NUCLEIC ACID VACCINES

RELATED APPLICATIONS

This Application is a continuation of U.S. application Ser. No. 16/036,318, filed Jul. 16, 2018, entitled "NUCLEIC ACID VACCINES: which is a continuation of U.S. application Ser. No. 15/089,050, filed Apr. 1, 2016, entitled "NUCLEIC ACID VACCINES", which is a continuation of International Patent Application Serial No. PCT/US2015/027400, filed Apr. 23, 2015, entitled "NUCLEIC ACID VACCINES", which is a Non-Prov of Prov (35 USC 119(e)) of U.S. Application Ser. No. 62/088,994, filed Dec. 8, 2014, entitled "NUCLEIC ACID VACCINES" and of U.S. Application Ser. No. 61/983,250, filed Apr. 23, 2014, entitled "NUCLEIC ACID VACCINES". The entire contents of these applications are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The invention relates to compositions, methods, processes, kits and devices for the selection, design, preparation, manufacture, formulation, and/or use of vaccines, specifically nucleic acid vaccines (NAVs). In particular, the invention relates to compositions, methods, processes, kits and devices for the selection, design, preparation, manufacture, formulation, and/or use of ribonucleic acid (RNA) vaccines, e.g., mRNA vaccines.

BACKGROUND OF THE INVENTION

Vaccination is an effective way to provide phrophylactic protection against infectious diseases, including, but not limited to, viral, bacterial, and/or parasitic diseases, such as influenza, AIDS, hepatotisis virus infection, cholera, malaria and tuberculosis, and many other diseases. For example, influenza infections are the seventh leading cause of death in the United States with 200, 000 hospitalizations and 40,000 deaths seen in the United States per year and cause about 3-5 million hospitalizations and about 300,000 to 500,000 deaths worldwide per year. Millions of people receive flu vaccines to protect them from seasonal flu each year. Vaccination can also rapidly prevent the spread of an emerging influenze pandemic.

A typical vaccine contains an agent that resembles a weakened or dead form of the disease-causing agent, which could be a microorganism, such as bacteria, virus, fungi, parasites, or one or more toxins and/or one or more proteins, for example, surface proteins, (i.e., antigens) of such a microorganism. The antigen or agent in the vaccine can stimulate the body's immune system to recognize the agent as a foreign invader, generate antibodies against it, destroy it and develop a memory of it. The vaccine-induced memory enables the immune system to act quickly to protect the body from any of these agents that it later encounters.

Vaccine production used in the art e.g., antigen vaccine production, has several stages, including the generation of antigens, antigen purification and inactivation, and vaccine formulation. First, the antigen is generated through culturing viruses in cell lines, growing bacteria in bioreactors, or producing recombinant proteins derived from viruses and bacteria in cell cultures, yeast or bacteria. Recombinant proteins are then purified and the viruses and bacteria are inactivated before they are formulated with adjuvants in

vaccines. It has been a challenge to drastically reduce the time and expense associated with current technologies in vaccine development.

Another obstacle to the development of new vaccine is the constant evolution of most infectious agents, such as viruses and bacteria. Viruses often mutate their surface proteins to generate new antigens which can help them skipping the active immune system that has been immunized by vaccines containing the viruses. In contrast, bacteria acquire and mutate key proteins to evade host defense and effective antibiotic applications.

For example, influenza A, B and C viruses are the etiological agents of influenza. Hemagglutinin (HA), the major envelop glycoprotein of influenza A and B viruses, or its homologue, hemagglutinin-esterase (HE) in influenza C virus, is the natural reservoir of the viruses. The rapid evolution of the hemagglutinin (HA) protein of the influenza virus results in the constant emergence of new strains, rendering the adaptive immune response of the host only partially protective to new infections. The biggest challenge for therapy and prophylaxis against influenza and other infections using traditional vaccines is the limitation of vaccines in breadth, providing protection only against closely related subtypes. In addition, today's length of the production process inhibits any fast reaction to develop and produce an adapted vaccine in a pandemic situation.

It is of great interest to develop new vaccines as well as new approaches to combatting infectious disease and infectious agents.

SUMMARY OF THE INVENTION

Described herein are compositions, methods, processes, kits and devices for the selection, design, preparation, manufacture, formulation, and/or use of nucleic acid vaccines (NAVs). In particular, described herein are compositions, methods, processes, kits and devices for the selection, design, preparation, manufacture, formulation, and/or use of nucleic acid vaccines, e.g., RNA vaccines and mRNA vaccines.

The present invention provides compositions, e.g., pharmaceutical compositions, comprising one or more onucleic acid vaccines or NAVs.

The NAVs or NAV compositions or the invention may be designed to comprise one or more nucleic acid molecules, e.g., polynucleotides, which encode one or more wild type or engineered proteins, peptides or polypeptides (e.g., antigens). In some embodiments, the nucleic acid molecule, e.g., polynucleotide, is RNA. In some embodiments the nucleic acid molecule, e.g., polynucleotide, is an mRNA. In some embodiments the NAV or NAV composition comprises a nucleic acid (e.g., a RNA polynucleotide) which is chemically modified. In some embodiments the infectious agent from which the antigen is derived or engineered includes, but is not limited to viruses, bacteria, fungi, protozoa, and/or parasites.

