

COMBINATION RNA VACCINE

RELATED APPLICATION DATA

The present application claims priority from United States Patent Application No. 5 63/511,332 filed 30 June 2023 entitled "Combination RNA Vaccine", the entire contents of which is hereby incorporated by reference.

SEQUENCE LISTING

10 The present application is filed together with a Sequence Listing in electronic form. The entire contents of the Sequence Listing are hereby incorporated by reference.

FIELD

The present disclosure relates to combination RNA vaccines and uses thereof. The present disclosure also relates to conventional mRNA vaccines and self-replicating RNA 15 vaccines for the treatment of diseases or conditions including respiratory syncytial virus (RSV).

BACKGROUND

Respiratory viral infections are a significant threat to human health. Infections, 20 such as those caused by the influenza virus and severe acute respiratory syndrome coronavirus (SARS-CoV) have been known to cause global pandemics, killing millions of people worldwide. Recently, SAR-CoV-2 has been responsible for causing the on-going worldwide pandemic of the severely infectious coronavirus disease 2019 (COVID-19). Moreover, respiratory syncytial virus (RSV) is the single most common cause of 25 respiratory hospitalization in infants, and reinfection remains common in later life. Whilst some vaccines are available for viral infections such as SARS-CoV-2 and RSV, such as mRNA vaccines developed to treat or prevent a SARS-CoV-2 infection, further improvements can be made to increase their efficacy and/or improve treatment strategies.

Currently, egg-based manufacturing processes are the most common way that 30 vaccines are produced. This process requires a significant amount of time to optimize virus growth in the eggs, as well as resources (i.e., eggs) to produce sufficient amounts of vaccine, particularly during a pandemic. Furthermore, given the long development time required, vaccine strain selection is conducted before the vaccine is made available, making it difficult to respond to changes in the virus. Vaccines have also been produced 35 using cell-based manufacturing processes involving cultured mammalian cells (e.g. Madin-Darby Canine Kidney, or MDCK cells) in place of eggs, and viral-based

platforms involving recombinant virus (e.g. baculovirus encoding an antigen of influenza) have also been utilised.

There remains a need for the development of specific and efficient viral vaccines that can be produced more rapidly and with broader utility than current egg-based techniques, for the treatment or prevention of respiratory viral infections, such as RSV and SARS-CoV-2. Nucleic acid-based vaccines offer distinct advantages over the current egg-based manufacturing platform, although some challenges remain. For example, the inherently labile nature of mRNA results in most RNA-based vaccines having limited ability to provide antigen at a dose and duration required to produce a strong, durable immune response.

Therefore, it will be apparent to the skilled person that there is a need in the art for compositions with broader utility and/or improved efficacy that are suitable for use as vaccines.

15 SUMMARY

The present disclosure is based on the inventors' identification of a RNA comprising an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, an antigen from influenza and an antigen from a respiratory syncytial virus (RSV) that is suitable as a vaccine for the treatment of a SARS-Cov-2 infection, coronavirus disease 2019 (COVID-19), influenza and/or RSV. Furthermore, the findings by the inventors provide basis for methods of treating or preventing or delaying progression of a disease or disorder such as a SARS-CoV-2 infection or COVID-19, influenza and/or RSV, as well as complications thereof including acute respiratory distress syndrome (ARDS), in a subject.

25 Accordingly, the present disclosure provides a polynucleotide comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

In an example, the polynucleotide comprises, in 5' to 3' order:

- (a) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 35 (b) the nucleotide sequence encoding an antigen from influenza, and

(c) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV).

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) the nucleotide sequence encoding an antigen from a respiratory syncytial virus
5 (RSV);

(b) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and

(c) the nucleotide sequence encoding an antigen from influenza.

In another example, the polynucleotide comprises, in 5' to 3' order:

10 (a) the nucleotide sequence encoding an antigen from influenza;
(b) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV); and

(c) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

15 In an example, the polynucleotide is RNA or DNA. In one example, the RNA is messenger RNA (mRNA). In one example, the mRNA is conventional mRNA (cRNA) or self-replicating RNA.

The present disclosure therefore also provides a RNA comprising:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

25 In an example, the RNA comprises, in 5' to 3' order:

(a) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) the nucleotide sequence encoding an antigen from influenza, and

30 (c) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV).

In another example, the RNA comprises, in 5' to 3' order:

(a) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

35 (b) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and

(c) the nucleotide sequence encoding an antigen from influenza.

In another example, the RNA comprises, in 5' to 3' order:

- (a) the nucleotide sequence encoding an antigen from influenza;
- (b) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV); and

- 5 (c) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure also provides a cRNA comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 10 (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

In an example, the cRNA comprises, in 5' to 3' order:

- 15 (a) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- (b) the nucleotide sequence encoding an antigen from influenza, and
- (c) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV).

- 20 In another example, the cRNA comprises, in 5' to 3' order:

- (a) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

- (b) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and

- 25 (c) the nucleotide sequence encoding an antigen from influenza.

In another example, the cRNA comprises, in 5' to 3' order:

- (a) the nucleotide sequence encoding an antigen from influenza;
- (b) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV); and

- 30 (c) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure also provides a self-replicating RNA comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 35 (b) a nucleotide sequence encoding a second antigen from influenza, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

In an example, the self-replicating RNA comprises, in 5' to 3' order:

5 (a) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) the nucleotide sequence encoding an antigen from influenza, and

(c) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV).

10 In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and

15 (c) the nucleotide sequence encoding an antigen from influenza.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) the nucleotide sequence encoding an antigen from influenza;

(b) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV); and

20 (c) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the regulatory element is operably linked to the 5' end of the first nucleotide sequence defined at (a). In one example, the regulatory element is selected from the group consisting of a Kozak consensus sequence, an IRES, a promoter and
25 combinations thereof. For example, the regulatory element is a Kozak consensus sequence. In another example, the regulatory element is an IRES. In another example, the promoter is a SG promoter.

In an example, the nucleotide sequences are operably linked to the same regulatory element (e.g., to the same SG promoter). In another example, the nucleotide
30 sequences are operably linked to different regulatory elements (e.g., to different SG promoters).

In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

35 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
5 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

10 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG
15 promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter.

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

20 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

In one example, the RNA comprises:

25 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

30 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

35 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
5 (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
10 to an IRES or a SG promoter.

In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory
15 syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
20 linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
25 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
30 virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
(RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute
35 respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter.

In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

5 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

In one example, the self-replicating RNA comprises:

10 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

15 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

20 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

25 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and

30 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and

35 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably
5 linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
10 syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES or a SG promoter.

15 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
(RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute
respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG
20 promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
to an IRES or a SG promoter.

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to
25 a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

30 In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter, and

35 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably
5 linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
10 (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
15 to an IRES or a SG promoter.

In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to
a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial
20 virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
25 syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

30 In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter, and

35 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter.

In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to
5 a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.

10 In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

15 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

20 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

25 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

30 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

35 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
5 linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
10 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

15 In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

20 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial
25 virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
30 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

35 In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

5 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

10 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the cRNA comprises, in 5' to 3' order:

15 (a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

20 In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

5 In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory
10 syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
15 linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

25 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
30 to an IRES.

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial
35 virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked an IRES.

In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
5 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES.

10 In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES, and

15 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
(RSV);

20 (b) a nucleotide sequence encoding a second antigen from a severe acute
respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
to an IRES.

In another example, the RNA comprises, in 5' to 3' order:

25 (a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

30 In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES, and

35 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
5 linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
10 (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

15 In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably
25 linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
30 syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

35 In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

5 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

10 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the polynucleotide comprises:

15 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

20 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the polynucleotide comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

5 In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

25 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the cRNA comprises:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

5 In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the cRNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

25 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

5 In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

10 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the polynucleotide comprises:

20 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the polynucleotide comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

5 In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

10 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the RNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

5 In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

25 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the self-replicating RNA comprises:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

5 In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the polynucleotide comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

5 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter,
10 and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

15 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked an IRES.

In one example, the RNA comprises:

20 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
5 to an IRES.

In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

10 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
25 virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute
30 respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the cRNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

5 In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

30 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the polynucleotide comprises:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

5 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

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(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked a SG promoter.

In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

5 In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter,
10 and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

15 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the cRNA comprises:

20 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
5 to a SG promoter.

In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

10 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
25 virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);

(b) a nucleotide sequence encoding a second antigen from a severe acute
30 respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from influenza;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

5 In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the polynucleotide comprises, in 5' to 3' order:

20 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the RNA comprises:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

5 In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

10 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

15 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

20 In another example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the cRNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

5 In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter,
10 and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES.

In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to
15 a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

20 In one example, the self-replicating RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

35 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked
5 to an IRES.

In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial
10 virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES.

In one example, the polynucleotide comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory
15 syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

20 In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

25 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

30 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

35 In another example, the polynucleotide comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

5 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the RNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

10 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

15 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the RNA comprises, in 5' to 3' order:

30 (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the cRNA comprises:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

5 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

10 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the cRNA comprises, in 5' to 3' order:

15 (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

20 (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

In another example, the cRNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

25 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises:

30 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

35 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from influenza, operably linked to a SG promoter, and

5 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter.

In one example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;

10 (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter, and

(c) a nucleotide sequence encoding a third antigen from influenza, operably linked to a SG promoter.

15 In another example, the self-replicating RNA comprises, in 5' to 3' order:

(a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;

(b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter, and

20 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter.

In one example, a polynucleotide, RNA, cRNA or self-replicating RNA of the disclosure comprises a Kozak consensus sequence. In an example, the Kozak consensus
25 sequence comprises or consists of a sequence set forth in SEQ ID NO: 6 (GCCACC). In one example, the Kozak consensus sequence consists of a sequence set forth in SEQ ID NO: 7 (ACCATGG).

In one example, the polynucleotide is a multicistronic RNA. For example, the polynucleotide is a multicistronic cRNA. For example, the cRNA is a multicistronic
30 cRNA. In another example, the polynucleotide is a multicistronic self-replicating mRNA. In another example, the self-replicating RNA is a monocistronic self-replicating mRNA. In another example, the polynucleotide is a multicistronic self-replicating RNA. In another example, the self-replicating RNA is a monocistronic self-replicating RNA.

In one example, the SG promoter is a native SG promoter. For example, a native
35 SG promoter is a promoter that is native to the RNA virus from which it is derived and/or

based on (e.g., an alphavirus). In one example, the native SG promoter is a native alphavirus SG promoter.

In one example, the SG promoter is a minimal SG promoter or an extended SG promoter.

5 In one example, the SG promoter is a minimal SG promoter. In one example, the native SG promoter is a minimal SG promoter. For example, the minimal SG promoter is the minimal sequence required for initiation of transcription. In one example, the minimal native SG promoter is 49 nucleotides in length. In one example, the minimal SG promoter is 49 nucleotides in length. In one example, the minimal native SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO:
10 1. In one example, the minimal SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 1.

In one example, the SG promoter is an extended SG promoter. In one example, the native SG promoter is an extended SG promoter. For example, the extended SG
15 promoter is extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein (e.g., NSP4) of the RNA virus (e.g., an alphavirus). In one example, the extended SG promoter is extended at the 5' end with nucleotides occurring in a sequence encoding an alphavirus NSP4. The addition of nucleotides to the 5' end of the SG promoter sequence did not interfere with expression of the non-structural protein
20 and viral replicase, e.g., alphavirus NSP4.

In one example, the SG promoter is extended at the 5' end by 51 or fewer nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is a minimal SG promoter extended at the 5' end by no more than 51 nucleotides occurring in a sequence encoding a non-
25 structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 1 extended at the 5' end by no more than 51 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 100 nucleotides in length. In one example, the extended
30 SG promoter is encoded by a sequence comprising or consisting of nucleotides 2 to 101 of SEQ ID NO: 5.

In one example, the SG promoter is extended at the 5' end by about 5 nucleotides to about 20 nucleotides, for example by about 5 nucleotides, or about 10 nucleotides, or about 12, or about 15 nucleotides, or about 20 nucleotides, occurring in a sequence
35 encoding a non-structural protein (e.g., an alphavirus NSP4). In another example, the SG promoter is extended at the 5' end by about 20 to about 35 nucleotides, for example,

by about 25 nucleotides or about 27 nucleotides, or about 30 nucleotides, or about 35 nucleotides, occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4).

In one example, the SG promoter is extended at the 5' end by about 12 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 1 extended at the 5' end by 12 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 61 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 41 to 101 of SEQ ID NO: 5. In another example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 2.

In one example, the SG promoter is extended at the 5' end by about 31 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 1 extended at the 5' end by 31 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 80 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 22 to 101 of SEQ ID NO: 5. In another example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 3.

In one example, the extended SG promoter comprises a repeat sequence corresponding to nucleotides 66 to 75 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence comprising nucleotides 50 to 75 of SEQ ID NO: 5 and nucleotides 66 to 101 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 15.

In one example, the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF- κ B-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a (FGF1A), GTX, or a combination thereof.

In one example, the IRES is a wild-type IRES derived from encephalomyocarditis virus (EMCV). For example, the wild-type EMCV IRES comprises a sequence set forth in SEQ ID NO: 4.

In one example, the first and/or second and/or third nucleotide sequences are
5 codon optimized.

In one example, the G/C content of the first and/or second and/or third nucleotide sequences are modified.

In one example, the G/C content of the first and/or second and/or third nucleotide sequences is increased by at least 5% compared to the G/C content of the unmodified
10 sequence. For example, the G/C content of the first and/or second and/or third nucleotide sequences is increased by at least 10%, or 15%, or 20%, or 25%, or 30%, or 35%, or 40% compared to the G/C content of the unmodified sequence.

In one example, the polynucleotide comprises at least one chemically modified nucleotide.

15 In one example, the chemically modified nucleotide is selected from the group consisting of N6,2'-O-dimethyl-adenosine (m6Am), 5-methyluridine (m5U), N4-acetylcytidine (ac4C), 2-thiocytidine (s2C), 2-thiouridine (s2U), 5-methylcytidine (m5C), N6-methyladenosine (m6a), pseudouridine (ψ), 1-methylpseudouridine (m1 ψ), and combinations thereof. For example, the chemically modified nucleotide is N6,2'-O-
20 dimethyl-adenosine (m6Am). For example, the chemically modified nucleotide is 5-methyluridine (m5U). For example, the chemically modified nucleotide is N4-acetylcytidine (ac4C). For example, the chemically modified nucleotide is 2-thiocytidine (s2C). For example, the chemically modified nucleotide is 2-thiouridine (s2U). For example, the chemically modified nucleotide is 5-methylcytidine (m5C). For example,
25 the chemically modified nucleotide is N6-methyladenosine (m6a). For example, the chemically modified nucleotide is pseudouridine (ψ). For example, the chemically modified nucleotide is 1-methylpseudouridine (m1 ψ).

In one example, the first nucleotide sequence comprises the 5'-UTR of haptoglobin (HP), fibrinogen beta chain (FGB), haptoglobin-related protein (HPR),
30 albumin (ALB), complement component 3 (C3), fibrinogen alpha chain (FGA), alpha 6 collagen (Col6A), alpha-1-antitrypsin (SERPINA1), alpha-1-antichymotrypsin (SERPINA3) a fragment and/or a variant thereof.

In one example, the 5'UTR is a 5'UTR of a Venezuelan equine encephalitis virus (VEEV) or modified forms thereof. For example, the 5'UTR comprises a sequence set
35 forth in SEQ ID NO: 13.

In one example, the 5'-UTR, the fragment and/or the variant thereof is between 40 and 2000 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 40 and 100 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 100 and 250 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 250 and 500 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 500 and 750 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 750 and 1000 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1000 and 1250 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1250 and 1500 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1500 and 1750 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1750 and 2000 nucleotides in length.

In one example, the 5'-UTR, the fragment and/or the variant thereof comprises a nucleotide sequence at least 90% identical to a nucleotide sequence set forth in any one of SEQ ID NO: 9 to 12. For example, the 5'-UTR, the fragment and/or the variant thereof comprises a nucleotide sequence 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identical to a nucleotide sequence set forth in any one of SEQ ID NO: 9 to 12.

In one example, the polynucleotide comprises a combination of two or more 5'-UTRs, fragments and/or variants thereof. In one example, the two or more 5'-UTRs are the same. In one example, the two or more 5'-UTRs are different.

In one example, the nucleotide sequence comprising the 5'UTR comprises at least one microRNA binding site, an AU rich element (ARE), a GC-rich element, a stem loop, and combinations thereof. In one example, the nucleotide sequence comprises a microRNA binding site. In one example, the nucleotide sequence comprises an AU rich element (ARE). In one example, the nucleotide comprises a GC-rich element. In one example, the nucleotide sequence comprises a stem loop. For example, the stem loop is a histone stem loop.

In one example, the polynucleotide further comprises a nucleotide sequence comprising a 3'UTR. In one example, the nucleotide sequence comprising the 3'UTR is located 3' of the second or the one or more additional nucleotide sequences. For example, the nucleotide sequence comprising the 3'UTR is located 3' of the second nucleotide sequence. In one example, the 3'UTR comprises a 3'-UTR of arachidonate 5-lipoxygenase (ALOX5), alpha I collagen (COL1A1), tyrosine hydroxylase (TH) gene,

amino-terminal enhancer of split (AES), human mitochondrial 12S rRNA (mtRNR1), a fragment and/or a variant thereof.

In one example, the 3'UTR is a 3'UTR of a Sindbis virus (SINV) or modified forms thereof. For example, the 3'UTR comprises a sequence set forth in SEQ ID NO:

5 14.

In one example, the 3'UTR, the fragment and/or the variant thereof is between 40 and 400 nucleotides in length. For example, the 3'-UTR is between 40 and 50, or 50 and 60, or 60 and 70, or 70 and 80, or 80 and 90, or 90 and 100, or 100 and 125, or 125 and 150, or 150 and 175, or 175 and 200, or 200 and 225, or 225 and 250, or 250 and 275, or 10 275 and 300, or 300 and 325, or 325 and 350, or 350 and 375, or 375 and 400 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 40 and 50 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 50 and 60 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 60 and 70 nucleotides in length. For 15 example, the 3'-UTR, the fragment and/or the variant thereof is between 70 and 80 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 80 and 90 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 90 and 100 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 100 and 125 nucleotides in 20 length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 125 and 150 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 150 and 175 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 175 and 200 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 200 and 225 25 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 225 and 250 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 250 and 275 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 275 and 300 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 300 and 325 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 325 and 350 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 350 and 375 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 375 and 400 nucleotides in length.

In one example, the polynucleotide comprises a combination of two or more 3'-UTRs, fragments and/or variants thereof. In one example, the two or more 3'-UTRs are the same. In one example, the two or more 3'-UTRs are different.

In one example, the nucleotide sequence comprising the 3'UTR, the fragment
5 and/or variant thereof comprises at least one microRNA binding site, an AU rich element (ARE), a GC-rich element, a triple helix, a stem loop, one or more stop codons and combinations thereof. In one example, the nucleotide sequence comprises a microRNA binding site. In one example, the nucleotide sequence comprises an AU rich element (ARE). In one example, the nucleotide sequence comprises a GC-rich element. In one
10 example, the nucleotide sequence comprises a triple helix. In one example, the nucleotide sequence comprises a stem loop. For example, the stem loop is a histone stem loop. In one example, the nucleotide sequence comprises one or more stop codons. For example, the one or more stop codons are located at the 5' end of the 3'-UTR.

In one example, the polynucleotide comprises a nucleotide sequence comprising
15 one or more 3' tailing sequences located at the 3' end of the nucleotide sequence comprising the 3'UTR. In one example, the one or more 3' tailing sequences are selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof. For example, the 3' tailing sequence comprises a poly-A sequence. In one example, the 3' tailing sequence
20 comprises a polyadenylation signal. In one example, the 3' tailing sequence comprises a G-quadruplex. In one example, the 3' tailing sequence comprises a poly-C sequence. In one example, the 3' tailing sequence comprises a stem loop. For example, the stem loop is a histone stem loop. In one example, the 3' tailing sequence comprises a poly-A sequence and a G-quadruplex. In one example, the 3' tailing sequence comprises a stem
25 loop (e.g., a histone stem loop) and a poly-A sequence.

In one example, the one or more 3' tailing sequences comprises one or more poly-A sequences each comprising between 10 and 300 consecutive adenosine nucleotides. For example, the poly-A sequences each comprises between 10 and 20, or 20 and 30, or 30 and 40, or 40 and 50, or 50 and 60, or 60 and 70, or 70 and 80, or 80 and 90, or 90
30 and 100, or 100 and 125, or 125 and 150, or 150 and 175, or 175 and 200, or 200 and 225, or 225 and 250, or 250 and 275, or 275 and 300 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 10 and 20 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 20 and 30 consecutive adenosine nucleotides. For example, the one
35 or more poly-A sequences each comprises between 30 and 40 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprise 36

consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 40 and 50 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 50 and 60 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 60 and 70 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 70 and 80 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 80 and 90 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 90 and 100 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 100 and 125 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 125 and 150 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 150 and 175 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 175 and 200 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 200 and 225 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 225 and 250 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 250 and 275 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 275 and 300 consecutive adenosine nucleotides.

In one example, the one or more poly-A sequence each comprises 10, or 20, or 30, or 40, or 50, or 60, or 70, or 80, or 90, or 100, or 125, or 150, or 175, or 200, or 225, or 250, or 275, or 300 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 10 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 20 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 30 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 40 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 50 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 60 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 70 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 80 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 90 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 100 consecutive adenosine nucleotides. For example, the one or more poly-

A sequence each comprises 125 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 150 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 175 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 200 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 225 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 250 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 275 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 300 consecutive adenosine nucleotides.

In one example, the poly-A sequence comprises 36 consecutive adenosine nucleotides. For example, the poly-A sequence comprises a sequence set forth in SEQ ID NO: 16.

In one example, the one or more poly-A sequences is separated by an interrupting linker. For example, the 3'tailing sequence comprises, in order of 5' to 3': a poly-A sequence comprising consecutive adenosine nucleotides, an interrupting linker, and a further poly-A sequence comprising consecutive adenosine nucleotides.

In one example, the interrupting linker is from 10 to 50, or 50 to 100, or 100 to 150 nucleotides in length. For example, the interrupting linker is from 10 to 50 nucleotides in length. For example, the interrupting linker is from 50 to 100 nucleotides in length. For example, the interrupting linker is from 100 to 150 nucleotides in length.

