

"Influenza vaccines"**RELATED APPLICATION DATA**

The present application claims priority from US Application No. 63/618,578 entitled "Influenza Vaccines" filed on 8 January 2024. The entire contents of that application are hereby incorporated by reference.

TECHNICAL FIELD

The present disclosure relates to vaccines and uses thereof. For example, the present disclosure relates to RNA vaccines, such as self-amplifying RNA vaccines for the treatment of diseases or conditions including influenza.

BACKGROUND

Influenza viruses are major human pathogens, causing a respiratory disease (commonly referred to as "influenza" or "the flu") that ranges in severity from sub-clinical infection to primary viral pneumonia, which can result in death. The clinical effects of infection vary with the virulence of the influenza strain and the exposure, history, age, and immune status of the host. Every year it is estimated that approximately 1 billion people worldwide undergo infection with influenza virus, leading to severe illness in 3-5 million cases and an estimated 300,000 to 500,000 of influenza related deaths.

Influenza viruses are enveloped RNA viruses that belong to the family of *Orthomyxoviridae*. Three broad types of influenza viruses are recognised, Type A, Type B and Type C, which are defined by the absence of serological cross-reactivity between their internal proteins. Influenza A viruses are widely distributed in nature and can infect a variety of birds and mammals. Their genomes consist of eight single-stranded RNA segments that code for 11 different proteins, one nucleoprotein (NP), three polymerase proteins (PA, PB1, and PB2), two matrix proteins (M1 and M2), three non-structural proteins (NS1, NS2, and PB1-F2), and two external glycoproteins: hemagglutinin (HA) and neuraminidase (NA). The viruses are classified on the basis of differences in antigenic structure of the HA and NA proteins, with their different combinations representing unique virus subtypes that are further classified into specific influenza virus strains.

The most efficient protection against influenza infection is vaccination against the circulating strain and it is important to produce influenza vaccines as quickly as possible. Influenza virus strains for use in vaccines change from season to season. For example, it is expected that the H5 subtype of influenza A virus may become prevalent in the near future. As the human population is immunologically naive to the new hemagglutinin subtype then this antigenic shift may cause a pandemic outbreak of influenza infections. The characteristics of an influenza strain that give it the potential to cause a pandemic

outbreak are: (a) it contains a new hemagglutinin compared to the HAs in currently-circulating human strains, i.e. one that has not been evident in the human population for over a decade, or has not previously been seen at all in the human population; (b) it is capable of being transmitted horizontally in the human population; and (c) it is pathogenic to humans.

The function of the HA protein in the virus is binding to the cell surface sialic acid and, after uptake in endosomes, mediating the fusion of viral and endosomal membranes leading to release of the viral RNA into the cell. An essential step in the fusion process is a large conformational change of the HA protein that rearranges the secondary structural elements of the protein so that the fusion peptide becomes exposed. Consequently, two conformations (pre- and post-fusion) of the HA protein exist that are very different in terms of their tertiary structure. Since the viral HA protein is primarily exposed to the immune system in the pre-fusion state, it is advantageous for the HA protein to be maintained in this conformation.

Hemagglutinin (HA) is the major glycoprotein encoded by the HA gene segment of an influenza virus and is integral to its infectivity. HA is a trimeric protein in which each monomer contains two polypeptide chains, HA1 and HA2, linked by a disulfide bond and anchored in the virus envelope by a C-terminus transmembrane domain. Each monomer is initially expressed as inactive HA0 which is subsequently cleaved by host proteases into HA1 and HA2 subunits which are linked via a disulfide bond to form a metastable pre-fusion state HA. This leads to internalization of the influenza virus by the cell into an endosome, which subsequently facilitates conformational rearrangement of the HA trimer. The HA protein then fuses with the endosomal membrane, thereby allowing for the release of viral gene segments, which are in the form of a ribonucleoprotein complex (RNP) together with nucleoproteins and a polymerase complex, into the cytoplasm of the host cell. The RNPs are transported into the host nucleus followed by transcription, and replication of the viral genome. The HA protein, together with the other newly generated viral proteins and a replicated genome, is then incorporated into the envelope of an influenza virion as it buds from an infected host cell. The HA protein on new viral particles remains attached to sialic acid groups of glycoproteins on the external cell surface and neuraminidase (NA) cleaves these groups and thereby allows for the efficient release of the newly formed virions.

An essential step in the fusion process is a large conformational change of the HA protein that rearranges the secondary structural elements of the protein so that the fusion peptide becomes exposed. Consequently, two conformations (pre- and post-fusion) of the HA protein exist that are very different in terms of their tertiary structure. Since the viral HA protein is primarily exposed to the immune system in the pre-fusion state, it is advantageous for the HA protein to be maintained in this conformation.

There is a need for the development of new vaccines that are immunologically efficacious and that target a subtype of influenza virus that has pandemic potential.

SUMMARY

In arriving at the present disclosure, the inventors considered there was a risk of influenza HA reassortment if a subject were to be immunized with a RNA vaccine encoding a HA from one influenza strain when infected with a HA from another strain. Since a cell could then contain RNA encoding HAs from two different strains and reassortment could occur. To address this problem, the inventors modified the HA in the RNA vaccine such that it could not mature and would thus prevent viral replication of any virus containing a nucleic acid encoding the modified HA. For example, the inventors modified an amino acid in a protease cleavage site in HA0 that is cleaved to produce HA1 and HA2. Such modification leads to a risk that the modified HA will no longer induce a protective immune response against an influenza expressing the wild type form of the modified HA since the HA contains a non-natural amino acid and is not cleaved to form HA1 and HA2, which are linked by disulfide bonds in mature HA but not linked by a peptide.

The present disclosure is also based on the inventors' identification increased stability of the pre-fusion conformation of the HA protein can be achieved by modifying specific amino acid residues within the HA proteolytic cleavage site and that this modified HA protein is suitable as a vaccine for the treatment or prevention of an influenza virus infection.

Accordingly, the present disclosure provides a RNA comprising a nucleotide sequence encoding a modified hemagglutinin (HA) protein from an influenza virus, wherein the modified HA protein comprises at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.

In one example, the HA protein is maintained in a HA0 form when expressed in a cell or in a subject.

In one example, the HA protein induces an immune response, e.g., a protective immune response against an influenza expressing the unmodified (wild type) form of the HA when administered to a subject.

In one example, the modified HA additionally comprises a deletion of an amino acid in a fusion peptide.

In one example, the HA cleavage site is a polybasic cleavage site.

For example, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, all the basic amino acids in the polybasic cleavage site except the most C-terminal R is/are deleted.

In another example, the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the sequence corresponding to the polybasic cleavage site after deletion of the basic amino acids consists of the sequence R-G-L (SEQ ID NO: 32).

In one example, the modified HA additionally has an amino acid in the fusion peptide deleted.

In one example, the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).

In a further example, the Q is deleted in the modified HA. For example, the Q in a fusion peptide at a position corresponding to residue 15 in the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33) is deleted.

In another example, the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from (R-)(R-)R-X₁-K/R-X₂-G-L (SEQ ID NO: 35), R-X₁-K/R-X₂-G-L (SEQ ID NO: 36), (R-)(R-)K-K/R-K/T-X₂-G-L (SEQ ID NO: 37) or K-K/R-K/T-X₂-G-L (SEQ ID NO: 38), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from R-X₁-X₂-X₂-G-L (SEQ ID NO: 39) or K-X₂-K/T-X₂-G-L (SEQ ID NO: 40) or K-K/R-X₂-X₂-G-L (SEQ ID NO: 41), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) and X₂ is any amino acid other than arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence K-X₂-X₂-X₂-G-L (SEQ ID NO: 42), wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44) or R-X-A-A-G-L (SEQ ID NO: 45) or K-A-K/T-A-G-L (SEQ ID NO: 46) or K-K/R-A-A-G-L (SEQ ID NO: 47) or K-A-A-A-G-L (SEQ ID NO: 48), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the HA cleavage site is a monobasic cleavage site. For example, the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue. In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence of X₁-X₂-G-L (SEQ ID NO: 50), wherein X₁ is any amino acid residue and wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In other examples, the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In further examples, the modified HA protein comprises the amino acid sequence of X-A-G-L (SEQ ID NO: 51), wherein X is any amino acid residue.

The present disclosure provides an RNA as described herein, wherein upon expression of the modified HA protein it is maintained in a pre-fusion stabilized conformation.

In one example, the modified HA is from a seasonal flu. For example, the HA cleavage site is a monobasic cleavage site.

In another example, the HA is from a pandemic flu. For example, the HA cleavage site is a polybasic cleavage site.

In one example, the modified HA is selected from a H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 subtype.

In one example, the modified HA is a H1 subtype.

In a one example, the modified HA is a H2 subtype.

In one example, the modified HA is a H5 subtype.

In one example, the RNA described herein comprises a further nucleotide sequence encoding a neuraminidase.

In some examples, the neuraminidase is selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes.

In one example, the neuraminidase is a N1 subtype. For example, the RNA encodes a H1 or H5 and a N1.

In one example, the neuraminidase is a N3 subtype. For example, the RNA encodes a H2 and a N3.

In other examples, the neuraminidase is from the same strain as the modified HA. In further examples, the neuraminidase is from a different strain as the modified HA.

Suitably, the influenza virus as described herein is an influenza A virus. In some examples, the influenza virus is of an avian or swine strain.

The present disclosure provides an RNA as described herein, wherein the RNA is operably linked to one or more regulatory sequences.

In one example, the RNA comprises:

- (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and
- (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES).

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

- (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a SG promoter; and
- (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an IRES.

In one example, the RNA is a monocistronic RNA.

In some examples, the RNA is a polycistronic RNA.

In further examples, the RNA is a bicistronic RNA.

In certain examples, the SG promoter is a minimal SG promoter or an extended SG promoter.

In one example, the extended promoter is a SGPv1 promoter, a SGPv2 promoter, a SGPv3 promoter or a SGPv4 promoter. For example, the SGPv1 promoter is comprises a sequence set forth in SEQ ID NO: 5. For example, the SGPv2 promoter comprises sequence set forth in SEQ ID NO: 6. For example, the SGPv3 promoter comprises a sequence set forth in SEQ ID NO: 7. For example, the SGPv4 promoter comprises a sequence set forth in SEQ ID NO:8.

In particular examples, the SG promoter is a SGPv2 promoter comprising a sequence set forth in SEQ ID NO: 6.

In one example, the IRES is derived 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.

Suitably, the EMCV IRES is a wild-type IRES encoded by a sequence set forth in SEQ ID NO: 9.

The present disclosure provides an RNA as described herein, wherein the RNA is a self-amplifying RNA.

Accordingly, the self-amplifying RNA comprises sequences from an alphavirus. For example, the sequence from the alphavirus comprises four non-structural proteins (NSP1, NSP2, NSP3 and NSP4) necessary for transcription and replication of viral RNA. 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.

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).

Suitably, 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).

The present disclosure provides an isolated polynucleotide encoding the RNA as described herein. In one example, the isolated polynucleotide is a recombinant DNA.

In another example, the recombinant DNA as described herein is a vector. For example, the vector is a Doggybone DNA. In other examples, the recombinant DNA is a plasmid.

In one example, the isolated polynucleotide encoding the RNA as described herein is operably linked to one or more regulatory sequences. For example, regulatory sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, polyadenylation sequences, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. In one example, the regulatory sequence is a promoter sequence. In another example, the regulatory sequence is an internal ribosome entry site (IRES).

In another example, the isolated polynucleotide comprises:

- (a) a first polynucleotide encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and
- (b) a second polynucleotide encoding the neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES).

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

- (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a SG promoter; and
- (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an IRES.

In a further example, the isolated polynucleotide comprises, consists of or consists essentially of a nucleotide sequence set forth in SEQ ID NO: 10 or SEQ ID NO: 11, or a fragment, variant or derivative thereof.

The present disclosure further provides a method of producing the RNA as described herein, the method comprising (i) providing a DNA template encoding the RNA as described herein, (ii) *in vitro* transcribing the RNA from the DNA template; and (iii) purifying the RNA from step (ii).

In one example, the method further comprises linearizing the DNA template prior to performing *in vitro* transcription.

In one example, the DNA template is a plasmid. In another example, the DNA template is a Doggybone DNA.

The present disclosure further provides a host cell comprising the RNA or the isolated polynucleotide as described herein.

The present disclosure further provides an immunogenic composition comprising the RNA as described herein. Suitably, the immunogenic composition elicits an immune response when administered to a subject.

In one example, the immunogenic composition comprises at least one further RNA encoding a modified HA protein comprising at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.

In one example, the modified HA additionally comprises a deletion of an amino acid in a fusion peptide.

In one example, the HA cleavage site is a polybasic cleavage site.

For example, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, all the basic amino acids in the polybasic cleavage site except the most C-terminal R is/are deleted.

In another example, the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the sequence corresponding to the polybasic cleavage site after deletion of the basic amino acids consists of the sequence R-G-L (SEQ ID NO: 32).

In one example, the modified HA additionally has an amino acid in the fusion peptide deleted.

In one example, the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).

In a further example, the Q is deleted in the modified HA. For example, the Q in a fusion peptide at a position corresponding to residue 15 in the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33) is deleted.

In another example, the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).

For example, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-R-G-L or K-K/R-K/T-R-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from (R-)(R-)R-X₁-K/R-X₂-G-L (SEQ ID NO: 35), R-X₁-K/R-X₂-G-L (SEQ ID NO: 36), (R-)(R-)K-K/R-K/T-X₂-G-L (SEQ ID NO: 37) or K-K/R-K/T-X₂-G-L (SEQ ID NO: 38), wherein X₁ is any basic amino acid

residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from R-X₁-X₂-X₂-G-L (SEQ ID NO: 39) or K-X₂-K/T-X₂-G-L (SEQ ID NO: 40) or K-K/R-X₂-X₂-G-L (SEQ ID NO: 41), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) and X₂ is any amino acid other than arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence K-X₂-X₂-X₂-G-L (SEQ ID NO: 42), wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44) or R-X-A-A-G-L (SEQ ID NO: 45) or K-A-K/T-A-G-L (SEQ ID NO: 46) or K-K/R-A-A-G-L (SEQ ID NO: 47) or K-A-A-A-G-L (SEQ ID NO: 48), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the HA cleavage site is a monobasic cleavage site. For example, the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue. In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence of X₁-X₂-G-L (SEQ ID NO: 50), wherein X₁ is any amino acid residue and wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In other examples, the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In further examples, the modified HA protein comprises the amino acid sequence of X-A-G-L (SEQ ID NO: 51), wherein X is any amino acid residue.

In another example, the immunogenic composition comprises at least one further RNA encoding a further HA from a further influenza virus, wherein the at least one further HA is a different subtype to the modified HA.

In a further example, the immunogenic composition comprises at least one further RNA encoding a neuraminidase selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes.

In one example, the neuraminidase is from the same strain as the modified HA. In another example, the neuraminidase is from a different strain as the modified HA.

In another example, the immunogenic composition as described herein is for use in treating or preventing a disease in a subject in need thereof.

Suitably, the disease is an influenza virus infection.

In one example, the RNA as described herein is contained in or is otherwise associated with a lipid-based carrier.

In one example, the lipid-based carrier is or comprises a lipid nanoparticle (LNP).

In a further example, the RNA is encapsulated in, bound to or adsorbed on the lipid nanoparticle.

The present disclosure provides a pharmaceutical composition comprising the RNA as described herein and a pharmaceutically acceptable carrier, diluent or excipient.

In one example, the RNA in the pharmaceutical composition is contained in or otherwise associated with a lipid-based carrier.

In one example, the lipid-based carrier is or comprises a LNP.

In a further example, the RNA is encapsulated in, bound to or adsorbed on the LNP.

In other examples, the immunogenic composition or the pharmaceutical composition as described herein is for use as a vaccine.

The present disclosure provides a vaccine comprising the RNA, the immunogenic composition or the pharmaceutical composition as described herein.

In one example, the vaccine elicits a protective immune response when administered to a subject. In another example, the vaccine elicits a therapeutic immune response for treating a disease, disorder and/or condition. In some examples, the disease, disorder and/or condition is an infectious disease. In a further example, the disease, disorder and/or condition is an influenza virus infection.

In one example, the vaccine is a monovalent vaccine.

In other examples, the vaccine is a multivalent vaccine.

In certain examples, the multivalent vaccine is a bivalent, trivalent or quadrivalent vaccine.

In another example, the vaccine comprises at least one further RNA comprising a nucleotide sequence encoding at least one further influenza antigen.

For example, the vaccine comprises at least two further RNA comprising a nucleotides sequence encoding at least two further influenza antigens.

Suitably, the vaccine comprises at least three further RNA comprising a nucleotides sequence encoding at least three further influenza antigens.

In other examples, the at least one further influenza antigen is derived from an influenza A virus.

In further examples, the at least one further influenza antigen is derived from an influenza B virus.

In another example, the vaccine comprises two influenza A virus strains and one influenza virus B strain. For example, the B strain is from a Victoria lineage.

In some examples, the vaccine comprises two influenza A virus strains and two influenza virus B strains. For example, the B strains are from a Victoria lineage and a Yamagata lineage.

In certain examples, the vaccine as described herein comprises at least one further RNA encoding a HA wherein the HA subtype is selected from H1, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16 subtypes. In one example, the HA is a H1 subtype. In one example, the HA is a H5 subtype.

In some examples, the vaccine as described herein comprises at least one further RNA encoding a modified HA protein comprising at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.

In one example, the modified HA additionally comprises a deletion of an amino acid in a fusion peptide.

In one example, the HA is from a pandemic flu.

In another example, the HA cleavage site is a polybasic cleavage site.

For example, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, all the basic amino acids in the polybasic cleavage site except the most C-terminal R is/are deleted.

In another example, the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the sequence corresponding to the polybasic cleavage site after deletion of the basic amino acids consists of the sequence R-G-L (SEQ ID NO: 32).

In one example, the modified HA additionally has an amino acid in the fusion peptide deleted.

In one example, the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).

In a further example, the Q is deleted in the modified HA. For example, the Q in a fusion peptide at a position corresponding to residue 15 in the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33) is deleted.

In another example, the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).

In certain examples, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-R-G-L (SEQ ID NO: 43) or K-K/R-K/T-R-G-L (SEQ ID NO: 44), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from (R-)(R-)R-X₁-K/R-X₂-G-L (SEQ ID NO: 35), R-X₁-K/R-X₂-G-L (SEQ ID NO: 36), (R-)(R-)K-K/R-K/T-X₂-G-L (SEQ ID

NO: 37) or K-K/R-K/T-X₂-G-L (SEQ ID NO: 38), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from R-X₁-X₂-X₂-G-L (SEQ ID NO: 39) or K-X₂-K/T-X₂-G-L (SEQ ID NO: 40) or K-K/R-X₂-X₂-G-L (SEQ ID NO: 41), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) and X₂ is any amino acid other than arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence K-X₂-X₂-X₂-G-L (SEQ ID NO: 42), wherein X₂ is any amino acid other than arginine (R) or lysine (K).

In another example, the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44) or R-X-A-A-G-L (SEQ ID NO: 45) or K-A-K/T-A-G-L (SEQ ID NO: 46) or K-K/R-A-A-G-L (SEQ ID NO: 47) or K-A-A-A-G-L (SEQ ID NO: 48), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the modified HA is from a seasonal flu.

In one example, the at least one further RNA comprised in the vaccine encodes a modified HA comprising a monobasic cleavage site.

In one example, the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue.

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence of X₁-X₂-G-L, wherein X₁ is any amino acid residue and wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In further examples, the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In some examples, the modified HA protein comprises the amino acid sequence of X-A-G-L, wherein X is any amino acid residue.

In one example, the modified HA is from a pandemic flu.

In further examples, the at least one further RNA comprised in the vaccine as described herein expresses a modified HA protein that it is maintained in a pre-fusion stabilized conformation.

Suitably, the at least one further RNA comprised in the vaccine as described herein is a neuraminidase selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes. In one example, the neuraminidase is a N1 subtype.

In one example, the neuraminidase is from the same strain as the modified HA.

In one example, the neuraminidase is from a different strain as the modified HA.

In some examples, the vaccine as described herein comprises the RNA contained in or otherwise associated with a lipid-based carrier.

Suitably, the lipid-based carrier is or comprises a LNP.

In one example, the RNA is encapsulated in, bound to or adsorbed on the LNP.

In particular examples of the vaccine, each RNA is formulated together in a LNP.

In other examples of the vaccine, each RNA is formulated separately in a LNP.

In certain examples, the vaccine as described herein comprises an adjuvant. Suitably, the adjuvant is selected from a group consisting of squalane and squalene (or other oils of plant or animal origin), inclusive of squalene oil-in-water emulsions (e.g., MF59, AS03 and AF03) 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, , 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 particular examples, the oil-in-water emulsions are squalene-in-water emulsions, and more particularly, submicron squalene-in-water emulsions. According to some examples, the vaccine composition comprises MF59. In other examples, the adjuvant is MF59.

In one example, the vaccine further comprises a recombinant protein antigen.

In a further example, the recombinant protein antigen is derived from a group comprising: influenza virus, coronavirus, respiratory syncytial virus (RSV), parainfluenza virus, rhinovirus, adenovirus or a combination thereof.

In one example, the present disclosure provides the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein for use in: (a) eliciting an immune response; and/or (b) preventing or treating a disease, disorder or condition in a subject.

In another example, the present disclosure provides a method of eliciting an immune response in a subject, said method including of step of administering a therapeutically effective amount of the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein to the subject to thereby elicit the immune response.