In some embodiments are provided methods of inducing, eliciting, boosting or triggering an immune response in a cell, tissue or organism, comprising contacting said cell, tissue or organism with any of the RNAVs described or taught herein.

Aspects of the invention provide nucleic acid vaccines (NAVs) comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, formulated within a cationic lipid nanoparticle. Some aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a

first antigenic polypeptide, formulated in a carrier having a molar ratio of about 20-60% cationic lipid: 5-25% non-cationic lipid: 25-55% sterol; and 0.5-15% PEG-modified lipid.

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 is selected from the group consisting of 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 has a molar ratio of about 20-60% cationic lipid: about 5-25% non-cationic lipid: about 25-55% sterol; and about 0.5-15% PEG-modified lipid. In some embodiments, the cationic lipid nanoparticle comprises a molar ratio of about 50% cationic lipid, about 1.5% PEG-modified lipid, about 38.5% cholesterol and about 10% non-cationic lipid. In some embodiments, the cationic lipid nanoparticle comprises a molar ratio of about 55% cationic lipid, about 2.5% PEG lipid, about 32.5% cholesterol and about 10% 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 nanoparticle has a molar ratio of 50:38.5:10:1.5 of cationic lipid: cholesterol: PEG2000-DMG:DSPC.

In some embodiments, the cationic lipid nanoparticle has a mean diameter of 50-150 nm. In some embodiments, the cationic lipid nanoparticle has a mean diameter of 80-100 nm. In some embodiments, the vaccine includes 1.5 mg/mL of RNA polynucleotide and 35-45 mg/mL lipids. In some embodiments, the NAV includes about 2 mg/mL of RNA polynucleotide and about 40 mg/mL lipids.

In some embodiments, the open reading frame is codon-optimized. In some embodiments, the first antigenic polypeptide is derived from an infectious agent. In some embodiments, the infectious agent is selected from a member of the group consisting of strains of viruses and strains of bacteria. In some embodiments, the one or more RNA polynucleotides encode a further antigenic polypeptide. In some embodiments, the further RNA polynucleotide comprises at least one chemical modification and a codon-optimized open reading frame, said open reading frame encoding an antigenic polypeptide.

In some embodiments, the one or more antigenic polypeptide is selected from those proteins listed in Tables 6-16, Tables 29-30, or fragments thereof. In some embodiments, the open reading frame of the one or more RNA polynucleotides and/or the open reading frame of the second RNA polynucleotide each, independently, encodes an antigenic polypeptide selected from Tables 6-16, Tables 29-30, or fragments thereof. In some embodiments, each of the open reading frame of the one or more RNA polynucleotides is selected from any of the RNA sequences Table 28, or fragments thereof.

In any of the embodiments provided herein, the infectious agent is a strain of virus selected from the group consisting of adenovirus; Herpes simplex, type 1; Herpes simplex, type 2; encephalitis virus, papillomavirus, Varicella-zoster virus; Epstein-barr virus; Human cytomegalovirus; Human herpes virus, type 8; Human papillomavirus; BK virus; JC virus; Smallpox; polio virus; Hepatitis B virus; Human bocavirus; Parvovirus B19; Human astrovirus; Norwalk virus; coxsackievirus; hepatitis A virus; poliovirus; rhinovirus; Severe acute respiratory syndrome virus; Hepatitis C virus; Yellow Fever virus; Dengue virus; West Nile virus; Rubella virus; Hepatitis E virus; Human Immunodeficiency virus (HIV); Influenza virus; Guanarito virus; Junin virus; Lassa virus; Machupo virus; Sabiá virus; Crimean-Congo hemorrhagic fever virus; Ebola virus; Marburg virus; Measles virus; Mumps virus; Parainfluenza virus; Respiratory syncytial virus; Human metapneumovirus; Hendra virus; Nipah virus; Rabies virus; Hepatitis D; Rotavirus; Orbivirus; Coltivirus; Banna virus; Human Enterovirus; Hanta virus; West Nile virus; Middle East Respiratory Syndrome Corona Virus; Japanese encephalitis virus; Vesicular exanthernavirus; and Eastern equine encephalitis.

In some embodiments, the virus is a strain of Influenza A or Influenza B or combinations thereof. In some embodiments, the strain of Influenza A or Influenza B is associated with birds, pigs, horses, dogs, humans or non-human primates. In some embodiments, the antigenic polypeptide encodes a hemagglutinin protein or fragment thereof. In some embodiments, the hemagglutinin protein is H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18, or a fragment thereof. In some embodiments, the hemagglutinin protein does not comprise a head domain (HA1). In some embodiments, the hemagglutinin protein comprises a portion of the head domain (HA1). In some embodiments, the hemagglutinin protein does not comprise a cytoplasmic domain. In some embodiments, the hemagglutinin protein comprises a portion of the cytoplasmic domain. In some embodiments, the truncated hemagglutinin protein. In some embodiments, the truncated hemagglutinin protein comprises a portion of the transmembrane domain. In some embodiments, the amino acid sequence of the hemagglutinin protein or fragment thereof comprises at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% 98%, or 99% identify with any of the amino acid sequences provided in Table 6-14. In some embodiments, the virus is selected from the group consisting of H1N1, H3N2, H7N9, and H10N8. In some embodiments, the antigenic polypeptide is selected from those proteins listed in Tables 6-14, or fragments thereof.