In one example, the interrupting linker is 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 25, or 30, or 35, or 40, or 45, or 50, or 55, or 60, or 65, or 70, or 75, or 80, or 85, or 90, or 95, or 100, or 110, or 120, or 130, or 140, or 150 nucleotides in length. For example, the interrupting linker is 1 nucleotide in length. For example, the interrupting linker is 2 nucleotides in length. For example, the interrupting linker is 3 nucleotides in length. For example, the interrupting linker is 4 nucleotides in length. For example, the interrupting linker is 5 nucleotides in length. For example, the interrupting linker is 6 nucleotides in length. For example, the interrupting linker is 7 nucleotides in length. For example, the interrupting linker is 8 nucleotides in length. For example, the interrupting linker is 9 nucleotides in length. For example, the interrupting linker is 10 nucleotides in length. For example, the interrupting linker is 11 nucleotides in length. For example, the interrupting linker is 12 nucleotides in length. For example, the interrupting linker is 13 nucleotides in length. For example, the interrupting linker is 14 nucleotides in length. For example, the interrupting linker is 15 nucleotides in length. For example, the

interrupting linker is 16 nucleotides in length. For example, the interrupting linker is 17 nucleotides in length. For example, the interrupting linker is 18 nucleotides in length. For example, the interrupting linker is 19 nucleotides in length. For example, the interrupting linker is 20 nucleotides in length. For example, the interrupting linker is 25 nucleotides in length. For example, the interrupting linker is 30 nucleotides in length. For example, the interrupting linker is 35 nucleotides in length. For example, the interrupting linker is 40 nucleotides in length. For example, the interrupting linker is 45 nucleotides in length. For example, the interrupting linker is 50 nucleotides in length. For example, the interrupting linker is 55 nucleotides in length. For example, the interrupting linker is 60 nucleotides in length. For example, the interrupting linker is 65 nucleotides in length. For example, the interrupting linker is 70 nucleotides in length. For example, the interrupting linker is 75 nucleotides in length. For example, the interrupting linker is 80 nucleotides in length. For example, the interrupting linker is 85 nucleotides in length. For example, the interrupting linker is 90 nucleotides in length. For example, the interrupting linker is 95 nucleotides in length. For example, the interrupting linker is 100 nucleotides in length. For example, the interrupting linker is 110 nucleotides in length. For example, the interrupting linker is 120 nucleotides in length. For example, the interrupting linker is 130 nucleotides in length. For example, the interrupting linker is 140 nucleotides in length. For example, the interrupting linker is 150 nucleotides in length.

In one example, the interrupting linker is 10 nucleotides in length. In one example, the interrupting linker comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 8. For example, the interrupting linker comprises or consists of a nucleotide sequence GCAUAUGACU.

In one example, the 3' tailing sequence comprises, in order of 5' to 3': a poly-A sequence comprising 30 consecutive adenosine nucleotides, an interrupting linker of 10 nucleotides, and a further poly-A sequence comprising 70 consecutive adenosine nucleotides.

In one example, the 3' tailing sequence comprises, in order of 5' to 3': a poly-A sequence comprising 30 consecutive adenosine nucleotides, an interrupting linker comprising or consisting of the nucleotide sequence set forth in SEQ ID NO: 8, and a further poly-A sequence comprising 70 consecutive adenosine nucleotides.

In one example, the RNA, optionally self-replicating RNA, comprises, in order from 5' to 3':

a) a 5'-UTR, fragment and/or variant thereof;

- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 5 d) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter;
- e) a nucleotide sequence encoding a third antigen from respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter;
- f) a 3'-UTR, fragment and/or variant thereof; and
- 10 g) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the RNA, optionally self-replicating RNA, comprises, in order from 5' to 3':

- 15 a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);
- 20 d) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter;
- e) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter;
- 25 f) a 3'-UTR, fragment and/or variant thereof; and
- g) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the RNA, optionally self-replicating RNA, comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) a nucleotide sequence encoding an antigen from influenza;
- 35 d) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter;

- e) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter;
- f) a 3'-UTR, fragment and/or variant thereof; and
- 5 g) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the RNA, optionally self-replicating RNA, of the present disclosure comprises, in order from 5' to 3':

- 10 a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from influenza, operably linked to a minimal SG promoter; and a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter; or
- 15 b) a nucleotide sequence an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from influenza, operably linked to an a minimal SG promoter; and a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an extended SG promoter; or
- 20 c) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from influenza, operably linked to a minimal SG promoter; and a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an a wild-type EMCV IRES.
- 25

In another example, the RNA, optionally self-replicating RNA, of the present disclosure comprises, in order from 5' to 3':

- 30 a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter; and a nucleotide sequence encoding a third antigen from influenza, operably linked to a minimal SG promoter; or
- 35

- b) a nucleotide sequence an antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an a minimal SG promoter; and a nucleotide sequence encoding a third antigen from influenza, operably linked to an extended SG promoter; or
- 5 c) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter; and a nucleotide sequence encoding a third antigen from influenza, operably linked to an a wild-type EMCV IRES.
- 10

In another example, the RNA, optionally self-replicating RNA, of the present disclosure comprises, in order from 5' to 3':

- 15 a) a nucleotide sequence encoding an antigen from influenza, operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter; and a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter; or
- 20 b) a nucleotide sequence an antigen from influenza, operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to an a minimal SG promoter; and a nucleotide sequence encoding a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an extended SG promoter; or
- 25 a nucleotide sequence encoding an antigen from influenza, operably linked to a minimal SG promoter; a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter; and a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an a wild-type EMCV IRES.
- 30

In one example, the RNA, optionally self-replicating RNA, of the present disclosure comprises, in order from 5' to 3': a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; a nucleotide sequence encoding an antigen from influenza operably linked to a minimal SG promoter

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comprising a sequence set forth in SEQ ID NO: 1; and a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1.

In one example, the RNA, optionally self-replicating RNA, of the present disclosure comprises, in order from 5' to 3': a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; a nucleotide sequence encoding an antigen from influenza, operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; and a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an extended SG promoter comprising a sequence set forth in SEQ ID NO: 5.

In one example, the RNA, optionally self-replicating RNA, of the present disclosure comprises, in order from 5' to 3': a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; a nucleotide sequence encoding an antigen from influenza, operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; and a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to a wild-type EMCV IRES comprising a sequence set forth in SEQ ID NO: 4.

In one example, the RNA further comprises a 5' terminal cap structure.

In one example, the 5' terminal cap structure is an endogenous cap or analogue thereof. For example, the 5' terminal cap structure is an endogenous cap. For example, the 5' terminal cap structure is an analogue of an endogenous cap.

In one example, the 5' terminal cap structure comprise a guanine or guanine analogue thereof. For example, the 5' terminal cap structure comprise a guanine. For example, the 5' terminal cap structure comprise a guanine analogue of a guanine.

In one example, the 5' terminal cap structure is selected from a group consisting of anti-reverse cap analogue (ARCA), N7,2'-O-dimethyl-guanosine (mCAP), inosine, N1-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, N6,2'-O-dimethyladenosine, 7-methylguanosine (m7G), Cap1, and Cap2. For example, the 5' terminal cap structure is anti-reverse cap analogue (ARCA). For example, the 5' terminal cap structure is N7,2'-O-dimethyl-guanosine (mCAP). For example, the 5' terminal cap structure is inosine. For example, the 5' terminal cap structure is N1-methyl-guanosine. For example, the 5' terminal cap structure is 2'-fluoro-guanosine. For example, the 5' terminal cap structure is 7-deaza-guanosine. For example, the 5' terminal cap structure is 8-oxo-guanosine.

For example, the 5' terminal cap structure is 2-amino-guanosine. For example, the 5' terminal cap structure is LNA-guanosine. For example, the 5' terminal cap structure is 2-azido-guanosine. For example, the 5' terminal cap structure is N6,2'-O-dimethyladenosine. For example, the 5' terminal cap structure is 7-methylguanosine (m7G). For example, the 5' terminal cap structure is Cap1. For example, the 5' terminal cap structure is Cap2.

In one example, the 5' terminal cap structure is linked to the 5' end of the RNA by a 5'-5'-triphosphate linkage or a 5'-5' phosphorothioate linkage. For example, the 5' terminal cap structure is linked to the 5' end of the RNA by a 5'-5'-triphosphate linkage. For example, the 5' terminal cap structure is linked to the 5' end of the RNA by a 5'-5' phosphorothioate linkage.

In one example, the antigens (i.e., a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respiratory syncytial virus (RSV) or influenza antigen) are expressed at substantially the same level. For example, the antigens have a level of expression within about 10%, or about 5% or about 1% of each other. In another example, the antigens are expressed at different levels. For example, the antigens have a level of expression greater than about 10%, or about 15% or about 20% of each other. Methods for determining the level of expression are known in the art and/or are described herein.

In one example, the self-replicating RNA is from an alphavirus. For example, the alphavirus is selected from the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan equine encephalitis virus (VEE) and combinations thereof.

In one example, the self-replicating RNA is from a Semliki Forest virus (SFV).

In one example, the self-replicating RNA is from a Sindbis virus (SIN).

In one example, the self-replicating RNA is from a Venezuelan equine encephalitis virus (VEE).

In one example, the antigen is selected from the group consisting of a respiratory syncytial virus (RSV), an influenza virus and/or a SARS-CoV-2.

In an example, the antigen is from a respiratory syncytial virus (RSV).

In an example, the antigen is from influenza.

In an example, the antigen is from a SARS-CoV-2.

In one example, the antigen is from a single strain of an influenza virus (i.e., monovalent) or from multiple strains (i.e., multivalent). For example, the immunogenic composition includes antigens from one or more (e.g., 1 or 2 or 3) influenza virus strains.

In one example, an antigen is from an influenza A virus strain. For example, the antigen is an influenza A virus hemagglutinin (HA) protein, a neuraminidase (NA)

protein, a matrix (M) protein, a nucleoprotein (NP), a non-structural (NS) protein, or an immunogenic fragment or variant thereof. In one example, the antigen encodes an influenza A hemagglutinin (HA) subtype H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 and/or an influenza A neuraminidase (NA) subtype
5 N1, N2, N3, N4, N5, N6, N7, N8 or N9 and/or an influenza A matrix (M) protein subtype M1 or M2 and/or an influenza A non-structural (NS) protein subtype NS1 or NS2.

The skilled person will be aware that pandemic strains of the influenza virus are commonly H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strains. For example, H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2,
10 strains.

In one example, the antigen is from a H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strain. For example, the antigen is a H1 hemagglutinin, or a H2 hemagglutinin, or a H3 hemagglutinin, or a H5 hemagglutinin, or a H6 hemagglutinin, or a H7 hemagglutinin or a H9 hemagglutinin. In another example, the antigen is a H5
15 subtype influenza A virus strain (i.e., a H5 hemagglutinin). In one example, the H5 hemagglutinin is an A/turkey/Turkey/1/2005 virus strain. In one example, the H3 hemagglutinin is an A/Delaware/39/2019 virus strain.

In one example, the antigen is a N1, N2, N3, N7 or N9 subtype influenza A virus strain. For example, the antigen is a N1 neuraminidase, or a N2 neuraminidase, or a N3
20 neuraminidase, or a N7 neuraminidase, or a N9 neuraminidase. For example, the antigens is a N1 neuraminidase subtype influenza A virus strain. In one example, the N1 neuraminidase is an A/turkey/Turkey/1/2005 strain. In one example, the N2 neuraminidase is an A/Delaware/39/2019 virus strain.

In one example, the antigen is a H5 hemagglutinin protein and/or a N1
25 neuraminidase protein. In one example, the antigen is a H5 hemagglutinin subtype influenza A virus strain. In another example, the antigen is a N1 neuraminidase subtype influenza A virus strain.

In an example, the antigen from an influenza protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about
30 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 19.

In an example, the antigen from an influenza protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at
35 least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 19.

In an example, the antigen from an influenza protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 20.

5 In an example, the antigen from an influenza protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 20.

10 In one example, the antigen is an influenza B virus strain. The skilled person will be aware that influenza B viruses are not divided into subtypes but are classified into two lineages, namely, B/Yamagata and B/Victoria.

In one example, the antigen is a B/Yamagata influenza B virus strain. For example, the influenza B virus strain is a B/Singapore/INFTT 16 0610/16 (By) virus strain. In another example, the antigen is a B/Victoria influenza B virus strain.

15 In one example, the antigen is an influenza B virus Hyam protein and/or a Nyam protein. For example, antigen is an influenza B virus Hyam protein. In another example, the antigen is an influenza B virus Nyam protein. In a further example, the antigen is an influenza B virus Hyam and Nyam protein. In one example, the antigen is a Hyam subtype influenza B virus strain. In another example, the antigen is a Nyam subtype influenza B virus strain.

In one example, the antigen is from influenza C.

In one example, the antigen is from the Alpha (B.1.1.7) strain, the Beta (B.1.351) strain, the Gamma (P1) strain, the Epsilon (B.1.429) strain, the Delta (B.1.617.2) mutant, 25 the Kappa (B.1.617.1) mutant, the Wuhan (original) strain and/or the Omicron (B.1.1.529) strain of a SARS-CoV-2.

In one example, the antigen is a spike (S) protein or a nucleocapsid (N) protein of a SARS-CoV-2. For example, the antigen is SARS-CoV-2 N protein or a S protein from the Alpha (B.1.1.7) strain, the Beta (B.1.351) strain, the Gamma (P1) strain, the Epsilon (B.1.429) strain, the Delta (B.1.617.2) mutant, the Kappa (B.1.617.1) mutant, the Wuhan (original) strain and/or the Omicron (B.1.1.529) strain. In one example, the antigen is a SARS-CoV-2 N protein or a S protein from the Wuhan (original) SARS-CoV-2 strain. In one example, the antigen is a SARS-CoV-2 N protein or a S protein from the delta SARS-CoV-2 strain. In one example, the antigen is a SARS-CoV-2 N protein or a S protein from the delta SARS-CoV-2 Omicron strain.

In an example, the antigen from a S protein of a SARS-CoV-2 comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 21.

In an example, the antigen from a S protein of a SARS-CoV-2 is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 21.

In an example, the antigen from a S protein of a SARS-CoV-2 comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 22.

In an example, the antigen from a S protein of a SARS-CoV-2 is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 22.

In one example, the antigen is a SARS-CoV-2 Nucleocapsid (N) protein. In another example, the antigen is a SARS-CoV-2 Envelope (E) protein. In one example, the antigen is a SARS-CoV-2 Matrix (M) protein.

In another example, the S protein is a mutant S protein.

In one example, a mutant S protein comprises a mutation in the receptor binding domain. For example, the mutation is selected from the group consisting of S438F, N439K, N440K, L441I, K444R, V445A, V445I, G446V, G446S, N450K, L452R, L452P, L455F, K458N, N460T, D467V, I468F, I468T, I468V, E471O, I472V, A475V, G476S, S477G, S477I, S477N, S477R, T478I, P479L, P479L, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484K, E484O, G485S, Y489H, Y489D, Y489F, Y489C, Y489N, F490L, F490S, P491R, Q493L, S494P, Y495N, T500N, N501S and Y505H, Y508H. In one example, a mutant S protein comprises a mutation in the receptor binding domain selected from the group consisting of N439K, N439L, L452R, S477N, T478I, V483A and E484D.

In one example, a mutant S protein comprises a mutation in the receptor binding domain. For example, the mutation is selected from the group consisting of R346K,

K417N, K417T, S438F, N439K, N440K, L441I, K444R, V445A, V445I, G446V, G446S, N450K, L452R, L452P, L455F, K458N, N460T, D467V, I468F, I468T, I468V, E471O, I472V, A475V, G476S, S477G, S477I, S477N, S477R, T478I, T478K, P479L, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484K, E484O, G485S, 5 Y489H, Y489D, Y489F, Y489C, Y489N, F490L, F490S, P491R, Q493L, S494P, Y495N, T500N, N501S, N501Y, Y505H and Y508H. In one example, a mutant S protein comprises a mutation in the receptor binding domain selected from the group consisting of R346K, K417N, K417T, N439K, N439L, L452R, S477N, T478I, V483A, E484D, E484K and N501Y.

10 In one example, a mutant S protein comprises a mutation selected from the group consisting of P337S, F338L, F338C, G339D, E340K, V341I, A344S, T345S, R346K, A348S, A348T, W353R, N354D, N354K, N354S, S359N, D364Y, V367F, S373L, V382L, P384L, P384S, T385A, T393P, V395I, F400C, R403K, R403S, D405V, R408I, Q414E, Q414K, Q414P, Q414R, T415S, K417R, K417N, I418V, Y421S, Y423C, 15 Y423F, Y423S, D427Y, R509K, V510L, V511E, V512L, L518I, H519O, A520S, A520V, P521R, P521S, A522P, A522S and D614G.

In one example, a mutant S protein comprises a mutation selected from the group consisting of L18F, D80A, T95I, Y144S, Y145N, D215G, P337S, F338L, F338C, G339D, E340K, V341I, A344S, T345S, R346K, A348S, A348T, W353R, N354D, 20 N354K, N354S, S359N, D364Y, V367F, S373L, V382L, P384L, P384S, T385A, T393P, V395I, F400C, R403K, R403S, D405V, R408I, Q414E, Q414K, Q414P, Q414R, T415S, K417N, K417T, K417R, I418V, Y421S, Y423C, Y423F, Y423S, D427Y, S438F, N439K, N440K, L441I, K444R, V445A, V445I, G446V, G446S, N450K, L452R, L452P, L455F, K458N, N460T, D467V, I468F, I468T, I468V, E471O, I472V, A475V, 25 G476S, S477G, S477I, S477N, S477R, T478I, T478K, P479L, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484K, E484O, G485S, Y489H, Y489D, Y489F, Y489C, Y489N, F490L, F490S, P491R, Q493L, S494P, Y495N, T500N, N501S, N501Y, Y505H, Y508H, R509K, V510L, V511E, V512L, L518I, H519O, A520S, A520V, P521R, P521S, A522P, A522S, A570D, D614G, P680H, P681H, A701V, T716I 30 and D950N.

In one example, the antigen from a RSV is a RSV surface glycoprotein selected from a Fusion (F), Glycoprotein (G), Small Hydrophobic protein (SH), the matrix proteins M and M2, the nucleocapsid proteins N, P and L, and the nonstructural proteins NS1 and NS2. In certain examples, the antigen is an RSV-F antigen.

35 In an example, the antigen from a RSV comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at

least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 17.

In an example, the antigen from a RSV is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at
5 least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 17.

In an example, the antigen from a RSV comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about
10 98%, at least about 99% or 100% identity to SEQ ID NO: 18.

In an example, the antigen from a RSV is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 18.

15 In an example, the composition further comprises one or more additional antigens. In some examples, the composition will therefore comprise a fourth and/or fifth antigen. In an example, the one or more additional antigens are viral antigens. In an example, the viral antigen is from a respiratory virus. In one example, the respiratory virus is selected from the group consisting of influenza virus, respiratory syncytial virus, parainfluenza
20 viruses, metapneumovirus, rhinovirus, coronaviruses, adenoviruses and bocaviruses.

In one example, the one or more additional viral antigens are from an influenza virus.

In one example, the one or more additional viral antigens are from a respiratory syncytial virus.

25 In one example, the one or more additional viral antigens are from a parainfluenza virus.

In one example, the one or more additional viral antigens are from a metapneumovirus.

In one example, the one or more additional viral antigens are from a rhinovirus.

30 In one example, the one or more additional viral antigens are from a coronavirus.

In one example, the one or more additional viral antigens are from an adenovirus.

In one example, the one or more additional viral antigens are from a bocavirus.

In one example, the one or more additional antigens are viral antigens from an influenza virus or a coronavirus.

35 The present disclosure provides an immunogenic composition comprising a polynucleotide of the present disclosure. The present disclosure further provides an

immunogenic composition comprising a RNA of the present disclosure. For example, the present disclosure provides an immunogenic composition comprising a cRNA of the present disclosure. The present disclosure also provides an immunogenic composition comprising a self-replicating RNA of the present disclosure. In an example, the composition of the present disclosure, when administered, is capable of inducing an immune response in the subject. For example, administration of the composition induces a humoral and/or a cell-mediated immune response. In one example, the composition induces a humoral immune response in the subject. For example, the humoral immune response is an antibody-mediated immune response. In another example, the composition induces a cell-mediated immune response. For example, the cell-mediated immune response includes activation of antigen-specific cytotoxic T cells.

In one example, an immunogenic composition of the disclosure comprises multiple polynucleotides, wherein each polynucleotide encodes different polypeptide antigen sequences. In another example, the immunogenic composition of the disclosure comprises multiple RNAs, wherein each RNA encodes different polypeptide antigen sequences. Thus, the present disclosure provides an immunogenic composition comprising:

- (a) an RNA comprising a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- (b) an RNA comprising a nucleotide sequence encoding a second antigen from influenza, and
- (c) an RNA comprising a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element. Each RNA may be cRNA, mRNA or self-replicating RNA, for example.

In a further example, the immunogenic composition of the disclosure comprises multiple cRNAs, wherein each cRNA encodes different polypeptide antigen sequences. In another example, the immunogenic composition of the disclosure comprises a plurality of self-replicating RNAs, wherein each self-replicating RNA encodes different polypeptide antigen sequences.

The present disclosure also provides a pharmaceutical composition comprising an immunogenic composition of the present disclosure and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein.

In one example, the pharmaceutical composition further comprises a lipid nanoparticle (LNP), a polymeric microparticle, and an oil-in-water emulsion. For

example, the polynucleotide, the RNA, the cRNA or the self-replicating RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. In one example, the polynucleotide is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. In another
5 example, the RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. For example, the cRNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. For example, the self-replicating RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion.

10 In one example, the pharmaceutical composition further comprises a LNP. For example, the polynucleotide is encapsulated in a LNP. In another example, the RNA is encapsulated in a LNP. For example, the cRNA is encapsulated in a LNP. For example, the self-replicating RNA is encapsulated in a LNP. For example, the polynucleotide is bound to a LNP. In another example, the RNA is bound to a LNP. For example, the
15 cRNA is bound to a LNP. In another example, the self-replicating RNA is bound to a LNP. For example, the polynucleotide is adsorbed on to a LNP. In another example, the RNA is adsorbed on to a LNP. For example, the cRNA is adsorbed on to a LNP. In a further example, the self-replicating RNA is adsorbed on to a LNP. In another example, wherein each RNA is formulated together in the LNP. In another example, wherein each
20 RNA is formulated separately in the LNP.