In another example, the present disclosure provides a method of treating and/or preventing an influenza-associated disease, disorder or condition in a subject, the method comprising administering a therapeutically or prophylactically effective amount of the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein to the subject.

In another example, the present disclosure provides the use of the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein in the manufacture of a medicament for treating and/or preventing a disease, disorder or condition in a subject in need thereof.

Suitably, the disease, disorder or condition is an influenza virus infection.

In one example, the present disclosure provides the use of the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein in the manufacture of a medicament for treating and/or preventing an influenza-associated disease, disorder or condition in a subject.

In one example, the present disclosure provides the use of the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein in the manufacture of a medicament for eliciting an immune response in a subject.

In another example, the immune response is a neutralizing antibody response.

In one example, the subject as described herein is a human subject.

In a further example, the present disclosure provides a kit comprising:

- a) the RNA, the immunogenic composition or the pharmaceutical composition or the vaccine as described herein;
- b) instructions for use thereof; and optionally
- c) a pharmaceutically acceptable carrier, excipient or diluent.

The present disclosure also provides a modified HA protein from an influenza virus, wherein the modified HA protein comprises at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.

In one example, the modified HA protein additionally comprises a deletion of an amino acid in a fusion peptide.

In another example, the HA cleavage site is a polybasic cleavage site.

For example, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue inserted from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, all the basic amino acids in the polybasic cleavage site except the most C-terminal R is/are deleted.

In another example, the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, the modified HA additionally has an amino acid in the fusion peptide deleted.

In one example, the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).

In a further example, the Q is deleted in the modified HA. For example, the Q in a fusion peptide at a position corresponding to residue 15 in the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33) is deleted.

In another example, the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from (R-)(R-)R-X₁-K/R-X₂-G-L, R-X₁-K/R-X₂-G-L (SEQ ID NO: 35), (R-)(R-)K-K/R-K/T-X₂-G-L (SEQ ID NO: 36) or K-K/R-K/T-X₂-G-L (SEQ ID NO: 37), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from R-X₁-X₂-X₂-G-L (SEQ ID NO: 38) or K-X₂-K/T-X₂-G-L (SEQ ID NO: 39) or K-K/R-X₂-X₂-G-L (SEQ ID NO: 40), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) and X₂ is any amino acid other than arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence K-X₂-X₂-X₂-G-L (SEQ ID NO: 42), wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44) or R-X-A-A-G-L (SEQ ID NO: 45) or K-A-K/T-A-G-L (SEQ ID NO: 46) or K-K/R-A-A-G-L (SEQ ID NO: 47) or K-A-A-A-G-L (SEQ ID NO: 48), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the modified HA protein comprises a monobasic cleavage site. For example, the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue. In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence of X₁-X₂-G-L (SEQ ID NO: 50), wherein X₁ is any amino acid residue and wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In other examples, the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In further examples, the modified HA protein comprises the amino acid sequence of X-A-G-L (SEQ ID NO: 51), wherein X is any amino acid residue.

The present disclosure provides an RNA as described herein, wherein upon expression of the modified HA protein it is maintained in a pre-fusion stabilized conformation.

In one example, the modified HA is from a seasonal flu. For example, the HA cleavage site is a monobasic cleavage site.

In another example, the HA is from a pandemic flu. For example, the HA cleavage site is a polybasic cleavage site.

In one example, the modified HA is selected from a H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 subtype.

In one example, the modified HA is a H1 subtype. In one example, the modified HA is a H2 subtype. In one example, the modified HA is a H5 subtype.

The present disclosure also provides a vaccine comprising the modified HA described herein.

The present disclosure also provides a recombinant influenza or virus like particle comprising the modified HA described herein. In one example, the recombinant influenza or virus like particle additionally comprises a NA, e.g., as described herein.

The present disclosure also provides a vaccine comprising the recombinant influenza or virus like particle described herein.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Self-amplifying RNA construct design: various constructs generated comprising an alphavirus self-amplifying RNA comprising a polynucleotide encoding a gene of interest derived from an influenza A virus (swine or avian strains), driven by a synthetic genomic promoter (SGP) v2. GenS, GenScript codon-optimized; GenA, GeneArt codon-optimized.

Figure 2: Effect of self-amplifying RNA constructs C1-C6 and control constructs comprising rH2 WT, rH2 Fu and rN3 WT on hemagglutinin inhibition (HAI) titers, fluorescent focus-based microneutralization (FFA MN) titers and N3 enzyme-linked lectin assay (N3 ELLA) titers in serological samples obtained from mice immunized with said constructs.

Figure 3: Effect of self-amplifying RNA constructs C7-C9, C11-C13, C3 and C5 on PV-hemagglutinin inhibition (PV-HAI) titers and N3 enzyme-linked lectin assay (N3 ELLA) titers in serological samples obtained from mice immunized with said constructs.

DETAILED DESCRIPTION

The inventors have shown that removing the cleavage sites of the influenza virus HA protein by modifying the amino acid sequence within this site leads to a modified

HA protein that is stabilized in its pre-fusion conformation. Stabilization of the pre-fusion conformation of the HA protein has been shown to be a key factor in the induction of an efficacious immune response. Furthermore, the removal of the cleavage site in the HA protein reduces the potential for viral reassortment in host cells.

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.

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).

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).

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).

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 “nucleotide sequence”, “polynucleotide” or “nucleic acid” refers to the arrangement of either deoxyribonucleotide or ribonucleotide residues in a polymer in either single- or double-stranded form. polynucleotide sequences can be composed of natural nucleotides of the following bases: T, A, C, G, and U, and/or synthetic analogs of the natural nucleotides. In the context of the present invention, adenosine is abbreviated as “A”, cytidine is abbreviated as “C”, guanosine is abbreviated as “G”, thymidine is abbreviated as “T”, and uridine is abbreviated as “U”. A polynucleotide can be a single-stranded or a double-stranded polynucleotide. Unless otherwise indicated, a polynucleotide is not defined by length and thus includes very large polynucleotides, as well as short ones, such as an oligonucleotide. In one example, the polynucleotide is a DNA. In another example, the polynucleotide is a RNA. In a particular example, the polynucleotide is a self-amplifying RNA.

As used herein, the term “encode”, “encodes” or “encoding” refers to a region of a polynucleotide capable of undergoing translation into a polypeptide.

As used herein, the term “influenza virus” refers to enveloped viruses belonging to the family *Orthomyxoviridae* and having a genome composed of eight negative-sense, single-stranded RNA (ribonucleic acid) segments. These influenza viruses are classified into types A, B, C and D, and the influenza A viruses are further divided into subtypes based on their major surface proteins HA and NA (neuraminidase).

As used herein, the terms “haemagglutinin”, “hemagglutinin” and “HA” refers to an envelope glycoprotein of an influenza virus. HA mediates the adsorption and penetration of influenza virus into a host cell. A native hemagglutinin protein typically

comprises a signal peptide, a stem domain, a globular head domain, a luminal domain, a transmembrane domain and a cytoplasmic domain. The haemagglutinin protein will be known to those of skill in the art to encompass, for example, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In some examples, the HA protein is derived from an influenza A virus.

As used herein, the term “modified protein”, e.g., “modified HA protein”, is to be understood as a protein which contains one or a plurality of modifications compared to a parent, consensus or wild-type protein, such as a wild-type HA protein. Wild type HA protein sequences can be determined experimentally or are publicly available on a number of databases. A suitable example is provided in the present disclosure as SEQ ID NO: 1 or SEQ ID NO: 2. The term “modification” or “modified” in the context of the present disclosure is to be understood as a substitution or replacement of one or more amino acid side chains, one or more substitutions, one or more deletions and/or one or more insertions in the protein of interest. Such modifications also encompass genetic manipulation of the DNA encoding the modified protein.

As used herein, the term “substitution” refers to the replacement of an amino acid at an identified position with a different amino acid to that found in a wild-type amino acid sequence.

Polynucleotides

Ribonucleic acids (RNA)

The present disclosure provides a RNA comprising a nucleotide sequence encoding a modified hemagglutinin (HA) protein from an influenza virus, wherein the modified HA protein comprises at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.

As used herein, the term “RNA” refers to a polymer of ribonucleotides. These molecules usually comprise adenosine-monophosphate, uridine-monophosphate, guanosine-monophosphate and cytidine-monophosphate monomers or analogues or modified versions thereof, which are connected to each other along a so-called backbone. The backbone is formed by phosphodiester bonds between the sugar (i.e., ribose) of a first monomer and a phosphate moiety of a second, adjacent monomer.

In one example, the RNA as described herein encodes a modified HA which additionally comprises a deletion of an amino acid in a fusion peptide. As used herein, the term “fusion peptide”, refers to a relatively hydrophobic sequence of amino acids positioned within the HA. In an unmodified, native HA protein, the fusion peptide is positioned at a N-terminus of a HA₂ subunit or within the HA between a C terminus of a HA₁ subunit and a N-terminus of a HA₂ subunit. In an example where the HA is modified as described herein, the HA is not cleaved into subunits.

In another example, the RNA as described herein encodes a HA protein comprising a polybasic cleavage site. As used herein, the term “polybasic cleavage site” refers to a proteolytic excision site in the amino acid sequence which comprises a motif comprising

multiple basic amino acid residues recognized by ubiquitous cellular proteases such as Furin, PC6 and site specific proteases such trypsin-like proteases which cleaves HA₀ into HA₁ and HA₂.

For example, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, all the basic amino acids in the polybasic cleavage site except a most C-terminal R is/are deleted.

In another example, the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).

Accordingly, the amino acid sequence within the HA cleavage site comprising RRRKK (SEQ ID NO: 52) is deleted.

In one example, the sequence corresponding to the polybasic cleavage site after deletion of the basic amino acids consists of the sequence R-G-L (SEQ ID NO: 32).

In one example, the modified HA additionally has an amino acid in the fusion peptide deleted.

In one example, the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).

In a further example, the Q is deleted in the modified HA. For example, the Q in a fusion peptide at a position corresponding to residue 15 in the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33) is deleted.

Accordingly, the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).

For example, the polybasic cleavage site as described herein, prior to the at least one amino acid substitution, comprises an amino acid sequence selected from R-X-K/R-R (SEQ ID NO: 53) or K-K/R-K/T-R (SEQ ID NO: 54), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K) and wherein the cleavage of HA₀ into HA₁ and HA₂ proteins occurs after the final "R" residue.

the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from (R-)(R-)R-X₁-K/R-X₂-G-L (SEQ ID NO:35), R-X₁-K/R-X₂-G-L (SEQ ID NO: 36), (R-)(R-)K-K/R-K/T-X₂-G-L (SEQ ID NO: 37) or K-K/R-K/T-X₂-G-L (SEQ ID NO: 38), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence selected from R-X₁-X₂-X₂-G-L (SEQ ID NO: 39) or K-X₂-K/T-X₂-G-L (SEQ ID NO: 40) or K-K/R-X₂-X₂-G-L (SEQ ID NO: 41), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) and X₂ is any amino acid other than arginine (R) or lysine (K).

In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence K-X₂-X₂-X₂-G-L (SEQ ID NO: 42), wherein X₂ is any amino acid other than arginine (R) or lysine (K).

Suitably, the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44) or R-X-A-A-G-L (SEQ ID NO: 45) or K-A-K/T-A-G-L (SEQ ID NO: 46) or K-K/R-A-A-G-L (SEQ ID NO: 47) or K-A-A-A-G-L (SEQ ID NO: 48), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In one example, the HA protein comprises a monobasic cleavage site. As used herein, the term “monobasic cleavage site” refers to a proteolytic excision site in the amino acid sequence which comprises a motif comprising a single basic amino acid residue, typically arginine (R), recognized by trypsin or trypsin-like proteases such as HAT, TMPRSS2 and TMPRSS4 which cleave HA₀ into HA₁ and HA₂.

Accordingly, the monobasic cleavage site as described herein, prior to the at least one amino acid substitution, comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue and wherein the cleavage of HA₀ into HA₁ and HA₂ proteins occurs after the “R” residue. In one example, the amino acid sequence following the amino acid substitution comprises an amino acid sequence of X₁-X₂-G-L (SEQ ID NO: 50), wherein X₁ is any amino acid residue and wherein X₂ is any amino acid other than arginine (R) or lysine (K).

In one example, the RNA as described herein encodes a modified HA protein comprising an amino acid substitution at position R340, wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2. In a further example, the RNA as described herein encodes a modified HA protein comprising a R340A substitution, wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2. Suitably, the RNA as described herein encodes a modified HA protein that comprises the amino acid sequence of X-A-G-L, wherein X is any amino acid residue.

Suitably, the modified HA protein described herein exhibits or possesses altered or modulated stability (e.g., increased stability) as compared to a wild-type or unmodified HA protein of a corresponding influenza virus. Accordingly, the present disclosure provides an RNA encoding a modified HA protein as described herein, wherein upon expression of the modified HA protein, the modified HA protein is maintained in a pre-fusion stabilized conformation.

Influenza A and B viruses cause seasonal epidemics of disease in people (known as flu season) with influenza A viruses being the only influenza viruses known to cause flu pandemics (i.e., global epidemics of flu disease). According to certain examples, the RNA encodes a modified HA protein, as described herein, which is derived from an influenza A virus or an influenza B virus. In some examples, the RNA encodes a modified HA protein derived from an influenza A virus.

In one example, the modified HA is from a seasonal flu. For example, the HA cleavage site is a monobasic cleavage site.

In another example, the HA is from a pandemic flu. For example, the HA cleavage site is a polybasic cleavage site.

Influenza A viruses are divided into subtypes based on two proteins on the surface of the virus: HA and NA. In one example, the RNA as described herein encodes a modified HA selected from a H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 subtype. In particular examples, the modified HA protein described herein is of a H2, H1, H5, H3, H7 or H9 influenza A subtype or more particularly of a H2, H1 or H5 influenza A subtype. In one example, the modified HA is a H1 subtype. In some examples, the modified HA protein described herein is of a H2 influenza A subtype. In one example, the modified HA is a H5 subtype.

The present disclosure further provides a RNA comprising a further nucleotide sequence encoding a neuraminidase (NA). Suitably, the neuraminidase is selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes. In one example, the neuraminidase is a N1 subtype. For example, the RNA encodes a H1 or H5 and a N1. In some examples, the NA is a N3 subtype. For example, the RNA encodes a H2 and a N3.

The HA and NA proteins may be derived from an influenza virus isolate from any host species. In various examples, the modified HA protein is derived from an avian influenza virus isolate or strain. In other examples, the modified HA protein is derived from or is derived at least partly from a swine influenza virus isolate or strain. In some examples, the NA is derived from the same strain as the modified HA. In other examples, the NA is derived from a different strain as the modified HA. In particular, the RNA described herein encodes a H2N3.

The present disclosure provides a RNA as described herein, wherein the RNA is operably linked to one or more regulatory sequences. In one example, the RNA as described here in comprises (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES). In a further example, the RNA as described herein comprises, in order from 5' to 3': (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a SG promoter; and (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an IRES.

As used herein, the term “operably linked to” refers to positioning of, for example, a subgenomic promoter or regulatory element (e.g., an IRES) relative to a polynucleotide such that expression of the polynucleotide is controlled or regulated by the element. For example, a subgenomic promoter can be operably linked to numerous polynucleotides, e.g., through another regulatory element, such as an internal ribosome entry site (IRES). As used herein, the term “subgenomic promoter” or “SGP” (also known as ‘junction region’ promoter) refers to a promoter that directs the expression of a heterologous nucleotide sequence, regulating protein expression. SG promoters suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein. A subgenomic promoter is generally necessary to drive the expression of genes using RNA as the template polynucleotide. The subgenomic promoter can be recognized by an RNA polymerase. The promoter itself may be a composite of segments derived from more than one source, naturally occurring or synthetic. 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 another 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 promoter is a SGPv1 promoter, a SGPv2 promoter, a SGPv3 promoter or a SGPv4 promoter. For example, the SGPv1 promoter is encoded by a sequence set forth in SEQ ID NO: 5. For example, the SGPv2 promoter is encoded by a sequence set forth in SEQ ID NO: 6. For example, the SGPv3 promoter is encoded by a sequence set forth in SEQ ID NO: 7. For example, the SGPv4 promoter is encoded by a sequence set forth in SEQ ID NO:8.

In other examples, the regulatory element is a SG promoter v2 (SGPv2). For example, the SGPv2 comprises a sequence set forth in SEQ ID NO: 6.

As used herein, the term “internal ribosome entry site” or “IRES” refers to a sequence of nucleotides within a RNA to which a ribosome or a component thereof, e.g., a 40S subunit of a ribosome, is capable of binding. 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 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 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). Suitably, 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: 9.

The present disclosure provides a RNA as described herein, wherein the RNA is a monocistronic RNA. As used herein, the term “monocistronic” refers to typically to an RNA, that comprises only one open reading frame (coding sequence or coding region). An open reading frame in this context is a sequence of several nucleotide triplets (codons) that can be translated into a peptide or protein. In another example, the RNA described herein is a polycistronic RNA. As used herein, the term “polycistronic” in this context refers to a RNA that encodes two or more polypeptides. The term encompasses “bicistronic” (i.e., encoding two polypeptides) molecules. Accordingly, the RNA provided herein is a bicistronic RNA, wherein the term “bicistronic” refers to a single polynucleotide that is capable of encoding two distinct polypeptides from different regions of the polynucleotide.

Self-amplifying RNA

The present disclosure provides a RNA as described herein, wherein the RNA is a self-amplifying RNA. As used herein, the term “self-amplifying RNA” refers to a construct based on an RNA virus that has been engineered to allow expression of heterologous RNA and proteins. Self-amplifying RNA can amplify in host cells leading to expression of the desired gene product in the host cell. Suitably, the self-amplifying RNA of the present disclosure suitably comprises one or more features of a RNA (e.g., a nucleotide sequence encoding a protein of interest), but further comprises nucleotide sequences encoding non-structural proteins, which enable the self-amplifying RNA to direct its self-amplification. Non-structural proteins can include a viral replicase (or viral polymerase), a viral protease, a viral helicase and optionally other non-structural viral proteins. The self-amplifying RNA of the present disclosure does not encode one or more alphavirus structural proteins (e.g., capsid and/or envelope glycoproteins). Accordingly, the self-amplifying RNA of the present disclosure cannot induce production of infectious viral particles.

Further, the intracellular replication of self-amplifying RNA is generally transient and produces a double-stranded RNA (dsRNA) intermediate during replication, which can induce interferon-mediated host-defense mechanisms by triggering pattern recognition receptors. This can result in strong antigen-specific immune responses against the encoded protein of interest.

The skilled person will understand that the self-amplifying 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-amplifying RNA is derived from or based on an alphavirus. Suitable alphaviruses will be apparent to the skilled person and/or described herein. In another example, the self-amplifying RNA is derived from or based on a virus other than an alphavirus, for example, a positive-stranded RNA virus. Suitable 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. In one example, the self-amplifying 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. In one example, the self-amplifying RNA comprises a viral replicase (or viral polymerase) such as an alphavirus protein NSP4.

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 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 contain genome sequences from more than one alphavirus. Suitably, the self-amplifying RNA provided herein comprises sequences obtained from an alphavirus selected from the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan equine encephalitis virus (VEE) and combinations thereof.

Deoxyribonucleotide acid (DNA)

As used herein, the term "DNA" is an abbreviation for deoxyribonucleic acid. The component nucleotides of DNA are usually deoxy-adenosine-monophosphate, deoxy-thymidine-monophosphate, deoxy-guanosine-monophosphate and deoxy-cytidine-monophosphate monomers or analogues thereof, which are composed of a sugar moiety (deoxyribose), a base moiety and a phosphate moiety, and polymerize by a characteristic

backbone structure. The backbone structure is, typically, formed by phosphodiester bonds between the sugar moiety of the nucleotide (i.e., deoxyribose) of a first monomer and a phosphate moiety of a second, adjacent monomer. The specific order of the monomers, *i.e.* the order of the bases linked to the sugar/phosphate-backbone, is called the DNA sequence. DNA may be single stranded or double stranded. In the double stranded form, the nucleotides of the first strand typically hybridize with the nucleotides of the second strand, such as by A/T-base-pairing and G/C-base-pairing. Although double stranded DNA comprises two opposing strands in terms of the 5' to 3' direction of the two single strands present in the double strand, it is common to nevertheless refer to a 5' end and a 3' end of the double stranded DNA, namely if the DNA comprises a coding sequence element that introduces a direction of the transcription into the double stranded DNA (and accordingly also a direction of the translation).

Isolated polynucleotides

For the purposes of the present disclosure, by “isolated” refers to material that has been removed from its natural state or otherwise been subjected to human manipulation. Isolated material may be substantially or essentially free from components that normally accompany it in its natural state or may be manipulated so as to be in an artificial state together with components that normally accompany it in its natural state.

In one example, the present disclosure provides an isolated polynucleotide encoding the RNA as described herein. In one example, the isolated polynucleotide is a DNA or more suitably, a recombinant DNA.

As used herein, the term “recombinant DNA” refers to a form of artificial DNA such as a synthetic DNA. Accordingly, the recombinant DNA is engineered to express an RNA that encodes the modified HA as described herein. Suitably, the recombinant DNA as described herein is a vector. As used herein, the term “vector” refers to any genetic element, such as a plasmid, which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. In one example, for the expression of the modified HA as described herein, the polynucleotide encoding the modified HA is incorporated by standard cloning techniques into an expression vector, suitable for expressing the modified HA in a host cell. The expression vector or plasmid provides all elements necessary for expression of the modified HA in the host cell. As used herein, the term “host cell” refers to any eukaryotic cell (e.g., mammalian cells), whether located *in vitro* or *in vivo*. Suitable expression vectors are commercially available and include standard plasmid vectors for expression in *E. coli*. In another example, the vector is a close-ended DNA, or a Doggybone DNA.