In some embodiments, the infectious agent is a strain of bacteria selected from Tuberculosis (*Mycobacterium tuberculosis*), clindamycin-resistant *Clostridium difficile*, fluoroquinolon-resistant *Clostridium difficile*, methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant *Enterococcus faecalis*, multidrug-resistant *Enterococcus faecium*, multidrug-resistance Pseudomonas aeruginosa, multidrug-resistant *Acinetobacter baumannii*, and vancomycin-resistant Staphylococcus aureus (VRSA). In some embodiments, the bacteria is *Clostridium difficile*.

In some embodiments, the NAV is multivalent. In some embodiments, the open reading frame of the one or more RNA polynucleotides encode at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 antigenic polypeptides. In some embodiments, the open reading frame of the one or more RNA polynucleotides encode at least 10, 15, 20 or 50 antigenic polypeptides. In some embodiments, the open reading frame of the one or more RNA polynucleotides encode 2-10, 10-15, 15-20, 20-50, 50-100 or 100-200 antigenic polypeptides.

In some embodiments, the RNA polynucleotide includes a chemical modification and the chemical modification is selected from any of those listed in Tables 22 and 23. In some embodiments, the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 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 RNA polynucleotide includes a second chemical modification wherein said second chemical modification is selected from any of those listed in Tables 22 and 23. In some embodiments, the combination of first and second chemical modification is selected from those listed in Table 25.

Other aspects provide a NAV comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, formulated within a nanoparticle, wherein the nanoparticle has a mean diameter of 50-200 nm. In some embodiments, the nanoparticle has a polydiversity value of less than 0.4. In some embodiments, the nanoparticle has a net neutral charge at a neutral pH. In some embodiments, the nanoparticle has a mean diameter of 80-100 nm. In some embodiments, the nanoparticle is a cationic lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some embodiments, the cationic lipid is selected from the group consisting of 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).

Other aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein at least 80% 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, the chemical modification is in the 5-position of the uracil. In some embodiments, the chemical modification is a N1-methyl pseudouridine. In some embodiments, the nucleic acid vaccine is formulated within a cationic lipid complex or cationic lipid nanoparticle.

Yet other aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, at least one 5' terminal cap and at least one chemical modification, formulated within a cationic lipid nanoparticle. In some embodiments, the 5' terminal cap is 7mG(5')ppp(5')NlmpNp. In some embodiments, the chemical modification is selected from any of those listed in Tables 22 and 23. In some embodiments, the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 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 RNA polynucleotide further comprises a second chemical modification wherein said second chemical modification is selected from any of those listed in Tables 22 and 23. In some embodiments, the combination of first and second chemical modification is selected from those listed in Table 25.

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 is selected from the group consisting of 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 has a molar ratio of about 20-60% cationic lipid: about 5-25% non-cationic lipid: about 25-55% sterol; and about 0.5-15% PEG-modified lipid.

Some aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a hemagglutinin protein and a pharmaceutically acceptable carrier or excipient, formulated within a cationic lipid nanoparticle. In some embodiments, the hemagglutinin protein is selected from HAL HA7 and HA10. In some embodiments, the RNA polynucleotide does not encode F protein. In some embodiments, the RNA polynucleotide further encodes neuraminidase protein. In some embodiments, the hemagglutinin protein is derived from a strain of Influenza A virus or Influenza B virus or combinations thereof. In some embodiments, the Influenza virus is selected from H1N1, H3N2, H7N9, and H10N8.

In some embodiments, the RNA polynucleotide includes a chemical modification and the chemical modification is selected from any of those listed in Tables 22 and 23. In some embodiments, the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 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 RNA polynucleotide further comprises a second chemical modification wherein said second chemical modification is selected from any of those listed in Tables 22 and 23. In some embodiments, the combination of first and second chemical modification is selected from those listed in Table 25.

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 is selected from the group consisting of 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 has a molar ratio of about 20-60% cationic lipid: about 5-25% non-cationic lipid: about 25-55% sterol; and about 0.5-15% PEG-modified lipid.

In some embodiments, the RNA polynucleotide comprises SEQ ID NOs 197-392. In some embodiments, the RNA polynucleotide comprises a polynucleotide having at least 80% sequence identity to SEQ ID NOs 197-392. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding an amino acid sequence having at least 90% sequence identity to SEQ ID NO 941. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding the amino acid sequence of SEQ ID NO941. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding an amino acid sequence having at least 90% sequence identity to SEQ ID NO 946. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding the amino acid sequence of SEQ ID NO 946. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding an amino acid sequence having at least 90% sequence identity to SEQ ID NO 1022. In some embodiments, the

RNA polynucleotide comprises a polynucleotide encoding the amino acid sequence of SEQ ID NO 1022. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding an amino acid sequence having at least 90% sequence identity to SEQ ID NO 1023. In some embodiments, the RNA polynucleotide comprises a polynucleotide encoding the amino acid sequence of SEQ ID NO 1023. In some embodiments, the RNA polynucleotide comprises SEQ ID NO 1024. In some embodiments, the RNA polynucleotide comprises a polynucleotide having 80-98% sequence identity to SEQ ID NO 1024. In some embodiments, the RNA polynucleotide comprises SEQ ID NO 1025. In some embodiments, the RNA polynucleotide comprises a polynucleotide having 80-98% sequence identity to SEQ ID NO 1025. In some embodiments, the RNA polynucleotide comprises SEQ ID NO 1026. In some embodiments, the RNA polynucleotide comprises a polynucleotide having 80-98% sequence identity to SEQ ID NO 1026. In some embodiments, the RNA polynucleotide comprises SEQ ID NO 1027. In some embodiments, the RNA polynucleotide comprises a polynucleotide having 80-98% sequence identity to SEQ ID NO 1027.

