(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 September 2018 (20.09.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/170347 A1

(51) International Patent Classification:
A61K 31/7105 (2006.01) *A61K 39/12* (2006.01)
A61K 31/7115 (2006.01) *A61P 37/04* (2006.01)

(21) International Application Number:
PCT/US2018/022777

(22) International Filing Date:
16 March 2018 (16.03.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/473,202	17 March 2017 (17.03.2017)	US
62/473,174	17 March 2017 (17.03.2017)	US
62/473,219	17 March 2017 (17.03.2017)	US

(71) Applicant: **MODERNATX, INC.** [US/US]; 320 Bent Street, Cambridge, MA 02141 (US).

(72) Inventors: **CIARAMELLA, Giuseppe**; 1 Hawes Road, Sudbury, MA 01776 (US). **HIMANSU, Sunny**; 23 Fernway, Winchester, MA 01890 (US). **PRESNYAK, Vladimir**; 502 West River Road #49, Hooksett, NH 03106 (US). **BENENATO, Kerry**; 415 Lincoln Road, Sudbury, MA 01776 (US). **KUMARASINGHE, Ellalahewage, Sathyajith**; 15 Littleton Road, 4B, Harvard, MA 01451 (US).

(74) Agent: **LOCKHART, Helen, C.** et al.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2018/170347 A1

(54) Title: ZOONOTIC DISEASE RNA VACCINES

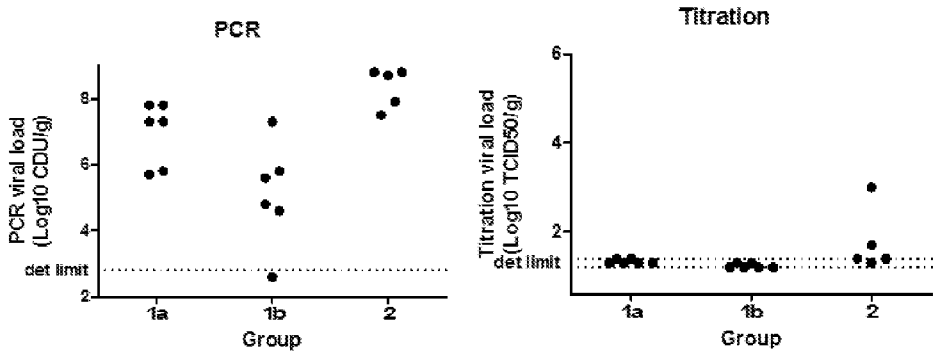


FIG. 15

(57) Abstract: The disclosure relates to Lassa virus, Nipah virus, and betacoronavirus ribonucleic acid vaccines as well as methods of using the vaccines and compositions comprising the vaccines.

ZOONOTIC DISEASE RNA VACCINES

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 62/473,174, filed March 17, 2017, U.S. provisional application number 62/473,202, filed March 17, 2017, and U.S. provisional application number 62/473,219, filed March 17, 2017, each of which is incorporated by reference herein in its entirety.

BACKGROUND

Zoonotic diseases are infectious diseases that are naturally transmitted from vertebrate animals to humans and vice versa. They are caused by all types of pathogenic agents, including bacteria, parasites, fungi, viruses and prions. In regions densely populated with both people and livestock, zoonotic diseases can spread very quickly. With changes in the environment, human behavior and habitat, increasingly these infections are emerging from wildlife species. Specific examples of zoonotic viruses include Lassa virus, Nipah virus, and betacoronaviruses.

Lassa Virus. Lassa virus (LASV), a segmented negative-sense RNA virus that belongs to the family Arenaviridae, is endemic to West Africa. Transmission typically occurs through contact with infected rodents or virus-contaminated rodent excreta, and person-to-person transmission. The LASV expresses just one protein on its surface, termed GPC, which mediates both attachment to and entry of host cells. GPC is a class I viral fusion protein that forms trimers on the viral surface. Each monomer in the trimer is assembled by distinct GP1 and GP2 subunits that mediate receptor binding and membrane fusion, respectively. Notably, on the viral surface, GP2 is coiled about the base of GP1 in a structure that is only metastable. The complex is prone to rapid disassembly of GP1 from GP2 and rearrangement of the GP2 into a much more stable six-helix bundle. The release of energy achieved by collapsing of the metastable viral-surface conformation to the much more stable six-helix bundle conformation drives fusion of viral and host membranes during infection. Because of its metastability, it is difficult to maintain GPC on its trimeric pre-fusion configuration when expressed recombinantly or even when expressed on some particle surfaces. Antibodies against the resulting separated subunits are not potently neutralizing. As a result, prior vaccine approaches that included natural GPC failed to elicit an effective antibody response, leading vaccine manufacturers to instead focus on induction of cell-mediated immunity as the most likely correlate of protection. Further, in the absence of knowledge about how to create or purify stabilized Lassa virus GPC trimeric, vaccine makers did not have the necessary reagents to evaluate the most ideal antibody responses.

The structure of the viral surface GP trimer remained unknown for Lassa and all other arenaviruses until this year. After a ten-year effort in engineering LASV GPC, using the GOC to evaluate human antibody responses from survivors, several high-resolution three-dimensional structures of the Lassa virus GPC in complex with these antibodies have been identified.

Nipah Virus. Nipah virus (NiV), of the genus henipahvirus (which includes Hendra virus) is part of the paramyxovirus family (see FIG. 7). Nipah first emerged in Malaysia in 1998, initially in domestic pigs and subsequently causing severe disease in humans, eventually killing over 1000 people. New outbreaks have occurred every year since, with fatality rates ranging from 40-70%. Nipah virus is classified as a BSL-4 agent and as a Category C priority pathogen by the CDC and NIAID. The primary reservoir is Pteropus bats; however, the virus is able to infect and replicate in many mammals (Luby et al 2013; Angeletti et al 2016).

There are no vaccines currently available against Nipah virus. Considering that the population of people that live in the same regions as pteropus bats is approximately 2 billion, the unmet need for a protective vaccine is high.

Coronavirus. Human Coronaviruses are highly contagious enveloped, positive single stranded RNA viruses of the Coronaviridae family. They are the common etiological agents of mild to moderate upper respiratory tract infections. However, novel coronaviruses such as Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) can result in severe lower respiratory tract infections and high mortality. MERS-CoV was first identified in 2012 within the Arabian Peninsula and since its initial outbreak, Sporadic MERS-CoV infections continue to appear within the Arabian Peninsula. The epidemiology of MERS-CoV infection in humans remains unclear and convoluted with Bats and Dromedary Camels being the major reservoirs for the virus. As of June 2016, the World Health Organization has reported a total of 1,769 MERS-CoV infections with a mortality rate of 36% and an ongoing risk of human to human transmission. The absence of a vaccine for MERS-CoV poses a severe global health threat due to its pandemic potential.

SUMMARY

Some aspects of the present disclosure provide zoonotic disease vaccines, comprising a ribonucleic acid (RNA) comprising an open reading frame (ORF) encoding an antigen selected from Lassa virus antigens, Nipah virus antigens, and betacoronavirus antigens, wherein intramuscular (IM) administration of a therapeutically effective amount of the vaccine to a subject induces an immune response in the subject.

In some embodiments, the ORF encodes a Lassa virus antigen.

In some embodiments, the Lassa virus antigen comprises a glycoprotein.

In some embodiments, the Lassa virus antigen comprises a Lassa virus glycoprotein precursor (GPC), a structurally stabilized Lassa virus GPC, an ectodomain of Lassa virus glycoprotein 1 (GP1), or a Lassa virus glycoprotein 2 (GP2).

In some embodiments, the Lassa virus antigen comprises amino acid residues 59-259 of a Lassa virus GPC.

In some embodiments, the Lassa virus antigen comprises a nucleocapsid protein (NP).

In some embodiments, the Lassa virus antigen has an amino acid sequence that has at least 90%, at least 95%, or at least 99% identity to an amino acid sequence identified by any one of SEQ ID NO: 1-3, but does not include wild-type protein sequence.

In some embodiments, the Lassa virus antigen has an amino acid sequence of any one of SEQ ID NO: 1-3.

In some embodiments, the RNA comprising an ORF sequence has at least 90%, at least 95%, or at least 99% identity to a nucleic acid sequence identified by any one of SEQ ID NO: 6, 7 or 9, but does not include wild-type protein sequence.

In some embodiments, the RNA comprising an ORF sequence comprises a nucleic acid sequence of any one of SEQ ID NO: 6, 7 or 9.

In some embodiments, the ORF encodes a Nipah virus antigen and/or a Hendra virus antigen.

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen comprises a hemagglutinin-neuraminidase protein (HN), a hemagglutinin protein (H), or a glycoprotein (G).

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen comprises an attachment glycoprotein, optionally a type II membrane protein.

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen comprises a fusion (F) glycoprotein.

In some embodiments, the F glycoprotein comprises a trimeric class I fusogenic envelope glycoprotein containing two heptad repeat (HR) regions and a hydrophobic fusion peptide.

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen is a Nipah virus antigen.

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen is a Hendra virus antigen.

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen has an amino acid sequence that has at least 90%, at least 95%, or at least 99% identity to an amino acid

sequence identified by any one of SEQ ID NO: 10-13 but does not include wild-type protein sequence.

In some embodiments, the Nipah virus antigen and/or a Hendra virus antigen has an amino acid sequence of any one of SEQ ID NO: 10-13.

In some embodiments, the RNA comprising an ORF sequence has at least 90%, at least 95%, or at least 99% identity to a nucleic acid sequence identified by SEQ ID NO: 16 or 17, but does not include wild-type protein sequence.

In some embodiments, the RNA comprising an ORF sequence comprises a nucleic acid sequence of SEQ ID NO: 16 or 17.

In some embodiments, the ORF encodes a middle east respiratory syndrome coronavirus (MERS-CoV) antigen and/or a severe acute respiratory syndrome-like coronavirus WIV1 (SL-CoV-WIV1) antigen.

In some embodiments, the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen comprises a betacoronavirus structural protein.

In some embodiments, the betacoronavirus structural protein is spike protein, envelope protein, nucleocapsid protein, or membrane protein.

In some embodiments, rein the betacoronavirus structural protein is spike protein.

In some embodiments, the betacoronavirus structural protein a S1 subunit of the spike protein or a S2 subunit of the spike protein.

In some embodiments, the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen is a MERS-CoV antigen.

In some embodiments, the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen is a SL-CoV-WIV1 antigen.

In some embodiments, wherein the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen has an amino acid sequence that has at least 90%, at least 95%, or at least 99% identity to an amino acid sequence identified SEQ ID NO: 18 but does not include wild-type protein sequence.

In some embodiments, the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen has an amino acid sequence of SEQ ID NO: 18.

In some embodiments, the RNA comprising an ORF sequence has at least 90%, at least 95%, or at least 99% identity to a nucleic acid sequence identified by SEQ ID NO: 18, but does not include wild-type protein sequence.

In some embodiments, the RNA comprising an ORF sequence comprises a nucleic acid sequence of SEQ ID NO: 18.

In some embodiments, IM administration of a therapeutically effective amount of the vaccine to a subject induces a neutralizing antibody titer in the subject.

In some embodiments, the neutralizing antibody titer is at least 100 neutralizing units per milliliter (NU/mL), at least 500 NU/mL, or at least 1000 NU/mL.

In some embodiments, the neutralizing antibody titer is sufficient to reduce viral infection of B cells by at least 50% relative to a neutralizing antibody titer of an unvaccinated control subject or relative to a neutralizing antibody titer of a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine.

In some embodiments, the neutralizing antibody titer is induced in the subject following fewer than three doses of the vaccine.

In some embodiments, a single dose is of 10 μg-100 μg.

In some embodiments, the neutralizing antibody titer and/or a T cell immune response is sufficient to reduce the rate of asymptomatic viral infection relative to the neutralizing antibody titer of unvaccinated control subjects.

In some embodiments, the neutralizing antibody titer and/or a T cell immune response is sufficient to prevent viral latency the subject.

In some embodiments, the neutralizing antibody titer is sufficient to block fusion of virus with epithelial cells and/or B cells of the subject.

In some embodiments, the neutralizing antibody titer is induced within 20 days following a single 10-100 μg of the vaccine, or within 40 days following a second 10-100 μg dose of the vaccine.

In some embodiments, IM administration of a therapeutically effective amount of the vaccine to a subject induces a T cell immune response in the subject.

In some embodiments, the T cell immune response comprises a $CD4^+$ T cell immune response and/or a $CD8^+$ T cell immune response.

In some embodiments, the antigen is expressed on the surface of cells of the subject.

In some embodiments, the vaccine comprises

(a) a ribonucleic acid (RNA) having an open reading frame (ORF) encoding two antigens, or (b) two RNAs, each having an ORF encoding an antigen.

In some embodiments, the vaccine comprises a RNA having an ORF encoding two antigens formulated in a lipid nanoparticle.

In some embodiments, the vaccine comprises two RNAs, each having an ORF encoding an antigen, wherein the two RNAs are formulated in a single lipid nanoparticle or wherein the each RNAs is formulated in a single lipid nanoparticle.

In some embodiments, the vaccine further comprises at least one additional RNA having an ORF encoding at least one additional antigen.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid, 25-55% sterol, and 0.5-15% PEG-modified lipid

In some embodiments, the antigen is fused to a signal peptide.

In some embodiments, the antigen is fused to a scaffold moiety.

In some embodiments, the scaffold moiety is selected from the group consisting of: ferritin, encapsulin, lumazine synthase, hepatitis B surface antigen, and hepatitis B core antigen.

In some embodiments, the RNA comprises messenger RNA (mRNA).

In some embodiments, the RNA further comprises a 5′ UTR and/or a 3′ UTR.

In some embodiments, the RNA is unmodified.

In some embodiments, the RNA comprise a modified nucleotide.

In some embodiments, at least 80% of the uracil in the ORF comprise 1-methyl-pseudouridine modification.

Some aspects of the present disclosure provide methods comprising administering to a subject the zoonotic disease vaccine in a therapeutically effective amount to induce an immune response in the subject.

In some embodiments, the therapeutically effective amount induces a neutralizing antibody titer and/or a T cell immune response in the subject.

In some embodiments, the vaccine is at least 80% relative to unvaccinated control subjects.

In some embodiments, detectable levels of the antigen are produced in the serum of the subject at 1-72 hours post administration of the vaccine.

In some embodiments, a neutralizing antibody titer of at least 100 NU/ml, at least 500 NU/ml, or at least 1000 NU/ml is produced in the serum of the subject at 1-72 hours post administration of the vaccine.

In some embodiments, the therapeutically effective amount is a total dose of 20 μg-200 μg or a total dose of 50 μg-100 μg.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

FIG. 1 shows the crystal structure of Lassa virus GPC in its trimeric, pre-fusion viral surface conformation. The three monomers are colored purple, orange and green, respectively, with the GP1 subunits in a light shade and GP2 subunits in a darker shade of each color. These structures illustrate the assembly surfaces of the trimer and quaternary epitopes at the base and apex that are formed only when the subunits assemble together in the trimer.

FIG. 2 shows anti-Ebola virus glycoprotein mouse IgG titers on 7 and 19 days post dose 2.

FIG. 3 shows the Ebola lethal challenge model study design. AG1 represents the designated Ebola GP mRNA vaccine, and AG2 represents the mRNA vaccine expressing wild type GP.

FIG. 4 shows mortality analysis of Guinea pigs in the Ebola challenge model.

FIG. 5 shows the average group weight loss post Ebola challenge.

FIG. 6 shows morbidity scores for individual animals.

FIG. 7 shows the paramyxovirus family.

FIG. 8 shows experimental design for the cotton rat challenge study.

FIG. 9 shows viral titers (top panel) and serum PIV3 neutralizing antibody titers (bottom panel) in cotton rats.

FIG. 10 shows viral titers (top panel) and serum PIV3 neutralizing antibody titers (bottom panel) in African green monkeys.

FIG. 11 shows VN titers in Balb/C mice after 2-dose immunization with MERS-CoV spike protein mRNA vaccine.

FIG. 12 shows VN titers against MERS-CoV after prime only (left), prime-boost (middle) or placebo (right) treatment. Individual values are shown as well as the geometric mean titer.

FIG. 13 shows MERS-CoV PCR and titration levels in nose swabs after challenge in prime only (left), prime-boost (middle) or placebo (right) treated animals. Panels A-C: Individual PCR values are shown as well as the lower limit of detection (1.2 log10 CDU/mL). Samples below the lower limit of detection are plotted as 1.1 log10 CDU/mL. Panels D-F:

Individual viral titration values are shown as well as the lower limit of detection (0.8 log10 TCID50/mL). Samples below the lower limit of detection are plotted as 0.7 log10 TCID50/mL.

FIG. 14 shows MERS-CoV PCR and titration levels in throat swabs after challenge in prime only (left), prime-boost (middle) or placebo (right) treated animals. Panels A-C: Individual PCR values are shown as well as the lower limit of detection (1.2 log10 CDU/mL). Samples below the lower limit of detection are plotted as 1.1 log10 CDU/mL. Panels D-F: Individual titration values are shown as well as the lower limit of detection (0.8 log10 TCID50/mL). Samples below the lower limit of detection are plotted as 0.7 log10 TCID50/mL.

FIG. 15 shows MERS-CoV PCR (left panel) and titration (right panel) results in pooled lung samples after challenge in prime only (1a), prime-boost (1b) or placebo (2) treated groups. Individual values are shown as well as the (range of the) lower limit of detection of PCR (2.8 log10 CDU/g) and virus titration (1.2 – 1.4 log10 TCID50/g).

DETAILED DESCRIPTION

Lassa Virus Vaccines

LASV (LASV) is an arenavirus (negative ssRNA) that represents a significant unmet global health care need. LASV expresses just one protein on its surface, termed GPC, which mediates both attachment to and entry of host cells. GPC is a class I viral fusion protein that forms trimers on the viral surface. Each monomer in the trimer is assembled by distinct GP1 and GP2 subunits that mediate receptor binding and membrane fusion, respectively. Notably, on the viral surface, GP2 is coiled about the base of GP1 in structure that is only metastable. The complex is prone to rapid disassembly of GP1 from GP2 and rearrangement of the GP2 into a much more stable six-helix bundle. The release of energy achieved by collapsing of the metastable viral-surface conformation to the much more stable six-helix bundle conformation drives fusion of viral and host membranes during infection. However, because of its metastability, it is difficult to maintain GPC on its trimeric pre-fusion configuration when expressed recombinantly or even when expressed on some particle surfaces. Antibodies against the resulting separated subunits are not potently neutralizing. As a result, prior vaccine approaches that included natural GPC failed to elicit an effective antibody response, leading vaccine manufacturers to instead focus on induction of cell-mediated immunity as the most likely correlate of protection. Further, in the absence of knowledge about how to create or purify stabilized LASV GPC trimeric, vaccine makers did not have the necessary reagents to evaluate the most ideal antibody responses.

The mRNA vaccines of the disclosure have been designed to express viral membrane bound proteins (B cell antigens) as well as intracellular proteins (T cell antigens). Arenaviruses

including LASV are pleomorphic enveloped viruses with membrane GP glycoprotein as the major surface antigen. In some respects the Lassa glycoprotein is a potent vaccine antigen with structural similarities to Ebola glycoproteins. The disclosure in some aspects includes, a mRNA vaccine expressing full length- membrane bound Lassa glycoprotein precursor GPC. The GPC precursor mRNA once translated will be matured through a natural process by the cellular proteases into the fully matured GP glycoprotein. The membrane anchored version of this protein will form trimers on cell surfaces and recognized by the immune system to generate humoral and cellular responses.

The most effective anti LASV antibodies are directed against a quaternary epitopes on GPC (those only formed when both GP1 and GP2 are intertwined, and three GP1-GP2 monomers form the proper trimer). Engineering and stabilization of GPC to firmly remain in this assembly allows recognition by the most potent human antibodies, and that the potent antibodies themselves are sufficient to provide post-exposure protection, even late in the disease course. The properly stabilized GPC trimer displays key quaternary epitopes that lead to broadly reactive, potent, and protective antibodies. The mRNA vaccines of the disclosure in some embodiments are designed to produce these unique stabilized GPCs in order to provoke production of the type and quality of neutralizing antibody necessary for eliminating the virus in the host.

Nipah Virus Vaccines

Nipah virus (NiV) and Hendra virus (HeV) are part of the paramyxovirus family. Virus-cell fusion by the paramyxoviruses is mediated by both an attachment protein (which can vary by genus) and a fusion (F) protein, which is well conserved throughout the family. There are currently no commercially available vaccines available against Nipah virus.

Parainfluenza virus 3 (PIV3, genus respirovirus), is closely related to Nipah virus. A mRNA vaccine against PIV3 encoding the PIV3 F protein, which exists functionally as a membrane bound trimer of two disulfide-linked subunits has been developed. Applicants have demonstrated that this PIV3 mRNA vaccine drives the efficient expression of this protein in its biologically relevant conformation, thus generating a robust neutralizing response.

Paramyxoviruses such as HeV and NiV possess two major membrane-anchored glycoproteins in the envelope of the viral particle. One glycoprotein is required for virion attachment to receptors on host cells and is designated as either hemagglutinin-neuraminidase protein (HN) or hemagglutinin protein (H), and the other is glycoprotein (G), which has neither hemagglutination nor neuraminidase activities. The attachment glycoproteins are type II

membrane proteins, where the molecule's amino (N) terminus is oriented toward the cytoplasm and the protein's carboxy (C) terminus is extracellular. The other major glycoprotein is the fusion (F) glycoprotein, which is a trimeric class I fusogenic envelope glycoprotein containing two heptad repeat (HR) regions and a hydrophobic fusion peptide. HeV and NiV infect cells though a pH-independent membrane fusion process into receptive host cells through the concerted action of their attachment G glycoprotein and F glycoprotein following receptor binding. The primary function of the HeV and NiV attachment G glycoprotein is to engage appropriate receptors on the surfaces of host cells, which for the majority of well-characterized paramyxoviruses are sialic acid moieties. The HeV and NiV G glycoproteins utilize the host cell protein receptors ephrin B2 and/or ephrin B3 and antibodies have been developed which block viral attachment by the G glycoprotein.

According to the disclosure, mRNA vaccines based on Nipah and Hendra F proteins have been developed. Additionally, soluble Nipah glycoprotein (G) vaccines and Hendra glycoprotein (G) vaccines are encompassed by the disclosure. In some aspects the vaccines may include F and G alone and/or in combination at different ratios.

The fusion glycoprotein (F) of Nipah virus mediates membrane fusion and is required for viral entry. Nipah F, like RSV F, is a class I fusion protein and they have similar structures and functions. The vaccines of the disclosure include stabilizing mutations to maintain the prefusion structure of Nipah F. Ideally stabilized mutants will maintain biophysical properties including structure and antigenicity.

Betacoronavirus Vaccines

Embodiments of the present disclosure provide RNA (e.g., mRNA) vaccines that include polynucleotide encoding a Middle East respiratory syndrome coronavirus (MERS-CoV) antigen and/or Bat SARS-like coronavirus WIV1, (SL-CoV-WIV1).

MERS-CoV is a positive-sense, single-stranded RNA virus of the genus Betacoronavirus. The genomes are phylogenetically classified into two clades, clade A and clade B. It has a strong tropism for non-ciliated bronchial epithelial cells, evades the innate immune response and antagonizes interferon (IFN) production in infected cells. Dipeptyl peptidase 4 (DDP4, also known as CD26) has been identified as a functional cellular receptor for MERS-CoV. Its enzymatic activity is not required for infection, although its amino acid sequence is highly conserved across species and is expressed in the human bronchial epithelium and kidneys. Most infected individuals develop severe acute respiratory illnesses, including fever, cough, and

shortness of breath, and the virus can be fatal. The disease may be transmitted among humans, generally among those in close contact.

Bat SARS-like coronavirus WIV1, (SL-CoV-WIV1) or SARS-like coronavirus WIV1 (WIV1), was isolated recently from Chinese rufous horseshoe bats. It is a single-stranded, enveloped, positive-sense RNA betacoronavirus. It has been demonstrated by phylogenetic analysis direct transmission of SARS from bats to humans may occur without intermediary Chinese civets.

The genome of MERS-CoV encodes at least four unique accessory proteins, such as 3, 4a, 4b and 5, two replicase proteins (open reading frame 1a and 1b), and four major structural proteins, including spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins (Almazan F et al. MBio 2013;4(5):e00650-13). The accessory proteins play nonessential roles in MERS-CoV replication, but they are likely structural proteins or interferon antagonists, modulating in vivo replication efficiency and/or pathogenesis, as in the case of SARS-CoV (Almazan F et al. MBio 2013;4(5):e00650-13; Totura AL et al. Curr Opin Virol 2012;2(3):264-75; Scobey T et al. Proc Natl Acad Sci USA 2013;110(40):16157-62). The other proteins of MERS-CoV maintain different functions in virus replication. The E protein, for example, involves in virulence, and deleting the E-coding gene results in replication-competent and propagation-defective viruses or attenuated viruses (Almazan F et al. MBio 2013;4(5):e00650-13). The S protein is particularly essential in mediating virus binding to cells expressing receptor dipeptidyl peptidase-4 (DPP4) through receptor-binding domain (RBD) in the S1 subunit, whereas the S2 subunit subsequently mediates virus entry via fusion of the virus and target cell membranes (Li F. J Virol 2015;89(4):1954-64; Raj VS et al. Nature 2013;495(7440):251-4).

In some aspects of the disclosure, the vaccine encodes the major antigenic component for MERS-CoV or SL-CoV-WIV1, the spike (S) glycoprotein. Spike protein is a typical type I viral fusion protein that exists as trimer on the viral surface with each monomer consisting of a Head (S1) and stem (S2) domain similar to influenza Hemagglutinin (HA). The S1 domain of the spike glycoprotein includes the receptor binding domain (RBD) that engages with the dipeptidyl peptidase-4 (DPP4) receptor and mediates viral fusion into the host cell, an N-terminal domain that may make initial contact with target cells, and 2 subdomains, all of which are susceptible to neutralizing antibodies. S2 domain consists of a six helix bundle fusion core involved in membrane fusion with the host endosomal membrane and is also a target for neutralization. Spike protein for betacoronaviruses has been shown to be an effective target for vaccines as antibodies against this protein are generated during natural infection and are protective in a

passive transfer animal model (REF). It has been demonstrated that mRNA vaccine for MERS-CoV elicits high levels of neutralizing antibodies and significantly reduces viral load in infected animals (see Examples).

The data demonstrate that expressing a stable trimeric Spike protein in its prefusion conformation (pre-S) (pre-S trimer) increases the magnitude and breadth of neutralizing activity against diverse strains of MERS CoV.

The zoonotic disease RNA vaccines described herein are superior to current vaccines in several ways. For example, the lipid nanoparticle (LNP) delivery system used herein increases the efficacy of RNA vaccines in comparison to other formulations, including a protamine-based approach described in the literature. The use of this LNP delivery system enables the effective delivery of chemically-modified RNA vaccines or unmodified RNA vaccines, without requiring additional adjuvant to produce a therapeutic result (e.g., production neutralizing antibody titer and/or a T cell response). In some embodiments, the zoonotic disease RNA vaccines disclosed herein are superior to conventional vaccines by a factor of at least 10 fold, 20, fold, 40, fold, 50 fold, 100 fold, 500 fold, or 1,000 fold when administered intramuscularly (IM) or intradermally (ID). These results can be achieved even when significantly lower doses of the RNA (e.g., mRNA) are administered in comparison with RNA doses used in other classes of lipid based formulations.

The LNP used in the studies described herein has been used previously to deliver siRNA in various animal models as well as in humans. In view of the observations made in association with the siRNA delivery of LNP formulations, the fact that LNP is useful in vaccines is quite surprising, particularly when immunity to an antigen has been hard to generate. It has been observed that therapeutic delivery of siRNA formulated in LNP causes an undesirable inflammatory response associated with a transient IgM response, typically leading to a reduction in antigen production and a compromised immune response. In contrast to the findings observed with siRNA, the LNP-mRNA formulations of the present disclosure are demonstrated herein to generate enhanced IgG levels, sufficient for prophylactic and therapeutic methods rather than transient IgM responses.

Exemplary Zoonotic Disease Antigens

Antigens are proteins capable of inducing an immune response (e.g., causing an immune system to produce antibodies against the antigens). Herein, use of the term antigen encompasses immunogenic proteins and immunogenic fragments (an immunogenic fragment that induces (or is capable of inducing) an immune response to a zoonotic disease antigen), unless otherwise

stated. It should be understood that the term "protein' encompasses peptides and the term "antigen" encompasses antigenic fragments.

A number of different antigens are associated with zoonotic diseases such as Lassa virus, Nipah virus, and betacoronavirus. Zoonotic disease vaccines, as provided herein, comprise at least one (one or more) ribonucleic acid (RNA, e.g., mRNA) having an open reading frame encoding at least one Lassa virus, Nipah virus, or betacoronavirus antigen. Non-limiting examples of zoonotic disease antigens are provided below.

Exemplary zoonotic disease antigens are provided in the Sequence Listing elsewhere herein. For example, the antigens may be encoded by (thus the RNA may comprise or consist of) any one of sequences set forth in SEQ ID NO: 6, 7, 9, 16, 17, or 20. In some embodiments, the antigens comprise a sequence set forth in SEQ ID NO: 1, 2, 3, 10, 11, 12, 13, or 18. In some embodiments, the aforementioned sequences may further comprise a 5' cap (e.g., 7mG(5')ppp(5')NlmpNp), a polyA tail, or a 5' cap and a polyA tail.

It should be understood that the zoonotic disease vaccines of the present disclosure may comprise any of the RNA open reading frames (ORFs), or encode any of the protein ORFs, described herein, with or without a signal sequence. It should also be understood that the zoonotic disease vaccines of the present disclosure may include any 5′ untranslated region (UTR) and/or any 3′ UTR. Any UTR sequence (e.g., of the prior art) may be used or exchanged for any of the UTR sequences described herein. UTRs may also be omitted from the vaccine constructs provided herein.

Nucleic Acids

The zoonotic disease vaccines of the present disclosure comprise at least one (one or more) ribonucleic acid (RNA) having an open reading frame encoding at least one zoonotic disease antigen. In some embodiments, the zoonotic disease antigen is a Lassa virus antigen. In some embodiments, the zoonotic disease antigen is a Nipah virus antigen. In some embodiments, the zoonotic disease antigen is a betcoronavirus antigen. In some embodiments, the RNA is a messenger RNA (mRNA) having an open reading frame encoding at least one zoonotic disease antigen. In some embodiments, the RNA (e.g., mRNA) further comprises a (at least one) 5′UTR, 3′UTR, a polyA tail and/or a 5′cap.

Nucleic acids comprise a polymer of nucleotides (nucleotide monomers), also referred to as polynucleotides. Nucleic acids may be or may include, for example, deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β- D-ribo

configuration, α-LNA having an α-L-ribo configuration (a diastereomer of LNA), 2′-amino-LNA having a 2′-amino functionalization, and 2′-amino- α-LNA having a 2′-amino functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) and/or chimeras and/or combinations thereof.

Messenger RNA (mRNA) is any ribonucleic acid that encodes a (at least one) protein (a naturally-occurring, non-naturally-occurring, or modified polymer of amino acids) and can be translated to produce the encoded protein *in vitro*, *in vivo*, *in situ* or *ex vivo*. The skilled artisan will appreciate that, except where otherwise noted, nucleic acid sequences set forth in the instant application may recite "T"s in a representative DNA sequence but where the sequence represents RNA (e.g., mRNA), the "T"s would be substituted for "U"s. Thus, any of the DNAs disclosed and identified by a particular sequence identification number herein also disclose the corresponding RNA (e.g., mRNA) sequence complementary to the DNA, where each "T" of the DNA sequence is substituted with "U."

It should be understood that the mRNA polynucleotides of the vaccines as provided herein are synthetic molecules, i.e., they are not naturally-occurring molecules. That is, the mRNA polynucleotides of the present disclosure are isolated mRNA polynucleotides. As is known in the art, "isolated polynucleotides" refer to polynucleotides that are substantially physically separated from other cellular material (e.g., separated from cells and/or systems that produce the polynucleotides) or from other material that hinders their use in the vaccines of the present disclosure. Isolated polynucleotides are substantially pure in that they have been substantially separated from the substances with which they may be associated in living or viral systems. Thus, mRNA polynucleotide vaccines are not associated with living or viral systems, such as cells or viruses. The mRNA polynucleotide vaccines do not include viral components (e.g., viral capsids, viral enzymes, or other viral proteins, for example, those needed for viral-based replication), and the mRNA polynucleotide vaccines are not packaged within, encapsulated within, linked to, or otherwise associated with a virus or viral particle. In some embodiments, the mRNA vaccines comprise a lipid nanoparticle that consists of, or consists essentially of, one or more mRNA polynucleotides (e.g., mRNA polynucleotides encoding one or more zoonotic viral antigen(s)).

An open reading frame (ORF) is a continuous stretch of DNA or RNA beginning with a start codon (e.g., methionine (ATG or AUG)) and ending with a stop codon (e.g., TAA, TAG or TGA, or UAA, UAG or UGA). An ORF typically encodes a protein. It will be understood that the sequences disclosed herein may further comprise additional elements, e.g., 5′ and 3′ UTRs,

but that those elements, unlike the ORF, need not necessarily be present in a vaccine of the present disclosure.

Variants

In some embodiments, an RNA of the present disclosure encodes a zoonotic disease antigen variant. Antigen or other polypeptide variants refers to molecules that differ in their amino acid sequence from a wild-type, native or reference sequence. The antigen/polypeptide variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants possess at least 50% identity to a wild-type, native or reference sequence. In some embodiments, variants share at least 80%, or at least 90% identity with a wild-type, native or reference sequence.

Variant antigens/polypeptides encoded by nucleic acids of the disclosure may contain amino acid changes that confer any of a number of desirable properties, e.g., that enhance their immunogenicity, enhance their expression, and/or improve their stability or PK/PD properties in a subject. Variant antigens/polypeptides can be made using routine mutagenesis techniques and assayed as appropriate to determine whether they possess the desired property. Assays to determine expression levels and immunogenicity are well known in the art and exemplary such assays are set forth in the Examples section. Similarly, PK/PD properties of a protein variant can be measured using art recognized techniques, e.g., by determining expression of antigens in a vaccinated subject over time and/or by looking at the durability of the induced immune response. The stability of protein(s) encoded by a variant nucleic acid may be measured by assaying thermal stability or stability upon urea denaturation or may be measured using in silico prediction. Methods for such experiments and *in silico* determinations are known in the art.

In some embodiments, a zoonotic disease vaccine comprises an mRNA ORF having a nucleotide sequence identified by any one of the sequences provided herein (see e.g., Sequence Listing), or having a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a nucleotide sequence identified by any one of the sequence provided herein.

The term “identity” refers to a relationship between the sequences of two or more polypeptides (e.g. antigens) or polynucleotides (nucleic acids), as determined by comparing the sequences. Identity also refers to the degree of sequence relatedness between or among sequences as determined by the number of matches between strings of two or more amino acid residues or nucleic acid residues. Identity measures the percent of identical matches between the

smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*e.g.*, "algorithms"). Identity of related antigens or nucleic acids can be readily calculated by known methods. "Percent (%) identity" as it applies to polypeptide or polynucleotide sequences is defined as the percentage of residues (amino acid residues or nucleic acid residues) in the candidate amino acid or nucleic acid sequence that are identical with the residues in the amino acid sequence or nucleic acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer programs for the alignment are well known in the art. It is understood that identity depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation. Generally, variants of a particular polynucleotide or polypeptide (e.g., antigen) have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F. Altschul, *et al* (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402). Another popular local alignment technique is based on the Smith-Waterman algorithm (Smith, T.F. & Waterman, M.S. (1981) "Identification of common molecular subsequences." *J. Mol. Biol.* 147:195-197). A general global alignment technique based on dynamic programming is the Needleman–Wunsch algorithm (Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." *J. Mol. Biol.* 48:443-453). More recently a Fast Optimal Global Sequence Alignment Algorithm (FOGSAA) has been developed that purportedly produces global alignment of nucleotide and protein sequences faster than other optimal global alignment methods, including the Needleman–Wunsch algorithm.

As such, polynucleotides encoding peptides or polypeptides containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences, in particular the polypeptide (e.g., antigen) sequences disclosed herein, are included within the scope of this disclosure. For example, sequence tags or amino acids, such as one or more lysines, can be added to peptide sequences (*e.g.*, at the N-terminal or C-terminal ends). Sequence tags can be used for peptide detection, purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (*e.g.*,

C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support. In some embodiments, sequences for (or encoding) signal sequences, termination sequences, transmembrane domains, linkers, multimerization domains (such as, *e.g.*, foldon regions) and the like may be substituted with alternative sequences that achieve the same or a similar function. In some embodiments, cavities in the core of proteins can be filled to improve stability, e.g., by introducing larger amino acids. In other embodiments, buried hydrogen bond networks may be replaced with hydrophobic resides to improve stability. In yet other embodiments, glycosylation sites may be removed and replaced with appropriate residues. Such sequences are readily identifiable to one of skill in the art. It should also be understood that some of the sequences provided herein contain sequence tags or terminal peptide sequences (*e.g.*, at the N-terminal or C-terminal ends) that may be deleted, for example, prior to use in the preparation of an RNA (*e.g.*, mRNA) vaccine.

As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of zoonotic disease antigens of interest. For example, provided herein is any protein fragment (meaning a polypeptide sequence at least one amino acid residue shorter than a reference antigen sequence but otherwise identical) of a reference protein, provided that the fragment is immunogenic and confers a protective immune response to the zoonotic disease pathogen. In addition to variants that are identical to the reference protein but are truncated, in some embodiments, an antigen includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations, as shown in any of the sequences provided or referenced herein. Antigens/antigenic polypeptides can range in length from about 4, 6, or 8 amino acids to full length proteins.

Stabilizing Elements

Naturally-occurring eukaryotic mRNA molecules can contain stabilizing elements, including, but not limited to untranslated regions (UTR) at their 5′-end (5′ UTR) and/or at their 3′-end (3′ UTR), in addition to other structural features, such as a 5′-cap structure or a 3′-poly(A) tail. Both the 5′ UTR and the 3′ UTR are typically transcribed from the genomic DNA and are elements of the premature mRNA. Characteristic structural features of mature mRNA, such as the 5′-cap and the 3′-poly(A) tail are usually added to the transcribed (premature) mRNA during mRNA processing.

In some embodiments, a vaccine includes at least one RNA polynucleotide having an open reading frame encoding at least one antigenic polypeptide having at least one modification,

at least one 5′ terminal cap, and is formulated within a lipid nanoparticle. 5′-capping of polynucleotides may be completed concomitantly during the in vitro-transcription reaction using the following chemical RNA cap analogs to generate the 5′-guanosine cap structure according to manufacturer protocols: 3′-O-Me-m7G(5')ppp(5') G [the ARCA cap];G(5')ppp(5')A; G(5')ppp(5')G; m7G(5')ppp(5')A; m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5′-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2′-O methyl-transferase to generate: m7G(5')ppp(5')G-2′-O-methyl. Cap 2 structure may be generated from the Cap 1 structure followed by the 2′-O-methylation of the 5′-antepenultimate nucleotide using a 2′-O methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2′-O-methylation of the 5′-preantepenultimate nucleotide using a 2′-O methyl-transferase. Enzymes may be derived from a recombinant source.