As used herein, the terms “close-ended DNA” or “Doggybone DNA” typically refer to linear DNA (e.g., a double stranded linear DNA) that includes at least one covalently closed end, and more particularly two covalently closed ends, such as single stranded hairpin loops or ends, where base pairing between complementary DNA strands is not present. Such hairpin loops suitably join the ends of complementary DNA strands.

The hairpin loops may themselves contain complementary sequences, particularly if the hairpin loops comprise part of a protelomerase target sequence. Because they have complementary internal sequences, such DNA can assume a “dumbbell” shape. It is envisaged, however, that the Doggybone DNA described herein may include additional secondary and tertiary polynucleotide structures, as are known in the art (e.g., one or more stem loop structures). The Doggybone DNA template provided herein is suitably an enzymatically-amplified close-ended DNA template or vector. To this end, the Doggybone DNA template has suitably been produced by a cell-free process and more particularly a bacterial cell-free process. As such, the Doggybone DNA template is suitably free from any bacterial propagation elements and antibiotic resistance elements or markers. Furthermore, the Doggybone DNA template provided herein is suitably not or does not comprise plasmid DNA.

According to particular examples, the Doggybone DNA template provided herein is a minimal, closed linear DNA vector developed by Touchlight Genetics Ltd, which can be rapidly produced, is plasmid-free and is synthesized through an enzymatic process using a DNA polymerase, Phi29, and the protelomerase enzyme, TelN. Such methods may yield a close-ended DNA template containing only the encoded sequence of interest, a promoter, a poly A tail and telomeric ends.

In one example, the present disclosure provides an isolated polynucleotide as described herein comprising: (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES). More suitably, the isolated polynucleotide comprises, in order from 5' to 3': (a) a first nucleotide sequence encoding the modified HA from an influenza virus, operably linked to a SG promoter; and (b) a second nucleotide sequences encoding the neuraminidase, operably linked to a SG promoter or an IRES.

The present disclosure provides an isolated polynucleotide as described herein that comprises, consists of or consists essentially of a nucleotide sequence set forth in SEQ ID NO: 10 or SEQ ID NO: 11, or a fragment, variant or derivative thereof.

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. 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 described herein. In one example, a variant may comprise a substitution. In another example, the substitution is a conservative substitution. A skilled person will appreciate that a conservative

substitution with reference to a polypeptide involves replacement of an amino acid in the polypeptide with a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size).

Codon optimization

In some examples, the isolated polynucleotide encoding the modified HA protein may be codon optimized. As used herein, the term “codon optimized” refers to modifying the codons of a gene sequence without altering the amino acid sequence of the protein or polypeptide encoded by said transgene. In another example, rare codons in the gene sequence are replaced by codons that are more abundant in the genes of the host organism.

Codon optimized sequences may be performed using suitable computer programs for codon optimization are available to the skilled person. (See e.g., Jayaraj et al., 2005, Nucl. Acids Res. 33(9):3011-3016; and on the internet). Commercially available tools for codon optimization are also available and these include those offered by GenScript (GenScript Biotech, NJ, USA) or GeneArt (Thermo Fisher Scientific, MA, USA). Codon optimization of nucleotide sequences aids in the generation of optimal vaccine constructs that demonstrate a high efficiency of protein folding, increased protein stability and increased antigen expression in the host cell.

Methods of producing RNA by *in vitro* transcription

The present disclosure a method of producing the RNA as described herein, the method comprising (i) providing a DNA template encoding the RNA as described herein, (ii) *in vitro* transcribing the RNA from the DNA template; and (ii) purifying the RNA from step (ii).

As used herein, the term “DNA template” refers to a polynucleotide template for RNA polymerase. Typically, a DNA template includes the sequence for a gene of interest operably linked to a RNA polymerase promoter sequence. A DNA template can be prepared for IVT from a number of sources with appropriate techniques which are well-known in the art (see, e.g., Linpinsel, J.L and Conn, G.L., General protocols for preparation of plasmid DNA template and Bowman, J.C., Azizi, B., Lenz, T.K., Ray, P., and Williams, L.D. in RNA IVT and RNA purification by denaturing PAGE. In: Recombinant and *in vitro* RNA synthesis. Methods in Molecular Biology, v. 941 Conn G.L. (ed), New York, N.Y. Humana Press, 2012). In one example, the DNA template is a plasmid DNA.

In one example, the RNA is produced using a plasmid DNA. 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 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). Restriction enzymes are removed using methods known in the art, including for example phenol/chloroform extraction and ethanol precipitation.

In another example, the DNA template is a close-ended, a closed linear DNA template or a Doggybone DNA as described herein.

As used herein, the term “*in vitro* transcription” or “IVT” refers to the process that allows for DNA template-directed synthesis of RNA performed outside of a host cell. For example, IVT may be performed in a bioreactor. The DNA template, and more particularly the plasmid or the close-ended DNA template, may be linearized prior to use in the IVT reaction. Accordingly, the present method may include the earlier or initial step of linearizing the DNA template. As used herein and in the context of DNA, the term “linearized” refers to DNA that comprises at least one free end (e.g., a free 5' end and/or a free 3' end) and more particularly two free ends. The close-ended DNA template (e.g., the dbDNA template) may also be linearized with a suitable restriction enzyme and optionally isolated or purified before it is subjected to IVT. RNA that are produced by IVT, particularly those for use in vaccines and in other therapeutics, are typically capped in order for the RNA to be translated. Typically, RNA that is ready for protein translation (‘mature’ RNA) bear a “cap” structure at their 5'-termini, which plays an important role in translation and stability. For example, the 5' cap plays a pivotal role in RNA metabolism, and is required to varying degrees for processing and maturation of an RNA transcript in the nucleus, transport of RNA from the nucleus to the cytoplasm, RNA stability, and efficient translation of the RNA to protein. It also helps protect the RNA from exonuclease degradation such that RNA lacking a 5' cap is rapidly degraded, is involved in recognition by the translational initiation factor eIF4E and promotes formation of the translation initiation machinery. In one example, the 5' cap comprises a 7-methyl guanosine (m7G) that is linked via a 5'-5'-triphosphate bridge to the 5'-end of the first transcribed nucleotide, resulting in a dinucleotide cap of m7GpppN, where N is any nucleoside (e.g. G, C, A or U) and is the first transcribed nucleotide. This is often referred to as cap0. Other examples include cap1 (m7GpppNmpN) which has additional methylation on the 2'O position of the first nucleotide and cap2 (m7GpppNmpNm) which has additional methylation on the 2'O position of both the first and second nucleotides.

The RNA produced by IVT may be further processed, for example by the addition of a poly(A) tail. The poly(A) tail may be included in the DNA template, added via PCR, or added post-transcriptionally by enzymatic polyadenylation. In particular examples, the close-ended DNA template may include a polyadenylation signal sequence. In further examples, the poly(A) tail is introduced by including a poly(dT) stretch at the end of the close-ended DNA template. In other examples, the poly(A) tail is added following IVT. In some examples, a 3' poly(A) tail is added following IVT through the addition of ATP in conjunction with a poly(A) polymerase.

Following IVT, the DNA template may be removed using any technique known to the person skilled in the art. In certain examples, following IVT, the DNA template is removed by treatment with a DNase. In various examples, the DNase is DNase I. The desired in vitro transcribed RNA may then be purified or isolated from the undesired components of the transcription or associated reactions. Various methods for purifying RNA will be apparent to the skilled person. For example, the RNA is purified using lithium chloride (LiCl) precipitation. In another example, the RNA is purified using tangential flow filtration (TFF). Following purification, the RNA is resuspended in e.g., nuclease- free water.

Host cells

The present disclosure further provides a host cell comprising the RNA or the isolated polynucleotide as described herein. As used herein, the term "host cell" refers to any eukaryotic cell (e.g., mammalian cells), whether located *in vitro* or *in vivo*.

Compositions

Immunogenic compositions

The present disclosure provides an immunogenic composition comprising the RNA as described herein. Suitably, the modified HA proteins expressed by the RNA described herein are immunogenic and are suitable for use as immunogens in a vaccine to treat or prevent an influenza virus infection in a subject in need thereof. In one example, the immunogenic composition as described herein elicits an immune response when administered to a subject.

As used herein, the term "immunogenic" will be understood to mean that the composition induces or generates an immune response. In particular examples, the modifications provided herein do not or do not substantially alter or modulate (i.e., increase or decrease) the immunogenicity/antigenicity of the modified HA protein (e.g., relative to or when compared to a wild-type or unmodified version thereof). The immunogenicity or antigenicity of the modified HA protein may be assessed by any means known in the art, such as by determining the presence or amount of a neutralizing antibody or an antibody recognizing the modified HA protein using a standard immunoassay and/or determining predicted or actual T cell reactivity known to the person skilled in the art. Alternatively, or in addition, a qualitative antibody measurement may be determined. For example, but without limitation, a measure of one or more functional features of antibodies elicited in a subject to which the compound or composition is administered, may be determined. Suitable functional features which can be measured are known in the art and include, without limitation, hemagglutinin agglutination-inhibition. A quantitative method for the determination of expression or secretion of cytokines or alteration in the phenotype of immune cells may also be used.

As used herein, the term "elicit an immune response" refers to generating or stimulating the production or activity of one or more elements of the immune system

inclusive of the cellular immune system, humoral immune system (i.e., antibodies) and/or the native immune system. Suitably, the immune response described herein includes one or more elements of the immune system, such as T lymphocytes, B lymphocytes, antibodies, neutrophils, dendritic cells inclusive of plasmacytoid dendritic cells, cytokines and/or chemokines. Non-limiting examples of cytokines include pro-inflammatory cytokines such as TNF- α , IL-2, IL-6, IL-8, IL-17A and IL-1 (e.g., IL-1 β).

In one example, the immunogenic composition as described herein comprises at least one further RNA encoding a further HA from a further influenza virus, wherein the at least one further HA is a different subtype to the modified HA. Accordingly, such a composition is suitable for use in treating or preventing a disease in a subject in need thereof. Particularly, the disease is an influenza virus infection.

Pharmaceutical compositions

The present disclosure provides a pharmaceutical composition comprising the RNA described herein, the pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent or excipient. As used herein, the term "composition" or "pharmaceutical composition" refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a subject.

As used herein, the term "pharmaceutically-acceptable carrier, diluent or excipient" refers to a solid or liquid filler, diluent or encapsulating substance. Pharmaceutically acceptable carriers, fillers and diluents will have sufficiently high purity and sufficiently low toxicity to make them suitable for administration to a subject. Depending upon the particular route of administration, a variety of carriers, well known in the art may be used. These carriers may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulfate, liposomes and other lipid-based carriers, vegetable oils, synthetic oils, polyols, alginate acid, phosphate buffered solutions, emulsifiers, isotonic saline and salts such as mineral acid salts including hydrochlorides, bromides and sulfates, organic acids such as acetates, propionates and malonates and pyrogen-free water. A useful reference describing pharmaceutically acceptable carriers, diluents and excipients is Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991), which is incorporated herein by reference.

The concentration of the RNA in the pharmaceutical composition can vary and will be selected based on fluid volumes, viscosities, body weight, type of RNA and other considerations in accordance with the particular mode of administration. The concentration of the RNA in the pharmaceutical composition will suitably be effective for prevention or treatment of a disease, disorder or condition, either in a single dose or as part of a series of doses. The amount may vary depending upon the health, physical condition, age and taxonomic group of the individual to be treated (e.g. non-human primate, primate, etc.), the capacity of the individual's immune system to react to the

encoded antigenic protein or peptide, the condition to be treated and other relevant factors.

Vaccines

As used herein, the term “vaccine” refers to a formulation which contains immunogenic antigens, which is in a form that is capable of being administered to a subject and which induces a protective immune response sufficient to induce immunity to prevent and/or ameliorate an infection and/or to reduce at least one symptom of an infection and/or to enhance the efficacy of another dose of the vaccine.

Accordingly, the present disclosure provides the immunogenic composition or the pharmaceutical composition comprising the RNA as described herein for use as a vaccine. Suitably, the vaccine elicits a protective immune response when administered to a subject.

As used herein, the term “protective immune response” refers to immunity or eliciting an immune response against an infectious agent, which is exhibited by a subject (e.g., a human), that prevents or ameliorates an infection or reduces at least one symptom thereof. Specifically, induction of a protective immune response from administration of the vaccine is evident by elimination or reduction of the presence of one or more symptoms of an influenza virus infection or a reduction in the duration or severity of such symptoms.

In some examples, the vaccine is a monovalent vaccine. According to the present invention, in one example, the vaccine as described herein is a monovalent vaccine. Viral vaccines, such as those for influenza, rely upon the induction of antibodies that protect against infection by neutralizing virions or blocking the virus's entry into cells. Humoral immune responses target viral surface proteins, however as these surface proteins are conserved within each strain, antibody-mediated protection is inadequate against strains with serologically distinct surface proteins. Furthermore, the surface proteins of many viruses are capable of rapid mutation. This means that it would be advantageous for most vaccines to be multivalent, i.e., include antigens from strains that are predicted to be most prevalent in a given time period.

Accordingly, the vaccine as described herein is a multivalent vaccine, such as a bivalent, or a trivalent or a quadrivalent vaccine. In one example, the vaccine comprises at least one strain that is associated with a pandemic or has the potential to be associated with a pandemic.

Suitably, the vaccine is a bivalent vaccine and comprises one further RNA comprising a nucleotide sequence encoding at least one further influenza antigen. For example, the vaccine as described herein comprises a further influenza antigen derived from an influenza A virus. In another example, the vaccine as described herein comprises a further influenza antigen derived from an influenza B virus.

In another example, the vaccine is a trivalent vaccine and comprises at least two further RNA comprising a nucleotides sequence encoding at least two further influenza

antigens. For example, the vaccine as described herein comprises two influenza A virus strains and one influenza virus B strain. In another example, the vaccine as described herein comprises two further influenza A virus strains or two further influenza virus B strains or a combination thereof.

In a further example, the vaccine is a quadrivalent vaccine and comprises at least three further RNA comprising a nucleotides sequence encoding at least three further influenza antigens. Typically, a quadrivalent vaccine comprises two influenza A virus strains and two influenza B virus strains.

In one example, the at least one further RNA comprised in the vaccine as described herein encodes a HA subtype selected from H1, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16 subtypes. In one example, the influenza viral antigens are 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 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.

Accordingly, in one example, the vaccine as described herein comprises at least one further RNA encodes a modified HA protein comprises at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.

In one example, the at least one further RNA comprised in the vaccine encodes a modified HA comprising a monobasic cleavage site. In some examples, the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L, wherein X is any amino acid residue. Suitably, the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2. In further examples, the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

In some examples, the modified HA protein encoded by the RNA comprised in the vaccine as described herein comprises the amino acid sequence of X-A-G-L, wherein X is any amino acid residue.

In other examples, the vaccine as described herein comprises at least one further RNA encoding a modified HA comprising a polybasic cleavage site. In certain examples, the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-R-G-L or K-K/R-K/T-R-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K). In another example, the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L or K-K/R-K/T-A-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

In further examples, the at least one further RNA comprised in the vaccine as described herein expresses a modified HA protein that it is maintained in a pre-fusion stabilized conformation.

In certain examples, the at least one further RNA is a neuraminidase selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes.

In one example, the neuraminidase is from the same strain as the modified HA. In other examples, the neuraminidase is from a different strain as the modified HA.

The skilled person will be aware that pandemic strains of the influenza virus are for example, but not limited to, H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2 strains.

In one example, the vaccine as described herein comprises an adjuvant. As used herein, the term “adjuvant” means a substance that is administered with an antigen and thereby increases the antigenicity of the antigen to facilitate the induction of immune response. The adjuvant may be any compound, which is suitable to support administration and delivery of the pharmaceutical composition. Such an adjuvant may be selected from any adjuvant known to a skilled person and suitable for the particular nature of the vaccine or immunogenic composition (i.e., for the induction of a suitable immune response in a mammal).

In certain examples, the adjuvant may be selected from the group consisting of: squalane and squalene (or other oils of plant or animal origin), inclusive of squalene oil-in-water emulsions (e.g., MF59, AS03 and AF03), TDM, MDP, muramyl dipeptide, pluronics, alum solution, aluminium hydroxide, ADJUMER™ (polyphosphazene); aluminium phosphate gel; glucans from algae; algammulin; aluminium hydroxide gel (alum); highly protein-adsorbing aluminium hydroxide gel; low viscosity aluminium hydroxide gel; AF or SPT (emulsion of squalane (5%), Tween 80 (0.2%), Pluronic L121 (1.25%), phosphate-buffered saline, pH 7.4); AVRIDINE™ (propanediamine); BAY R1005™ ((N-(2-deoxy-2-L-leucylamino-b-D-glucopyranosyl)-N-octadecyl-dodecanoyl-amide hydroacetate); CALCITRIOL™ (1-alpha,25-dihydroxy-vitamin D3); calcium phosphate gel; CAP™ (calcium phosphate nanoparticles); cholera holotoxin, cholera-toxin-A1-protein-A-D-fragment fusion protein, sub-unit B of the cholera toxin; CRL 1005 (block copolymer P1205); cytokine-containing liposomes; DDA (dimethyldioctadecylammonium bromide); DHEA (dehydroepiandrosterone); DMPC (dimyristoylphosphatidylcholine); DMPG (dimyristoylphosphatidylglycerol); DOC/alum complex (deoxycholic acid sodium salt); Freund's complete adjuvant; Freund's incomplete adjuvant; gamma inulin; Gerbu adjuvant (mixture of: i) N-acetylglucosaminyl-(PI-4)-N-acetylmuramyl-L-alanyl-D-glutamine (GMDP), ii) dimethyldioctadecylammonium chloride (DDA), iii) zinc-L-proline salt complex (ZnPro-8); GM-CSF; GMDP (N-acetylglucosaminyl-(bl-4)-N-acetylmuramyl-L-alanyl-D-isoglutamine); imiquimod (1-(2-methoxypropyl)-1H-imidazoquinoline-4-amine); ImmTher™ (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate); DRVs (immunoliposomes prepared from dehydration-rehydration

vesicles); interferon-gamma; interleukin-1 β ; interleukin-2; interleukin-7; interleukin-12; ISCOMSTM; ISCOPREP 7.0.3.TM; liposomes; LOXORIBINETM (7-allyl-8-oxoguanosine); LT oral adjuvant (E.coli labile enterotoxin-prototoxin); microspheres and microparticles of any composition;; MONTANIDE ISA 51TM (purified incomplete Freund's adjuvant); MONTANIDE ISA 720TM (metabolisable oil adjuvant); MPLTM (3-Q-desacyl-4'-monophosphoryl lipid A); MTP-PE and MTP-PE liposomes ((N-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxyphosphoryloxy))-ethylamide, monosodium salt); MURAMETIDETM (Nac-Mur-L-Ala-D-Gln-OCH₃); MURAPALMITINETM and D-MURAPALMITINETM (Nac-Mur-L-Thr-D-isoGln-sn-glyceroldipalmitoyl); NAGO (neuraminidase-galactose oxidase); nanospheres or nanoparticles of any composition; NISVs (non-ionic surfactant vesicles); PLEURANTM (β -glucan); PLGA, PGA and PLA (homo- and co-polymers of lactic acid and glycolic acid; microspheres/nanospheres); PLURONIC L121TM; PMMA (polymethyl methacrylate); PODDSTM (proteinoid microspheres); polyethylene carbamate derivatives; poly-rA: poly-rU (polyadenylic acid-polyuridylic acid complex); polysorbate 80 (Tween 80); protein cochleates (Avanti Polar Lipids, Inc., Alabaster, AL); STIMULONTM (QS-21); Quil-A (Quil-A saponin); S-28463 (4-amino-otec-dimethyl-2-ethoxymethyl-1H-imidazoquinoline-1-ethanol); SAF-1TM ("Syntex adjuvant formulation"); Sendai proteoliposomes and Sendai-containing lipid matrices; Span-85 (sorbitan trioleate); Specol (emulsion of Marcol 52, Span 85 and Tween 85); squalene or Robane® (2,6,10,15,19,23-hexamethyltetracosan and 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane); stearyltyrosine (octadecyltyrosine hydrochloride); Theramid® (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-dipalmitoxypropylamide); Theronyl-MDP (TermurtideTM or -MDP; N-acetylmuramyl-L-threonyl-D-isoglutamine); Ty particles (Ty-VLPs or virus-like particles); Walter-Reed liposomes (liposomes containing lipid A adsorbed on aluminium hydroxide), and lipopeptides, including Pam3Cys, in particular aluminium salts, such as Adju-phos, Alhydrogel, Rehydrogel; emulsions, including CFA, SAF, IFA, MF59, Provax, TiterMax, Montanide, Vaxfectin; copolymers, including Optivax (CRL1005), L121, Poloaxmer4010), etc.; liposomes, including Stealth, cochleates, including BIORAL; plant derived adjuvants, including QS21, Quil A, Iscomatrix, ISCOM; adjuvants suitable for costimulation including Tomatine, biopolymers, including PLG, PMM, Inulin; microbe derived adjuvants, including Romurtide, DETOX, MPL, CWS, Mannose, CpG polynucleotide sequences, CpG7909, ligands of human TLR 1-10, ligands of murine TLR 1-13, ISS-1018, IC31, Imidazoquinolines, Ampligen, Ribi529, IMOxine, IRIVs, VLPs, cholera toxin, heat-labile toxin, Pam3Cys, Flagellin, GPI anchor, LNFPIII/Lewis X, antimicrobial peptides, UC-1V150, RSV fusion protein, cdiGMP; and adjuvants suitable as antagonists including CGRP neuropeptide.