Aspects of the invention provide nucleic acids comprising 80-95% sequence identity to SEQ ID NO 1027 or SEQ ID NO 1026. Other aspects provide a nucleic acid comprising SEQ ID NO: 395.

Yet other aspects provide a method of inducing an antigen specific immune response in a subject comprising administering any of the vaccines described herein to the subject in an effective amount to produce an antigen specific immune response. In some embodiments, the antigen specific immune response comprises a T cell response. In some embodiments, the antigen specific immune response comprises a B cell response. In some embodiments, the method of producing an antigen specific immune response involves a single administration of the vaccine. In some embodiments, the method further comprises administering a booster dose of the vaccine. In some embodiments, the vaccine is administered to the subject by intradermal or intramuscular injection.

In some embodiments, the booster dose of the vaccine is administered to the subject on day twenty one. In some embodiments, a dosage of between 10 ug/kg and 400 ug/kg of the vaccine is administered to the subject. In some embodiments, a dosage of 25 micrograms of the RNA polynucleotide is included in the vaccine administered to the subject. In some embodiments, a dosage of 100 micrograms of the RNA polynucleotide is included in the vaccine administered to the subject. In some embodiments, a dosage of 400 micrograms of the RNA polynucleotide is included in the vaccine administered to the subject. In some embodiments, the RNA polynucleotide accumulates at a 100 fold higher level in the local lymph node in comparison with the distal lymph node.

Aspects provide methods of preventing or treating influenza viral infection comprising administering to a subject any of the vaccines described herein. In some embodiments, the antigen specific immune response comprises a T cell response. In some embodiments, the antigen specific immune response comprises a B cell response. In some embodiments, the method of producing an antigen specific immune response involves a single administration of the vaccine. In some embodiments, the vaccine is administered to the subject by intradermal or intramuscular injection.

Yet other aspects provide methods of vaccinating a subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not coformulated or co-administered with the vaccine. In some embodiments, a dosage of between 10 ug/kg and 400 ug/kg of the nucleic acid vaccine is administered to the subject. In some embodiments, the nucleic acid vaccine is administered to the subject by intradermal or intramuscular injection. In some embodiments, the nucleic acid vaccine is administered to the subject on day zero. In some embodiments, a second dose of the nucleic acid vaccine is administered to the subject on day twenty one.

In some embodiments, a dosage of 25 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 100 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 400 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, the RNA polynucleotide accumulates at a 100 fold higher level in the local lymph node in comparison with the distal lymph node.

Aspects of the invention provide a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and a pharmaceutically acceptable carrier or excipient, wherein an adjuvant is not included in the vaccine. In some embodiments, the stabilization element is a histone stem-loop. In some embodiments, the stabilization element is a nucleic acid sequence having decreased GC content relative to wild type sequence.

Aspects of the invention provide NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide is present in the formulation for in vivo administration to a host, which confers an antibody titer superior to the criterion for seroprotection for the first antigen for an acceptable percentage of human subjects. In some embodiments, the antibody titer is a neutralizing antibody titer.

Also provided are NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide is present in a formulation for in vivo administration to a host for eliciting a longer lasting high antibody titer than an antibody titer elicited by an mRNA vaccine having a stabilizing element or formulated with an adjuvant and encoding the first antigenic polypeptide. In some embodiments, the RNA polynucleotide is formulated to produce a neutralizing antibodies within one week of a single administration. In some embodiments, the adjuvant is selected from a cationic peptide and an immunostimulatory nucleic acid. In some embodiments, the cationic peptide is protamine.

Aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification, the open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide is present in the formulation for in vivo administration to a host such that the level of antigen expression in the host significantly exceeds a level of antigen expression produced by an mRNA vaccine having a stabilizing element or formulated with an adjuvant and encoding the first antigenic polypeptide.

Other aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification, the open reading

frame encoding a first antigenic polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is required for an unmodified mRNA vaccine to produce an equivalent antibody titer. In some embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

Aspects of the invention also provide a unit of use vaccine, comprising between 10 ug and 400 ug of one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification, the open reading frame encoding a first antigenic polypeptide, and a pharmaceutically acceptable carrier or excipient, formulated for delivery to a human subject. In some embodiments, the vaccine further comprises a cationic lipid nanoparticle.

Aspects of the invention provide methods of creating, maintaining or restoring antigenic memory to an influenza strain in an individual or population of individuals comprising administering to said individual or population an antigenic memory booster nucleic acid vaccine comprising (a) at least one RNA polynucleotide, said polynucleotide comprising at least one chemical modification and two or more codon-optimized open reading frames, said open reading frames encoding a set of reference antigenic polypeptides, and (b) optionally a pharmaceutically acceptable carrier or excipient. In some embodiments, the vaccine is administered to the individual via a route selected from the group consisting of intramuscular administration, intradermal administration and subcutaneous administration. In some embodiments, the administering step comprises contacting a muscle tissue of the subject with a device suitable for injection of the composition. In some embodiments, the administering step comprises contacting a muscle tissue of the subject with a device suitable for injection of the composition in combination with electroporation.

