. An RNA molecule comprising:
   (a) a first polynucleotide encoding one or more viral replication proteins, wherein one or more miRNA binding sites in the first polynucleotide have been modified as compared to a reference polynucleotide; and
   (b) a second polynucleotide comprising a first transgene encoding a first antigenic protein or a fragment thereof.
2. The RNA molecule of claim 1, wherein;
   (i) modification of the one or more miRNA binding sites reduces or eliminates miRNA binding;
   (ii) two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, or 15 miRNA binding sites in the first polynucleotide have been modified; and/or
   (iii) the one or more miRNA binding sites are selected from regions that bind a miRNA having a sequence of SEQ ID NOs:58, 59, 72, 80, 81, 83, 101, 102, 103, 112, 113, 114, 128, 131, 142, 156, 157, 171, 175, and any combination thereof.
3. The RNA molecule of claim 1 or 2, wherein the one or more viral replication proteins are alphavirus proteins, and/or wherein the first polynucleotide encodes a polyprotein comprising an alphavirus nsP1 protein, an alphavirus nsP2 protein, an alphavirus nsP3 protein, and an alphavirus nsP4 protein.
4. The RNA molecule of claim 1, wherein the first polynucleotide:
   (i) comprises a sequence having at least 80%, at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:6; and/or
   (ii) encodes a polyprotein comprising a sequence having at least 80%, at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:187.
5. The RNA molecule of any one of claims 1-4, further comprising:
   (i) a 5' untranslated region (UTR), optionally wherein the 5' UTR comprises a

sequence selected from the group consisting of: an alphavirus 5' UTR sequence, a sequence of SEQ ID NO: 5, and combinations thereof; and/or

(ii) a 3' UTR, optionally wherein the 3' UTR comprises a sequence selected from the group consisting of: an alphavirus 3' UTR sequence, a sequence of SEQ ID NO: 9, a poly-A sequence, and combinations thereof.

6. The RNA molecule of any one of claims 1-5, wherein the first antigenic protein is:

(i) a viral protein, optionally wherein the viral protein is a coronavirus protein, an orthomyxovirus protein, a paramyxovirus protein, a picornavirus protein, a flavivirus protein, a filovirus protein, a rhabdovirus protein, a togavirus protein, an arterivirus protein, a bunyavirus protein, an arenavirus protein, a reovirus protein, a bornavirus protein, a retrovirus protein, an adenovirus protein, a herpesvirus protein, a polyomavirus protein, a papillomavirus protein, a poxvirus protein, or a hepadnavirus protein,

optionally wherein the viral protein is (a) a SARS-CoV-2 spike glycoprotein, optionally wherein the SARS-CoV-2 spike glycoprotein comprises an amino acid sequence having at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, or

(b) an influenza virus glycoprotein;

(ii) a bacterial protein;

(iii) a fungal protein;

(iv) a protozoan protein; or

(v) a parasite protein.

7. The RNA molecule of any one of claims 1-6, wherein the first transgene of the second polynucleotide comprises a sequence having at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to a sequence of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13, and/or wherein the first transgene is expressed from a first subgenomic promoter.

8. The RNA molecule of any one of claims 1-7, wherein the first polynucleotide is located 5' of the second polynucleotide, and/or

wherein the RNA molecule further comprises an intergenic region located between the first polynucleotide and the second polynucleotide, and optionally wherein the intergenic region comprises a sequence having at least 85% identity to a sequence of SEQ ID NO:7.

9. The RNA molecule of claim 8, wherein the RNA molecule is a self-replicating RNA molecule, and/or wherein the RNA molecule comprises a sequence having at least 80%, at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to

(i) a sequence of SEQ ID NO:1;

(ii) a sequence of SEQ ID NO:2;

(iii) a sequence of SEQ ID NO:3;

(iv) a sequence of SEQ ID NO:4; or

(v) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 9;

(vi) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 11 and SEQ ID NO: 9;

(vii) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12 and SEQ ID NO: 9; or

(viii) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 13 and SEQ ID NO: 9,

wherein T is substituted with U.

10. The RNA molecule of claim 9, wherein the RNA molecule further comprises a 5' cap, optionally wherein the 5' cap has a Cap 1 structure, a Cap 1 (^{m6}A) structure, a Cap 2 structure, or a Cap 0 structure.
11. A DNA molecule encoding the RNA molecule of any one of claims 1-10.
12. A composition comprising the RNA molecule of any one of claims 1-10, and a lipid formulation 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.
13. The composition of claim 12, wherein

(i) the lipid formulation is a lipid nanoparticle having a size of less than about 200nm;