Oil-in-water emulsions have been found to be particularly suitable for use in adjuvanting influenza virus vaccines. Various such emulsions are known, and they typically include at least one oil and at least one surfactant, with the oil(s) and

surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5 µm in diameter, and may even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with an average size less than 220 nm are preferred as they can be subjected to filter sterilization.

In particular examples, the oil-in-water emulsions are squalene-in-water emulsions, and more particularly, submicron squalene-in-water emulsions. According to some examples, the vaccine composition comprises MF59. In other examples, the adjuvant is or comprises MF-59.

The present disclosure also provides a vaccine as described herein, wherein the vaccine further comprises a recombinant protein antigen. Additional recombinant protein antigens will be apparent to the skilled person and include, for example, viral antigens derived from *Oxomyxoviridae* viruses (Influenza A, B or C), *Paramyxoviridae* viruses (Pneumoviruses (e.g., Respiratory syncytial virus (RSV), Bovine respiratory syncytial virus, Pneumonia virus of mice, and Turkey rhinotracheitis virus), Paramyxovirus types 1-4 (PIV), Mumps, Sendai viruses, Simian virus 5)), Metapneumoviruses, such as human metapneumovirus (hMPV) and avian metapneumoviruses (aMPV)), Coronaviruses (e.g., severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), SARS coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), Avian infectious bronchitis (IBV), Mouse hepatitis virus (MHV), Cytomegalovirus (CMV), Papovaviruses (e.g., Papillomaviruses and Polyomaviruses), Adenoviruses and Arenaviruses.

In some examples, the recombinant protein antigen is derived from a group comprising: influenza virus, coronavirus, respiratory syncytial virus (RSV), parainfluenza virus, rhinovirus, adenovirus or a combination thereof.

Lipid carriers

The present disclosure provides an immunogenic composition, a pharmaceutical composition or a vaccine comprising the RNA as described herein, wherein the RNA is contained in or otherwise associated with a lipid-based carrier.

Suitably, the lipid-based carrier may be cationic lipid, a lipid nanoparticle, a liposome, a cochleate, a virosome, an immune- stimulating complex, a microparticle, a microsphere, a nanosphere, a unilamellar vesicle, a multilamellar vesicle, an oil-in-water emulsion, a water-in-oil emulsion, an emulsome, and a polycationic peptide, a cationic nano-emulsion or combinations thereof. The lipid-based carrier suitably comprises any lipid or mixture of lipids capable of forming a lipid bilayer structure. These include a phospholipids, sterols inclusive of cholesterol, cholesterol-esters and phytosterols, fatty acids and/or triglycerides. Non-limiting examples of phospholipids include phosphatidylcholine (PC) (lecithin), phosphatidic acid, phosphatidylethanolamine (PE) (cephalin), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM) or natural or synthetic derivatives thereof known in the art.

Suitably, the lipid-based carrier is or comprises a lipid nanoparticle. In one example, the RNA as described herein is encapsulated in, bound to or adsorbed on the lipid nanoparticle (LNP). 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.

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 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 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, 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-didocosaheptaenoyl-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-didocosaheptaenoyl-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-dioleoyloxy-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.

In one example, each of the RNA comprised in the vaccine as described herein are formulated separately in a LNP. In those examples, the separately formed RNA-LNPs are combined together during vaccine formulation. In other examples, each of the RNA comprised in the vaccine as described herein are formulated together in a LNP.

Formulation of LNPs to be administered may vary according to the route of administration and formulation selected. An appropriate pharmaceutical composition comprising an LNP to be administered can be prepared in a physiologically acceptable carrier. For solutions or emulsions, suitable carriers include, 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 adjusting and buffering agents and toxicity adjusting agents, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The LNPs 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.

The carrier in the LNP composition may be water, typically pyrogen-free water, isotonic saline or buffered (aqueous) solutions, such as phosphate or citrate buffered solutions. For injection of an LNP vaccine composition, water or more particularly a buffer, even more particularly an aqueous buffer, may be used, containing a sodium salt, preferably at least about 50 mM of a sodium salt, preferably at least about 0.01 mM of a calcium salt, and optionally a potassium salt, such as at least about 3 mM of a potassium

salt. In an example, the sodium, calcium and, optionally, potassium salts may be present as their chloride, iodide, or bromide form or in the form of their hydroxides, carbonates, hydrogen carbonates or sulfates. Non-limiting examples of sodium salts include e.g. NaCl, NaI, NaBr, Na₂CO₃, NaHCO₃, Na₂SO₄, examples of the optional potassium salts include e.g. KCl, KI, KBr, K₂CO₃, KHCO₃, K₂SO₄, and examples of calcium salts include e.g. CaCl₂, CaI₂, CaBr₂, CaCO₃, CaSO₄, Ca(OH)₂. Furthermore, organic anions of the aforementioned cations may be contained in the buffer. In certain examples, the buffer suitable for injection purposes, may contain salts selected from sodium chloride (NaCl), calcium chloride (CaCl₂) and optionally potassium chloride (KCl), wherein further anions may be present additional to the chlorides. In one example, the salts in the injection buffer are present in a concentration of at least about 50 mM sodium chloride (NaCl), at least about 3mM potassium chloride (KCl) and at least about 0.01mM calcium chloride (CaCl₂). The injection buffer may be hypertonic, isotonic or hypotonic with reference to the specific reference medium.

Methods of treatment

The present disclosure provides an RNA as described herein and compositions comprising said RNA for use in therapy, such as vaccines, for use in eliciting an immune response; and/or treating a disease, disorder or condition in a subject.

Accordingly, in one form, the present disclosure provides a method of eliciting an immune response in a subject, said method including of step of administering a therapeutically effective amount of the isolated RNA disclosed herein, the immunogenic composition, the pharmaceutical composition or the vaccine disclosed herein to the subject to thereby elicit the immune response.

As used herein, the term “subject” shall be taken to mean any animal including humans, for example a mammal. Exemplary subjects include but are not limited to humans and non-human primates. For example, the subject is a human.

As used herein, “treating”, “treat” or “treatment” refers to a therapeutic intervention that at least partly ameliorates, eliminates or reduces a symptom or pathological sign of a disease, disorder or condition, such as an infectious disease, disorder or condition (e.g., a viral infection), after it has begun to develop. Treatment need not be absolute to be beneficial to the subject. The beneficial effect can be determined using any methods or standards known to the skilled person.

As used herein, “preventing”, “prevent” or “prevention” refers to a course of action initiated prior to infection by, or exposure to, a pathogen (e.g., a virus) or molecular components thereof and/or before the onset of a symptom or pathological sign of the disease, disorder or condition, so as to prevent infection and/or reduce the symptom or pathological sign. It is to be understood that such preventing need not be absolute to be beneficial to a subject. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of the disease, disorder or condition, or exhibits only early

signs for the purpose of decreasing the risk of developing a symptom or pathological sign of the disease, disorder or condition.

As used herein, the terms “disease”, “disorder” or “condition” refers to a disruption of or interference with a normal physiological or biological function of a subject. Diseases, disorders, and/or conditions that may be treated by the immunogenic compositions as described herein include infectious diseases. In particular, the disease is an influenza virus infection.

In one example, the present disclosure provides the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein for use in: (a) eliciting an immune response; and/or (b) preventing or treating a disease, disorder or condition in a subject.

The present disclosure also provides a method of eliciting an immune response in a subject, said method including of step of administering a therapeutically or prophylactically effective amount of the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein to the subject to thereby elicit the immune response.

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.

Accordingly, provided herein is a method of treating and/or preventing an influenza-associated disease, disorder or condition in a subject, the method comprising administering a therapeutically or prophylactically effective amount of the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein, to the subject.

In a further example, the method as described herein comprises administering the vaccine and administering the recombinant protein antigen as described herein.

Suitably, provided herein is the use of the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein in the manufacture of a medicament for eliciting an immune response in a subject.

In one example, the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein induces a humoral and/or a cell-mediated immune response. In one example, the compositions induce a humoral immune response in the subject. For example, the humoral immune response is an antibody-mediated immune response. For example, production of neutralizing antibodies. Viral vaccines, such as those for influenza, rely upon the induction of antibodies that protect against infection by neutralizing virions or blocking the virus's entry into cells. In one example, the immune response is a neutralizing antibody response.

In a further example, provided herein is the use of the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein in the manufacture of a medicament for treating and/or preventing a disease, disorder or condition in a subject in need thereof. In a particular example, the disease, disorder or condition is an influenza virus infection. Accordingly, the present specification also provides the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein in the manufacture of a medicament for treating and/or preventing an influenza-associated disease, disorder or condition in a subject.

The present disclosure provides that the subject as described herein, is human. As used herein, the term “subject” includes any human or non-human animal that is susceptible to infection by an influenza virus. A subject of this invention can be a mammal and in particular embodiments is a human, which can be an infant, a child, an adult or an elderly adult. This also includes a subject at risk of infection by an influenza virus or a subject at risk of an influenza infection is any subject who may be or has been exposed to an influenza virus. The subject may be a primary contact of an individual diagnosed with an influenza infection.

Any safe route of administration may be employed for providing a patient with the composition of the present disclosure. For example, oral, rectal, parenteral, sublingual, buccal, intravenous, intra-articular, intra-muscular, intra-dermal, subcutaneous,

inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed.

Dosage forms include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release of the therapeutic agent may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, the controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

Formulation of the compositions to be administered will vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. 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 adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate.

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 therapeutically/prophylactically effective. The dosage ranges for the administration of the RNA of the disclosure are those large enough to produce the desired effect. For example, the composition comprises an effective amount of the 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 physician in the event of any complication.

Administration of the 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 of the administration is therapeutic or prophylactic, and

other factors known to skilled practitioners. The administration of the 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.

Kits

In one example, the present disclosure provides a kit comprising: (a) a container comprising the RNA, immunogenic composition, pharmaceutical composition or the vaccine as described herein; (b) a pharmaceutically acceptable carrier, excipient or diluent, and optionally (c) a package insert with instructions for use thereof.

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 RNA as described herein. 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 an influenza virus infection, 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 (BWFI), 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.

The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: Generation of the self-amplifying RNA constructs

The inventors generated several H2N3 (from both swine and avian strains) constructs and evaluated their immunogenicity in standard laboratory inbred mice.

Briefly, DNA templates encoding self-amplifying RNAs (saRNAs) described herein were produced in competent *Escherichia coli* cells transformed with a DNA plasmid. Individual bacterial colonies were isolated and the resultant plasmid DNA amplified in *E. coli* cultures. Following fermentation, the plasmid DNA was isolated using Maxiprep DNA kit and linearized by restriction digest. Restriction enzymes were removed using phenol/chloroform extraction and ethanol precipitation.

RNA was made by in vitro transcription from the linearized DNA template using a T7 RNA polymerase. Subsequently, the DNA template was removed by DNase digestion. Enzymatic capping was performed with Cap0 to provide functional RNA. The RNA from IVT reactions was purified by tangential flow filtration (TFF) and frozen at

–80°C. The saRNA constructs utilised in the experiments are outlined in **Figure 1** and comprise:

1. C1 (nucleotide sequence of HA protein set forth in SEQ ID NO: 12) which includes an alphavirus saRNA comprising a polynucleotide encoding a wildtype H2N3 antigen of an influenza A virus [swine strain; A/swine/Missouri/2124514/2006 (H2N3)], driven by a synthetic genomic promoter (SGP) v2.
2. C2 (nucleotide sequence of HA protein set forth in SEQ ID NO: 13) which includes an alphavirus saRNA comprising a polynucleotide encoding a H2N3 antigen comprising a R340A mutation in the H2 region (fusion-stabilized; Fu) of an influenza A virus (swine strain), driven by a synthetic genomic promoter (SGP) v2.
3. C3 (nucleotide sequence of HA protein set forth in SEQ ID NO: 14) which includes an alphavirus saRNA comprising a polynucleotide encoding a GenScript codon-optimized wildtype H2N3 antigen of an influenza A virus (swine strain), driven by a synthetic genomic promoter (SGP) v2.
4. C4 (nucleotide sequence of HA protein set forth in SEQ ID NO: 15) which includes an alphavirus saRNA comprising a polynucleotide encoding a GenScript codon-optimized H2N3 antigen comprising a R340A mutation in the H2 region (fusion-stabilized; Fu) of an influenza A virus (swine strain), driven by a synthetic genomic promoter (SGP) v2.
5. C5 (nucleotide sequence of HA protein set forth in SEQ ID NO: 16) which includes an alphavirus saRNA comprising a polynucleotide encoding a GeneArt codon-optimized H2N3 antigen comprising a R340A mutation in the H2 region (fusion-stabilized; Fu) of an influenza A virus (swine strain), driven by a synthetic genomic promoter (SGP) v2.
6. C6 (nucleotide sequence of HA protein set forth in SEQ ID NO: 17) which includes an alphavirus saRNA comprising a polynucleotide encoding a GenScript codon-optimized wildtype H2N3 antigen of an influenza A virus (swine strain), driven by a synthetic genomic promoter (SGP) v2.
7. C7 (nucleotide sequence of HA protein set forth in SEQ ID NO: 18) which includes an alphavirus saRNA comprising a polynucleotide encoding a wildtype H2N3 antigen of an influenza A virus [avian strain; A/Chicken/OH/494832/2007 (H2N3)], driven by a synthetic genomic promoter (SGP) v2.
8. C8 (nucleotide sequence of HA protein set forth in SEQ ID NO: 19) which includes an alphavirus saRNA comprising a polynucleotide encoding a H2N3 antigen comprising a R340A mutation in the H2 region (fusion-stabilized; Fu) of an influenza A virus (avian strain), driven by a synthetic genomic promoter (SGP) v2.

9. C9 (nucleotide sequence of HA protein set forth in SEQ ID NO: 20) which includes an alphavirus saRNA comprising a polynucleotide encoding a GenScript codon-optimized wildtype H2N3 antigen of an influenza A virus (avian strain), driven by a synthetic genomic promoter (SGP) v2.
10. C10 (nucleotide sequence of HA protein set forth in SEQ ID NO: 21) which includes an alphavirus saRNA comprising a polynucleotide encoding a GenScript codon-optimized H2N3 antigen comprising a R340A mutation in the H2 region (fusion-stabilized; Fu) of an influenza A virus (avian strain), driven by a synthetic genomic promoter (SGP) v2.
11. C11 (nucleotide sequence of HA protein set forth in SEQ ID NO: 22) which includes an alphavirus saRNA comprising a polynucleotide encoding a GeneArt codon-optimized wildtype H2N3 antigen of an influenza A virus (avian strain), driven by a synthetic genomic promoter (SGP) v2.
12. C12 (nucleotide sequence of HA protein set forth in SEQ ID NO: 23) which includes an alphavirus saRNA comprising a polynucleotide encoding a GeneArt codon-optimized H2N3 antigen comprising a R340A mutation in the H2 region (fusion-stabilized; Fu) of an influenza A virus (avian strain), driven by a synthetic genomic promoter (SGP) v2.
13. C13 (nucleotide sequence of HA protein set forth in SEQ ID NO: 24) which includes an alphavirus saRNA comprising a GenScript polynucleotide manually mutated from C3.

Example 2: Evaluation of immunogenicity of bicistronic H2N3 (swine strain) saRNA constructs in mice

A pre-clinical animal study showed that modifications to the HA cleavage site does not substantially impact antigenicity at the tested dose level regardless of whether the antigen was membrane-bound or soluble (Freyn A *et al.* (2021) Mol. Ther). Here, the inventors sought to evaluate the immunogenicity of the various bicistronic H2N3 (swine strain) saRNA constructs, C1-C6 as shown in **Figure 1**, in mice.

Methods: The constructs were injected into 6 to 8 weeks old female BLAB/c mice. 160 mice were employed and divided into high (1 ug/animal) and low (0.01 ug/animal) dose treatment groups per construct. Wildtype and fusion-stabilized recombinant H2 and wildtype recombinant N3 were employed as controls. Mice were vaccinated intramuscularly at Day 0 and Day 21, following a prime-boost regime.

To assess antibody response against the various constructs, serum was collected from the mice at the end of study and tested using several assays including the hemagglutinin inhibition assay (HAI), the fluorescent focus-based microneutralization assay (FFA MN) and the N3 enzyme-linked lectin assay (N3 ELLA).

Hemagglutinin inhibition assay (HAI): Sera were evaluated for HAI as previously described (Heeringa et al. *J. Clin. Microbiol.* 2020; 58 (e00750-20).32 Briefly, sera were treated with receptor-destroying enzyme (RDE) (Denka Seiken Co.,

Ltd., Tokyo, Japan) and diluted with PBS to an initial dilution of 1:10. Serially diluted (2-fold), heat-inactivated, and RDE-treated sera from immunized mice were incubated with an equal volume of viruses (4 hemagglutinin units [HAU] per well) at room temperature for 30 min. After incubation, an equivalent volume of TRBC (Lampire Biological Labs, Pipersville, PA, USA) diluted to 0.5% in PBS was added, and the plates were incubated at room temperature for 45 min. Hemagglutination inhibition was determined by visual inspection, and HAI titer was expressed as the reciprocal of the highest dilution of the samples with hemagglutination inhibition.

Fluorescent focus-based microneutralization assay (FFA MN): To assess MN by anti-NA and anti-HA antibodies, an HA quantification-based MN assay with a longer incubation time was developed. Two-fold serial dilution of RDE and heat-treated test samples were mixed with an equal volume of influenza virus solution containing 100 TCID₅₀/well in neutralization medium (33016 MDCK protein free medium (PFM) [GIBCO, Thermo Fisher Scientific]), incubated for 1 h at 37°C with 5% CO₂. The MDCK 33016-PFM cells had been seeded on the preceding day as 2.5E4/well (2.5E6/plate) in cell growth medium (DM134; Irvine Scientific, Santa Ana, CA, USA). Next, 100 µL of serum-virus mixture was transferred from each well onto the confluent cell monolayer and incubated for 1 h at 37°C with 5% CO₂. The antibody/virus mixture was removed, and cells were washed twice and incubated for 5 days (37°C, 5% CO₂) with neutralizing media (100 µL/well) containing 2-fold serially diluted serum samples supplemented with TPCK-trypsin working stock (Sigma-Aldrich). The plates were examined by HA assay for MN titers. Subsequently, 50 µL of supernatant was transferred to each well and an equal volume of 0.5% TRBC was added and incubated at room temperature for 30 min. The presence of TRBC agglutination (no neutralization) or absence (neutralization) was observed and the highest serum dilution that protected cells from infection was taken used as the neutralization titer.

N3 enzyme-linked lectin assay (N3 ELLA): Sera were examined for NAI activity by ELLA as previously described (Couzens L. et al. *J. Virol. Methods*. 2014; 210: 7-14). Briefly, NA from the homologous or heterologous vaccine strains was mixed with serial dilutions of heat-inactivated sera in buffer containing 33.3 mM MES (pH 6.5; Alfa Aesar, Haverhill, MA, USA), 4 mM calcium chloride (K-D Medical, Columbia, MD, USA), 0.5% Tween-20 (Thermo Fisher Scientific), and 1% BSA fraction V (Calbiochem, Sigma-Aldrich) in plates coated with fetuin (25 µg/mL in PBS). Following overnight incubation at 37°C, the cleavage of sialic acid was detected by peanut agglutinin-HRP conjugate (1 µg/mL in PBS, Thermo Fisher Scientific), developed with TMB (Thermo Fisher Scientific), and stopped with 2 N sulfuric acid (Sigma-Aldrich). Absorbance was measured on a Synergy H1 plate reader (BioTek Instruments, now Agilent Technologies, Winooski, VT, USA). NAI titer was determined by generating a nonlinear regression in GraphPad Prism (GraphPad Software) and calculating the reciprocal of the dilution that resulted in a 50% reduction in NA activity versus the no-serum controls.

Results: As shown in **Figure 2**, constructs C1-C6 are highly immunogenic in mice. This is demonstrated by high antibody titers observed in HAI, FFA and N3 ELLA assays in sera obtained from mice immunized with the constructs.

In particular, the GenS and GenA codon optimized constructs comprising the stabilized pre-fusion form of the HA protein (constructs C4 and C5) demonstrated greater immunogenicity as compared to constructs comprising the native form of HA.

The constructs also demonstrated greater immunogenicity as compared to that raised by recombinant H2 wildtype or fusion proteins (rH2 WT, rH2 Fu). Similar results were observed in the N3 ELLA assay when compared to recombinant N3 wildtype (rN3 WT) protein.

These data indicate that the pre-fusion stabilized form of the HA protein is highly immunogenic in mice and that modifications to the HA cleavage site to produce a pre-fusion stable conformation do not impact antigenicity or immunogenicity of the immunogen *in vivo*.

Example 3: Evaluation of immunogenicity of bicistronic H2N3 (avian and swine strain) saRNA constructs using a pseudotyped virus

The inventors sought to evaluate the immunogenicity of the various bicistronic H2N3 (avian strain) saRNA constructs, C7-C9 and C11-C13 as shown in **Figure 1** using a replication-deficient surrogate virus pseudotyped with the influenza antigens expressed by the recited constructs. These viral pseudotypes (PV) possess stable activity and can be utilized as target antigens in *in vitro* viral entry assays to assess vaccine immunogenicity.