Aspects of the invention provide methods of vaccinating a subject comprising administering to the subject a single dosage of between 25 ug/kg and 400 ug/kg of a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide in an effective amount to vaccinate the subject.

Aspects provide NAVs comprising one or more RNA polynucleotides having an open reading frame encoding a hemagglutinin protein fragment, wherein the hemagglutinin protein includes only a portion of at least one of: a head domain (HA1), a cytoplasmic domain, or a transmembrane domain. In some embodiments, the hemagglutinin protein is H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18. In some embodiments, the hemagglutinin protein does not comprise the head domain (HA1). In some embodiments, the hemagglutinin protein does not comprise the cytoplasmic domain. In some embodiments, the truncated hemagglutinin protein does not comprise the transmembrane domain. In some embodiments, the amino acid sequence of the hemagglutinin protein comprises at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% 98%, or 99% identify with any of the amino acid sequences provided in Tables 6-16.

In some embodiments, the vaccine is formulated within a cationic lipid complex or cationic lipid nanoparticle. In some embodiments, the polynucleotide comprises at least one 5' terminal cap and at least one chemical modification.

Aspects also provide any of the vaccines described herein for use in a method of inducing an antigen specific immune response in a subject. In some embodiments, the method comprises administering the vaccine to the subject in an effective amount to produce an antigen specific immune response.

Other aspects provide a use of any of the vaccines described herein in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the vaccine to the subject in an effective amount to produce an antigen specific immune response.

Aspects also provide for any of the vaccines described herein for use in a method of preventing or treating influenza viral infection, the method comprising administering the vaccine to a subject.

Other aspects provide a use any of the vaccines described herein in the manufacture of a medicament for use in a method of preventing or treating influenza viral infection, the method comprising administering the vaccine to a subject.

Other aspects provide nucleic acid vaccines for use in a method of vaccinating a subject wherein the nucleic acid vaccine comprises a first RNA polynucleotide having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not coformulated or co-administered with the vaccine. In some embodiments, the method further comprises administering the vaccine to the subject.

Other aspects provide a use of a nucleic acid vaccine in the manufacture of a medicament for use in a method of vaccinating a subject wherein the nucleic acid vaccine comprises a first RNA polynucleotide having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not coformulated or co-administered with the vaccine. In some embodiments, the method further comprises administering the vaccine to the subject.

Aspects of the invention provide an antigenic memory booster nucleic acid vaccine for use in a method of creating, maintaining or restoring antigenic memory to an influenza strain in an individual or population of individuals. In some embodiments, the antigenic memory booster nucleic acid vaccine comprises (a) at least one RNA polynucleotide, said polynucleotide comprising at least one chemical modification and two or more codon-optimized open reading frames, said open reading frames encoding a set of reference antigenic polypeptides, and (b) optionally a pharmaceutically acceptable carrier or excipient; and wherein the method comprises administering to said individual or population the antigenic memory booster nucleic acid vaccine.

Other aspects provide a use of an antigenic memory booster nucleic acid vaccine in the manufacture of a medicament for use in a method of creating, maintaining or restoring antigenic memory to an influenza strain in an individual or population of individuals, wherein the antigenic memory booster nucleic acid vaccine comprises (a) at least one RNA polynucleotide, said polynucleotide comprising at least one chemical modification and two or more codon-optimized open reading frames, said open reading frames encoding a set of reference antigenic polypeptides, and (b) optionally a pharmaceutically acceptable carrier or excipient; and wherein the method comprises administering to said individual or population the antigenic memory booster nucleic acid vaccine.

Other aspects provide a nucleic acid vaccine for use in a method of vaccinating a subject, wherein the nucleic acid vaccine comprises a first RNA polynucleotide having an open reading frame encoding a first antigenic polypeptide, and wherein the method comprises administering to the

subject a single dosage of between 25 ug/kg and 400 ug/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject.

Other aspects provide a use of a nucleic acid vaccine in the manufacture of a medicament for use in a method of vaccinating a subject, wherein the nucleic acid vaccine comprises a first RNA polynucleotide having an open reading frame encoding a first antigenic polypeptide, and wherein the method comprises administering to the subject a single dosage of between 25 ug/kg and 400 ug/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject.

In some embodiments, the NAV polynucleotides may encode one or more polypeptides of an influenza strain as an antigen. Such antigens include, but are not limited to those antigens encoded by the polynucleotides listed in the Tables presented herein. In the table, the GenBank Accession Number or GI Accession Number represents either the complete or partial CDS of the encoded antigen. The NAV polynucleotides may comprise a region of any of the sequences listed in the tables or entire coding region of the mRNA listed. They may comprise hybrid or chimeric regions, or mimics or variants.

The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

FIGS. **1**A and **1**B are schematics of a polynucleotide construct. FIG. **1**A is a schematic of a polynucleotide construct taught in commonly owned co-pending U.S. patent application Ser. No. 13/791,922 filed Mar. 9, 2013, the contents of which are incorporated herein by reference. FIG. **1**B is a schematic of a linear polynucleotide construct.

FIG. **2** is a schematic of a series of chimeric polynucleotides of the present invention.