The 3′-poly(A) tail is typically a stretch of adenine nucleotides added to the 3′-end of the transcribed mRNA. It can, in some instances, comprise up to about 400 adenine nucleotides. In some embodiments, the length of the 3′-poly(A) tail may be an essential element with respect to the stability of the individual mRNA.

In some embodiments, zoonotic disease RNA vaccines may include one or more stabilizing elements. Stabilizing elements may include for instance a histone stem-loop. A stem-loop binding protein (SLBP), a 32 kDa protein has been identified. It is associated with the histone stem-loop at the 3'-end of the histone messages in both the nucleus and the cytoplasm. Its expression level is regulated by the cell cycle; it peaks during the S-phase, when histone mRNA levels are also elevated. The protein has been shown to be essential for efficient 3'-end processing of histone pre-mRNA by the U7 snRNP. SLBP continues to be associated with the stem-loop after processing, and then stimulates the translation of mature histone mRNAs into histone proteins in the cytoplasm. The RNA binding domain of SLBP is conserved through metazoa and protozoa; its binding to the histone stem-loop depends on the structure of the loop. The minimum binding site includes at least three nucleotides 5' and two nucleotides 3′ relative to the stem-loop.

In some embodiments, zoonotic disease RNA vaccines include a coding region, at least one histone stem-loop, and optionally, a poly(A) sequence or polyadenylation signal. The poly(A) sequence or polyadenylation signal generally should enhance the expression level of the encoded protein. The encoded protein, in some embodiments, is not a histone protein, a reporter

protein (*e.g.* Luciferase, GFP, EGFP, β-Galactosidase, EGFP), or a marker or selection protein (*e.g.* alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT)).

In some embodiments, the combination of a poly(A) sequence or polyadenylation signal and at least one histone stem-loop, even though both represent alternative mechanisms in nature, acts synergistically to increase the protein expression beyond the level observed with either of the individual elements. The synergistic effect of the combination of poly(A) and at least one histone stem-loop does not depend on the order of the elements or the length of the poly(A) sequence.

In some embodiments, zoonotic disease RNA vaccines do not comprise a histone downstream element (HDE). "Histone downstream element" (HDE) includes a purine-rich polynucleotide stretch of approximately 15 to 20 nucleotides 3′ of naturally occurring stem-loops, representing the binding site for the U7 snRNA, which is involved in processing of histone pre-mRNA into mature histone mRNA. In some embodiments, the nucleic acid does not include an intron.

In some embodiments, zoonotic disease RNA vaccines may or may not contain an enhancer and/or promoter sequence, which may be modified or unmodified or which may be activated or inactivated. In some embodiments, the histone stem-loop is generally derived from histone genes, and includes an intramolecular base pairing of two neighbored partially or entirely reverse complementary sequences separated by a spacer, consisting of a short sequence, which forms the loop of the structure. The unpaired loop region is typically unable to base pair with either of the stem loop elements. It occurs more often in RNA, as is a key component of many RNA secondary structures, but may be present in single-stranded DNA as well. Stability of the stem-loop structure generally depends on the length, number of mismatches or bulges, and base composition of the paired region. In some embodiments, wobble base pairing (non-Watson-Crick base pairing) may result. In some embodiments, the at least one histone stem-loop sequence comprises a length of 15 to 45 nucleotides.

In some embodiments, zoonotic disease RNA vaccines may have one or more AU-rich sequences removed. These sequences, sometimes referred to as AURES are destabilizing sequences found in the 3'UTR. The AURES may be removed from the RNA vaccines. Alternatively the AURES may remain in the RNA vaccine.

Signal Peptides

In some embodiments, a zoonotic disease vaccine comprises a RNA having an ORF that encodes a signal peptide fused to the zoonotic disease antigen. Signal peptides, comprising the

N-terminal 15-60 amino acids of proteins, are typically needed for the translocation across the membrane on the secretory pathway and, thus, universally control the entry of most proteins both in eukaryotes and prokaryotes to the secretory pathway. In eukaryotes, the signal peptide of a nascent precursor protein (pre-protein) directs the ribosome to the rough endoplasmic reticulum (ER) membrane and initiates the transport of the growing peptide chain across it for processing. ER processing produces mature proteins, wherein the signal peptide is cleaved from precursor proteins, typically by a ER-resident signal peptidase of the host cell, or they remain uncleaved and function as a membrane anchor. A signal peptide may also facilitate the targeting of the protein to the cell membrane.

A signal peptide may have a length of 15-60 amino acids. For example, a signal peptide may have a length of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids. In some embodiments, a signal peptide has a length of 20-60, 25-60, 30-60, 35-60, 40-60, 45- 60, 50-60, 55-60, 15-55, 20-55, 25-55, 30-55, 35-55, 40-55, 45-55, 50-55, 15-50, 20-50, 25-50, 30-50, 35-50, 40-50, 45-50, 15-45, 20-45, 25-45, 30-45, 35-45, 40-45, 15-40, 20-40, 25-40, 30-40, 35-40, 15-35, 20-35, 25-35, 30-35, 15-30, 20-30, 25-30, 15-25, 20-25, or 15-20 amino acids.

Signal peptides from heterologous genes (which regulate expression of genes other than zoonotic disease antigens in nature) are known in the art and can be tested for desired properties and then incorporated into a nucleic acid of the disclosure. In some embodiments, the signal peptide may comprise one of the following sequences: MDSKGSSQKGSRLLLLLVVSNLLLPQGVVG (SEQ ID NO: 21), MDWTWILFLVAAATRVHS (SEQ ID NO: 22); METPAQLLFLLLLWLPDTTG (SEQ ID NO: 23); MLGSNSGQRVVFTILLLLVAPAYS (SEQ ID NO: 24); MKCLLYLAFLFIGVNCA (SEQ ID NO: 25); MWLVSLAIVTACAGA (SEQ ID NO: 26).

Fusion Proteins

In some embodiments, a zoonotic disease RNA vaccine of the present disclosure includes an RNA encoding an antigenic fusion protein. Thus, the encoded antigen or antigens may include two or more proteins (e.g., protein and/or protein fragment) joined together. Alternatively, the protein to which a protein antigen is fused does not promote a strong immune response to itself, but rather to the zoonotic disease antigen. Antigenic fusion proteins, in some embodiments, retain the functional property from each original protein.

Scaffold Moieties

The RNA (e.g., mRNA) vaccines as provided herein, in some embodiments, encode fusion proteins which comprise zoonotic disease antigens linked to scaffold moieties. In some embodiments, such scaffold moieties impart desired properties to an antigen encoded by a nucleic acid of the disclosure. For example scaffold proteins may improve the immunogenicity of an antigen, e.g., by altering the structure of the antigen, altering the uptake and processing of the antigen, and/or causing the antigen to bind to a binding partner.

In some embodiments, the scaffold moiety is protein that can self-assemble into protein nanoparticles that are highly symmetric, stable, and structurally organized, with diameters of 10–150 nm, a highly suitable size range for optimal interactions with various cells of the immune system. In some embodiments, viral proteins or virus-like particles can be used to form stable nanoparticle structures. Examples of such viral proteins are known in the art. For example, in some embodiments, the scaffold moiety is a hepatitis B surface antigen (HBsAg). HBsAg forms spherical particles with an average diameter of ~22 nm and which lacked nucleic acid and hence are non-infectious (Lopez-Sagaseta, J. et al. *Computational and Structural Biotechnology Journal* 14 (2016) 58–68). In some embodiments, the scaffold moiety is a hepatitis B core antigen (HBcAg) self-assembles into particles of 24–31 nm diameter, which resembled the viral cores obtained from HBV-infected human liver. HBcAg produced in self-assembles into two classes of differently sized nanoparticles of 300 Å and 360 Å diameter, corresponding to 180 or 240 protomers. In some embodiments a zoonotic disease antigen is fused to HBsAG or HBcAG to facilitate self-assembly of nanoparticles displaying the zoonotic disease antigen.

In another embodiment, bacterial protein platforms may be used. Non-limiting examples of these self-assembling proteins include ferritin, lumazine and encapsulin.

Ferritin is a protein whose main function is intracellular iron storage. Ferritin is made of 24 subunits, each composed of a four-alpha-helix bundle, that self-assemble in a quaternary structure with octahedral symmetry (Cho K.J. et al. *J Mol Biol.* 2009;390:83–98). Several high-resolution structures of ferritin have been determined, confirming that *Helicobacter pylori* ferritin is made of 24 identical protomers, whereas in animals, there are ferritin light and heavy chains that can assemble alone or combine with different ratios into particles of 24 subunits (Granier T. et al. *J Biol Inorg Chem.* 2003;8:105–111; Lawson D.M. et al. *Nature.* 1991;349:541–544). Ferritin self-assembles into nanoparticles with robust thermal and chemical stability. Thus, the ferritin nanoparticle is well-suited to carry and expose antigens.

Lumazine synthase (LS) is also well-suited as a nanoparticle platform for antigen display. LS, which is responsible for the penultimate catalytic step in the biosynthesis of riboflavin, is an

enzyme present in a broad variety of organisms, including archaea, bacteria, fungi, plants, and eubacteria (Weber S.E. *Flavins and Flavoproteins*. Methods and Protocols, Series: Methods in Molecular Biology. 2014). The LS monomer is 150 amino acids long, and consists of beta-sheets along with tandem alpha-helices flanking its sides. A number of different quaternary structures have been reported for LS, illustrating its morphological versatility: from homopentamers up to symmetrical assemblies of 12 pentamers forming capsids of 150 Å diameter. Even LS cages of more than 100 subunits have been described (Zhang X. et al. *J Mol Biol.* 2006;362:753–770).

Encapsulin, a novel protein cage nanoparticle isolated from thermophile *Thermotoga maritima*, may also be used as a platform to present antigens on the surface of self-assembling nanoparticles. Encapsulin is assembled from 60 copies of identical 31 kDa monomers having a thin and icosahedral T = 1 symmetric cage structure with interior and exterior diameters of 20 and 24 nm, respectively (Sutter M. et al. *Nat Struct Mol Biol.* 2008, 15: 939-947). Although the exact function of encapsulin in *T. maritima* is not clearly understood yet, its crystal structure has been recently solved and its function was postulated as a cellular compartment that encapsulates proteins such as DyP (Dye decolorizing peroxidase) and Flp (Ferritin like protein), which are involved in oxidative stress responses (Rahmanpour R. et al. *FEBS J.* 2013, 280: 2097-2104).

Linkers and Cleavable Peptides

In some embodiments, the mRNAs of the disclosure encode more than one polypeptide, referred to herein as fusion proteins. In some embodiments, the mRNA further encodes a linker located between at least one or each domain of the fusion protein. The linker can be, for example, a cleavable linker or protease-sensitive linker. In some embodiments, the linker is selected from the group consisting of F2A linker, P2A linker, T2A linker, E2A linker, and combinations thereof. This family of self-cleaving peptide linkers, referred to as 2A peptides, has been described in the art (see for example, Kim, J.H. et al. (2011) *PLoS ONE* 6:e18556). In some embodiments, the linker is an F2A linker. In some embodiments, the linker is a GGGS linker. In some embodiments, the fusion protein contains three domains with intervening linkers, having the structure: domain-linker-domain-linker-domain.

Cleavable linkers known in the art may be used in connection with the disclosure. Exemplary such linkers include: F2A linkers,T2A linkers, P2A linkers, E2A linkers (See, e.g., WO2017/127750). The skilled artisan will appreciate that other art-recognized linkers may be suitable for use in the constructs of the disclosure (e.g., encoded by the nucleic acids of the disclosure). The skilled artisan will likewise appreciate that other polycistronic constructs

(mRNA encoding more than one antigen/polypeptide separately within the same molecule) may be suitable for use as provided herein.

Sequence Optimization

In some embodiments, an ORF encoding an antigen of the disclosure is codon optimized. Codon optimization methods are known in the art. For example, an ORF of any one or more of the sequences provided herein may be codon optimized. Codon optimization, in some embodiments, may be used to match codon frequencies in target and host organisms to ensure proper folding; bias GC content to increase mRNA stability or reduce secondary structures; minimize tandem repeat codons or base runs that may impair gene construction or expression; customize transcriptional and translational control regions; insert or remove protein trafficking sequences; remove/add post translation modification sites in encoded protein (*e.g.*, glycosylation sites); add, remove or shuffle protein domains; insert or delete restriction sites; modify ribosome binding sites and mRNA degradation sites; adjust translational rates to allow the various domains of the protein to fold properly; or reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art – non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA) and/or proprietary methods. In some embodiments, the open reading frame (ORF) sequence is optimized using optimization algorithms.

In some embodiments, a codon optimized sequence shares less than 95% sequence identity to a naturally-occurring or wild-type sequence ORF (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen). In some embodiments, a codon optimized sequence shares less than 90% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen). In some embodiments, a codon optimized sequence shares less than 85% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen). In some embodiments, a codon optimized sequence shares less than 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen). In some embodiments, a codon optimized sequence shares less than 75% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen).

In some embodiments, a codon optimized sequence shares between 65% and 85% (*e.g.*, between about 67% and about 85% or between about 67% and about 80%) sequence identity to a

naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen). In some embodiments, a codon optimized sequence shares between 65% and 75% or about 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a zoonotic disease antigen).

In some embodiments, a codon-optimized sequence encodes an antigen that is as immunogenic as, or more immunogenic than (*e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 100%, or at least 200% more), than a zoonotic disease antigen encoded by a non-codon-optimized sequence. In some embodiments, a codon-optimized sequence shares between 65% and 85% (e.g., between about 67% and about 85%, or between about 67% and about 80%) sequence identity to a naturally-occurring sequence or a wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (e.g., an antigenic protein or polypeptide)). In some embodiments, a codon-optimized sequence shares between 65% and 75%, or about 80% sequence identity to a naturally-occurring sequence or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (e.g., an antigenic protein or polypeptide)).

When transfected into mammalian host cells, the modified mRNAs have a stability of between 12-18 hours, or greater than 18 hours, *e.g.*, 24, 36, 48, 60, 72, or greater than 72 hours and are capable of being expressed by the mammalian host cells.

In some embodiments, a codon optimized RNA may be one in which the levels of G/C are enhanced. The G/C-content of nucleic acid molecules (*e.g.*, mRNA) may influence the stability of the RNA. RNA having an increased amount of guanine (G) and/or cytosine (C) residues may be functionally more stable than RNA containing a large amount of adenine (A) and thymine (T) or uracil (U) nucleotides. As an example, WO02/098443 discloses a pharmaceutical composition containing an mRNA stabilized by sequence modifications in the translated region. Due to the degeneracy of the genetic code, the modifications work by substituting existing codons for those that promote greater RNA stability without changing the resulting amino acid. The approach is limited to coding regions of the RNA.

Chemically Unmodified Nucleotides

In some embodiments, at least one RNA (e.g., mRNA) of a zoonotic disease vaccines of the present disclosure is not chemically modified and comprises the standard ribonucleotides consisting of adenosine, guanosine, cytosine and uridine. In some embodiments, nucleotides and

nucleosides of the present disclosure comprise standard nucleoside residues such as those present in transcribed RNA (e.g. A, G, C, or U). In some embodiments, nucleotides and nucleosides of the present disclosure comprise standard deoxyribonucleosides such as those present in DNA (e.g. dA, dG, dC, or dT).

Chemical Modifications

Zoonotic disease RNA vaccines of the present disclosure comprise, in some embodiments, at least one nucleic acid (e.g., RNA) having an open reading frame encoding at least one zoonotic disease antigen, wherein the nucleic acid comprises nucleotides and/or nucleosides that can be standard (unmodified) or modified as is known in the art. In some embodiments, nucleotides and nucleosides of the present disclosure comprise modified nucleotides or nucleosides. Such modified nucleotides and nucleosides can be naturally-occurring modified nucleotides and nucleosides or non-naturally occurring modified nucleotides and nucleosides. Such modifications can include those at the sugar, backbone, or nucleobase portion of the nucleotide and/or nucleoside as are recognized in the art.

In some embodiments, a naturally-occurring modified nucleotide or nucleotide of the disclosure is one as is generally known or recognized in the art. Non-limiting examples of such naturally occurring modified nucleotides and nucleotides can be found, inter alia, in the widely recognized MODOMICS database.

In some embodiments, a non-naturally occurring modified nucleotide or nucleoside of the disclosure is one as is generally known or recognized in the art. Non-limiting examples of such non-naturally occurring modified nucleotides and nucleosides can be found, inter alia, in published US application Nos. PCT/US2012/058519; PCT/US2013/075177; PCT/US2014/058897; PCT/US2014/058891; PCT/US2014/070413; PCT/US2015/36773; PCT/US2015/36759; PCT/US2015/36771; or PCT/IB2017/051367 all of which are incorporated by reference herein.

Hence, nucleic acids of the disclosure (*e.g.*, DNA nucleic acids and RNA nucleic acids, such as mRNA nucleic acids) can comprise standard nucleotides and nucleosides, naturally-occurring nucleotides and nucleosides, non-naturally-occurring nucleotides and nucleosides, or any combination thereof.

Nucleic acids of the disclosure (*e.g.*, DNA nucleic acids and RNA nucleic acids, such as mRNA nucleic acids), in some embodiments, comprise various (more than one) different types of standard and/or modified nucleotides and nucleosides. In some embodiments, a particular

region of a nucleic acid contains one, two or more (optionally different) types of standard and/or modified nucleotides and nucleosides.

In some embodiments, a modified RNA nucleic acid (*e.g.*, a modified mRNA nucleic acid), introduced to a cell or organism, exhibits reduced degradation in the cell or organism, respectively, relative to an unmodified nucleic acid comprising standard nucleotides and nucleosides.

In some embodiments, a modified RNA nucleic acid (*e.g.*, a modified mRNA nucleic acid), introduced into a cell or organism, may exhibit reduced immunogenicity in the cell or organism, respectively (*e.g.*, a reduced innate response) relative to an unmodified nucleic acid comprising standard nucleotides and nucleosides.

Nucleic acids (*e.g.*, RNA nucleic acids, such as mRNA nucleic acids), in some embodiments, comprise non-natural modified nucleotides that are introduced during synthesis or post-synthesis of the nucleic acids to achieve desired functions or properties. The modifications may be present on internucleotide linkages, purine or pyrimidine bases, or sugars. The modification may be introduced with chemical synthesis or with a polymerase enzyme at the terminal of a chain or anywhere else in the chain. Any of the regions of a nucleic acid may be chemically modified.

The present disclosure provides for modified nucleosides and nucleotides of a nucleic acid (*e.g.*, RNA nucleic acids, such as mRNA nucleic acids). A "nucleoside" refers to a compound containing a sugar molecule (*e.g.*, a pentose or ribose) or a derivative thereof in combination with an organic base (*e.g.*, a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase"). A "nucleotide" refers to a nucleoside, including a phosphate group. Modified nucleotides may by synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides. Nucleic acids can comprise a region or regions of linked nucleosides. Such regions may have variable backbone linkages. The linkages can be standard phosphodiester linkages, in which case the nucleic acids would comprise regions of nucleotides.

Modified nucleotide base pairing encompasses not only the standard adenosine-thymine, adenosine-uracil, or guanosine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures, such as, for example, in those nucleic acids having at least one chemical modification. One example of such non-standard base pairing is the base pairing between the

modified nucleotide inosine and adenine, cytosine or uracil. Any combination of base/sugar or linker may be incorporated into nucleic acids of the present disclosure.

In some embodiments, modified nucleobases in nucleic acids (*e.g.*, RNA nucleic acids, such as mRNA nucleic acids) comprise 1-methyl-pseudouridine (m1ψ), 1-ethyl-pseudouridine (e1ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), and/or pseudouridine (ψ). In some embodiments, modified nucleobases in nucleic acids (*e.g.*, RNA nucleic acids, such as mRNA nucleic acids) comprise 5-methoxymethyl uridine, 5-methylthio uridine, 1-methoxymethyl pseudouridine, 5-methyl cytidine, and/or 5-methoxy cytidine. In some embodiments, the polyribonucleotide includes a combination of at least two (*e.g.*, 2, 3, 4 or more) of any of the aforementioned modified nucleobases, including but not limited to chemical modifications.

In some embodiments, a RNA nucleic acid of the disclosure comprises 1-methyl-pseudouridine (m1ψ) substitutions at one or more or all uridine positions of the nucleic acid.

In some embodiments, a RNA nucleic acid of the disclosure comprises 1-methyl-pseudouridine (m1ψ) substitutions at one or more or all uridine positions of the nucleic acid and 5-methyl cytidine substitutions at one or more or all cytidine positions of the nucleic acid.

In some embodiments, a RNA nucleic acid of the disclosure comprises pseudouridine (ψ) substitutions at one or more or all uridine positions of the nucleic acid.

In some embodiments, a RNA nucleic acid of the disclosure comprises pseudouridine (ψ) substitutions at one or more or all uridine positions of the nucleic acid and 5-methyl cytidine substitutions at one or more or all cytidine positions of the nucleic acid..

In some embodiments, a RNA nucleic acid of the disclosure comprises uridine at one or more or all uridine positions of the nucleic acid.

In some embodiments, nucleic acids (*e.g.*, RNA nucleic acids, such as mRNA nucleic acids) are uniformly modified (*e.g.*, fully modified, modified throughout the entire sequence) for a particular modification. For example, a nucleic acid can be uniformly modified with 1-methyl-pseudouridine, meaning that all uridine residues in the mRNA sequence are replaced with 1-methyl-pseudouridine. Similarly, a nucleic acid can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as those set forth above.

The nucleic acids of the present disclosure may be partially or fully modified along the entire length of the molecule. For example, one or more or all or a given type of nucleotide (*e.g.*, purine or pyrimidine, or any one or more or all of A, G, U, C) may be uniformly modified in a nucleic acid of the disclosure, or in a predetermined sequence region thereof (*e.g.*, in the mRNA including or excluding the polyA tail). In some embodiments, all nucleotides X in a nucleic acid

of the present disclosure (or in a sequence region thereof) are modified nucleotides, wherein X may be any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

The nucleic acid may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, *i.e.*, any one or more of A, G, U or C) or any intervening percentage (*e.g.*, from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%). It will be understood that any remaining percentage is accounted for by the presence of unmodified A, G, U, or C.

The nucleic acids may contain at a minimum 1% and at maximum 100% modified nucleotides, or any intervening percentage, such as at least 5% modified nucleotides, at least 10% modified nucleotides, at least 25% modified nucleotides, at least 50% modified nucleotides, at least 80% modified nucleotides, or at least 90% modified nucleotides. For example, the nucleic acids may contain a modified pyrimidine such as a modified uracil or cytosine. In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the uracil in the nucleic acid is replaced with a modified uracil (*e.g.*, a 5-substituted uracil). The modified uracil can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures). In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the cytosine in the nucleic acid is replaced with a modified cytosine (*e.g.*, a 5-substituted cytosine). The modified cytosine can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures).

Untranslated Regions (UTRs)

The nucleic acids of the present disclosure may comprise one or more regions or parts which act or function as an untranslated region. Where nucleic acids are designed to encode at

least one antigen of interest, the nucleic may comprise one or more of these untranslated regions (UTRs). Wild-type untranslated regions of a nucleic acid are transcribed but not translated. In mRNA, the 5′ UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3′ UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the polynucleotides of the present disclosure to, among other things, enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites. A variety of 5'UTR and 3'UTR sequences are known and available in the art.

A 5' UTR is region of an mRNA that is directly upstream (5') from the start codon (the first codon of an mRNA transcript translated by a ribosome). A 5' UTR does not encode a protein (is non-coding). Natural 5′UTRs have features that play roles in translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG (SEQ ID NO: 27), where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'.5′UTR also have been known to form secondary structures which are involved in elongation factor binding.

In some embodiments of the disclosure, a 5' UTR is a heterologous UTR, i.e., is a UTR found in nature associated with a different ORF. In another embodiment, a 5' UTR is a synthetic UTR, i.e., does not occur in nature. Synthetic UTRs include UTRs that have been mutated to improve their properties, e.g., which increase gene expression as well as those which are completely synthetic. Exemplary 5' UTRs include *Xenopus* or human derived a-globin or b-globin (US8278063; US9012219), human cytochrome b-245 a polypeptide, and hydroxysteroid (17b) dehydrogenase, and Tobacco etch virus (US8278063, US9012219). CMV immediate-early 1 (IE1) gene (US2014/0206753, WO2013/185069), the sequence GGGAUCCUACC (SEQ ID NO: 28) (WO2014/144196) may also be used. In another embodiment, 5' UTR of a TOP gene is a 5' UTR of a TOP gene lacking the 5' TOP motif (the oligopyrimidine tract) (e.g., WO2015/101414, WO2015/101415, WO2015/062738, WO2015/024667, WO2015/024667; 5' UTR element derived from ribosomal protein Large 32 (L32) gene (WO2015/101414, WO2015/101415, WO2015/062738), 5' UTR element derived from the 5'UTR of an hydroxysteroid (17-β) dehydrogenase 4 gene (HSD17B4) (WO2015/024667), or a 5' UTR

element derived from the 5' UTR of ATP5A1 (WO2015/024667) can be used. In some embodiments, an internal ribosome entry site (IRES) is used instead of a 5' UTR.

A 3' UTR is region of an mRNA that is directly downstream (3') from the stop codon (the codon of an mRNA transcript that signals a termination of translation). A 3' UTR does not encode a protein (is non-coding). Natural or wild type 3′ UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) (SEQ ID NO: 29) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′ UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

Introduction, removal or modification of 3′ UTR AU rich elements (AREs) can be used to modulate the stability of nucleic acids (e.g., RNA) of the disclosure. When engineering specific nucleic acids, one or more copies of an ARE can be introduced to make nucleic acids of the disclosure less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using nucleic acids of the disclosure and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hour, 12 hour, 24 hour, 48 hour, and 7 days post-transfection.

3′ UTRs may be heterologous or synthetic. With respect to 3' UTRs, globin UTRs, including Xenopus β-globin UTRs and human β-globin UTRs are known in the art (US8278063, US9012219, US2011/0086907). A modified β-globin construct with enhanced stability in some cell types by cloning two sequential human β-globin 3'UTRs head to tail has been developed and is well known in the art (US2012/0195936, WO2014/071963). In addition a2-globin, a1-globin,

UTRs and mutants thereof are also known in the art (WO2015/101415, WO2015/024667). Other 3′ UTRs described in the mRNA constructs in the non-patent literature include CYBA (Ferizi et al., 2015) and albumin (Thess et al., 2015). Other exemplary 3′ UTRs include that of bovine or human growth hormone (wild type or modified) (WO2013/185069, US2014/0206753, WO2014/152774), rabbit β globin and hepatitis B virus (HBV), α-globin 3′ UTR and Viral VEEV 3' UTR sequences are also known in the art. In some embodiments, the sequence UUUGAAUU (WO2014/144196) is used. In some embodiments, 3′ UTRs of human and mouse ribosomal protein are used. Other examples include rps9 3'UTR (WO2015/101414), FIG4 (WO2015/101415), and human albumin 7 (WO2015/101415).

Those of ordinary skill in the art will understand that 5'UTRs that are heterologous or synthetic may be used with any desired 3' UTR sequence. For example, a heterologous 5'UTR may be used with a synthetic 3'UTR with a heterologous 3" UTR.

Non-UTR sequences may also be used as regions or subregions within a nucleic acid. For example, introns or portions of introns sequences may be incorporated into regions of nucleic acid of the disclosure. Incorporation of intronic sequences may increase protein production as well as nucleic acid levels.

Combinations of features may be included in flanking regions and may be contained within other features. For example, the ORF may be flanked by a 5' UTR which may contain a strong Kozak translational initiation signal and/or a 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail. 5′ UTR may comprise a first polynucleotide fragment and a second polynucleotide fragment from the same and/or different genes such as the 5′ UTRs described in US Patent Application Publication No. 2010/0293625 and PCT/US2014/069155, herein incorporated by reference in its entirety.

It should be understood that any UTR from any gene may be incorporated into the regions of a nucleic acid. Furthermore, multiple wild-type UTRs of any known gene may be utilized. It is also within the scope of the present disclosure to provide artificial UTRs which are not variants of wild type regions. These UTRs or portions thereof may be placed in the same orientation as in the transcript from which they were selected or may be altered in orientation or location. Hence a 5′ or 3′ UTR may be inverted, shortened, lengthened, made with one or more other 5′ UTRs or 3′ UTRs. As used herein, the term "altered" as it relates to a UTR sequence, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3′ UTR or 5′ UTR may be altered relative to a wild-type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional

nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3′ or 5′) comprise a variant UTR.

In some embodiments, a double, triple or quadruple UTR such as a 5′ UTR or 3′ UTR may be used. As used herein, a "double" UTR is one in which two copies of the same UTR are encoded either in series or substantially in series. For example, a double beta-globin 3′ UTR may be used as described in US Patent publication 20100129877, the contents of which are incorporated herein by reference in its entirety.

It is also within the scope of the present disclosure to have patterned UTRs. As used herein "patterned UTRs" are those UTRs which reflect a repeating or alternating pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than 3 times. In these patterns, each letter, A, B, or C represent a different UTR at the nucleotide level.

In some embodiments, flanking regions are selected from a family of transcripts whose proteins share a common function, structure, feature or property. For example, polypeptides of interest may belong to a family of proteins which are expressed in a particular cell, tissue or at some time during development. The UTRs from any of these genes may be swapped for any other UTR of the same or different family of proteins to create a new polynucleotide. As used herein, a "family of proteins" is used in the broadest sense to refer to a group of two or more polypeptides of interest which share at least one function, structure, feature, localization, origin, or expression pattern.

The untranslated region may also include translation enhancer elements (TEE). As a non-limiting example, the TEE may include those described in US Application No. 2009/0226470, herein incorporated by reference in its entirety, and those known in the art.

In Vitro Transcription of RNA

cDNA encoding the polynucleotides described herein may be transcribed using an in vitro transcription (IVT) system. *In vitro* transcription of RNA is known in the art and is described in International Publication WO2014/152027, which is incorporated by reference herein in its entirety.

In some embodiments, the RNA transcript is generated using a non-amplified, linearized DNA template in an *in vitro* transcription reaction to generate the RNA transcript. In some embodiments, the template DNA is isolated DNA. In some embodiments, the template DNA is cDNA. In some embodiments, the cDNA is formed by reverse transcription of a RNA polynucleotide, for example, but not limited to Lassa virus, Nipah virus, or betacoronavirus

RNA, *e.g.* mRNA. In some embodiments, cells, *e.g.*, bacterial cells, *e.g.*, *E. coli*, *e.g.*, DH-1 cells are transfected with the plasmid DNA template. In some embodiments, the transfected cells are cultured to replicate the plasmid DNA which is then isolated and purified. In some embodiments, the DNA template includes a RNA polymerase promoter, *e.g.*, a T7 promoter located 5 ' to and operably linked to the gene of interest.

In some embodiments, an *in vitro* transcription template encodes a 5′ untranslated (UTR) region, contains an open reading frame, and encodes a 3′ UTR and a polyA tail. The particular nucleic acid sequence composition and length of an *in vitro* transcription template will depend on the mRNA encoded by the template.

A "5′ untranslated region" (UTR) refers to a region of an mRNA that is directly upstream (*i.e.*, 5′) from the start codon (*i.e.*, the first codon of an mRNA transcript translated by a ribosome) that does not encode a polypeptide. When RNA transcripts are being generated, the 5' UTR may comprise a promoter sequence. Such promoter sequences are known in the art. It should be understood that such promoter sequences will not be present in a vaccine of the disclosure.

A "3′ untranslated region" (UTR) refers to a region of an mRNA that is directly downstream (*i.e.*, 3′) from the stop codon (*i.e.*, the codon of an mRNA transcript that signals a termination of translation) that does not encode a polypeptide.

An "open reading frame" is a continuous stretch of DNA beginning with a start codon (*e.g.*, methionine (ATG)), and ending with a stop codon (*e.g.*, TAA, TAG or TGA) and encodes a polypeptide.

A "polyA tail" is a region of mRNA that is downstream, *e.g.*, directly downstream (*i.e.*, 3′), from the 3′ UTR that contains multiple, consecutive adenosine monophosphates. A polyA tail may contain 10 to 300 adenosine monophosphates. For example, a polyA tail may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 adenosine monophosphates. In some embodiments, a polyA tail contains 50 to 250 adenosine monophosphates. In a relevant biological setting (*e.g.*, in cells, *in vivo*) the poly(A) tail functions to protect mRNA from enzymatic degradation, *e.g.*, in the cytoplasm, and aids in transcription termination, and/or export of the mRNA from the nucleus and translation.

In some embodiments, a nucleic acid includes 200 to 3,000 nucleotides. For example, a nucleic acid may include 200 to 500, 200 to 1000, 200 to 1500, 200 to 3000, 500 to 1000, 500 to 1500, 500 to 2000, 500 to 3000, 1000 to 1500, 1000 to 2000, 1000 to 3000, 1500 to 3000, or 2000 to 3000 nucleotides).

An *in vitro* transcription system typically comprises a transcription buffer, nucleotide triphosphates (NTPs), an RNase inhibitor and a polymerase.

The NTPs may be manufactured in house, may be selected from a supplier, or may be synthesized as described herein. The NTPs may be selected from, but are not limited to, those described herein including natural and unnatural (modified) NTPs.

Any number of RNA polymerases or variants may be used in the method of the present disclosure. The polymerase may be selected from, but is not limited to, a phage RNA polymerase, *e.g.*, a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, and/or mutant polymerases such as, but not limited to, polymerases able to incorporate modified nucleic acids and/or modified nucleotides, including chemically modified nucleic acids and/or nucleotides. Some embodiments exclude the use of DNase.

In some embodiments, the RNA transcript is capped *via* enzymatic capping. In some embodiments, the RNA comprises 5' terminal cap, for example, 7mG(5')ppp(5')NlmpNp.

Chemical Synthesis

Solid-phase chemical synthesis. Nucleic acids the present disclosure may be manufactured in whole or in part using solid phase techniques. Solid-phase chemical synthesis of nucleic acids is an automated method wherein molecules are immobilized on a solid support and synthesized step by step in a reactant solution. Solid-phase synthesis is useful in site-specific introduction of chemical modifications in the nucleic acid sequences.

Liquid Phase Chemical Synthesis. The synthesis of nucleic acids of the present disclosure by the sequential addition of monomer building blocks may be carried out in a liquid phase.

Combination of Synthetic Methods. The synthetic methods discussed above each has its own advantages and limitations. Attempts have been conducted to combine these methods to overcome the limitations. Such combinations of methods are within the scope of the present disclosure. The use of solid-phase or liquid-phase chemical synthesis in combination with enzymatic ligation provides an efficient way to generate long chain nucleic acids that cannot be obtained by chemical synthesis alone.

Ligation of Nucleic Acid Regions or Subregions

Assembling nucleic acids by a ligase may also be used. DNA or RNA ligases promote intermolecular ligation of the 5' and 3' ends of polynucleotide chains through the formation of a phosphodiester bond. Nucleic acids such as chimeric polynucleotides and/or circular nucleic acids may be prepared by ligation of one or more regions or subregions. DNA fragments can be

joined by a ligase catalyzed reaction to create recombinant DNA with different functions. Two oligodeoxynucleotides, one with a 5' phosphoryl group and another with a free 3' hydroxyl group, serve as substrates for a DNA ligase.

Purification

Purification of the nucleic acids described herein may include, but is not limited to, nucleic acid clean-up, quality assurance and quality control. Clean-up may be performed by methods known in the arts such as, but not limited to, AGENCOURT® beads (Beckman Coulter Genomics, Danvers, MA), poly-T beads, LNATM oligo-T capture probes (EXIQON® Inc, Vedbaek, Denmark) or HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC). The term "purified" when used in relation to a nucleic acid such as a "purified nucleic acid" refers to one that is separated from at least one contaminant. A "contaminant" is any substance that makes another unfit, impure or inferior. Thus, a purified nucleic acid (e.g., DNA and RNA) is present in a form or setting different from that in which it is found in nature, or a form or setting different from that which existed prior to subjecting it to a treatment or purification method.

A quality assurance and/or quality control check may be conducted using methods such as, but not limited to, gel electrophoresis, UV absorbance, or analytical HPLC.

In some embodiments, the nucleic acids may be sequenced by methods including, but not limited to reverse-transcriptase-PCR.

Quantification

In some embodiments, the nucleic acids of the present disclosure may be quantified in exosomes or when derived from one or more bodily fluid. Bodily fluids include peripheral blood, serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, and umbilical cord blood. Alternatively, exosomes may be retrieved from an organ selected from the group consisting of lung, heart, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colon, breast, prostate, brain, esophagus, liver, and placenta.

Assays may be performed using construct specific probes, cytometry, qRT-PCR, real-time PCR, PCR, flow cytometry, electrophoresis, mass spectrometry, or combinations thereof while the exosomes may be isolated using immunohistochemical methods such as enzyme linked immunosorbent assay (ELISA) methods. Exosomes may also be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

These methods afford the investigator the ability to monitor, in real time, the level of nucleic acids remaining or delivered. This is possible because the nucleic acids of the present disclosure, in some embodiments, differ from the endogenous forms due to the structural or chemical modifications.

In some embodiments, the nucleic acid may be quantified using methods such as, but not limited to, ultraviolet visible spectroscopy (UV/Vis). A non-limiting example of a UV/Vis spectrometer is a NANODROP® spectrometer (ThermoFisher, Waltham, MA). The quantified nucleic acid may be analyzed in order to determine if the nucleic acid may be of proper size, check that no degradation of the nucleic acid has occurred. Degradation of the nucleic acid may be checked by methods such as, but not limited to, agarose gel electrophoresis, HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), liquid chromatography-mass spectrometry (LCMS), capillary electrophoresis (CE) and capillary gel electrophoresis (CGE).

Lipid Nanoparticles (LNPs)

In some embodiments, zoonotic disease RNA (e.g., mRNA) vaccines of the disclosure are formulated in a lipid nanoparticle (LNP). Lipid nanoparticles typically comprise ionizable cationic lipid, non-cationic lipid, sterol and PEG lipid components along with the nucleic acid cargo of interest. The lipid nanoparticles of the disclosure can be generated using components, compositions, and methods as are generally known in the art, see for example PCT/US2016/052352; PCT/US2016/068300; PCT/US2017/037551; PCT/US2015/027400; PCT/US2016/047406; PCT/US2016000129; PCT/US2016/014280; PCT/US2016/014280; PCT/US2017/038426; PCT/US2014/027077; PCT/US2014/055394; PCT/US2016/52117; PCT/US2012/069610; PCT/US2017/027492; PCT/US2016/059575 and PCT/US2016/069491, all of which are incorporated by reference herein in their entirety.

