

the poly(A)-tail to assure higher maximum protein levels and prolonged persistence of the mRNA may be used. These were identified by an *ex vivo* selection process for sequences that confer RNA stability and augment total protein expression (see WO 2017/060314, herein incorporated by reference). Alternatively, the 3'-UTR may be two re-iterated 3'-UTRs of the human beta-globin mRNA. Furthermore, a poly(A)-tail measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a 10 nucleotide linker sequence (of random nucleotides) and another 70 adenosine residues may be used. This poly(A)-tail sequence was designed to enhance RNA stability and translational efficiency.

Furthermore, a secretory signal peptide (sec) may be fused to the antigen-encoding regions preferably in a way that the sec is translated as N terminal tag. In one example, sec corresponds to the secretory signal peptide of the S protein. Sequences coding for short linker peptides predominantly consisting of the amino acids glycine (G) and serine (S), as commonly used for fusion proteins may be used as GS/Linkers.

The vaccine RNA described herein may be complexed with proteins and/or lipids, preferably lipids, to generate RNA-particles for administration. If a combination of different RNAs is used, the RNAs may be complexed together or complexed separately with proteins and/or lipids to generate RNA-particles for administration.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising an RNA, wherein said RNA comprises, from a 5' to 3' direction:

- (i) a 5' UTR that comprises a human alpha-globin 5'-UTR;
- (ii) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 9, wherein said nucleotide sequence:
 - (a) includes modified uridines in place of uridines; and
 - (b) encodes a SARS-CoV-2 Spike (S) polypeptide that is at least 95% identical to SEQ ID NO: 7 and is prefusion stabilized in that it includes at least one proline

substitution at position(s) corresponding to position(s) 986 and/or 987 in SEQ ID NO: 7; and

(iii) a 3'-UTR that comprises a first sequence from the amino terminal enhancer of split (AES) messenger RNA and a second sequence from the mitochondrial encoded 12S ribosomal RNA.

In another aspect, the present disclosure refers to a use of a pharmaceutical composition in the manufacture of a medicament for vaccinating humans known to have been exposed and/or infected by SARS-CoV-2, and/or humans not known to have been exposed to SARS-CoV-2, the pharmaceutical composition comprising an RNA, wherein said RNA comprises, from a 5' to 3' direction:

(i) a 5'-UTR that comprises a human alpha-globin 5'-UTR;

(ii) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 9, wherein said nucleotide sequence:

(a) includes modified uridines in place of uridines; and

(b) encodes a SARS-CoV-2 Spike (S) polypeptide that is at least 95% identical to SEQ ID NO: 7 and is prefusion stabilized in that it includes at least one proline substitution at position(s) corresponding to position(s) 986 and/or 987 in SEQ ID NO: 7; and

(iii) a 3'-UTR that comprises a first sequence from the amino terminal enhancer of split (AES) messenger RNA and a second sequence from the mitochondrial encoded 12S ribosomal RNA.

In another aspect, the present disclosure refers to a method of manufacturing the pharmaceutical composition as disclosed herein, comprising combining the RNA with a

cationically ionizable lipid, a sterol, a neutral lipid, and a polymer conjugated lipid to form lipid nanoparticles that encapsulate the RNA.

In one example, the disclosure relates to a composition or medical preparation comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one example, an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

Claims

1. A pharmaceutical composition comprising an RNA, wherein said RNA comprises, from a 5' to 3' direction:
 - (i) a 5' UTR that comprises a human alpha-globin 5'-UTR;
 - (ii) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 9, wherein said nucleotide sequence:
 - (a) includes modified uridines in place of uridines; and
 - (b) encodes a SARS-CoV-2 Spike (S) polypeptide that is at least 95% identical to SEQ ID NO: 7 and is prefusion stabilized in that it includes at least one proline substitution at position(s) corresponding to position(s) 986 and/or 987 in SEQ ID NO: 7; and
 - (iii) a 3'-UTR that comprises a first sequence from the amino terminal enhancer of split (AES) messenger RNA and a second sequence from the mitochondrial encoded 12S ribosomal RNA.
2. The pharmaceutical composition of claim 1, wherein the RNA comprises a 5'-cap that comprises $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$.
3. The pharmaceutical composition of claim 1, wherein the RNA comprises a polyA sequence, wherein the polyA sequence comprises a sequence that is at least 90% identical to SEQ ID NO: 14, which optionally comprises a modified uridine in place of one or more uridines.
4. The pharmaceutical composition of claim 3, wherein the 5'-UTR comprises a sequence that is at least 90% identical to SEQ ID NO: 12, which optionally comprises a modified uridine in place of one or more uridines.
5. The pharmaceutical composition of claim 4, wherein the 3'-UTR comprises a sequence that is at least 90% identical to SEQ ID NO: 13, which optionally comprises a modified uridine in place of one or more uridines.