FIG. **3** is a schematic of a series of chimeric polynucleotides illustrating various patterns of positional modifications and showing regions analogous to those regions of an mRNA polynucleotide.

FIG. **4** is a schematic of a series of chimeric polynucleotides illustrating various patterns of positional modifications based on Formula I.

FIG. **5** is a is a schematic of a series of chimeric polynucleotides illustrating various patterns of positional modifications based on Formula I and further illustrating a blocked or structured 3' terminus.

FIGS. **6**A and **6**B are schematics of circular constructs of the present invention.

FIGS. **7**A-**7**B are schematics of circular constructs of the present invention.

FIGS. **8**A-**8**B are schematics of a circular constructs of the present invention. FIG. **8**A shows a circular construct comprising at least one sensor region and a spacer region. FIG. **8**B shows a non-coding circular construct.

FIG. **9** is a schematic of a non-coding circular construct of the present invention.

FIG. **10** shows HA neutralization titres of a chemically modified mRNA influenza vaccine in comparison with protein and unmodified mRNA vaccines.

FIG. **11** shows hemagglutinin inhibition titers in mice following vaccination with different doses and formulations of mRNA encoding the hemagglutinin protein of the H1N1 virus.

FIGS. **12**A-**12**D show percent survival of mice after vaccination and challenge with influenza A/PR/8/34 virus. FIG. **12**A shows percent survival at 1 week post challenge. FIG. **12**B shows percent survival at 2 weeks post challenge. FIG. **12**C shows percent survival at 3 weeks post challenge. FIG. **12**D shows percent survival at 4 weeks post challenge.

FIG. **13** shows the mean hemaglutination inhibition titers of mice after vaccination and challenge with influenza A/PR/8/34 virus

FIGS. **14**A-**14**C shows CD4 T cell IFNγ cytokine responses. FIG. **14**A shows IFNγ production upon H1 protein/peptide stimulation. FIG. **14**B shows IFNγ production upon H7 protein/peptide stimulation. FIG. **14**C shows IFNγ production upon PMA+ionomycin stimulation.

FIGS. **15**A-**15**D shows IgG production following H1 and H7 protein/peptide stimulation.

FIG. **16** is a graph showing hemagglutinin inhibition titers (HAI) against H10 following administration of the H10N8/N1-methyl pseudouridine/C0 formulation MC3 vaccine at the indicated dosages.

FIG. **17** is a graph showing hemagglutinin inhibition titers (HAI) against H10 following administration of the H10N8/N1-methyl pseudouridine/C1 formulation MC3 vaccine at the indicated dosages.

FIG. **18** is a graph comparing hemagglutinin inhibition titers (HAI) against H7 following administration of 10 μg/dose of the H7N9/C0 formulation compared to the H7N9/C1 formulation.

FIG. **19** is a graph of the mean hemagglutinin inhibition titers (HAI) in serum samples from cynomolgus monkey at various time points prior to and after administration of the indicated formulations and dosages.

FIG. **20** is a graph showing the H7N9 viral load in ferrets challenged at day 21 after receiving a single immunization.

FIGS. **21**A-**21**D present mouse survival and HAI titers in mice challenged with a lethal dose following administration of a single dose of mRNA NAV encoding H7N9. FIG. **21**A shows survival at day 7 post challenge. FIG. **21**B shows survival at day 21 post challenge. FIG. **21**C shows survival at day 84 post challenge. FIG. **21**D shows HAI titers.

FIGS. **22-1** through **22-51** show an alignment of amino acid sequences of hemagglutinin proteins from influenza A H7N9 strains relative to a consensus sequence. The sequences, from top to bottom, correspond to SEQ ID NOs: 1020, 8, 13, 7, 98, 99, 100, 96, 89, 92, 24, 29, 90, 57, 42, 27, 78, 74, 95, 30, 31, 32, 25, 39, 38, 35, 36, 37, 82, 91, 83, 104, 93, 94, 178, 133, 134, 132, 135, 136, 173, 175, 174, 73, 70, 33, 34, 41, 40, 76, 75, 72, 79, 80, 86, 105, 77, 11, 12, 4, 164, 150, 151, 171, 152, 165, 121, 166, 167, 156, 157, 168, 143, 169, 144, 145, 122, 113, 117, 119, 138, 137, 146, 147, 148, 158, 159, 160, 161, 162, 62, 129, 130, 141, 123, 124, 176, 125, 182, 183, 179, 180, 184, 181, 185, 186, 187, 188, 189, 190, 193, 177, 140, 49, 47, 48, 97, 50, 51, 191, 195, 196, 128, 131, 170, 111, 112, 118, 108, 120, 109, 107, 26, 106, 52, 53, 28, 192, 45, 46, 6, 44, 43, 126, 19, 21, 20, 59, 54, 71, 102, 10, 101, 2, 58, 23, 55, 5, 22, 103, 153, 154, 155, 172, 142, 114, 115, 116, 127, 149, 163, 139, 194, 110, 17, 81, 3, 85, 87, 88, 84, 61, 60, 1, 9, 14, 15, 16, 64, 63, 66, 65, 67, 68, 69, 18, and 56.

FIGS. **23-1** through **23-14** (SEQ ID NOS: 1021, 967-1019) shows an alignment of amino acid sequences of hemagglutinin proteins from influenza A H10N8 strains relative to a consensus sequence.