Vaccines of the present disclosure are typically formulated in lipid nanoparticle. In some embodiments, the lipid nanoparticle comprises at least one ionizable cationic lipid, at least one non-cationic lipid, at least one sterol, and/or at least one polyethylene glycol (PEG)-modified lipid.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 20-60% ionizable cationic lipid. For example, the lipid nanoparticle may comprise a molar ratio of 20-50%, 20-40%, 20-30%, 30-60%, 30-50%, 30-40%, 40-60%, 40-50%, or 50-60% ionizable cationic lipid. In some embodiments, the lipid nanoparticle comprises a molar ratio of 20%, 30%, 40%, 50, or 60% ionizable cationic lipid.

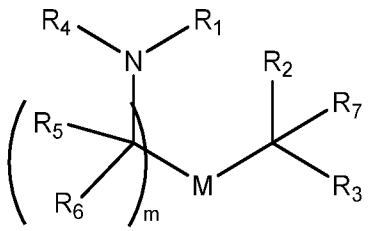
In some embodiments, the lipid nanoparticle comprises a molar ratio of 5-25% non-cationic lipid. For example, the lipid nanoparticle may comprise a molar ratio of 5-20%, 5-15%, 5-10%, 10-25%, 10-20%, 10-25%, 15-25%, 15-20%, or 20-25% non-cationic lipid. In some embodiments, the lipid nanoparticle comprises a molar ratio of 5%, 10%, 15%, 20%, or25% non-cationic lipid.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 25-55% sterol. For example, the lipid nanoparticle may comprise a molar ratio of 25-50%, 25-45%, 25-40%, 25-35%, 25-30%, 30-55%, 30-50%, 30-45%, 30-40%, 30-35%, 35-55%, 35-50%, 35-45%, 35-40%, 40-55%, 40-50%, 40-45%, 45-55%, 45-50%, or 50-55% sterol. In some embodiments, the lipid nanoparticle comprises a molar ratio of 25%, 30%, 35%, 40%, 45%, 50%, or 55% sterol.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 0.5-15% PEG-modified lipid. For example, the lipid nanoparticle may comprise a molar ratio of 0.5-10%, 0.5-5%, 1-15%, 1-10%, 1-5%, 2-15%, 2-10%, 2-5%, 5-15%, 5-10%, or 10-15%. In some embodiments, the lipid nanoparticle comprises a molar ratio of 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% PEG-modified lipid.

In some embodiments, the lipid nanoparticle comprises a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid, 25-55% sterol, and 0.5-15% PEG-modified lipid.

In some embodiments, an ionizable cationic lipid of the disclosure comprises a compound of Formula (I):



(I),

or a salt or isomer thereof, wherein:

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and

-R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$, $-C(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, -N(OR)C(O)R, $-N(OR)S(O)_2R$, -N(OR)C(O)OR, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

In some embodiments, a subset of compounds of Formula (I) includes those in which when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, –CHQR, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, -N(OR)C(O)R, $-N(OR)S(O)_2R$, -N(OR)C(O)OR, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C_{1-3} alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)R$, -C(O)N(R)OR, and $-C(=NR_9)N(R)_2$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R_4 is $-(CH_2)_nQ$ in which n is 1 or 2, or (ii) R_4 is $-(CH_2)_nCHQR$ in which n is 1, or (iii) R_4 is -CHQR, and $-CQ(R)_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -$S(O)_2$-, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, -$S(O)_2$R, -$S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, -$(CH_2)_nQ$, -$(CH_2)_nCHQR$, -CHQR, -$CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -$O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, -CX_3, -CX_2H, -CXH_2, -CN, -$C(O)N(R)_2$, -N(R)C(O)R, -$N(R)S(O)_2$R, -$N(R)C(O)N(R)_2$, -$N(R)C(S)N(R)_2$, -$CRN(R)_2C(O)OR$, -$N(R)R_8$, -$O(CH_2)_nOR$, -$N(R)C(=NR_9)N(R)_2$, -$N(R)C(=CHR_9)N(R)_2$, -$OC(O)N(R)_2$, -N(R)C(O)OR,

-N(OR)C(O)R, -N(OR)S(O)$_2$R, -N(OR)C(O)OR, -N(OR)C(O)N(R)$_2$, -N(OR)C(S)N(R)$_2$, -N(OR)C(=NR$_9$)N(R)$_2$, -N(OR)C(=CHR$_9$)N(R)$_2$, -C(=NR$_9$)R, -C(O)N(R)OR, and -C(=NR$_9$)N(R)$_2$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)$_2$-, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, -S(O)$_2$R, -S(O)$_2$N(R)$_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{2-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is -$(CH_2)_n$Q or -$(CH_2)_n$CHQR, where Q is -N(R)$_2$, and n is selected from 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -$S(O)_2$-, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';

R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of -$(CH_2)_nQ$, -$(CH_2)_nCHQR$, -CHQR, and -$CQ(R)_2$, where Q is -$N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -$S(O)_2$-, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;

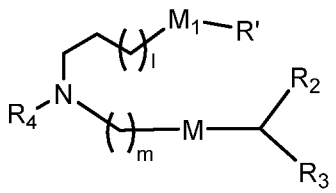
each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

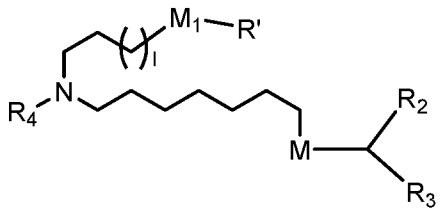
In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IA):



(IA),

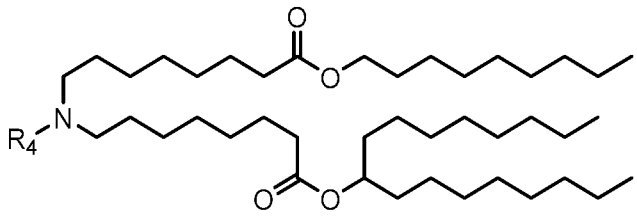
or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or -$(CH_2)_nQ$, in which Q is OH, -NHC(S)$N(R)_2$, -NHC(O)$N(R)_2$, -N(R)C(O)R, -N(R)$S(O)_2$R, -N(R)R_8, -NHC(=NR_9)$N(R)_2$, -NHC(=CHR_9)$N(R)_2$, -OC(O)$N(R)_2$, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (II):

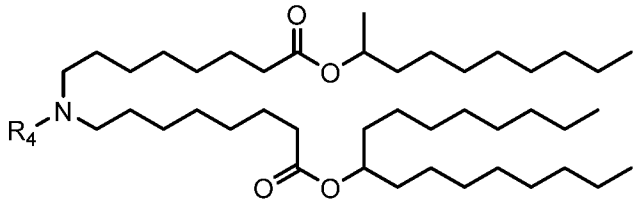


(II) or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which n is 2, 3, or 4, and Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

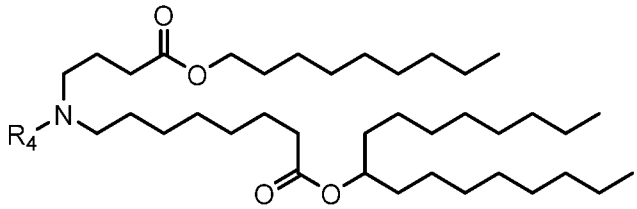
In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IIa), (IIb), (IIc), or (IIe):



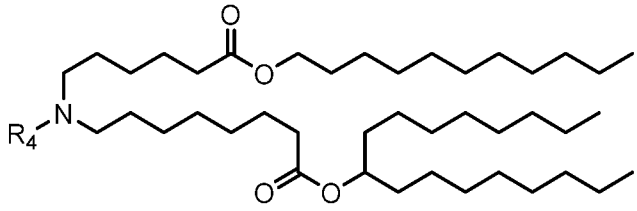
(IIa),



(IIb),



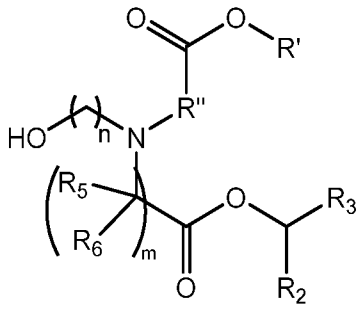
(IIc), or



(IIe),

or a salt or isomer thereof, wherein R_4 is as described herein.

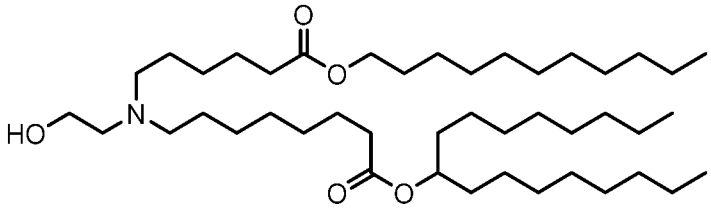
In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IId):



(IId),

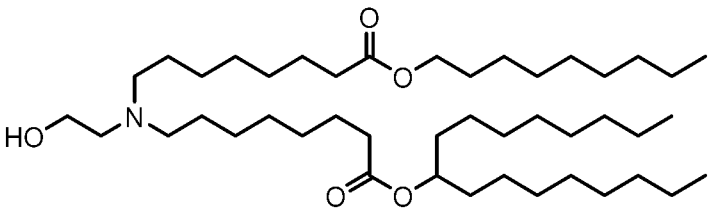
or a salt or isomer thereof, wherein n is 2, 3, or 4; and m, R', R", and R_2 through R_6 are as described herein. For example, each of R_2 and R_3 may be independently selected from the group consisting of C_{5-14} alkyl and C_{5-14} alkenyl.

In some embodiments, an ionizable cationic lipid of the disclosure comprises a compound having structure:



(Compound 1).

In some embodiments, an ionizable cationic lipid of the disclosure comprises a compound having structure:



(Compound 2).

In some embodiments, a non-cationic lipid of the disclosure comprises 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-gly cero-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 (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine,1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-

didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and mixtures thereof.

In some embodiments, a PEG modified lipid of the disclosure comprises a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, and mixtures thereof. In some embodiments, the PEG-modified lipid is PEG-DMG, PEG-c-DOMG (also referred to as PEG-DOMG), PEG-DSG and/or PEG-DPG.

In some embodiments, a sterol of the disclosure comprises cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and mixtures thereof.

In some embodiments, a LNP of the disclosure comprises an ionizable cationic lipid of Compound 1, wherein the non-cationic lipid is DSPC, the structural lipid that is cholesterol, and the PEG lipid is PEG-DMG.

In some embodiments, a LNP of the disclosure comprises an N:P ratio of from about 2:1 to about 30:1.

In some embodiments, a LNP of the disclosure comprises an N:P ratio of about 6:1.

In some embodiments, a LNP of the disclosure comprises an N:P ratio of about 3:1.

In some embodiments, a LNP of the disclosure comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of from about 10:1 to about 100:1.

In some embodiments, a LNP of the disclosure comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of about 20:1.

In some embodiments, a LNP of the disclosure comprises a wt/wt ratio of the ionizable cationic lipid component to the RNA of about 10:1.

In some embodiments, a LNP of the disclosure has a mean diameter from about 50 nm to about 150 nm.

In some embodiments, a LNP of the disclosure has a mean diameter from about 70 nm to about 120 nm.

Multivalent Vaccines

The zoonotic disease vaccines, as provided herein, may include an RNA (e.g. mRNA) or multiple RNAs encoding two or more antigens of the same or different zoonotic disease species. In some embodiments, a zoonotic disease vaccine includes an RNA or multiple RNAs encoding two or more antigens. In some embodiments, the RNA (at least one RNA) of a zoonotic disease vaccine may encode 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more antigens.

In some embodiments, two or more different RNA (e.g., mRNA) encoding antigens may be formulated in the same lipid nanoparticle. In other embodiments, two or more different RNA encoding antigens may be formulated in separate lipid nanoparticles (each RNA formulated in a single lipid nanoparticle). The lipid nanoparticles may then be combined and administered as a single vaccine composition (e.g., comprising multiple RNA encoding multiple antigens) or may be administered separately.

Broad spectrum RNA (e.g., mRNA) vaccines

There may be situations where persons are at risk for infection with more than one strain of LASV. RNA (*e.g.*, mRNA) therapeutic vaccines are particularly amenable to combination vaccination approaches due to a number of factors including, but not limited to, speed of manufacture, ability to rapidly tailor vaccines to accommodate perceived geographical threat, and the like. Moreover, because the vaccines utilize the human body to produce the antigenic protein, the vaccines are amenable to the production of larger, more complex antigenic proteins, allowing for proper folding, surface expression, antigen presentation, *etc.* in the human subject. To protect against more than one strain of a viral infection, a combination vaccine can be administered that includes RNA (*e.g.*, mRNA) encoding at least one antigenic polypeptide protein (or antigenic portion thereof) of a first virus and further includes RNA encoding at least one antigenic polypeptide protein (or antigenic portion thereof) of a second virus. RNA (*e.g.*, mRNA) can be co-formulated, for example, in a single lipid nanoparticle (LNP) or can be formulated in separate LNPs for co-administration.

Combination Vaccines

The zoonotic disease vaccines, as provided herein, may include an RNA or multiple RNAs encoding two or more antigens of the same or different viral strains. Also provided herein are combination vaccines that include RNA encoding one or more zoonotic disease antigen(s) and one or more antigen(s) of a different organisms (e.g., bacterial and/or viral organism). Thus, the vaccines of the present disclosure may be combination vaccines that target one or more antigens of the same strain/species, or one or more antigens of different strains/species, e.g., antigens which induce immunity to organisms which are found in the same geographic areas where the risk of Lassa virus, Nipah virus, or betacoronavirus infection is high or organisms to which an individual is likely to be exposed to when exposed to the virus.

Flagellin Adjuvants

Flagellin is an approximately 500 amino acid monomeric protein that polymerizes to form the flagella associated with bacterial motion. Flagellin is expressed by a variety of flagellated bacteria (*Salmonella typhimurium* for example) as well as non-flagellated bacteria (such as *Escherichia coli*). Sensing of flagellin by cells of the innate immune system (dendritic cells, macrophages, *etc.*) is mediated by the Toll-like receptor 5 (TLR5) as well as by Nod-like receptors (NLRs) Ipaf and Naip5. TLRs and NLRs have been identified as playing a role in the activation of innate immune response and adaptive immune response. As such, flagellin provides an adjuvant effect in a vaccine.

The nucleotide and amino acid sequences encoding known flagellin polypeptides are publicly available in the NCBI GenBank database. The flagellin sequences from *S. Typhimurium, H. Pylori, V. Cholera, S. marcesens, S. flexneri, T. Pallidum, L. pneumophila, B. burgdorferei, C. difficile, R. meliloti, A. tumefaciens, R. lupini, B. clarridgeiae, P. Mirabilis, B. subtilus, L. monocytogenes, P. aeruginosa*, and *E. coli*, among others are known.

A flagellin polypeptide, as used herein, refers to a full length flagellin protein, immunogenic fragments thereof, and peptides having at least 50% sequence identify to a flagellin protein or immunogenic fragments thereof. Exemplary flagellin proteins include flagellin from *Salmonella typhi* (UniPro Entry number: Q56086), *Salmonella typhimurium* (A0A0C9DG09), *Salmonella enteritidis* (A0A0C9BAB7), and *Salmonella choleraesuis* (Q6V2X8). In some embodiments, the flagellin polypeptide has at least 60%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, or 99% sequence identify to a flagellin protein or immunogenic fragments thereof.

In some embodiments, the flagellin polypeptide is an immunogenic fragment. An immunogenic fragment is a portion of a flagellin protein that provokes an immune response. In some embodiments, the immune response is a TLR5 immune response. An example of an immunogenic fragment is a flagellin protein in which all or a portion of a hinge region has been deleted or replaced with other amino acids. For example, an antigenic polypeptide may be inserted in the hinge region. Hinge regions are the hypervariable regions of a flagellin. Hinge regions of a flagellin are also referred to as "D3 domain or region, "propeller domain or region," "hypervariable domain or region" and "variable domain or region." "At least a portion of a hinge region," as used herein, refers to any part of the hinge region of the flagellin, or the entirety of the hinge region. In other embodiments an immunogenic fragment of flagellin is a 20, 25, 30, 35, or 40 amino acid C-terminal fragment of flagellin.

The flagellin monomer is formed by domains D0 through D3. D0 and D1, which form the stem, are composed of tandem long alpha helices and are highly conserved among different bacteria. The D1 domain includes several stretches of amino acids that are useful for TLR5 activation. The entire D1 domain or one or more of the active regions within the domain are immunogenic fragments of flagellin. Examples of immunogenic regions within the D1 domain include residues 88-114 and residues 411-431 (in *Salmonella* typhimurium FliC flagellin). Within the 13 amino acids in the 88-100 region, at least 6 substitutions are permitted between *Salmonella* flagellin and other flagellins that still preserve TLR5 activation. Thus, immunogenic fragments of flagellin include flagellin like sequences that activate TLR5 and contain a 13 amino acid motif that is 53% or more identical to the *Salmonella* sequence in 88-100 of FliC (LQRVRELAVQSAN; SEQ ID NO: 31).

Pharmaceutical Formulations

Provided herein are compositions (*e.g.*, pharmaceutical compositions), methods, kits and reagents for prevention or treatment of zoonotic disease in humans and other mammals, for example. zoonotic disease RNA (*e.g.*, mRNA) vaccines can be used as therapeutic or prophylactic agents. They may be used in medicine to prevent and/or treat infectious disease.

In some embodiments, a zoonotic disease vaccine containing RNA polynucleotides as described herein can be administered to a subject (*e.g.*, a mammalian subject, such as a human subject), and the RNA polynucleotides are translated *in vivo* to produce an antigenic polypeptide (antigen).

An "effective amount" of a zoonotic disease vaccine is based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the RNA (e.g., length, nucleotide composition, and/or extent of modified nucleosides), other components of the vaccine, and other determinants, such as age, body weight, height, sex and general health of the subject. Typically, an effective amount of a zoonotic disease vaccine provides an induced or boosted immune response as a function of antigen production in the cells of the subject. In some embodiments, an effective amount of the zoonotic disease RNA vaccine containing RNA polynucleotides having at least one chemical modifications are more efficient than a composition containing a corresponding unmodified polynucleotide encoding the same antigen or a peptide antigen. Increased antigen production may be demonstrated by increased cell transfection (the percentage of cells transfected with the RNA vaccine), increased protein translation and/or expression from the polynucleotide, decreased nucleic acid degradation (as demonstrated, for

example, by increased duration of protein translation from a modified polynucleotide), or altered antigen specific immune response of the host cell.

The term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*. A "pharmaceutically acceptable carrier," after administered to or upon a subject, does not cause undesirable physiological effects. The carrier in the pharmaceutical composition must be "acceptable" also in the sense that it is compatible with the active ingredient and can be capable of stabilizing it. One or more solubilizing agents can be utilized as pharmaceutical carriers for delivery of an active agent. Examples of a pharmaceutically acceptable carrier include, but are not limited to, biocompatible vehicles, adjuvants, additives, and diluents to achieve a composition usable as a dosage form. Examples of other carriers include colloidal silicon oxide, magnesium stearate, cellulose, and sodium lauryl sulfate. Additional suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences.

In some embodiments, RNA vaccines (including polynucleotides and their encoded polypeptides) in accordance with the present disclosure may be used for treatment or prevention of zoonotic disease. Zoonotic disease RNA vaccines may be administered prophylactically or therapeutically as part of an active immunization scheme to healthy individuals or early in infection during the incubation phase or during active infection after onset of symptoms. In some embodiments, the amount of RNA vaccines of the present disclosure provided to a cell, a tissue or a subject may be an amount effective for immune prophylaxis.

Zoonotic disease RNA (*e.g.*, mRNA) vaccines may be administered with other prophylactic or therapeutic compounds. As a non-limiting example, a prophylactic or therapeutic compound may be an adjuvant or a booster. As used herein, when referring to a prophylactic composition, such as a vaccine, the term “booster” refers to an extra administration of the prophylactic (vaccine) composition. A booster (or booster vaccine) may be given after an earlier administration of the prophylactic composition. The time of administration between the initial administration of the prophylactic composition and the booster may be, but is not limited to, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 10 days, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7

months, 8 months, 9 months, 10 months, 11 months, 1 year, 18 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 25 years, 30 years, 35 years, 40 years, 45 years, 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years or more than 99 years. In exemplary embodiments, the time of administration between the initial administration of the prophylactic composition and the booster may be, but is not limited to, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 6 months or 1 year.

In some embodiments, zoonotic disease RNA vaccines may be administered intramuscularly, intranasally or intradermally, similarly to the administration of inactivated vaccines known in the art.

The zoonotic disease RNA vaccines may be utilized in various settings depending on the prevalence of the infection or the degree or level of unmet medical need. As a non-limiting example, the RNA vaccines may be utilized to treat and/or prevent a variety of infectious disease. RNA vaccines have superior properties in that they produce much larger antibody titers, better neutralizing immunity, produce more durable immune responses, and/or produce responses earlier than commercially available vaccines.

Provided herein are pharmaceutical compositions including zoonotic disease RNA vaccines and RNA vaccine compositions and/or complexes optionally in combination with one or more pharmaceutically acceptable excipients.

Zoonotic disease RNA (*e.g.*, mRNA) vaccines may be formulated or administered alone or in conjunction with one or more other components. For instance, zoonotic disease RNA vaccines (vaccine compositions) may comprise other components including, but not limited to, adjuvants.

In some embodiments, zoonotic disease RNA vaccines do not include an adjuvant (they are adjuvant free).

Zoonotic disease RNA (*e.g.*, mRNA) vaccines may be formulated or administered in combination with one or more pharmaceutically-acceptable excipients. In some embodiments, vaccine compositions comprise at least one additional active substances, such as, for example, a therapeutically-active substance, a prophylactically-active substance, or a combination of both. Vaccine compositions may be sterile, pyrogen-free or both sterile and pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents, such as vaccine compositions, may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

In some embodiments, zoonotic disease RNA vaccines are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers to the RNA vaccines or the polynucleotides contained therein, for example, RNA polynucleotides (*e.g.*, mRNA polynucleotides) encoding antigens.

Formulations of the vaccine compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient (*e.g.*, mRNA polynucleotide) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

In some embodiments, zoonotic disease RNA vaccines are formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (*e.g.*, from a depot formulation); (4) alter the biodistribution (*e.g.*, target to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; and/or (6) alter the release profile of encoded protein (antigen) *in vivo*. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with zoonotic disease RNA vaccines (*e.g.*, for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof.

Dosing/Administration

Provided herein are compositions (*e.g.*, pharmaceutical compositions), methods, kits and reagents for prevention and/or treatment of zoonotic disease in humans and other mammals. zoonotic disease RNA vaccines can be used as therapeutic or prophylactic agents. In some aspects, the RNA vaccines of the disclosure are used to provide prophylactic protection from zoonotic disease. In some aspects, the RNA vaccines of the disclosure are used to treat a

zoonotic disease infection. In some embodiments, the zoonotic disease vaccines of the present disclosure are used in the priming of immune effector cells, for example, to activate peripheral blood mononuclear cells (PBMCs) *ex vivo*, which are then infused (re-infused) into a subject.

A subject may be any mammal, including non-human primate and human subjects. Typically, a subject is a human subject.

In some embodiments, the zoonotic disease vaccines are administered to a subject (e.g., a mammalian subject, such as a human subject) in an effective amount to induce an antigen-specific immune response. The RNA encoding the zoonotic disease antigen is expressed and translated *in vivo* to produce the antigen, which then stimulates an immune response in the subject.

Prophylactic protection from zoonotic disease can be achieved following administration of a zoonotic disease RNA vaccine of the present disclosure. Vaccines can be administered once, twice, three times, four times or more but it is likely sufficient to administer the vaccine once (optionally followed by a single booster). It is possible, although less desirable, to administer the vaccine to an infected individual to achieve a therapeutic response. Dosing may need to be adjusted accordingly.

A method of eliciting an immune response in a subject against zoonotic disease is provided in aspects of the present disclosure. The method involves administering to the subject a zoonotic disease RNA vaccine comprising at least one RNA (e.g., mRNA) having an open reading frame encoding at least one zoonotic disease antigen, thereby inducing in the subject an immune response specific to zoonotic disease antigen, wherein anti-antigen antibody titer in the subject is increased following vaccination relative to anti-antigen antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the zoonotic disease. An "anti-antigen antibody" is a serum antibody the binds specifically to the antigen.

A prophylactically effective dose is an effective dose that prevents infection with the virus at a clinically acceptable level. In some embodiments, the effective dose is a dose listed in a package insert for the vaccine. A traditional vaccine, as used herein, refers to a vaccine other than the mRNA vaccines of the present disclosure. For instance, a traditional vaccine includes, but is not limited, to live microorganism vaccines, killed microorganism vaccines, subunit vaccines, protein antigen vaccines, DNA vaccines, virus like particle (VLP) vaccines, *etc*. In exemplary embodiments, a traditional vaccine is a vaccine that has achieved regulatory approval and/or is registered by a national drug regulatory body, for example the Food and Drug Administration (FDA) in the United States or the European Medicines Agency (EMA).

In some embodiments, the anti-antigen antibody titer in the subject is increased 1 log to 10 log following vaccination relative to anti-antigen antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the zoonotic disease or an unvaccinated subject. In some embodiments, the anti-antigen antibody titer in the subject is increased 1 log, 2 log, 3 log, 4 log, 5 log, or 10 log following vaccination relative to anti-antigen antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the zoonotic disease or an unvaccinated subject.

A method of eliciting an immune response in a subject against a zoonotic disease is provided in other aspects of the disclosure. The method involves administering to the subject a zoonotic disease RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one zoonotic disease antigen, thereby inducing in the subject an immune response specific to zoonotic disease antigen, wherein the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine against the zoonotic disease at 2 times to 100 times the dosage level relative to the RNA vaccine.

In some embodiments, the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at twice the dosage level relative to the zoonotic disease RNA vaccine. In some embodiments, the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at three times the dosage level relative to the zoonotic disease RNA vaccine. In some embodiments, the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 4 times, 5 times, 10 times, 50 times, or 100 times the dosage level relative to the zoonotic disease RNA vaccine. In some embodiments, the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 10 times to 1000 times the dosage level relative to the zoonotic disease RNA vaccine. In some embodiments, the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 100 times to 1000 times the dosage level relative to the zoonotic disease RNA vaccine.

In other embodiments, the immune response is assessed by determining [protein] antibody titer in the subject. In other embodiments, the ability of serum or antibody from an immunized subject is tested for its ability to neutralize viral uptake or reduce zoonotic disease transformation of human B lymphocytes. In other embodiments, the ability to promote a robust T cell response(s) is measured using art recognized techniques.

Other aspects the disclosure provide methods of eliciting an immune response in a subject against a zoonotic disease by administering to the subject a zoonotic disease RNA

vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one zoonotic disease antigen, thereby inducing in the subject an immune response specific to zoonotic disease antigen, wherein the immune response in the subject is induced 2 days to 10 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the zoonotic disease. In some embodiments, the immune response in the subject is induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine at 2 times to 100 times the dosage level relative to the RNA vaccine.

In some embodiments, the immune response in the subject is induced 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 5 weeks, or 10 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

Also provided herein are methods of eliciting an immune response in a subject against a zoonotic disease by administering to the subject a zoonotic disease RNA vaccine having an open reading frame encoding a first antigen, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not co-formulated or co-administered with the vaccine.

Zoonotic disease RNA (*e.g.*, mRNA) vaccines may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited, to intradermal, intramuscular, intranasal, and/or subcutaneous administration. The present disclosure provides methods comprising administering RNA vaccines to a subject in need thereof. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. zoonotic disease RNA (*e.g.*, mRNA) vaccines compositions are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of zoonotic disease RNA (*e.g.*, mRNA)vaccines compositions may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

The effective amount of a zoonotic disease vaccine, as provided herein, may be as low as 20 μg, administered for example as a single dose or as two 10 μg doses. In some embodiments, the effective amount is a total dose of 20 μg-200 μg. For example, the effective amount may be a total dose of 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 55 μg, 60 μg, 65 μg, 70 μg, 75 μg, 80 μg, 85 μg, 90 μg, 95 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg, 150 μg, 160 μg, 170 μg, 180 μg, 190 μg or 200 μg. In some embodiments, the effective amount is a total dose of 25 μg-200 μg. In some embodiments, the effective amount is a total dose of 50 μg-200 μg.

In some embodiments, zoonotic disease RNA (*e.g.*, mRNA) vaccines compositions may be administered at dosage levels sufficient to deliver 0.0001 mg/kg to 100 mg/kg, 0.001 mg/kg to 0.05 mg/kg, 0.005 mg/kg to 0.05 mg/kg, 0.001 mg/kg to 0.005 mg/kg, 0.05 mg/kg to 0.5 mg/kg, 0.01 mg/kg to 50 mg/kg, 0.1 mg/kg to 40 mg/kg, 0.5 mg/kg to 30 mg/kg, 0.01 mg/kg to 10 mg/kg, 0.1 mg/kg to 10 mg/kg, or 1 mg/kg to 25 mg/kg, of subject body weight per day, one or more times a day, per week, per month, etc. to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect (*see e.g.*, the range of unit doses described in International Publication No. WO2013/078199, herein incorporated by reference in its entirety). The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, every four weeks, every 2 months, every three months, every 6 months, *etc*. In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. In exemplary embodiments, zoonotic disease RNA (*e.g.*, mRNA) vaccines compositions may be administered at dosage levels sufficient to deliver 0.0005 mg/kg to 0.01 mg/kg, *e.g.*, about 0.0005 mg/kg to about 0.0075 mg/kg, *e.g.*, about 0.0005 mg/kg, about 0.001 mg/kg, about 0.002 mg/kg, about 0.003 mg/kg, about 0.004 mg/kg or about 0.005 mg/kg.

In some embodiments, zoonotic disease RNA (*e.g.*, mRNA) vaccine compositions may be administered once or twice (or more) at dosage levels sufficient to deliver 0.025 mg/kg to 0.250 mg/kg, 0.025 mg/kg to 0.500 mg/kg, 0.025 mg/kg to 0.750 mg/kg, or 0.025 mg/kg to 1.0 mg/kg.

In some embodiments, zoonotic disease RNA (*e.g.*, mRNA) vaccine compositions may be administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5

years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.0100 mg, 0.025 mg, 0.050 mg, 0.075 mg, 0.100 mg, 0.125 mg, 0.150 mg, 0.175 mg, 0.200 mg, 0.225 mg, 0.250 mg, 0.275 mg, 0.300 mg, 0.325 mg, 0.350 mg, 0.375 mg, 0.400 mg, 0.425 mg, 0.450 mg, 0.475 mg, 0.500 mg, 0.525 mg, 0.550 mg, 0.575 mg, 0.600 mg, 0.625 mg, 0.650 mg, 0.675 mg, 0.700 mg, 0.725 mg, 0.750 mg, 0.775 mg, 0.800 mg, 0.825 mg, 0.850 mg, 0.875 mg, 0.900 mg, 0.925 mg, 0.950 mg, 0.975 mg, or 1.0 mg. Higher and lower dosages and frequency of administration are encompassed by the present disclosure. For example, a zoonotic disease RNA (*e.g.*, mRNA) vaccine composition may be administered three or four times.

In some embodiments, zoonotic disease RNA (*e.g.*, mRNA) vaccine compositions may be administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.010 mg, 0.025 mg, 0.100 mg or 0.400 mg.

In some embodiments, the zoonotic disease RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 μg/kg and 400 μg/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments, the RNA vaccine for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 μg and 400 μg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments, a zoonotic disease RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered to the subject as a single dosage of 25-1000 μg (*e.g.*, a single dosage of mRNA encoding a zoonotic disease antigen). In some embodiments, a zoonotic disease RNA vaccine is administered to the subject as a single dosage of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 μg. For example, a zoonotic disease RNA vaccine may be administered to a subject as a single dose of 25-100, 25-500, 50-100, 50-500, 50-1000, 100-500, 100-1000, 250-500, 250-1000, or 500-1000 μg. In some embodiments, a zoonotic disease RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered to the subject as two dosages, the combination of which equals 25-1000 μg of the zoonotic disease RNA (*e.g.*, mRNA) vaccine.

AN zoonotic disease RNA (*e.g.*, mRNA) vaccine pharmaceutical composition described herein can be formulated into a dosage form described herein, such as an intranasal,

intratracheal, or injectable (*e.g.*, intravenous, intraocular, intravitreal, intramuscular, intradermal, intracardiac, intraperitoneal, and subcutaneous).

Vaccine Efficacy

Some aspects of the present disclosure provide formulations of the zoonotic disease RNA (*e.g.*, mRNA) vaccine, wherein the zoonotic disease RNA vaccine is formulated in an effective amount to produce an antigen specific immune response in a subject (*e.g.*, production of antibodies specific to an anti-zoonotic disease antigen). "An effective amount" is a dose of an zoonotic disease RNA (*e.g.*, mRNA) vaccine effective to produce an antigen-specific immune response. Also provided herein are methods of inducing an antigen-specific immune response in a subject.

As used herein, an immune response to a vaccine or LNP of the present disclosure is the development in a subject of a humoral and/or a cellular immune response to a (one or more) zoonotic disease protein(s) present in the vaccine. For purposes of the present disclosure, a "humoral" immune response refers to an immune response mediated by antibody molecules, including, e.g., secretory (IgA) or IgG molecules, while a "cellular" immune response is one mediated by T-lymphocytes (e.g., CD4+ helper and/or CD8+ T cells (e.g., CTLs) and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells (CTLs). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves and antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A cellular immune response also leads to the production of cytokines, chemokines, and other such molecules produced by activated T-cells and/or other white blood cells including those derived from CD4+ and CD8+ T-cells.

In some embodiments, the antigen-specific immune response is characterized by measuring an anti-zoonotic disease antigen antibody titer produced in a subject administered an zoonotic disease RNA (*e.g.*, mRNA) vaccine as provided herein. An antibody titer is a measurement of the amount of antibodies within a subject, for example, antibodies that are specific to a particular antigen (*e.g.*, an anti-zoonotic disease antigen) or epitope of an antigen. Antibody titer is typically expressed as the inverse of the greatest dilution that provides a

positive result. Enzyme-linked immunosorbent assay (ELISA) is a common assay for determining antibody titers, for example.

In some embodiments, an antibody titer is used to assess whether a subject has had an infection or to determine whether immunizations are required. In some embodiments, an antibody titer is used to determine the strength of an autoimmune response, to determine whether a booster immunization is needed, to determine whether a previous vaccine was effective, and to identify any recent or prior infections. In accordance with the present disclosure, an antibody titer may be used to determine the strength of an immune response induced in a subject by the zoonotic disease RNA (*e.g.*, mRNA) vaccine.

In some embodiments, an anti-zoonotic disease antigen antibody titer produced in a subject is increased by at least 1 log relative to a control. For example, anti-zoonotic disease antigen antibody titer produced in a subject may be increased by at least 1.5, at least 2, at least 2.5, or at least 3 log relative to a control. In some embodiments, the anti-zoonotic disease antigen antibody titer produced in the subject is increased by 1, 1.5, 2, 2.5 or 3 log relative to a control. In some embodiments, the anti-zoonotic disease antigen antibody titer produced in the subject is increased by 1-3 log relative to a control. For example, the anti-zoonotic disease antigen antibody titer produced in a subject may be increased by 1-1.5, 1-2, 1-2.5, 1-3, 1.5-2, 1.5-2.5, 1.5-3, 2-2.5, 2-3, or 2.5-3 log relative to a control.

In some embodiments, the anti-zoonotic disease antigen antibody titer produced in a subject is increased at least 2 times relative to a control. For example, the anti-zoonotic disease antigen antibody titer produced in a subject may be increased at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times relative to a control. In some embodiments, the anti-zoonotic disease antigen antibody titer produced in the subject is increased 2, 3, 4, 5, 6, 7, 8, 9, or 10 times relative to a control. In some embodiments, the anti-zoonotic disease antigen antibody titer produced in a subject is increased 2-10 times relative to a control. For example, the anti-zoonotic disease antigen antibody titer produced in a subject may be increased 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10 times relative to a control.

A control, in some embodiments, is the anti-zoonotic disease antigen antibody titer produced in a subject who has not been administered an zoonotic disease RNA (*e.g.*, mRNA) vaccine. In some embodiments, a control is an anti-zoonotic disease antigen antibody titer produced in a subject administered a recombinant or purified zoonotic disease protein vaccine. Recombinant protein vaccines typically include protein antigens that either have been produced

in a heterologous expression system (*e.g.*, bacteria or yeast) or purified from large amounts of the pathogenic organism.

In some embodiments, the ability of an zoonotic disease vaccine to be effective is measured in a murine model. For example, the zoonotic disease vaccines may be administered to a murine model and the murine model assayed for induction of neutralizing antibody titers. Viral challenge studies may also be used to assess the efficacy of a vaccine of the present disclosure. For example, the zoonotic disease vaccines may be administered to a murine model, the murine model challenged with zoonotic disease antigen, and the murine model assayed for survival and/or immune response (e.g., neutralizing antibody response, T cell response (e.g., cytokine response)).

In some embodiments, an effective amount of an zoonotic disease RNA (*e.g.*, mRNA) vaccine is a dose that is reduced compared to the standard of care dose of a recombinant zoonotic disease protein vaccine. A "standard of care," as provided herein, refers to a medical or psychological treatment guideline and can be general or specific. "Standard of care" specifies appropriate treatment based on scientific evidence and collaboration between medical professionals involved in the treatment of a given condition. It is the diagnostic and treatment process that a physician/ clinician should follow for a certain type of patient, illness or clinical circumstance. A "standard of care dose," as provided herein, refers to the dose of a recombinant or purified zoonotic disease protein vaccine, or a live attenuated or inactivated zoonotic disease vaccine, or an zoonotic disease VLP vaccine, that a physician/clinician or other medical professional would administer to a subject to treat or prevent a zoonotic disease, or a zoonotic disease-related condition, while following the standard of care guideline for treating or preventing a zoonotic disease, or a zoonotic disease related condition.

In some embodiments, the anti-zoonotic disease antigen antibody titer produced in a subject administered an effective amount of a zoonotic disease RNA vaccine is equivalent to an anti-zoonotic disease antigen antibody titer produced in a control subject administered a standard of care dose of a recombinant or purified zoonotic disease protein vaccine, or a live attenuated or inactivated zoonotic disease vaccine, or a zoonotic disease VLP vaccine.