Methods: Briefly, a HIV reporter virus pseudotyped with influenza antigens is produced by co-transfection of the recited influenza H2N3 constructs, and HAT (Human airway trypsin protease and TMPRSS2) plasmids together with a third-generation pseudotyping lentiviral viral backbone plasmid (e.g., HIV-1 based pHAGE Δenv ZsGreen/LUC) into HEK-293T cells. Pseudovirus is harvested post transfection and clarified and concentrated by filtration and ultracentrifugation in a 30% sucrose suspension for 2 hours at 32,000 RPM at 4°C. HA binding and NA activity were then assessed using the HA assay and ELLA assay as described in Example 2.

Results: As shown in **Fig 3**, the H2N3 constructs (both avian and swine strains) were shown to be highly immunogenic as demonstrated by the high HAI and ELLA titers observed.

In the HAI assay, all constructs were shown to be highly immunogenic with no statistically significant differences between the various constructs. Construct C9 (GenScript codon-optimized wildtype avian H2N3) and fusion-stabilised constructs such as C8 (avian H2N3 antigen comprising a R340A mutation in the H2 region) and C12 (GeneArt codon-optimized avian H2N3) demonstrated higher HAI titers (absolute values) for both the avian and swine strain as compared to the other constructs employed in a matched setting. Conversely, C8 and C11 (GeneArt codon-optimized wildtype avian

H2N3) demonstrated the lowest HAI titers (absolute values) in the group for the closely related swine strain. In the ELLA assay, constructs C9 and C12 were similarly amongst the highest-performing constructs, with C3 (GenScript codon-optimized wildtype swine H2N3) and C13 (GenScript polynucleotide codon-optimized manually mutated from C3) demonstrating the lowest ELLA titers (absolute values).

These data indicate that the avian H2N3 are highly immunogenic and cross-reactive.

Numbered paragraphs

1. A RNA comprising a nucleotide sequence encoding a modified hemagglutinin (HA) protein from an influenza virus, wherein the modified HA protein comprises at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.
2. The RNA of paragraph 1, wherein the modified HA additionally comprises a deletion of an amino acid in a fusion peptide
3. The RNA of paragraph 1 or paragraph 2, wherein the HA cleavage site is a polybasic cleavage site.
4. The RNA of paragraph 3, wherein the polybasic cleavage site prior to the at least one amino acid substitution or deletion comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.
5. The RNA of paragraph 4, wherein the modified HA protein has one or more basic amino acids deleted from the sequence X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-) K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.
6. The RNA of any one of paragraphs 3 to 5, wherein all the basic amino acids in the polybasic cleavage site except a most C-terminal R is/are deleted.
7. The RNA of any one of paragraphs 3 to 6, wherein the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).
8. The RNA of any one of paragraphs 3 to 7, wherein the sequence corresponding to the polybasic cleavage site after deletion of the basic amino acids consists of the sequence R-G-L (SEQ ID NO: 32).

9. The RNA of any one of paragraphs 3 to 7, wherein the modified HA additionally has an amino acid in the fusion peptide deleted.
10. The RNA of paragraph 9, wherein the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).
11. The RNA of paragraph 10, wherein the Q is deleted in the modified HA.
12. The RNA of paragraph 11, wherein the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).
13. The RNA of paragraph 3, wherein the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).
14. The RNA of paragraph 1, wherein the HA cleavage site is a monobasic cleavage site.
15. The RNA of paragraph 14, wherein the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue.
16. The RNA of paragraph 14 or paragraph 15, wherein the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
17. The RNA of any one of paragraphs 14 to 16, wherein the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
18. The RNA of any one of paragraphs 14 to 17, wherein the modified HA protein comprises the amino acid sequence of X-A-G-L (SEQ ID NO: 51), wherein X is any amino acid residue.
19. The RNA of any one of paragraphs 1 to 18, wherein upon expression of the modified HA protein it is maintained in a pre-fusion stabilized conformation.

20. The RNA of any one of paragraphs 1 to 19, wherein the modified HA is selected from a H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 subtype.
21. The RNA of paragraph 20, wherein the modified HA is a H2 subtype.
22. The RNA of any one of paragraphs 1 to 21, wherein the RNA comprises a further polynucleotide sequence encoding a neuraminidase.
23. The RNA of paragraph 22, wherein the neuraminidase is selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes.
24. The RNA of paragraph 22 or 23, wherein the neuraminidase is a N3 subtype.
25. The RNA of any one of paragraphs 22 to 24, wherein the neuraminidase is from the same strain as the modified HA.
26. The RNA of any one of paragraphs 22 to 25, wherein the neuraminidase is from a different strain as the modified HA.
27. The RNA of any one of paragraphs 1 to 26, wherein the influenza virus is an influenza A virus.
28. The RNA of any one of paragraphs 1 to 27, wherein the influenza virus is a chicken or swine strain.
29. The RNA of any one of paragraphs 22 to 28, wherein the RNA is operably linked to one or more regulatory sequences.
30. The RNA of paragraph 29, the RNA comprising:
 - (a) a first polynucleotide encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and
 - (b) a second polynucleotide encoding the neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES).
31. The RNA of paragraph 29 or paragraph 30, wherein the RNA comprises, in order from 5' to 3':
 - (a) a first polynucleotide encoding the modified HA from an influenza virus, operably linked to a SG promoter; and
 - (b) a second polynucleotide encoding the neuraminidase, operably linked to a SG promoter or an IRES.

32. The RNA of any one of paragraphs 29 to 31, wherein the RNA encodes a H2N3.
33. The RNA of any one of paragraphs 1 to 32, wherein the RNA is a monocistronic RNA.
34. The RNA of any one of paragraphs 1 to 32, wherein the RNA is a polycistronic RNA.
35. The RNA of paragraph 34, wherein the RNA is a bicistronic RNA.
36. The RNA of paragraph 30 or paragraph 31, wherein the SG promoter is a minimal SG promoter or an extended SG promoter.
37. The RNA of paragraph 36, wherein the SG promoter is a SGPv2 promoter.
38. The RNA of paragraph 37, wherein the SGPv2 promoter is encoded by a sequence set forth in SEQ ID NO: 6.
39. The RNA of anyone of paragraphs 30 to 38, 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- 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.
40. The RNA of paragraph 39 wherein the EMCV IRES is a wild-type IRES encoded by a sequence set forth in SEQ ID NO: 9.
41. The RNA of any one of paragraphs 1 to 40, wherein the RNA is a self-amplifying RNA.
42. The RNA of paragraph 41, wherein the self-replicating RNA comprises sequences from an alphavirus.

43. The RNA of paragraph 42, 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.
44. An isolated polynucleotide encoding the RNA of any one of paragraphs 1 to 43.
45. The isolated polynucleotide of paragraph 44, wherein the isolated polynucleotide is a recombinant DNA.
46. The isolated polynucleotide of paragraph 45 wherein the recombinant DNA is a vector.
47. The isolated polynucleotide of paragraph 46, wherein the vector is a Doggybone DNA.
48. The isolated polynucleotide of paragraph 45 or paragraph 46, wherein the recombinant DNA is a plasmid.
49. The isolated polynucleotide of any one of paragraphs 44 to 48, wherein the isolated polynucleotide encoding the RNA of any one of paragraphs 1 to 34 is operably linked to one or more regulatory sequences.
50. The isolated polynucleotide of paragraph 49, comprising:
 - (a) a first polynucleotide encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and
 - (b) a second polynucleotide encoding the neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES).
51. The isolated polynucleotide of paragraph 50, comprising, in order from 5' to 3':
 - (a) a first polynucleotide encoding the modified HA from an influenza virus, operably linked to a SG promoter; and
 - (b) a second polynucleotide encoding the neuraminidase, operably linked to a SG promoter or an IRES.
52. The isolated polynucleotide of any one of paragraphs 44 to 51, wherein the isolated polynucleotide comprises, consists of or consists essentially of a polynucleotide sequence set forth in SEQ ID NO: 10 or SEQ ID NO: 11, or a fragment, variant or derivative thereof.
53. A method of producing the RNA of any one of paragraphs 1 to 43, the method comprising (i) providing a DNA template encoding the RNA of any one of

paragraphs 1 to 43, (ii) in vitro transcribing the RNA from the DNA template; and (ii) purifying the RNA from step (ii).

54. The method of paragraph 53, wherein the DNA template is a Doggybone DNA.
55. A host cell comprising the RNA of any one of paragraphs 1 to 43, the isolated polynucleotide of any one of paragraphs 44 to 52.
56. An immunogenic composition comprising the RNA of any one of paragraphs 1 to 43.
57. The immunogenic composition of paragraph 56, wherein the immunogenic composition elicits an immune response when administered to a subject.
58. The immunogenic composition of paragraph 57, wherein the immunogenic composition comprises at least one further RNA encoding a further HA from a further influenza virus, wherein the at least one further HA is a different subtype to the modified HA.
59. The immunogenic composition of any one of paragraphs 56 to 58 for use in treating or preventing a disease in a subject in need thereof.
60. The immunogenic composition of paragraph 59, wherein the disease is an influenza virus infection.
61. The immunogenic composition of any one of paragraphs 56 to 60 wherein the RNA is contained in or otherwise associated with a lipid-based carrier.
62. The immunogenic composition of paragraph 61, wherein the lipid-based carrier is or comprises a lipid nanoparticle.
63. The immunogenic composition of paragraph 62, wherein the RNA is encapsulated in, bound to or adsorbed on the lipid nanoparticle.
64. A pharmaceutical composition comprising the RNA of any one of paragraphs 1 to 43 and a pharmaceutically acceptable carrier, diluent or excipient.
65. The pharmaceutical composition of paragraph 64, wherein the RNA is contained in or otherwise associated with a lipid-based carrier.

66. The pharmaceutical composition of paragraph 65, wherein the lipid-based carrier is or comprises a lipid nanoparticle (LNP).
67. The pharmaceutical composition of paragraph 66, wherein the RNA is encapsulated in, bound to or adsorbed on the LNP.
68. The immunogenic composition of any one of paragraphs 56 to 63 or the pharmaceutical composition of any one of paragraphs 64 to 67, for use as a vaccine.
69. A vaccine comprising the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 44 to 52 or the pharmaceutical composition of any one of paragraphs 64 to 67.
70. The vaccine of paragraph 69, wherein the vaccine elicits a protective immune response when administered to a subject.
71. The vaccine of paragraph 69 or paragraph 70, wherein the vaccine is a monovalent vaccine.
72. The vaccine of paragraph 69 or paragraph 70, wherein the vaccine is a multivalent vaccine.
73. The vaccine of paragraph 72, wherein the multivalent vaccine is a bivalent, trivalent or quadrivalent vaccine.
74. The vaccine of paragraph 72 or paragraph 73, wherein the vaccine comprises at least one further RNA comprising a polynucleotide encoding at least one further influenza antigen.
75. The vaccine of paragraph 72 or paragraph 73, wherein the vaccine comprises at least two further RNA comprising a polynucleotide encoding at least two further influenza antigens.
76. The vaccine of paragraph 72 or paragraph 73, wherein the vaccine comprises at least three further RNA comprising a polynucleotide encoding at least three further influenza antigens.
77. The vaccine of paragraph 74, wherein the at least one further influenza antigen is derived from an influenza A virus.

78. The vaccine of paragraph 74, wherein the at least one further influenza antigen is derived from an influenza B virus.
79. The vaccine of paragraph 75, wherein the vaccine comprises two influenza A virus strains and one influenza virus B strain.
80. The vaccine of paragraph 75, wherein the vaccine comprises two influenza A virus strains and two influenza virus B strains.
81. The vaccine of any one of paragraphs 69 to 80, wherein the at least one further RNA is a HA subtype selected from H1, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16 subtypes.
82. The vaccine of paragraph 81, wherein the at least one further RNA encodes a modified HA protein comprising at least one amino acid substitution relative to a native HA protein within a HA cleavage site.
83. The vaccine of paragraph 82, wherein the at least one further RNA encodes a modified HA protein additionally having an amino acid in a fusion peptide deleted.
84. The vaccine of paragraph 83, wherein the at least one further RNA encodes a modified HA comprising a polybasic cleavage site.
85. The vaccine of paragraph 84, wherein the polybasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.
86. The vaccine of paragraph 85, wherein the modified HA protein has one or more basic amino acids deleted from the sequence X₁-X₁-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-) K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.
87. The vaccine of any one of paragraphs 84 to 86, wherein all the basic amino acids in the polybasic cleavage site except a most C-terminal R is/are deleted.

88. The vaccine any one of paragraphs 84 to 87, wherein the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).
89. The vaccine of any one of paragraphs 84 to 88, wherein the modified HA additionally has an amino acid in the fusion peptide deleted.
90. The vaccine of paragraph 89, wherein the fusion peptide prior to the deletion comprises the sequence GLFGAIAAGFIEGGWQGMVDG (SEQ ID NO: 33).
91. The vaccine of paragraph 90, wherein the Q is deleted in the modified HA.
92. The vaccine of paragraph 91, wherein the fusion peptide following the deletion comprises the sequence GLFGAIAAGFIEGGWGMVDG (SEQ ID NO: 34).
93. The vaccine of paragraph 84, wherein the polybasic cleavage site of the modified HA protein comprising the at least one amino acid substitution comprises an amino acid sequence selected from R-X-K/R-A-G-L (SEQ ID NO: 43) or K-K/R-K/T-A-G-L (SEQ ID NO: 44), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).
94. The vaccine of paragraph 84, wherein the at least one further RNA encodes a modified HA comprising a monobasic cleavage site.
95. The vaccine of paragraph 94, wherein the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue.
96. The vaccine of paragraph 94 or 95, wherein the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
97. The vaccine of any one of paragraphs 94 to 96, wherein the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.

98. The vaccine of any one of paragraphs 94 to 97, wherein the modified HA protein comprises the amino acid sequence of X-A-G-L (SEQ ID NO: 51), wherein X is any amino acid residue.
99. The vaccine of any one of paragraphs 69 to 98, wherein upon expression of the at least one further RNA encoding a modified HA protein it is maintained in a pre-fusion stabilized conformation.
100. The vaccine of paragraph 99, wherein the at least one further RNA is a neuraminidase selected from N1, N2, N3, N4, N5, N6, N7, N8 and N9 subtypes.
101. The vaccine of paragraph 100, wherein the neuraminidase is from the same strain as the modified HA.
102. The vaccine of paragraph 101, wherein the neuraminidase is from a different strain as the modified HA.
103. The vaccine of any one of paragraphs 69 to 102 wherein the RNA is contained in or otherwise associated with a lipid-based carrier.
104. The vaccine of paragraph 103, wherein the lipid-based carrier is or comprises a lipid nanoparticle (LNP).
105. The vaccine of paragraph 104, wherein the RNA is encapsulated in, bound to or adsorbed on the LNP.
106. The vaccine of any one of paragraphs 69 to 105, wherein each RNA is formulated together in a LNP.
107. The vaccine of any one of paragraphs 69 to 105, wherein each RNA is formulated separately in a LNP.
108. The vaccine of any one of paragraphs 69 to 107, wherein the vaccine comprises an adjuvant.
109. The vaccine of paragraph 108, wherein the adjuvant is selected from a group consisting of Freund's adjuvant, incomplete Freund's adjuvants, aluminum phosphate, aluminum hydroxide, GMCSF, 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, squalane and squalene (or other oils of plant or animal origin), inclusive of squalene oil-in-water emulsions (e.g., MF59, AS03 and AF03), glycolipid analogs, octodecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, ODA, cytokines, and other adjuvants and derivatives thereof.

110. The vaccine of paragraph 108 or paragraph 109, wherein the adjuvant is MF59.
111. The vaccine of any one of paragraphs 69 to 110, wherein the vaccine further comprises a recombinant protein antigen.
112. The vaccine of paragraph 111, wherein the recombinant protein antigen is derived from a group comprising: influenza virus, coronavirus, respiratory syncytial virus (RSV), parainfluenza virus, rhinovirus, adenovirus or a combination thereof.
113. The RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112 for use in: (a) eliciting an immune response; and/or (b) preventing or treating a disease, disorder or condition in a subject.
114. A method of eliciting an immune response in a subject, said method including of step of administering a therapeutically or prophylactically effective amount of the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112, to the subject to thereby elicit the immune response.
115. A method of treating and/or preventing an influenza-associated disease, disorder or condition in a subject, the method comprising administering a therapeutically or prophylactically effective amount of the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112, to the subject.
116. Use of the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112 in the manufacture of a medicament for eliciting an immune response in a subject.

117. The use of paragraph 116, wherein the immune response is a neutralizing antibody response.
118. Use of the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112 in the manufacture of a medicament for treating and/or preventing a disease, disorder or condition in a subject in need thereof.
119. The use of paragraph 118, wherein the disease, disorder or condition is an influenza virus infection.
120. Use of the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112 in the manufacture of a medicament for treating and/or preventing an influenza-associated disease, disorder or condition in a subject.
121. The RNA of paragraph 113, the method of paragraph 114 or paragraph 115, or the use of any one of paragraphs 116 to 120, wherein the subject is human.
122. A kit comprising:
 - a) the RNA of any one of paragraphs 1 to 43, the immunogenic composition of any one of paragraphs 56 to 63, the pharmaceutical composition of any one of paragraphs 64 to 67 or the vaccine of any one of paragraphs 69 to 112;
 - b) instructions for use thereof; and optionally
 - c) a pharmaceutically acceptable carrier, excipient or diluent.

Sequence listing (for any discrepancy between this sequence listing and the filed ST26 file, this sequence listing is to take precedence)

SEQ ID NO. 1: Amino acid sequence of HA protein of A/Chicken/Ohio/494832/2007
MTITFLILLFTVVKGDQICIGYHANNSTEKVDTILERNVTVTTHAKDILEKTHNGK
LCRLSGIPPLELGDCSIAGWLLGNPECDRLLSVPEWSYIVEKENPVGGLCYPGSF
NDYEELKHLTSTVTHFEKVKILPRDQWTQHNTTGGSRACAVFGNPSFFRNMV
WLTKKGSNYPIAKRSYNNTSGEQMLIIVGVHHPNDDAEQRTLYQNVGTYYVSV
GTSTLNKRSIPEIATRPKVNGQGGRMEFSWTLLETWDVINFEETGNLIAPEYGF
KISKRGSSGIMKTEKTLNCECTRCQTPLGAINTTLPFHNIHPLTIGECPKYVKSDR
LVLATGLRNVPQIESRGLFGAIAFGIEGGWQGMVDGWYGYHHSNDQGSYAA
DKESTQKAIDGITNKVNSVIEKMNTQFEAVGKEFNLERLENLNKKMEDGFL
DVWTYNAELLVLMENERTLDFHDSNVRNLYDKVRMQLRDNAKEIGNGCFF
YHKCDDECMNSVRNGTYDYPKYEEESKLNREIKGVKLSDMGVYQILAIYAT
VAGSLSLAIMIAGISFWMCNGLSLQCRICI

SEQ ID NO. 2: Amino acid sequence of HA protein of A/Swine/Missouri/2124514/2006
MTITFLILLFTVVKGDQICIGYHANNSTEKVDTILERNVTVTTHAKNILEKTHNGK
LCRLSGIPPLELGDCSIAGWLLGNPECDRLLSVPEWSYIVEKENPVGGLCYPGSF
NDYEELKHLTSTVTHFEKVKILPRDQWTQHNTTGGSRACAVSGNPSFFRNMV
WLTKKGSNYPIAKRSYNNTSGEQMLVIWGIHHPNDDAEQRTLYQNVGTYYVSV
GTSTLNKRSIPEIATRPKVNLGGRMEFSWTLLETWDVINFEETGNLIAPEYGF
ISKRGSSGIMKTEKILENCECTKCQTPLGAINTTLPFHNIHPLTIGECPKYVKSDRLI
LATGLRNVPQIESRGLFGAIAFGIEGGWQGMVDGWYGYHHSNDQGSYAAD
KESTQKAIDGITNKVNSVIEKMNTQFEAVGKEFNLERLENLNKKMEDGFID
VWTYNAELLVLMENERTLDFHDSNVKNLYDKVRMQLRDNAKEIGNGCFFY
HKCDDECMNSVRNGTYDYIKYEEESKLNREIKGVKLSNMGVYQILAIYATV
AGSLSLAIMIAGISFWMCNGLSLQCRICI

SEQ ID NO. 3: Amino acid sequence of the modified HA protein of A/Chicken/Ohio/494832/2007
MTITFLILLFTVVKGDQICIGYHANNSTEKVDTILERNVTVTTHAKDILEKTHNGK
LCRLSGIPPLELGDCSIAGWLLGNPECDRLLSVPEWSYIVEKENPVGGLCYPGSF
NDYEELKHLTSTVTHFEKVKILPRDQWTQHNTTGGSRACAVFGNPSFFRNMV
WLTKKGSNYPIAKRSYNNTSGEQMLIIVGVHHPNDDAEQRTLYQNVGTYYVSV
GTSTLNKRSIPEIATRPKVNGQGGRMEFSWTLLETWDVINFEETGNLIAPEYGF
KISKRGSSGIMKTEKTLNCECTRCQTPLGAINTTLPFHNIHPLTIGECPKYVKSDR
LVLATGLRNVPQIESAGLFGAIAFGIEGGWQGMVDGWYGYHHSNDQGSYAA
DKESTQKAIDGITNKVNSVIEKMNTQFEAVGKEFNLERLENLNKKMEDGFL
DVWTYNAELLVLMENERTLDFHDSNVRNLYDKVRMQLRDNAKEIGNGCFF

YHKCDDECMNSVRNGTYDYPKYEEESKLNREIKGVKLSDMGVYQILAIYAT
VAGSLSLAIMIAGISFWMCSNGSLQCRICI

SEQ ID NO. 4: Amino acid sequence of the modified HA protein of A/Swine/Missouri/2124514/2006