In one example, the LNP comprises a PEG-lipid, a structural lipid and/or a neutral lipid. For example, the LNP comprises a PEG-lipid, a structural lipid and a neutral lipid. In another example, the LNP comprises a PEG-lipid, a structural lipid or a neutral lipid.

In one example, the LNP further comprises a cationic lipid. In another example,
25 the LNP does not comprise a cationic lipid.

In one example, the pharmaceutical composition further comprises a polymeric microparticle. For example, the polynucleotide is encapsulated in a polymeric microparticle. In another example, the RNA is encapsulated in a polymeric microparticle. For example, the cRNA is encapsulated in a polymeric microparticle. For
30 example, the self-replicating RNA is encapsulated in a polymeric microparticle. For example, the polynucleotide is bound to a polymeric microparticle. In another example, the RNA is bound to a polymeric microparticle. For example, the cRNA is bound to a polymeric microparticle. In another example, the self-replicating RNA is bound to a polymeric microparticle. For example, the polynucleotide is adsorbed on to a polymeric
35 microparticle. In another example, the RNA is adsorbed on to a polymeric microparticle.

For example, the cRNA is adsorbed on to a polymeric microparticle. In a further example, the self-replicating RNA is adsorbed on to a polymeric microparticle.

In one example, the pharmaceutical composition further comprises an oil-in-water emulsion. For example, the polynucleotide is encapsulated in an oil-in-water emulsion.

5 In another example, the RNA is encapsulated in an oil-in-water emulsion. For example, the cRNA is encapsulated in an oil-in-water emulsion. For example, the self-replicating RNA is encapsulated in an oil-in-water emulsion. For example, the polynucleotide is bound to an oil-in-water emulsion. In another example, the RNA is bound to an oil-in-water emulsion. For example, the cRNA is bound to an oil-in-water emulsion. In another
10 example, the self-replicating RNA is bound to an oil-in-water emulsion. In a further example, the self-replicating RNA is adsorbed on to an oil-in-water emulsion. In a further example, the self-replicating RNA is resuspended in an oil-in-water emulsion.

The present disclosure also provides the immunogenic composition or the pharmaceutical composition of the disclosure for use as a vaccine.

15 In one example, the polynucleotide is DNA. In one example, the disclosure provides a DNA encoding a cRNA vaccine of the disclosure. In one example, the disclosure provides a DNA encoding a self-replicating RNA vaccine of the disclosure.

In one example, the DNA is a plasmid.

The present disclosure provides a method of treating or preventing or delaying
20 progression of a disease or condition in a subject, the method comprising administering an immunogenic composition, a pharmaceutical composition or a vaccine of the present disclosure to a subject in need thereof. In one example, the disclosure provides a method of treating a disease or condition in a subject, the method comprising administering an immunogenic composition, a pharmaceutical composition or a vaccine of the present
25 disclosure to a subject in need thereof. In another example, the disclosure provides a method of preventing a disease or condition in a subject, the method comprising administering an immunogenic composition, a pharmaceutical composition or a vaccine of the present disclosure to a subject in need thereof. In a further example, the disclosure provides a method of delaying progression of a disease or condition in a subject, the
30 method comprising administering an immunogenic composition, a pharmaceutical composition or a vaccine of the present disclosure to a subject in need thereof.

In one example, the present disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the
35 disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In another

example, the disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for delaying progression of a disease or
5 condition in a subject in need thereof.

In one example, the present disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for treating a disease
10 or condition in a subject in need thereof. In another example, the disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

In one example, the present disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for treating a disease
15 or condition in a subject in need thereof. In another example, the disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

In one example, the present disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In another
25 example, the disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

In another example, the present disclosure provides use of an immunogenic
35 composition, a pharmaceutical composition or a vaccine of the disclosure in the preparation of a medicament for treating or preventing or delaying progression of a

disease or condition in a subject in need thereof. For example, the disclosure provides use an immunogenic composition, a pharmaceutical composition or a vaccine of the disclosure in the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In another example, the disclosure provides use of an
5 immunogenic composition, a pharmaceutical composition or a vaccine of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of an immunogenic composition, a pharmaceutical composition or a vaccine of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a
10 subject in need thereof.

In one example, the present disclosure provides a polynucleotide of the disclosure for use in treating or preventing or delaying progression of a disease or condition in a subject in need thereof. In another example, the present disclosure provides a RNA of the disclosure for use in treating or preventing or delaying progression of a disease or
15 condition in a subject in need thereof. In another example, the present disclosure provides a cRNA of the disclosure for use in treating or preventing or delaying progression of a disease or condition in a subject in need thereof. In yet another example, the present disclosure provides a self-replicating RNA of the disclosure for use in treating or preventing or delaying progression of a disease or condition in a subject in need thereof.
20 In yet another example, the present disclosure provides an immunogenic composition or vaccine disclosed herein for use in treating or preventing or delaying progression of a disease or condition in a subject in need thereof.

In one example, the subject suffers from a disease or condition. In one example, the subject has been diagnosed as suffering from a disease or condition. In one example,
25 the subject is receiving treatment for a disease or condition.

In one example, the disease or condition is selected from the group consisting of influenza, COVID-19, a SARS-CoV-2 infection, RSV and acute respiratory distress syndrome (ARDS) or a combination thereof. In another example, the disease or condition is influenza, a SARS-CoV-2 infection and RSV. In another example, the disease or
30 condition is influenza and COVID-19. In another example, the disease or condition is a SARS-CoV-2 and influenza. In another example, the disease or condition is a RSV and influenza. In another example, the disease or condition is a SARS-CoV-2 and RSV. In another example, the disease or condition is a RSV and COVID-19. In another example, the disease or condition is a SARS-CoV-2. In another example, the disease or condition
35 is a RSV. In another example, the disease or condition is influenza. In another example,

the disease or condition is COVID-19. In another example, the disease or condition is ARDS.

In one example, a composition or vaccine of the present disclosure is administered in an amount sufficient to reduce the severity of or prevent onset of one or more
5 symptoms of a SARS-CoV-2 infection, COVID-19, influenza, RSV and/or ARDS. Symptoms thereof will be apparent to the skilled person and/or are described herein.

In an example, the present disclosure provides a method of inducing an immune response in a subject, the method comprising administering a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a
10 vaccine disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

15 In an example, the present disclosure provides a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in inducing an immune response in a subject in need thereof.

In one example, the composition induces a humoral immune response in the subject. For example, the humoral immune response is an antibody-mediated immune
20 response. For example, production of neutralizing antibodies. In another example, the composition induces a cell-mediated immune response. For example, the cell-mediated immune response includes activation of antigen-specific cytotoxic T cells. For example, the T cells are CD4 T cells and/or CD8 T cells. In one example, the T cells are CD4 T cells. In another example, the T cells are CD8 T cells. In a further example, the T cells
25 are CD4 and CD8 T cells.

In one example, administration of a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine of the present disclosure induces a CD4 T cell mediated immune response.

In one example, administration of a pharmaceutical composition, an
30 immunogenic composition disclosed herein or a vaccine of the present disclosure induces a CD8 T cell mediated immune response.

In one example, administration of a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine of the present disclosure induces a CD4 and a CD8 T cell mediated immune response.

35 In one example, the CD4 T cell mediated immune response is a Th0, a Th1 and/or a Th2 response. For example, the CD4 T cell mediated immune response is a Th0

response. In another example, the CD4 T cell mediated immune response is a Th1 response. In a further example, the CD4 T cell mediated immune response is a Th2 response. In one example, the CD4 T cell mediated immune response is a Th0 and Th1 response. In another example, the CD4 T cell mediated immune response is a Th0 and Th2 response. In a further example, the CD4 T cell mediated immune response is a Th1 and Th2 response. In another example, the CD4 T cell mediated immune response is a Th0, Th1 and Th2 response.

In one example, the Th0 response cytokines express interleukin 2 (IL2+) and/or tumor necrosis factor alpha (TNFa+); and/or are negative for interferon gamma (IFNg-), IL5- and/or IL13-. For example, the cytokine is IL2+. In another example, the cytokine is TNFa+. In one example, the cytokine is IFNg-. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th1 response cytokines express interferon gamma (IFNg+); and/or are negative for IL5- and/or IL13-. For example, the cytokine is IFNg+. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th2 response cytokines express IL5+ and/or IL13+; and/or are negative for IFNg. For example, the cytokine is IL5+. In a further example, the cytokine is IL13+. For example, the cytokine is IFNg-.

In an example, the present disclosure provides a method for reducing viral load in a subject having a viral infection comprising administering a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject having a viral infection.

In an example, the present disclosure provides use of a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for reducing viral load in a subject having a viral infection.

In an example, the present disclosure provides a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in reducing viral load in a subject having a viral infection.

In an example, the subject is a human of 18 years of age or older. In another example, the subject is a human of any age, e.g., from about 1 month to 100 years old, e.g., from about 2 months to about 80 years old, from about 6 months of age to about 3 years old, from about 3 years to about 18 years old, from about 12 years to about 18 years old, from about 18 years to about 55 years old, from about 50 years to about 75 years old, from about 40 years to about 65 years old. In another example, the subject is a human from 2 years of age. In another example, subject is a human from 18 years of age, a

human from 30 years of age, a human from 40 years of age, a human from 50 years of age, a human from 60 years of age, a human from 70 years of age, a human from 80 years of age or a human from about 90 years of age. In another example, the subject is less than 2 years of age, less than 18 months of age, less than 12 months of age, less than 6 months of age or less than 3 months of age.

In an example, the immunogenic compositions described herein are administered to an infant subject. In an example, the infant subject has, or is suspected of having RSV, influenza and/or a SARS-CoV-2 infection.

In an example, the infant is between about 1 month and 12 months of age, or between 2 months and 11 months of age, or between 3 months and 10 months of age, or between 4 months and 9 months of age, or between 5 months and 8 months of age or between 6 months and 7 months of age. In an example, the infant is between about 1 month and 11 months of age, between about 1 month and 10 months of age, between about 1 month and 9 months of age, between about 1 month and 8 months of age, between about 1 month and 7 months of age, between about 1 month and 6 months of age, between about 1 month and 5 months of age, between about 1 month and 4 months of age, between about 1 month and 3 months of age or between about 1 month and 2 months of age. In another example the infant is about 1 month of age, about 2 months of age, about 3 months of age, about 4 months of age, about 5 months of age, about 6 months of age, about 7 months of age, about 8 months of age, about 9 months of age, about 10 months of age, about 11 months of age or about 12 months of age.

Thus, in an example, there is provided a method of treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant comprising administering an immunogenic composition, pharmaceutical composition or vaccine disclosed herein to the infant.

In another example, there is provided use of an immunogenic composition, pharmaceutical composition or vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant.

In another example, there is provided an immunogenic composition, pharmaceutical composition or vaccine disclosed herein for use in treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant.

In another example, there is provided a method of inducing an immune response to a respiratory syncytial virus (RSV) in an infant comprising administering an immunogenic composition, pharmaceutical composition or vaccine disclosed herein to the infant.

In another example, there is provided use of an immunogenic composition, pharmaceutical composition or vaccine disclosed herein in the preparation of a medicament for inducing an immune reponse to a respiratory syncytial virus (RSV) in an infant.

5 In another example, there is provided an immunogenic composition, pharmaceutical composition or vaccine disclosed herein for use in inducing an immune reponse to a respiratory syncytial virus (RSV) in an infant.

Where treatment, prevention or delaying progression of RSV in an infant is contemplated, a skilled person will be aware of suitable immunogenic compositions
10 described herein for administration to the infant.

In an example, a composition or vaccine described herein is administered in a one dose regimen. In another example, the composition is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.

The present disclosure also provides a kit comprising at least one RNA of the
15 disclosure (e.g., a self-replicating RNA), optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions for use in treating or preventing or delaying progression of a disease or disorder (e.g., an influenza virus infection, a RSV infection, a SARS-CoV-2 infection, COVID-19 and/or ARDS) in a subject.

20 In one example, the RNA, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a vial. In another example, the RNA, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a syringe.

Any discussion of documents, acts, materials, devices, articles or the like which
25 has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

30 **KEY TO SEQUENCE LISTING**

Sequence	SEQ ID NO
Nucleotide sequence of alphavirus native subgenomic promoter (v1)	1
Nucleotide sequence of extended subgenomic promoter (v2)	2
Nucleotide sequence of extended subgenomic promoter (v3)	3
Nucleotide sequence of wild-type EMCV IRES	4

Nucleotide sequence of extended subgenomic promoter (v4)	5
Nucleotide sequence of a Kozak consensus sequence	6
Nucleotide sequence of a Kozak consensus sequence	7
Nucleotide sequence of an interrupting linker	8
Nucleotide sequence of a GC-rich element	9
Nucleotide sequence of a GC-rich element	10
Nucleotide sequence of a GC-rich element	11
Nucleotide sequence of a histone stem loop	12
Nucleotide sequence of 5'UTR of VEEV	13
Nucleotide sequence of 3'UTR of SINV	14
Nucleotide sequence of extended subgenomic promoter	15
Poly-A sequence	16
mRNA sequence of RSV F protein antigen	17
mRNA sequence of RSV Pre F protein antigen	18
mRNA sequence of influenza antigen H1N1	19
mRNA sequence of influenza antigen H5N1	20
mRNA sequence of Omicron strain of SARS-CoV-2 S protein ectodomain antigen	21
mRNA sequence of Omicron strain of SARS-CoV-2 S protein ectodomain antigen	22

DETAILED DESCRIPTION

General

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter.

Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

5 Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* to any other example of the disclosure unless specifically stated otherwise. Stated another way, any specific example of the present disclosure may be combined with any other specific example of the disclosure (except where mutually exclusive).

10 Any example of the present disclosure disclosing a specific feature or group of features or method or method steps will be taken to provide explicit support for disclaiming the specific feature or group of features or method or method steps.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

15 Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan *et al.* (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

20 The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein the term “derived from” shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source. Similarly, the term “based on” shall be taken to indicate that a specified integer may be developed or used from a particular source albeit not necessarily directly from that source.

Selected Definitions

As used herein, the term “monocistronic” in reference to the polynucleotide, RNA, cRNA and/or self-replicating RNA, refers to a RNA that encodes one polypeptide.

As used herein, the term “multicistronic” (also known as “polycistronic”) in reference to the polynucleotide, RNA, cRNA and/or self-replicating RNA, refers to a RNA that encodes two or more polypeptides. The term encompasses “bicistronic” (or “dicistronic”; i.e., encoding two polypeptides) and “tricistronic” (i.e., encoding three polypeptides) molecules. By “bicistronic” is meant a single nucleic acid that is capable of encoding two distinct polypeptides from different regions of the nucleic acid.

As used herein, the term “conventional mRNA” or “cRNA” or “non-amplifying RNA” refers to a construct that allows expression of heterologous RNA and proteins but the RNA that cannot amplify in host cells.

As used herein, the term “self-replicating RNA” refers to a construct based on an RNA virus that has been engineered to allow expression of heterologous mRNA and proteins. Self-replicating RNA (e.g., in the form of naked RNA) can amplify in host cells leading to expression of the desired gene product in the host cell.

The term “naked” as used herein refers to nucleic acids that are substantially free of other macromolecules, such as lipids, polymers and proteins. A “naked” nucleic acid, such as a self-replicating RNA, is not formulated with other macromolecules to improve cellular uptake. Accordingly, a naked nucleic acid is not encapsulated in, absorbed on, or bound to a lipid nanoparticle (LNP), a liposome, a polymeric microparticle or an oil-in-water emulsion.

As used herein, the term “nucleotide sequence” or “nucleic acid sequence” will be understood to mean a series of contiguous nucleotides (or bases) covalently linked to a phosphodiester backbone. By convention, sequences are presented from the 5' end to the 3' end, unless otherwise specified. To facilitate a clear description of the nucleic acids, particular sequence components are referred to as e.g., a “first nucleotide sequence” and a “second nucleotide sequence”. It is to be understood that the first and second sequences can appear in any desired order or orientation, unless otherwise specified, and that no particular order or orientation is intended by the words “first”, “second” etc.

As used herein, the term “antigen” refers to a molecule or structure containing one or more epitopes that induce, elicit, augment or boost a cellular and/or humoral immune response. Antigens can include, for example, proteins and peptides from a pathogen such as a virus, bacteria, fungus, protozoan, plant or from a tumour.

5 As used herein the term "adjuvant" refers to a compound that, when used in combination with a specific immunogen (e.g. a VLP) in a formulation, augments or otherwise alters or modifies the resultant immune response. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean
10 decreasing or suppressing certain antigen-specific immune responses.

As used herein, the term “operably linked to” means positioning a subgenomic promoter or regulatory element (e.g., an IRES) relative to a nucleic acid such that expression of the nucleic acid is controlled or regulated by the element. For example, a subgenomic promoter can be operably linked to numerous nucleic acids, e.g., through
15 another regulatory element, such as an internal ribosome entry site (IRES).

As used herein, the term “subgenomic promoter” (also known as ‘junction region’ promoter) refers to a promoter that directs the expression of a heterologous nucleotide sequence, regulating protein expression.

As used herein, the term “internal ribosome entry site” or “IRES” refers to a
20 sequence of nucleotides within a mRNA to which a ribosome or a component thereof, e.g., a 40S subunit of a ribosome, is capable of binding. An IRES need not necessarily comprise nucleic acid that induces translation of a mRNA (e.g., a start codon; AUG).

The term “polypeptide” or “polypeptide chain” will be understood to mean a series of contiguous amino acids linked by peptide bonds. For example, a protein shall
25 be taken to include a single polypeptide chain i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). The series of polypeptide chains can be covalently linked using a suitable chemical or a disulfide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and
30 hydrophobic interactions.

The term “recombinant” shall be understood to mean the product of artificial genetic recombination.

As used herein the term “substantially the same” in reference to the level of expression is meant that the first and second antigens (at least) have a level of expression
35 within about 10% or less of each other unless the context implies otherwise.

As used herein, the terms “disease”, “disorder” or “condition” refers to a disruption of or interference with normal function, and is not to be limited to any specific condition, and will include diseases or disorders.

As used herein, a subject “at risk” of having developed or developing a SARS-CoV-2 infection may have nor may not have detectable disease or symptoms of a SARS-CoV-2 infection, and may have nor may not have displayed detectable disease or symptoms of a SARS-CoV-2 infection prior to the treatment according to the present disclosure. “At risk” denotes that a subject has one or more risk factors, which are measurable parameters that correlate with development of a SARS-CoV-2 infection, as known in the art and/or described herein.

As used herein, the terms “treatment” or “treating” of a subject includes the application or administration of a compound or composition of the disclosure to a subject (or application or administration of a compound of the disclosure to a cell or tissue from a subject) with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term “treating” refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating.

As used herein, “preventing” or “prevention” is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are also well known by physicians.

As used herein, the phrase “delaying progression of” includes reducing or slowing down the progression of the disease or condition in an individual and/or at least one symptom of a disease or condition.

An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired result. For example, the desired result may be a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect treatment of a disease or condition as

hereinbefore described. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect a change associated with a disease or condition as hereinbefore described. The effective amount may vary according to the disease or condition to be treated or factor to be altered and also according to the weight, age, racial background, sex, health and/or physical condition and other factors relevant to the mammal being treated. Typically, the effective amount will fall within a relatively broad range (e.g. a “dosage” range) that can be determined through routine trial and experimentation by a medical practitioner. Accordingly, this term is not to be construed to limit the disclosure to a specific quantity, e.g., weight or number of RNA. The effective amount can be administered in a single dose or in a dose repeated once or several times over a treatment period.

A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disease or condition. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the RNA of the present disclosure to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the RNA are outweighed by the therapeutically beneficial effects.

As used herein, the term “prophylactically effective amount” shall be taken to mean a sufficient quantity of the RNA of the disclosure to prevent or inhibit or delay the onset of one or more detectable symptoms of a disease or disorder as described herein.

A “subject” can also be any animal that is susceptible to infection for example by a SARS-CoV-2, a RSV and/or influenza. A subject of this disclosure can be a mammal and in particular embodiments is a human, which can be an infant, a child, an adult or an elderly adult. A “subject at risk of infection” is any subject who may be or has been exposed to the infection. The subject may be a primary contact of an individual diagnosed with the infection. “Subject” includes any human or non-human animal. Thus, in addition to being useful for human treatment, the compounds of the present disclosure may also be useful for veterinary treatment of mammals, including companion animals and farm animals, such as, but not limited to dogs, cats, horses, cows, sheep, and pigs.

As used herein, the term “lipid nanoparticle” or “LNP” shall be understood to refer to lipid-based particles having at least one dimension on the order of nanometers (e.g., 1-1,000 nm) and which comprises a compound of any formulae described herein. In embodiments, LNPs are formulated in a composition for delivery of a polynucleotide to a desired target such as a cell, tissue, organ, tumor, and the like. For example, the lipid nanoparticle or LNP any lipid composition, including, may be selected from, but not

limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles, wherein solid lipid nanoparticles lack lipid bilayers.

5

Antigens

Antigens suitable for use in the compositions described herein will be apparent to the skilled person.

In an example, the immunogenic compositions described herein comprise:

- 10 (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

15 wherein each nucleotide sequence is operably linked to a regulatory element.

Influenza

Influenza, also known as "the flu", is an infectious disease caused by an influenza virus. It will be apparent to the skilled person that there are currently four influenza
20 viruses – A, B, C and D. Influenza A virus is the most common flu virus infecting humans, animals, and birds, whilst influenza B virus infection mostly occurs in humans. Infection of influenza C virus does not cause any severe symptom in human or mammals and influenza D, to date, has only infected pigs and cattle.

In one example, the antigen is from an influenza A virus strain. For example, the
25 antigen is an influenza A virus hemagglutinin (HA) protein, a neuraminidase (NA) protein, a matrix (M) protein, a nucleoprotein (NP), a non-structural (NS) protein, or an immunogenic fragment or variant thereof. In one example, the antigen is an influenza A hemagglutinin (HA) subtype H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 and/or an influenza A neuraminidase (NA) subtype N1, N2, N3, N4,
30 N5, N6, N7, N8 or N9 and/or an influenza A matrix (M) protein subtype M1 or M2 and/or an influenza A non-structural (NS) protein subtype NS1 or NS2.