In some embodiments, an effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a dose equivalent to an at least 2-fold reduction in a standard of care dose of a recombinant or purified zoonotic disease protein vaccine. For example, an effective amount of a zoonotic disease RNA vaccine may be a dose equivalent to an at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold reduction in a standard of care dose of a recombinant or purified zoonotic disease protein

vaccine. In some embodiments, an effective amount of a zoonotic disease RNA vaccine is a dose equivalent to an at least at least 100-fold, at least 500-fold, or at least 1000-fold reduction in a standard of care dose of a recombinant or purified zoonotic disease protein vaccine. In some embodiments, an effective amount of a zoonotic disease RNA vaccine is a dose equivalent to a 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 20-, 50-, 100-, 250-, 500-, or 1000-fold reduction in a standard of care dose of a recombinant or purified zoonotic disease protein vaccine. In some embodiments, the anti-zoonotic disease antigen antibody titer produced in a subject administered an effective amount of a zoonotic disease RNA vaccine is equivalent to an anti-zoonotic disease antigen antibody titer produced in a control subject administered the standard of care dose of a recombinant or protein zoonotic disease protein vaccine, or a live attenuated or inactivated zoonotic disease vaccine, or a zoonotic disease VLP vaccine. In some embodiments, an effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a dose equivalent to a 2-fold to 1000-fold (*e.g.*, 2-fold to 100-fold, 10-fold to 1000-fold) reduction in the standard of care dose of a recombinant or purified zoonotic disease protein vaccine, wherein the anti-zoonotic disease antigen antibody titer produced in the subject is equivalent to an anti-zoonotic disease antigen antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified zoonotic disease protein vaccine, or a live attenuated or inactivated zoonotic disease vaccine, or a zoonotic disease VLP vaccine.

In some embodiments, the effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a dose equivalent to a 2 to 1000-, 2 to 900-, 2 to 800-, 2 to 700-, 2 to 600-, 2 to 500-, 2 to 400-, 2 to 300-, 2 to 200-, 2 to 100-, 2 to 90-, 2 to 80-, 2 to 70-, 2 to 60-, 2 to 50-, 2 to 40-, 2 to 30-, 2 to 20-, 2 to 10-, 2 to 9-, 2 to 8-, 2 to 7-, 2 to 6-, 2 to 5-, 2 to 4-, 2 to 3-, 3 to 1000-, 3 to 900-, 3 to 800-, 3 to 700-, 3 to 600-, 3 to 500-, 3 to 400-, 3 to 3 to 00-, 3 to 200-, 3 to 100-, 3 to 90-, 3 to 80-, 3 to 70-, 3 to 60-, 3 to 50-, 3 to 40-, 3 to 30-, 3 to 20-, 3 to 10-, 3 to 9-, 3 to 8-, 3 to 7-, 3 to 6-, 3 to 5-, 3 to 4-, 4 to 1000-, 4 to 900-, 4 to 800-, 4 to 700-, 4 to 600-, 4 to 500-, 4 to 400-, 4 to 300-, 4 to 200-, 4 to 100-, 4 to 90-, 4 to 80-, 4 to 70-, 4 to 60-, 4 to 50, 4 to 40-, 4 to 30-, 4 to 20-, 4 to 10-, 4 to 9-, 4 to 8-, 4 to 7-, 4 to 6-, 4 to 5-, 4 to 4-, 5 to 1000-, 5 to 900-, 5 to 800-, 5 to 700-, 5 to 600-, 5 to 500-, 5 to 400-, 5 to 300-, 5 to 200-, 5 to 100-, 5 to 90-, 5 to 80-, 5 to 70-, 5 to 60-, 5 to 50-, 5 to 40-, 5 to 30-, 5 to 20-, 5 to 10-, 5 to 9-, 5 to 8, 5 to 7-, 5 to 6-, 6 to 1000-, 6 to 900-, 6 to 800-, 6 to 700-, 6 to 600-, 6 to 500-, 6 to 400-, 6 to 300-, 6 to 200-, 6 to 100-, 6 to 90-, 6 to 80-, 6 to 70-, 6 to 60-, 6 to 50-, 6 to 40-, 6 to 30-, 6 to 20-, 6 to 10-, 6 to 9-, 6 to 8-, 6 to 7-, 7 to 1000-, 7 to 900-, 7 to 800-, 7 to 700-, 7 to 600-, 7 to 500-, 7 to 400-, 7 to 300-, 7 to 200-, 7 to 100-, 7 to 90-, 7 to 80-, 7 to 70-, 7 to 60-, 7 to 50-, 7 to 40-, 7 to 30-, 7 to 20-, 7 to 10-, 7 to 9-, 7 to 8-, 8 to 1000-, 8 to 900-, 8 to 800-, 8 to 700-, 8 to 600-, 8 to 500-, 8 to 400-, 8 to

300-, 8 to 200-, 8 to 100-, 8 to 90-, 8 to 80-, 8 to 70-, 8 to 60-, 8 to 50-, 8 to 40-, 8 to 30-, 8 to 20-, 8 to 10-, 8 to 9-, 9 to 1000-, 9 to 900-, 9 to 800-, 9 to 700-, 9 to 600-, 9 to 500-, 9 to 400-, 9 to 300-, 9 to 200-, 9 to 100-, 9 to 90-, 9 to 80-, 9 to 70-, 9 to 60-, 9 to 50-, 9 to 40-, 9 to 30-, 9 to 20-, 9 to 10-, 10 to 1000-, 10 to 900-, 10 to 800-, 10 to 700-, 10 to 600-, 10 to 500-, 10 to 400-, 10 to 300-, 10 to 200-, 10 to 100-, 10 to 90-, 10 to 80-, 10 to 70-, 10 to 60-, 10 to 50-, 10 to 40-, 10 to 30-, 10 to 20-, 20 to 1000-, 20 to 900-, 20 to 800-, 20 to 700-, 20 to 600-, 20 to 500-, 20 to 400-, 20 to 300-, 20 to 200-, 20 to 100-, 20 to 90-, 20 to 80-, 20 to 70-, 20 to 60-, 20 to 50-, 20 to 40-, 20 to 30-, 30 to 1000-, 30 to 900-, 30 to 800-, 30 to 700-, 30 to 600-, 30 to 500-, 30 to 400-, 30 to 300-, 30 to 200-, 30 to 100-, 30 to 90-, 30 to 80-, 30 to 70-, 30 to 60-, 30 to 50-, 30 to 40-, 40 to 1000-, 40 to 900-, 40 to 800-, 40 to 700-, 40 to 600-, 40 to 500-, 40 to 400-, 40 to 300-, 40 to 200-, 40 to 100-, 40 to 90-, 40 to 80-, 40 to 70-, 40 to 60-, 40 to 50-, 50 to 1000-, 50 to 900-, 50 to 800-, 50 to 700-, 50 to 600-, 50 to 500-, 50 to 400-, 50 to 300-, 50 to 200-, 50 to 100-, 50 to 90-, 50 to 80-, 50 to 70-, 50 to 60-, 60 to 1000-, 60 to 900-, 60 to 800-, 60 to 700-, 60 to 600-, 60 to 500-, 60 to 400-, 60 to 300-, 60 to 200-, 60 to 100-, 60 to 90-, 60 to 80-, 60 to 70-, 70 to 1000-, 70 to 900-, 70 to 800-, 70 to 700-, 70 to 600-, 70 to 500-, 70 to 400-, 70 to 300-, 70 to 200-, 70 to 100-, 70 to 90-, 70 to 80-, 80 to 1000-, 80 to 900-, 80 to 800-, 80 to 700-, 80 to 600-, 80 to 500-, 80 to 400-, 80 to 300-, 80 to 200-, 80 to 100-, 80 to 90-, 90 to 1000-, 90 to 900-, 90 to 800-, 90 to 700-, 90 to 600-, 90 to 500-, 90 to 400-, 90 to 300-, 90 to 200-, 90 to 100-, 100 to 1000-, 100 to 900-, 100 to 800-, 100 to 700-, 100 to 600-, 100 to 500-, 100 to 400-, 100 to 300-, 100 to 200-, 200 to 1000-, 200 to 900-, 200 to 800-, 200 to 700-, 200 to 600-, 200 to 500-, 200 to 400-, 200 to 300-, 300 to 1000-, 300 to 900-, 300 to 800-, 300 to 700-, 300 to 600-, 300 to 500-, 300 to 400-, 400 to 1000-, 400 to 900-, 400 to 800-, 400 to 700-, 400 to 600-, 400 to 500-, 500 to 1000-, 500 to 900-, 500 to 800-, 500 to 700-, 500 to 600-, 600 to 1000-, 600 to 900-, 600 to 800-, 600 to 700-, 700 to 1000-, 700 to 900-, 700 to 800-, 800 to 1000-, 800 to 900-, or 900 to 1000-fold reduction in the standard of care dose of a recombinant zoonotic disease protein vaccine. In some embodiments, such as the foregoing, the anti-zoonotic disease antigen antibody titer produced in the subject is equivalent to an anti-zoonotic disease antigen antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified zoonotic disease protein vaccine, or a live attenuated or inactivated zoonotic disease vaccine, or a zoonotic disease VLP vaccine. In some embodiments, the effective amount is a dose equivalent to (or equivalent to an at least) 2-, 3 -,4 -,5 -,6-, 7-, 8-, 9-, 10-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-, 110-, 120-, 130-, 140-, 150-, 160-, 170-, 1280-, 190-, 200-, 210-, 220-, 230-, 240-, 250-, 260-, 270-, 280-, 290-, 300-, 310-, 320-, 330-, 340-, 350-, 360-, 370-, 380-, 390-, 400-, 410-, 420-, 430-, 440-, 450-, 4360-, 470-, 480-, 490-, 500-, 510-, 520-, 530-, 540-, 550-, 560-,

5760-, 580-, 590-, 600-, 610-, 620-, 630-, 640-, 650-, 660-, 670-, 680-, 690-, 700-, 710-, 720-, 730-, 740-, 750-, 760-, 770-, 780-, 790-, 800-, 810-, 820--, 830-, 840-, 850-, 860-, 870-, 880-, 890-, 900-, 910-, 920-, 930-, 940-, 950-, 960-, 970-, 980-, 990-, or 1000-fold reduction in the standard of care dose of a recombinant zoonotic disease protein vaccine. In some embodiments, such as the foregoing, an anti-zoonotic disease antigen antibody titer produced in the subject is equivalent to an anti-zoonotic disease antigen antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified zoonotic disease protein vaccine, or a live attenuated or inactivated zoonotic disease vaccine, or a zoonotic disease VLP vaccine.

In some embodiments, the effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a total dose of 50-1000 μg. In some embodiments, the effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a total dose of 50-1000, 50-900, 50-800, 50-700, 50-600, 50-500, 50-400, 50-300, 50-200, 50-100, 50-90, 50-80, 50-70, 50-60, 60-1000, 60- 900, 60-800, 60-700, 60-600, 60-500, 60-400, 60-300, 60-200, 60-100, 60-90, 60-80, 60-70, 70-1000, 70-900, 70-800, 70-700, 70-600, 70-500, 70-400, 70-300, 70-200, 70-100, 70-90, 70-80, 80-1000, 80- 900, 80-800, 80-700, 80-600, 80-500, 80-400, 80-300, 80-200, 80-100, 80-90, 90-1000, 90-900, 90-800, 90-700, 90-600, 90-500, 90-400, 90-300, 90-200, 90-100, 100-1000, 100- 900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, 200-300, 300-1000, 300-900, 300-800, 300-700, 300-600, 300-500, 300-400, 400-1000, 400-900, 400-800, 400-700, 400-600, 400-500, 500-1000, 500-900, 500-800, 500-700, 500-600, 600-1000, 600-900, 600-900, 600-700, 700-1000, 700-900, 700-800, 800-1000, 800-900, or 900-1000 μg. In some embodiments, the effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a total dose of 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 μg. In some embodiments, the effective amount is a dose of 25-500 μg administered to the subject a total of two times. In some embodiments, the effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a dose of 25-500, 25-400, 25-300, 25-200, 25-100, 25-50, 50-500, 50-400, 50-300, 50-200, 50-100, 100-500, 100-400, 100-300, 100-200, 150-500, 150-400, 150-300, 150-200, 200-500, 200-400, 200-300, 250-500, 250-400, 250-300, 300-500, 300-400, 350-500, 350-400, 400-500 or 450-500 μg administered to the subject a total of two times. In some embodiments, the effective amount of a zoonotic disease RNA (*e.g.*, mRNA) vaccine is a total dose of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 μg administered to the subject a total of two times.

Vaccine efficacy may be assessed using standard analyses (see, e.g., Weinberg et al., J Infect Dis. 2010 Jun 1;201(11):1607-10). For example, vaccine efficacy may be measured by

double-blind, randomized, clinical controlled trials. Vaccine efficacy may be expressed as a proportionate reduction in disease attack rate (AR) between the unvaccinated (ARU) and vaccinated (ARV) study cohorts and can be calculated from the relative risk (RR) of disease among the vaccinated group with use of the following formulas:

Efficacy = (ARU – ARV)/ARU x 100; and

Efficacy = (1-RR) x 100.

Likewise, vaccine effectiveness may be assessed using standard analyses (see, e.g., Weinberg et al., J Infect Dis. 2010 Jun 1;201(11):1607-10). Vaccine effectiveness is an assessment of how a vaccine (which may have already proven to have high vaccine efficacy) reduces disease in a population. This measure can assess the net balance of benefits and adverse effects of a vaccination program, not just the vaccine itself, under natural field conditions rather than in a controlled clinical trial. Vaccine effectiveness is proportional to vaccine efficacy (potency) but is also affected by how well target groups in the population are immunized, as well as by other non-vaccine-related factors that influence the 'real-world' outcomes of hospitalizations, ambulatory visits, or costs. For example, a retrospective case control analysis may be used, in which the rates of vaccination among a set of infected cases and appropriate controls are compared. Vaccine effectiveness may be expressed as a rate difference, with use of the odds ratio (OR) for developing infection despite vaccination:

Effectiveness = (1 – OR) x 100.

In some embodiments, efficacy of the zoonotic disease vaccine is at least 60% relative to unvaccinated control subjects. For example, efficacy of the zoonotic disease vaccine may be at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 98%, or 100% relative to unvaccinated control subjects.

Sterilizing Immunity. Sterilizing immunity refers to a unique immune status that prevents effective pathogen infection into the host. In some embodiments, the effective amount of a zoonotic disease vaccine of the present disclosure is sufficient to provide sterilizing immunity in the subject for at least 1 year. For example, the effective amount of a zoonotic disease vaccine of the present disclosure is sufficient to provide sterilizing immunity in the subject for at least 2 years, at least 3 years, at least 4 years, or at least 5 years. In some embodiments, the effective amount of a zoonotic disease vaccine of the present disclosure is sufficient to provide sterilizing immunity in the subject at an at least 5-fold lower dose relative to control. For example, the effective amount may be sufficient to provide sterilizing immunity in the subject at an at least 10-fold lower, 15-fold, or 20-fold lower dose relative to a control.

Detectable Antigen. In some embodiments, the effective amount of a zoonotic disease vaccine of the present disclosure is sufficient to produce detectable levels of zoonotic disease antigen as measured in serum of the subject at 1-72 hours post administration.

Titer. An antibody titer is a measurement of the amount of antibodies within a subject, for example, antibodies that are specific to a particular antigen (e.g., an anti-zoonotic disease antigen). Antibody titer is typically expressed as the inverse of the greatest dilution that provides a positive result. Enzyme-linked immunosorbent assay (ELISA) is a common assay for determining antibody titers, for example.

In some embodiments, the effective amount of a zoonotic disease vaccine of the present disclosure is sufficient to produce a 1,000-10,000 neutralizing antibody titer produced by neutralizing antibody against the zoonotic disease antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the effective amount is sufficient to produce a 1,000-5,000 neutralizing antibody titer produced by neutralizing antibody against the zoonotic disease antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the effective amount is sufficient to produce a 5,000-10,000 neutralizing antibody titer produced by neutralizing antibody against the zoonotic disease antigen as measured in serum of the subject at 1-72 hours post administration.

In some embodiments, the neutralizing antibody titer is at least 100 NT_{50}. For example, the neutralizing antibody titer may be at least 200, 300, 400, 500, 600, 700, 800, 900 or 1000 NT_{50}. In some embodiments, the neutralizing antibody titer is at least 10,000 NT_{50}.

In some embodiments, the neutralizing antibody titer is at least 100 neutralizing units per milliliter (NU/mL). For example, the neutralizing antibody titer may be at least 200, 300, 400, 500, 600, 700, 800, 900 or 1000 NU/mL. In some embodiments, the neutralizing antibody titer is at least 10,000 NU/mL.

In some embodiments, an anti-zoonotic disease antigen antibody titer produced in the subject is increased by at least 1 log relative to a control. For example, an anti-zoonotic disease antigen antibody titer produced in the subject may be increased by at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 log relative to a control.

In some embodiments, an anti-zoonotic disease antigen antibody titer produced in the subject is increased at least 2 times relative to a control. For example, an anti-zoonotic disease antigen antibody titer produced in the subject is increased by at least 3, 4, 5, 6, 7, 8, 9 or 10 times relative to a control.

In some embodiments, a geometric mean, which is the nth root of the product of n numbers, is generally used to describe proportional growth. Geometric mean, in some embodiments, is used to characterize antibody titer produced in a subject.

A control may be, for example, an unvaccinated subject, or a subject administered a live attenuated zoonotic disease vaccine, an inactivated zoonotic disease vaccine, or a protein subunit zoonotic disease vaccine.

Additional Embodiments

One aspect of the disclosure is a Lassa virus (LASV) vaccine, comprising at least one RNA polynucleotide having an open reading frame encoding at least one LASV antigenic polypeptide. In some embodiments, the LASV antigenic polypeptide is a Lassa glycoprotein precursor GPC. In some embodiments, the LASV antigenic polypeptide is a structurally stabilized GPC. In some embodiments, the LASV antigenic polypeptide is a ectodomain of LASV glycoprotein 1 (GP1). In some embodiments, the LASV antigenic polypeptide is a glycoprotein. In some embodiments, the glycoprotein comprises amino acid residues 59-259 of the LASV glycoprotein precursor (GPC). In some embodiments, the LASV antigenic polypeptide is glycoprotein 2 (GP2). In some embodiments, the LASV antigenic polypeptide is a nucleocapsid protein (NP). In some embodiments, the LASV antigenic polypeptide is fused to a signal peptide.

In some embodiments, the LASV antigenic has an amino acid sequence that has at least 90% identity to an amino acid sequence identified by any one of SEQ ID NO: 1-3, but does not include wild-type protein sequence. In some embodiments, the LASV antigenic has an amino acid sequence that has at least 95% identity to an amino acid sequence identified by any one of SEQ ID NO: 1-3, but does not include wild-type protein sequence. In some embodiments, the LASV antigenic has an amino acid sequence that has at least 99% identity to an amino acid sequence identified by any one of SEQ ID NO: 1-3, but does not include wild-type protein sequence. In some embodiments, the LASV antigenic polypeptide has an amino acid sequence of any one of SEQ ID NO: 1-3.

In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence that has at least 80% identity to any one of SEQ ID NO: 6, 7, or 9, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence that has at least 85% identity to any one of SEQ ID NO: 6, 7, or 9, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence that has at least 90% identity to any one of SEQ ID NO: 6, 7, or 9, but

does not include wild-type mRNA sequence. In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence that has at least 95% identity to any one of SEQ ID NO: 6, 7, or 9, but does not include wild-type mRNA sequence.

In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence that has at least 98% identity to any one of SEQ ID NO: 4-9, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence of any one of SEQ ID NO: 6, 7, or 9. In some embodiments, the LASV antigenic polypeptide has membrane fusion activity, attaches to cell receptors, causes fusion of viral and cellular membranes, and/or is responsible for binding of the virus to a cell being infected. In some embodiments, the at least one RNA polynucleotide having an open reading frame encoding at least one LASV antigenic polypeptide comprises at least one chemical modification. In some embodiments, the chemical modification is selected from pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 5-methyluridine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine and 2'-O-methyl uridine. In some embodiments, the chemical modification is in the carbon 5-position of the uracil. In some embodiments, the chemical modification is a N1-methylpseudouridine or N1-ethylpseudouridine. In some embodiments, at least 80% of the uracil in the open reading frame have a chemical modification. In some embodiments, at least 90% of the uracil in the open reading frame have a chemical modification. In some embodiments, 100% of the uracil in the open reading frame have a chemical modification. In some embodiments, 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil. In some embodiments, at least one RNA polynucleotide having an open reading frame encoding at least one LASV antigenic polypeptide further encodes at least one 5' terminal cap. In some embodiments, the 5' terminal cap is 7mG(5')ppp(5')NlmpNp.

In some embodiments, the RNA polynucleotide having an open reading frame encoding at least one LASV antigenic polypeptide is formulated in a cationic lipid nanoparticle. In some embodiments, the cationic lipid nanoparticle has a mean diameter of 50-200 nm. In some embodiments, the cationic lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some embodiments, the cationic lipid nanoparticle comprises a molar ratio of about 20-60% cationic lipid, 0.5-15% PEG-modified lipid, 25-55% sterol, and 5-25% non-cationic lipid. In some embodiments, the cationic lipid is an ionizable cationic lipid and

the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol. In some embodiments, the cationic lipid is selected from 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319). In some embodiments, the cationic lipid nanoparticle comprises a compound of Formula (I), optionally Compound 3, 18, 20, 25, 26, 29, 30, 60, 108-112, or 122. In some embodiments, the cationic lipid nanoparticle comprises a compound of Formula (II). In some embodiments, the cationic lipid nanoparticle has a polydispersity value of less than 0.4. In some embodiments, the cationic lipid nanoparticle has a net neutral charge at a neutral pH value. In some embodiments, further comprising an adjuvant.

In some embodiments, the open reading frame encoding at least one LASV antigenic polypeptide is codon-optimized. In some embodiments, the LASV vaccine is multivalent. In some embodiments, the LASV vaccine is formulated in an effective amount to produce an antigen-specific immune response. In some embodiments, the LASV vaccine is for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the LASV vaccine in an amount effective to produce an antigen specific immune response in the subject.

One aspect of the disclosure is a pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the LASV vaccine as described herein, wherein the effective dose is sufficient to produce detectable levels of antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the cut off index of the antigen is 1-2.

One aspect of the disclosure is a pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the LASV vaccine as described herein, wherein the effective dose is sufficient to produce a 1,000- 10,000 neutralization titer produced by neutralizing antibody against said antigen as measured in serum of the subject at 1-72 hours post administration.

One aspect of the disclosure is a composition comprising the LASV vaccine as described herein formulated in a lipid nanoparticle comprising compounds of Formula (I), (IA) and/or Formula (II), discussed below.

One aspect of the disclosure is a method of inducing an immune response in a subject, the method comprising administering to the subject the LASV vaccine as described herein in an amount effective to produce an antigen-specific immune response in the subject. In some embodiments, the antigen specific immune response comprises a T cell response or a B cell response. In some embodiments, the subject is administered a single dose of the vaccine. In some

embodiments, the subject is administered a booster dose of the vaccine. In some embodiments, the vaccine is administered to the subject by intradermal injection or intramuscular injection. In some embodiments, an anti-antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control. In some embodiments, an anti-antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control. In some embodiments, the anti-antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control. In some embodiments, the anti-antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a control. In some embodiments, the control is an anti-antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine against the virus.

One aspect of the disclosure is a paramyxovirus vaccine, comprising: at least one RNA polynucleotide having an open reading frame encoding at least one Nipah virus (NiV) and/or Hendra virus (HeV) antigenic polypeptide. In some embodiments, the NiV and/or HeV antigenic polypeptide is a hemagglutinin-neuraminidase protein (HN) or hemagglutinin protein (H). In some embodiments, the NiV and/or HeV antigenic polypeptide is a glycoprotein (G). In some embodiments, the NiV and/or HeV antigenic polypeptide is an attachment glycoproteins which is a type II membrane protein. In some embodiments, the NiV and/or HeV antigenic polypeptide is a fusion (F) glycoprotein. In some embodiments, the F glycoprotein comprises a trimeric class I fusogenic envelope glycoprotein containing two heptad repeat (HR) regions and a hydrophobic fusion peptide.

In some embodiments, the NiV and/or HeV antigenic polypeptide is NiV antigenic polypeptide. In some embodiments, the NiV and/or HeV antigenic polypeptide is HeV antigenic polypeptide. In some embodiments, the NiV and/or HeV antigenic polypeptide is fused to a signal peptide. In some embodiments, the NiV and/or HeV antigenic has an amino acid sequence that has at least 90% identity to an amino acid sequence identified by any one of SEQ ID NO: 10-13, but does not include wild-type protein sequence. In some embodiments, the NiV and/or HeV antigenic has an amino acid sequence that has at least 95% identity to an amino acid sequence identified by any one of SEQ ID NO: 10-13, but does not include wild-type protein sequence. In some embodiments, the NiV and/or HeV antigenic has an amino acid sequence that has at least 99% identity to an amino acid sequence identified by any one of SEQ ID NO: 10-13, but does not include wild-type protein sequence. In some embodiments, the NiV and/or HeV antigenic polypeptide has an amino acid sequence of any one of SEQ ID NO: 10-13.

In some embodiments, the at least one RNA polynucleotide has a nucleic acid sequence that has at least 80% identity to any one of SEQ ID NO: 16 or 17, but does not include wild-type mRNA sequence.

In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence that has at least 85% identity to SEQ ID NO: 16 or 17, but does not include wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence that has at least 90% identity to SEQ ID NO: 16 or 17, but does not include wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence that has at least 95% identity to SEQ ID NO: 16 or 17, but does not include wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence that has at least 98% identity to SEQ ID NO: 16 or 17, but does not include wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence of SEQ ID NO: 16 or 17.

In some embodiments, the antigenic polypeptide has membrane fusion activity, attaches to cell receptors, causes fusion of viral and cellular membranes, and/or is responsible for binding of the virus to a cell being infected. In some embodiments, at least one RNA polynucleotide comprises at least one chemical modification. In some embodiments, the chemical modification is selected from pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 5-methyluridine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine and 2'-O-methyl uridine. In some embodiments, the chemical modification is in the 5-position of the uracil.

In some embodiments, the chemical modification is a N1-methylpseudouridine or N1-ethylpseudouridine. In some embodiments, at least 80% of the uracil in the open reading frame have a chemical modification. In some embodiments, at least 90% of the uracil in the open reading frame have a chemical modification. In some embodiments, 100% of the uracil in the open reading frame have a chemical modification. In some embodiments, 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil. In some embodiments, at least one RNA polynucleotide further encodes at least one 5' terminal cap. In some embodiments, the 5' terminal cap is 7mG(5')ppp(5')NlmpNp. In some embodiments, the RNA polynucleotide is formulated in a cationic lipid nanoparticle. In some embodiments, the cationic lipid nanoparticle has a mean diameter of 50-200 nm. In some

embodiments, the cationic lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some embodiments, the cationic lipid nanoparticle comprises a molar ratio of about 20-60% cationic lipid, 0.5-15% PEG-modified lipid, 25-55% sterol, and 5-25% non-cationic lipid. In some embodiments, the cationic lipid is an ionizable cationic lipid and the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol.

In some embodiments, the cationic lipid is selected from 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319). In some embodiments, the cationic lipid nanoparticle comprises a compound of Formula (I), optionally Compound 3, 18, 20, 25, 26, 29, 30, 60, 108-112, or 122. In some embodiments, the cationic lipid nanoparticle comprises a compound of Formula (II). In some embodiments, the cationic lipid nanoparticle has a polydispersity value of less than 0.4. In some embodiments, the cationic lipid nanoparticle has a net neutral charge at a neutral pH value. Some embodiments further comprise an adjuvant. In some embodiments, the open reading frame is codon-optimized. In some embodiments, the vaccine is multivalent. Some embodiments are formulated in an effective amount to produce an antigen-specific immune response. Some embodiments are for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the vaccine in an amount effective to produce an antigen specific immune response in the subject.

One aspect of the disclosure is a pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the paramyxovirus vaccine as described herein, wherein the effective dose is sufficient to produce detectable levels of antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the cut off index of the antigen is 1-2.

One aspect of the disclosure is a pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the paramyxovirus vaccine as described herein, wherein the effective dose is sufficient to produce a 1,000- 10,000 neutralization titer produced by neutralizing antibody against said antigen as measured in serum of the subject at 1-72 hours post administration.

One aspect of the disclosure is a composition comprising the paramyxovirus vaccine as described herein formulated in a lipid nanoparticle comprising compounds of Formula (I), (IA), and/or Formula (II), discussed below.

One aspect of the disclosure is a method of inducing an immune response in a subject, the method comprising administering to the subject the paramyxovirus vaccine as described herein in an amount effective to produce an antigen-specific immune response in the subject. In some embodiments, the antigen specific immune response comprises a T cell response or a B cell response. In some embodiments, the subject is administered a single dose of the vaccine. In some embodiments, the subject is administered a booster dose of the vaccine. In some embodiments, the vaccine is administered to the subject by intradermal injection or intramuscular injection. In some embodiments, an anti-antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control. In some embodiments, an anti-antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control. In some embodiments, the anti-antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control. In some embodiments, the anti-antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a control. In some embodiments, the control is an anti-antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine against the virus.

One aspect of the invention is a betacoronavirus vaccine, comprising: at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one MERS-CoV or SARS-like coronavirus WIV1 (SL-CoV-WIV1) antigenic polypeptide. In some embodiments, the antigenic polypeptide is a betacoronavirus structural protein. In some embodiments, the betacoronavirus structural protein is spike protein (S), envelope protein (E), nucleocapsid protein (N) or membrane protein (M). In some embodiments, the betacoronavirus structural protein is spike protein (S). In some embodiments, the antigenic polypeptide is a S1 subunit of the spike protein (S). In some embodiments, the antigenic polypeptide is a S2 subunit of the spike protein (S). In some embodiments, the antigenic polypeptide is an SL-CoV-WIV1 antigenic polypeptide. In some embodiments, the antigenic polypeptide is a MERS-CoV antigenic polypeptide. In some embodiments, the open reading from is codon-optimized. In some embodiments, the vaccine is multivalent. In some embodiments, at least one RNA polynucleotide encodes at least 2 antigenic polypeptides. In some embodiments, at least one RNA polynucleotide encodes at least 10 antigenic polypeptides. In some embodiments, at least one RNA polynucleotide encodes at least 100 antigenic polypeptides. In some embodiments, at least one RNA polynucleotide encodes 2-100 antigenic polypeptides.

In some embodiments, the MERS-CoV or SL-CoV-WIV1 antigenic polypeptide has an amino acid sequence that has at least 90% identity to an amino acid sequence identified by SEQ ID NO: 18, but does not include wild-type protein sequence. In some embodiments, the MERS-

CoV or SL-CoV-WIV1 antigenic polypeptide has an amino acid sequence that has at least 95% identity to an amino acid sequence identified by SEQ ID NO: 18, but does not include wild-type protein sequence. In some embodiments, the MERS-CoV or SL-CoV-WIV1 antigenic polypeptide has an amino acid sequence of SEQ ID NO: 18.

In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence that has at least 80% identity to SEQ ID NO: 19 or 20, but does not include wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence that has at least 90% identity to SEQ ID NO: 19 or 20, but does not include wild-type mRNA sequence. In some embodiments, at least one RNA polynucleotide has a nucleic acid sequence of SEQ ID NO: 19 or 20.

In some embodiments, at least one RNA polynucleotide comprises at least one chemical modification. In some embodiments, the chemical modification is selected from pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 5-methyluridine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine and 2'-O-methyl uridine. In some embodiments, the chemical modification is in the 5-position of the uracil. In some embodiments, the chemical modification is a N1-methylpseudouridine or N1-ethylpseudouridine. In some embodiments, at least 80% of the uracil in the open reading frame have a chemical modification. In some embodiments, at least 90% of the uracil in the open reading frame have a chemical modification. In some embodiments, 100% of the uracil in the open reading frame have a chemical modification. In some embodiments, 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil. In some embodiments, at least one RNA polynucleotide further encodes at least one 5' terminal cap. In some embodiments, the 5' terminal cap is 7mG(5')ppp(5')NlmpNp.

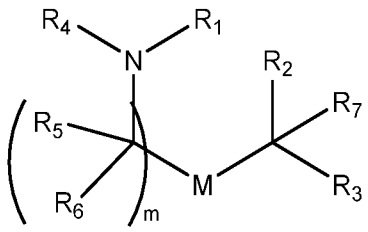
In some embodiments, the RNA polynucleotide is formulated in a cationic lipid nanoparticle. In some embodiments, the cationic lipid nanoparticle has a mean diameter of 50-200 nm. In some embodiments, the cationic lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some embodiments, the cationic lipid nanoparticle comprises a molar ratio of about 20-60% cationic lipid, 0.5-15% PEG-modified lipid, 25-55% sterol, and 5-25% non-cationic lipid. In some embodiments, the cationic lipid is an ionizable cationic lipid and the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol. In some embodiments, the cationic lipid is selected from 2,2-dilinoleyl-4-dimethylaminoethyl-

[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319). In some embodiments, the cationic lipid nanoparticle comprises a compound of Formula (I), optionally Compound 3, 18, 20, 25, 26, 29, 30, 60, 108-112, or 122. In some embodiments, the cationic lipid nanoparticle comprises a compound of Formula (II). In some embodiments, the cationic lipid nanoparticle has a polydispersity value of less than 0.4. In some embodiments, the cationic lipid nanoparticle has a net neutral charge at a neutral pH value. Some embodiments further comprise an adjuvant. In some embodiments, the open reading frame is codon-optimized. In some embodiments, the vaccine is multivalent. Some embodiments are formulated in an effective amount to produce an antigen-specific immune response. Some embodiments are for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the vaccine in an amount effective to produce an antigen specific immune response in the subject.

One aspect of the invention is a pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the betacoronavirus vaccine as described herein, wherein the effective dose is sufficient to produce detectable levels of antigen as measured in serum of the subject at 1-72 hours post administration. In some embodiments, the cut off index of the antigen is 1-2.

One aspect of the invention is a pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the betacoronavirus vaccine as described herein, wherein the effective dose is sufficient to produce a 1,000- 10,000 neutralization titer produced by neutralizing antibody against said antigen as measured in serum of the subject at 1-72 hours post administration.

One aspect of the invention is a composition comprising the betacoronavirus vaccine as described herein formulated in a lipid nanoparticle comprising compounds of Formula (I):



(I),

or a salt or isomer thereof, wherein: R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R'; R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is selected from

the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, $-OR$, $-O(CH_2)_nN(R)_2$, $-C(O)OR$, $-OC(O)R$, $-CX_3$, $-CX_2H$, $-CXH_2$, $-CN$, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, $-C(O)N(R)OR$, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle; R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

In some embodiments, a subset of compounds of Formula (I) includes those in which when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2. In some embodiments, a subset of compounds of Formula (I) includes those in which R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R'; R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, $-O(CH_2)_nN(R)_2$, $-C(O)OR$, $OC(O)R$, $-CX_3$, $-CX_2H$, $-CXH_2$, $-CN$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-CRN(R)_2C(O)OR$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$,

-N(OR)C(O)R, -N(OR)S(O)$_2$R, -N(OR)C(O)OR, -N(OR)C(O)N(R)$_2$, -N(OR)C(S)N(R)$_2$, -N(OR)C(=NR$_9$)N(R)$_2$, -N(OR)C(=CHR$_9$)N(R)$_2$, -C(=NR$_9$)N(R)$_2$, -C(=NR$_9$)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C_{1-3} alkyl, and each n is independently selected from 1, 2, 3, 4, and 5; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)$_2$-, -S-S-, an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle; R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, -S(O)$_2$R, -S(O)$_2$N(R)$_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R'; R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is selected from the group consisting of a C_{3-6} carbocycle, -(CH$_2$)$_n$Q, -(CH$_2$)$_n$CHQR, -CHQR, -CQ(R)$_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH$_2$)$_n$N(R)$_2$, -C(O)OR, -OC(O)R, -CX$_3$, -CX$_2$H, -CXH$_2$, -CN, -C(O)N(R)$_2$,-N(R)C(O)R, -N(R)S(O)$_2$R, -N(R)C(O)N(R)$_2$, -N(R)C(S)N(R)$_2$, -CRN(R)$_2$C(O)OR, -N(R)R$_8$, -O(CH$_2$)$_n$OR, -N(R)C(=NR$_9$)N(R)$_2$, -N(R)C(=CHR$_9$)N(R)$_2$, -OC(O)N(R)$_2$, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)$_2$R, -N(OR)C(O)OR, -N(OR)C(O)N(R)$_2$, -N(OR)C(S)N(R)$_2$, -N(OR)C(=NR$_9$)N(R)$_2$, -N(OR)C(=CHR$_9$)N(R)$_2$, -C(=NR$_9$)R, -C(O)N(R)OR, and -C(=NR$_9$)N(R)$_2$, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R_4 is -(CH$_2$)$_n$Q in which n

is 1 or 2, or (ii) R_4 is $-(CH_2)_nCHQR$ in which n is 1, or (iii) R_4 is -CHQR, and $-CQ(R)_2$, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle; R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

In some embodiments, the subset of compounds of Formula (I) includes those in which R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R'; R_2 and R_3 are independently selected from the group consisting of H, C_{2-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is $-(CH_2)_nQ$ or $-(CH_2)_nCHQR$, where Q is $-N(R)_2$, and n is selected from 3, 4, and 5; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R'; R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle; R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, and $-CQ(R)_2$, where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5; each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group; R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H; each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl; each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl; each Y is independently a C_{3-6} carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IA): (IA), or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M'; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, -N(R)C(O)R, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

One aspect of the invention is a method of inducing an immune response in a subject, the method comprising administering to the subject the betacoronavirus vaccine as described herein in an amount effective to produce an antigen-specific immune response in the subject. In some embodiments, the antigen specific immune response comprises a T cell response or a B cell response. In some embodiments, the subject is administered a single dose of the vaccine. In some embodiments, the subject is administered a booster dose of the vaccine. In some embodiments, the vaccine is administered to the subject by intradermal injection or intramuscular injection.

In some embodiments, an anti-antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control. In some embodiments, an anti-antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control. In some embodiments, the anti-antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control. In some embodiments, the anti-antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a control. In some embodiments, the control is an anti-antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine against the virus.

EXAMPLES

Example 1: Ebola vaccine immunogenicity study

8-10 week old female Balb/c mice were immunized intramuscularly with 10 µg of Ebola mRNA vaccines or recombinant Zaire ebolavirus Glycoprotein on day 0 and 14. Serum samples were collected on day 21, 33, 52 and 77 to measure antibody response.

Animals receiving 2 doses of the Ebola mRNA vaccine antigens had high levels of GP specific IgG titers 1 week after 2nd dose (FIG. 2). The results from this study were used to select a lead vaccine candidate to be tested in a Guinea pig challenge model.

Example 2: Guinea pig challenge model

Guinea pigs (n=5) were immunized intramuscularly with 20 µg of select mRNA vaccine constructs (AG1 and AG2) on Day 0 and 21. Three weeks after the second dose, animals were challenged with 1000pfu (XLD50) through Intraperitoneal injection of Guinea pig adapted Zaire Ebola virus strain Mayinga-76. Serial bleeds were collected 3, 6, 9 and 12 days after challenge to measure viremia. Animals were monitored for morbidity and mortality for 4 weeks after challenge (FIG. 3).