MTITFLILLFTVVKGDQICIGYHANNSTEKVDITLERNVTVTTHAKNILEKTHNGK
LCRLSGIPPELGDCSIAGWLLGNPECDRLLSVPEWSYIVEKENPVNGLCYPGSF
NDYEELKHLLTSVTHFEKVILPRDQWTQHHTTGGSRACAVSGNPSFFRNMV
WLTKKGSNYPIAKRSYNNTSGEQMLVIWGIHPNDDAEQRTLYQNVGTYVSV
GTSTLNKRISPEIATRPKVNGLGGRMEFSWTLLTWDVINFEISTGNLIAPEYGFK
ISKRGSSGIMKTEKILENCETKCQTPLGAINTTLPFHNIHPLTIGECPKYVKSDRLI
LATGLRNVPQIESAGLFGAIAAGFIEGGWQGMVDGWYGYHHSNDQSGSYAAD
KESTQKAIDGITNKVNSVIEKMNTQFEAVGKEFNLERLENLNKKMEDGFID
VWTYNAELLVLMENERTLDFHDSNVKNLYDKVRMQLRDNAKEIGNGCFEFY
HKCDDECMNSVRNGTYDYIKYEEESKLNREIKGVKLSNMGVYQILAIYATV
AGSLSLAIMIAGISFWMCSNGSLQCRICI

SEQ ID NO. 5: Nucleotide sequence of extended subgenomic promoter (v1)
CTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAG

SEQ ID NO. 6: Nucleotide sequence of extended subgenomic promoter (v2)
GGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCT
AGTCCGCCAAG

SEQ ID NO. 7: Nucleotide sequence of extended subgenomic promoter (v3)
GGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTA
GTCCGCCAAG

SEQ ID NO. 8: Nucleotide sequence of extended subgenomic promoter (v4)
ACTCTAGCTAGCAGTGTTAAATCATTACGCTACCTGAGAGGGGCCCTATA
ACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAG

SEQ ID NO. 9: Nucleotide sequence of wild-type EMCV IRES
CCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTG
CGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGG
GCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTTTCCC
CTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTC
CTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGC
AGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTG
TATAAGATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTT

GGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGG
GGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGC
CTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGG
CCCCCGAACCACGGGGACGTGGTTTTTCCTTTGAAAAACACGATGATAATA
TGGCCACAACC

SEQ ID NO. 10: Nucleotide sequence of the modified HA protein of
A/Chicken/Ohio/494832/2007

ATGACCATTACCTTCCTGATCCTGCTGTTACCGTGGTGAAGGGCGACCAG
ATCTGCATCGGATACCACGCCAACAATAGCACCGAGAAGGTGGACACCATC
CTGGAGAGAAACGTGACCGTCAACCCACGCCAAGGACATCCTGGAAAAGAC
CCACAACGGCAAGCTGTGCCGGCTGAGCGGCATCCCTCCTCTGGAAGTGGG
AGATTGCTCTATCGCCGGCTGGCTGCTGGGCAATCCTGAGTGTGACCGGCT
GCTGAGCGTGCCAGAATGGAGCTACATAGTGGAAAAGGAAAACCCAGTGA
ACGGCCTGTGTTACCCCGGCTCTTTTAACGACTACGAGGAACTGAAGCACC
TGCTGACCAGCGTGACACATTTTCGAGAAGGTTAAAATCCTGCCTAGAGATC
AGTGGACCCAACACACCACAACCGGCGGAAGCAGAGCCTGCGCCGTGTTT
GGCAACCCCAGCTTCTTCAGAAATATGGTGTGGCTGACCAAGAAGGGTAGC
AACTACCCCATCGCTAAGCGGAGCTACAACAACACAAGCGGAGAGCAGAT
GCTGATCATCTGGGGCGTGACCAACCCCAACGACGACGCCGAACAGAGAA
CCCTGTACCAGAACGTGGGCACATATGTGTCCGTCGGCACCAGCACCCCTGA
ACAAAAGAAGCATCCCCGAGATCGCTACAAGACCTAAGGTGAATGGCCAG
GGCGGTAGAATGGAATTTAGCTGGACCCTGCTGGAGACTTGGGACGTGATC
AACTTCGAGAGCACAGGCAACCTGATCGCCCCTGAGTACGGCTTTAAGATC
AGCAAGAGGGGCAGTTCTGGAATCATGAAAACAGAGAAAACCCCTCGAAAA
TTGCGAGACAAGATGTCAGACCCCTCTGGGCGCTATCAACACAACACTGCC
CTTCCACAATATCCACCCCTTTACAATCGGCGAGTGTCTTAAGTACGTGAA
GTCTGATAGACTGGTCCTGGCTACAGGGCTGAGAAACGTGCCTCAGATCGA
GTCTGCAGGCCTTTTCGGCGCCATCGCCGGATTCATCGAGGGCGGTTGGCA
GGGCATGGTCGACGGCTGGTATGGCTACCACCACAGCAACGACCAGGGCA
GCGGCTATGCCGCTGATAAGGAATCCACACAGAAGGCCATCGACGGCATC
ACCAACAAGGTGAACAGCGTGATCGAGAAGATGAACACCCAATTTGAGGC
CGTGGGAAAAGAGTTCAACAACCTGGAGCGCAGACTGGAAAACCTGAACA
AGAAAATGGAAGATGGCTTCCTGGACGTGTGGACATACAACGCCGAGCTG
CTGGTGCTGATGGAAAACGAGCGGACCTTGGACTTCCACGACAGCAATGTG
CGGAATCTGTACGACAAGGTGCGGATGCAGCTGCGAGACAACGCCAAGGA
AATCGGCAACGGCTGCTTCGAGTTCTACCATAAGTGCGACGATGAGTGCAT
GAACAGCGTGCGGAATGGAACCTACGATTACCCTAAGTACGAGGAAGAAT
CTAAGCTGAATAGAAACGAGATTAAGGGAGTGAAACTGTCCGATATGGGC
GTGTACCAGATTCTGGCCATCTACGCCACCGTGGCCGGCTCCCTGAGCCTG

GCCATCATGATCGCCGGCATCAGCTTTTGGATGTGCAGCAACGGCTCTCTG
CAGTGCAGAATCTGTATCTGA

SEQ ID NO. 11: Nucleotide sequence of the modified HA protein of A/Swine/Missouri/2124514/2006

ATGACCATCACTTTTCTCATCCTCCTGTTACAGTAGTGAAAGGGGACCAA
ATATGCATCGGATACCATGCCAACAATTCCACAGAAAAAGTTGACACAATC
TTGGAACGAAACGTCACCGTGACTCATGCCAAGAACATTCTTGAAAAGACG
CATAATGGAAAGTTGTGCAGATTGAGCGGGATCCCTCCATTGGAAGTGGG
GATTGCAGCATTGCAGGTTGGCTCCTTGGAATCCGGAATGTGACCGGCTC
TTAAGTGTACCTGAATGGTCCTATATAGTGGAAAAGGAAAACCCGGTGAAT
GGTCTGTGCTATCCAGGCAGTTTCAATGATTATGAGGAATTGAAACATCTTC
TCACCAGTGTGACACACTTTGAGAAAGTTAAGATTCTGCCCAGAGATCAAT
GGACCCAGCACACAACAACCTGGTGGTTCTCGGGCCTGTGCAGTATCTGGAA
ACCCGTCATTCTTTAGGAACATGGTTTGGCTTACAAAGAAAGGGTCAAAC
ACCCAATTGCTAAAAGGTCATACAACAACACAAGTGGGGAGCAAATGCTG
GTAATATGGGGGATACATCACCCCAATGACGATGCGGAACAGAGGACACT
GTACCAGAATGTGGGAACATATGTTTCCGTTGGAACATCAACACTAAATAA
GAGGTCAATCCCTGAAATAGCAACAAGGCCCAAAGTCAATGGACTGGGAG
GAAGAATGGAATTCTCTTGGAAGTCTATTGGAGACATGGGATGTCATAAAT
TTGAGAGCACTGGTAATCTGATTGCACCAGAATACGGATTTAAAATATCAA
AGAGAGGAAGCTCAGGAATTATGAAGACAGAGAAAATACTTGAAAATTGT
GAAACCAAATGTCAGACCCCCTTGGGGGCAATAAATACAACATTGCCCTTT
CACAACATTCACCCATTGACAATAGGTGAGTGCCCCAAGTATGTAAAGTCA
GATAGACTGATTTTGGCGACAGGACTAAGAAATGTCCCCCAGATTGAATCA
GCCGGATTGTTTGGAGCAATAGCTGGGTTTATAGAAGGCGGATGGCAAGGG
ATGGTTGATGGCTGGTATGGGTACCATCACAGCAATGATCAAGGATCAGGA
TATGCAGCAGACAAAGAATCCACTCAAAAGGCAATTGATGGGATAACTAA
CAAAGTAAATTCTGTGATTGAAAAGATGAACACTCAGTTTGAGGCTGTTGG
GAAAGAGTTCAACAACCTAGAGAGAAGACTGGAAAACCTTAAATAAAAAGA
TGGAAGACGGCTTTATTGATGTATGGACATATAATGCCGAACCTCCTAGTTCT
AATGGAAAATGAGAGGACACTTGATTTCCATGATTCTAATGTGAAGAATCT
GTACGATAAGGTCAGAATGCAATTGAGAGACAATGCTAAGGAAATAGGGA
ACGGATGCTTTGAGTTTTATCATAAATGTGATGATGAATGCATGAATAGTG
TCAGGAATGGGACATATGATTATATCAAATATGAGGAAGAGTCCAAGCTGA
ACAGGAACGAAATCAAAGGAGTGAAATTGAGCAATATGGGGGTTTATCAA
ATACTTGCTATATACGCTACAGTTGCAGGCTCTTTGTCACTGGCAATCATGA
TAGCTGGGATTTCTTTCTGGATGTGTTCTAATGGGTCTCTGCAATGCAGAAT
TTGCATATGA

SEQ ID NO. 12: Nucleotide sequence of HA protein in construct C1

ATGACCATCACTTTTCTCATCCTCCTGTTACAGTAGTGAAAGGGGACCAA
ATATGCATCGGATACCATGCCAACAATTCCACAGAAAAAGTTGACACAATC
TTGGAACGAAACGTCACCGTGACTCATGCCAAGAACATTCTTGAAAAGACG
CATAATGGAAAGTTGTGCAGATTGAGCGGGATCCCTCCATTGGAACCTGGGG
GATTGCAGCATTGCAGGTTGGCTCCTTGGAATCCGGAATGTGACCGGCTC
TTAAGTGTACCTGAATGGTCCTATATAGTGGAAAAGGAAAACCCGGTGAAT
GGTCTGTGCTATCCAGGCAGTTTCAATGATTATGAGGAATTGAAACATCTTC
TCACCAGTGTGACACACTTTGAGAAAGTTAAGATTCTGCCCAGAGATCAAT
GGACCCAGCACACAACAACTGGTGGTTCTCGGGCCTGTGCAGTATCTGGAA
ACCCGTCATTCTTTAGGAACATGGTTTGGCTTACAAAGAAAGGGTCAAAC
ACCCAATTGCTAAAAGGTCATACAACAACACAAGTGGGGAGCAAATGCTG
GTAATATGGGGGATACATCACCCCAATGACGATGCGGAACAGAGGACACT
GTACCAGAATGTGGGAACATATGTTTCCGTTGGAACATCAACACTAAATAA
GAGGTCAATCCCTGAAATAGCAACAAGGCCCAAAGTCAATGGACTGGGAG
GAAGAATGGAATTCTCTTGGACTCTATTGGAGACATGGGATGTCATAAATT
TTGAGAGCACTGGTAATCTGATTGCACCAGAATACGGATTTAAAATATCAA
AGAGAGGAAGCTCAGGAATTATGAAGACAGAGAAAATACTTGAAAATTGT
GAAACCAAATGTCAGACCCCCCTGGGGGCAATAAATACAACATTGCCCTTT
CACAACATTACCCATTGACAATAGGTGAGTGCCCCAAGTATGTAAAGTCA
GATAGACTGATTTTGGCGACAGGACTAAGAAATGTCCCCCAGATTGAATCA
AGGGGATTGTTTGGAGCAATAGCTGGGTTTATAGAAGGCGGATGGCAAGG
GATGGTTGATGGCTGGTATGGGTACCATCACAGCAATGATCAAGGATCAGG
ATATGCAGCAGACAAAGAATCCACTCAAAAGGCAATTGATGGGATAACTA
ACAAAGTAAATTCTGTGATTGAAAAGATGAACACTCAGTTTGAGGCTGTTG
GGAAAGAGTTCAACAACCTAGAGAGAAGACTGGAAAACCTAAATAAAAAAG
ATGGAAGACGGCTTTATTGATGTATGGACATATAATGCCGAACCTCCTAGTT
CTAATGGAAAATGAGAGGACACTTGATTTCCATGATTCTAATGTGAAGAAT
CTGTACGATAAGGTCAGAATGCAATTGAGAGACAATGCTAAGGAAATAGG
GAACGGATGCTTTGAGTTTTATCATAAATGTGATGATGAATGCATGAATAG
TGTCAGGAATGGGACATATGATTATATCAAATATGAGGAAGAGTCCAAGCT
GAACAGGAACGAAATCAAAGGAGTGAAATTGAGCAATATGGGGGTTTATC
AAATACTTGCTATATACGCTACAGTTGCAGGCTCTTTGTCACTGGCAATCAT
GATAGCTGGGATTTCTTTCTGGATGTGTTCTAATGGGTCTCTGCAATGCAGA
ATTTGCATATGA

SEQ ID NO. 13: Nucleotide sequence of HA protein in construct C2

ATGACCATCACTTTTCTCATCCTCCTGTTACAGTAGTGAAAGGGGACCAA
ATATGCATCGGATACCATGCCAACAATTCCACAGAAAAAGTTGACACAATC
TTGGAACGAAACGTCACCGTGACTCATGCCAAGAACATTCTTGAAAAGACG
CATAATGGAAAGTTGTGCAGATTGAGCGGGATCCCTCCATTGGAACCTGGGG
GATTGCAGCATTGCAGGTTGGCTCCTTGGAATCCGGAATGTGACCGGCTC
TTAAGTGTACCTGAATGGTCCTATATAGTGGAAAAGGAAAACCCGGTGAAT
GGTCTGTGCTATCCAGGCAGTTTCAATGATTATGAGGAATTGAAACATCTTC
TCACCAGTGTGACACACTTTGAGAAAGTTAAGATTCTGCCCAGAGATCAAT
GGACCCAGCACACAACAACTGGTGGTTCTCGGGCCTGTGCAGTATCTGGAA
ACCCGTCATTCTTTAGGAACATGGTTTGGCTTACAAAGAAAGGGTCAAAC
ACCCAATTGCTAAAAGGTCATACAACAACACAAGTGGGGAGCAAATGCTG

GTAATATGGGGGATACATCACCCCAATGACGATGCGGAACAGAGGACACT
GTACCAGAATGTGGGAACATATGTTTCCGTTGGAACATCAACACTAAATAA
GAGGTCAATCCCTGAAATAGCAACAAGGCCCAAAGTCAATGGACTGGGAG
GAAGAATGGAATTCTCTTGGACTCTATTGGAGACATGGGATGTCATAAATT
TTGAGAGCACTGGTAATCTGATTGCACCAGAATACGGATTTAAAATATCAA
AGAGAGGAAGCTCAGGAATTATGAAGACAGAGAAAATACTTGAAAATTGT
GAAACCAAATGTCAGACCCCCCTTGGGGGCAATAAATACAACATTGCCCTTT
CACAACATTACCCATTGACAATAGGTGAGTGCCCCAAGTATGTAAAGTCA
GATAGACTGATTTTGGCGACAGGACTAAGAAATGTCCCCCAGATTGAATCA
GCCGGATTGTTTGGAGCAATAGCTGGGTTTATAGAAGGCGGATGGCAAGGG
ATGGTTGATGGCTGGTATGGGTACCATCACAGCAATGATCAAGGATCAGGA
TATGCAGCAGACAAAGAATCCACTCAAAAGGCAATTGATGGGATAACTAA
CAAAGTAAATTCTGTGATTGAAAAGATGAACACTCAGTTTGAGGCTGTTGG
GAAAGAGTTCAACAACCTAGAGAGAAGACTGGAAAACCTTAAATAAAAAGA
TGGAAGACGGCTTTATTGATGTATGGACATATAATGCCGAACCTCCTAGTTCT
AATGGAAAATGAGAGGACACTTGATTTCCATGATTCTAATGTGAAGAATCT
GTACGATAAGGTCAGAATGCAATTGAGAGACAATGCTAAGGAAATAGGGA
ACGGATGCTTTGAGTTTATCATAAATGTGATGATGAATGCATGAATAGTG
TCAGGAATGGGACATATGATTATATCAAATATGAGGAAGAGTCCAAGCTGA
ACAGGAACGAAATCAAAGGAGTGAAATTGAGCAATATGGGGGTTTATCAA
ATACTTGCTATATACGCTACAGTTGCAGGCTCTTTGTCACTGGCAATCATGA
TAGCTGGGATTTCCTTCTGGATGTGTTCTAATGGGTCTCTGCAATGCAGAAT
TTGCATATGA

SEQ ID NO. 14: Nucleotide sequence of HA protein in construct C3

ATGACCATCACCTTCCTGATCCTGCTGTTTACAGTGGTGAAGGGTGATCAG
ATCTGCATCGGCTATCACGCCAACAACAGCACCGAGAAAGTTGACACCATC
CTGGAAGGAACGTCACCGTTACTCATGCCAAAAACATCCTGGAAAAGACC
CACAATGGCAAGCTGTGTCCGGCTGAGCGGGATCCCCCCCCCTGGAGCTGGGA
GATTGCTCTATCGCCGGCTGGCTGCTCGGCAATCCAGAGTGCGACCGGCTG
CTGAGCGTGCCTGAGTGGTCTACATCGTGGAAAAAGAGAACCTGTGAAC
GGCCTTTGCTACCCCGGCTCTTTCAATGACTACGAGGAACTGAAGCACCTG
CTGACAAGCGTGACCCACTTCGAGAAAGTCAAAATTCTGCCTAGAGACCAG
TGGACCCAGCACACCACACAGGCGGCAGCAGAGCCTGCGCCGTGTCCGG
CAATCCTAGCTTCTTCAGAAACATGGTGTGGCTGACAAAGAAGGGCTCTAA
CTACCCTATCGCCAAGAGGAGCTACAACAATACCAGCGGAGAGCAGATGC
TGGTGATCTGGGGCATCCACCACCCTAACGATGATGCCGAGCAACGGACCC
TGTACCAGAACGTGGGCACCTATGTGTCCGTGGGAACGAGCACACTGAACA
AGCGGAGCATCCCTGAAATTGCCACAAGACCTAAGGTGAATGGCCTGGGA
GGAAGAATGGAATTCAGCTGGACACTGCTGGAGACATGGGACGTGATAAA
CTTCGAGAGCACAGGCAACCTGATCGCGCCTGAGTACGGCTTCAAGATCTC
TAAGCGGGGCAGCAGCGGCATTATGAAAACAGAGAAAATCCTGGAAAAC
GCGAAACCAAGTGCCAAACACCACTGGGCGCTATCAACACAACCTCCCCT
TCCACAACATCCACCCTCTGACCATCGGCGAGTGTCTAAGTACGTGAAGT
CTGACAGACTGATCCTGGCTACCGGACTGAGAAATGTGCCCCAGATCGAGT
CTAGAGGCCTCTTCGGCGCCATTGCTGGCTTCATCGAAGGCGGCTGGCAGG
GCATGGTCGACGGCTGGTACGGCTACCATCACAGCAACGACCAGGGCTCCG

GATACGCCGCTGATAAGGAGAGCACCCAGAAAGCCATCGATGGCATCACC
AACAAGGTGAACTCTGTGATCGAAAAGATGAACACTCAATTCGAGGCTGTG
GGCAAGGAATTTAACAACCTGGAGCGCAGACTGGAAAACCTGAACAAGAA
GATGGAGGACGGCTTTATCGACGTGTGGACCTACAACGCCGAGCTGCTGGT
GCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAAGA
ACCTGTACGACAAGGTGCGGATGCAGCTGCGGGACAACGCCAAGGAAATC
GGCAACGGTTGTTTTGAATTCTACCACAAGTGTGACGACGAGTGCATGAAC
AGCGTGAGAAACGGAACCTACGATTACATCAAGTATGAGGAAGAGAGCAA
GCTGAATAGAAATGAGATCAAGGGAGTGAAGCTGTCCAACATGGGCGTGT
ACCAGATCCTGGCCATCTACGCCACCGTGGCCGGCTCCCTGTCTCTGGCCAT
CATGATCGCCGGAATCAGCTTCTGGATGTGCAGTAACGGCAGCCTGCAGTG
CAGAATCTGCATCTGA