DETAILED DESCRIPTION

It is of great interest in the fields of therapeutics, diagnostics, reagents and for biological assays to be able design, synthesize and deliver a nucleic acid, e.g., a ribonucleic acid (RNA) for example, a messenger RNA (mRNA) encoding a peptide or polypeptide of interest inside a cell, whether in vitro, in vivo, in situ or ex vivo, such as to effect physiologic outcomes which are beneficial to the cell, tissue or organ and ultimately to an organism. One beneficial outcome is to cause intracellular translation of the nucleic acid and production of at least one encoded peptide or polypeptide of interest.

Of particular interest, is the ability to design, synthesize and deliver a nucleic acid, e.g., a ribonucleic acid (RNA), for example, a messenger RNA (mRNA), which encodes an antigen, e.g., an antigen derived from an infectious microorganism, for the purpose of vaccination.

Described herein are compositions (including pharmaceutical compositions) and methods for the selection, design, preparation, manufacture, formulation, and/or use of nucleic acid vaccines (NAVs) where at least one component of the NAV is a nucleic acid molecule, e.g., a polynucleotide. In particular, described herein are compositions (including pharmaceutical compositions) and methods for the selection, design, preparation, manufacture, formulation, and/or use of nucleic acid vaccines (NAVs) where at least one component of the NAV is a polynucleotide. In particular, described herein are compositions (including pharmaceutical compositions) and methods for the selection, design, preparation, manufacture, formulation, and/or use of nucleic acid vaccines (NAVs) where at least one component of the NAV is a RNA polynucleotide, e.g., an mRNA polynucleotide which encodes an antigen, e.g., an antigen derived from an infectious microorganism. In certain embodiments, the invention relates to compositions (including pharmaceutical compositions) and methods for the selection, design, preparation, manufacture, formulation, and/or use of ribonucleic acid vaccines (RNAVs) where at least one component of the RNAV is a ribonucleic acid molecule, e.g., a mRNA which encodes an antigen, e.g., an antigen derived from an infectious microorganism. As such the present invention is directed, in part, to polynucleotides, specifically in vitro transcription (IVT) polynucleotides, chimeric polynucleotides and/or circular polynucleotides which may function as a vaccine or component of a vaccine.

Also provided are systems, processes, devices and kits for the selection, design and/or utilization of the NAVs described herein.

According to the present invention, the polynucleotides may be modified in a manner as to avoid the deficiencies of or provide improvements over other polynucleotide molecules of the art.

Although attempts have been made to produce functional RNA vaccines, including mRNA vaccines and self-replicating RNA vaccines, the therapeutic efficacy of these RNA vaccines have not yet been fully established. Quite surprisingly, the inventors have discovered a class of formulations for delivering mRNA vaccines in vivo that results in significantly enhanced, and in many respects synergistic, immune responses including enhanced antigen generation and functional antibody production with neutralization capability. These results are achieved even when significantly lower doses of the mRNA are administered in comparison with mRNA doses used in other classes of lipid based formulations. The formulations of the invention have demonstrated significant unexpected in vivo immune responses sufficient to establish the efficacy of functional mRNA vaccines as prophylactic and therapeutic agents.

The invention involves, in some aspects, the surprising finding that lipid nanoparticle (LNP) formulations significantly enhance the effectiveness of mRNA vaccines, including chemically modified and unmodified mRNA vaccines. The efficacy of mRNA vaccines formulated in LNP was examined in vivo using several distinct viral antigens and in a variety of different animal models. The results presented herein demonstrate the unexpected superior efficacy of the mRNA vaccines formulated in LNP over other mRNA vaccines formulated in other lipid based carriers as well as over protein antigens.

In addition to providing an enhanced immune response, the formulations of the invention generate a more rapid immune response following a single dose of antigen than other mRNA or protein based vaccines tested. A study described herein involved intravenous (IV), intramuscular (IM), or intradermal (ID) vaccination of mice, followed by challenge with a lethal dose of virus. In addition to all of the vaccinated animals surviving the lethal dose, significantly stronger early immune responses were observed (anti-viral activity via virus neutralization assay and HA inhibition (HAI)) in comparison to protein antigen and other lipid based formulations (lipoplex). The data demonstrates that as early as 1 week after vaccination two groups of animals receiving a mRNA-LNP formulation (ID or IM) displayed HAI titers over 40, at 60 and 114, respectively. An HAI titer of greater than 40 is deemed sufficient to protect from a lethal challenge of influenza. The rapid response was unexpected, particularly when compared to the response seen with protein antigen and mRNA vaccines formulated in other lipid carriers (lipoplex), which at one week and even at three weeks following vaccination continued to show ineffective HAI titers of less than 40.

At each of the later time points (3 weeks and 5 weeks), the formulations of the invention continued to provide significantly stronger therapeutic responses than did protein antigen. In fact both chemically unmodified and modified mRNA-LNP formulation administered by IV route had enhanced HAI titers with respect to the protein antigen. By week 3, all of the animals receiving an mRNA-LNP formulation by IM or ID administration displayed HAI activity over 40, as compared to protein antigen, which at one week and three weeks continued to show HAI titers of less than 40. By week 5 a mRNA-LNP formulation administered by ID route had a surprising HAI activity of greater than 10,000, in contrast to the HAI titer of around 400 for protein antigen at that time point. Mice receiving a mRNA-LNP formulation also displayed neutralizing activity of 79-250 (IM) and 250 (ID) by microneutralization assay, in comparison to protein antigen, which had undetectable neutralization activity at that time point. By week 5 following vaccination, five of the six LNP formulated groups showed high neutralizing activity between 789 and 24892. In contrast, the mice vaccinated with protein antigen displayed neutralizing activity in only 3 of 5 mice and ranging only between 79 and 250.