All animals receiving the mRNA vaccine (AG1 and AG2) were completely protected in the lethal challenge model while all placebo treated animals succumbed to infection by day 10. Furthermore, animals receiving Ebola GP mRNA vaccines did not demonstrate any significant morbidity or weight loss after challenge.

Example 3: Product development strategy

Two Phase 1/2 clinical trials are planned and will be a safety, immunogenicity and dose-selection studies in non-endemic and endemic settings. The first clinical study (FIH) will be

initiated in the US and will include approximately 90 subjects. Three dose levels of investigational vaccine will be tested compared to placebo in a staggered manner. To mitigate risk of different immunogenicity in subjects from endemic and non-endemic setting, the second clinical study in endemic setting will be initiated in collaboration with a local clinical study site. Following evaluation of immunogenicity and safety data from both clinical studies (at 1 month post-vaccination), a dose of vaccine for further development will be selected.

Example 4: PIV3 mRNA vaccine as a demonstration

An mRNA vaccine was designed based on the PIV3 fusion protein and tested in two animal models, cotton rat and African green monkey, for immunogenicity and protection from viral challenge.

First, cotton rats were dosed 10 μg, or 25 μg of the mRNA PIV3 vaccine, placebo, or formalin inactivated (FI) PIV3 vaccine at days 0 and 28. Blood was collected pre-dose and on days 27 and 56 (28 days post dose 2) for immunogenicity testing by viral neutralization assay. On day 57 the animals were challenged with PIV3 and viral titer measured 5 days post challenge on lung and nose samples.

As shown in FIG. 9, both the 10 μg and 25 μg doses of mRNA vaccine completely protected cotton rats from a challenge that results in viral loads of 4 to 5 logs in lung and nose respectively, while FI vaccine showed no significant protection. The right panel shows that this protection was the result of neutralizing titers in the range of 7 to 9 logs.

The second model used to assess our mRNA PIV3 vaccine was African green monkey, which were screened as PIV3 seronegative before the experiment. The design was similar to the cotton rat study, but with animals dosed at 5, 25, or 50ug of the vaccine. As shown in FIG. 10, absolute neutralizing titers in serum were lower than in the cotton rat model, however the 25 and 50 μg doses still conferred complete protection from detectable viral load. The 5ug dose resulted in a reduction in viral load at 5 days post challenge of approximately 1.5 to 2 logs in nose and lung, respectively, relative to placebo.

While the results above suggest a high probability of success in generating an mRNA vaccine based on Nipah F protein, soluble Nipah glycoprotein (G) vaccines have also been shown to be protective *in vivo*. Leveraging the flexibility of this mRNA platform we will design and test constructs of the Nipah and Hendra glycoprotein G protein as well, ultimately testing the efficacy of F and G alone and in combination at different ratios. This flexible mRNA technology allows multiple constructs to be combined and administered as one vaccine. It also enables selection of the ideal ratio of antigens to elicit the optimal immune response.

Example 5: MERS-CoV spike protein mRNA vaccine

Mouse Immunogenicity

To determine the immunogenicity of MERS-CoV spike protein mRNA vaccine, female balb/c mice were immunized intramuscularly with 10 μg of the vaccine on Day 0 and 28. Virus neutralizing (VN) antibody titers in the mouse sera in response to MERS spike protein mRNA vaccine measured on Day 0, 21, 42 and 56 using an *in vitro* neutralization assay. All animals were confirmed to be seronegative at the beginning of the study.

As shown in FIG. 11, a single dose of the mRNA vaccine induced neutralizing antibodies with an average serum titer of 1:320 on day 21. After the second dose on day 21, the VN antibody titers were boosted to 1:3000 by day 42 and further boosted up to 1:4800 by day 56. In contrast, placebo treated mice had no detectable VN antibody titer throughout the study.

Rabbit Challenge

Oryctolagus cuniculus (Rabbit) has been recently identified as a suitable animal model for MERS-CoV infection. The sequence homology for the receptor gene for MERS-CoV, DPP4(dipeptidyl peptidase 4), between humans and rabbits is such that it allows proficient infection of rabbits with MERS-CoV (Ra et al., J Virol 2014; Haagmans et al., J Virol, 2015). Nevertheless, replication of MERS-CoV in rabbits require a very high viral inoculum administered through the intra-nasal and intra-tracheal route.

In order to assess the efficacy of MERS-CoV spike protein mRNA vaccine, 6 month old New Zealand white rabbits were challenged 6 weeks after prime with EMC/2012 MERS-CoV. The vaccine was tested in a one or two dose regimen, with the boost spaced 3 weeks apart on day 21 for group 2, and each dose was 20 μg. Nasal and Throat swabs were collected from one day prior to challenge; to the end of study, 4 days post challenge. Serum from animals was collected on Day 0, 21, 35, 42 and 47 for measuring virus neutralizing antibody titers.

In the Single dose group (prime only), all animals became VN positive two weeks after the vaccination and remained equally high until one week before challenge. At the time of challenge (day 0) a minor decrease in VN antibodies was observed, which was boosted upon challenge virus MERS-CoV (see FIG. 12). Similarly, all animals receiving 2 doses (prime-boost) of the vaccine became VN positive two weeks after the first vaccination and responses were boosted after the second vaccination on day -21. VN antibody responses remained high until the time of challenge and were not further boosted upon challenge on day 0. No VN

antibody responses could be detected in any of the placebo treated animals during the vaccination and challenge phase of the study (FIG. 12).

Analysis of PCR and virus titration in rabbit nose swabs

In the prime only group (1a), virus could be detected by PCR on day 1 after challenge in all animals. Three animals remained PCR positive until the end of follow up, while 3 animals became PCR negative in within 2 to 4 days post challenge (FIG. 13, Panel A). None of the PCR positive signals detected after challenge could be confirmed by virus titration (FIG. 13, Panel D).

In the prime-boost group (1b), virus could be detected by PCR on day 1 after challenge in three out of six animals, which remained positive on day 2 after challenge and were PCR negative by day 3 post challenge (FIG. 13, Panel B). None of the PCR positive signals detected after challenge could be confirmed by virus titration (FIG. 13, Panel E).

Analysis of PCR and virus titration in rabbit throat swabs

In the prime only group (1a), virus could be detected by PCR on day 1 after challenge in all animals. One animal remained PCR positive until day 3 after challenge, however all animals were PCR negative day 4 post challenge (FIG. 14, Panel A). None of the PCR positive signals detected after challenge could be confirmed by virus titration (FIG. 14, Panel D).

In the prime-boost group (1b), virus could be detected by PCR day 1 after challenge in three out of six animals and all were PCR negative the following day. Additionally, two of these animals were PCR positive on the last day of follow up (FIG. 14, Panel B). PCR signals could not be detected in any of the other three animals. None of the PCR positive signals detected after challenge could be confirmed by virus titration (FIG. 14, Panel E).

In all placebo animals (group 2) virus could be detected by PCR on day 1 after challenge and remained PCR positive until the last sample that was analyzed. Only three PCR positive signals could be confirmed by virus titration (FIG. 14, Panels C and F).

Viral loads were also measured in the right nasal turbinates post mortem at the day of scheduled euthanasia (4 dpi). Levels of viral RNA were measured using a MERS-CoV-specific TaqMan PCR and levels of infectious (replication competent) virus using Vero cell culture.

Of the prime only group, samples from 2 out of 6 animals were positive by PCR, but all were undetectable by virus titration. The remaining four animals of group 1a were negative in PCR and virus titration. In the prime-boost group, 1 of 6 animals was positive by PCR, which again could not be detected by virus titration. The remaining five animals of group 1b were

negative in PCR and virus titration. Finally all placebo animals were positive by PCR and in four animals the PCR positive signal could be confirmed by virus titration.

Analysis of viral load in rabbit lungs

Rabbit lungs were dissected into 9 separated regions post mortem for individual for assessment of viral load by region of the lung. For determining the viral load in the total lung the different sections of the lungs were pooled (equal amount of material for each section) and these samples were tested by both PCR and titration (FIG. 15). Results by PCR showed that only one animal in the prime-boost group was PCR negative in the lungs. In contrast, while PCR positive signals could be detected in almost all animals, virus titration on the total lung samples resulted in only two positive animals, both in the placebo group.

Summary

The patterns of viral load observed by PCR and by titration observed in each of the sample types in the rabbit challenge model are suggestive of a high level of protection from viral replication. The lack of any replicating virus in most of the vaccinated animal samples indicates that any PCR signal found in those same samples is likely due to the detection of residual nucleic acid sequences from input virus during the challenge itself.

The body of the immunogenicity and viral challenge data indicate that the vaccines of the invention generate robust immunologic responses with high neutralizing titers that are protective from viral replication upon challenge.

EQUIVALENTS

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”
It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but

not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

The terms "about" and "substantially" preceding a numerical value mean ±10% of the recited numerical value.

Where a range of values is provided, each value between the upper and lower ends of the range are specifically contemplated and described herein.

The entire contents of International Application Nos. PCT/US2015/027400, PCT/US2016/043348, PCT/US2016/043332, PCT/US2016/058327, PCT/US2016/058324, PCT/US2016/058314, PCT/US2016/058310, PCT/US2016/058321, PCT/US2016/058297, PCT/US2016/058319, and PCT/US2016/058314 are incorporated herein by reference.

SEQUENCES

It should be understood that any of the mRNA sequences described herein may include a 5′ UTR and/or a 3′ UTR. The UTR sequences may be selected from the following sequences, or other known UTR sequences may be used. It should also be understood that any of the mRNA constructs described herein may further comprise a polyA tail and/or cap (e.g., 7mG(5')ppp(5')NlmpNp). Further, while many of the mRNAs and encoded antigen sequences described herein include a signal peptide and/or a peptide tag (e.g., C-terminal His tag), it should be understood that the indicated signal peptide and/or peptide tag may be substituted for a different signal peptide and/or peptide tag, or the signal peptide and/or peptide tag may be omitted.

Exemplary Sequences: Human IgG kappa signal sequence included in protein and nucleic acid sequences are underlined

Lassa_GPC protein

MGQIVTFFQEVPHVIEEVMNIVLIALSLLAILKGIYNVATCGLFGLVSFLLLCGRSCSTTYKGVYELQTLELD
MASLNMTMPLSCTKNNSHHYIMVGNETGLELTLTNTSIINHKFCNLSDAHKKDLYDHALMSIISTFHLSIPN
FNQYEAMSCDFNGGKISVQYNLSHTYAVDAANHCGTIANGVLQTFMRMAWGGSYIALDSGKGSWDCIM
TSYQYLIIQNTTWEDHCQFSRPSPIGYLGLLSQRTRDIYISRRLLGTFTWTLSDSEGNETPGGYCLTRWMLIE
AELKCFGNTAVAKCNEKHDEEFCDMLRLFDFNKQAIMRLKTEAQMSIQLINKAVNALINDQLIMKNHLRD
IMGIPYCNYSKYWYLNHTVTGKTSLPRCWLVSNGSYLNETRFSDDIEQQADNMITEMLQKEYLDRQGKTP
LGLVDLFVFSTSFYLISIFLHLVKIPTHRHIIGKPCPKPHRLNHMGICSCGLYKHPGVPVKWKR (SEQ ID NO: 1)

Lassa_GPC nucleic acid

ATGGGCCAGATCGTGACATTCTTCCAAGAGGTGCCCCACGTGATCGAGGAAGTGATGAACATCGTCCT
GATCGCCCTGAGCCTGCTGGCCATCCTGAAGGGCATCTACAACGTGGCCACCTGTGGCCTGTTTGGCC
TGGTGTCATTCCTGCTGCTGTGCGGCAGAAGCTGCAGCACCACATACAAGGGCGTGTACGAGCTGCAG
ACCCTGGAACTGGATATGGCCAGCCTGAACATGACCATGCCTCTGAGCTGCACCAAGAACAACAGCC
ACCACTACATCATGGTCGGAAACGAGACAGGACTGGAACTGACCCTGACCAACACCAGCATCATCAA
CCACAAGTTCTGCAACCTGAGCGACGCCCACAAGAAGGACCTGTACGATCACGCCCTGATGAGCATCA
TCTCCACCTTCCACCTGAGCATCCCCAACTTCAACCAGTACGAGGCCATGAGCTGCGACTTCAACGGC
GGCAAGATCAGCGTGCAGTACAATCTGAGCCACACCTACGCCGTGGACGCCGCCAATCACTGTGGCAC
AATTGCCAATGGCGTGCTGCAGACATTCATGCGGATGGCCTGGGGCGGCTCTTATATCGCCCTGGATT
CTGGCAAAGGCAGCTGGGACTGCATCATGACCAGCTACCAGTACCTGATCATCCAGAACACCACCTGG
GAAGATCACTGCCAGTTCAGCAGACCCTCTCCTATCGGCTATCTGGGCCTGCTGAGCCAGAGAACCCG
GGACATCTACATCAGCAGAAGGCTGCTGGGCACCTTCACCTGGACACTGTCTGACAGCGAGGGCAAC
GAAACACCTGGCGGCTACTGCCTGACCAGATGGATGCTGATTGAGGCCGAGCTGAAGTGCTTCGGCAA
TACCGCCGTGGCCAAGTGCAACGAGAAGCACGACGAGGAATTCTGCGACATGCTGCGGCTGTTCGATT
TCAACAAGCAGGCCATCATGCGGCTCAAGACCGAGGCTCAGATGTCCATCCAGCTGATCAACAAGGC
CGTGAATGCCCTGATCAACGATCAGCTCATCATGAAGAACCACCTCCGGGATATCATGGGCATCCCTT
ACTGCAACTACAGCAAGTACTGGTATCTCAACCACACCGTGACCGGCAAGACCAGCCTGCCTAGATGT
TGGCTGGTGTCCAACGGCAGCTACCTGAACGAGACACGGTTCAGCGACGACATCGAGCAGCAGGCCG
ACAACATGATCACCGAGATGCTGCAGAAAGAGTACCTGGACCGGCAGGGCAAGACACCTCTGGGACT
CGTGGATCTGTTCGTGTTCAGCACCAGCTTCTACCTGATCTCTATCTTCCTGCACCTGGTCAAGATCCC
CACACACCGGCACATCATCGGCAAGCCCTGTCCTAAGCCTCACCGGCTGAACCACATGGGAATCTGTA
GCTGCGGCCTGTACAAGCACCCTGGCGTGCCAGTGAAGTGGAAGAGA (SEQ ID NO: 4)

Lassa_GPC mRNA

AUGGGCCAGAUCGUGACAUUCUUCCAAGAGGUGCCCCACGUGAUCGAGGAAGUGAUGAACAUCGU
CCUGAUCGCCCUGAGCCUGCUGGCCAUCCUGAAGGGCAUCUACAACGUGGCCACCUGUGGCCUGUU
UGGCCUGGUGUCAUUCCUGCUGCUGUGCGGCAGAAGCUGCAGCACCACAUACAAGGGCGUGUACGA
GCUGCAGACCCUGGAACUGGAUAUGGCCAGCCUGAACAUGACCAUGCCUCUGAGCUGCACCAAGAA
CAACAGCCACCACUACAUCAUGGUCGGAAACGAGACAGGACUGGAACUGACCCUGACCAACACCAG
CAUCAUCAACCACAAGUUCUGCAACCUGAGCGACGCCCACAAGAAGGACCUGUACGAUCACGCCCU
GAUGAGCAUCAUCUCCACCUUCCACCUGAGCAUCCCCAACUUCAACCAGUACGAGGCCAUGAGCUG
CGACUUCAACGGCGGCAAGAUCAGCGUGCAGUACAAUCUGAGCCACACCUACGCCGUGGACGCCGC
CAAUCACUGUGGCACAAUUGCCAAUGGCGUGCUGCAGACAUUCAUGCGGAUGGCCUGGGGCGGCUC
UUAUAUCGCCCUGGAUUCUGGCAAAGGCAGCUGGGACUGCAUCAUGACCAGCUACCAGUACCUGAU
CAUCCAGAACACCACCUGGGAAGAUCACUGCCAGUUCAGCAGACCCUCUCCUAUCGGCUAUCUGGG
CCUGCUGAGCCAGAGAACCCGGGACAUCUACAUCAGCAGAAGGCUGCUGGGCACCUUCACCUGGAC
ACUGUCUGACAGCGAGGGCAACGAAACACCUGGCGGCUACUGCCUGACCAGAUGGAUGCUGAUUGA
GGCCGAGCUGAAGUGCUUCGGCAAUACCGCCGUGGCCAAGUGCAACGAGAAGCACGACGAGGAAUU
CUGCGACAUGCUGCGGCUGUUCGAUUUCAACAAGCAGGCCAUCAUGCGGCUCAAGACCGAGGCUCA
GAUGUCCAUCCAGCUGAUCAACAAGGCCGUGAAUGCCCUGAUCAACGAUCAGCUCAUCAUGAAGAA
CCACCUCCGGGAUAUCAUGGGCAUCCCUUACUGCAACUACAGCAAGUACUGGUAUCUCAACCACAC
CGUGACCGGCAAGACCAGCCUGCCUAGAUGUUGGCUGGUGUCCAACGGCAGCUACCUGAACGAGAC
ACGGUUCAGCGACGACAUCGAGCAGCAGGCCGACAACAUGAUCACCGAGAUGCUGCAGAAAGAGUA
CCUGGACCGGCAGGGCAAGACACCUCUGGGACUCGUGGAUCUGUUCGUGUUCAGCACCAGCUUCUA
CCUGAUCUCUAUCUUCCUGCACCUGGUCAAGAUCCCCACACACCGGCACAUCAUCGGCAAGCCCUG
UCCUAAGCCUCACCGGCUGAACCACAUGGGAAUCUGUAGCUGCGGCCUGUACAAGCACCCUGGCGU
GCCAGUGAAGUGGAAGAGA (SEQ ID NO: 6)

Lassa_Nucleoprotein with or without signal sequence - protein

MSASKEVKSFLWTQSLRRELSGYCSNIKLQVVKDAQALLHGLDFSEVSNVQRLMRKQKRDDGDLKRLRD
LNQAVNNLVELKSTQQKSVLRVGTLSSDDLLVLAADLEKLKSKVVRTERPLSSGIYMGNLSSQQLDQRKA
LLNMIGMTGGNGGRNTTSDGIVRVWDVKNAELLNNQFGTMPSLTLACLTKQGQVDLNDAVQALTDLGLI
YTAKYPNSSDLDRLAQSHPILNMIDTKKSSLNISGYNFSLGAAVKAGACMLDGGNMLETIKVSPQTMDGIL
KSILKVKRSLGMFISDTPGERNPYENILYKICLSGDGWPYIASRTSIVGRAWENTVVDLESDNKPQKTGNGG
SNKSLQSAGFAAGLTYSQLMTLKDSMLQLDPNAKTWMDIEGRPEDPVEIALYQPSSGCYIHFFREPTDLKQ
FKQDAKYSHGIDVTDLFAAQPGLTSAVIEALPRNMVITCQGSEDIRKLLESQGRRDIKLIDISLSKVDSRKFE
NAVWDQFKDLCHMHTGIVVEKKKRGGKEEITPHCALMDCIMFDAAVSGGVDAKVLRAVLPRDMVFRTS
TPKVVL (SEQ ID NO: 2)

METPAQLLFLLLLWLPDTTGMSASKEVKSFLWTQSLRRELSGYCSNIKLQVVKDAQALLHGLDFSEVSNV
QRLMRKQKRDDGDLKRLRDLNQAVNNLVELKSTQQKSVLRVGTLSSDDLLVLAADLEKLKSKVVRTERP
LSSGIYMGNLSSQQLDQRKALLNMIGMTGGNGGRNTTSDGIVRVWDVKNAELLNNQFGTMPSLTLACLT
KQGQVDLNDAVQALTDLGLIYTAKYPNSSDLDRLAQSHPILNMIDTKKSSLNISGYNFSLGAAVKAGACM
LDGGNMLETIKVSPQTMDGILKSILKVKRSLGMFISDTPGERNPYENILYKICLSGDGWPYIASRTSIVGRAW
ENTVVDLESDNKPQKTGNGGSNKSLQSAGFAAGLTYSQLMTLKDSMLQLDPNAKTWMDIEGRPEDPVEIA
LYQPSSGCYIHFFREPTDLKQFKQDAKYSHGIDVTDLFAAQPGLTSAVIEALPRNMVITCQGSEDIRKLLESQ
GRRDIKLIDISLSKVDSRKFENAVWDQFKDLCHMHTGIVVEKKKRGGKEEITPHCALMDCIMFDAAVSGG
VDAKVLRAVLPRDMVFRTSTPKVVL (SEQ ID NO: 3)

Lassa_Nucleoprotein with or without signal sequence – nucleic acid

ATGGAGACTCCTGCCCAGCTCTTGTTCCTTTTGCTATTGTGGCTTCCCGACACCACCGGCATGAGCGCC
AGCAAGGAGGTCAAGAGCTTCCTCTGGACCCAGAGCCTAAGAAGAGAGCTTAGCGGCTACTGCAGCA
ACATCAAGCTTCAGGTGGTGAAGGACGCCCAGGCCCTGCTGCACGGCCTGGACTTCAGCGAGGTGAG
CAACGTGCAGAGACTGATGAGAAAGCAGAAGCGAGACGACGGCGACCTGAAGCGTCTGCGGGACCTG
AACCAGGCCGTGAACAACCTGGTGGAGCTTAAGAGCACCCAGCAGAAGTCTGTGCTGAGAGTGGGCA
CCCTGAGCAGCGACGACCTGCTGGTGCTGGCCGCCGACCTGGAGAAGCTGAAGTCTAAGGTCGTCAG
AACCGAGCGGCCATTGAGCTCAGGCATCTACATGGGCAACCTTAGCAGTCAGCAGCTGGACCAGAGA
AAGGCCTTGCTGAACATGATCGGCATGACCGGCGGCAACGGCGGCAGAAACACCACCAGCGACGGCA
TCGTGAGAGTGTGGGACGTGAAGAACGCCGAGCTACTCAACAACCAGTTCGGCACCATGCCCAGCCT
GACCCTGGCCTGCCTGACCAAGCAGGGCCAGGTGGACCTCAATGACGCCGTGCAGGCACTAACCGAC
CTTGGCCTGATCTACACCGCCAAGTACCCCAACTCTTCAGACCTGGACAGACTGGCGCAGTCCCACCC
CATCTTAAATATGATTGACACCAAGAAGTCATCCCTTAACATCAGTGGCTACAACTTCAGCCTGGGCG
CCGCCGTGAAGGCCGGCGCCTGCATGCTGGACGGCGGAAATATGCTGGAAACTATCAAGGTGAGCCC
TCAGACCATGGACGGTATCCTGAAGTCCATTTTGAAGGTTAAGAGATCCCTGGGTATGTTCATCAGCG
ACACCCCAGGCGAGAGAAACCCCTACGAGAACATCCTGTACAAGATCTGCCTGAGTGGCGACGGCTG

GCCCTACATCGCGAGCAGAACCAGCATCGTGGGAAGGGCCTGGGAGAACACCGTGGTGGATCTTGAG
AGCGACAACAAGCCCCAGAAGACCGGAAATGGCGGTTCAAACAAGAGCCTGCAGAGCGCCGGCTTCG
CCGCCGGCCTGACCTACAGCCAGCTGATGACCCTGAAGGACAGCATGCTACAATTGGATCCCAACGCC
AAGACTTGGATGGACATCGAGGGCAGACCCGAGGACCCCGTGGAGATCGCCCTGTACCAGCCCTCAT
CCGGCTGCTACATCCACTTCTTCAGAGAGCCCACAGATCTGAAGCAGTTCAAGCAGGACGCGAAGTAT
AGCCATGGCATAGACGTCACCGATTTATTCGCGGCCCAGCCGGGCCTTACGAGCGCCGTGATCGAGGC
GCTGCCCAGAAACATGGTGATCACCTGCCAGGGCAGCGAGGACATCAGAAAGCTCCTTGAATCTCAA
GGCCGGAGAGATATTAAGCTGATAGATATCAGCTTATCTAAGGTTGACAGCAGAAAGTTCGAGAACG
CTGTATGGGACCAATTCAAGGACCTGTGCCACATGCATACGGGCATAGTGGTAGAGAAGAAGAAGCG
TGGCGGAAAGGAGGAGATCACACCTCACTGCGCCCTGATGGACTGCATCATGTTCGACGCGGCAGTCT
CCGGCGGCGTCGACGCAAAGGTCCTCCGGGCCGTGCTGCCAAGGGACATGGTGTTCCGGACAAGCAC
CCCTAAGGTAGTGCTG (SEQ ID NO: 5)

Lassa_Nucleoprotein with or without signal sequence - mRNA

AUGGAGACUCCUGCCCAGCUCUUGUUCCUUUUGCUAUUGUGGCUUCCCGACACCACCGGCAUGAGC
GCCAGCAAGGAGGUCAAGAGCUUCCUCUGGACCCAGAGCCUAAGAAGAGAGCUUAGCGGCUACUGC
AGCAACAUCAAGCUUCAGGUGGUGAAGGACGCCCAGGCCCUGCUGCACGGCCUGGACUUCAGCGAG
GUGAGCAACGUGCAGAGACUGAUGAGAAAGCAGAAGCGAGACGACGGCGACCUGAAGCGUCUGCG
GGACCUGAACCAGGCCGUGAACAACCUGGUGGAGCUUAAGAGCACCCAGCAGAAGUCUGUGCUGAG
AGUGGGCACCCUGAGCAGCGACGACCUGCUGGUGCUGGCCGCCGACCUGGAGAAGCUGAAGUCUAA
GGUCGUCAGAACCGAGCGGCCAUUGAGCUCAGGCAUCUACAUGGGCAACCUUAGCAGUCAGCAGCU
GGACCAGAGAAAGGCCUUGCUGAACAUGAUCGGCAUGACCGGCGGCAACGGCGGCAGAAACACCAC
CAGCGACGGCAUCGUGAGAGUGUGGGACGUGAAGAACGCCGAGCUACUCAACAACCAGUUCGGCAC
CAUGCCCAGCCUGACCCUGGCCUGCCUGACCAAGCAGGGCCAGGUGGACCUCAAUGACGCCGUGCA
GGCACUAACCGACCUUGGCCUGAUCUACACCGCCAAGUACCCCAACUCUUCAGACCUGGACAGACU
GGCGCAGUCCCACCCCAUCUUAAAUAUGAUUGACACCAAGAAGUCAUCCCUUAACAUCAGUGGCUA
CAACUUCAGCCUGGGCGCCGCCGUGAAGGCCGGCGCCUGCAUGCUGGACGGCGGAAAUAUGCUGGA
AACUAUCAAGGUGAGCCCUCAGACCAUGGACGGUAUCCUGAAGUCCAUUUUGAAGGUUAAGAGAU
CCCUGGGUAUGUUCAUCAGCGACACCCCAGGCGAGAGAAACCCCUACGAGAACAUCCUGUACAAGA
UCUGCCUGAGUGGCGACGGCUGGCCCUACAUCGCGAGCAGAACCAGCAUCGUGGGAAGGGCCUGGG
AGAACACCGUGGUGGAUCUUGAGAGCGACAACAAGCCCCAGAAGACCGGAAAUGGCGGUUCAAAC
AAGAGCCUGCAGAGCGCCGGCUUCGCCGCCGGCCUGACCUACAGCCAGCUGAUGACCCUGAAGGAC
AGCAUGCUACAAUUGGAUCCCAACGCCAAGACUUGGAUGGACAUCGAGGGCAGACCCGAGGACCCC
GUGGAGAUCGCCCUGUACCAGCCCUCAUCCGGCUGCUACAUCCACUUCUUCAGAGAGCCCACAGAU
CUGAAGCAGUUCAAGCAGGACGCGAAGUAUAGCCAUGGCAUAGACGUCACCGAUUUAUUCGCGGCC
CAGCCGGGCCUUACGAGCGCCGUGAUCGAGGCGCUGCCCAGAAACAUGGUGAUCACCUGCCAGGGC
AGCGAGGACAUCAGAAAGCUCCUUGAAUCUCAAGGCCGGAGAGAUAUUAAGCUGAUAGAUAUCAG
CUUAUCUAAGGUUGACAGCAGAAAGUUCGAGAACGCUGUAUGGGACCAAUUCAAGGACCUGUGCC
ACAUGCAUACGGGCAUAGUGGUAGAGAAGAAGAAGCGUGGCGGAAAGGAGGAGAUCACACCUCAC
UGCGCCCUGAUGGACUGCAUCAUGUUCGACGCGGCAGUCUCCGGCGGCGUCGACGCAAAGGUCCUC
CGGGCCGUGCUGCCAAGGGACAUGGUGUUCCGGACAAGCACCCCUAAGGUAGUGCUG (SEQ ID NO: 7)

ATGAGCGCCAGCAAGGAGGTCAAGAGCTTCCTCTGGACCCAGAGCCTAAGAAGAGAGCTTAGCGGCT
ACTGCAGCAACATCAAGCTTCAGGTGGTGAAGGACGCCCAGGCCCTGCTGCACGGCCTGGACTTCAGC
GAGGTGAGCAACGTGCAGAGACTGATGAGAAAGCAGAAGCGAGACGACGGCGACCTGAAGCGTCTG
CGGGACCTGAACCAGGCCGTGAACAACCTGGTGGAGCTTAAGAGCACCCAGCAGAAGTCTGTGCTGA
GAGTGGGCACCCTGAGCAGCGACGACCTGCTGGTGCTGGCCGCCGACCTGGAGAAGCTGAAGTCTAA
GGTCGTCAGAACCGAGCGGCCATTGAGCTCAGGCATCTACATGGGCAACCTTAGCAGTCAGCAGCTGG
ACCAGAGAAAGGCCTTGCTGAACATGATCGGCATGACCGGCGGCAACGGCGGCAGAAACACCACCAG
CGACGGCATCGTGAGAGTGTGGGACGTGAAGAACGCCGAGCTACTCAACAACCAGTTCGGCACCATG
CCCAGCCTGACCCTGGCCTGCCTGACCAAGCAGGGCCAGGTGGACCTCAATGACGCCGTGCAGGCACT
AACCGACCTTGGCCTGATCTACACCGCCAAGTACCCCAACTCTTCAGACCTGGACAGACTGGCGCAGT
CCCACCCCATCTTAAATATGATTGACACCAAGAAGTCATCCCTTAACATCAGTGGCTACAACTTCAGC
CTGGGCGCCGCCGTGAAGGCCGGCGCCTGCATGCTGGACGGCGGAAATATGCTGGAAACTATCAAGG
TGAGCCCTCAGACCATGGACGGTATCCTGAAGTCCATTTTGAAGGTTAAGAGATCCCTGGGTATGTTC
ATCAGCGACACCCCAGGCGAGAGAAACCCCTACGAGAACATCCTGTACAAGATCTGCCTGAGTGGCG
ACGGCTGGCCCTACATCGCGAGCAGAACCAGCATCGTGGGAAGGGCCTGGGAGAACACCGTGGTGGA
TCTTGAGAGCGACAACAAGCCCCAGAAGACCGGAAATGGCGGTTCAAACAAGAGCCTGCAGAGCGCC
GGCTTCGCCGCCGGCCTGACCTACAGCCAGCTGATGACCCTGAAGGACAGCATGCTACAATTGGATCC
CAACGCCAAGACTTGGATGGACATCGAGGGCAGACCCGAGGACCCCGTGGAGATCGCCCTGTACCAG
CCCTCATCCGGCTGCTACATCCACTTCTTCAGAGAGCCCACAGATCTGAAGCAGTTCAAGCAGGACGC

GAAGTATAGCCATGGCATAGACGTCACCGATTTATTCGCGGCCCAGCCGGGCCTTACGAGCGCCGTGA
TCGAGGCGCTGCCCAGAAACATGGTGATCACCTGCCAGGGCAGCGAGGACATCAGAAAGCTCCTTGA
ATCTCAAGGCCGGAGAGATATTAAGCTGATAGATATCAGCTTATCTAAGGTTGACAGCAGAAAGTTCG
AGAACGCTGTATGGGACCAATTCAAGGACCTGTGCCACATGCATACGGGCATAGTGGTAGAGAAGAA
GAAGCGTGGCGGAAAGGAGGAGATCACACCTCACTGCGCCCTGATGGACTGCATCATGTTCGACGCG
GCAGTCTCCGGCGGCGTCGACGCAAAGGTCCTCCGGGCCGTGCTGCCAAGGGACATGGTGTTCCGGAC
AAGCACCCCTAAGGTAGTGCTG (SEQ ID NO: 8)

mRNA

AUGAGCGCCAGCAAGGAGGUCAAGAGCUUCCUCUGGACCCAGAGCCUAAGAAGAGAGCUUAGCGGC
UACUGCAGCAACAUCAAGCUUCAGGUGGUGAAGGACGCCCAGGCCCUGCUGCACGGCCUGGACUUC
AGCGAGGUGAGCAACGUGCAGAGACUGAUGAGAAAGCAGAAGCGAGACGACGGCGACCUGAAGCG
UCUGCGGGACCUGAACCAGGCCGUGAACAACCUGGUGGAGCUUAAGAGCACCCAGCAGAAGUCUGU
GCUGAGAGUGGGCACCCUGAGCAGCGACGACCUGCUGGUGCUGGCCGCCGACCUGGAGAAGCUGAA
GUCUAAGGUCGUCAGAACCGAGCGGCCAUUGAGCUCAGGCAUCUACAUGGGCAACCUUAGCAGUCA
GCAGCUGGACCAGAGAAAGGCCUUGCUGAACAUGAUCGGCAUGACCGGCGGCAACGGCGGCAGAAA
CACCACCAGCGACGGCAUCGUGAGAGUGUGGGACGUGAAGAACGCCGAGCUACUCAACAACCAGUU
CGGCACCAUGCCCAGCCUGACCCUGGCCUGCCUGACCAAGCAGGGCCAGGUGGACCUCAAUGACGC
CGUGCAGGCACUAACCGACCUUGGCCUGAUCUACACCGCCAAGUACCCCAACUCUUCAGACCUGGA
CAGACUGGCGCAGUCCCACCCCAUCUUAAAUAUGAUUGACACCAAGAAGUCAUCCCUUAACAUCAG
UGGCUACAACUUCAGCCUGGGCGCCGCCGUGAAGGCCGGCGCCUGCAUGCUGGACGGCGGAAAUAU
GCUGGAAACUAUCAAGGUGAGCCCUCAGACCAUGGACGGUAUCCUGAAGUCCAUUUUGAAGGUUA
AGAGAUCCCUGGGUAUGUUCAUCAGCGACACCCCAGGCGAGAGAAACCCCUACGAGAACAUCCUGU
ACAAGAUCUGCCUGAGUGGCGACGGCUGGCCCUACAUCGCGAGCAGAACCAGCAUCGUGGGAAGGG
CCUGGGAGAACACCGUGGUGGAUCUUGAGAGCGACAACAAGCCCCAGAAGACCGGAAAUGGCGGU
UCAAACAAGAGCCUGCAGAGCGCCGGCUUCGCCGCCGGCCUGACCUACAGCCAGCUGAUGACCCUG
AAGGACAGCAUGCUACAAUUGGAUCCCAACGCCAAGACUUGGAUGGACAUCGAGGGCAGACCCGAG
GACCCCGUGGAGAUCGCCCUGUACCAGCCCUCAUCCGGCUGCUACAUCCACUUCUUCAGAGAGCCC
ACAGAUCUGAAGCAGUUCAAGCAGGACGCGAAGUAUAGCCAUGGCAUAGACGUCACCGAUUUAUU
CGCGGCCCAGCCGGGCCUUACGAGCGCCGUGAUCGAGGCGCUGCCCAGAAACAUGGUGAUCACCUG
CCAGGGCAGCGAGGACAUCAGAAAGCUCCUUGAAUCUCAAGGCCGGAGAGAUAUUAAGCUGAUAG
AUAUCAGCUUAUCUAAGGUUGACAGCAGAAAGUUCGAGAACGCUGUAUGGGACCAAUUCAAGGAC
CUGUGCCACAUGCAUACGGGCAUAGUGGUAGAGAAGAAGAAGCGUGGCGGAAAGGAGGAGAUCAC
ACCUCACUGCGCCCUGAUGGACUGCAUCAUGUUCGACGCGGCAGUCUCCGGCGGCGUCGACGCAAA
GGUCCUCCGGGCCGUGCUGCCAAGGGACAUGGUGUUCCGGACAAGCACCCCUAAGGUAGUGCUG
(SEQ ID NO: 9)

Nipah_G

METPAQLLFLLLLWLPDTTGMPAENKKVRFENTTSDKGKNPSKVIKSYYGTMDIKKINEGLLDSKILSAFN
TVIALLGSIVIIVMNIMIIQNYTRSTDNQAVIKDALQGIQQQIKGLADKIGTEIGPKVSLIDTSSTITIPANIGLL
GSKISQSTASINENVNEKCKFTLPPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPDNICLQKTSNQILKPK
LISYTLPVVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTP
PNPNTVYHCSAVYNNEFYYVLCAVSTVGDPILNSTYWSGSLMMTRLAVKPKSNGGGYNQHQLALRSIEK
GRYDKVMPYGPSGIKQGDTLYFPAVGFLVRTEFKYNDSNCPITKCQYSKPENCRLSMGIRPNSHYILRSGLL
KYNLSDGENPKIVFIEISDQRLSIGSPSKVYDSLGQPVFYQASFSWDTMIKFGDVQTVNPLVVNWRDNTVIS
RPGQSQCPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNEILYRAQLASEDTNA
QKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT (SEQ ID NO: 10)

MPAENKKVRFENTTSDKGKNPSKVIKSYYGTMDIKKINEGLLDSKILSAFNTVIALLGSIVIIVMNIMIIQNYT
RSTDNQAVIKDALQGIQQQIKGLADKIGTEIGPKVSLIDTSSTITIPANIGLLGSKISQSTASINENVNEKCKFT
LPPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPDNICLQKTSNQILKPKLISYTLPVVGQSGTCITDPLLA
MDEGYFAYSHLERIGSCSRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYYV
LCAVSTVGDPILNSTYWSGSLMMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDTLY
FPAVGFLVRTEFKYNDSNCPITKCQYSKPENCRLSMGIRPNSHYILRSGLLKYNLSDGENPKIVFIEISDQRLS
IGSPSKVYDSLGQPVFYQASFSWDTMIKFGDVQTVNPLVVNWRDNTVISRPGQSQCPRFNTCPEICWEGVY
NDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNEILYRAQLASEDTNAQKTITNCFLLKNKIWCISLVEIY
DTGDNVIRPKLFAVKIPEQCT (SEQ ID NO: 11)

ATGGAAACCCCTGCTCAGCTGCTGTTCCTGCTGCTGCTGTGGCTGCCTGATACAACAGGCATGCCCGCC
GAGAACAAGAAAGTTCGCTTCGAGAACACCACCAGCGACAAGGGCAAGAACCCCAGCAAAGTGATCA
AGAGCTACTACGGCACCATGGACATCAAGAAGATCAACGAGGGCCTGCTGGACAGCAAGATCCTGAG