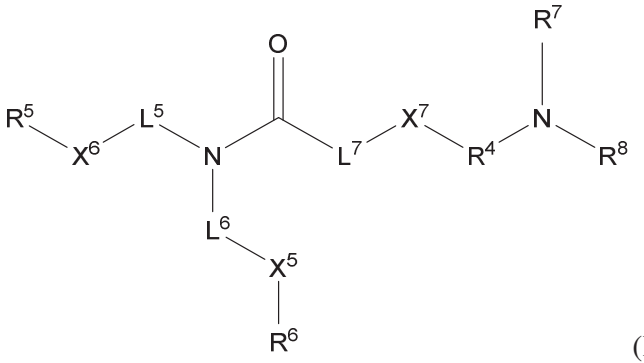
(ii) the lipid formulation encapsulates the nucleic acid molecule or is complexed to

the nucleic acid molecule;

(iii) the composition has a total lipid:nucleic acid molecule weight ratio of about 50:1 to about 10:1; or

(iv) combinations thereof.

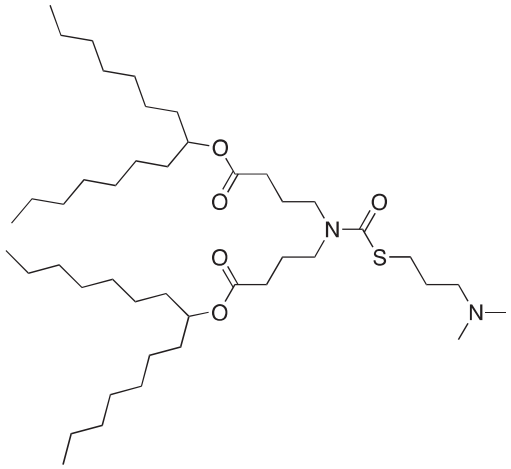
14. The composition of claim 12 or 13, wherein the lipid formulation comprises an ionizable cationic lipid, optionally wherein the ionizable cationic lipid has a structure of Formula I:



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein R^5 and R^6 are each independently selected from the group consisting of a linear or branched C_1-C_{31} alkyl, C_2-C_{31} alkenyl or C_2-C_{31} alkynyl and cholesteryl; L^5 and L^6 are each independently selected from the group consisting of a linear C_1-C_{20} alkyl and C_2-C_{20} alkenyl; X^5 is -C(O)O-, whereby -C(O)O-R^6 is formed or -OC(O)- whereby -OC(O)-R^6 is formed; X^6 is -C(O)O- whereby -C(O)O-R^5 is formed or -OC(O)- whereby -OC(O)-R^5 is formed; X^7 is S or O; L^7 is absent or lower alkyl; R^4 is a linear or branched C_1-C_6 alkyl; and R^7 and R^8 are each independently selected from the group consisting of a hydrogen and a linear or branched C_1-C_6 alkyl;

optionally wherein the ionizable cationic lipid has the following structure:



15. The composition of any one of claims 12-14, wherein the lipid formulation further comprises:

(i) a helper lipid, optionally wherein the helper lipid is a phospholipid, optionally wherein the helper lipid selected from dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidyl choline (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), and phosphatidylcholine (PC);

(ii) a polyethylene glycol (PEG)-lipid conjugate, optionally wherein the PEG-lipid conjugate is a PEG-DMG;

(iii) cholesterol; or

(iv) combinations thereof.

16. The composition of any one of claims 12-15, wherein:

(i) the lipid portion of the lipid formulation comprises about 40 mol% to about 60 mol% of an ionizable cationic lipid, about 4 mol% to about 16 mol% DSPC, about 30 mol% to about 47 mol% cholesterol, and about 0.5 mol% to about 3 mol% PEG2000-DMG; and/or

(ii) the composition is a lyophilized composition.

17. The composition of any one of claims 12-16, wherein the RNA molecule comprises a sequence having at least 80%, at least 85%, 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 97.5%, at least 98%, at least 98.5%, at least 99%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, at least 99.9%, or 100% identity to:

(A) a sequence of SEQ ID NO:1;

(B) a sequence of SEQ ID NO:2;

(C) a sequence of SEQ ID NO:3;

(D) a sequence of SEQ ID NO:4;

(E) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 9;

(F) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 11 and SEQ ID NO: 9;

(G) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 12 and SEQ ID NO: 9; or

(H) a sequence comprising a sequence of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 13 and SEQ ID NO: 9,

wherein T is substituted with U.

18. The composition of any of claims 12-17, wherein the composition is to be administered to a subject in need thereof, and/or wherein the composition is:

(i) a lyophilized composition and is to be reconstituted prior to administration;

(ii) to be administered as a booster dose to a subject who was previously vaccinated against coronavirus; and/or

(iii) to be administered at a dosage of about 0.01 μg to about 1,000 μg of nucleic acid.

19. Use of a composition of any of claims 12-18 in the manufacture of a medicament for preventing or ameliorating COVID-19.

20. Use of an RNA molecule of any of 1-10, or a composition of any one of claims 12-18, in the manufacture of a medicament for inducing an immune response in a subject.