In one example, the influenza viral antigen is from different subtypes of the influenza virus. For example, different hemagglutinin subtypes and/or different neuraminidase subtypes and/or matrix protein subtypes, and/or nucleoprotein subtypes
35 and/or non-structural protein subtypes.

The skilled person will be aware that pandemic strains of the influenza virus are commonly H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strains. For example, H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2, strains.

5 In one example, the antigen is a H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strain. For example, the antigen is a H1 hemagglutinin, or a H2 hemagglutinin, or a H3 hemagglutinin, or a H5 hemagglutinin, or a H6 hemagglutinin, or a H7 hemagglutinin or a H9 hemagglutinin. For example, the antigen is a H5 subtype influenza A virus strain (i.e., a H5 hemagglutinin). In one example, the H5 hemagglutinin is an
10 A/turkey/Turkey/1/2005 virus strain. In one example, the H3 hemagglutinin is an A/Delaware/39/2019 virus strain.

In one example, the antigen is an N1, N2, N3, N7 or N9 subtype influenza A virus strain. For example, the antigen is a N1 neuraminidase, or a N2 neuraminidase, or a N3 neuraminidase, or a N7 neuraminidase, or a N9 neuraminidase. For example, the antigen
15 is a N1 neuraminidase subtype influenza A virus strain. In one example, the N1 neuraminidase is an A/turkey/Turkey/1/2005 strain. In one example, the N2 neuraminidase is an A/Delaware/39/2019 virus strain.

In one example, the antigen is a H5 hemagglutinin protein and/or a N1 neuraminidase protein. For example, the antigen is a H5 hemagglutinin subtype influenza
20 A virus strain. In another example, the second antigen is a N1 neuraminidase subtype influenza A virus strain.

In one example, the antigen is an influenza B virus strain. The skilled person will be aware that influenza B viruses are not divided into subtypes but are classified into two lineages, namely, B/Yamagata and B/Victoria.

25 In one example, the antigen is a B/Yamagata influenza B virus strain. For example, the influenza B virus strain is a B/Singapore/INFTT 16 0610/16 (By) virus strain. In another example, the antigen is a B/Victoria influenza B virus strain.

In one example, the antigen is an influenza B virus Hyam protein and/or a Nyam protein. For example, antigen is an influenza B virus Hyam protein. In another example,
30 the antigen is an influenza B virus Nyam protein. In a further example, the antigen is an influenza B virus Hyam and Nyam protein.

In an example, the antigen from an influenza protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at
35 least about 98%, at least about 99% or 100% identity to SEQ ID NO: 19.

In an example, the antigen from an influenza protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 19.

In an example, the antigen from an influenza protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 20.

In an example, the antigen from an influenza protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 20.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

COVID-19 is an infectious disease caused by SARS-CoV-2. It was first identified in December 2019 in Wuhan, Hubei, China, and has resulted in an ongoing pandemic.

In one example, the antigen is from a single strain of a SARS-CoV-2 (i.e., monovalent). In another example, the antigen is from the Alpha (B.1.1.7) strain, the Beta (B.1.351) strain, the Gamma (P1) strain, the Epsilon (B.1.429) strain, the Delta (B.1.617.2) mutant, the Kappa (B.1.617.1) mutant, the Wuhan (original) strain or the Omicron (B.1.1.529) strain of a SARS-CoV-2.

In one example, the antigen is a spike (S) protein or a nucleocapsid (N) protein of a SARS-CoV-2. For example, the antigen is a SARS-CoV-2 N protein or a S protein from the Alpha (B.1.1.7) strain, the Beta (B.1.351) strain, the Gamma (P1) strain, the Epsilon (B.1.429) strain, the Delta (B.1.617.2) mutant, the Kappa (B.1.617.1) mutant, the Wuhan (original) strain or the Omicron (B.1.1.529) strain. In one example, the antigen is a SARS-CoV-2 N protein or a S protein from the Wuhan (original) SARS-CoV-2 strain. In one example, the antigen is a SARS-CoV-2 N protein or a S protein from the delta SARS-CoV-2 strain. In one example, the antigen is a SARS-CoV-2 N protein or a S protein from the delta SARS-CoV-2 omicron strain.

In an example, the antigen from a S protein of a SARS-CoV-2 comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at

least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 21.

In an example, the antigen from a S protein of a SARS-CoV-2 is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 21.

In an example, the antigen from a S protein of a SARS-CoV-2 comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 22.

In an example, the antigen from a S protein of a SARS-CoV-2 is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 22.

Respiratory syncytial virus (RSV)

RSV is an enveloped non-segmented negative-strand RNA virus in the family Paramyxoviridae, genus *Pneumovirus*. To infect a host cell, paramyxoviruses such as RSV, like other enveloped viruses such as influenza virus require fusion of the viral membrane with a host cell's membrane. For RSV, the conserved fusion protein (RSV-F glycoprotein) fuses the viral and cellular membranes by coupling irreversible protein refolding with juxtaposition of the membranes. Based on paramyxovirus studies, the RSV-F protein initially folds into a metastable pre-fusion conformation. During cell entry, the pre-fusion conformation undergoes refolding and conformational changes to its stable post-fusion conformation.

In an example, the antigen is from a RSV surface glycoprotein selected from the Fusion (F), Glycoprotein (G), Small Hydrophobic protein (SH), the matrix proteins M and M2, the nucleocapsid proteins N, P and L, and the nonstructural proteins NS1 and NS2. In certain examples, the antigen is an RSV-F antigen.

The F glycoprotein of RSV is a type I single-pass integral membrane protein having four general domains: N-terminal ER-translocating signal sequence (SS), ectodomain (ED), transmembrane domain (TM), and a cytoplasmic tail (CT). CT contains a single palmitoylated cysteine residue. The sequence of F protein is highly

conserved among RSV isolates but evolves over time. Unlike most paramyxoviruses, the F protein in RSV can mediate entry and syncytium formation independent of the other viral proteins (HN is usually necessary in addition to F in other paramyxoviruses).

The RSV-F glycoprotein is translated from mRNA into an approximately 574 amino acid protein designated F0. Post-translational processing of F0 includes removal of an N-terminal signal peptide by a signal peptidase in the endoplasmic reticulum. F0 is also cleaved at two sites (approximately 109/110 and approximately 136/137) by cellular proteases (in particular furin) in the trans-Golgi. This cleavage results in the removal of a short intervening sequence and generates two subunits designated F1 (~50 kDa; C-terminal; approximately residues 137-574) and F2 (~20 kDa; N-terminal; approximately residues 1-109) that remain associated with each other. F1 contains a hydrophobic fusion peptide at its N-terminus and also two amphipathic heptad-repeat regions (HRA and HRB). HRA is near the fusion peptide and HRB is near the transmembrane domain. Three F1-F2 heterodimers are assembled as homotrimers of F1-F2 in the virion.

RSV-F antigens suitable for inclusion in the immunogenic compositions described herein include RSV-F glycoprotein and RSV-F glycoprotein variants. Suitable RSV-F glycoprotein variants include, for example, full length F protein and truncated variants such as soluble ecto-domains, each optionally containing one or more mutations, such as furin-cleavage mutations, trypsin-cleavage mutations, fusion peptide mutations (e.g., deletions in whole or in part), mutations that stabilize the HRB trimer, and mutations that destabilize the HRA trimer.

Full length and truncated RSV-F glycoproteins, including those with one or more such mutations in a variety of combinations are well known in the art and are disclosed for example in WO2011/008974, the disclosure of which is incorporated herein by reference in its entirety.

In one example, the antigen is from a Pre F or F protein of a RSV. In one example, the antigen is from a G protein of a RSV. In an example, the composition described herein comprises additional antigens. In an example, the additional antigen is a virus, bacteria, a fungus or a protozoan.

In an example, the antigen from a RSV comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 17.

In an example, the antigen from a RSV is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at

least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 17.

In an example, the antigen from a RSV comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at
 5 least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 18.

In an example, the antigen from a RSV is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at
 10 least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% identity to SEQ ID NO: 18.

Viral antigens

In one example, the polynucleotides of the present disclosure further comprise an additional viral antigen.

15 Additional viral antigens that can be encoded by a RNA according to the present disclosure will be apparent to the skilled person and include, for example, proteins and peptides from a *Orthomyxoviruses* (e.g., Influenza A, B and C), *Paramyxoviridae* viruses (Pneumoviruses (e.g., Respiratory syncytial virus (RSV), Bovine respiratory syncytial virus, Pneumonia virus of mice, and Turkey rhinotracheitis virus), Paramyxovirus types
 20 1-4 (PIV), Mumps, Sendai viruses, Simian virus 5)), Bovine parainfluenza virus, Nipahvirus, Henipavirus and Newcastle disease virus), Poxviridae (e.g., Variola vera, including but not limited to, Variola major and Variola minor, Metapneumoviruses, such as human metapneumovirus (hMPV) and avian metapneumoviruses (aMPV)), Morbilliviruses (e.g., Measles), Picornaviruses (e.g., Enteroviruses, Rhinoviruses,
 25 Heparnavirus, Parechovirus, Cardioviruses and Aphthoviruses), Enteroviruses (e.g., Poliovirus types 1, 2 or 3, Coxsackie A virus types 1 to 22 and 24, Coxsackie B virus types 1 to 6, Echovirus (ECHO) virus types 1 to 9, 11 to 27 and 29 to 34 and Enterovirus 68 to 71), Bunyaviruses (e.g., California encephalitis virus), Phlebovirus (e.g., Rift Valley Fever virus), Nairovirus (e.g., Crimean-Congo hemorrhagic fever virus),
 30 Heparnaviruses (e.g., Hepatitis A virus (HAV)), Togaviruses (e.g., Rubivirus, an Alphavirus, or an Arterivirus), Flaviviruses (e.g., Tick-borne encephalitis (TBE) virus, Dengue (types 1, 2, 3 or 4) virus, Yellow Fever virus, Japanese encephalitis virus, Kyasanur Forest Virus, West Nile encephalitis virus, St. Louis encephalitis virus, Russian spring-summer encephalitis virus, Powassan encephalitis virus), Pestiviruses
 35 (e.g., Bovine viral diarrhea (BVDV), Classical swine fever (CSFV) or Border disease (BDV)), Hepadnaviruses (e.g., Hepatitis B virus, Hepatitis C virus), Rhabdoviruses (e.g.,

Lyssavirus (Rabies virus) and Vesiculovirus (VSV)), Caliciviridae (e.g., Norwalk virus, and Norwalk-like Viruses (e.g., Hawaii Virus and Snow Mountain Virus); Coronaviruses (e.g., severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), SARS coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome (MERS) coronavirus
 5 (MERS-CoV), Avian infectious bronchitis (IBV), Mouse hepatitis virus (MHV), and Porcine transmissible gastroenteritis virus (TGEV)), Retroviruses (e.g., Oncovirus, a Lentivirus or a Spumavirus), Reoviruses (e.g., Orthoreo virus, a Rotavirus, an Orbivirus, or a Coltivirus), Parvoviruses (e.g., Parvovirus B 19), Delta hepatitis virus (HDV), Hepatitis E virus (HEV), Human Herpesviruses (e.g., Herpes Simplex Viruses (HSV),
 10 Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human Herpesvirus 6 (HHV6), Human Herpesvirus 7 (HHV7), and Human Herpesvirus 8 (HHV8)), Papovaviruses (e.g., Papillomaviruses and Polyomaviruses), Adenoviruses and Arenaviruses.

In one example, the additional viral antigen is from a parainfluenza virus.

15 In one example, the additional viral antigen is from a metapneumovirus.

In one example, the additional viral antigen is from a rhinovirus.

In one example, the additional viral antigen is from a coronavirus. In this example, the coronavirus may be SARS-CoV2 and the antigen is a RNA encoding a SARS-CoV2 spike glycoprotein peptide or fragment thereof, a RNA encoding a SARS-CoV2
 20 nucleocapsid phosphoprotein peptide or fragment thereof, a RNA encoding a SARS-CoV2 membrane glycoprotein peptide or fragment thereof, a RNA encoding a SARS-CoV2 orf3a or fragment thereof, a RNA encoding a SARS-CoV2 orflab or fragment thereof or a variant of the above.

In one example, the additional viral antigen is from an adenovirus.

25 In one example, the additional viral antigen is from a bocavirus.

In one example, the additional antigen is from a single strain of an influenza virus (i.e., monovalent) or from multiple strains (i.e., multivalent).

In one example, the additional antigen is from an influenza A, B and/or C virus strain.

30 In one example, the additional antigen is from an influenza A virus strain. For example, the antigen is an influenza A virus hemagglutinin (HA) protein, a neuraminidase (NA) protein, a matrix (M) protein, a nucleoprotein (NP), a non-structural (NS) protein, or an immunogenic fragment or variant thereof. In one example, the additional antigen is an influenza A hemagglutinin (HA) subtype H1, H2, H3, H4, H5,
 35 H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 and/or an influenza A neuraminidase (NA) subtype N1, N2, N3, N4, N5, N6, N7, N8 or N9 and/or an influenza

A matrix (M) protein subtype M1 or M2 and/or an influenza A non-structural (NS) protein subtype NS1 or NS2.

The skilled person will be aware that pandemic strains of the influenza virus are commonly H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strains. For example,
5 H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2, strains.

In one example, the antigen is H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strain. For example, the antigen is a H1 hemagglutinin, or a H2 hemagglutinin, or a H3 hemagglutinin, or a H5 hemagglutinin, or a H6 hemagglutinin, or a H7
10 hemagglutinin or a H9 hemagglutinin. For example, the antigen is a H5 subtype influenza A virus strain (i.e., a H5 hemagglutinin). In one example, the H5 hemagglutinin is an A/turkey/Turkey/1/2005 virus strain. In one example, the H3 hemagglutinin is an A/Delaware/39/2019 virus strain.

In one example, the antigen is N1, N2, N3, N7 or N9 subtype influenza A virus strain. For example, the antigen is a N1 neuraminidase, or a N2 neuraminidase, or a N3 neuraminidase, or a N7 neuraminidase, or a N9 neuraminidase. For example, the antigen
15 is a N1 neuraminidase subtype influenza A virus strain. In one example, the N1 neuraminidase is an A/turkey/Turkey/1/2005 strain. In one example, the N2 neuraminidase is an A/Delaware/39/2019 virus strain.

20 In an example, the antigen is a H5 hemagglutinin protein or a N1 neuraminidase protein. For example, the antigen is a H5 hemagglutinin subtype influenza A virus strain or a N1 neuraminidase subtype influenza A virus strain.

Infections such as influenza and coronavirus infection are leading causes of ARDS. Accordingly, in one example of the present disclosure, the ARDS is associated
25 with an influenza, RSV or a SARS-CoV-2 infection. In one example, the ARDS is associated with a SARS-CoV-2 infection. Thus, a skilled person will understand that antigens targeting a SARS-CoV-2 infection or influenza, including those listed above, may be antigens suitable for the treatment of ARDS.

30 Bacterial antigens

In one example, the additional antigen of the present disclosure is a bacterial antigen.

Bacterial antigens that can be encoded by the RNA of the present disclosure will be apparent to the skilled person and include, for example, proteins and peptides from a
35 *Neisseria meningitides*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *Bordetella pertussis*, *Burkholderia sp.* (e.g., *Burkholderia mallei*,

Burkholderia pseudomallei and *Burkholderia cepacia*), *Staphylococcus aureus*, *Haemophilus influenzae*, *Clostridium tetani* (Tetanus), *Clostridium perfringens*, *Clostridium botulinum*, *Corynebacterium diphtheriae* (Diphtheria), *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Coxiella burnetii*, *Brucella sp.* (e.g., *B. abortus*, *B. canis*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. suis* and *B. pinnipediae*), *Francisella sp.* (e.g., *F. novicida*, *F. philomiragia* and *F. tularensis*), *Streptococcus agalactiae*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum* (Syphilis), *Haemophilus ducreyi*, *Enterococcus faecalis*, *Enterococcus faecium*, *Helicobacter pylori*, *Staphylococcus saprophyticus*, *Yersinia enterocolitica*, *E. coli*, *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Mycobacterium tuberculosis*, *Rickettsia*, *Listeria*, *Chlamydia pneumoniae*, *Vibrio cholerae*, *Salmonella typhi* (typhoid fever), *Borrelia burgdorferi*, *Porphyromonas sp.*, *Klebsiella sp.*

Fungal antigens

15 In one example, the additional antigen of the present disclosure is a fungal antigen.

Fungal antigens that can be encoded by a RNA according to the present disclosure or provided in the form of a polypeptide will be apparent to the skilled person and include, for example, proteins and peptides from *Dermatophytes* (including

20 *Epidermophyton floccosum*, *Microsporum audouinii*, *Microsporum canis*, *Microsporum distortum*, *Microsporum equinum*, *Microsporum gypsum*, *Microsporum nanum*, *Trichophyton concentricum*, *Trichophyton equinum*, *Trichophyton gallinae*, *Trichophyton gypseum*, *Trichophyton megnini*, *Trichophyton mentagrophytes*, *Trichophyton quinckeanum*, *Trichophyton rubrum*, *Trichophyton schoenleini*,

25 *Trichophyton tonsurans*, *Trichophyton verrucosum*, *T. verrucosum* var. *album*, var. *discoideus*, var. *ochraceum*, *Trichophyton violaceum*, and/or *Trichophyton faviforme*), *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus sydowi*, *Aspergillus flavatus*, *Aspergillus glaucus*, *Blastoschizomyces capitatus*, *Candida albicans*, *Candida enolase*, *Candida tropicalis*,

30 *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida kusei*, *Candida parakwsei*, *Candida lusitanae*, *Candida pseudotropicalis*, *Candida guilliermondi*, *Cladosporium carrionii*, *Coccidioides immitis*, *Blastomyces dermatidis*, *Cryptococcus neoformans*, *Geotrichum clavatum*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Microsporidia*, *Encephalitozoon spp.*, *Septata intestinalis* and

35 *Enterocytozoon bieneusi*.

Protazoan antigens

In one example, the additional antigen of the present disclosure is a protazoan antigen.

Protazoan antigens that can be encoded by a RNA according to the present disclosure, or provided in the form of a polypeptide will be apparent to the skilled person and include, for example, proteins and peptides from *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Cyclospora cayatanensis* and *Toxoplasma*.

Polynucleotides

As used herein, the term “polynucleotide” refers a molecular chain of nucleotides chemically bonded by a series of ester linkages between the phosphoryl group of one nucleotide and the hydroxyl group of the sugar in an adjacent nucleotide. In one example, the polynucleotide is a DNA. In one example, the polynucleotide is a RNA, e.g., mRNA. For example, the mRNA is a conventional mRNA (cRNA) or a self-replicating RNA.

As used herein, the term “fragment” refers to a portion of a nucleotide sequence or polypeptide of a reference nucleotide sequence or polypeptide disclosed herein which maintains a defined activity of the full length nucleotide sequence or polypeptide.

By way of non-limiting example, where the conventional mRNA, self-replicating RNA comprises a polynucleotide encoding more than one antigen, wherein said more than one antigen may be expressed by a monocistronic polynucleotide, or each of said antigens may be expressed by polycistronic (or multicistronic) polynucleotides. For example, the antigens may be expressed by a monocistronic polynucleotide or by polycistronic polynucleotides.

As used herein, the term “variant” refers to a nucleotide sequence with one or more substitutions, insertions, deletions and/or other modifications compared to the unmodified sequence. It will be apparent to the skilled person that any variant described herein will have the same or similar expression of the encoded protein. For example, the variant is a functional variant. Exemplary modifications to the nucleotide sequence and/or polypeptide will be apparent to the skilled person and/or described herein.

In one example, a modification is a chemical modification of one or more nucleotide(s) of the nucleotide sequence. For example, at least one naturally occurring nucleotide of the polynucleotide is replaced with a chemically modified nucleotide (e.g. pseudouridine (ψ), and 1-methylpseudouridine (m1 ψ)).

In one example, the modification comprises increasing the G/C content of the nucleotide sequence.

In one example, the modification comprises codon optimization of the nucleotide sequence.

In one example, the substitution is a conservative substitution. A skilled person will appreciate that a conservative substitution with reference to a polypeptide involves
5 replacement of an amino acid in the polypeptide with a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size). In one example, the substitution is a non-conservative substitution.

As used herein, the term “encode”, “encodes” or “encoding” refers to a region of a polynucleotide capable of undergoing translation into a polypeptide.

10 The polynucleotide of the present disclosure includes DNA and RNA (e.g. mRNA).

Deoxyribonucleic acid (DNA)

In one example, the polynucleotide is a DNA (e.g. DNA vector).

15 It will be apparent to the skilled person that a DNA of the present disclosure further comprises an endonuclease restriction site at the 3' end of the 3'UTR. The skilled person will appreciate that endonuclease restriction site allows for the insertion of one or more nucleotide sequence(s) (e.g. encoding an antigen of interest, a fragment and/or a variant thereof) without disrupting the remainder of the DNA.

20 As used herein, the term “restriction endonuclease site” refers to a sequence of DNA that binds to a restriction endonuclease. Typically, the restriction endonuclease site is short sequence (e.g. of approximately 4-8 base pairs) recognised and cleaved by the restriction endonuclease.

As used herein, the term “restriction enzymes” or “restriction endonucleases”
25 refers to a class of enzyme that occur naturally in bacteria and in some viruses. Restriction endonuclease bind specifically to and cleave double-stranded DNA at specific sites within or adjacent to a restriction endonuclease site. Exemplary restriction endonuclease include, for example, BciVI (Bful), BcuI (SpeI), EcoRI, AatII, AgeI (BshTI), ApaI, BamHI, BglII, BlnI (Bpu1102I), BsrGI (Bsp1407), ClaI (Bsu15I), EcoRI, EcoRV (Eco32I), Eam1104I (EarI), HindIII, KpnI, MluI, NcoI, NdeI, NheI, NotI, NsiI,
30 Mph1103I), PstI, PvuI, PvuII, SacI, SalI, ScaI, SpeI, XbaI, XhoI, SacII (Cfr42I) and XbaI.

In one example, the present disclosure provides a transcribable polynucleotide comprising nucleotide sequences encoding antigens of interest operably linked to a regulatory element such as a SG promoter and an IRES. For example, the polynucleotide
35 is the DNA plasmid comprising the first and second nucleotide sequences.