CGCCTTCAACACCGTGATTGCCCTGCTGGGCTCTATCGTGATCATCGTGATGAACATCATGATCATCCA
GAACTACACCCGGTCCACCGACAACCAGGCCGTGATTAAGGATGCTCTGCAGGGAATCCAGCAGCAG
ATCAAAGGCCTGGCCGACAAGATCGGCACAGAGATCGGCCCTAAGGTGTCCCTGATCGACACCAGCA
GCACCATCACAATCCCCGCCAATATCGGACTGCTGGGATCCAAGATCAGCCAGAGCACCGCCAGCATC
AACGAGAACGTGAACGAGAAGTGCAAGTTCACCCTGCCTCCACTGAAGATCCACGAGTGCAACATCA
GCTGCCCCAATCCTCTGCCATTCAGAGAGTACAGACCCCAGACAGAGGGCGTGTCCAATCTCGTGGGC
CTGCCTGACAATATCTGCCTGCAGAAGACCAGCAACCAGATCCTGAAGCCTAAGCTGATCTCCTACAC
ACTGCCCGTCGTGGGCCAGAGCGGCACCTGTATTACAGATCCTCTGCTGGCCATGGACGAGGGCTACT
TTGCCTACAGCCACCTGGAAAGAATCGGCAGCTGTAGCCGGGGAGTGTCCAAGCAGAGAATCATCGG
CGTGGGCGAAGTGCTGGATAGAGGCGACGAAGTGCCCAGCCTGTTCATGACCAATGTGTGGACCCCTC
CTAATCCTAACACCGTGTACCACTGCAGCGCCGTGTACAACAACGAGTTCTACTACGTGCTGTGCGCC
GTGTCCACAGTGGGCGACCCTATCCTGAACAGCACCTATTGGAGCGGCAGCCTGATGATGACCAGACT
GGCCGTGAAGCCCAAGAGCAATGGCGGCGGATACAACCAGCATCAGCTGGCCCTGCGGTCCATCGAG
AAGGGCAGATACGACAAAGTGATGCCTTACGGCCCCAGCGGCATCAAGCAAGGCGATACCCTGTACT
TTCCCGCCGTGGGATTTCTCGTGCGGACCGAGTTCAAGTACAACGACAGCAACTGCCCCATCACCAAG
TGCCAGTACAGCAAGCCCGAGAACTGCAGACTGAGCATGGGCATCAGACCCAACAGCCACTACATCC
TGAGAAGCGGCCTGCTGAAGTACAACCTGAGCGACGGCGAGAACCCCAAGATCGTGTTCATCGAGAT
CAGCGACCAGCGGCTGTCTATCGGCAGCCCTAGCAAGGTGTACGACTCTCTGGGACAGCCAGTGTTCT
ACCAGGCCTCCTTCAGCTGGGACACCATGATCAAGTTCGGCGACGTGCAGACCGTGAATCCCCTGGTG
GTCAACTGGCGGGACAATACCGTGATCAGCAGACCTGGCCAGTCTCAGTGCCCCAGATTCAACACATG
CCCCGAGATCTGTTGGGAAGGCGTGTACAATGACGCCTTCCTGATCGATCGGATCAACTGGATCTCTG
CCGGCGTGTTCCTGGACTCCAATCAGACAGCCGAGAATCCTGTGTTCACCGTGTTCAAGGACAATGAG
ATCCTGTATCGGGCCCAGCTGGCCTCCGAGGATACAAATGCCCAGAAGACAATCACCAACTGCTTTCT
GCTCAAGAACAAGATCTGGTGCATCAGCCTGGTGGAAATCTACGACACCGGCGACAACGTGATCAGG
CCCAAGCTGTTCGCCGTGAAGATCCCTGAGCAGTGCACA (SEQ ID NO: 14)

mRNA

AUGGAAACCCCUGCUCAGCUGCUGUUCCUGCUGCUGCUGUGGCUGCCUGAUACAACAGGCAUGCCC
GCCGAGAACAAGAAAGUUCGCUUCGAGAACACCACCAGCGACAAGGGCAAGAACCCCAGCAAAGUG
AUCAAGAGCUACUACGGCACCAUGGACAUCAAGAAGAUCAACGAGGGCCUGCUGGACAGCAAGAUC
CUGAGCGCCUUCAACACCGUGAUUGCCCUGCUGGGCUCUAUCGUGAUCAUCGUGAUGAACAUCAUG
AUCAUCCAGAACUACACCCGGUCCACCGACAACCAGGCCGUGAUUAAGGAUGCUCUGCAGGGAAUC
CAGCAGCAGAUCAAAGGCCUGGCCGACAAGAUCGGCACAGAGAUCGGCCCUAAGGUGUCCCUGAUC
GACACCAGCAGCACCAUCACAAUCCCCGCCAAUAUCGGACUGCUGGGAUCCAAGAUCAGCCAGAGC
ACCGCCAGCAUCAACGAGAACGUGAACGAGAAGUGCAAGUUCACCCUGCCUCCACUGAAGAUCCAC
GAGUGCAACAUCAGCUGCCCCAAUCCUCUGCCAUUCAGAGAGUACAGACCCCAGACAGAGGGCGUG
UCCAAUCUCGUGGGCCUGCCUGACAAUAUCUGCCUGCAGAAGACCAGCAACCAGAUCCUGAAGCCU
AAGCUGAUCUCCUACACACUGCCCGUCGUGGGCCAGAGCGGCACCUGUAUUACAGAUCCUCUGCUG
GCCAUGGACGAGGGCUACUUUGCCUACAGCCACCUGGAAAGAAUCGGCAGCUGUAGCCGGGGAGUG
UCCAAGCAGAGAAUCAUCGGCGUGGGCGAAGUGCUGGAUAGAGGCGACGAAGUGCCCAGCCUGUU
CAUGACCAAUGUGUGGACCCCUCCUAAUCCUAACACCGUGUACCACUGCAGCGCCGUGUACAACAA
CGAGUUCUACUACGUGCUGUGCGCCGUGUCCACAGUGGGCGACCCUAUCCUGAACAGCACCUAUUG
GAGCGGCAGCCUGAUGAUGACCAGACUGGCCGUGAAGCCCAAGAGCAAUGGCGGCGGAUACAACCA
GCAUCAGCUGGCCCUGCGGUCCAUCGAGAAGGGCAGAUACGACAAAGUGAUGCCUUACGGCCCCAG
CGGCAUCAAGCAAGGCGAUACCCUGUACUUUCCCGCCGUGGGAUUUCUCGUGCGGACCGAGUUCAA
GUACAACGACAGCAACUGCCCCAUCACCAAGUGCCAGUACAGCAAGCCCGAGAACUGCAGACUGAG
CAUGGGCAUCAGACCCAACAGCCACUACAUCCUGAGAAGCGGCCUGCUGAAGUACAACCUGAGCGA
CGGCGAGAACCCCAAGAUCGUGUUCAUCGAGAUCAGCGACCAGCGGCUGUCUAUCGGCAGCCCUAG
CAAGGUGUACGACUCUCUGGGACAGCCAGUGUUCUACCAGGCCUCCUUCAGCUGGGACACCAUGAU
CAAGUUCGGCGACGUGCAGACCGUGAAUCCCCUGGUGGUCAACUGGCGGGACAAUACCGUGAUCAG
CAGACCUGGCCAGUCUCAGUGCCCCAGAUUCAACACAUGCCCCGAGAUCUGUUGGGAAGGCGUGUA
CAAUGACGCCUUCCUGAUCGAUCGGAUCAACUGGAUCUCUGCCGGCGUGUUCCUGGACUCCAAUCA
GACAGCCGAGAAUCCUGUGUUCACCGUGUUCAAGGACAAUGAGAUCCUGUAUCGGGCCCAGCUGGC
CUCCGAGGAUACAAAUGCCCAGAAGACAAUCACCAACUGCUUUCUGCUCAAGAACAAGAUCUGGUG
CAUCAGCCUGGUGGAAAUCUACGACACCGGCGACAACGUGAUCAGGCCCAAGCUGUUCGCCGUGAA
GAUCCCUGAGCAGUGCACA (SEQ ID NO: 16)

Nipah_F

METPAQLLFLLLLWLPDTTGILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPNVSNMSQCTGSVMEN
YKTRLNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMKNADNINKLKS
SIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGPNLQDPV

SNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSGYYIIVRVYFPILTEIQQAYIQELL
PVSFNNDNSEWISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSS
HVPRFALSNGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIA
IGPPVFTDKVDISSQISSMNQSLQQSKDYIKEAQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFISFIIVEKK
RNTYSRLEDRRVRPTSSGDLYYIGT (SEQ ID NO: 12)

ILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPNVSNMSQCTGSVMENYKTRLNGILTPIKGALEIYKN
NTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMKNADNINKLKSSIESTNEAVVKLQETAEKTV
YVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGPNLQDPVSNSMTIQAISQAFGGNYETL
LRTLGYATEDFDDLLESDSITGQIIYVDLSGYYIIVRVYFPILTEIQQAYIQELLPVSFNNDNSEWISIVPNFILV
RNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSSHVPRFALSNGVLFANCISVT
CQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGPPVFTDKVDISSQISSMNQ
SLQQSKDYIKEAQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFISFIIVEKKRNTYSRLEDRRVRPTSSGDL
YYIGT (SEQ ID NO: 13)

ATGGAAACCCCTGCTCAGCTGCTGTTCCTGCTGCTGCTGTGGCTGCCTGATACAACAGGCATGCCCGCC
GAGAACAAGAAAGTTCGCTTCGAGAACACCACCAGCGACAAGGGCAAGAACCCCAGCAAAGTGATCA
AGAGCTACTACGGCACCATGGACATCAAGAAGATCAACGAGGGCCTGCTGGACAGCAAGATCCTGAG
CGCCTTCAACACCGTGATTGCCCTGCTGGGCTCTATCGTGATCATCGTGATGAACATCATGATCATCCA
GAACTACACCCGGTCCACCGACAACCAGGCCGTGATTAAGGATGCTCTGCAGGGAATCCAGCAGCAG
ATCAAAGGCCTGGCCGACAAGATCGGCACAGAGATCGGCCCTAAGGTGTCCCTGATCGACACCAGCA
GCACCATCACAATCCCCGCCAATATCGGACTGCTGGGATCCAAGATCAGCCAGAGCACCGCCAGCATC
AACGAGAACGTGAACGAGAAGTGCAAGTTCACCCTGCCTCCACTGAAGATCCACGAGTGCAACATCA
GCTGCCCCAATCCTCTGCCATTCAGAGAGTACAGACCCCAGACAGAGGGCGTGTCCAATCTCGTGGGC
CTGCCTGACAATATCTGCCTGCAGAAGACCAGCAACCAGATCCTGAAGCCTAAGCTGATCTCCTACAC
ACTGCCCGTCGTGGGCCAGAGCGGCACCTGTATTACAGATCCTCTGCTGGCCATGGACGAGGGCTACT
TTGCCTACAGCCACCTGGAAAGAATCGGCAGCTGTAGCCGGGGAGTGTCCAAGCAGAGAATCATCGG
CGTGGGCGAAGTGCTGGATAGAGGCGACGAAGTGCCCAGCCTGTTCATGACCAATGTGTGGACCCCTC
CTAATCCTAACACCGTGTACCACTGCAGCGCCGTGTACAACAACGAGTTCTACTACGTGCTGTGCGCC
GTGTCCACAGTGGGCGACCCTATCCTGAACAGCACCTATTGGAGCGGCAGCCTGATGATGACCAGACT
GGCCGTGAAGCCCAAGAGCAATGGCGGCGGATACAACCAGCATCAGCTGGCCCTGCGGTCCATCGAG
AAGGGCAGATACGACAAAGTGATGCCTTACGGCCCCAGCGGCATCAAGCAAGGCGATACCCTGTACT
TTCCCGCCGTGGGATTTCTCGTGCGGACCGAGTTCAAGTACAACGACAGCAACTGCCCCATCACCAAG
TGCCAGTACAGCAAGCCCGAGAACTGCAGACTGAGCATGGGCATCAGACCCAACAGCCACTACATCC
TGAGAAGCGGCCTGCTGAAGTACAACCTGAGCGACGGCGAGAACCCCAAGATCGTGTTCATCGAGAT
CAGCGACCAGCGGCTGTCTATCGGCAGCCCTAGCAAGGTGTACGACTCTCTGGGACAGCCAGTGTTCT
ACCAGGCCTCCTTCAGCTGGGACACCATGATCAAGTTCGGCGACGTGCAGACCGTGAATCCCCTGGTG
GTCAACTGGCGGGACAATACCGTGATCAGCAGACCTGGCCAGTCTCAGTGCCCCAGATTCAACACATG
CCCCGAGATCTGTTGGGAAGGCGTGTACAATGACGCCTTCCTGATCGATCGGATCAACTGGATCTCTG
CCGGCGTGTTCCTGGACTCCAATCAGACAGCCGAGAATCCTGTGTTCACCGTGTTCAAGGACAATGAG
ATCCTGTATCGGGCCCAGCTGGCCTCCGAGGATACAAATGCCCAGAAGACAATCACCAACTGCTTTCT
GCTCAAGAACAAGATCTGGTGCATCAGCCTGGTGGAAATCTACGACACCGGCGACAACGTGATCAGG
CCCAAGCTGTTCGCCGTGAAGATCCCTGAGCAGTGCACA (SEQ ID NO: 15)

mRNA

AUGGAAACCCCUGCUCAGCUGCUGUUCCUGCUGCUGCUGUGGCUGCCUGAUACAACAGGCAUGCCC
GCCGAGAACAAGAAAGUUCGCUUCGAGAACACCACCAGCGACAAGGGCAAGAACCCCAGCAAAGUG
AUCAAGAGCUACUACGGCACCAUGGACAUCAAGAAGAUCAACGAGGGCCUGCUGGACAGCAAGAUC
CUGAGCGCCUUCAACACCGUGAUUGCCCUGCUGGGCUCUAUCGUGAUCAUCGUGAUGAACAUCAUG
AUCAUCCAGAACUACACCCGGUCCACCGACAACCAGGCCGUGAUUAAGGAUGCUCUGCAGGGAAUC
CAGCAGCAGAUCAAAGGCCUGGCCGACAAGAUCGGCACAGAGAUCGGCCCUAAGGUGUCCCUGAUC
GACACCAGCAGCACCAUCACAAUCCCCGCCAAUAUCGGACUGCUGGGAUCCAAGAUCAGCCAGAGC
ACCGCCAGCAUCAACGAGAACGUGAACGAGAAGUGCAAGUUCACCCUGCCUCCACUGAAGAUCCAC
GAGUGCAACAUCAGCUGCCCCAAUCCUCUGCCAUUCAGAGAGUACAGACCCCAGACAGAGGGCGUG
UCCAAUCUCGUGGGCCUGCCUGACAAUAUCUGCCUGCAGAAGACCAGCAACCAGAUCCUGAAGCCU
AAGCUGAUCUCCUACACACUGCCCGUCGUGGGCCAGAGCGGCACCUGUAUUACAGAUCCUCUGCUG
GCCAUGGACGAGGGCUACUUUGCCUACAGCCACCUGGAAAGAAUCGGCAGCUGUAGCCGGGGAGUG
UCCAAGCAGAGAAUCAUCGGCGUGGGCGAAGUGCUGGAUAGAGGCGACGAAGUGCCCAGCCUGUU
CAUGACCAAUGUGUGGACCCCUCCUAAUCCUAACACCGUGUACCACUGCAGCGCCGUGUACAACAA
CGAGUUCUACUACGUGCUGUGCGCCGUGUCCACAGUGGGCGACCCUAUCCUGAACAGCACCUAUUG
GAGCGGCAGCCUGAUGAUGACCAGACUGGCCGUGAAGCCCAAGAGCAAUGGCGGCGGAUACAACCA

GCAUCAGCUGGCCCUGCGGUCCAUCGAGAAGGGCAGAUACGACAAAGUGAUGCCUUACGGCCCCAG
CGGCAUCAAGCAAGGCGAUACCCUGUACUUUCCCGCCGUGGGAUUUCUCGUGCGGACCGAGUUCAA
GUACAACGACAGCAACUGCCCCAUCACCAAGUGCCAGUACAGCAAGCCCGAGAACUGCAGACUGAG
CAUGGGCAUCAGACCCAACAGCCACUACAUCCUGAGAAGCGGCCUGCUGAAGUACAACCUGAGCGA
CGGCGAGAACCCCAAGAUCGUGUUCAUCGAGAUCAGCGACCAGCGGCUGUCUAUCGGCAGCCCUAG
CAAGGUGUACGACUCUCUGGGACAGCCAGUGUUCUACCAGGCCUCCUUCAGCUGGGACACCAUGAU
CAAGUUCGGCGACGUGCAGACCGUGAAUCCCCUGGUGGUCAACUGGCGGGACAAUACCGUGAUCAG
CAGACCUGGCCAGUCUCAGUGCCCCAGAUUCAACACAUGCCCCGAGAUCUGUUGGGAAGGCGUGUA
CAAUGACGCCUUCCUGAUCGAUCGGAUCAACUGGAUCUCUGCCGGCGUGUUCCUGGACUCCAAUCA
GACAGCCGAGAAUCCUGUGUUCACCGUGUUCAAGGACAAUGAGAUCCUGUAUCGGGCCCAGCUGGC
CUCCGAGGAUACAAAUGCCCAGAAGACAAUCACCAACUGCUUUCUGCUCAAGAACAAGAUCUGGUG
CAUCAGCCUGGUGGAAAUCUACGACACCGGCGACAACGUGAUCAGGCCCAAGCUGUUCGCCGUGAA
GAUCCCUGAGCAGUGCACA (SEQ ID NO: 17)

>gi|940378825|gb|ALK02457.1|spikeprotein[SARS-likecoronavirusWIV16]

MFIFLFFLTLTSGSDLESCTTFDDVQAPNYPQHSSSRRGVYYPDEIFRSDTLYLTQDLFLPFYSNVTGFHTINH
RFDNPVIPFKDGVYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPTGT
QTHTMIFDNAFNCTFEYISDSFSLDVAEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNILKPI
FKLPLGINITNFRAILTAFLPAQDTWGTSAAAYFVGYLKPATFMLKYDENGTITDAVDCSQNPLAELKCSV
KSFEIDKGIYQTSNFRVAPSKEVVRFPNITNLCPFGEVFNATTFPSVYAWERKRISNCVADYSVLYNSTSFST
FKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFTGCVLAWNTRNIDATQT
GNYNYKYRSLRHGKLRPFERDISNVPFSPDGKPCTPPAFNCYWPLNDYGFYITNGIGYQPYRVVVLSFELLN
APATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVLDFTDSVRDPKTSEILDISPCSF
GGVSVITPGTNTSSEVAVLYQDVNCTDVPVAIHADQLTPSWRVYSTGNNVFQTQAGCLIGAEHVDTSYEC
DIPIGAGICASYHTVSSLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNM
YICGDSTECANLLLQYGSFCTQLNRALSGIAVEQDRNTREVFAQVKQMYKTPTLKDFGGFNFSQILPDPLKP
TKRSFIEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTAT
AGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVN
QNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLA
ATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVF
VFNGTSWFITQRNFFSPQIITTDNTFVSGSCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDI
SGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCC
SCLKGACSCGSCCKFDEDDSEPVLKGVKLHYT(SEQ ID NO: 18)

>WIV16_FL_CO_Spike

ATGTTTATCTTCCTGTTCTTCCTGACCCTGACCAGCGGCAGCGACCTGGAAAGCTGCACCACCTTCGAC
GACGTGCAGGCCCCCAACTACCCTCAGCACAGCTCTAGCAGACGGGGCGTGTACTACCCCGACGAGAT
CTTCAGAAGCGACACCCTGTACCTGACCCAGGACCTGTTCCTGCCCTTCTACAGCAACGTGACCGGCTT
CCACACCATCAACCACAGATTCGACAACCCCGTGATCCCCTTCAAGGACGGGGTGTACTTTGCCGCCA
CCGAGAAGTCCAATGTCGTGCGGGGATGGGTGTTCGGCAGCACCATGAACAACAAGAGCCAGAGCGT
GATCATCATCAACAACAGCACCAACGTCGTGATCCGGGCCTGCAACTTCGAGCTGTGCGACAACCCAT
TCTTCGCCGTGTCCAAGCCCACCGGCACCCAGACCCACACCATGATCTTCGACAACGCCTTCAACTGC
ACCTTCGAGTACATCAGCGACAGCTTCAGCCTGGACGTGGCCGAGAAAAGCGGCAACTTCAAGCACCT
GAGAGAATTCGTGTTCAAGAACAAGGACGGCTTCCTGTACGTGTACAAGGGCTACCAGCCCATCGACG
TCGTGCGCGATCTGCCCAGCGGCTTCAACATCCTGAAGCCCATCTTCAAGCTGCCCCTGGGCATCAAC
ATCACCAACTTCCGGGCTATCCTGACCGCCTTCCTGCCCGCCCAGGATACCTGGGGAACAAGCGCCGC
TGCCTACTTCGTGGGCTACCTGAAGCCTGCCACCTTCATGCTGAAGTACGACGAGAACGGCACCATCA
CCGACGCCGTGGACTGCAGCCAGAATCCTCTGGCCGAGCTGAAGTGCAGCGTGAAGTCCTTCGAGATC
GACAAGGGCATCTACCAGACCAGCAACTTCAGAGTGGCCCCCAGCAAAGAAGTCGTGCGGTTCCCCA
ATATCACCAACCTGTGCCCCTTCGGCGAGGTGTTCAACGCCACCACCTTTCCCAGCGTGTACGCCTGGG
AGCGGAAGCGGATCAGCAACTGCGTGGCCGACTACAGCGTGCTGTACAACTCCACCAGCTTCTCCACC
TTCAAGTGCTACGGCGTGTCCGCCACCAAGCTGAACGACCTGTGCTTCAGCAATGTGTACGCCGACTC
CTTCGTCGTGAAGGGCGACGATGTGCGCCAGATCGCCCCTGGACAGACAGGCGTGATCGCCGATTACA
ACTACAAGCTGCCTGACGACTTCACCGGCTGCGTGCTGGCCTGGAACACCAGAAACATCGACGCCACC
CAGACAGGCAACTACAATTACAAGTACAGAAGCCTGCGGCACGGCAAGCTGCGGCCCTTCGAGAGGG
ACATCTCCAACGTGCCCTTCAGCCCCGACGGCAAGCCTTGTACCCCCCCTGCCTTTAACTGCTACTGGC
CCCTGAACGACTACGGCTTCTACATCACAAACGGCATCGGCTATCAGCCCTACCGGGTGGTGGTGCTG
TCCTTTGAGCTGCTGAATGCCCCTGCCACCGTGTGCGGCCCTAAGCTGAGCACCGACCTGATCAAGAA
CCAGTGCGTGAACTTCAACTTCAACGGCCTGACCGGCACCGGCGTGCTGACACCTAGCAGCAAGAGAT
TCCAGCCCTTCCAGCAGTTCGGCCGGGACGTGCTGGATTTCACCGACAGCGTGCGGGACCCCAAGACC
AGCGAGATCCTGGACATCAGCCCCTGCAGCTTCGGCGGAGTGTCCGTGATCACCCCCGGCACCAATAC

CAGCTCTGAGGTGGCCGTGCTGTATCAGGACGTGAACTGCACCGATGTGCCCGTGGCCATCCACGCCG
ATCAGCTGACCCCATCTTGGCGGGTGTACTCCACCGGCAACAACGTGTTCCAGACACAAGCCGGCTGC
CTGATCGGAGCCGAGCACGTGGACACCAGCTACGAGTGCGACATCCCTATCGGCGCTGGCATCTGCGC
CAGCTACCACACCGTGTCCAGCCTGAGAAGCACCAGCCAGAAATCTATCGTGGCCTACACCATGAGCC
TGGGCGCCGACAGCTCTATCGCCTACTCCAACAACACAATCGCCATCCCCACCAATTTCAGCATCTCC
ATCACCACCGAAGTGATGCCCGTGTCCATGGCCAAGACCTCCGTGGATTGCAACATGTACATCTGCGG
CGACAGCACCGAGTGCGCCAACCTGCTGCTGCAGTACGGCAGCTTCTGCACCCAGCTGAACAGAGCCC
TGAGCGGAATCGCCGTGGAACAGGACAGAAACACCCGGGAAGTGTTCGCCCAAGTGAAGCAGATGTA
TAAGACCCCCACCCTGAAGGATTTCGGCGGCTTTAACTTCAGCCAGATCCTGCCCGACCCTCTGAAGC
CTACCAAGCGGAGCTTCATCGAGGACCTGCTGTTCAACAAAGTGACCCTGGCCGACGCCGGCTTTATG
AAGCAGTATGGCGAGTGCCTGGGCGACATCAACGCCCGGGATCTGATCTGCGCCCAGAAGTTTAACG
GACTGACCGTGCTGCCCCCTCTGCTGACCGACGATATGATCGCCGCCTACACAGCCGCCCTGGTGTCT
GGCACAGCTACCGCCGGATGGACATTTGGAGCTGGCGCCGCTCTGCAGATCCCCTTTGCCATGCAGAT
GGCCTACCGGTTCAATGGCATCGGCGTGACCCAGAATGTGCTGTACGAGAACCAGAAGCAGATCGCC
AACCAGTTCAACAAGGCCATTAGCCAGATTCAGGAAAGCCTGACCACCACCAGCACCGCCCTGGGCA
AACTGCAGGACGTCGTGAACCAGAACGCCCAGGCCCTGAACACCCTCGTGAAGCAGCTGAGCAGCAA
TTTCGGCGCCATCAGCTCCGTGCTGAACGATATCCTGAGCAGACTGGACAAGGTGGAAGCAGAGGTGC
AGATCGACCGGCTGATCACCGGCAGACTGCAGAGCCTGCAGACCTACGTGACACAGCAGCTGATTAG
AGCCGCCGAGATCAGGGCCAGCGCCAATCTGGCCGCCACAAAGATGAGCGAGTGTGTGCTGGGCCAG
AGCAAGCGGGTGGACTTCTGCGGCAAGGGCTATCACCTGATGAGCTTCCCCCAGGCCGCTCCTCACGG
CGTGGTGTTTCTGCACGTGACATACGTGCCCAGCCAGGAACGGAACTTCACCACCGCCCCAGCCATCT
GCCACGAGGGCAAGGCCTACTTCCCCCGGGAAGGCGTGTTCGTGTTTAACGGCACCTCCTGGTTTATC
ACCCAGCGGAATTTCTTCAGTCCGCAGATCATCACCACAGACAACACCTTCGTGTCCGGCAGCTGCGA
CGTCGTGATTGGCATCATTAACAACACCGTGTACGACCCCCTGCAGCCCGAGCTGGACAGCTTCAAAG
AGGAACTGGACAAGTACTTCAAGAACCACACCTCCCCCGACGTGGACCTGGGCGATATCTCCGGCATC
AATGCCAGCGTCGTGAATATCCAGAAAGAGATCGATCGCCTGAACGAGGTGGCCAAGAACCTGAATG
AGAGCCTGATCGACCTGCAGGAACTGGGGAAGTACGAGCAGTACATCAAGTGGCCTTGGTACGTGTG
GCTGGGCTTTATCGCCGGCCTGATCGCCATCGTGATGGTCACCATCCTGCTGTGCTGCATGACCAGCTG
TTGCAGCTGTCTGAAGGGCGCCTGCAGCTGTGGCTCCTGCTGCAAGTTCGATGAGGACGACAGCGAGC
CTGTGCTGAAAGGCGTGAAGCTGCACTACACC (SEQ ID NO: 19)

AUGUUUAUCUUCCUGUUCUUCCUGACCCUGACCAGCGGCAGCGACCUGGAAAGCUGCACCACCUUC
GACGACGUGCAGGCCCCCAACUACCCUCAGCACAGCUCUAGCAGACGGGGCGUGUACUACCCCGAC
GAGAUCUUCAGAAGCGACACCCUGUACCUGACCCAGGACCUGUUCCUGCCCUUCUACAGCAACGUG
ACCGGCUUCCACACCAUCAACCACAGAUUCGACAACCCCGUGAUCCCCUUCAAGGACGGGGUGUAC
UUUGCCGCCACCGAGAAGUCCAAUGUCGUGCGGGGAUGGGUGUUCGGCAGCACCAUGAACAACAAG
AGCCAGAGCGUGAUCAUCAUCAACAACAGCACCAACGUCGUGAUCCGGGCCUGCAACUUCGAGCUG
UGCGACAACCCAUUCUUCGCCGUGUCCAAGCCCACCGGCACCCAGACCCACACCAUGAUCUUCGAC
AACGCCUUCAACUGCACCUUCGAGUACAUCAGCGACAGCUUCAGCCUGGACGUGGCCGAGAAAAGC
GGCAACUUCAAGCACCUGAGAGAAUUCGUGUUCAAGAACAAGGACGGCUUCCUGUACGUGUACAA
GGGCUACCAGCCCAUCGACGUCGUGCGCGAUCUGCCCAGCGGCUUCAACACCUGAAGCCCAUCUU
CAAGCUGCCCCUGGGCAUCAACAUCACCAACUUCCGGGCUAUCCUGACCGCCUUCCUGCCCGCCCAG
GAUACCUGGGGAACAAGCGCCGCUGCCUACUUCGUGGGCUACCUGAAGCCUGCCACCUUCAUGCUG
AAGUACGACGAGAACGGCACCAUCACCGACGCCGUGGACUGCAGCCAGAAUCCUCUGGCCGAGCUG
AAGUGCAGCGUGAAGUCCUUCGAGAUCGACAAGGGCAUCUACCAGACCAGCAACUUCAGAGUGGCC
CCCAGCAAAGAAGUCGUGCGGUUCCCCAAUAUCACCAACCUGUGCCCCUUCGGCGAGGUGUUCAAC
GCCACCACCUUUCCCAGCGUGUACGCCUGGGAGCGGAAGCGGAUCAGCAACUGCGUGGCCGACUAC
AGCGUGCUGUACAACUCCACCAGCUUCUCCACCUUCAAGUGCUACGGCGUGUCCGCCACCAAGCUG
AACGACCUGUGCUUCAGCAAUGUGUACGCCGACUCCUUCGUCGUGAAGGGCGACGAUGUGCGCCAG
AUCGCCCCUGGACAGACAGGCGUGAUCGCCGAUUACAACUACAAGCUGCCUGACGACUUCACCGGC
UGCGUGCUGGCCUGGAACACCAGAAACAUCGACGCCACCCAGACAGGCAACUACAAUUACAAGUAC
AGAAGCCUGCGGCACGGCAAGCUGCGGCCCUUCGAGAGGGACAUCUCCAACGUGCCCUUCAGCCCC
GACGGCAAGCCUUGUACCCCCCCUGCCUUUAACUGCUACUGGCCCCUGAACGACUACGGCUUCUAC
AUCACAAACGGCAUCGGCUAUCAGCCCUACCGGGUGGUGGUGCUGUCCUUUGAGCUGCUGAAUGCC
CCUGCCACCGUGUGCGGCCCUAAGCUGAGCACCGACCUGAUCAAGAACCAGUGCGUGAACUUCAAC
UUCAACGGCCUGACCGGCACCGGCGUGCUGACACCUAGCAGCAAGAGAUUCCAGCCCUUCCAGCAG
UUCGGCCGGGACGUGCUGGAUUUCACCGACAGCGUGCGGGACCCCAAGACCAGCGAGAUCCUGGAC
AUCAGCCCCUGCAGCUUCGGCGGAGUGUCCGUGAUCACCCCCGGCACCAAUACCAGCUCUGAGGUG
GCCGUGCUGUAUCAGGACGUGAACUGCACCGAUGUGCCCGUGGCCAUCCACGCCGAUCAGCUGACC
CCAUCUUGGCGGGUGUACUCCACCGGCAACAACGUGUUCCAGACACAAGCCGGCUGCCUGAUCGGA
GCCGAGCACGUGGACACCAGCUACGAGUGCGACAUCCCUAUCGGCGCUGGCAUCUGCGCCAGCUAC
CACACCGUGUCCAGCCUGAGAAGCACCAGCCAGAAAUCUAUCGUGGCCUACACCAUGAGCCUGGGC

GCCGACAGCUCUAUCGCCUACUCCAACAACACAAUCGCCAUCCCCACCAAUUUCAGCAUCUCCAUC
ACCACCGAAGUGAUGCCCGUGUCCAUGGCCAAGACCUCCGUGGAUUGCAACAUGUACAUCUGCGGC
GACAGCACCGAGUGCGCCAACCUGCUGCUGCAGUACGGCAGCUUCUGCACCCAGCUGAACAGAGCC
CUGAGCGGAAUCGCCGUGGAACAGGACAGAAACACCCGGGAAGUGUUCGCCCAAGUGAAGCAGAU
GUAUAAGACCCCCACCCUGAAGGAUUUCGGCGGCUUUAACUUCAGCCAGAUCCUGCCCGACCCUCU
GAAGCCUACCAAGCGGAGCUUCAUCGAGGACCUGCUGUUCAACAAAGUGACCCUGGCCGACGCCGG
CUUUAUGAAGCAGUAUGGCGAGUGCCUGGGCGACAUCAACGCCCGGGAUCUGAUCUGCGCCCAGAA
GUUUAACGGACUGACCGUGCUGCCCCCUCUGCUGACCGACGAUAUGAUCGCCGCCUACACAGCCGC
CCUGGUGUCUGGCACAGCUACCGCCGGAUGGACAUUUGGAGCUGGCGCCGCUCUGCAGAUCCCCUU
UGCCAUGCAGAUGGCCUACCGGUUCAAUGGCAUCGGCGUGACCCAGAAUGUGCUGUACGAGAACCA
GAAGCAGAUCGCCAACCAGUUCAACAAGGCCAUUAGCCAGAUUCAGGAAAGCCUGACCACCACCAG
CACCGCCCUGGGCAAACUGCAGGACGUCGUGAACCAGAACGCCCAGGCCCUGAACACCCUCGUGAA
GCAGCUGAGCAGCAAUUUCGGCGCCAUCAGCUCCGUGCUGAACGAUAUCCUGAGCAGACUGGACAA
GGUGGAAGCAGAGGUGCAGAUCGACCGGCUGAUCACCGGCAGACUGCAGAGCCUGCAGACCUACGU
GACACAGCAGCUGAUUAGAGCCGCCGAGAUCAGGGCCAGCGCCAAUCUGGCCGCCACAAAGAUGAG
CGAGUGUGUGCUGGGCCAGAGCAAGCGGGUGGACUUCUGCGGCAAGGGCUAUCACCUGAUGAGCU
UCCCCCAGGCCGCUCCUCACGGCGUGGUGUUUCUGCACGUGACAUACGUGCCCAGCCAGGAACGGA
ACUUCACCACCGCCCCAGCCAUCUGCCACGAGGGCAAGGCCUACUUCCCCCGGGAAGGCGUGUUCG
UGUUUAACGGCACCUCCUGGUUUAUCACCCAGCGGAAUUUCUUCAGUCCGCAGAUCAUCACCACAG
ACAACACCUUCGUGUCCGGCAGCUGCGACGUCGUGAUUGGCAUCAUUAACAACACCGUGUACGACC
CCCUGCAGCCCGAGCUGGACAGCUUCAAAGAGGAACUGGACAAGUACUUCAAGAACCACACCUCCC
CCGACGUGGACCUGGGCGAUAUCUCCGGCAUCAAUGCCAGCGUCGUGAAUAUCCAGAAAGAGAUCG
AUCGCCUGAACGAGGUGGCCAAGAACCUGAAUGAGAGCCUGAUCGACCUGCAGGAACUGGGGAAG
UACGAGCAGUACAUCAAGUGGCCUUGGUACGUGUGGCUGGGCUUUAUCGCCGGCCUGAUCGCCAUC
GUGAUGGUCACCAUCCUGCUGUGCUGCAUGACCAGCUGUUGCAGCUGUCUGAAGGGCGCCUGCAGC
UGUGGCUCCUGCUGCAAGUUCGAUGAGGACGACAGCGAGCCUGUGCUGAAAGGCGUGAAGCUGCA
CUACACC (SEQ ID NO: 20)

CLAIMS

What is claimed is:

1. A zoonotic disease vaccine, comprising a ribonucleic acid (RNA) comprising an open reading frame (ORF) encoding an antigen selected from Lassa virus antigens, Nipah virus antigens, and betacoronavirus antigens, wherein intramuscular (IM) administration of a therapeutically effective amount of the vaccine to a subject induces an immune response in the subject.

2. The zoonotic disease vaccine of claim 1, wherein the ORF encodes a Lassa virus antigen.

3. The zoonotic disease vaccine of claim 2, wherein the Lassa virus antigen comprises a glycoprotein.

4. The zoonotic disease vaccine of claim 3, wherein the Lassa virus antigen comprises a Lassa virus glycoprotein precursor (GPC), a structurally stabilized Lassa virus GPC, an ectodomain of Lassa virus glycoprotein 1 (GP1), or a Lassa virus glycoprotein 2 (GP2).

5. The zoonotic disease vaccine of claim 4, wherein the Lassa virus antigen comprises amino acid residues 59-259 of a Lassa virus GPC.

6. The zoonotic disease vaccine of claim 2, wherein the Lassa virus antigen comprises a nucleocapsid protein (NP).

7. The zoonotic disease vaccine of claim 2, wherein the Lassa virus antigen has an amino acid sequence that has at least 90%, at least 95%, or at least 99% identity to an amino acid sequence identified by any one of SEQ ID NO: 1-3, but does not include wild-type protein sequence.

8. The zoonotic disease vaccine of claim 2, wherein the Lassa virus antigen has an amino acid sequence of any one of SEQ ID NO: 1-3.

9. The zoonotic disease vaccine of claim 2, wherein the RNA comprising an ORF sequence has at least 90%, at least 95%, or at least 99% identity to a nucleic acid sequence

identified by any one of SEQ ID NO: 6, 7 or 9, but does not include wild-type protein sequence.

10. The zoonotic disease vaccine of claim 2, wherein the RNA comprising an ORF sequence comprises a nucleic acid sequence of any one of SEQ ID NO: 6, 7 or 9.

11. The zoonotic disease vaccine of claim 1, wherein the ORF encodes a Nipah virus antigen and/or a Hendra virus antigen.

12. The zoonotic disease vaccine of claim 11, wherein the Nipah virus antigen and/or a Hendra virus antigen comprises a hemagglutinin-neuraminidase protein (HN), a hemagglutinin protein (H), or a glycoprotein (G).

13. The zoonotic disease vaccine of claim 12, wherein the Nipah virus antigen and/or a Hendra virus antigen comprises an attachment glycoprotein, optionally a type II membrane protein.

14. The zoonotic disease vaccine of claim 12, wherein the Nipah virus antigen and/or a Hendra virus antigen comprises a fusion (F) glycoprotein.

15. The zoonotic disease vaccine of claim 14, wherein the F glycoprotein comprises a trimeric class I fusogenic envelope glycoprotein containing two heptad repeat (HR) regions and a hydrophobic fusion peptide.

