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 alphavirus replication proteins, wherein one or more miRNA binding sites a sequence encoding the one or more alphavirus replication proteins have been modified as compared to a reference polynucleotide encoding the one or more alphavirus replication proteins; and (b) a second polynucleotide comprising a first transgene encoding a first antigenic protein. 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 prophylactic treatment of COVID-19, wherein (i) the one or more alphavirus replication proteins comprise an nsP1 protein, an nsP2 protein, an nsP3 protein, and an nsP4 protein; and (ii) the first antigenic protein comprises a SARS-CoV-2 antigenic protein. 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 prophylactic treatment of an infection by an infectious agent, wherein (i) the one or more alphavirus replication proteins comprise an nsP1 protein, an nsP2 protein, an nsP3 protein, and an nsP4 protein; and (ii) the first antigenic protein comprises a protein of the infectious agent.

[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.

CLAIMS

What is claimed is:

1. An RNA molecule comprising:
   (a) a first polynucleotide encoding one or more alphavirus replication proteins, wherein one or more miRNA binding sites in a sequence encoding the one or more alphavirus replication proteins have been modified as compared to a reference polynucleotide encoding the one or more alphavirus replication proteins; and
   (b) a second polynucleotide comprising a first transgene encoding a first antigenic protein .
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:
   (i) the one or more alphavirus replication proteins comprise an alphavirus nsP1 protein, an alphavirus nsP2 protein, an alphavirus nsP3 protein, and an alphavirus nsP4 protein; or
   (ii) 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

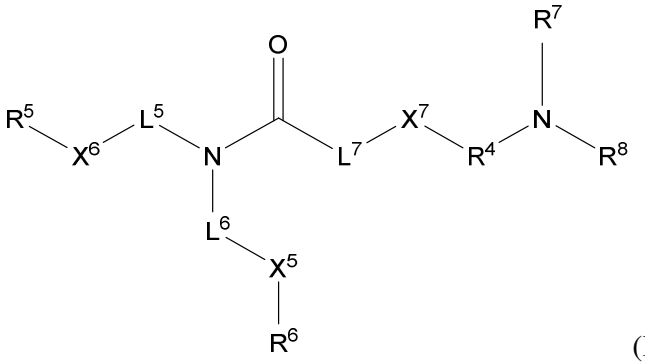
(i) the lipid formulation is a lipid nanoparticle having a size of less than 200nm;

(ii) the lipid formulation encapsulates the RNA molecule or is complexed to the RNA molecule;

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

(iv) combinations thereof.

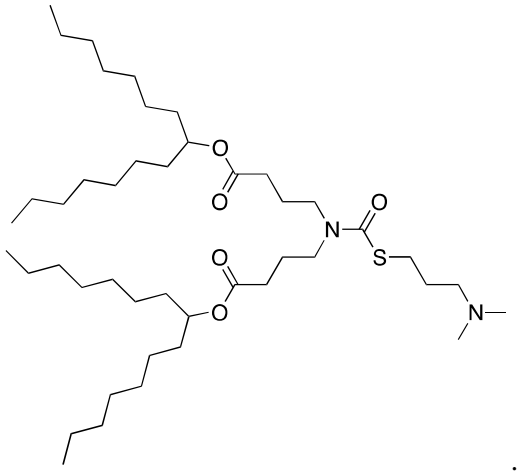
14. The composition of claim 12 or 13, wherein the lipid formulation comprises an ionizable cationic lipid, optionally wherein the ionizable cationic lipid has a structure of Formula I:



(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 40 mol% to 60 mol% of an ionizable cationic lipid, 4 mol% to 16 mol% DSPC, 30 mol% to 47 mol% cholesterol, and 0.5 mol% to 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 0.01 μg to 1,000 μg of nucleic acid.

19. Use of a composition of any of claims 12-18 in the manufacture of a medicament for prophylactic treatment of COVID-19, wherein:

(i) the one or more alphavirus replication proteins comprise an nsP1 protein, an nsP2 protein, an nsP3 protein, and an nsP4 protein; and

(ii) the first antigenic protein comprises a SARS-CoV-2 antigenic protein.

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 prophylactic treatment of an infection by an infectious agent, wherein:

(i) the one or more alphavirus replication proteins comprise an nsP1 protein, an nsP2 protein, an nsP3 protein, and an nsP4 protein; and

(ii) the first antigenic protein comprises a protein of the infectious agent.