SEQ ID NO. 15: Nucleotide sequence of HA protein in construct C4

ATGACCATCACCTTCCTGATCCTGCTGTTTACAGTGGTGAAGGGTGATCAG
ATCTGCATCGGCTATCACGCCAACAACAGCACCGAGAAAGTTGACACCATC
CTGGAAGGAACGTCACCGTTACTCATGCCAAAAACATCCTGGAAAAGACC
CACAATGGCAAGCTGTGTGCGGCTGAGCGGGATCCCCCCCCTGGAGCTGGGA
GATTGCTCTATCGCCGGCTGGCTGCTCGGCAATCCAGAGTGCGACCGGCTG
CTGAGCGTGCCTGAGTGGTCTACATCGTGGAAAAAGAGAACCCTGTGAAC
GGCCTTTGCTACCCCGGCTCTTTCAATGACTACGAGGAACTGAAGCACCTG
CTGACAAGCGTGACCCACTTCGAGAAAGTCAAAATTCTGCCTAGAGACCAG
TGGACCCAGCACACCACACAGGCGGCAGCAGAGCCTGCGCCGTGTCCGG
CAATCCTAGCTTCTTCAGAAACATGGTGTGGCTGACAAAGAAGGGCTCTAA
CTACCCTATCGCCAAGAGGAGCTACAACAATACCAGCGGAGAGCAGATGC
TGGTGATCTGGGGCATCCACCACCCTAACGATGATGCCGAGCAACGGACCC
TGTACCAGAACGTGGGGCACCTATGTGTCCGTGGGAACGAGCACACTGAACA
AGCGGAGCATCCCTGAAATTGCCACAAGACCTAAGGTGAATGGCCTGGGA
GGAAGAATGGAATTCAGCTGGACACTGCTGGAGACATGGGACGTGATAAA
CTTCGAGAGCACAGGCAACCTGATCGCGCCTGAGTACGGCTTCAAGATCTC
TAAGCGGGGCAGCAGCGGCATTATGAAAACAGAGAAAATCCTGGAAAAC
GCGAAACCAAGTGCCAAACACCACTGGGCGCTATCAACACAACCCTCCCCT
TCCACAACATCCACCCTCTGACCATCGGCGAGTGTCTTAAGTACGTGAAGT
CTGACAGACTGATCCTGGCTACCGGACTGAGAAATGTGCCCCAGATCGAGT
CTGCCGGCCTCTTCGGCGCCATTGCTGGCTTCATCGAAGGCGGCTGGCAGG
GCATGGTCGACGGCTGGTACGGCTACCATCACAGCAACGACCAGGGCTCCG
GATACGCCGCTGATAAGGAGAGCACCCAGAAAGCCATCGATGGCATCACC
AACAAGGTGAACTCTGTGATCGAAAAGATGAACACTCAATTCGAGGCTGTG
GGCAAGGAATTTAACAACCTGGAGCGCAGACTGGAAAACCTGAACAAGAA
GATGGAGGACGGCTTTATCGACGTGTGGACCTACAACGCCGAGCTGCTGGT
GCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAAGA
ACCTGTACGACAAGGTGCGGATGCAGCTGCGGGACAACGCCAAGGAAATC
GGCAACGGTTGTTTTGAATTCTACCACAAGTGTGACGACGAGTGCATGAAC
AGCGTGAGAAACGGAACCTACGATTACATCAAGTATGAGGAAGAGAGCAA
GCTGAATAGAAATGAGATCAAGGGAGTGAAGCTGTCCAACATGGGCGTGT
ACCAGATCCTGGCCATCTACGCCACCGTGGCCGGCTCCCTGTCTCTGGCCAT

CATGATCGCCGGAATCAGCTTCTGGATGTGCAGTAACGGCAGCCTGCAGTG
CAGAATCTGCATCTGA

SEQ ID NO. 16: Nucleotide sequence of HA protein in construct C5

ATGACGATCACCTTCCTGATCCTGCTGTTACCGTGGTCAAGGGCGACCAG
ATCTGCATCGGCTACCACGCCAACAACAGCACCGAGAAGGTGGACACCATC
CTGGAACGGAACGTGACCGTGACACACGCCAAGAATATCCTGGAAAAGAC
CCACAACGGCAAGCTGTGCCGGCTGTCTGGAATCCCTCCACTGGAAGTGGG
CGACTGTTCTATCGCTGGATGGCTGCTGGGCAACCCCGAGTGTGATAGACT
GCTGTCTGTGCCCCGAGTGGTCCTACATCGTGGAAGAAAGAAAACCCCGTGAA
CGGCCTGTGCTACCCCGGCAGCTTCAACGACTACGAGGAACTGAAGCACCT
CCTGACCAGCGTGACCCACTTCGAGAAAGTGAAGATCCTGCCTCGGGACCA
GTGGACCCAGCACACAACAACAGGCGGATCTAGAGCCTGTGCCGTGTCCGG
CAATCCAGCTTCTTCCGGAATATGGTCTGGCTGACCAAGAAGGGCAGCAA
CTACCCTATCGCCAAGCGGAGCTACAACAACACCAGCGGCGAGCAGATGCT
GGTCATCTGGGGAATCCACCATCCTAACGACGACGCCGAGCAGAGAACCCT
GTACCAGAATGTGGGCACCTACGTGTCCGTGGGCACCAGCACACTGAACAA
GAGATCTATCCCGAGATCGCCACCAGGCCTAAAGTGAATGGCCTCGGCGG
CAGAATGGAATTTCAGCTGGACCCTGCTGGAAACCTGGGACGTGATCAACTT
CGAGAGCACCGGCAACCTGATCGCCCCTGAGTACGGCTTCAAGATCAGCAA
GAGAGGCAGCAGCGGCATCATGAAGACCGAGAAGATTCTGGAAGAACTGCG
AGACAAAGTGTGAGACCCCTCTGGGCGCCATCAACACCACACTGCCCTTCC
ACAACATTACCCCTCTGACCATCGGCGAGTGCCCCAAATACGTGAAGTCCG
ACAGACTGATCCTGGCCACCGGCCTGAGAAATGTGCCCCAGATTGAATCTG
CCGGCCTGTTCCGAGCCATTGCCGGCTTTATTGAAGGCGGCTGGCAAGGCA
TGGTGGACGGATGGTACGGCTATCACACAGCAACGATCAAGGCAGCGGC
TACGCCGCCGACAAAGAGTCTACACAGAAGGCCATCGACGGCATCACCAA
CAAAGTGAACAGCGTGATCGAGAAGATGAACACCCAGTTCGAGGCCGTGG
GCAAAGAGTTCAACAACCTGGAAAGACGGCTGGAAAACCTCAACAAGAAA
ATGGAAGATGGCTTCATCGACGTGTGGACCTACAACGCCGAAGTGTGGTG
CTGATGGAAAACGAGCGGACCCTGGACTTCCACGACAGCAACGTGAAGAA
CCTGTACGACAAAGTGCGGATGCAGCTGCGGGACAACGCCAAAGAGATCG
GCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACGACGAGTGCATGAAGT
CCGTGCGGAACGGCACATACGACTACATTAAGTACGAGGAAGAGAGCAAG
CTGAACCGGAACGAGATCAAGGGCGTGAAGCTGAGCAACATGGGCGTGTA
CCAGATTCTGGCCATCTACGCCACAGTGGCCGGATCTCTGAGCCTGGCCAT
CATGATCGCCGGAATCAGCTTCTGGATGTGCAGCAACGGCAGCCTGCAGTG
CAGAATCTGCATCTGA

SEQ ID NO. 17: Nucleotide sequence of HA protein in construct C6

ATGACGATCACCTTCCTGATCCTGCTGTTACCGTGGTCAAGGGCGACCAG
ATCTGCATCGGCTACCACGCCAACAACAGCACCGAGAAGGTGGACACCATC
CTGGAACGGAACGTGACCGTGACACACGCCAAGAATATCCTGGAAAAGAC
CCACAACGGCAAGCTGTGCCGGCTGTCTGGAATCCCTCCACTGGAAGTGGG
CGACTGTTCTATCGCTGGATGGCTGCTGGGCAACCCCGAGTGTGATAGACT
GCTGTCTGTGCCCCGAGTGGTCCTACATCGTGGAAGAAAGAAAACCCCGTGAA
CGGCCTGTGCTACCCCGGCAGCTTCAACGACTACGAGGAACTGAAGCACCT

CCTGACCAGCGTGACCCACTTCGAGAAAGTGAAGATCCTGCCTCGGGACCA
GTGGACCCAGCACACAACAACAGGCGGATCTAGAGCCTGTGCCGTGTCCGG
CAATCCCAGCTTCTTCCGGAATATGGTCTGGCTGACCAAGAAGGGCAGCAA
CTACCCTATCGCCAAGCGGAGCTACAACAACACCAGCGGCGAGCAGATGCT
GGTCATCTGGGGAATCCACCATCCTAACGACGACGCCGAGCAGAGAACCCT
GTACCAGAATGTGGGCACCTACGTGTCCGTGGGCACCAGCACACTGAACAA
GAGATCTATCCCCGAGATCGCCACCAGGCCTAAAGTGAATGGCCTCGGCGG
CAGAATGGAATTCAGCTGGACCCTGCTGGAAACCTGGGACGTGATCAACTT
CGAGAGCACCGGCAACCTGATCGCCCCTGAGTACGGCTTCAAGATCAGCAA
GAGAGGCAGCAGCGGCATCATGAAGACCGAGAAGATTCTGGAAAACCTGCG
AGACAAAGTGTGACACCCCTCTGGGCGCCATCAACACCACACTGCCCTTCC
ACAACATTACCCCTCTGACCATCGGCGAGTGCCCCAAATACGTGAAGTCCG
ACAGACTGATCCTGGCCACCGGCCTGAGAAATGTGCCCCAGATTGAATCTA
GAGGCCTGTTTCGGAGCCATTGCCGGCTTTATTGAAGGCGGCTGGCAAGGCA
TGGTGGACGGATGGTACGGCTATCACACAGCAACGATCAAGGCAGCGGC
TACGCCGCCGACAAAGAGTCTACACAGAAGGCCATCGACGGCATCACCAA
CAAAGTGAACAGCGTGATCGAGAAGATGAACACCCAGTTTCGAGGCCGTGG
GCAAAGAGTTCAACAACCTGGAAAGACGGCTGGAAAACCTCAACAAGAAA
ATGGAAGATGGCTTCATCGACGTGTGGACCTACAACGCCGAACCTGCTGGTG
CTGATGGAAAACGAGCGGACCCTGGACTTCCACGACAGCAACGTGAAGAA
CCTGTACGACAAAGTGCGGATGCAGCTGCGGGACAACGCCAAAGAGATCG
GCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACGACGAGTGCATGAAC
CCGTGCGGAACGGCACATACGACTACATTAAGTACGAGGAAGAGAGCAAG
CTGAACCGGAACGAGATCAAGGGCGTGAAGCTGAGCAACATGGGCGTGTA
CCAGATTCTGGCCATCTACGCCACAGTGGCCGATCTCTGAGCCTGGCCAT
CATGATCGCCGGAATCAGCTTCTGGATGTGCAGCAACGGCAGCCTGCAGTG
CAGAATCTGCATCTGA

SEQ ID NO. 18: Nucleotide sequence of HA protein in construct C7

ATGACCATCACTTTTCTCATCCTCCTGTTTACAGTAGTGAAAGGGGACCAA
ATATGTATCGGATACCATGCCAACAATTCCACAGAAAAAGTTGACACAATC
TTGGAACGAAACGTCACCGTGACTCATGCCAAGGACATTCTTGAAAAGACG
CATAATGGAAGTTGTGCAGATTAAGCGGGATCCCTCCATTGGAACCTGGGG
GATTGCAGCATTGCAGGTTGGCTCCTTGGAATCCAGAGTGTGACCGGCTC
TTAAGTGTACCTGAATGGTCTATATAGTGGAAAAGGAAAACCCGGTGAAT
GGTCTGTGCTACCCAGGCAGTTTCAATGACTATGAGGAATTGAAACATCTC
CTCACCAGTGTGACACACTTTGAGAAAGTTAAGATCCTGCCTAGAGATCAA
TGGACCCAGCACACAACAACCTGGTGGTTCTCGGGCCTGTGCAGTATTTGGG
AATCCATCATTCTTCAGAAACATGGTCTGGCTTACAAAGAAGGGGTCAAAC
TACCCAATCGCTAAAAGGTCATACAACAACAAGTGGGGAGCAAATGCT
GATAATCTGGGGGGTACATCATCCAATGACGATGCAGAACAAAGGACAC
TGTACCAGAATGTGGGAACATATGTTTCCGTTGGAACATCAACACTAAATA
AGAGGTCAATCCCTGAAATAGCGACAAGGCCCAAAGTCAATGGACAAGGA
GGGAGAATGGAATTCTCTTGACTCTATTAGAGACATGGGATGTCATAAAT
TTTGAGAGCACTGGTAATCTGATTGCACCAGAATATGGTTTTAAATATCA
AAGAGAGGAAGCTCAGGAATTATGAAGACAGAGAAAACACTTGAAAATTG
TGAGACCAGATGCCAGACCCCCTTAGGGGCAATAAATACAACATTGCCCTT

TCACAACATTACCCATTGACAATAGGTGAGTGCCCCAAGTATGTCAAGTC
AGATAGACTGGTCTTGGCGACAGGACTAAGAAATGTTCCCTCAGATTGAATC
AAGGGGATTGTTTGGAGCAATAGCTGGGTTTATAGAAGGCGGATGGCAAG
GGATGGTTGATGGCTGGTATGGGTATCATCACAGCAATGATCAAGGATCAG
GATATGCAGCAGACAAAGAATCCACTCAAAAGGCAATCGATGGGATAACT
AACAAAGGTGAACTCTGTGATTGAAAAGATGAACACTCAGTTTGAGGCTGTT
GGGAAAGAGTTCAACAATCTAGAGAGAAGACTAGAAAACCTTAAATAAGAA
AATGGAAGATGGATTTCTCGATGTATGGACATACAATGCCGAACCTCCTAGT
TCTAATGGAAAATGAGAGGACACTTGATTTCATGACTCTAATGTGAGGAA
TCTGTACGATAAGGTCAGAATGCAACTGAGGGACAATGCTAAGGAAATAG
GGAACGGATGCTTTGAGTTTTATCATAAATGTGATGATGAATGCATGAACA
GTGTCAGGAATGGAACATATGATTATCCCAAATATGAAGAAGAGTCCAAGC
TGAACAGGAACGAAATCAAAGGGGTGAAATTGAGCGATATGGGGGTCTAT
CAAATACTTGCTATATACGCTACAGTTGCAGGCTCCTTGTCAGTGGCAATCA
TGATAGCTGGGATTTCTTTCTGGATGTGTTCTAATGGGTCTCTGCAATGCAG
AATTTGCATTTGA

SEQ ID NO. 19: Nucleotide sequence of HA protein in construct C8

ATGACCATCACTTTTCTCATCCTCTGTTTACAGTAGTGAAAGGGGACCAA
ATATGTATCGGATACCATGCCAACAATTCCACAGAAAAAGTTGACACAATC
TTGGAACGAAACGTCACCGTGACTCATGCCAAGGACATTCTTGAAAAGACG
CATAATGGAAAGTTGTGCAGATTAAGCGGGATCCCTCCATTGGAACCTGGGG
GATTGCAGCATTGCAGGTTGGCTCCTTGGAATCCAGAGTGTGACCGGCTC
TTAAGTGTACCTGAATGGTCCTATATAGTGGAAAAGGAAAACCCGGTGAAT
GGTCTGTGCTACCCAGGCAGTTTCAATGACTATGAGGAATTGAAACATCTC
CTCACCAGTGTGACACACTTTGAGAAAAGTTAAGATCCTGCCTAGAGATCAA
TGGACCCAGCACACAACAACCTGGTGGTTCTCGGGCCTGTGCAGTATTTGGG
AATCCATCATTCTTCAGAAACATGGTCTGGCTTACAAAGAAGGGGTCAAAC
TACCCAATCGCTAAAAGGTCATACAACAACAAGTGGGGGAGCAAATGCT
GATAATCTGGGGGGTACATCATCCCAATGACGATGCAGAACAAAGGACAC
TGTACCAGAATGTGGGAACATATGTTTCCGTTGGAACATCAACACTAAATA
AGAGGTCAATCCCTGAAATAGCGACAAGGCCCAAAGTCAATGGACAAGGA
GGGAGAATGGAATTCTCTTGACTCTATTAGAGACATGGGATGTCATAAAT
TTTGAGAGCACTGGTAATCTGATTGCACCAGAATATGGTTTTAAATATCA
AAGAGAGGAAGCTCAGGAATTATGAAGACAGAGAAAACACTTGAAAATTG
TGAGACCAGATGCCAGACCCCTTAGGGGCAATAAATACAACATTGCCCTT
TCACAACATTACCCATTGACAATAGGTGAGTGCCCCAAGTATGTCAAGTC
AGATAGACTGGTCTTGGCGACAGGACTAAGAAATGTTCCCTCAGATTGAATC
AGCCGGATTGTTTGGAGCAATAGCTGGGTTTATAGAAGGCGGATGGCAAGG
GATGGTTGATGGCTGGTATGGGTATCATCACAGCAATGATCAAGGATCAGG
ATATGCAGCAGACAAAGAATCCACTCAAAAGGCAATCGATGGGATAACTA
ACAAGGTGAACTCTGTGATTGAAAAGATGAACACTCAGTTTGAGGCTGTTG
GGAAAGAGTTCAACAATCTAGAGAGAAGACTAGAAAACCTTAAATAAGAAA
ATGGAAGATGGATTTCTCGATGTATGGACATACAATGCCGAACCTCCTAGTT
CTAATGGAAAATGAGAGGACACTTGATTTCATGACTCTAATGTGAGGAAT
CTGTACGATAAGGTCAGAATGCAACTGAGGGACAATGCTAAGGAAATAGG
GAACGGATGCTTTGAGTTTTATCATAAATGTGATGATGAATGCATGAACAG

TGTCAGGAATGGAACATATGATTATCCCAAATATGAAGAAGAGTCCAAGCT
GAACAGGAACGAAATCAAAGGGGTGAAATTGAGCGATATGGGGGTCTATC
AAATACTTGCTATATACGCTACAGTTGCAGGCTCCTTGTCAGTGGCAATCAT
GATAGCTGGGATTTCTTTCTGGATGTGTTCTAATGGGTCTCTGCAATGCAGA
ATTTGCATTTGA

SEQ ID NO. 20: Nucleotide sequence of HA protein in construct C9

ATGACCATTACCTTCCTGATCCTGCTGTTACCGTGGTGAAGGGCGACCAG
ATCTGCATCGGATACCACGCCAACAAATAGCACCGAGAAGGTGGACACCATC
CTGGAGAGAAACGTGACCGTCACCCACGCCAAGGACATCCTGAAAAAGAC
CCACAACGGCAAGCTGTGCCGGCTGAGCGGCATCCCTCCTCTGGAAGTGGG
AGATTGCTCTATCGCCGGCTGGCTGCTGGGCAATCCTGAGTGTGACCGGCT
GCTGAGCGTGCCAGAATGGAGCTACATAGTGGAAAAGGAAAACCCAGTGA
ACGGCCTGTGTTACCCCGGCTCTTTTAACGACTACGAGGAAGTGAAGCACC
TGCTGACCAGCGTGACACATTTTCGAGAAGGTTAAAATCCTGCCTAGAGATC
AGTGGACCCAACACACCACAACCGGCGGAAGCAGAGCCTGCGCCGTGTTT
GGCAACCCCAAGCTTCTTCAGAAATATGGTGTGGCTGACCAAGAAGGGTAGC
AACTACCCCATCGCTAAGCGGAGCTACAACAACACAAGCGGAGAGCAGAT
GCTGATCATCTGGGGCGTGACCAACCCCAACGACGACGCCGAACAGAGAA
CCCTGTACCAGAACGTGGGCACATATGTGTCCGTCCGCCACCAGCACCTGA
ACAAAAGAAGCATCCCCGAGATCGCTACAAGACCTAAGGTGAATGGCCAG
GGCGGTAGAATGGAATTTAGCTGGACCCTGCTGGAGACTTGGGACGTGATC
AACTTCGAGAGCACAGGCAACCTGATCGCCCCTGAGTACGGCTTTAAGATC
AGCAAGAGGGGCAGTTCTGGAATCATGAAAACAGAGAAAACCCTCGAAAA
TTGCGAGACAAGATGTCAGACCCCTCTGGGCGCTATCAACACAACACTGCC
CTTCCACAATATCCACCCTCTTACAATCGGCGAGTGTCTAAGTACGTGAA
GTCTGATAGACTGGTCCTGGCTACAGGGCTGAGAAACGTGCCTCAGATCGA
GTCTAGAGGCCTTTTCGGCGCCATCGCCGGATTTCATCGAGGGCGGTTGGCA
GGGCATGGTCGACGGCTGGTATGGCTACCACCACAGCAACGACCAGGGCA
GCGGCTATGCCGCTGATAAGGAATCCACACAGAAGGCCATCGACGGCATC
ACCAACAAGGTGAACAGCGTGATCGAGAAGATGAACACCCAATTTGAGGC
CGTGGGAAAAGAGTTCAACAACCTGGAGCGCAGACTGGAAAACCTGAACA
AGAAAATGGAAGATGGCTTCCTGGACGTGTGGACATACAACGCCGAGCTG
CTGGTGCTGATGGAAAACGAGCGGACCTTGGACTTCCACGACAGCAATGTG
CGAATCTGTACGACAAGGTGCGGATGCAGCTGCGAGACAACGCCAAGGA
AATCGGCAACGGCTGCTTCGAGTTCTACCATAAGTGCGACGATGAGTGCAT
GAACAGCGTGCGGAATGGAACCTACGATTACCCTAAGTACGAGGAAGAAT
CTAAGCTGAATAGAAACGAGATTAAGGGAGTGAACTGTCCGATATGGGC
GTGTACCAGATTCTGGCCATCTACGCCACCGTGGCCGGCTCCCTGAGCCTG
GCCATCATGATCGCCGGCATCAGCTTTTGGATGTGCAGCAACGGCTCTCTG
CAGTGCAGAATCTGTATCTGA