16. The zoonotic disease vaccine of any one of claims 11-15, wherein the Nipah virus antigen and/or a Hendra virus antigen is a Nipah virus antigen.

17. The zoonotic disease vaccine of any one of claims 11-15, wherein the Nipah virus antigen and/or a Hendra virus antigen is a Hendra virus antigen.

18. The zoonotic disease vaccine of claim 11, wherein the Nipah virus antigen and/or a Hendra virus antigen has an amino acid sequence that has at least 90%, at least 95%, or at least 99% identity to an amino acid sequence identified by any one of SEQ ID NO: 10-13 but does not include wild-type protein sequence.

19. The zoonotic disease vaccine of claim 11, wherein the Nipah virus antigen and/or a Hendra virus antigen has an amino acid sequence of any one of SEQ ID NO: 10-13.

20. The zoonotic disease vaccine of claim 11, wherein the RNA comprising an ORF sequence has at least 90%, at least 95%, or at least 99% identity to a nucleic acid sequence identified by SEQ ID NO: 16 or 17, but does not include wild-type protein sequence.

21. The zoonotic disease vaccine of claim 11, wherein the RNA comprising an ORF sequence comprises a nucleic acid sequence of SEQ ID NO: 16 or 17.

22. The zoonotic disease vaccine of claim 1, wherein the ORF encodes a middle east respiratory syndrome coronavirus (MERS-CoV) antigen and/or a severe acute respiratory syndrome-like coronavirus WIV1 (SL-CoV-WIV1) antigen.

23. The zoonotic disease vaccine of claim 22, wherein the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen comprises a betacoronavirus structural protein.

24. The zoonotic disease vaccine of claim 23, wherein the betacoronavirus structural protein is spike protein, envelope protein, nucleocapsid protein, or membrane protein.

25. The zoonotic disease vaccine of claim 24, wherein the betacoronavirus structural protein is spike protein.

26. The zoonotic disease vaccine of claim 25, wherein the betacoronavirus structural protein a S1 subunit of the spike protein or a S2 subunit of the spike protein.

27. The zoonotic disease vaccine of any one of claims 22-26, wherein the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen is a MERS-CoV antigen.

28. The zoonotic disease vaccine of any one of claims 22-26, wherein the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen is a SL-CoV-WIV1 antigen.

29. The zoonotic disease vaccine of claim 22, wherein the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen has an amino acid sequence that has at least 90%, at least 95%, or at least 99% identity to an amino acid sequence identified SEQ ID NO: 18 but does not include wild-type protein sequence.

30. The zoonotic disease vaccine of claim 22, wherein the MERS-CoV antigen and/or a SL-CoV-WIV1 antigen has an amino acid sequence of SEQ ID NO: 18.

31. The zoonotic disease vaccine of claim 22, wherein the RNA comprising an ORF sequence has at least 90%, at least 95%, or at least 99% identity to a nucleic acid sequence identified by SEQ ID NO: 18, but does not include wild-type protein sequence.

32. The zoonotic disease vaccine of claim 22, wherein the RNA comprising an ORF sequence comprises a nucleic acid sequence of SEQ ID NO: 18.

33. The zoonotic disease vaccine of any one of claims 1-32, wherein IM administration of a therapeutically effective amount of the vaccine to a subject induces a neutralizing antibody titer in the subject.

34. The zoonotic disease vaccine of claim 33, wherein the neutralizing antibody titer is at least 100 neutralizing units per milliliter (NU/mL), at least 500 NU/mL, or at least 1000 NU/mL.

35. The zoonotic disease vaccine of claim 33 or 34, wherein the neutralizing antibody titer is sufficient to reduce viral infection of B cells by at least 50% relative to a neutralizing antibody titer of an unvaccinated control subject or relative to a neutralizing antibody titer of a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine.

36. The zoonotic disease vaccine of any one of claims 33-35, wherein the neutralizing antibody titer is induced in the subject following fewer than three doses of the vaccine.

37. The zoonotic disease vaccine of any one of claims 1-36, wherein a single dose is of 10 μg-100 μg.

38. The zoonotic disease vaccine of any one of claims 33-37, wherein the neutralizing antibody titer and/or a T cell immune response is sufficient to reduce the rate of asymptomatic viral infection relative to the neutralizing antibody titer of unvaccinated control subjects.

39. The zoonotic disease vaccine of any one of claims 33-38, wherein the neutralizing antibody titer and/or a T cell immune response is sufficient to prevent viral latency the subject.

40. The zoonotic disease vaccine of any one of claims 33-39, wherein the neutralizing antibody titer is sufficient to block fusion of virus with epithelial cells and/or B cells of the subject.

41. The zoonotic disease vaccine of any one of claims 33-40, wherein the neutralizing antibody titer is induced within 20 days following a single 10-100 μg of the vaccine, or within 40 days following a second 10-100 μg dose of the vaccine.

42. The zoonotic disease vaccine of any one of claims 33-40, wherein IM administration of a therapeutically effective amount of the vaccine to a subject induces a T cell immune response in the subject.

43. The zoonotic disease vaccine of claim 42, wherein the T cell immune response comprises a $CD4^+$ T cell immune response and/or a $CD8^+$ T cell immune response.

44. The zoonotic disease vaccine of any one of claims 1-43, wherein the antigen is expressed on the surface of cells of the subject.

45. The zoonotic disease vaccine of any one of claims 1-44, wherein the vaccine comprises (a) a ribonucleic acid (RNA) having an open reading frame (ORF) encoding two antigens, or (b) two RNAs, each having an ORF encoding an antigen.

46. The zoonotic disease vaccine of any one of claims 1-45, wherein the vaccine comprises a RNA having an ORF encoding two antigens formulated in a lipid nanoparticle.

47. The zoonotic disease vaccine of any one of claims 1-46, wherein the vaccine comprises two RNAs, each having an ORF encoding an antigen, wherein the two RNAs are formulated in a single lipid nanoparticle or wherein the each RNAs is formulated in a single lipid nanoparticle.

48. The zoonotic disease vaccine of any one of claims 1-47, further comprising at least one additional RNA having an ORF encoding at least one additional antigen.

49. The zoonotic disease vaccine of any one of claims 46-48, wherein the lipid nanoparticle comprises a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid, 25-55% sterol, and 0.5-15% PEG-modified lipid

50. The zoonotic disease vaccine of any one of claims 1-49, wherein the antigen is fused to a signal peptide.

51. The zoonotic disease vaccine of any one of claims 1-50, wherein the antigen is fused to a scaffold moiety.

52. The zoonotic disease vaccine of claim 51, wherein the scaffold moiety is selected from the group consisting of: ferritin, encapsulin, lumazine synthase, hepatitis B surface antigen, and hepatitis B core antigen.

53. The zoonotic disease vaccine of any one of claims 1-52, wherein the RNA comprises messenger RNA (mRNA).

54. The zoonotic disease vaccine of any one of claims 1-53, wherein the RNA further comprises a 5′UTR and/or a 3′UTR.

55. The zoonotic disease vaccine of any one of claims 1-54, wherein the RNA is unmodified.

56. The zoonotic disease vaccine of any one of claims 1-54, wherein the RNA comprise a modified nucleotide.

57. The zoonotic disease vaccine of claim 56, wherein at least 80% of the uracil in the ORF comprise 1-methyl-pseudouridine modification.

58. A method comprising administering to a subject the zoonotic disease vaccine of any one of claims 1-57 in a therapeutically effective amount to induce an immune response in the subject.

59. The method of claim 58, wherein the therapeutically effective amount induces a neutralizing antibody titer and/or a T cell immune response in the subject.

60. The method of claim 59, wherein efficacy of the vaccine is at least 80% relative to unvaccinated control subjects.

61. The method of any one of claims 58-60, wherein detectable levels of the antigen are produced in the serum of the subject at 1-72 hours post administration of the vaccine.

62. The method of any one of claims 59-61, wherein a neutralizing antibody titer of at least 100 NU/ml, at least 500 NU/ml, or at least 1000 NU/ml is produced in the serum of the subject at 1-72 hours post administration of the vaccine.

63. The method of any one of claims 58-62, wherein the therapeutically effective amount is a total dose of 20 μg-200 μg or a total dose of 50 μg-100 μg.

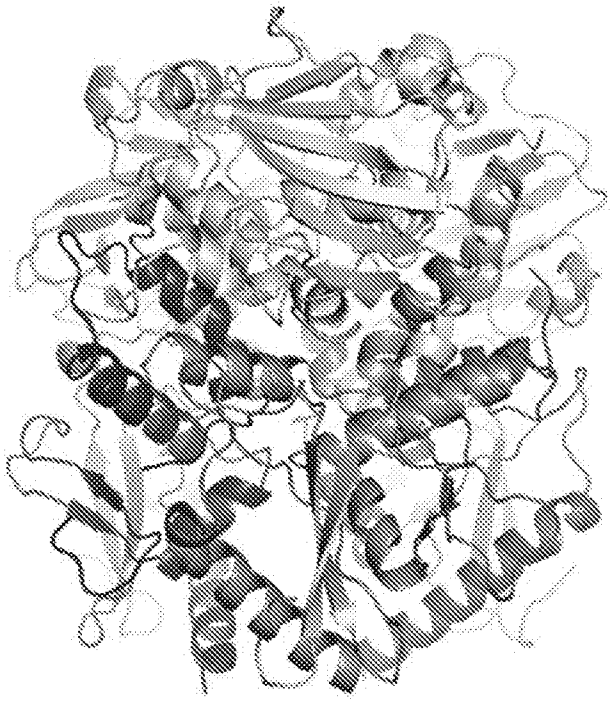


FIG. 1

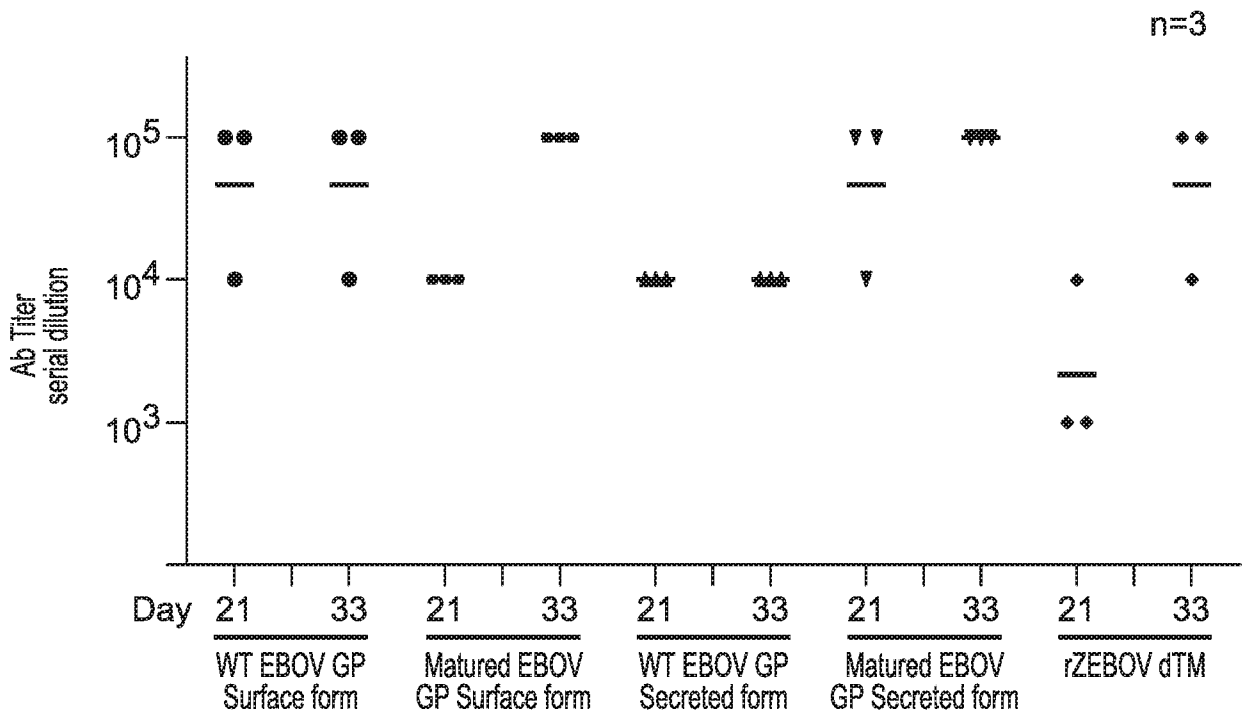

n=3
Ab Titer
serial dilution
10^5
10^4
10^3
Day
21
33
WT EBOV GP
Surface form
Matured EBOV
GP Surface form
WT EBOV GP
Secreted form
Matured EBOV
GP Secreted form
rZEBOV dTM

FIG. 2

Group	N animals	Vaccine	Dose	Route
A	5	Ag 1	20 ug/100 ul	IM
B	5	Ag 2	20 ug/100 ul	IM
C	5	PBS	100 ul	IM

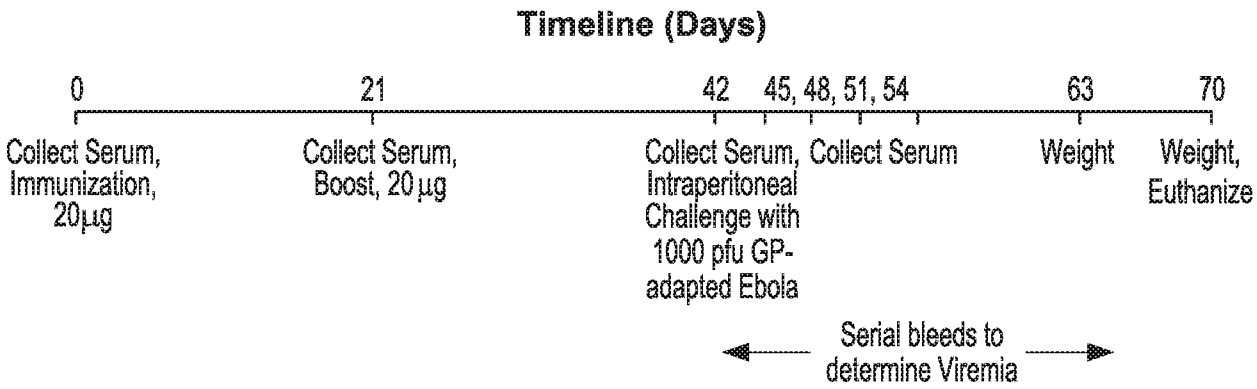


FIG. 3

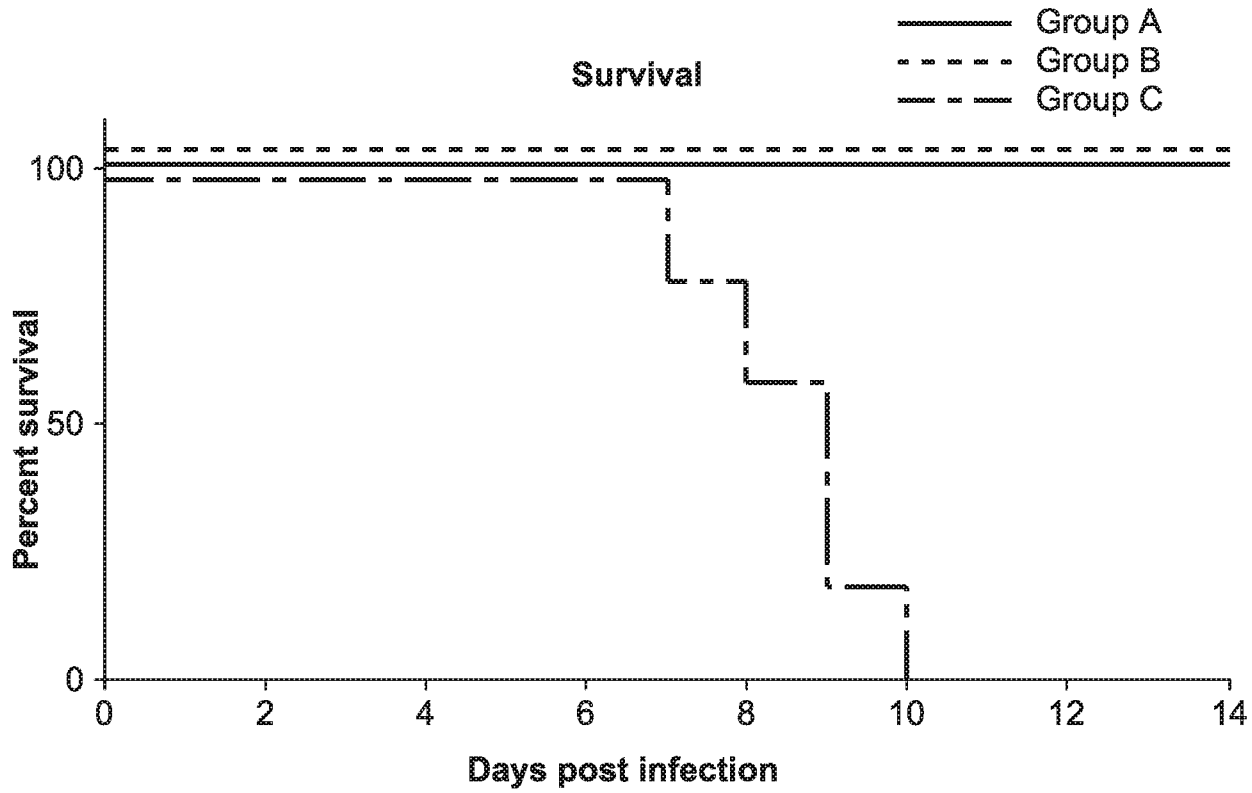


FIG. 4

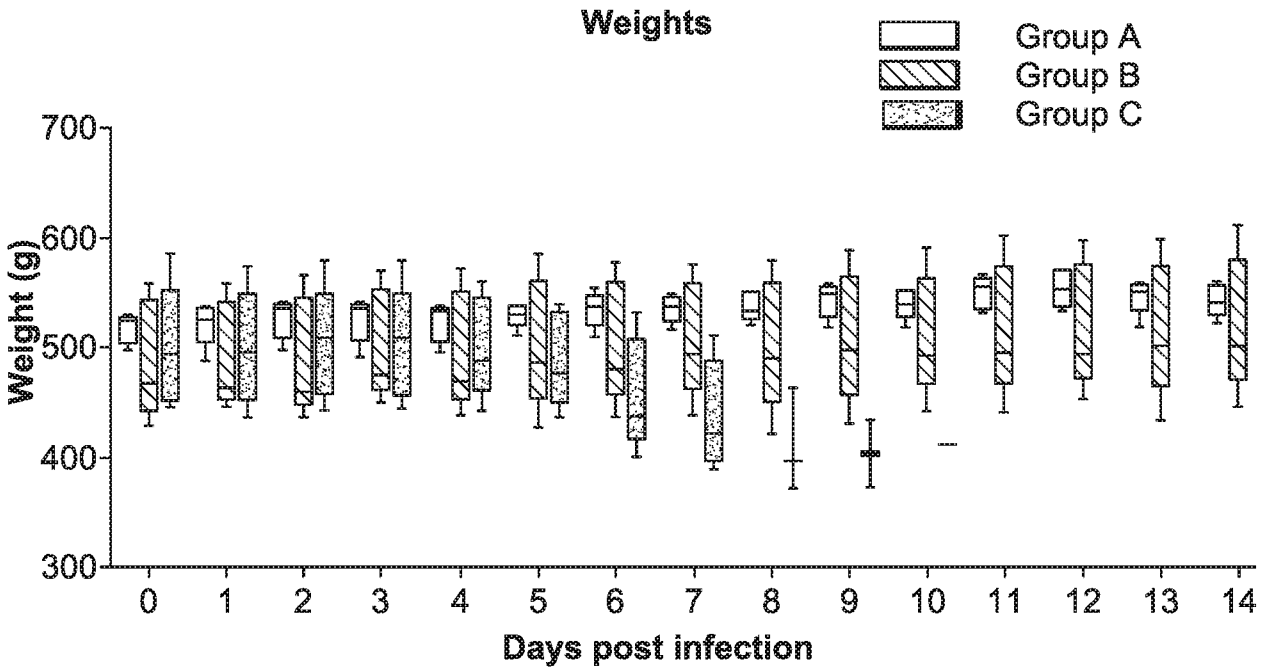

Weights
Group A
Group B
Group C
Weight (g)
700
600
500
400
300
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post infection

FIG. 5

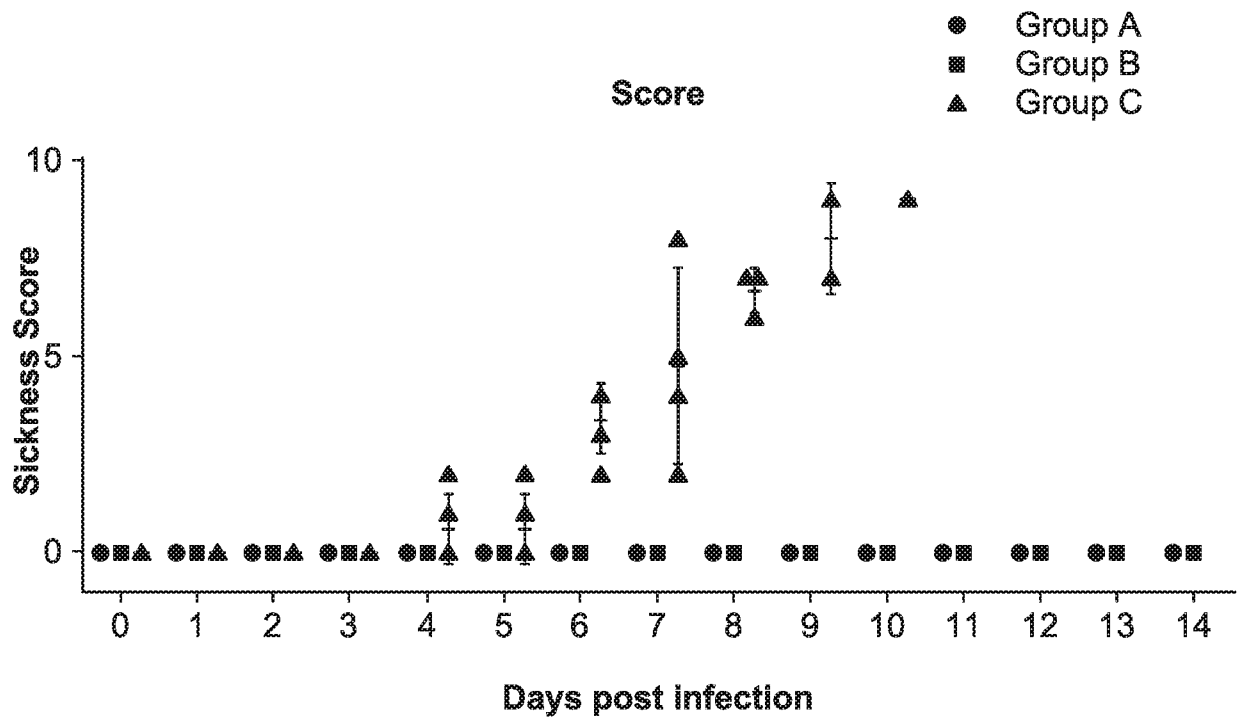

Score
Group A
Group B
Group C
Sickness Score
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post infection

FIG. 6

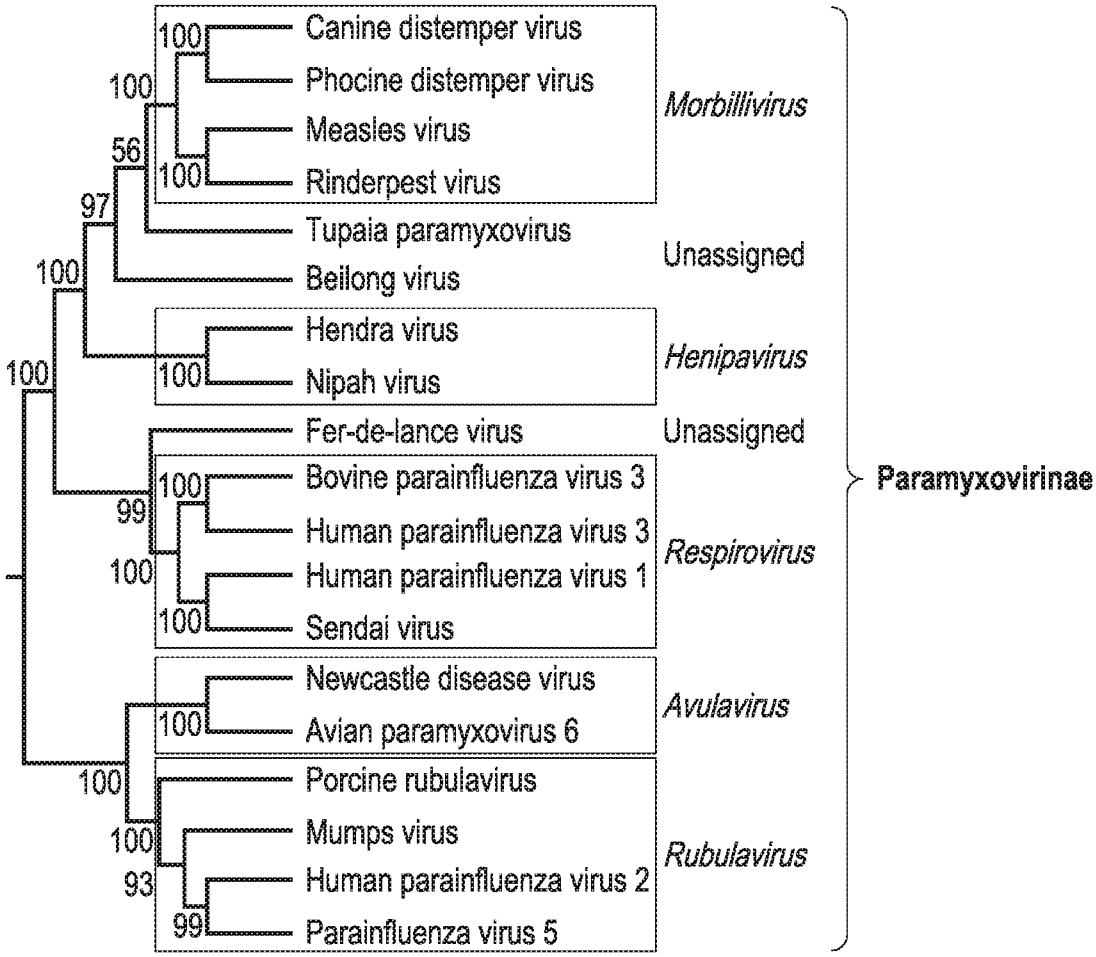

Canine distemper virus
Phocine distemper virus
Measles virus
Rinderpest virus
Morbillivirus
Tupaia paramyxovirus
Beilong virus
Unassigned
Hendra virus
Nipah virus
Henipavirus
Fer-de-lance virus
Unassigned
Bovine parainfluenza virus 3
Human parainfluenza virus 3
Human parainfluenza virus 1
Sendai virus
Respirovirus
Newcastle disease virus
Avian paramyxovirus 6
Avulavirus
Porcine rubulavirus
Mumps virus
Human parainfluenza virus 2
Parainfluenza virus 5
Rubulavirus
Paramyxovirinae
100
100
56
100
97
100
100
100
99
100
100
100
100
100
93
99

FIG. 7

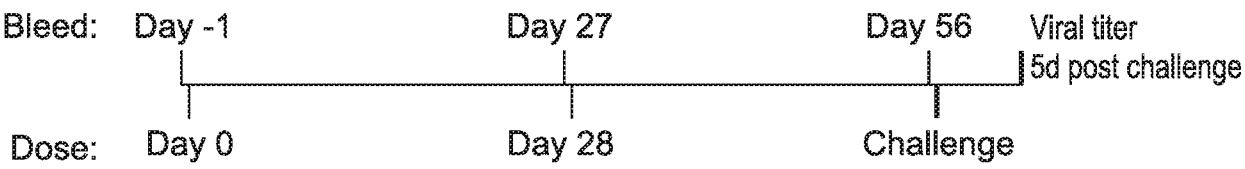

Bleed:
Day -1
Day 27
Day 56
Viral titer
5d post challenge
Dose:
Day 0
Day 28
Challenge

FIG. 8

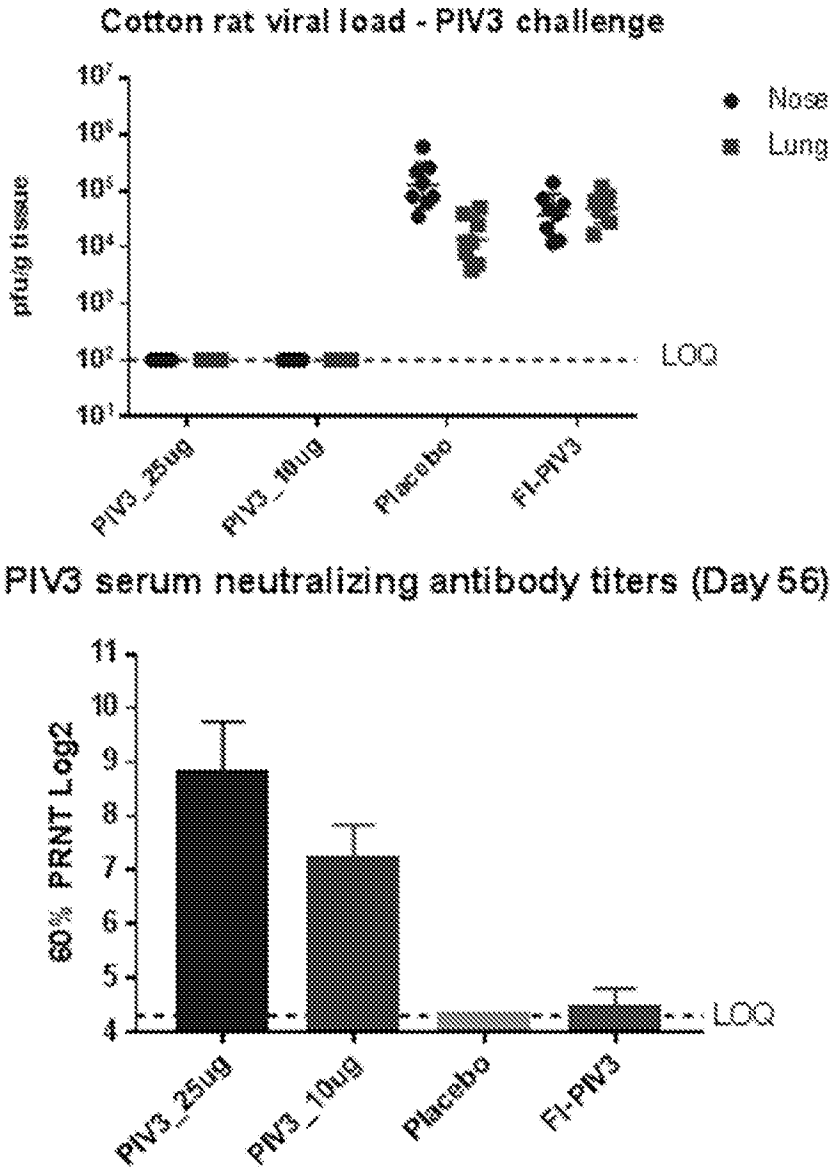

Cotton rat viral load - PIV3 challenge
Nose
Lung
pfu/g tissue
LOQ
PIV3_25ug
PIV3_10ug
Placebo
FI-PIV3
PIV3 serum neutralizing antibody titers (Day 56)
60% PRNT Log2
LOQ
PIV3_25ug
PIV3_10ug
Placebo
FI-PIV3

FIG. 9

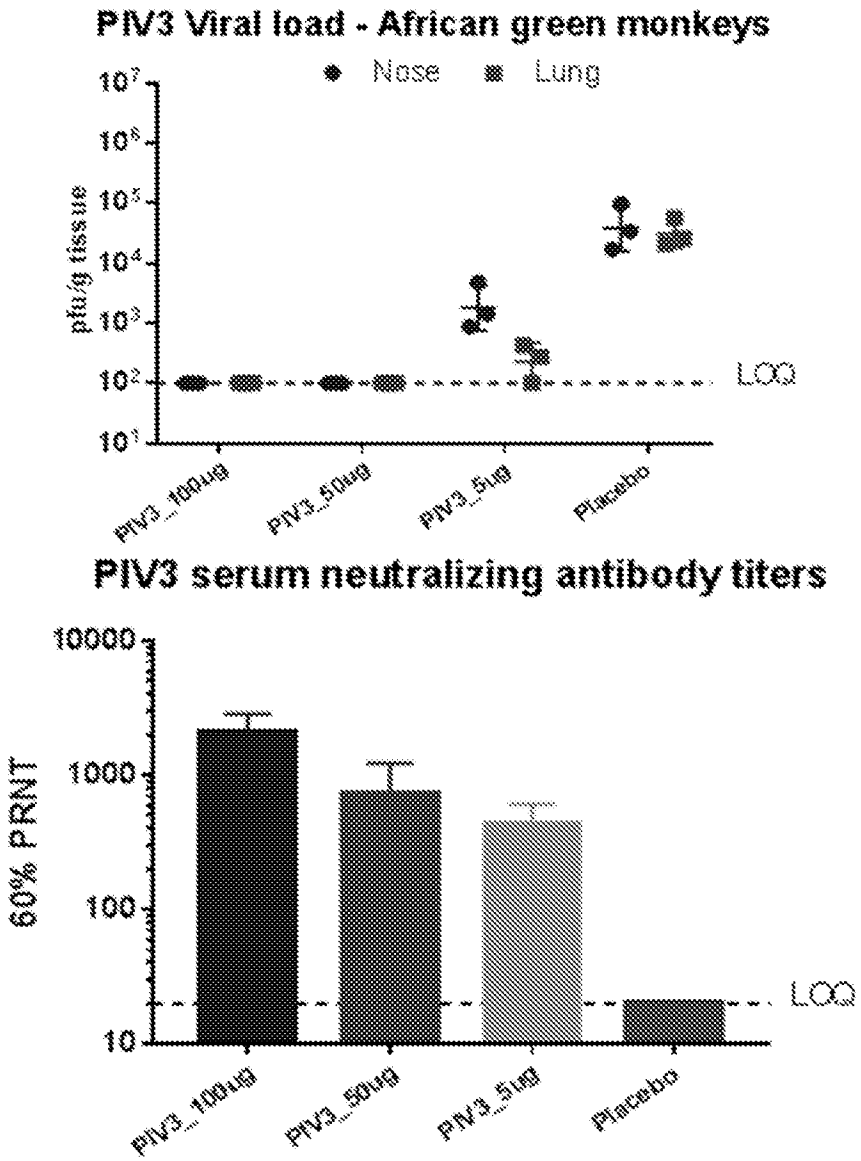

PIV3 Viral load - African green monkeys
Nose
Lung
pfu/g tissue
LOQ
PIV3_100ug
PIV3_50ug
PIV3_5ug
Placebo
PIV3 serum neutralizing antibody titers
60% PRNT
10000
1000
100
10
LOQ
PIV3_100ug
PIV3_50ug
PIV3_5ug
Placebo

FIG. 10

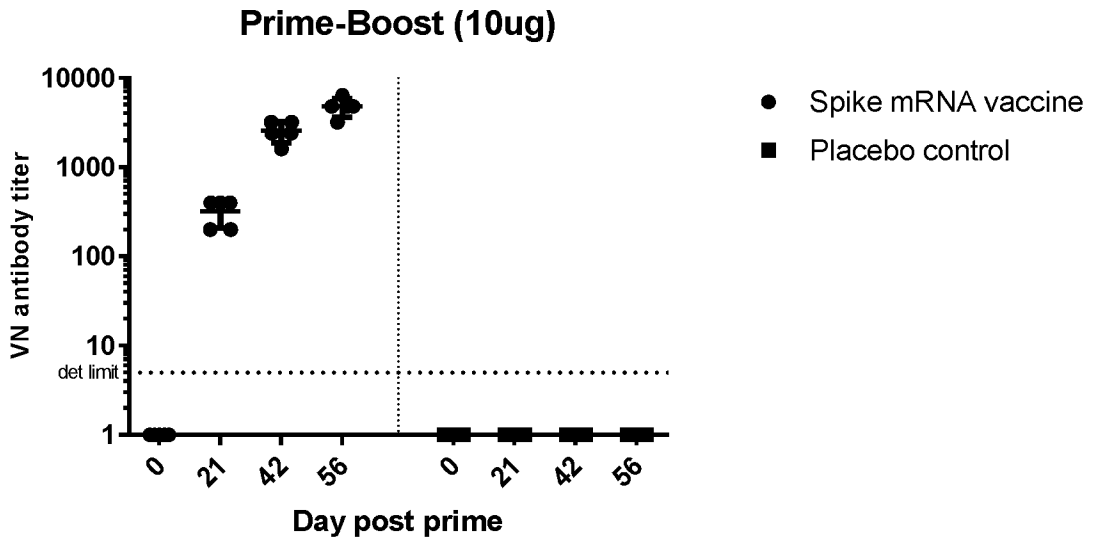

Prime-Boost (10ug)
VN antibody titer
10000
1000
100
10
det limit
1
0
21
42
56
0
21
42
56
Day post prime
Spike mRNA vaccine
Placebo control

FIG. 11

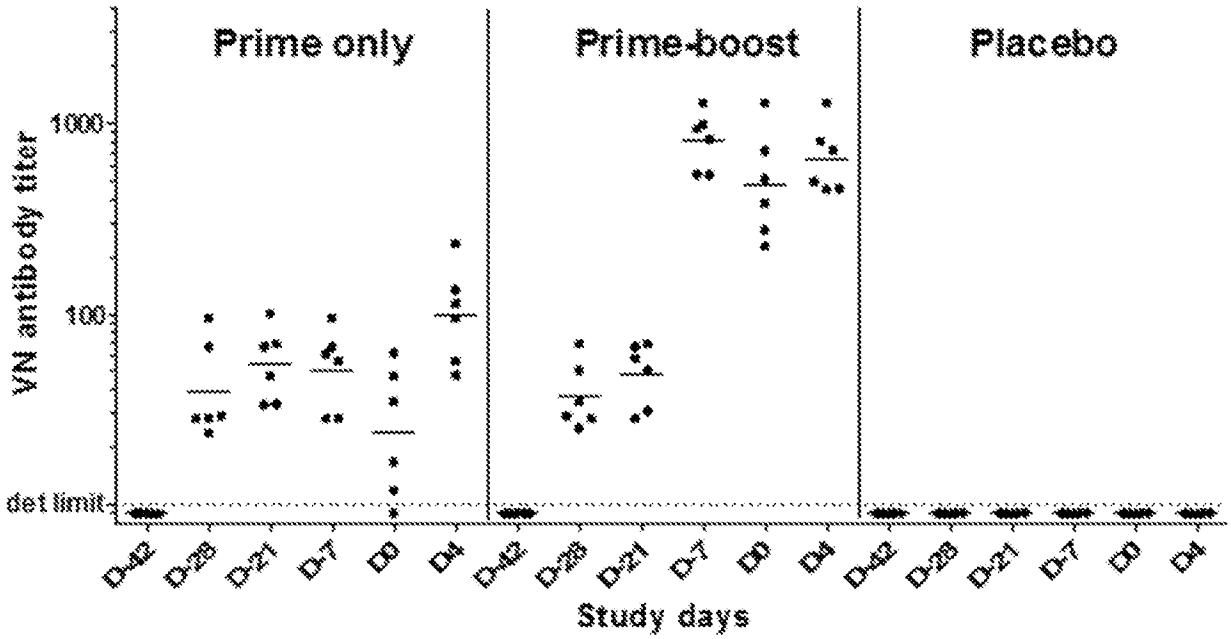

Prime only
Prime-boost
Placebo
VN antibody titer
1000
100
det limit
D-42
D-28
D-21
D-7
D0
D4
Study days