In one example, the DNA comprises a nucleotide sequence comprising a restriction endonuclease site located 3' of the 3'UTR. The presence of the restriction endonuclease site located 3' of the 3'UTR allows for production of a linearised DNA. Linearisation of DNA ensures defined termination of *in vitro* transcribed DNA to
5 produce mRNA.

Ribonucleic acid (RNA)

As used herein, the term "RNA (ribonucleic acid)" refers to a single stranded molecular chain of nucleotides chemically bonded by a series of ester linkages between
10 the phosphoryl group of one nucleotide and the hydroxyl group of the sugar in an adjacent nucleotide. Suitable forms of RNA will be apparent to the skilled person. In one example, the RNA is messenger RNA (mRNA). In one example, the mRNA encoding the chemoattract and/or the antigen is a monocistronic mRNA. For example, the monocistronic mRNA is a conventional mRNA (cRNA) or a self-replicating RNA. In
15 another example, the mRNA encoding the chemoattract and/or the antigen is a multicistronic mRNA. For example, the multicistronic mRNA is a conventional mRNA (cRNA) or a self-replicating RNA.

In one example, the polynucleotide is a mRNA comprising comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2);
- (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

25 The mRNA of the present disclosure encompasses a non-replicating mRNA (also referred to as conventional mRNA (cRNA) or non-amplifying) in addition to a self-replicating RNA (also known as self-amplifying RNA or sa-mRNA).

Conventional (non-replicating) RNA

30 In one example, the polynucleotide is a cRNA comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory
syndrome coronavirus 2 (SARS-CoV-2);
- (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial
35 virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

In an example, the cRNA of the present disclosure comprises in order from 5' to 3': a 5' cap structure, a 5'-UTR, a fragment and/or a variant thereof, a first nucleotide sequence encoding a first antigen of interest, a second nucleotide sequence encoding a second antigen of interest, a third nucleotide sequence encoding a second antigen of interest, a 3'-UTR and a 3' tailing sequence (e.g. a polyadenylation signal or one or more poly-A tails). The cRNA of the present disclosure may further comprise an translation internal ribosome entry site (e.g. Kozak consensus sequence or IRES) operably linked to the antigen of interest.

10 *Self-replicating RNA*

The present disclosure provides a self-replicating RNA (also known as a replicon) comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 15 (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

The skilled person will understand that the self-replicating RNA of the present disclosure is based on the genomic RNA of RNA viruses. The RNA should be positive (+)-stranded so that it can be directly translated after delivery to a cell without the need for intervening replication steps (e.g., reverse transcription). Translation of the RNA results in the production of non-structural proteins (NSPs) which combine to form a replicase complex (i.e., an RNA-dependent RNA polymerase). The complex then amplifies the original RNA, producing both antisense and sense transcripts, resulting in production of multiple daughter RNAs which may subsequently be translated and transcribed, enhancing overall protein expression.

In one example, the self-replicating RNA of the present disclosure comprises the non-structural proteins of the RNA virus, the 5' and 3' untranslated regions (UTRs) and the native subgenomic promoter.

In one example, the self-replicating RNA comprises one or more non-structural proteins of the RNA virus. For example, the RNA comprises at least one or more genes selected from the group consisting of a viral replicase (or viral polymerase), a viral protease, a viral helicase and other non-structural viral proteins. For example, the self-replicating RNA comprises a viral replicase (or viral polymerase).

In another example, the self-replicating RNA comprises a 5'- and a 3'-end UTR of the RNA virus. It will be apparent to the skilled person that the terms 5' and a 3'UTR also encompasses the terms 5' and 3' conserved sequence elements (CSE). In one example, the self-replicating RNA comprises a 5'- and a 3'-end CSE.

5 The self-replicating RNA of the present disclosure cannot induce production of infectious viral particles. For example, the self-replicating RNA of the present disclosure does not comprise viral genes encoding structural proteins necessary for production of viral particles.

10 In one example, the self-replicating RNA is derived from or based on an alphavirus. Suitable alphaviruses will be apparent to the skilled person and/or described herein.

15 In another example, the self-replicating RNA is derived from or based on a virus other than an alphavirus, for example, a positive-stranded RNA virus. Positive-stranded RNA viruses suitable for use in the present disclosure will be apparent to the skilled person and include, for example, a picornavirus, a flavivirus, a rubivirus, a pestivirus, a hepacivirus, a calicivirus, or a coronavirus.

Alphavirus

20 In one example, the self-replicating RNA of the present disclosure is derived from (or based on) an alphavirus.

Alphaviruses are the sole genus in the *Togaviridae* family and are an enveloped virus with a positive-sense, single-stranded RNA genome. The skilled person will understand that the alphavirus genome comprises two open reading frames (ORFs), non-structural and structural. The first ORF encodes four non-structural proteins (NSP1, NSP2, NSP3 and NSP4) necessary for transcription and replication of viral RNA. The second encodes three structural proteins: the core nucleocapsid protein C, and the envelope proteins P62 and E1, which associate as a heterodimer. The viral membrane-anchored surface glycoproteins are responsible for receptor recognition and entry into target cells through membrane fusion.

30 In one example, the self-replicating RNA of the present disclosure comprises a viral replicase (or viral polymerase). For example, the viral replicase is an alphavirus replicase, such as an alphavirus protein NSP4.

35 In one example, the self-replicating RNA of the present disclosure does not encode one or more alphavirus structural proteins (e.g., capsid and/or envelope glycoproteins). For example, the self-replicating RNA is unable to produce RNA-containing alphavirus virions (i.e., infectious viral particles).

In one example, the self-replicating RNA comprises a native alphavirus SG promoter. For example, the native alphavirus SG promoter is a minimal SG promoter (i.e., the minimal sequence required for initiation of transcription) and comprises a sequence set forth in SEQ ID NO: 1.

5 The skilled person will be aware of alphaviruses suitable for use in the present disclosure. Exemplary alphaviruses include, but are not limited to, Venezuelan equine encephalitis virus (VEE; e.g., Trinidad donkey, TC83CR), Semliki Forest virus (SFV), Sindbis virus (SIN), Ross River virus, Western equine encephalitis virus, Eastern equine encephalitis virus, Chikungunya virus, S.A. AR86 virus, Everglades virus, Mucambo
10 virus, Barmah Forest virus, Middelburg virus, Pixuna virus, O'nyong-nyong virus, Getah virus, Sagiyama virus, Bebaru virus, Mayaro virus, Una virus, Aura virus, Whataroa virus, Banbanki virus, Kyzylagach virus, Highlands J virus, Fort Morgan virus, Ndumu virus, and Buggy Creek virus. The term alphavirus may also include chimeric alphaviruses (e.g., as described by Perri et al, (2003) J. Virol. 77(19): 10394-403) that
15 contain genome sequences from more than one alphavirus.

Regulatory elements

The present disclosure provides a polynucleotide comprising:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2);
- (b) a nucleotide sequence encoding a second antigen from influenza, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),

wherein each nucleotide sequence is operably linked to a regulatory element.

25 In an example, the regulatory element is selected from the group consisting of a subgenomic (SG) promoter and an internal ribosome entry site (IRES) and a Kozak consensus sequence or a combination thereof. In an example, the regulatory element is a SG promoter.

Kozak consensus sequence

As used herein, the term "Kozak consensus sequence" refers to a nucleotide sequence identified in eukaryotic genes that facilitates the translation of the gene by containing a start codon (also referred to as a translation initiation codon) which is recognised by a ribosome.

35 Exemplary Kozak consensus sequence are known in the art and/or described herein. In one example, the Kozak consensus sequence is set forth in SEQ ID NO: 6

(GCCACC). In another example, the Kozak consensus sequence is set forth in SEQ ID NO: 7 (ACCATGG).

Subgenomic Promoters

5 SG promoters (also known as 'junction region' promoters) suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein.

In one example, the SG promoter is derived from or based on an alphavirus SG promoter. For example, the SG promoter is a native alphavirus SG promoter. In one example, the native SG promoter is a minimal SG promoter. For example, the minimal
10 SG promoter is the minimal sequence required for initiation of transcription. In one example, the native SG promoter is an extended SG promoter. For example, the extended SG promoter is a minimal SG promoter extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein (e.g., NSP4) of the RNA virus (e.g., an alphavirus). In one example, the extended SG promoter is a minimal SG promoter
15 extended at the 5' end with nucleotides occurring in a sequence encoding an alphavirus NSP4.

In one example, the SG promoter is extended at the 5' end by about 31 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence set forth in SEQ ID
20 NO: 1 extended at the 5' end by 31 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 80 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 22 to 101 of SEQ ID NO: 5. In another example, the extended SG promoter is encoded by a sequence comprising
25 or consisting of a sequence set forth in SEQ ID NO: 3.

In one example, the extended SG promoter comprises a repeat sequence corresponding to nucleotides 66 to 75 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence comprising nucleotides 50 to 75 of SEQ ID NO: 5 and nucleotides 66 to 101 of SEQ ID NO: 5. For example, the extended SG promoter is
30 encoded by a sequence set forth in SEQ ID NO: 15.

In one example, the polynucleotide of the disclosure comprises a SG promoter from any alphavirus. For example, the RNA of the disclosure (e.g., cRNA or self-replicating RNA) comprises a SG promoter from any alphavirus.

In one example, the self-replicating RNA comprises a SG promoter from any
35 alphavirus.

The polynucleotide of the present disclosure comprises three nucleotide sequences encoding three antigens of interest. In one example, the three nucleotide sequences are each operably linked to SG promoters. When three nucleotide sequences are present in the RNA of the present disclosure, the promoters operably linked can be the same or different. For example, the three SG promoters are derived from the same alphavirus. In another example, the three SG promoters are derived from different alphaviruses.

Internal Ribosomal Entry Site (IRES)

IRES suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein.

In one example, the IRES is derived from encephalomyocarditis virus (EMCV). For example, the IRES is a wild-type IRES from EMCV.

In one example, the IRES is derived from a fibroblast growth factor 1A (FGF1A) IRES.

In addition, synthetic IRES elements have been described, which can be designed, according to methods known in the art to mimic the function of naturally occurring IRES elements (see Chappell, SA et al. Proc. Natl Acad. Sci. USA (2000) 97(4): 1536-41).

In one example, the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF-κB-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a (FGF1A), GTX, or a combination thereof.

In one example, the IRES is a wild-type IRES derived from encephalomyocarditis virus (EMCV). For example, the wild-type EMCV IRES comprises a sequence set forth in SEQ ID NO: 4.

5'untranslated region (5'-UTR)

In an example, the polynucleotides described herein comprise a 5'-untranslated region (5'-UTR).

As used herein, the term “5′-untranslated region” or “5′-UTR” refers to a non-coding region of an mRNA located at the 5′ end of the translation initiation sequence (AUG).

Exemplary 5′-UTRs include, for example, 5′-UTR of haptoglobin (HP),
5 fibrinogen beta chain (FGB), haptoglobin-related protein (HPR), albumin (ALB),
complement component 3 (C3), fibrinogen alpha chain (FGA), alpha 6 collagen (Col6A),
alpha-1-antitrypsin (SERPINA1), alpha-1-antichymotrypsin (SERPINA3) a fragment
and/or a variant thereof.

In one example, the 5′UTR is a 5′UTR of a Venezuelan equine encephalitis virus
10 (VEEV) or modified forms thereof. For example, the 5′UTR comprises a sequence set
forth in SEQ ID NO: 13.

In one example, the 5′UTR comprises at least one microRNA binding site, an AU
rich element (ARE), a GC-rich element, a stem loop, and combinations thereof.

15 microRNA binding site

As used herein, the term “microRNA binding site” refers to a sequence within a
polynucleotide (e.g. within a DNA or RNA transcript) that has sufficient complementarity
to all or one region of a miRNA to interact, associate or bind to the microRNA (miRNA).

As used herein, the term “microRNA” or “miRNA” refers to 19-25 nucleotide
20 long non-coding RNAs that bind to the 5′-UTR of polynucleotides and down-regulate
gene expression (e.g. by inhibiting translation). The presence of microRNA binding
site(s) in the 5′UTR of the present disclosure can function to inhibit translation of the 5′-
UTR.

Suitable miRNA binding sites for use in the present disclosure will be apparent to
25 the skilled person and/or described herein.

In one example, the miRNA binding site comprises a binding site for tissue
specific microRNA or those regulating biological processes. For example, miRNA of
the liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-
92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223,
30 miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-id, miR-149), kidney
(miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126). For
example, microRNA that regulate biological processes such as angiogenesis (miR-132).
Further exemplifying miRNA and miRNA binding sites are disclosed in US patent
application US14/043,927.

35

AU rich element (ARE)

As used herein, the term “AU rich element (ARE)” or “AU rich elements (AREs)” refers to a region of a nucleotide sequence comprising stretches of Adenine (A) and Uridine (U). Exemplary AREs include, for example, ARE from cytoplasmic myc (c-myc), myoblast determination protein 1 (myoD), c-Jun, Myogenin, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor alpha (TNF- α), or a combination thereof.

In one example, the ARE comprises a human antigen R or “HuR” (also known as Elavl1) specific binding site. HuR is known to bind AREs increasing the stability of the mRNA.

10

GC-rich element

As used herein, the term “GC-rich element” refers to a nucleotide sequence with a high amount of Guanine (G) and/or Cytosine (C) compared to Adenine (A) and Thymine(T)/Uracil(U). The presence of GC-rich elements in a polynucleotide (e.g. mRNA) can stabilise the mRNA.

15

In one example, the GC-rich element comprises a sequence of 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 21, or 22, or 23, or 24, or 25, or 26, or 27, or 28, or 29, or 30 nucleotides in length.

In one example, the GC-rich element comprises between 30% and 40%, or 40% and 50%, or 50% and 60%, or 60% and 70% cytosine. For example, the GC-rich element comprises between 30% and 40% cytosine. For example, the GC-rich element comprises between 40% and 50% cytosine. For example, the GC-rich element comprises between 50% and 60% cytosine. For example, the GC-rich element comprises between 60% and 70% cytosine.

In one example, the GC-rich element comprises 30%, or 40%, or 50%, or 60%, or 70% cytosine. For example, the GC-rich element comprises 30% cytosine. For example, the GC-rich element comprises 40% cytosine. For example, the GC-rich element comprises 50% cytosine. For example, the GC-rich element comprises 60% cytosine. For example, the GC-rich element comprises 70% cytosine.

25

In one example, the GC-rich element is at least 50% cytosine.

In one example, the GC-rich element is at least 60% cytosine.

In one example, the GC-rich element is at least 70% cytosine.

In one example, the GC-rich element comprises a nucleotide sequence according to SEQ ID NO:9. In one example, the GC-rich element comprises a nucleotide sequence

30

CCCCGGCGCC (SEQ ID NO:10). In another example, the GC-rich element comprises a nucleotide sequence CCCCCGC (SEQ ID NO:11).

Stem loop

5 As used herein, the term “stem loop” refers to a nucleotide sequence comprising an intramolecular base pairing of two neighbored entirely or partially reverse complementary sequences to form a stem-loop. A stem-loop can occur in single-stranded DNA or, more commonly, in RNA. The stem loop can also be referred to as a hairpin or hairpin loop which usually consists of a stem and a terminal loop within a consecutive
10 sequence, wherein the stem is formed by two neighbored entirely or partially reverse complementary sequences separated by a short sequence which builds the loop into a stem-loop structure.

The stability of the paired stem loop is determined by the length, the number of mismatched or bulges it contains, and the nucleotide composition of the paired region.

15 In one example, a loop of the stem loop is between 3 and 10 nucleotides in length. For example, the loop of the stem loop is between 3 and 8, or 3 and 7, or 3 and 6, or 4 and 5 nucleotides in length.

In one example, the loop of the stem loop is 4 nucleotides in length.

In one example, the stem loop is a histone stem loop. For example, the histone
20 stem loop comprises or consist of a nucleotide sequence set for in SEQ ID NO: 12.

3'untranslated region (3'-UTR)

In an example, the polynucleotides of the present disclosure comprise a 3'-untranslated region (3'-UTR).

25 As used herein, the term “3'-UTR” refers to a region of an mRNA located at the 3'end of the the translation termination codon (i.e. stop codon).

Exemplary 3'-UTRs include, for example, a 3'-UTR of arachidonate 5-lipoxygenase (ALOX5), alpha I collagen (COL1A1), tyrosine hydroxylase (TH) gene, amino-terminal enhancer of split (AES), human mitochondrial 12S rRNA (mtRNR1), a
30 fragment and/or a variant thereof.

In one example, the 3'UTR is a 3'UTR of a Sindbis virus (SINV) or modified forms thereof. For example, the 3'UTR comprises a sequence set forth in SEQ ID NO: 14.

In one example, the 3'-UTR comprises or consists of a nucleotide sequence
35 derived from a 3'-UTR of an albumin gene. In one example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of a vertebrate α -globin gene.

For example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of a mammalian α -globin gene. For example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of a human α -globin gene.

In one example, the 3'-UTR of the present disclosure further comprises at least one microRNA binding site, an AU rich element (ARE), a GC-rich element, a triple helix, a stem loop, one or more stop codons or a combination thereof.

Stop codon

As used herein, the term "stop codon" refers to a trinucleotide sequence within a mRNA that signals the stop of protein synthesis by a ribosome.

In one example, the polynucleotide of the present disclosure comprises at least one stop codon at the 5' end of a 3'-UTR. For example, the stop codon is selected from UAG, UAA, and UGA.

In one example, the polynucleotide comprises two consecutive stop codons comprising a sequence UGAUGA.

In one example, the polynucleotide comprises two consecutive stop codons comprising a sequence UAAUAG.

3' tailing sequence

In an example, the polynucleotide of the present disclosure comprises one or more 3' tailing sequences located at the 3' end of the 3'UTR.

As described herein, the term "3' tailing sequence" or "3' tailing sequences" refers to a nucleotide sequence (e.g. polyadenylation signal) which induces the addition of non-encoded nucleotides to the 3' end of a mRNA or a nucleotide sequence (e.g. poly-A sequence) located at the 3' end of a mRNA. A skilled person will appreciate that the 3' tailing sequence and/or products of the 3' tailing sequence in a mRNA functions to stabilise the mRNA and/or prevent the mRNA from degradation.

As used herein, the term "interrupting linker" in reference to a poly-A or poly-C sequence of the present disclosure refers to a single nucleotide or nucleotide sequence which are linked to, and interrupt, a stretch of consecutive adenosine or cytosine nucleotides in the poly-A or poly-C sequence. For example, the interrupting linker in a poly-A sequence is a single nucleotide or a nucleotide sequence consisting or comprising a nucleotide other than an adenosine nucleotide. For example, the interrupting linker in a poly-C sequence is a single nucleotide or a nucleotide sequence consisting or comprising a nucleotide other than an cytosine nucleotide.

In one example, the one or more 3' tailing sequences are selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

5 Poly-A sequence

As used herein, the term "polyA sequence" refers to a nucleotide sequence of Adenine (A) located at the 3' end of a mRNA. In the context of the present disclosure, the polyA sequence may be located within the mRNA or DNA (e.g. a DNA plasmid serving as a template for generating the mRNA by transcription of the vector).

10 Suitable poly-A sequence for use in the present disclosure will be apparent to the skilled person and/or are described herein. In one example, the poly-A sequence comprises consecutive (i.e. one after the other) adenosine nucleotides of any length (e.g. to 10 to 300). In one example, the poly-A sequence comprises consecutive adenosine nucleotides separated by one or more interrupting linkers. In one example, the poly-A
15 sequence comprises consecutive adenosine nucleotides without an interrupting linker.

Polyadenylation signal

As used herein, the term "polyadenylation signal" refers to a nucleotide sequence which induces polyadenylation. Polyadenylation is typically understood to be the
20 addition of a polyA sequence to a RNA (e.g. to a premature mRNA to generate a mature mRNA). The polyadenylation signal may be located within a nucleotide sequence at the 3'-end of the polynucleotide (e.g. mRNA) to be polyadenylated.

Suitable polyadenylation signal for use in the present disclosure will be apparent to the skilled person and/or described herein.

25 In one example, the polyadenylation signal comprises a hexamer consisting of Adenine and Uracil/Thymidine nucleotides. In one example, the hexamer sequence comprises or consists of AAUAAA.

In one example, the 3'tailing sequence comprises a polyadenylation signal but does not comprise a polyA sequence.

30

G-quadruplex

As used herein, the term "G-quadruplex" or "G4" refers to a nucleotide sequence rich in guanine residues which forms a four stranded secondary structure. For example, the G-quadruplex is a cyclic hydrogen bonded array of four guanine nucleotides formed
35 by G-rich sequences in both DNA and RNA.

In one example, the 3' tailing sequence comprises a polyA sequence and a G-quadruplex. For example, the 3' tailing sequence comprises a polyA sequence linked to a G-quadruplex to produce a polyA-G quartet.

5 Poly-C sequence

As used herein, the term "poly-C sequence" refers to a nucleotide sequence of Cytosine (C) located at the 3' end of a mRNA. In the context of the present disclosure, the polyC sequence may be located within the mRNA or DNA (e.g. a DNA plasmid serving as a template for generating the mRNA by transcription of the vector).

10 Suitable poly-C sequence for use in the present disclosure will be apparent to the skilled person and/or are described herein.

In one example, the one or more 3' tailing sequences comprises one or more poly-C sequences each comprising between 10 and 300 consecutive cytosine nucleotides. For example, the one or more poly-C sequences each comprises between 10 and 20, or 20
15 and 30, or 30 and 40, or 40 and 50, or 50 and 60, or 60 and 70, or 70 and 80, or 80 and 90, or 90 and 100, or 100 and 125, or 125 and 150, or 150 and 175, or 175 and 200, or 200 and 225, or 225 and 250, or 250 and 275, or 275 and 300 consecutive cytosine nucleotides. For example, the one or more poly-C sequence each comprises 10, or 20, or 30, or 40, or 50, or 60, or 70, or 80, or 90, or 100, or 125, or 150, or 175, or 200, or
20 225, or 250, or 275, or 300 consecutive cytosine nucleotides.

In one example, the one or more poly-C sequences is separated by an interrupting linker. For example, the fourth nucleotide sequence comprising the one or more 3' tailing sequences comprises, in order of 5' to 3': consecutive cytosine nucleotides, an interrupting linker, and further consecutive cytosine nucleotides.