6. The pharmaceutical composition of claim 1, wherein the nucleotide sequence of (ii) is characterized in that its G/C content is increased by about 55% as compared to the G/C content of the nucleotide sequence of SEQ ID NO: 2.
7. The pharmaceutical composition of claim 1, wherein the RNA is formulated in lipid nanoparticles comprising:
 - (A) a cationically ionizable lipid;
 - (B) a sterol;
 - (C) a neutral lipid; and
 - (D) a polymer conjugated lipid.
8. The pharmaceutical composition of claim 7, further comprising at least one salt and/or a cryoprotectant.
9. The pharmaceutical composition of claim 8, wherein the cryoprotectant comprises sucrose.
10. The pharmaceutical composition of claim 7, wherein the composition is formulated for intramuscular administration.
11. The pharmaceutical composition of claim 7, wherein the RNA is present in the composition in an amount of about 3 µg per dose, about 10 µg per dose, or about 30 µg per dose.
12. The pharmaceutical composition of claim 1, wherein the RNA comprises a polyA sequence, wherein the polyA sequence comprises at least 100 adenine nucleotides.
13. Use of a pharmaceutical composition in the manufacture of a medicament for vaccinating humans known to have been exposed and/or infected by SARS-CoV-2, and/or humans not known to have been exposed to SARS-CoV-2, the pharmaceutical composition comprising an RNA, wherein said RNA comprises, from a 5' to 3' direction:
 - (i) a 5'-UTR that comprises a human alpha-globin 5'-UTR;
 - (ii) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 9, wherein said nucleotide sequence:

- (a) includes modified uridines in place of uridines; and
 - (b) encodes a SARS-CoV-2 Spike (S) polypeptide that is at least 95% identical to SEQ ID NO: 7 and is prefusion stabilized in that it includes at least one proline substitution at position(s) corresponding to position(s) 986 and/or 987 in SEQ ID NO: 7; and
 - (iii) a 3'-UTR that comprises a first sequence from the amino terminal enhancer of split (AES) messenger RNA and a second sequence from the mitochondrial encoded 12S ribosomal RNA.
14. The use of claim 13, wherein more than one dose of the pharmaceutical composition is to be administered.
15. The use of claim 14, wherein the more than one dose comprises a first dose and at least a second dose, and wherein the first and second doses are spaced apart by a time period of about 21 days.
16. The use of claim 13, wherein at least one dose of the pharmaceutical composition is to be administered, each of which independently comprises RNA in an amount within a range of 1 µg to 100 µg.
17. The use of claim 13, wherein at least two doses of the pharmaceutical composition are to be administered, each of which comprises about 30 µg of the RNA.
18. The use of claim 13, wherein the RNA comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 20.
19. The pharmaceutical composition of claim 1 or the use of claim 18, wherein the RNA includes modified uridines in place of all uridines.
20. The pharmaceutical composition or use of claim 13 or 19, wherein the modified uridines are each N1-methyl-pseudouridine.
21. The use of claim 13, wherein the nucleotide sequence of (ii) is SEQ ID NO: 9.

22. The use of claim 13, wherein at least one dose of the pharmaceutical composition is to be administered, each of which independently comprises RNA in an amount within a range of 10 µg to 30 µg.
23. The use of claim 13, wherein administration is by intramuscular administration.
24. The use of claim 13, wherein the RNA comprises:
(A) a 5'-cap that comprises $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$; or
(B) a polyA sequence comprising 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence; or
(C) a combination of (A) and (B).
25. The use of claim 13, wherein the RNA is formulated in lipid nanoparticles comprising:
(A) a cationically ionizable lipid;
(B) a sterol;
(C) a neutral lipid; and
(D) a polymer conjugated lipid.
26. The use of claim 24, wherein at least two doses of the pharmaceutical composition are to be administered.
27. The use of claim 26, wherein a subsequent dose of the pharmaceutical composition is to be administered after the second dose, wherein the subsequent dose is to be administered at least about 3 months after administration of the second dose.
28. The use of claim 13, further comprising that a second vaccine is to be administered.
29. The use of claim 28, wherein the second vaccine is an influenza vaccine.
30. A method of manufacturing the pharmaceutical composition of claim 1, comprising combining the RNA with a cationically ionizable lipid, a sterol, a neutral lipid, and a polymer conjugated lipid to form lipid nanoparticles that encapsulate the RNA.