FIG. 12

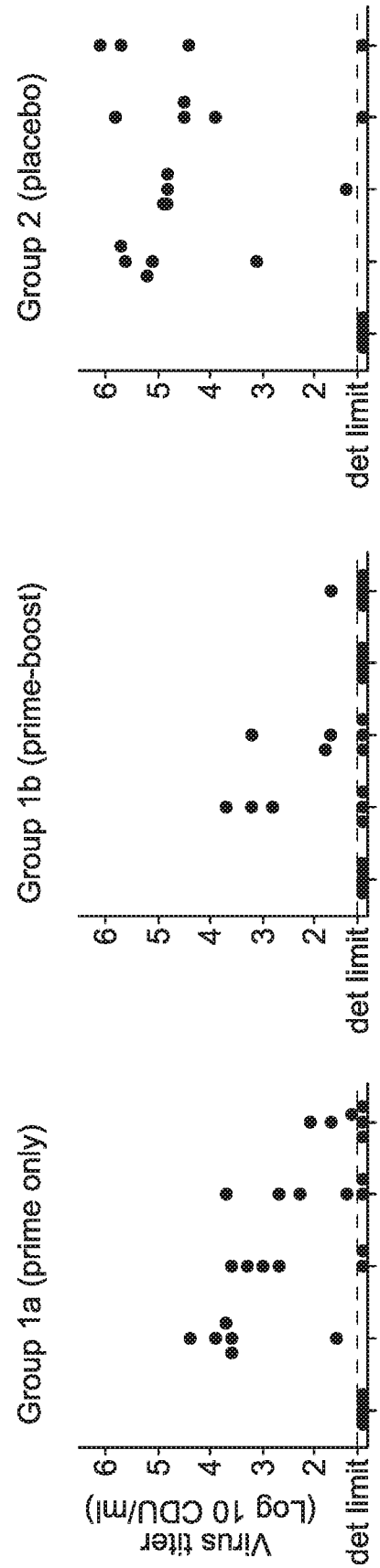


FIG. 13A

FIG. 13B

FIG. 13C

FIG. 13D

FIG. 13E

FIG. 13F

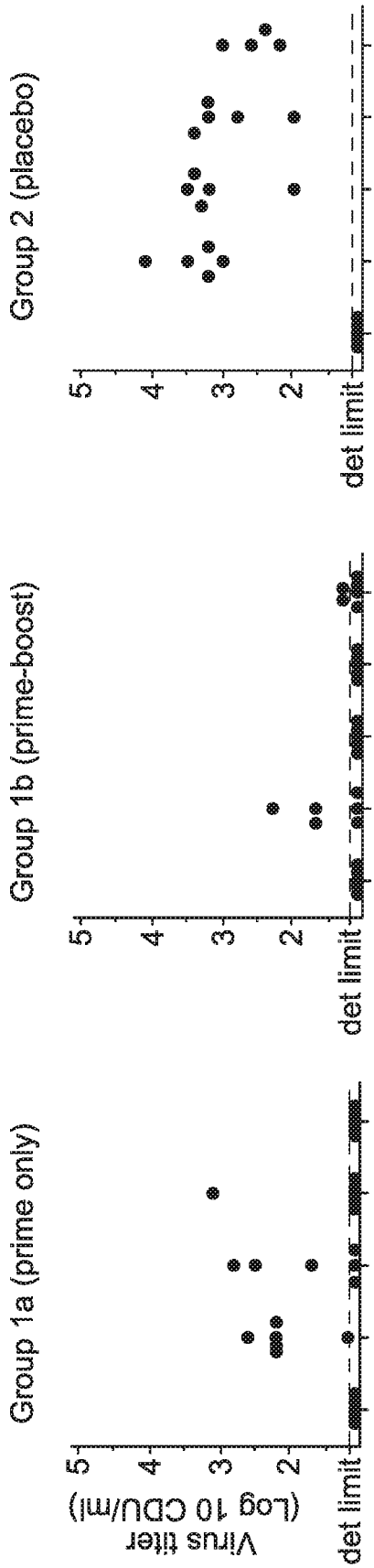


FIG. 14A

FIG. 14B

FIG. 14C

FIG. 14D

FIG. 14E

FIG. 14F

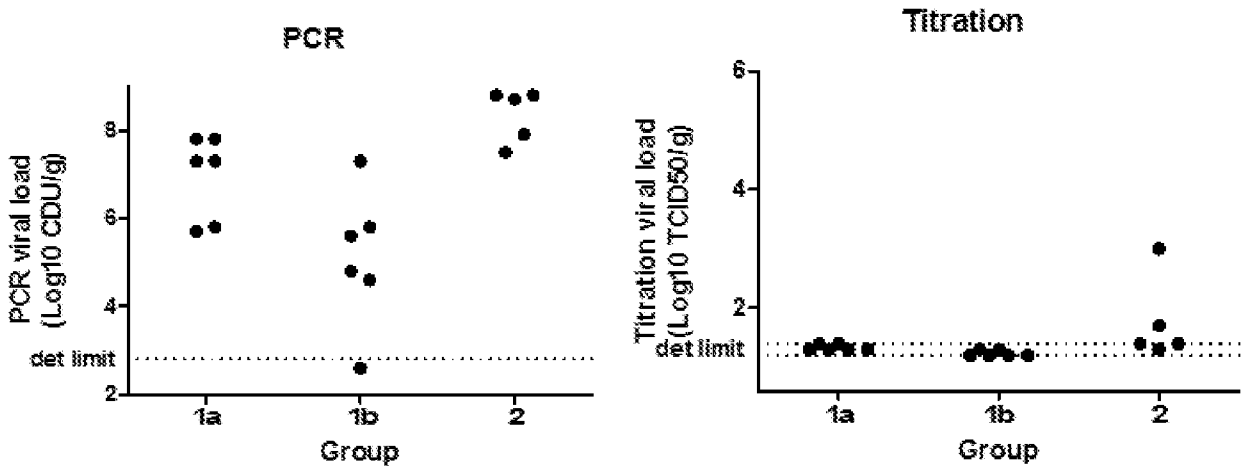

PCR
PCR viral load
(Log10 CDU/g)
8
6
4
det limit
2
1a
1b
2
Group
Titration
Titration viral load
(Log10 TCID50/g)
6
4
2
det limit
1a
1b
2
Group

FIG. 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/022777

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/7105 (2006.01) ***A61K 31/7115 (2006.01)*** ***A61K 39/12 (2006.01)*** ***A61P 37/04 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: PATENW = EPODOC, WPIAP, TXPEA, TXPEB, TXPEC, TXPEE, TXPEF, TXPEH, TXPEI, TXPEP, TXPES, TXPWOEA, TXPUSE0A, TXPUSE1A, TXPUSEA, TXPUSEB, TXPEPEA, TXPEPEB; MEDLINE, BIOSIS, CAPLUS, EMBASE; Google; GenomeQuest, Patent Scope and internal databases.

Search terms: RNA, Lassa Virus, Nipah virus, betacoronoavirus, vaccine, RNA vaccine, zoonosis, zoonotic, Modernatx, KUMARASINGHE, Ellalahewage Sathyajith, BENENATO, Kerry, PRESNYAK, Vladimir, CIARAMELLA, Giuseppe, HIMANSU, Sunny and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2018	29 June 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Ann Le AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832745

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2018/022777**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 8754062 B2 (Moderna Therapeutics) 17 June 2014	
A	US 2013/0156849 A1 (Moderna Therapeutics) 20 June 2013	
A	Greer, C.E et al.: "Long-term Protection in Hamsters against Human Parainfluenza Virus Type 3 Following Mucosal or Combinations of Mucosal and Systemic Immunizations with Chimeric Alphavirus-based Replicon Particles", (2007) Scand J Immunol 66 (6), pages 645-653	
A	Editorial: "Taking forward a 'One Health' approach for turning the tide against the Middle East respiratory syndrome coronavirus and other zoonotic pathogens with epidemic potential", Editorial / International Journal of Infectious Diseases 47 (2016) pages 5–9	
T	CDC: Centers for Disease Control and Prevention: "Coronavirus"; "Prevention and Treatment", Page last reviewed: November 9, 2017 Page last updated: November 9, 2017Content source:Content source: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases [Internet] Internet URL: https://www.cdc.gov/coronavirus/about/prevention.html (page viewed 27 June 2018) (whole document) whole document	1-63
A	CDC: Centers for Disease Control and Prevention: "Lassa Fever"; "Prevention", Page last reviewed: March 25, 2014 Page last updated: March 25, 2014 source:Content source:Centers for Disease Control and Prevention National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) Division of High-Consequence Pathogens and Pathology (DHCPP) Viral Special Pathogens Branch (VSPB) [Internet] Internet URL: https://www.cdc.gov/vhf/lassa/prevention/index.html (page viewed 27 June 2018) (whole document)	

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2018/022777**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CDC: Centers for Disease Control and Prevention: "Nipah Virus (NiV)"; "Prevention", Page last reviewed: March 20, 2014 Page last updated: March 20, 2014 Content source: Centers for Disease Control and Prevention National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) Division of High-Consequence Pathogens and Pathology (DHCPP) Viral Special Pathogens Branch (VSPB) [Internet] Internet URL: https://www.cdc.gov/vhf/nipah/prevention/index.html (page viewed 27 June 2018) (whole document)	
L	Chahal, J.S et al.:"Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose", (2016), PNAS pages E4133-E4142 Lack of unity a posteriori with citation	
P,X	WO 2017/070626 A2 (MODERNATX, INC) 27 April 2018 (see whole document, specifically pages 5, 8-11 18-19, 82-83 of D9)	1 and 22-63

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/022777

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2018/022777

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Invention 1:
- Claims 1, 33-63 (in part) and claims 2-10 are directed to RNA vaccines against Lassa Virus. The feature of a vaccine comprising RNA comprising ORF encoding a Lassa Virus antigen is specific to this group of claims.

- Invention 2:
- Claims 1, 33-63 (in part) and claims 11-21 are directed to RNA vaccines against Nipah Virus
- . The feature of a vaccine comprising RNA comprising ORF encoding a Nipah Virus antigen is specific to this group of claims.

- Invention 3:
- Claims 1, 33-63 (in part) and claims 22-32 are directed to RNA vaccines against Betacoronoavirus
- . The feature of a vaccine comprising RNA comprising ORF encoding a Betacoronoavirus antigen is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the feature of a RNA vaccine against a zoonotic disease causing pathogen

However this feature does not make a contribution over the prior art because it is disclosed in:

D8: Chahal, J.S et al.:"Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose", (2016), PNAS pages E4133-E4142

D8 discloses RNA vaccines comprising RNA encoding the antigen of zoonotic pathogen, Ebola (see whole document).

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 8754062 B2	17 June 2014	US 2013245105 A1	19 Sep 2013
		US 8754062 B2	17 Jun 2014
		AU 2012318752 A1	24 Apr 2014
		AU 2012318752 B2	31 Aug 2017
		AU 2012352180 A1	31 Jul 2014
		AU 2013243834 A1	30 Oct 2014
		AU 2013243946 A1	30 Oct 2014
		AU 2013243947 A1	30 Oct 2014
		AU 2013243948 A1	30 Oct 2014
		AU 2013243949 A1	30 Oct 2014
		AU 2013243950 A1	30 Oct 2014
		AU 2013243951 A1	30 Oct 2014
		AU 2013243952 A1	30 Oct 2014
		AU 2013243953 A1	30 Oct 2014
		AU 2013243954 A1	30 Oct 2014
		AU 2013243955 A1	30 Oct 2014
		AU 2013243955 B2	22 Feb 2018
		AU 2013348363 A1	11 Jun 2015
		AU 2016231503 A1	06 Oct 2016
		AU 2017202228 A1	27 Apr 2017
		AU 2017213503 A1	31 Aug 2017
		AU 2017232121 A1	12 Oct 2017
		AU 2018200373 A1	22 Mar 2018
		AU 2018200374 A1	22 Mar 2018
		AU 2018200375 A1	22 Mar 2018
		AU 2018200377 A1	22 Mar 2018
		AU 2018200379 A1	22 Mar 2018
		AU 2018200380 A1	22 Mar 2018
		AU 2018200381 A1	01 Feb 2018
		BR 112014007852 A2	18 Apr 2017
		CA 2850624 A1	11 Apr 2013
		CA 2859387 A1	20 Jun 2013
		CA 2866919 A1	03 Oct 2013
		CA 2868391 A1	10 Oct 2013
		CA 2868393 A1	10 Oct 2013
		CA 2868398 A1	10 Oct 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		CA 2868418 A1	10 Oct 2013
		CA 2868422 A1	10 Oct 2013
		CA 2868429 A1	10 Oct 2013
		CA 2868434 A1	10 Oct 2013
		CA 2868438 A1	10 Oct 2013
		CA 2868440 A1	10 Oct 2013
		CA 2868996 A1	10 Oct 2013
		CA 2869005 A1	10 Oct 2013
		CA 2892529 A1	30 May 2014
		CA 2894462 A1	19 Jun 2014
		CN 103974724 A	06 Aug 2014
		CN 104114572 A	22 Oct 2014
		CN 104411338 A	11 Mar 2015
		CN 104870022 A	26 Aug 2015
		EP 2763701 A1	13 Aug 2014
		EP 2791160 A1	22 Oct 2014
		EP 2833892 A2	11 Feb 2015
		EP 2833894 A1	11 Feb 2015
		EP 2833920 A2	11 Feb 2015
		EP 2833921 A2	11 Feb 2015
		EP 2833922 A2	11 Feb 2015
		EP 2833923 A1	11 Feb 2015
		EP 2834259 A1	11 Feb 2015
		EP 2834260 A1	11 Feb 2015
		EP 2834358 A2	11 Feb 2015
		EP 2847329 A1	18 Mar 2015
		EP 2849799 A2	25 Mar 2015
		EP 2922554 A1	30 Sep 2015
		EP 2931319 A1	21 Oct 2015
		EP 2964234 A1	13 Jan 2016
		HK 1200730 A1	14 Aug 2015
		HK 1203077 A1	16 Oct 2015
		HK 1206601 A1	15 Jan 2016
		HK 1206612 A1	15 Jan 2016
		HK 1206635 A1	15 Jan 2016
		HK 1206636 A1	15 Jan 2016

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		HK 1206638 A1	15 Jan 2016
		HK 1206639 A1	15 Jan 2016
		HK 1206748 A1	15 Jan 2016
		HK 1206749 A1	15 Jan 2016
		HK 1206779 A1	15 Jan 2016
		HK 1206780 A1	12 Feb 2016
		HK 1207306 A1	29 Jan 2016
		HK 1214515 A1	29 Jul 2016
		HK 1220122 A1	28 Apr 2017
		JP 2014530601 A	20 Nov 2014
		JP 6113737 B2	12 Apr 2017
		JP 2015535430 A	14 Dec 2015
		JP 6144355 B2	07 Jun 2017
		JP 2015519040 A	09 Jul 2015
		JP 6189415 B2	30 Aug 2017
		JP 2015501844 A	19 Jan 2015
		JP 2015513912 A	18 May 2015
		JP 2015513913 A	18 May 2015
		JP 2015513914 A	18 May 2015
		JP 2015513916 A	18 May 2015
		JP 2015516143 A	08 Jun 2015
		JP 2015517995 A	25 Jun 2015
		JP 2015518704 A	06 Jul 2015
		JP 2015518705 A	06 Jul 2015
		JP 2015518816 A	06 Jul 2015
		JP 2015519881 A	16 Jul 2015
		JP 2017113029 A	29 Jun 2017
		JP 2017121239 A	13 Jul 2017
		JP 2017121240 A	13 Jul 2017
		JP 2017121241 A	13 Jul 2017
		JP 2017121243 A	13 Jul 2017
		JP 2017121244 A	13 Jul 2017
		JP 2017123847 A	20 Jul 2017
		JP 2017123853 A	20 Jul 2017
		JP 2017140048 A	17 Aug 2017
		JP 2017141230 A	17 Aug 2017

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		JP 2017197545 A	02 Nov 2017
		JP 2018023373 A	15 Feb 2018
		KR 20140068245 A	05 Jun 2014
		KR 20140102759 A	22 Aug 2014
		MX 2014003979 A	26 Aug 2014
		MX 2014007233 A	04 Feb 2015
		NZ 623476 A	30 Sep 2016
		RU 2014117701 A	10 Nov 2015
		RU 2014129004 A	10 Feb 2016
		SG 10201602654S A	30 May 2016
		SG 10201604896T A	30 Aug 2016
		SG 11201401196W A	29 May 2014
		SG 11201402666W A	30 Oct 2014
		US 2013245104 A1	19 Sep 2013
		US 8664194 B2	04 Mar 2014
		US 2013253043 A1	26 Sep 2013
		US 8680069 B2	25 Mar 2014
		US 2013259924 A1	03 Oct 2013
		US 8999380 B2	07 Apr 2015
		US 2014161873 A1	12 Jun 2014
		US 9050297 B2	09 Jun 2015
		US 2014170594 A1	19 Jun 2014
		US 9055992 B2	16 Jun 2015
		US 2014249208 A1	04 Sep 2014
		US 9061059 B2	23 Jun 2015
		US 2014221465 A1	07 Aug 2014
		US 9089604 B2	28 Jul 2015
		US 2014105965 A1	17 Apr 2014
		US 9095552 B2	04 Aug 2015
		US 2014199371 A1	17 Jul 2014
		US 9107886 B2	18 Aug 2015
		US 2014186432 A1	03 Jul 2014
		US 9114113 B2	25 Aug 2015
		US 2014141067 A1	22 May 2014
		US 9149506 B2	06 Oct 2015
		US 2013237594 A1	12 Sep 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 9186372 B2	17 Nov 2015
		US 2013259923 A1	03 Oct 2013
		US 9192651 B2	24 Nov 2015
		US 2014113960 A1	24 Apr 2014
		US 9216205 B2	22 Dec 2015
		US 2014147432 A1	29 May 2014
		US 9220755 B2	29 Dec 2015
		US 2014105964 A1	17 Apr 2014
		US 9220792 B2	29 Dec 2015
		US 2015111248 A1	23 Apr 2015
		US 9221891 B2	29 Dec 2015
		US 2014200262 A1	17 Jul 2014
		US 9233141 B2	12 Jan 2016
		US 2015064236 A1	05 Mar 2015
		US 9254311 B2	09 Feb 2016
		US 2014141068 A1	22 May 2014
		US 9255129 B2	09 Feb 2016
		US 2013244278 A1	19 Sep 2013
		US 9271996 B2	01 Mar 2016
		US 2015246139 A1	03 Sep 2015
		US 9283287 B2	15 Mar 2016
		US 2013252281 A1	26 Sep 2013
		US 9295689 B2	29 Mar 2016
		US 2014107189 A1	17 Apr 2014
		US 9301993 B2	05 Apr 2016
		US 2015086614 A1	26 Mar 2015
		US 9303079 B2	05 Apr 2016
		US 2013242738 A1	19 Sep 2013
		US 9332400 B2	03 May 2016
		US 2013257822 A1	03 Oct 2013
		US 9360966 B2	07 Jun 2016
		US 2013115272 A1	09 May 2013
		US 9428535 B2	30 Aug 2016
		US 2016114011 A1	28 Apr 2016
		US 9504734 B2	29 Nov 2016
		US 2015376648 A1	31 Dec 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 9572896 B2	21 Feb 2017
		US 2016158385 A1	09 Jun 2016
		US 9572897 B2	21 Feb 2017
		US 2016152678 A1	02 Jun 2016
		US 9587003 B2	07 Mar 2017
		US 2014147454 A1	29 May 2014
		US 9597380 B2	21 Mar 2017
		US 2014105966 A1	17 Apr 2014
		US 9675668 B2	13 Jun 2017
		US 2016199513 A1	14 Jul 2016
		US 9782462 B2	10 Oct 2017
		US 2016075733 A1	17 Mar 2016
		US 9814760 B2	14 Nov 2017
		US 2016151516 A1	02 Jun 2016
		US 9827332 B2	28 Nov 2017
		US 2015368665 A1	24 Dec 2015
		US 9828416 B2	28 Nov 2017
		US 2015166616 A1	18 Jun 2015
		US 9878056 B2	30 Jan 2018
		US 2016234846 A1	11 Aug 2016
		US 9936504 B2	03 Apr 2018
		US 2013123481 A1	16 May 2013
		US 2013156849 A1	20 Jun 2013
		US 2013236974 A1	12 Sep 2013
		US 2013237592 A1	12 Sep 2013
		US 2013237593 A1	12 Sep 2013
		US 2013244279 A1	19 Sep 2013
		US 2013245106 A1	19 Sep 2013
		US 2013245107 A1	19 Sep 2013
		US 2013266640 A1	10 Oct 2013
		US 2014010861 A1	09 Jan 2014
		US 2014113959 A1	24 Apr 2014
		US 2014148502 A1	29 May 2014
		US 2014155472 A1	05 Jun 2014
		US 2014155473 A1	05 Jun 2014
		US 2014155474 A1	05 Jun 2014

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2014155475 A1	05 Jun 2014
		US 2014171485 A1	19 Jun 2014
		US 2014179771 A1	26 Jun 2014
		US 2014193482 A1	10 Jul 2014
		US 2014194494 A1	10 Jul 2014
		US 2014200263 A1	17 Jul 2014
		US 2014200264 A1	17 Jul 2014
		US 2014206755 A1	24 Jul 2014
		US 2014255467 A1	11 Sep 2014
		US 2014255468 A1	11 Sep 2014
		US 2014275229 A1	18 Sep 2014
		US 2015044277 A1	12 Feb 2015
		US 2015050354 A1	19 Feb 2015
		US 2015064235 A1	05 Mar 2015
		US 2015307542 A1	29 Oct 2015
		US 2016022840 A1	28 Jan 2016
		US 2016193299 A1	07 Jul 2016
		US 2016289674 A1	06 Oct 2016
		US 2016348099 A1	01 Dec 2016
		US 2016354490 A1	08 Dec 2016
		US 2016354491 A1	08 Dec 2016
		US 2016354492 A1	08 Dec 2016
		US 2016354493 A1	08 Dec 2016
		US 2016375134 A1	29 Dec 2016
		US 2017028085 A1	02 Feb 2017
		US 2017056528 A1	02 Mar 2017
		US 2017252461 A1	07 Sep 2017
		US 2017348436 A1	07 Dec 2017
		US 2017368200 A1	28 Dec 2017
		US 2018000910 A1	04 Jan 2018
		US 2018002393 A1	04 Jan 2018
		US 2018086807 A1	29 Mar 2018
		US 2018125937 A1	10 May 2018
		US 2018170990 A1	21 Jun 2018
		WO 2013052523 A1	11 Apr 2013
		WO 2013090648 A1	20 Jun 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		WO 2013142958 A1	03 Oct 2013
		WO 2013151663 A1	10 Oct 2013
		WO 2013151664 A1	10 Oct 2013
		WO 2013151665 A2	10 Oct 2013
		WO 2013151666 A2	10 Oct 2013
		WO 2013151667 A1	10 Oct 2013
		WO 2013151668 A2	10 Oct 2013
		WO 2013151669 A1	10 Oct 2013
		WO 2013151670 A2	10 Oct 2013
		WO 2013151671 A1	10 Oct 2013
		WO 2013151672 A2	10 Oct 2013
		WO 2013151736 A2	10 Oct 2013
		WO 2014081507 A1	30 May 2014
		WO 2014093924 A1	19 Jun 2014
		WO 2014093926 A1	19 Jun 2014
		WO 2014164253 A1	09 Oct 2014
		ZA 201403783 B	30 Sep 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2013/0156849 A1	20 June 2013	US 2013156849 A1	20 Jun 2013
		AU 2012318752 A1	24 Apr 2014
		AU 2012318752 B2	31 Aug 2017
		AU 2012352180 A1	31 Jul 2014
		AU 2013243834 A1	30 Oct 2014
		AU 2013243946 A1	30 Oct 2014
		AU 2013243947 A1	30 Oct 2014
		AU 2013243948 A1	30 Oct 2014
		AU 2013243949 A1	30 Oct 2014
		AU 2013243950 A1	30 Oct 2014
		AU 2013243951 A1	30 Oct 2014
		AU 2013243952 A1	30 Oct 2014
		AU 2013243953 A1	30 Oct 2014
		AU 2013243954 A1	30 Oct 2014
		AU 2013243955 A1	30 Oct 2014
		AU 2013243955 B2	22 Feb 2018
		AU 2013348363 A1	11 Jun 2015
		AU 2016231503 A1	06 Oct 2016
		AU 2017202228 A1	27 Apr 2017
		AU 2017213503 A1	31 Aug 2017
		AU 2017232121 A1	12 Oct 2017
		AU 2018200373 A1	22 Mar 2018
		AU 2018200374 A1	22 Mar 2018
		AU 2018200375 A1	22 Mar 2018
		AU 2018200377 A1	22 Mar 2018
		AU 2018200379 A1	22 Mar 2018
		AU 2018200380 A1	22 Mar 2018
		AU 2018200381 A1	01 Feb 2018
		BR 112014007852 A2	18 Apr 2017
		CA 2850624 A1	11 Apr 2013
		CA 2859387 A1	20 Jun 2013
		CA 2866919 A1	03 Oct 2013
		CA 2868391 A1	10 Oct 2013
		CA 2868393 A1	10 Oct 2013
		CA 2868398 A1	10 Oct 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		CA 2868418 A1	10 Oct 2013
		CA 2868422 A1	10 Oct 2013
		CA 2868429 A1	10 Oct 2013
		CA 2868434 A1	10 Oct 2013
		CA 2868438 A1	10 Oct 2013
		CA 2868440 A1	10 Oct 2013
		CA 2868996 A1	10 Oct 2013
		CA 2869005 A1	10 Oct 2013
		CA 2892529 A1	30 May 2014
		CA 2894462 A1	19 Jun 2014
		CN 103974724 A	06 Aug 2014
		CN 104114572 A	22 Oct 2014
		CN 104411338 A	11 Mar 2015
		CN 104870022 A	26 Aug 2015
		EP 2763701 A1	13 Aug 2014
		EP 2791160 A1	22 Oct 2014
		EP 2833892 A2	11 Feb 2015
		EP 2833894 A1	11 Feb 2015
		EP 2833920 A2	11 Feb 2015
		EP 2833921 A2	11 Feb 2015
		EP 2833922 A2	11 Feb 2015
		EP 2833923 A1	11 Feb 2015
		EP 2834259 A1	11 Feb 2015
		EP 2834260 A1	11 Feb 2015
		EP 2834358 A2	11 Feb 2015
		EP 2847329 A1	18 Mar 2015
		EP 2849799 A2	25 Mar 2015
		EP 2922554 A1	30 Sep 2015
		EP 2931319 A1	21 Oct 2015
		EP 2964234 A1	13 Jan 2016
		HK 1200730 A1	14 Aug 2015
		HK 1203077 A1	16 Oct 2015
		HK 1206601 A1	15 Jan 2016
		HK 1206612 A1	15 Jan 2016
		HK 1206635 A1	15 Jan 2016
		HK 1206636 A1	15 Jan 2016

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		HK 1206638 A1	15 Jan 2016
		HK 1206639 A1	15 Jan 2016
		HK 1206748 A1	15 Jan 2016
		HK 1206749 A1	15 Jan 2016
		HK 1206779 A1	15 Jan 2016
		HK 1206780 A1	12 Feb 2016
		HK 1207306 A1	29 Jan 2016
		HK 1214515 A1	29 Jul 2016
		HK 1220122 A1	28 Apr 2017
		JP 2014530601 A	20 Nov 2014
		JP 6113737 B2	12 Apr 2017
		JP 2015535430 A	14 Dec 2015
		JP 6144355 B2	07 Jun 2017
		JP 2015519040 A	09 Jul 2015
		JP 6189415 B2	30 Aug 2017
		JP 2015501844 A	19 Jan 2015
		JP 2015513912 A	18 May 2015
		JP 2015513913 A	18 May 2015
		JP 2015513914 A	18 May 2015
		JP 2015513916 A	18 May 2015
		JP 2015516143 A	08 Jun 2015
		JP 2015517995 A	25 Jun 2015
		JP 2015518704 A	06 Jul 2015
		JP 2015518705 A	06 Jul 2015
		JP 2015518816 A	06 Jul 2015
		JP 2015519881 A	16 Jul 2015
		JP 2017113029 A	29 Jun 2017
		JP 2017121239 A	13 Jul 2017
		JP 2017121240 A	13 Jul 2017
		JP 2017121241 A	13 Jul 2017
		JP 2017121243 A	13 Jul 2017
		JP 2017121244 A	13 Jul 2017
		JP 2017123847 A	20 Jul 2017
		JP 2017123853 A	20 Jul 2017
		JP 2017140048 A	17 Aug 2017
		JP 2017141230 A	17 Aug 2017

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		JP 2017197545 A	02 Nov 2017
		JP 2018023373 A	15 Feb 2018
		KR 20140068245 A	05 Jun 2014
		KR 20140102759 A	22 Aug 2014
		MX 2014003979 A	26 Aug 2014
		MX 2014007233 A	04 Feb 2015
		NZ 623476 A	30 Sep 2016
		RU 2014117701 A	10 Nov 2015
		RU 2014129004 A	10 Feb 2016
		SG 10201602654S A	30 May 2016
		SG 10201604896T A	30 Aug 2016
		SG 11201401196W A	29 May 2014
		SG 11201402666W A	30 Oct 2014
		US 2013245104 A1	19 Sep 2013
		US 8664194 B2	04 Mar 2014
		US 2013253043 A1	26 Sep 2013
		US 8680069 B2	25 Mar 2014
		US 2013245105 A1	19 Sep 2013
		US 8754062 B2	17 Jun 2014
		US 2013259924 A1	03 Oct 2013
		US 8999380 B2	07 Apr 2015
		US 2014161873 A1	12 Jun 2014
		US 9050297 B2	09 Jun 2015
		US 2014170594 A1	19 Jun 2014
		US 9055992 B2	16 Jun 2015
		US 2014249208 A1	04 Sep 2014
		US 9061059 B2	23 Jun 2015
		US 2014221465 A1	07 Aug 2014
		US 9089604 B2	28 Jul 2015
		US 2014105965 A1	17 Apr 2014
		US 9095552 B2	04 Aug 2015
		US 2014199371 A1	17 Jul 2014
		US 9107886 B2	18 Aug 2015
		US 2014186432 A1	03 Jul 2014
		US 9114113 B2	25 Aug 2015
		US 2014141067 A1	22 May 2014

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 9149506 B2	06 Oct 2015
		US 2013237594 A1	12 Sep 2013
		US 9186372 B2	17 Nov 2015
		US 2013259923 A1	03 Oct 2013
		US 9192651 B2	24 Nov 2015
		US 2014113960 A1	24 Apr 2014
		US 9216205 B2	22 Dec 2015
		US 2014147432 A1	29 May 2014
		US 9220755 B2	29 Dec 2015
		US 2014105964 A1	17 Apr 2014
		US 9220792 B2	29 Dec 2015
		US 2015111248 A1	23 Apr 2015
		US 9221891 B2	29 Dec 2015
		US 2014200262 A1	17 Jul 2014
		US 9233141 B2	12 Jan 2016
		US 2015064236 A1	05 Mar 2015
		US 9254311 B2	09 Feb 2016
		US 2014141068 A1	22 May 2014
		US 9255129 B2	09 Feb 2016
		US 2013244278 A1	19 Sep 2013
		US 9271996 B2	01 Mar 2016
		US 2015246139 A1	03 Sep 2015
		US 9283287 B2	15 Mar 2016
		US 2013252281 A1	26 Sep 2013
		US 9295689 B2	29 Mar 2016
		US 2014107189 A1	17 Apr 2014
		US 9301993 B2	05 Apr 2016
		US 2015086614 A1	26 Mar 2015
		US 9303079 B2	05 Apr 2016
		US 2013242738 A1	19 Sep 2013
		US 9332400 B2	03 May 2016
		US 2013257822 A1	03 Oct 2013
		US 9360966 B2	07 Jun 2016
		US 2013115272 A1	09 May 2013
		US 9428535 B2	30 Aug 2016
		US 2016114011 A1	28 Apr 2016

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 9504734 B2	29 Nov 2016
		US 2015376648 A1	31 Dec 2015
		US 9572896 B2	21 Feb 2017
		US 2016158385 A1	09 Jun 2016
		US 9572897 B2	21 Feb 2017
		US 2016152678 A1	02 Jun 2016
		US 9587003 B2	07 Mar 2017
		US 2014147454 A1	29 May 2014
		US 9597380 B2	21 Mar 2017
		US 2014105966 A1	17 Apr 2014
		US 9675668 B2	13 Jun 2017
		US 2016199513 A1	14 Jul 2016
		US 9782462 B2	10 Oct 2017
		US 2016075733 A1	17 Mar 2016
		US 9814760 B2	14 Nov 2017
		US 2016151516 A1	02 Jun 2016
		US 9827332 B2	28 Nov 2017
		US 2015368665 A1	24 Dec 2015
		US 9828416 B2	28 Nov 2017
		US 2015166616 A1	18 Jun 2015
		US 9878056 B2	30 Jan 2018
		US 2016234846 A1	11 Aug 2016
		US 9936504 B2	03 Apr 2018
		US 2013123481 A1	16 May 2013
		US 2013236974 A1	12 Sep 2013
		US 2013237592 A1	12 Sep 2013
		US 2013237593 A1	12 Sep 2013
		US 2013244279 A1	19 Sep 2013
		US 2013245106 A1	19 Sep 2013
		US 2013245107 A1	19 Sep 2013
		US 2013266640 A1	10 Oct 2013
		US 2014010861 A1	09 Jan 2014
		US 2014113959 A1	24 Apr 2014
		US 2014148502 A1	29 May 2014
		US 2014155472 A1	05 Jun 2014
		US 2014155473 A1	05 Jun 2014

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2014155474 A1	05 Jun 2014
		US 2014155475 A1	05 Jun 2014
		US 2014171485 A1	19 Jun 2014
		US 2014179771 A1	26 Jun 2014
		US 2014193482 A1	10 Jul 2014
		US 2014194494 A1	10 Jul 2014
		US 2014200263 A1	17 Jul 2014
		US 2014200264 A1	17 Jul 2014
		US 2014206755 A1	24 Jul 2014
		US 2014255467 A1	11 Sep 2014
		US 2014255468 A1	11 Sep 2014
		US 2014275229 A1	18 Sep 2014
		US 2015044277 A1	12 Feb 2015
		US 2015050354 A1	19 Feb 2015
		US 2015064235 A1	05 Mar 2015
		US 2015307542 A1	29 Oct 2015
		US 2016022840 A1	28 Jan 2016
		US 2016193299 A1	07 Jul 2016
		US 2016289674 A1	06 Oct 2016
		US 2016348099 A1	01 Dec 2016
		US 2016354490 A1	08 Dec 2016
		US 2016354491 A1	08 Dec 2016
		US 2016354492 A1	08 Dec 2016
		US 2016354493 A1	08 Dec 2016
		US 2016375134 A1	29 Dec 2016
		US 2017028085 A1	02 Feb 2017
		US 2017056528 A1	02 Mar 2017
		US 2017252461 A1	07 Sep 2017
		US 2017348436 A1	07 Dec 2017
		US 2017368200 A1	28 Dec 2017
		US 2018000910 A1	04 Jan 2018
		US 2018002393 A1	04 Jan 2018
		US 2018086807 A1	29 Mar 2018
		US 2018125937 A1	10 May 2018
		US 2018170990 A1	21 Jun 2018
		WO 2013052523 A1	11 Apr 2013

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		WO 2013090648 A1	20 Jun 2013
		WO 2013142958 A1	03 Oct 2013
		WO 2013151663 A1	10 Oct 2013
		WO 2013151664 A1	10 Oct 2013
		WO 2013151665 A2	10 Oct 2013
		WO 2013151666 A2	10 Oct 2013
		WO 2013151667 A1	10 Oct 2013
		WO 2013151668 A2	10 Oct 2013
		WO 2013151669 A1	10 Oct 2013
		WO 2013151670 A2	10 Oct 2013
		WO 2013151671 A1	10 Oct 2013
		WO 2013151672 A2	10 Oct 2013
		WO 2013151736 A2	10 Oct 2013
		WO 2014081507 A1	30 May 2014
		WO 2014093924 A1	19 Jun 2014
		WO 2014093926 A1	19 Jun 2014
		WO 2014164253 A1	09 Oct 2014
		ZA 201403783 B	30 Sep 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2017/070626 A2	27 April 2018	WO 2017070626 A2	27 Apr 2017
		AR 106463 A1	17 Jan 2018
		AR 106464 A1	17 Jan 2018
		AR 106465 A1	17 Jan 2018
		AU 2016341309 A1	07 Jun 2018
		AU 2016341311 A1	07 Jun 2018
		AU 2016342045 A1	07 Jun 2018
		AU 2016342048 A1	07 Jun 2018
		AU 2016342049 A1	07 Jun 2018
		AU 2016342371 A1	07 Jun 2018
		AU 2016342376 A1	07 Jun 2018
		CA 3002819 A1	27 Apr 2017
		CA 3002820 A1	27 Apr 2017
		CA 3002822 A1	27 Apr 2017
		CA 3002912 A1	27 Apr 2017
		CA 3002922 A1	27 Apr 2017
		CA 3003090 A1	27 Apr 2017
		CA 3003103 A1	27 Apr 2017
		SG 11201803360U A	30 May 2018
		SG 11201803363Y A	30 May 2018
		SG 11201803365R A	30 May 2018
		TW 201729835 A	01 Sep 2017
		TW 201729836 A	01 Sep 2017
		TW 201729838 A	01 Sep 2017
		US 2017340724 A1	30 Nov 2017

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/022777**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2017340725 A1	30 Nov 2017
		US 2018008694 A1	11 Jan 2018
		US 2018028645 A1	01 Feb 2018
		WO 2017070601 A1	27 Apr 2017
		WO 2017070613 A1	27 Apr 2017
		WO 2017070616 A2	27 Apr 2017
		WO 2017070618 A1	27 Apr 2017
		WO 2017070620 A2	27 Apr 2017
		WO 2017070622 A1	27 Apr 2017
		WO 2017070623 A1	27 Apr 2017
		WO 2017070624 A1	27 Apr 2017

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.