25 In one example, the interrupting linker is from 10 to 50, or 50 to 100, or 100 to 150 nucleotides in length. For example, the interrupting linker is 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 25, or 30, or 35, or 40, or 45, or 50, or 55, or 60, or 65, or 70, or 75, or 80, or 85, or 90, or 95, or 100, or 110, or 120, or 130, or 140, or 150 nucleotides in length.

30

5' cap structure

In one example, the present disclosure provides a mRNA comprising a 5' terminal cap structure.

35 As used herein, the term "5' cap structure" refers to a structure at the 5' terminal end of a mRNA involved in nuclear export and binds a mRNA Cap Binding Protein (CBP). The 5' cap structure is known to stabilise mRNA through association of CBP

with poly(A) binding protein to form a mature mRNA. Accordingly, the presence of a 5' cap structure in the mRNA of the present disclosure can further increase the stability of the mRNA compared to a mRNA without the 5' cap.

Exemplary 5' cap structure includes, for example, anti-reverse cap analogue (ARCA), N7,2'-O-dimethyl-guanosine (mCAP), inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, N6,2'-O-dimethyladenosine, 7-methylguanosine (m7G), Cap1, and Cap2.

Typically, an endogenous mRNA is 5' capped with a guanosine through a (5')-ppp-(5')-triphosphate linkage attached to the 5' terminal nucleotide of the mRNA. The guanosine cap can then be methylated to a 7-methylguanosine (m7G) generating a 7mG(5')ppp(5')N,pN2p (Cap0 structure), where N represents the first and second 5' terminal nucleotide of the mRNA. The cap0 structure can be further 2'-O-methylated to produce 7mG(5')ppp(5')NlmpNp (Cap1), and/or 7mG(5')-ppp(5')NlmpN2mp (Cap2).

In one example, the polynucleotide of the present disclosure comprises an endogenous cap.

As used herein, the term "endogenous cap" refers to a 5' cap synthesised in a cell. For example, endogenous cap is a natural 5' cap or a wild-type 5' cap. For example, the endogenous cap is a Cap0, Cap1, or Cap2 structure.

In one example, the polynucleotide of the present disclosure comprises an analog of an endogenous cap (also referred to as cap analog).

As used herein, the term "analogue thereof" in the context of an endogenous cap or "cap analog" refers to a synthetic 5' cap. The cap analog can be used to produce 5' capped mRNA in *in vitro* transcription reactions. Cap analogs may be chemically (i.e. non-enzymatically) or enzymatically synthesized and/or linked to a nucleotide (e.g. 5' terminal nucleotide of an mRNA). Exemplary cap analogs are commercially available and include, for example, 3''-O-Me-m7G(5')ppp(5')G, G(5')ppp(5')A, G(5')ppp(5')G, m7G(5')ppp(5')A, m7G(5')ppp(5')G (New England BioLabs). In one example, the cap analog is N7,3'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine (i.e. anti-reverse cap analogue (ARCA)).

In one example, the 5' cap structure is a non-hydrolyzable cap structure. The non-hydrolyzable cap structure can prevent decapping of the mRNA and increase the half-life of the mRNA.

In one example, the non-hydrolyzable cap structure comprises a modified nucleotide selected from a group consisting of a α -thio-guanosine nucleotide, α -methyl-phosphonate, seleno-phosphate, and a combination thereof. In one example, the modified

nucleotide is linked to the 5' end of the mRNA through an α -phosphorothiate linkage. Methods of linking the modified nucleotide to the 5' end of the mRNA will be apparent to the skilled person. For example, using a Vaccinia Capping Enzyme (New England Biolabs).

5

Modifications

In one example, polynucleotides of the present disclosure comprises one or more modification(s). Typically, modifications are introduced into a polynucleotide (e.g. mRNA) to increase the translation efficiency and/or stability of the polynucleotide.

10 Suitable modifications to the polynucleotide will be apparent to the skilled person and/or described herein.

In one example, the nucleotide sequence comprising the 5'-UTR and/or the fragment thereof is modified. Modification of the nucleotide sequences comprising the 5'-UTR and/or the fragment thereof results in a variant of the 5'-UTR and/or the

15 fragment thereof.

In one example, one or more nucleotide sequence(s) of the polynucleotide are codon optimized. Method of codon optimization will be apparent to the skilled person and/or described herein. For example, tools for codon optimization of polynucleotide include, for example, GeneArt GeneOptimizer (ThermoFisher®) or GenSmart®

20 (GeneScript®).

In one example, the polynucleotide is modified to increase the amount of Guanine (G) and/or Cytosine (C) in the polynucleotide. The amount of G/C in the polynucleotide (i.e. G/C content) can influence the stability of the polynucleotide. Accordingly, polynucleotide comprising an increased amount of G/C nucleotides can be functionally

25 more stable than polynucleotides containing a large amount of Adenine (A) and Thymine (T) or Uracil (U) nucleotides. The G/C content is increased by substituting A or T nucleotides with G or C nucleotides.

In one example, the G/C content is increased in the nucleotide sequences encoding the antigens of interest. The modification(s) in the nucleotide sequences takes advantage

30 of the ability of substituting codons that contain less favourable combinations of nucleotides (in terms of mRNA stability) with alternative codons encoding the same amino acid, or encoding amino acid(s) of similar chemistry (e.g. conserved amino acid substitution). For example, the G/C content is increased by substituting codons containing A or T nucleotides with codons containing G or C nucleotides that encode for

35 the same amino acid. For example, the G/C content is increased by substituting codons

containing A or T nucleotides with codons containing G or C nucleotides that encode for an amino acid of similar chemistry.

In one example, the G/C content is increased in the nucleotide sequences of the polynucleotide which do not encode the antigen of interest. For example, the G/C
5 content is increased in the 5'-UTR, the fragment and/or the variant thereof. For example, the G/C content is increased in the 3'-UTR, the fragment and/or the variant thereof.

In one example, the polynucleotide comprises at least one chemically modified nucleotide.

As used herein, the term "chemical modification" or "chemical modified" in the
10 context of a nucleotide refers to a naturally occurring nucleotides (i.e. A, T, C, G, U) which are modified by replacement, insertion or removal of individual or several atoms or atomic groups compared to the naturally occurring nucleotides. In one example, at least one naturally occurring nucleotide of the polynucleotide is replaced with a chemically modified nucleotide. In one example, at least 10%, or 20%, or 30%, or 40%,
15 or 50%, or 60%, or 70%, or 80%, or 90%, or 100% of naturally occurring nucleotides of the polynucleotide is replaced with a chemically modified nucleotides. Suitable chemical modified nucleotides for use in the present disclosure will be apparent to the skilled person and/or described herein. Exemplary chemically modified nucleotides include, for example, N6,2'-O-dimethyl-adenosine (m6Am), 5-methyluridine (m5U), N4-acetylcytidine (ac4C), 2-thiocytidine (s2C), 2-thiouridine (s2U), 5-methylcytidine
20 (m5C), N6-methyladenosine (m6a), pseudouridine (ψ), and 1-methylpseudouridine (m1 ψ).

Methods of Production

25 Suitable methods for the production of a polynucleotide, a cRNA and/or a self-replicating RNA of the present disclosure will be apparent to the skilled person and/or described herein.

In one example, the polynucleotide is DNA. For example, the polynucleotide is a plasmid DNA.

30 In one example, the cRNA is produced using a plasmid DNA. In one example, the self-replicating RNA is produced using a plasmid DNA. The skilled person will understand that plasmid DNA is relatively stable. Briefly, competent bacterial cells (e.g., *Escherichia coli*) cells are transformed with a DNA plasmid encoding a self-replicating RNA of the present disclosure. Individual bacterial colonies are isolated and the resultant
35 plasmid DNA amplified in *E. coli* cultures.

In one example, the plasmid DNA is isolated following fermentation. For example, the plasmid DNA is isolated using a commercially available kit (e.g., Maxiprep DNA kit), or other routine methods known to the skilled person. Following isolation, plasmid DNA is linearized by restriction digest (i.e., using a restricting enzyme).
5 Restriction enzymes are removed using methods known in the art, including for example phenol/chloroform extraction and ethanol precipitation.

In one example, mRNA is made by *in vitro* transcription from a linearized DNA template using an RNA polymerase (e.g., T7 RNA polymerase). Following *in vitro* transcription, the DNA template is removed by DNase digestion. The skilled person will
10 understand that synthetic mRNA capping is performed to correct mRNA processing and contribute to stabilization of the mRNA. In one example, the mRNA is enzymatically 5'-capped. For example, the 5' cap is a cap0 structure or a cap1 structure. In one example, the 5' cap is a cap0 structure, for example, the 5'-cap (i.e., cap0) consists of an inverted 7-methylguanosine connected to the rest of the mRNA via a 5'-5' triphosphate
15 bridge. In one example, the 5' cap is a cap1 structure, for example, the 5'-cap (i.e., cap1) consists of the cap0 with an additional methylation of the 2'O position of the initiating nucleotide.

In one example, the mRNA is purified. Various methods for purifying mRNA will be apparent to the skilled person. For example, the mRNA is purified using lithium
20 chloride (LiCl) precipitation. In another example, the mRNA is purified using tangential flow filtration (TFF). Following purification, the mRNA is resuspended in e.g., nuclease-free water.

Compositions

25 The present disclosure provides an immunogenic composition comprising a polynucleotide of the present disclosure.

The present disclosure also provides an immunogenic composition comprising a RNA of the present disclosure.

The present disclosure also provides an immunogenic composition comprising a
30 cRNA of the present disclosure.

The present disclosure further provides an immunogenic composition comprising a self-replicating RNA of the present disclosure.

The present disclosure also provides a pharmaceutical composition comprising an immunogenic composition of the present disclosure and a pharmaceutically acceptable
35 carrier.

It will be apparent to the skilled person and/or described herein, that the polynucleotide, RNA, cRNA and/or self-replicating RNA of the present disclosure may be present as naked RNA or in combination with lipids, polymers or other delivery system that facilitates entry into the cells.

5

Delivery systems

In one example, the pharmaceutical composition of the present disclosure further comprises a LNP, a polymeric microparticle and an oil-in-water emulsion. For example, the polynucleotide, the cRNA and/or the self-replicating RNA is encapsulated in, bound
10 to or adsorbed on a LNP, a polymeric microparticle, or an oil-in-water emulsion.

Lipid Nanoparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a LNP.

15 It will be apparent that the term “lipid nanoparticle” or “LNP” refers to any lipid composition, including, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar) micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles, wherein solid lipid nanoparticles lack lipid bilayers.

20 Lipid nanoparticles suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein. The lipids can have an anionic, cationic or zwitterionic hydrophilic head group.

In one example, the lipid nanoparticle comprises a PEG-lipid, a sterol structural lipid and/or a neutral lipid. In one example, the lipid nanoparticle further comprises a
25 cationic lipid. In one example, the lipid nanoparticle does not comprise a cationic lipid.

In one example, the LNP comprises a PEG-lipid. For example, the PEG-lipid is selected from the group consisting of PEG-c-DMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid and combinations thereof.

In one example, the LNP comprises a structural lipid. For example, the structural
30 lipid is selected from the group consisting of cholesterol fecosterol, sitosterol, campesterol, stigmasterol, brassicasterol, ergosterol, tomatidine, tomatine, ursolic acid and alpha-tocopherol and combinations thereof.

In one example, the LNP comprises a neutral lipid. Exemplary phospholipids (anionic or zwitterionic) for use in the present disclosure include, for example,
35 phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, and phosphatidylglycerols. For example, the neutral lipid is selected from the group

consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin and combinations thereof.

In one example, the LNP comprises a cationic lipid. Exemplary cationic lipids include, but are not limited to, dioleoyl trimethylammonium propane (DOTAP), 1,2-distearoyloxy-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), 2,5-bis((9Z,12Z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). In one example, the phospholipid is 2,5-bis((9Z,12Z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). Exemplary zwitterionic lipids include, but are not limited to, acyl zwitterionic lipids and ether zwitterionic lipids, such as dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and dodecylphosphocholine. The lipids can be saturated or unsaturated.

Polymeric microparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a polymeric microparticle.

The skilled person will be aware that various polymers can form microparticles to encapsulate or adsorb the polynucleotide, the RNA, the cRNA and/or the self-replicating RNA of the present disclosure. It will be apparent that use of a substantially non-toxic polymer means that particles are safe, and the use of a biodegradable polymer means that the particles can be metabolised after delivery to avoid long-term persistence.

Useful polymers are also sterilisable, to assist in the preparation of pharmaceutical grade formulations.

Exemplary non-toxic and biodegradable polymers include, but are not limited to, poly(α - hydroxy acids), polyhydroxy butyric acids, polylactones (including
5 polycaprolactones), polydioxanones, polyvalerolactone, polyorthoesters, polyanhydrides, polycyanoacrylates, tyrosine-derived polycarbonates, polyvinyl-pyrrolidinones or polyester-amides, and combinations thereof.

Oil-in-water cationic emulsions

10 In one example, the pharmaceutical composition of the present disclosure further comprises an oil-in-water cationic emulsion.

Suitable oils for use in an oil-in-water emulsion will be apparent to the skilled person and/or are described herein. For example, the emulsion comprises one or more oils derived, for example, from an animal (e.g., fish) or a vegetable source (e.g., nuts,
15 seeds, grains). The skilled person will recognise that biocompatible and biodegradable oils are preferentially used. Exemplary animal oils (i.e., fish oils) include cod liver oil, shark liver oils, and whale oil. Exemplary vegetable oils include peanut oil, coconut oil, olive oil, soybean oil, jojoba oil, safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil, corn oil.

20 In addition to the oil, the oil-in-water emulsion also comprises a cationic lipid to facilitate formation and stabilisation of the emulsion. Suitable cationic lipids will be apparent to the skilled person and/or are described herein. Exemplary cationic lipids include, but are not limited to, limited to: 1, 2-dioleoyloxy-3-(trimethylammonio)propane (DOTAP), 3'-[N-(N',N'-Dimethylaminoethane)-carbonyl] Cholesterol (DC
25 Cholesterol), dimethyldioctadecyl-ammonium (DDA), 1,2-Dimyristoyl-3-Trimethyl-AmmoniumPropane (DMTAP), dipalmitoyl[C16:0]trimethyl ammonium propane (DPTAP) and distearoyltrimethylammonium propane (DSTAP).

In some examples, the oil-in-water emulsion also comprises a non-ionic surfactant and/or a zwitterionic surfactant. The skilled person will be aware of surfactants suitable
30 for use in the present disclosure. Exemplary surfactants include, but are not limited to: the polyoxyethylene sorbitan esters surfactants (e.g., polysorbate 20 and polysorbate 80) and copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO).

35 *Pharmaceutically acceptable carrier*

Suitably, in compositions or methods for administration of the polynucleotides, RNA, cRNA and/or the self-replicating RNA of the disclosure to a subject, the polynucleotides, RNA, cRNA and/or the self-replicating RNA is combined with a pharmaceutically acceptable carrier as is understood in the art. Accordingly, one example of the present disclosure provides a composition (e.g., a pharmaceutical composition) comprising a RNA (e.g., self-replicating RNA) of the disclosure (and any delivery system) combined with a pharmaceutically acceptable carrier. Another example of the present disclosure provides a composition (e.g., a pharmaceutical composition) comprising a cRNA of the disclosure (and any delivery system) combined with a pharmaceutically acceptable carrier.

In general terms, by “carrier” is meant a solid or liquid filler, binder, diluent, encapsulating substance, emulsifier, wetting agent, solvent, suspending agent, coating or lubricant that may be safely administered to any subject, e.g., a human. Depending upon the particular route of administration, a variety of acceptable carriers, known in the art may be used, as for example described in Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991).

The polynucleotides, RNA, cRNA and/or the self-replicating RNA of the present disclosure is useful for parenteral, topical, oral, or local administration, intramuscular administration, aerosol administration, or transdermal administration, for prophylactic or for therapeutic treatment. In one example, the polynucleotide, RNA, cRNA or the self-replicating RNA is administered parenterally, such as intramuscularly, subcutaneously or intravenously. For example, the polynucleotide RNA, cRNA or the self-replicating RNA is administered intramuscularly. In another example, the cRNA is administered parenterally, such as intramuscularly, subcutaneously or intravenously. For example, the cRNA is administered intramuscularly.

Formulation of a polynucleotide, RNA, cRNA or the self-replicating RNA to be administered will vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. An appropriate pharmaceutical composition comprising a polynucleotide, RNA, cRNA or self-replicating RNA to be administered can be prepared in a physiologically acceptable carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), dextrose solution and glycine. Intravenous

vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH
5 adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The polynucleotide, RNA, cRNA and/or the self-replicating RNA can be stored in the liquid stage or can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques.

10 The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures known to the skilled artisan, and will depend on the ultimate pharmaceutical formulation desired.

Upon formulation, compositions of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is
15 therapeutically/prophylactically effective. The dosage ranges for the administration of the polynucleotide, RNA, cRNA or self-replicating RNA of the disclosure are those large enough to produce the desired effect. For example, the composition comprises an effective amount of the self-replicating RNA. In one example, the composition comprises a therapeutically effective amount of the polynucleotide, RNA, cRNA or the
20 self-replicating RNA. In another example, the composition comprises a prophylactically effective amount of the polynucleotide, RNA, cRNA or the self-replicating RNA.

The dosage should not be so large as to cause adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual
25 physician in the event of any complication.

Dosage can vary from about 0.1 mg/kg to about 300 mg/kg, e.g., from about 0.2 mg/kg to about 200 mg/kg, such as, from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

In some examples, the polynucleotide, RNA, cRNA and/or the self-replicating
30 RNA is administered at an initial (or loading) dose which is higher than subsequent (maintenance doses). For example, the polynucleotide, RNA, cRNA or the self-replicating RNA is administered at an initial dose of between about 10mg/kg to about 30mg/kg. The polynucleotide, RNA, cRNA or the self-replicating RNA is then administered at a maintenance dose of between about 0.0001mg/kg to about 10mg/kg.
35 The maintenance doses may be administered every 7-35 days, such as, every 7 or 14 or 28 days.

In some examples, a dose escalation regime is used, in which the polynucleotide, RNA, cRNA or the self-replicating RNA is initially administered at a lower dose than used in subsequent doses. This dosage regime is useful in the case of subject's initially suffering adverse events

5 In the case of a subject that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

A subject may be retreated with the polynucleotide, RNA, cRNA or the self-replicating RNA of the present disclosure. A subject may be retreated with the
10 polynucleotide, RNA, cRNA or the self-replicating RNA, by being given more than one exposure or set of doses, such as at least about two exposures of the binding protein, for example, from about 2 to 60 exposures, and more particularly about 2 to 40 exposures, most particularly, about 2 to 20 exposures.

In one example, any retreatment may be given when signs or symptoms of disease
15 return.

In another example, any retreatment may be given at defined intervals. For example, subsequent exposures may be administered at various intervals, such as, for example, about 24-28 weeks or 48-56 weeks or longer. For example, such exposures are administered at intervals each of about 24-26 weeks or about 38-42 weeks, or about 50-
20 54 weeks.

In the case of a subject that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

In another example, for subjects experiencing an adverse reaction, the initial (or
25 loading) dose may be split over numerous days in one week or over numerous consecutive days.

Administration of a polynucleotide, RNA, cRNA or the self-replicating RNA according to the methods of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose
30 of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the polynucleotide, RNA, cRNA or the self-replicating RNA may be essentially continuous over a preselected period of time or may be in a series of spaced doses, e.g., either during or after development of a condition.

35 *Adjuvants and additional components*

Pharmaceutical compositions according to the present disclosure can comprise additional adjuvants or immunopotentiating agents. An adjuvant or immunopotentiating agent is a substance that modifies the action of the principal ingredient. Thus, a skilled person will understand that the terms “adjuvant” or “immunopotentiating agent” may be used interchangeably. In an example, an adjuvant or immunopotentiating agent is a substance that enhances a subject’s immune response to an antigen. Suitable adjuvants or immunopotentiating agents will be apparent to the skilled person and include, for example, aluminium-containing adjuvants (e.g. amorphous aluminum hydroxyphosphate sulfate, aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate), AS04, MF59, AS01_B, or CpG 1018.

In an example, the immunogenic compositions described herein comprise an adjuvant for enhancing immunogenicity. In an example, the adjuvant is selected from the group consisting of Freund's adjuvant, incomplete Freund's adjuvants, aluminum phosphate, aluminum hydroxide, GMCSP, BCG, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, monophosphoryl lipid A (MPL), RIBI, MPL, trehalose dimycolate (TDM), Novasomes®, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, MHC antigens, PolyI:C, MF59, glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, ODA, cytokines, and other adjuvants and derivatives thereof. In an example, the adjuvant is MF59. In an example, MF59 is administered at the same time as the administration of a composition or vaccine of the disclosure. In another example, MF59 is administered sequentially to, preceding, or proceeding the administration of a composition or vaccine of the disclosure.

In an example, an adjuvant has one or more of the following effects:

- increasing the magnitude or function of the antibody response;
- increasing cell mediated immunity;
- inducing mucosal immunity; and/or
- reducing antigen dose.

In one example, compositions according to the disclosure comprise an additional RNA encoding including: (i) one or more antigens; (ii) one or more immunopotentiating agents; (iii) one or more chemoattractants; and/or (iv) one or more targeting molecules.

As used herein, “immunopotentiating agent” means any molecule capable of enhancing an immune response in a subject. Examples of immunopotentiating agents include cytokines, chemokines, and immune potentiators. In example, the one or more immunopotentiating agents are selected from the group consisting of interleukin 12, interleukin 7, interleukin 15, and interleukin 21.

As used herein, “targeting molecule” means any molecule capable of targeting an immune cell surface marker, for example, a dendritic cell or an antigen presenting cell. In one example, the targeting molecule targets a dendritic cell surface marker. In an example, the targeting molecule is selected from the group consisting of DEC-205, Clec-
5 9A, DC-SIGN, CD11c, DCIR2, Dectin-1/2, CD80/86, F4/80, CIRE, mannose, and CD36. In an example, the targeting molecule is DEC-205.

In another example, the targeting molecule is an antibody. In one example, the targeting molecule is a monoclonal antibody.

10 Screening Assays

Suitable methods for selecting a polynucleotide, RNA, cRNA or the self-replicating RNA of the present disclosure are available to those skilled in the art. Assays may be conducted to assess the efficiency and efficacy of the polynucleotide, RNA, cRNA or the self-replicating RNA including, for example, serology and immune
15 responses.