SEQ ID NO. 21: Nucleotide sequence of HA protein in construct C10

ATGACCATTACCTTCCTGATCCTGCTGTTACCGTGGTGAAGGGCGACCAG
ATCTGCATCGGATACCACGCCAACAAATAGCACCGAGAAGGTGGACACCATC
CTGGAGAGAAACGTGACCGTCACCCACGCCAAGGACATCCTGAAAAAGAC
CCACAACGGCAAGCTGTGCCGGCTGAGCGGCATCCCTCCTCTGGAAGTGGG

AGATTGCTCTATCGCCGGCTGGCTGCTGGGCAATCCTGAGTGTGACCGGCT
GCTGAGCGTGCCAGAATGGAGCTACATAGTGGAAAAGGAAAACCCAGTGA
ACGGCCTGTGTTACCCCGGCTCTTTTAACGACTACGAGGAACTGAAGCACC
TGCTGACCAGCGTGACACATTTTCGAGAAGGTTAAAATCCTGCCTAGAGATC
AGTGGACCCAACACACCACAACCGGCGGAAGCAGAGCCTGCGCCGTGTTT
GGCAACCCCAAGCTTCTTCAGAAATATGGTGTGGCTGACCAAGAAGGGTAGC
AACTACCCCATCGCTAAGCGGAGCTACAACAACACAAGCGGAGAGCAGAT
GCTGATCATCTGGGGCGTGACCAACCCCAACGACGACGCCGAACAGAGAA
CCCTGTACCAGAACGTGGGCACATATGTGTCCGTCCGGCACCAGCACCTGA
ACAAAAGAAGCATCCCCGAGATCGCTACAAGACCTAAGGTGAATGGCCAG
GGCGGTAGAATGGAATTTAGCTGGACCCTGCTGGAGACTTGGGACGTGATC
AACTTCGAGAGCACAGGCAACCTGATCGCCCCTGAGTACGGCTTTAAGATC
AGCAAGAGGGGCAGTTCTGGAATCATGAAAACAGAGAAAACCCTCGAAAA
TTGCGAGACAAGATGTCAGACCCCTCTGGGCGCTATCAACACAACACTGCC
CTTCCACAATATCCACCCTCTTACAATCGGCGAGTGTCTAAGTACGTGAA
GTCTGATAGACTGGTCCTGGCTACAGGGCTGAGAAACGTGCCTCAGATCGA
GTCTGCAGGCCTTTTCGGCGCCATCGCCGATTTCATCGAGGGCGGTTGGCA
GGGCATGGTCGACGGCTGGTATGGCTACCACCACAGCAACGACCAGGGCA
GCGGCTATGCCGCTGATAAGGAATCCACACAGAAGGCCATCGACGGCATC
ACCAACAAGGTGAACAGCGTGATCGAGAAGATGAACACCCAATTTGAGGC
CGTGGGAAAAGAGTTCAACAACCTGGAGCGCAGACTGGAAAACCTGAACA
AGAAAATGGAAGATGGCTTCCTGGACGTGTGGACATACAACGCCGAGCTG
CTGGTGCTGATGGAAAACGAGCGGACCTTGGACTTCCACGACAGCAATGTG
CGGAATCTGTACGACAAGGTGCGGATGCAGCTGCGAGACAACGCCAAGGA
AATCGGCAACGGCTGCTTCGAGTTCTACCATAAGTGCGACGATGAGTGCAT
GAACAGCGTGCGGAATGGAACCTACGATTACCCTAAGTACGAGGAAGAAT
CTAAGCTGAATAGAAACGAGATTAAGGGAGTGAAACTGTCCGATATGGGC
GTGTACCAGATTCTGGCCATCTACGCCACCGTGGCCGGCTCCCTGAGCCTG
GCCATCATGATCGCCGGCATCAGCTTTTGGATGTGCAGCAACGGCTCTCTG
CAGTGCAGAATCTGTATCTGA

SEQ ID NO. 22: Nucleotide sequence of HA protein in construct C11

ATGACGATCACCTTCCTGATCCTGCTGTTACCGTGGTCAAGGGCGACCAG
ATCTGCATCGGCTACCACGCCAACAACAGCACCGAGAAGGTGGACACCATC
CTGGAACGGAACGTGACCGTGACACACGCCAAGGACATTCTGGAAGAAC
CCACAACGGCAAGCTGTGCCGGCTGTCTGGAATCCCTCCACTGGAAGTGG
CGACTGTTCTATCGCTGGATGGCTGCTGGGCAACCCCGAGTGTGATAGACT
GCTGTCTGTGCCCCGAGTGGTCCTACATCGTGGAAAAGAAAACCCCGTGAA
CGGCCTGTGCTACCCCGGCAGCTTCAACGACTACGAGGAACTGAAGCACCT
CCTGACCAGCGTGACCCACTTCGAGAAAGTGAAGATCCTGCCTCGGGACCA
GTGGACCCAGCACACAACAACAGGCGGATCTAGAGCCTGCGCCGTGTTCCG
CAATCCCAGCTTCTTCCGGAACATGGTCTGGCTGACCAAGAAGGGCAGCAA
CTACCCTATCGCCAAGCGGAGCTACAACAACACCAGCGCGAGCAGATGCT
GATCATCTGGGGAGTGCATACCCCAACGACGACGCCGAACAGAGAACCC
TGTACCAGAACGTGGGCACCTATGTGTCCGTGGGCACAAGCACACTGAACA
AGCGCAGCATCCCCGAGATCGCCACCAGACCTAAAGTGAATGGCCAAGGC
GGCAGAATGGAATTCAGCTGGACCCTGCTGGAAACCTGGGACGTGATCAAC

TTCGAGAGCACCGGCAACCTGATCGCCCCTGAGTACGGCTTCAAGATCAGC
AAGAGAGGCAGCAGCGGCATCATGAAGACCGAGAAAACCCTGGAAAACCTG
CGAGACACGGTGTCTAGACACCTCTGGGCGCCATCAACACCACACTGCCCTT
CCACAACATTACCCCTCTGACCATCGGCGAGTGCCCCAAATACGTGAAGTC
CGACAGACTGGTGCTGGCCACCGGCCTGAGAAATGTGCCTCAGATCGAGAG
CAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGG
CATGGTGGACGGATGGTACGGCTATCACCACAGCAACGATCAAGGCAGCG
GCTACGCCGCCGACAAAGAGTCTACACAGAAGGCCATCGACGGCATCACC
AACAAAGTGAACAGCGTGATCGAGAAGATGAACACCCAGTTCGAGGCCGT
GGGCAAAGAGTTCAACAACCTGGAAAGACGGCTGGAAAACCTGAACAAGA
AAATGGAAGATGGCTTCCTGGACGTGTGGACCTACAACGCCGAACCTGCTGG
TGCTGATGGAAAACGAGCGGACCTGGACTTCCACGACAGCAACGTGCGG
AACCTGTACGACAAAGTGC GGATGCAGCTGCGGGACAACGCCAAAGAGAT
CGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACGACGAGTGCATGAA
CTCCGTGCGCAACGGCACCTACGACTACCCTAAGTACGAGGAAGAGAGCA
AGCTGAACCGGAACGAGATCAAGGGCGTGAAGCTGAGCGACATGGGCGTG
TACCAGATCCTGGCCATCTATGCCACAGTGGCCGGATCTCTGTCTCTGGCCA
TCATGATCGCCGAATCAGCTTCTGGATGTGCAGCAACGGCTCCCTGCAGT
GCAGAATCTGCATCTGA

SEQ ID NO. 23: Nucleotide sequence of HA protein in construct C12

ATGACGATCACCTTCCTGATCCTGCTGTTACCGTGGTCAAGGGCGACCAG
ATCTGCATCGGCTACCACGCCAACAACAGCACCGAGAAGGTGGACACCATC
CTGGAACGGAACGTGACCGTGACACACGCCAAGGACATTCTGGAAAAGAC
CCACAACGGCAAGCTGTGCCGGCTGTCTGGAATCCCTCCACTGGAACCTGGG
CGACTGTTCTATCGCTGGATGGCTGCTGGGCAACCCCGAGTGTGATAGACT
GCTGTCTGTGCCCCGAGTGGTCTACATCGTGGA AAAAGAAAACCCCGTGAA
CGGCCTGTGCTACCCCGGCAGCTTCAACGACTACGAGGAACCTGAAGCACCT
CCTGACCAGCGTGACCCACTTCGAGAAAGTGAAGATCCTGCCTCGGGACCA
GTGGACCCAGCACACAACAACAGGCGGATCTAGAGCCTGCGCCGTGTTCCGG
CAATCCCAGCTTCTTCCGGAACATGGTCTGGCTGACCAAGAAGGGCAGCAA
CTACCCTATCGCCAAGCGGAGCTACAACAACACCAGCGGCGAGCAGATGCT
GATCATCTGGGGAGTGCATCACCCCAACGACGACGCCGAACAGAGAACCC
TGTACCAGAACGTGGGCACCTATGTGTCCGTGGGCACAAGCACACTGAACA
AGCGCAGCATCCCCGAGATCGCCACCAGACCTAAAGTGAATGGCCAAGGC
GGCAGAATGGAATTCAGCTGGACCCTGCTGGAAACCTGGGACGTGATCAAC
TTCGAGAGCACCGGCAACCTGATCGCCCCTGAGTACGGCTTCAAGATCAGC
AAGAGAGGCAGCAGCGGCATCATGAAGACCGAGAAAACCCTGGAAAACCTG
CGAGACACGGTGTCTAGACACCTCTGGGCGCCATCAACACCACACTGCCCTT
CCACAACATTACCCCTCTGACCATCGGCGAGTGCCCCAAATACGTGAAGTC
CGACAGACTGGTGCTGGCCACCGGCCTGAGAAATGTGCCTCAGATCGAGAG
CGCAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGG
CATGGTGGACGGATGGTACGGCTATCACCACAGCAACGATCAAGGCAGCG
GCTACGCCGCCGACAAAGAGTCTACACAGAAGGCCATCGACGGCATCACC
AACAAAGTGAACAGCGTGATCGAGAAGATGAACACCCAGTTCGAGGCCGT
GGGCAAAGAGTTCAACAACCTGGAAAGACGGCTGGAAAACCTGAACAAGA
AAATGGAAGATGGCTTCCTGGACGTGTGGACCTACAACGCCGAACCTGCTGG

TGCTGATGGAAAACGAGCGGACCCTGGACTTCCACGACAGCAACGTGCGG
AACCTGTACGACAAAGTGCGGATGCAGCTGCGGGACAACGCCAAAGAGAT
CGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACGACGAGTGCATGAA
CTCCGTGCGCAACGGCACCTACGACTACCCTAAGTACGAGGAAGAGAGCA
AGCTGAACCGGAACGAGATCAAGGGCGTGAAGCTGAGCGACATGGGCGTG
TACCAGATCCTGGCCATCTATGCCACAGTGGCCGGATCTCTGTCTCTGGCCA
TCATGATCGCCGAATCAGCTTCTGGATGTGCAGCAACGGCTCCCTGCAGT
GCAGAATCTGCATCTGA

SEQ ID NO. 24: Nucleotide sequence of HA protein in construct C13

ATGACCATCACCTTCCTGATCCTGCTGTTTACAGTGGTGAAGGGTGATCAG
ATCTGCATCGGCTATCACGCCAACACAGCACCGAGAAAGTTGACACCATC
CTGGAAGGAACGTCACCGTTACTCATGCCAAAGACATCCTGGAAGAGACC
CACAATGGCAAGCTGTGTGCGGCTGAGCGGGATCCCCCCCCCTGGAGCTGGGA
GATTGCTCTATCGCCGGCTGGCTGCTCGGCAATCCAGAGTGCGACCGGCTG
CTGAGCGTGCCTGAGTGGTCTACATCGTGGAAAAAGAGAACCTGTGAAC
GGCCTTTGCTACCCCGGCTCTTTCAATGACTACGAGGAAGTGAAGCACCTG
CTGACAAGCGTGACCCACTTCGAGAAAGTCAAAATTCTGCCTAGAGACCAG
TGGACCCAGCACACCACACAGGCGGCAGCAGAGCCTGCGCCGTGTTCTGGC
AATCCTAGCTTCTTCAGAAACATGGTGTGGCTGACAAAGAAGGGCTCTAAC
TACCCTATCGCCAAGAGGAGCTACAACAATACCAGCGGAGAGCAGATGCT
GATCATCTGGGGCGTCCACCACCCTAACGATGATGCCGAGCAACGGACCCT
GTACCAGAACGTGGGCACCTATGTGTCCGTGGGAACGAGCACACTGAACA
AGCGGAGCATCCCTGAAATTGCCACAAGACCTAAGGTGAATGGCCAGGGA
GGAAGAATGGAATTCAGCTGGACACTGCTGGAGACATGGGACGTGATAAA
CTTCGAGAGCACAGGCAACCTGATCGCGCCTGAGTACGGCTTCAAGATCTC
TAAGCGGGGCAGCAGCGGCATTATGAAAACAGAGAAAACCTGGAAAACCT
GCGAAACCAGATGCCAAACACCACTGGGCGCTATCAACACAACCTCCCCT
TCCACAACATCCACCCTCTGACCATCGGCGAGTGTCTTAAGTACGTGAAGT
CTGACAGACTGGTGTGCTGGCTACCGGACTGAGAAATGTGCCCCAGATCGAGT
CTAGAGGCCTCTTCGGCGCCATTGCTGGCTTCATCGAAGGCGGCTGGCAGG
GCATGGTCGACGGCTGGTACGGCTACCATCACAGCAACGACCAGGGCTCCG
GATACGCCGCTGATAAGGAGAGCACCCAGAAAGCCATCGATGGCATCACC
AACAAGGTGAACTCTGTGATCGAAAAGATGAACACTCAATTTCGAGGCTGTG
GGCAAGGAATTTAACAACCTGGAGCGCAGACTGGAAAACCTGAACAAGAA
GATGGAGGACGGCTTTCTCGACGTGTGGACCTACAACGCCGAGCTGCTGGT
GCTGATGGAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAGAA
ACCTGTACGACAAGGTGCGGATGCAGCTGCGGGACAACGCCAAGGAAATC
GGCAACGGTTGTTTTGAATTCTACCACAAGTGTGACGACGAGTGCATGAAC
AGCGTGAGAAACGGAACCTACGATTACCCCAAGTATGAGGAAGAGAGCAA
GCTGAATAGAAATGAGATCAAGGGAGTGAAGCTGTCCGACATGGGCGTGT
ACCAGATCCTGGCCATCTACGCCACCGTGGCCGGCTCCCTGTCTCTGGCCAT
CATGATCGCCGAATCAGCTTCTGGATGTGCAGTAACGGCAGCCTGCAGTG
CAGAATCTGCATCTGA

SEQ ID NO. 25: Blank

SEQ ID NO. 26: Polybasic cleavage site

X₁-X₁-X₂-X₂-X₂-R-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO. 27: Polybasic cleavage site

(R-)(R-)R-X₂-K/R-R-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

SEQ ID NO. 28: Polybasic cleavage site

(R-)(R-)K-K/R-K/T-R-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

SEQ ID NO. 29: deleted sequence

X₁-X₁-X₂-X₂-X₂, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO. 30: deleted sequence

(R-)(R-)R-X₂-K/R, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

SEQ ID NO. 31: deleted sequence

(R-)(R-) K-K/R-K/T, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

SEQ ID NO. 32: mutant polybasic cleavage site

R-G-L

SEQ ID NO. 33: fusion peptide sequence

GLFGAIAGFIEGGWQGMVDG

SEQ ID NO. 34: mutant fusion peptide sequence

GLFGAIAGFIEGGWGMVDG

SEQ ID NO. 35: mutant polybasic cleavage site

(R-)(R-)R-X₁-K/R-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

SEQ ID NO. 36: mutant polybasic cleavage site

R-X₁-K/R-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 37: mutant polybasic cleavage site

(R-)(R-)K-K/R-K/T-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.

SEQ ID NO: 38: mutant polybasic cleavage site

K-K/R-K/T-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 39: mutant polybasic cleavage site

R-X₁-X₂-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 40: mutant polybasic cleavage site

K-X₂-K/T-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 41: mutant polybasic cleavage site

K-K/R-X₂-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 42: mutant polybasic cleavage site

K-X₂-X₂-X₂-G-L, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K), X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 43: mutant polybasic cleavage site

R-X-K/R-A-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 44: mutant polybasic cleavage site

K-K/R-K/T-A-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 45: mutant polybasic cleavage site

R-X-A-A-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 46: mutant polybasic cleavage site

K-A-K/T-A-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 47: mutant polybasic cleavage site

K-K/R-A-A-G-L, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 48: mutant polybasic cleavage site

K-A-A-A-G-L), wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 49: monobasic cleavage site

X-R-G-L, wherein X is any amino acid residue.

SEQ ID NO: 50: mutant monobasic cleavage site

X₁-X₂-G-L, wherein X₁ is any amino acid residue and wherein X₂ is any amino acid other than arginine (R) or lysine (K).

SEQ ID NO: 51: mutant monobasic cleavage site

X-A-G-L, wherein X is any amino acid residue.

SEQ ID NO: 52: deleted site

RRRKK

SEQ ID NO: 53: polybasic cleavage site

R-X-K/R-R, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

SEQ ID NO: 54: polybasic cleavage site

K-K/R-K/T-R, wherein X is any basic amino acid residue selected from arginine (R) or lysine (K).

CLAIMS:

1. A RNA comprising a nucleotide sequence encoding a modified hemagglutinin (HA) protein from an influenza virus, wherein the modified HA protein comprises at least one amino acid substitution or deletion relative to a native HA protein within a HA cleavage site.
2. The RNA of claim 1, wherein the modified HA additionally comprises a deletion of an amino acid in a fusion peptide
3. The RNA of claim 1, wherein the HA cleavage site is a polybasic cleavage site.
4. The RNA of claim 3, wherein the polybasic cleavage site prior to the at least one amino acid substitution or deletion comprises an amino acid sequence selected from X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-)K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent.
5. The RNA of claim 4, wherein:
 - (i) the modified HA protein has one or more basic amino acids deleted from the sequence X₁-X₁-X₂-X₂-X₂-R-G-L (SEQ ID NO: 26) or (R-)(R-)R-X₂-K/R-R-G-L (SEQ ID NO: 27) or (R-)(R-) K-K/R-K/T-R-G-L (SEQ ID NO: 28), wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K) and bracketed amino acids may individually or collectively be absent; or
 - (ii) all the basic amino acids in the polybasic cleavage site except a most C-terminal R is/are deleted; or
 - (iii) the amino acids X₁-X₁-X₂-X₂-X₂ (SEQ ID NO: 29) or (R-)(R-)R-X₂-K/R (SEQ ID NO: 30) or (R-)(R-) K-K/R-K/T (SEQ ID NO: 31) are deleted, wherein X₁ is any basic amino acid residue selected from arginine (R) or lysine (K) or is absent, X₂ is any basic amino acid residue selected from arginine (R) or lysine (K).
6. The RNA of claim 3, wherein the sequence corresponding to the polybasic cleavage site after deletion of the basic amino acids consists of the sequence R-G-L.
7. The RNA of claim 2, wherein the fusion peptide prior to the deletion comprises the sequence GLFGAIAGFIEGGWQGMVDG (SEQ ID NO: 33).

8. The RNA of claim 7, wherein the Q is deleted in the modified HA and/or wherein the fusion peptide following the deletion comprises the sequence GLFGAIAGFIEGGWGMVDG (SEQ ID NO: 34).
9. The RNA of claim 1, wherein the HA cleavage site is a monobasic cleavage site.
10. The RNA of claim 9, wherein the monobasic cleavage site prior to the at least one amino acid substitution comprises an amino acid sequence of X-R-G-L (SEQ ID NO: 49), wherein X is any amino acid residue.
11. The RNA of claim 9, wherein the modified HA protein comprises a substitution at position R340 wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2 and/or wherein the modified HA protein comprises a R340A substitution wherein the numbering is based on the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 2.
12. The RNA of claim 9, wherein the modified HA protein comprises the amino acid sequence of X-A-G-L (SEQ ID NO: 51), wherein X is any amino acid residue.
13. The RNA of claim 1, wherein the modified HA is selected from a H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 subtype.
14. The RNA of claim 13, wherein the modified HA is a H2 subtype.
15. The RNA of claim 1, wherein the RNA comprises a further polynucleotide sequence encoding a neuraminidase.
16. The RNA of claim 15, wherein the neuraminidase is a N3 subtype.
17. The RNA of claim 1, wherein the influenza virus is an influenza A virus.
18. The RNA of claim 1, wherein the influenza virus is a chicken or swine strain.
19. The RNA of any claim, wherein the RNA is operably linked to one or more regulatory sequences.
20. The RNA of claim 1, wherein the RNA is a self-amplifying RNA.
21. The RNA of claim 20, the RNA comprising:
 - (a) a first polynucleotide encoding the modified HA from an influenza virus, operably linked to a subgenomic (SG) promoter; and

(b) a second polynucleotide encoding a neuraminidase, operably linked to a SG promoter or an internal ribosome entry site (IRES).

22. A pharmaceutical composition comprising the RNA of claim 1 and a pharmaceutically acceptable carrier, diluent or excipient.
23. The pharmaceutical composition of claim 22, wherein the RNA is contained in or otherwise associated with a lipid-based carrier.
24. A method of eliciting an immune response in a subject, said method including of step of administering a therapeutically or prophylactically effective amount of the RNA of claim 1 to the subject to thereby elicit the immune response.
25. Use of the RNA of claim 1 the manufacture of a medicament for eliciting an immune response in a subject.