Antigen expression

In one example, the self-replicating RNA is assessed for expression of the polynucleotide/s of interest. In another example, the cRNA is assessed for expression of
20 the polynucleotide/s of interest. In another example, the RNA is assessed for expression of the polynucleotide/s of interest

For example, antigen expression is detected using antibodies against the polynucleotide/s of interest. In one example, the number of cells positive for antigen expression is measured by e.g., fluorescence-activated cell sorting (FACS). In another
25 example the mean fluorescence intensity (MFI) is determined using e.g., FACS. In a further example, the specific potency value or the probability of successful transfection per unit mass of RNA is calculated.

Microneutralization Assay

30 In one example, the self-replicating RNA (naked and/or formulated) is assessed for antibody responses. In one example, the cRNA (naked and/or formulated) is assessed for antibody responses. For example, the cRNA and/or the self-replicating RNA is assessed using a microneutralisation assay. Methods of performing a microneutralization assay will be apparent to the skilled person. In one example, the microneutralization
35 assay is a short form assay. For one example, a virus fluorescent focus-based

microneutralization assay is performed. In another example, the microneutralization assay is a long form assay.

Hemagglutination inhibition (HAI) assay

- 5 In one example, the self-replicating RNA (naked and/or formulated) is assessed for antibody responses. In one example, the cRNA (naked and/or formulated) is assessed for antibody responses. For example, the cRNA and/or self-replicating RNA is assessed using a hemagglutination inhibition (HAI) assay. Methods of performing a HAI assay will be apparent to the skilled person and/or described, for example, in WHO (2011)
- 10 *Manual for the laboratory diagnosis and virological surveillance of influenza*: WHO Press, World Health Organization.

Antigen Specific T cell Responses

- 15 In one example, the self-replicating RNA is assessed for its ability to induce antigen specific T cell responses. In one example, the cRNA is assessed for its ability to induce antigen specific T cell responses. Methods of assessing induction of antigen specific T cell responses will be apparent to the skilled person and/or are described herein.

- 20 For example, antigen-specific T cell detection is performed on splenic cultures. Briefly, splenocyte cultures are established in T cell medium and cell cultures are either stimulated with antigenic peptides or unstimulated. In one example, antigen-specific T cell responses are determined using flow cytometry.

Neutralising assays

- 25 The self-replicating RNA of the disclosure may be screened *in vitro* for their ability to bind to a SARS-CoV-2 S protein and neutralises binding of the S protein to ACE2. Suitable assays will be apparent to the skilled person and include, for example, a Vero microneutralisation assay, a sVNT assay, or a psuedovirus neutralisation assay (using e.g., HEK-293T cells or HeLa-ACE2 cells).
- 30 In one example, the neutralization assay is a Vero microneutralization assay. Briefly, SARS-Cov-2 wild-type virus is passaged in Vero cells (i.e., the Vero lineage isolated from kidney epithelial cells extracted from an African green monkey). Serial two-fold dilutions of a test protein are incubated with 100 TCID₅₀ (i.e., median tissue culture infectious dose) of SARS-CoV-2 for 1 hour and residual virus infectivity is
- 35 assessed in Vero cells; viral cytopathic effect is read, for example, on day 5. The

neutralising antibody titre is calculated using the Reed/Muench method as previously described (Houser et al., 2016; Subbarao et al 2004).

In one example, the neutralization assay is a surrogate neutralization test (sVNT). Briefly, the wells of a plate are coated with hACE2 protein in carbonate-bicarbonate coating buffer (e.g., pH 9.6). HRP-conjugated SARS-CoV-2 and HRP-conjugated SARS-CoV-2 pre-incubated with test proteins is added to the hACE2 at different concentrations and incubated, for example, for 1h at room temperature. Unbound HRP conjugated antigens are removed by washing. Colorimetric signal is developed on the enzymatic reaction of HRP with chromogenic substrate, e.g., 3,3',5,5'-tetramethylbenzidine (TMB). In one example, the absorbance reading at 450 nm and 570 nm is acquired.

In one example, the neutralisation is a pseudovirus neutralisation assay. Briefly, HIV reporter virus pseudotyped with SARS-Cov-2 S protein is produced by co-transfection of SARS-2-CoV-2 spike plasmids together with a viral backbone plasmid (e.g., pDR-NL Δenv FLUC) into e.g., HEK-293T cells. Pseudovirus is harvested post transfection and clarified by filtration. Virus stock titres, reported as Relative Luciferase Units infectious dose (RLU), are calculated by limiting dilution infections in Hela-hACE2 cells measuring luciferase activity as a read-out for viral infection.

20 **Methods of Treatment or Prevention**

The present disclosure provides methods of using the immunogenic composition or the pharmaceutical composition of the present disclosure as a vaccine.

The present disclosure also provides methods of treating or preventing a disease or condition in a subject comprising administering the immunogenic composition or the pharmaceutical composition of the present disclosure. For example, the disease or condition is a respiratory virus infection, such as influenza, a SARS-CoV-2 infection, COVID-19, or respiratory syncytial virus (RSV). In another example, the disease or condition is acute respiratory distress syndrome (ARDS).

30 Influenza

Influenza, also known as "the flu", is an infectious disease caused by an influenza virus. Symptoms can be mild to severe and the most common symptoms include high fever, runny nose, sore throat, muscle and joint pain, headache, coughing, and feeling tired. Symptoms typically begin two days after exposure to the virus and most last less than a week. Complications of influenza may include viral pneumonia, secondary bacterial pneumonia, sinus infections, and worsening of previous health problems such

as asthma or heart failure. Viral pneumonia may also lead to acute respiratory distress syndrome (ARDS).

It will be apparent to the skilled person that there are currently four influenza viruses – A, B, C and D. Influenza A virus is the most common flu virus infecting
5 humans, animals, and birds, whilst influenza B virus infection mostly occurs in humans. Infection of influenza C virus does not cause any severe symptom in human or mammals and influenza D, to date, has only infected pigs and cattle.

Thus, in some examples of the present disclosure, the subject has an influenza virus infection. In one example, the subject has influenza. In some examples, the
10 influenza is associated with ARDS. In one example, the methods of the present disclosure can be used to treat or prevent ARDS in a subject suffering from an influenza virus infection. In one example, the methods of the present disclosure can be used to treat or prevent ARDS in a subject suffering from influenza.

In an example, the methods described herein comprise the identification of a
15 subject having or suspected of having influenza. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe influenza.

Coronavirus Disease 2019 (COVID-19)

20 The present disclosure provides, for example, methods of treating or preventing COVID-19. The present disclosure also provides, for example, methods of treating or preventing SARS-CoV-2 infection. In some examples of the present disclosure the subject has a SARS-CoV-2 infection but does not have clinically diagnosed COVID-19.

COVID-19 is an infectious disease caused by SARS-CoV-2. It was first identified
25 in December 2019 in Wuhan, Hubei, China, and has resulted in an ongoing pandemic. Common symptoms include fever, cough, fatigue, shortness of breath, and loss of smell and taste. While the majority of cases result in mild symptoms, some progress to ARDS. The time from exposure to onset of symptoms is typically around five days, but may range from two to fourteen days.

30 Thus, in some examples, the subject has a SARS-CoV-2 infection. In one example, the subject has COVID-19. In particular, severe COVID-19 often results in ARDS. The methods of the present disclosure can be used to treat or prevent ARDS in a subject suffering from COVID-19.

Complications associated with COVID-19 also include sepsis or pneumonia.
35 Complications including sepsis or pneumonia are therefore also contemplated for

treatment by the RNA (e.g., self-replicating RNA, compositions or vaccines of the disclosure.

Complications associated with COVID-19 also include sepsis or pneumonia. Complications including sepsis or pneumonia are therefore also contemplated for
5 treatment by the RNA (e.g., self-replicating RNA, compositions or vaccines of the disclosure.

In an example, the methods described herein comprise the identification of a subject having or suspected of having SARS-CoV-2. In this example, the subject may have one or more of the above symptoms and may be classified as having SARS-CoV-
10 2.

Acute Respiratory Distress Syndrome (ARDS)

The present disclosure provides, for example, methods of treating or preventing ARDS in a subject.

15 ARDS is a life-threatening condition characterized by bilateral pulmonary infiltrates, severe hypoxemia, and disruption of the alveolar-capillary membrane barrier (i.e., pulmonary vascular leak), leading to non-cardiogenic pulmonary edema. There is currently no effective pharmacological therapy.

Infectious etiologies, including influenza and coronavirus infection, are leading
20 causes of ARDS. Accordingly, in one example of the present disclosure, the ARDS is associated with an influenza or a coronavirus infection. For example, the ARDS is associated with influenza. In another example, the ARDS is associated with a coronavirus infection, such as a SARS-COV-2 infection. In one example, the ARDS is associated with a SARS-CoV-2 infection.

25 ARDS is classified according to the Berlin Definition, which includes:

- (1) presentation within 1 week of clinical insult or onset of respiratory symptoms;
- (2) acute hypoxemic respiratory failure, as determined by a $\text{PaO}_2/\text{FiO}_2$ ratio of 300 mmHg or less on at least 5 cm of continuous positive airway pressure (CPAP) or positive end expiratory pressure (PEEP), where PaO_2 is the partial pressure of
30 oxygen in arterial blood and the FiO_2 is the fraction of inspired oxygen;
- (3) bilateral opacities on lung radiographs not fully explained by effusions, consolidation, or atelectasis; and
- (4) edema/respiratory failure not fully explained by cardiac failure or fluid overload.

In one example, the subject has or suffers from ARDS (i.e., the subject satisfies
35 the Berlin definition of ARDS). For example, the subject is in need of treatment (i.e., in need thereof).

In one example, the subject has or suffers from a symptom associated with ARDS. Symptoms associated with ARDS and methods of identifying subjects at risk of developing ARDS will be apparent to the skilled person and/or are described herein. For example, the subject has one or more or all of the following symptoms:

- 5 a) a respiratory frequency of greater than 30 breaths per minute;
- b) an oxygen saturation (SpO_2) of 93% or less on room air;
- c) a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) of less than 300 mmHg;
- d) a $\text{SpO}_2/\text{FiO}_2$ ratio of less than 218; and
- 10 e) radiographic lung infiltrates in an amount of greater than 50%.

Currently, ARDS is classified as mild, moderate or severe with an associated increased mortality. The severity of ARDS can be categorized according to the Berlin definition as follows:

- (i) Mild ARDS: $\text{PaO}_2/\text{FiO}_2$ of 200-300 mmHg on at least 5 cm CPAP or PEEP;
- 15 (ii) Moderate ARDS: $\text{PaO}_2/\text{FiO}_2$ of 100-200 mmHg on at least 5 cm PEEP; and
- (iii) Severe ARDS: $\text{PaO}_2/\text{FiO}_2$ of less than or equal to 100 mmHg on at least 5 cm PEEP.

In one example, the ARDS is mild ARDS. In another example, the ARDS is moderate ARDS. In a further example, the ARDS is severe ARDS.

- 20 In an example, the methods described herein comprise the identification of a subject having or suspected of having ARDS. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe ARDS.

- The methods of the present disclosure can, in addition to treatment of existing ARDS, be used to prevent the onset of ARDS. Thus, in one example, the subject does
25 not have ARDS.

Respiratory Syncytial Virus (RSV)

- The present disclosure provides, for example, methods of treating or preventing or delaying progress of RSV. RSV is an enveloped non-segmented negative-strand RNA
30 virus in the family Paramyxoviridae, genus *Pneumovirus*. To infect a host cell, paramyxoviruses such as RSV, like other enveloped viruses such as influenza virus, require fusion of the viral membrane with a host cell's membrane.

- In one example, the subject has or suffers from a symptom associated with RSV. Symptoms associated with RSV and methods of identifying subjects at risk of developing
35 RSV will be apparent to the skilled person and/or are described herein. For example, the subject has one or more or all of the following symptoms indicative of mild RSV:

- a) congested or runny nose;
b) dry cough;
c) low-grade fever;
d) sore throat;
5 e) sneezing;
f) headache;
- or in severe cases:
- a) short, shallow and rapid breathing;
b) struggling to breathe i.e., chest muscles and skin pull inward with each breath;
10 c) cough;
d) poor feeding;
e) unusual tiredness (lethargy);
f) irritability.
- Thus, in one example, the RSV is mild RSV. In a further example, the RSV is
15 severe RSV.
- In an example, the methods described herein comprise the identification of a subject having or suspected of having RSV. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe RSV.
- The methods of the present disclosure can, in addition to treatment of existing
20 RSV, be used to prevent the onset of RSV. Thus, in one example, the subject does not have RSV.
- In another example, there is provided a method of treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant comprising administering an immunogenic composition, pharmaceutical composition or vaccine disclosed herein to
25 the infant.
- An infant having or suspected of having RSV may display one or more or all of the following symptoms indicative of RSV:
- a) irritability;
b) decreased activity;
30 c) decreased appetite;
d) apnea;
e) fever;
f) runny nose;
g) cough, which progressed to wheezing or difficulty breathing.

In an example, the methods described herein comprise the identification of an infant having or suspected of having RSV. In this example, the infant may have one or more of the above symptoms.

5 Kits

An example of the disclosure provides kits containing a self-replicating RNA of the present disclosure useful for the treatment or prevention of a disease or disorder as described above. Another example of the disclosure provides kits containing a cRNA of the present disclosure useful for the treatment or prevention of a disease or disorder as described above. Another example of the disclosure provides kits containing a RNA of the present disclosure useful for the treatment or prevention of a disease or disorder as described above. Another example of the disclosure provides kits containing a polynucleotide of the present disclosure useful for the treatment or prevention of a disease or disorder as described above.

In one example, the kit comprises (a) a container comprising a self-replicating RNA optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent; and (b) a package insert with instructions for treating or preventing a disease or disorder (e.g., a SARS-CoV-2 infection, COVID-19, RSV, influenza, ARDS) in a subject.

In one example, the kit comprises (a) a container comprising a RNA optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent; and (b) a package insert with instructions for treating or preventing a disease or disorder (e.g., a SARS-CoV-2 infection, COVID-19, RSV, influenza, ARDS) in a subject.

In accordance with this example of the disclosure, the package insert is on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for a disease or disorder of the disclosure and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the polynucleotide, RNA, self-replicating RNA and/or the cRNA. The label or package insert indicates that the composition is used for treating a subject eligible for treatment, e.g., one having or predisposed to developing a SARS-CoV-2 infection, COVID-19, RSV, influenza, ARDS, or sepsis or pneumonia associated with COVID-19, with specific guidance regarding dosing amounts and intervals of treatment and any other medicament being provided. The kit may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection

(BWF), phosphate-buffered saline, Ringer's solution, and/or dextrose solution. The kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

5 The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: Generation of the self-replicating RNA

10 DNA templates encoding a self-replicating RNAs can be produced in competent *Escherichia coli* cells transformed with a DNA plasmid. Individual bacterial colonies can be isolated and the resultant plasmid DNA amplified in *E. coli* cultures. Following fermentation, the plasmid DNA can be isolated using Maxiprep DNA kit and linearized by restriction digest. Restriction enzymes can then be removed using phenol/chloroform extraction and ethanol precipitation.

15 mRNA can be made by *in vitro* transcription from the linearized DNA template using a T7 RNA polymerase. Subsequently, the DNA template can be removed by DNase digestion. Enzymatic capping can be performed with Cap0 to provide functional mRNA. The resultant mRNA can then be purified and resuspended in nuclease-free water.

20

Example 2: *In vitro* characterisation of the self-replicating RNA

 The self-replicating RNAs produced in Example 1 can then be assessed for expression of the genes of interest that are expressed in the form of an antigen.

25 Two-fold serial dilutions of unformulated (naked) or LNP-formulated self-amplifying mRNA constructs can be either electroporated or transfected into a Baby Hamster Kidney (BHK) cell line. After about 17-19 hrs, cells can be harvested and stained for antigen expression using anti-antigen antibodies. The number of cells positive for antigen expression and the mean fluorescence intensities (MFIs) can be measured by FACS. Data are analysed to calculate the specific potency values (the probability of successful transfection per unit of mass of RNA) and the MFI generated.

30 *In vitro* activity and potency of unformulated RNA and LNPs can be determined by FACS based on antigen co-expression and expressed in read-outs such as FACS potency, encapsulation efficiency, SAM recovery, size, PDI, Zeta potential, conductivity, concentration and endotoxin levels.

35

Antibody responses

To assess antibody responses, serum can be collected from immunized mice and tested by microneutralization assays and e.g., hemagglutination inhibition assays.

For all serological assays sera can be treated in the same way, with *Vibrio cholerae* neuraminidase, also known as receptor-destroying enzyme (RDE) (Denka Seiken Co. Ltd., Tokyo, Japan) and diluted to a starting dilution of 1:10 with PBS. Sheep serum to H5N1 virus (FDA/CBER Kensington lot nu. H5-Ag-1115) can be used as positive control sera for influenza antigen, for example.

Microneutralization assays

Microneutralization assays, short and long form are performed in a qualified mammalian cell line (proprietary 33016-PF Madin-Darby Canine Kidney (MDCK)).

Microneutralization assay short form (MN Assay SF)

Virus fluorescent focus-based microneutralization (FFA MN) assay can be performed using an in house developed protocol. RDE treated test mouse samples and positive control sera can be heat inactivated, diluted to a starting dilution of 1:40 with PBS, and fourfold serial diluted using the U-Bottom 96 well plate (BD Falcon) in neutralization medium (comprised of minimum essential medium D-MEM (GIBCO), supplemented with 1% BSA (Rockland, BSA-30), 100 U/mL penicillin and 100 ug/mL streptomycin (GIBCO)). Virus can be diluted to ~ 1,000 – 1,500 fluorescent focus-forming units (FFU)/well (20,000 – 30,000 FFU/mL) in neutralization medium and added in a 1:1 ratio to diluted serum.

After incubation for 2 h at 37°C, 5% CO₂, plates (Half Area 96 well plate, Corning) containing MDCK 33016-PF cells can be inoculated with this mixture and incubated overnight for 16 – 18 h at 37°C with 5% CO₂. MDCK 33016-PF cells are seeded as 3.0E4/well (3.0E6/plate) at 6-8h earlier in the cell growth medium (comprised of D-MEM, supplemented with 10% HyClone fetal bovine serum - FBS (Gibco), 100 U/mL penicillin and 100 ug/mL streptomycin). Following the overnight incubation and prior to immunostaining, cells can then be fixed with cold mixture of acetone and methanol.

The virus can then be visualized using separate 1 h incubations at room temperature of monoclonal antibodies specific to the virus proteins of interest and Alexa Fluor 488 Goat Anti-Mouse IgG (H+L) Ab (Invitrogen cat. no. A11001) diluted in PBS buffer containing 0.05% tween-20 (Sigma) and 2% BSA (Fraction V, Calbiochem, 2960, 1194C175). Viral protein can be quantified by a CTL Immunospot analyzer (Cellular Technology Limited, Shaker Heights, Cleveland, OH), using a fluorescein isothiocyanate

(FITC) fluorescence filter set with excitation and emission wavelengths of 482 and 536 nm. Fluorescent foci can be enumerated by use of software Immunospot 7.0.12.1 professional analyzer DC, using a custom analysis module.

5 Microneutralization assay long form (MN Assay LF)

MN assay LF is performed using an in house developed protocol. RDE treated test mouse samples and positive control sera are heat inactivated, diluted to a starting dilution of 1:40 with PBS, and twofold serial diluted using the U-Bottom 96 well plate (BD Falcon) in neutralization medium (comprised of the 30% spent growth media (Irvine Scientific) and 70% infective media (protein free media – 33016 MDCK PFM; GIBCO) 10 supplemented with 100 U/mL penicillin, 100 ug/mL streptomycin (GIBCO), and 0.33 ug/mL TPCK-trypsin (TPCK treated, Tosyl phenylalanyl chloromethyl ketone, Sigma). A virus of interest is diluted to 100TCID (tissue culture infectious dose) per well in neutralization medium and added in a 1:1 ratio to diluted serum. Serially pre-diluted 15 serum samples are incubated with the virus and allowed to react for 1h at 37°C, 5% CO₂. In the inoculation step, plates (Cell Culture 96-well plate, Costar) containing MDCK 33016-PF cells are seeded at 3.0^{E4}/well (3.0^{E6}/plate) a day before in the antibiotic free cell growth medium (Irvine Scientific) and washed with sterile PBS, then infected with this mixture and incubated for 1h at 37°C with 5% CO₂. Infection is stopped by aspiration 20 of antibody/virus mixture and cells washed with sterile PBS, inoculated with neutralizing media (100ul/well) containing twofold serially diluted antibodies and then incubated for 5 days at 37°C with 5% CO₂. In the final “read-out” step, detection of virus is performed by quantification of the virus using 0.5% turkey red blood cells (Lampire Biological Laboratories). The absence of infectivity constitutes a positive neutralization reaction 25 and indicates the presence of virus-specific antibodies in the serum sample.

NUMBERED STATEMENTS OF THE DISCLOSURE

The present disclosure provides at least the following numbered statements:

- 30 1. A RNA comprising:
- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
 - (b) a nucleotide sequence encoding a second antigen from influenza, and
 - (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial 35 virus (RSV),
- wherein each nucleotide sequence is operably linked to a regulatory element.

2. The RNA of statement 1, wherein the the RNA comprises, in 5' to 3' order:
 - (a) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
 - 5 (b) the nucleotide sequence encoding an antigen from influenza, and
 - (c) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV).
3. The RNA of statement 1, wherein the RNA comprises in 5' to 3' order:
 - 10 (a) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV);
 - (b) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and
 - (c) the nucleotide sequence encoding an antigen from influenza.
- 15 4. The RNA of statement 1, wherein the RNA comprises in 5' to 3' order:
 - (a) the nucleotide sequence encoding an antigen from influenza;
 - (b) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV); and
 - 20 (c) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
5. The RNA of any one of statements 1 to 4, wherein the regulatory element is selected from the group consisting of a promoter, a Kozak consensus sequence and an
25 IRES.
6. The RNA of statement 1, wherein the RNA comprises:
 - (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;
 - 30 (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and
 - (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
- 35 7. The RNA of statement 6, wherein the RNA comprises, in 5' to 3' order:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;
- (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and
- 5 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
8. The RNA of statement 6, wherein the RNA comprises, in 5' to 3' order:
- (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus
- 10 (RSV), operably linked to a SG promoter;
- (b) a nucleotide sequence encoding a second antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and
- (c) a nucleotide sequence encoding a third antigen from influenza, operably linked
- 15 to an IRES or a SG promoter.
9. The RNA of statement 6, wherein the RNA comprises, in 5' to 3' order:
- (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;
- 20 (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and
- (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.
- 25 10. The RNA of any one of statements 1 to 9, wherein the RNA is a monocistronic RNA.
11. The RNA of any one of statements 1 to 9, wherein the RNA is a polycistronic RNA.
- 30 12. The RNA of statement 5, wherein the promoter is a subgenomic (SG) promoter.
13. The RNA of statement 12, wherein the SG promoter is a minimal SG promoter or an extended SG promoter.
- 35

14. The RNA of statement 13, wherein the extended SG promoter is extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein of an RNA virus.
- 5 15. The RNA of statement 13, wherein the minimal SG promoter is encoded by a sequence set forth in SEQ ID NO: 1.
16. The RNA of statement 13, wherein the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 5.
- 10 17. The RNA of any one of statements 5 to 9, wherein the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV),
- 15 Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF- κ B-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a
- 20 (FGF1A), GTX, or a combination thereof.
18. The RNA of statement 17, wherein the EMCV IRES is a wild-type IRES encoded by a sequence set forth in SEQ ID NO: 4.
- 25 19. The RNA of any one of statements 1 to 18, wherein the antigens are expressed at substantially the same level.
20. The RNA of any one of statements 1 to 19, wherein the antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a nucleocapsid (N) or spike
- 30 (S) protein, optionally encoded by the sequence set forth in SEQ ID NO:21 or 22.
21. The RNA of any one of statements 1 to 20, wherein the antigen from influenza is from influenza A, influenza B or influenza C, optionally encoded by the sequence set forth in SEQ ID NO:19 or 20.

22. The RNA of any one of statements 1 to 21, wherein the antigen from a RSV is a RSV surface glycoprotein selected from a Fusion (F), Pre Fusion (Pre F), Glycoprotein (G), Small Hydrophobic protein (SH), the matrix proteins M and M2, the nucleocapsid proteins N, P and L, and the nonstructural proteins NS1 and NS2, optionally encoded by
5 the sequence set forth in SEQ ID NO:17 or 18.
23. The RNA of any one of statements 1 to 22, wherein the RNA is a self-replicating RNA or a cRNA.
- 10 24. The RNA of statement 23, wherein the self-replicating RNA is from an alphavirus.
25. The RNA of statement 24, wherein the alphavirus is selected from the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan equine
15 encephalitis virus (VEE) and combinations thereof.
26. An immunogenic composition comprising the RNA of any one of statements 1 to 25.
- 20 27. The immunogenic composition of statement 26, wherein the RNA is self-replicating RNA and the composition comprises a plurality of self-replicating RNAs, wherein each self-replicating RNA encodes different polypeptide antigen sequences.
28. The immunogenic composition of statement 27, wherein the RNA is self-replicating RNA and the composition comprises a plurality of self-replicating RNAs,
25 wherein each self-replicating RNA encodes the same polypeptide antigen sequences.
29. A pharmaceutical composition comprising an immunogenic composition of any one of statements 26 to 28 and a pharmaceutically acceptable carrier.
30
30. The pharmaceutical composition of statement 29, further comprising a lipid nanoparticle (LNP), a polymeric microparticle or an oil-in-water emulsion.
31. The pharmaceutical composition of statement 30, wherein the RNA is
35 encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle or an oil-in-water emulsion.

32. The pharmaceutical composition of any one of statements 29 to 31, wherein each RNA is formulated together in the LNP.
- 5 33. The pharmaceutical composition of any one of statements 29 to 31, wherein each RNA is formulated separately in the LNP.
34. The immunogenic composition of any one of statements 26 to 28 or the pharmaceutical composition of any one of statements 29 to 33, for use as a vaccine.
- 10 35. A vaccine comprising the immunogenic composition of any one of statements 26 to 28 or the pharmaceutical composition of any one of statements 29 to 33.
36. A polynucleotide encoding the RNA of any one of statements 1 to 25.
- 15 37. The polynucleotide of statement 36, wherein the polynucleotide is a recombinant DNA.
38. The polynucleotide of statement 37, wherein the recombinant DNA is a plasmid.
- 20 39. A polynucleotide comprising:
a) a nucleotide sequence encoding a first antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
b) a nucleotide sequence encoding a second antigen from influenza, operably
25 linked to an IRES or a SG promoter, and
c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
40. The polynucleotide of statement 39, wherein the polynucleotide comprises, in order
30 from 5' to 3':
a) the nucleotide sequence encoding a first antigen from a SARS-CoV-2; and
b) the nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter; and
c) the nucleotide sequence encoding a third antigen from a RSV, operably
35 linked to an IRES or a SG promoter.

41. The polynucleotide of statement 39, wherein the polynucleotide comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a third antigen from a RSV; and
 - b) the nucleotide sequence encoding a first antigen from a SARS-CoV-2,
- 5 operably linked to an IRES or a SG promoter; and
- c) the nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter.
42. The polynucleotide of statement 39, wherein the polynucleotide comprises, in order
- 10 from 5' to 3':
- a) the nucleotide sequence encoding a second antigen from influenza; and
 - b) the nucleotide sequence encoding a third antigen from a RSV, operably linked to an IRES or a SG promoter; and
 - c) the nucleotide sequence encoding a first antigen from a SARS-CoV-2,
- 15 operably linked to an IRES or a SG promoter.
43. A conventional mRNA (cRNA) comprising:
- a) a nucleotide sequence encoding a first antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 20 b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and
- c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
- 25 44. The cRNA of statement 43, wherein the cRNA comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a first antigen from a SARS-CoV-2; and
 - b) the nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter; and
 - c) the nucleotide sequence encoding a third antigen from RSV, operably
- 30 linked to an IRES or a SG promoter.
45. The cRNA of statement 43, wherein the cRNA comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a third antigen from RSV; and
 - b) the nucleotide sequence encoding a first antigen from a SARS-CoV-2
- 35 operably linked to an IRES or a SG promoter; and

- c) the nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter.
46. The cRNA of statement 43, wherein the cRNA comprises, in order from 5' to 3':
- 5 a) the nucleotide sequence encoding a second antigen from influenza; and
- b) the nucleotide sequence encoding a third antigen from RSV operably linked to an IRES or a SG promoter; and
- c) the nucleotide sequence encoding a first antigen from a SARS-CoV-2, operably linked to an IRES or a SG promoter.
- 10
47. The polynucleotide of any one of statements 39 to 42, or the cRNA of any one of statements 43 to 56, wherein the nucleotide sequence of (a) is operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof.
- 15
48. A method of treating or preventing or delaying progression of a disease or condition in a subject in need thereof, the method comprising administering the RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33, or the vaccine
- 20 of statement 35 to the subject.
49. Use of the RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28 the pharmaceutical composition of any one of statements 29 to 33, or the vaccine of statement 35 in the manufacture of a medicament for treating
- 25 or preventing or delaying progression of a disease or condition in a subject in need thereof.
50. The RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29
- 30 to 33, or the vaccine of statement 35 for use in treating or preventing or delaying progression of a disease or condition in a subject in need thereof.
51. The method of statement 48, the use of statement 49 or the RNA, the pharmaceutical composition, the immunogenic composition or the vaccine for use of
- 35 statement 50, wherein the disease or condition is selected from the group consisting of influenza, RSV, a SARS-CoV-2 infection and/or COVID-19.

52. A method of inducing an immune response in a subject, the method comprising administering the RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28, the pharmaceutical composition of any one of statements
5 29 to 33, or the vaccine of statement 35 to the subject in need thereof.

53. Use of the RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33, or the vaccine of statement 35 in the manufacture of a medicament for inducing
10 an immune response in a subject in need thereof.

54. The RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33, or the vaccine of statement 35 for use in inducing an immune response in a subject
15 in need thereof.

55. The method of statement 52, the use of statement 53, or the pharmaceutical composition, immunogenic composition or vaccine for use of statement 54, wherein the immune response is a humoral and/or a cell-mediated immune response.
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56. A method for reducing viral load in a subject comprising administering the RNA of any one of statements 1 to 25, the immunogenic composition of any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33 or the vaccine of statement 35 to the subject in need thereof.
25

57. Use of the RNA of any one of statements 1 to 25, the immunogenic composition of any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33 or the vaccine of statement 35 in the preparation of a medicament for reducing viral load in a subject in need thereof.
30

58. The RNA of any one of statements 1 to 25, the immunogenic composition of any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33 or the vaccine of statement 35 for use in reducing viral load in a subject in need thereof.
35

59. The method of any one of statements 48, 51, 52, 55 or 56, the use of any one of statements 49, 51, 53, 55 or 57, or the pharmaceutical composition, immunogenic composition or vaccine for use of any one of statements 50, 51, 54, 55 or 58, wherein the subject is a human of 18 years of age or older.

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60. A method of treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant comprising administering the RNA of any one of statements 1 to 25, the immunogenic composition of any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33 or the vaccine of statement 35, to the infant.

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61. Use of the RNA of any one of statements 1 to 25, the immunogenic composition of any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33 or the vaccine of statement 35 in the preparation of a medicament for treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant.

15

62. The RNA of any one of statements 1 to 25, the immunogenic composition of any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33 or the vaccine of statement 35 for use in treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant.

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63. The method of statement 60, the use of statement 61 or the immunogenic composition, pharmaceutical composition or vaccine for use of statement 62, wherein the infant is between about 1 month and 12 months of age.

25

64. The method of any one of statements 48, 51, 52, 55, 56, 59 or 60, the use of any one of statements 49, 51, 53, 55, 57, 59 or 61 or the pharmaceutical composition, immunogenic composition or vaccine for use of any one of statements 50, 51, 54, 55, 58, 59 or 62, wherein the vaccine or composition is administered in a one dose regimen.

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65. The method of any one of statements 48, 51, 52, 55, 56, 59 or 60, the use of any one of statements 49, 51, 53, 55, 57, 59 or 61 or the pharmaceutical composition, immunogenic composition or vaccine for use of any one of statements 50, 51, 54, 55, 58, 59 or 62, wherein the composition or vaccine is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.

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66. A kit comprising:
- (a) the RNA of any one of statements 1 to 25, the immunogenic composition any one of statements 26 to 28, the pharmaceutical composition of any one of statements 29 to 33, or the vaccine of statement 35;
 - (b) instructions for use thereof; and optionally
 - (c) a pharmaceutically acceptable carrier, excipient or diluent.

CLAIMS

1. A RNA comprising:
 - (a) a nucleotide sequence encoding an antigen from a severe acute respiratory
 - 5 syndrome coronavirus 2 (SARS-CoV-2);
 - (b) a nucleotide sequence encoding a second antigen from influenza, and
 - (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV),wherein each nucleotide sequence is operably linked to a regulatory element.
- 10 2. The RNA of claim 1, wherein the the RNA comprises, in 5' to 3' order:
 - (a) the nucleotide sequence encoding an antigen from a severe acute respiratory
 - sndrome coronavirus 2 (SARS-CoV-2);
 - (b) the nucleotide sequence encoding an antigen from influenza, and
 - 15 (c) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV).
3. The RNA of claim 1, wherein the RNA comprises in 5' to 3' order:
 - (a) the nucleotide sequence encoding an antigen from a respiratory syncytial virus
 - 20 (RSV);
 - (b) the nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and
 - (c) the nucleotide sequence encoding an antigen from influenza.
- 25 4. The RNA of claim 1, wherein the RNA comprises in 5' to 3' order:
 - (a) the nucleotide sequence encoding an antigen from influenza;
 - (b) the nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV); and
 - (c) the nucleotide sequence encoding an antigen from a severe acute respiratory
 - 30 syndrome coronavirus 2 (SARS-CoV-2).
5. The RNA of claim 1, wherein the regulatory element is selected from the group consisting of a promoter, a Kozak consensus sequence and an IRES.
- 35 6. The RNA of claim 1, wherein the RNA comprises:

- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;
- (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and
- 5 (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
7. The RNA of claim 6, wherein the RNA comprises, in 5' to 3' order:
- (a) a nucleotide sequence encoding an antigen from a severe acute respiratory
- 10 syndrome coronavirus 2 (SARS-CoV-2), operably linked to a SG promoter;
- (b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and
- (c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
- 15
8. The RNA of claim 6, wherein the RNA comprises, in 5' to 3' order:
- (a) a nucleotide sequence encoding an antigen from a respiratory syncytial virus (RSV), operably linked to a SG promoter;
- (b) a nucleotide sequence encoding a second antigen from a severe acute
- 20 respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter, and
- (c) a nucleotide sequence encoding a third antigen from influenza, operably linked to an IRES or a SG promoter.
- 25 9. The RNA of claim 6, wherein the RNA comprises, in 5' to 3' order:
- (a) a nucleotide sequence encoding an antigen from influenza, operably linked to a SG promoter;
- (b) a nucleotide sequence encoding a second antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter, and
- 30 (c) a nucleotide sequence encoding a third antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to an IRES or a SG promoter.
10. The RNA of claim 1, wherein the RNA is a monocistronic RNA.
- 35 11. The RNA of claim 1, wherein the RNA is a polycistronic RNA.

12. The RNA of claim 5, wherein the promoter is a subgenomic (SG) promoter.
13. The RNA of claim 12, wherein the SG promoter is a minimal SG promoter or an extended SG promoter.
- 5 14. The RNA of claim 13, wherein the extended SG promoter is extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein of an RNA virus.
- 10 15. The RNA of claim 13, wherein the minimal SG promoter is encoded by a sequence set forth in SEQ ID NO: 1.
16. The RNA of claim 13, wherein the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 5.
- 15 17. The RNA of claim 5, wherein the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF-κB-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a (FGF1A), GTX, or a combination thereof.
- 20 25 18. The RNA of claim 17, wherein the EMCV IRES is a wild-type IRES encoded by a sequence set forth in SEQ ID NO: 4.
19. The RNA of claim 1, wherein the antigens are expressed at substantially the same level.
- 30 20. The RNA of claim 1, wherein the antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a nucleocapsid (N) or spike (S) protein, optionally encoded by the sequence set forth in SEQ ID NO:21 or 22.
- 35

21. The RNA of claim 1, wherein the antigen from influenza is from influenza A, influenza B or influenza C, optionally encoded by the sequence set forth in SEQ ID NO:19 or 20.
- 5 22. The RNA of claim 1, wherein the antigen from a RSV is a RSV surface glycoprotein selected from a Fusion (F), Pre Fusion (Pre F), Glycoprotein (G), Small Hydrophobic protein (SH), the matrix proteins M and M2, the nucleocapsid proteins N, P and L, and the nonstructural proteins NS1 and NS2, optionally encoded by the sequence set forth in SEQ ID NO:17 or 18.
- 10 23. The RNA of claim 1, wherein the RNA is a self-replicating RNA or a cRNA.
24. The RNA of claim 23, wherein the self-replicating RNA is from an alphavirus.
- 15 25. The RNA of claim 24, wherein the alphavirus is selected from the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan equine encephalitis virus (VEE) and combinations thereof.
26. An immunogenic composition comprising the RNA of claim 1.
- 20 27. The immunogenic composition of claim 26, wherein the RNA is self-replicating RNA and the composition comprises a plurality of self-replicating RNAs, wherein each self-replicating RNA encodes different polypeptide antigen sequences.
- 25 28. The immunogenic composition of claim 27, wherein the RNA is self-replicating RNA and the composition comprises a plurality of self-replicating RNAs, wherein each self-replicating RNA encodes the same polypeptide antigen sequences.
29. A pharmaceutical composition comprising an immunogenic composition of claim
- 30 26 and a pharmaceutically acceptable carrier.
30. The pharmaceutical composition of claim 29, further comprising a lipid nanoparticle (LNP), a polymeric microparticle or an oil-in-water emulsion.
- 35 31. The pharmaceutical composition of claim 30, wherein the RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle or an oil-in-water emulsion.

32. The pharmaceutical composition of claim 29, wherein each RNA is formulated together in the LNP.
- 5 33. The pharmaceutical composition of claims 29, wherein each RNA is formulated separately in the LNP.
34. The immunogenic composition of claim 26 or the pharmaceutical composition of claim 29, for use as a vaccine.
- 10 35. A vaccine comprising the immunogenic composition of claim 26 or the pharmaceutical composition of claim 29.
36. A polynucleotide encoding the RNA of claim 1.
- 15 37. The polynucleotide of claim 36, wherein the polynucleotide is a recombinant DNA.
38. The polynucleotide of claim 37, wherein the recombinant DNA is a plasmid.
- 20 39. A polynucleotide comprising:
a) a nucleotide sequence encoding a first antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
b) a nucleotide sequence encoding a second antigen from influenza, operably
25 linked to an IRES or a SG promoter, and
c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
40. The polynucleotide of claim 39, wherein the polynucleotide comprises, in order
30 from 5' to 3':
a) the nucleotide sequence encoding a first antigen from a SARS-CoV-2; and
b) the nucleotide sequence encoding a second antigen from influenza, operably
linked to an IRES or a SG promoter; and
c) the nucleotide sequence encoding a third antigen from a RSV, operably
35 linked to an IRES or a SG promoter.

41. The polynucleotide of claim 39, wherein the polynucleotide comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a third antigen from a RSV; and
 - b) the nucleotide sequence encoding a first antigen from a SARS-CoV-2, operably linked to an IRES or a SG promoter; and
 - c) the nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter.
42. The polynucleotide of claim 39, wherein the polynucleotide comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a second antigen from influenza; and
 - b) the nucleotide sequence encoding a third antigen from a RSV, operably linked to an IRES or a SG promoter; and
 - c) the nucleotide sequence encoding a first antigen from a SARS-CoV-2, operably linked to an IRES or a SG promoter.
43. A conventional mRNA (cRNA) comprising:
- a) a nucleotide sequence encoding a first antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
 - b) a nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter, and
 - c) a nucleotide sequence encoding a third antigen from a respiratory syncytial virus (RSV), operably linked to an IRES or a SG promoter.
44. The cRNA of claim 43, wherein the cRNA comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a first antigen from a SARS-CoV-2; and
 - b) the nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter; and
 - c) the nucleotide sequence encoding a third antigen from RSV, operably linked to an IRES or a SG promoter.
45. The cRNA of claim 43, wherein the cRNA comprises, in order from 5' to 3':
- a) the nucleotide sequence encoding a third antigen from RSV; and
 - b) the nucleotide sequence encoding a first antigen from a SARS-CoV-2 operably linked to an IRES or a SG promoter; and

c) the nucleotide sequence encoding a second antigen from influenza, operably linked to an IRES or a SG promoter.

46. The cRNA of claim 43, wherein the cRNA comprises, in order from 5' to 3':

- 5 a) the nucleotide sequence encoding a second antigen from influenza; and
 b) the nucleotide sequence encoding a third antigen from RSV operably linked to an IRES or a SG promoter; and
 c) the nucleotide sequence encoding a first antigen from a SARS-CoV-2, operably linked to an IRES or a SG promoter.

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47. The polynucleotide of claim 39, or the cRNA of claim 43, wherein the nucleotide sequence of (a) is operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof.

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48. A method of treating or preventing or delaying progression of a disease or condition in a subject in need thereof, the method comprising administering the RNA of claim 1 to the subject.

20 49. Use of the RNA of claim 1 in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof.

50. The RNA of claim 1 for use in treating or preventing or delaying progression of a disease or condition in a subject in need thereof.

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51. The method of claim 48, the use of claim 49 or the RNA, the pharmaceutical composition, the immunogenic composition or the vaccine for use of claim 50, wherein the disease or condition is selected from the group consisting of influenza, RSV, a SARS-CoV-2 infection and/or COVID-19.

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52. A method of inducing an immune response in a subject, the method comprising administering the RNA of claim 1 to the subject in need thereof.

53. Use of the RNA of claims 1 in the manufacture of a medicament for inducing an
35 immune response in a subject in need thereof.

54. The RNA of claim 1 for use in inducing an immune response in a subject in need thereof.

55. The method of claim 52, the use of claim 53, or the pharmaceutical composition,
5 immunogenic composition or vaccine for use of claim 54, wherein the immune response is a humoral and/or a cell-mediated immune response.

56. A method for reducing viral load in a subject comprising administering the RNA of claim 1 to the subject in need thereof.

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57. Use of the RNA of claim 1 in the preparation of a medicament for reducing viral load in a subject in need thereof.

58. The RNA of claim 1 for use in reducing viral load in a subject in need thereof.

15

59. The method of any one of claims 48, 51, 52, 55 or 56, the use of any one of claims 49, 51, 53, 55 or 57, or the pharmaceutical composition, immunogenic composition or vaccine for use of any one of claims 50, 51, 54, 55 or 58, wherein the subject is a human of 18 years of age or older.

20

60. A method of treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant comprising administering the RNA of claim 1 to the infant.

61. Use of the RNA of 1 in the preparation of a medicament for treating, preventing
25 or delaying progress of a respiratory syncytial virus (RSV) in an infant.

62. The RNA of claim 1 for use in treating, preventing or delaying progress of a respiratory syncytial virus (RSV) in an infant.

30 63. The method of claim 60, the use of claim 61 or the immunogenic composition, pharmaceutical composition or vaccine for use of claim 62, wherein the infant is between about 1 month and 12 months of age.

64. The method of any one of claims 48, 51, 52, 55, 56, 59 or 60, the use of any one
35 of claims 49, 51, 53, 55, 57, 59 or 61 or the pharmaceutical composition, immunogenic

composition or vaccine for use of any one of claims 50, 51, 54, 55, 58, 59 or 62, wherein the vaccine or composition is administered in a one dose regimen.

65. The method of any one of claims 48, 51, 52, 55, 56, 59 or 60, the use of any one
5 of claims 49, 51, 53, 55, 57, 59 or 61 or the pharmaceutical composition, immunogenic composition or vaccine for use of any one of claims 50, 51, 54, 55, 58, 59 or 62, wherein the composition or vaccine is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.

- 10 66. A kit comprising:
(a) the RNA of claim 1;
(b) instructions for use thereof; and optionally
(c) a pharmaceutically acceptable carrier, excipient or diluent.