The mRNA-LNP formulations of the invention also produced quantitatively and qualitatively better immune responses than did mRNA vaccines formulated in a different lipid carrier (lipoplex). At week 5 the mRNA-lipoplex

vaccine produced HAI titers of 197, in comparison to those achieved by the mRNA-LNP formulations of the invention (HAI titers of 635-10,152). At all other time points and for all of the mRNA-lipoplex vaccines, none of the HAI titers reached the critical level of greater than 40. Additionally, the mRNA-lipoplex vaccines did not result in any detectable neutralizing activity in the microneutralization activity, even as late as five weeks after vaccination.

The data described herein demonstrate that the formulations of the invention produced significant unexpected improvements over both existing protein antigen vaccines and mRNA vaccine formulations, including: 100% rescue from lethal influenza challenge with rapid onset of protective antibody titers after 1 week and high antibody titers, i.e., 50 fold over unmodified mRNA and 20 fold over the protein vaccine.

Additionally, the mRNA-LNP formulations of the invention were superior to other lipid formulations even when the dose of mRNA was significantly lower than in the other lipid formulations. For instance, the data described above was generated using 10 μg of mRNA in the mRNA-LNP formulations in contrast to 80 μg of mRNA in the mRNA-lipoplex formulation.

The formulations of the invention also showed strong efficacy in several non-rodent animal models, including non-human primates. Highly effective vaccination was observed in cynomoglus monkeys and ferrets. Cynomoglus monkeys were vaccinated with various doses of mRNA-LNP formulations (50 μg/dose, 200 μg/dose, 400 μg/dose). Quite surprisingly, the vaccine formulations of the invention at all doses measured significantly reduced viral titers in the lungs of ferrets when exposed to virus just 7 days following single vaccination. Statistically significant increases in antibody titer as measured by HAI and microneutralization were detected as early as 7 days following vaccination and through the entire length of the study (84 days). A single vaccination was able to eliminate all virus in most animals.

The LNP used in the studies described herein has been used previously to deliver siRNA various in 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. 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 invention are demonstrated herein to generate enhanced IgG levels, sufficient for prophylactic and therapeutic methods rather than transient IgM responses.

I. Nucleic Acid Vaccines (NAVs)

Nucleic Acid Vaccines (NAVs) of the present invention comprise one or more polynucleotides, e.g., polynucleotide constructs, which encode one or more wild type or engineered antigens. Exemplary polynucleotides, e.g., polynucleotide constructs, include antigen-encoding RNA polynucleotides, e.g., mRNAs. In exemplary aspect, polynucleotides of the invention, e.g., antigen-encoding RNA polynucleotides, may include at least one chemical modification.

NAV compositions of the invention may comprise other components including, but not limited to, tolerizing agents or adjuvants.

Tolerizing Agent or Composition

Where auto-immunity mediated side effects occur, tolerizing mRNA and/or such as any of those taught for example in U.S. Ser. No. 61/892,556 filed Oct. 18, 2013, and PCT/US2014/61104 filed Oct. 17, 2014, the contents of which are incorporated herein by reference in their entirety) are co-administered with the NAV to induce antigen specific tolerance.

Adjuvants

Adjuvants or immune potentiators, may also be administered with or in combination with one or more NAVs.

In one embodiment, an adjuvant acts as a co-signal to prime T-cells and/or B-cells and/or NK cells as to the existence of an infection.

Advantages of adjuvants include the enhancement of the immunogenicity of antigens, modification of the nature of the immune response, the reduction of the antigen amount needed for a successful immunization, the reduction of the frequency of booster immunizations needed and an improved immune response in elderly and immunocompromised vaccinees. These may be co-administered by any route, e.g., intramusculary, subcutaneous, IV or intradermal injections.

Adjuvants useful in the present invention may include, but are not limited to, natural or synthetic. They may be organic or inorganic.

Aduvants may be selected from any of the classes (1) mineral salts, e.g., aluminium hydroxide and aluminium or calcium phosphate gels; (2) emulsions including: oil emulsions and surfactant based formulations, e.g., microfluidised detergent stabilised oil-in-water emulsion, purified saponin, oil-in-water emulsion, stabilised water-in-oil emulsion; (3) particulate adjuvants, e.g., virosomes (unilamellar liposomal vehicles incorporating influenza haemagglutinin), structured complex of saponins and lipids, polylactide co-glycolide (PLG); (4) microbial derivatives; (5) endogenous human immunomodulators; and/or (6) inert vehicles, such as gold particles; (7) microorganism derived adjuvants; (8) tensoactive compunds; (9) carbohydrates; or combinations thereof.

Adjuvants for DNA nucleic acid vaccines (DNA) have been disclosed in, for example, Kobiyama, et al Vaccines, 2013, 1(3), 278-292, the contents of which are incorporated herein by reference in their entirety. Any of the adjuvants disclosed by Kobiyama may be used in the RNAVs of the present invention.

Other adjuvants which may be utilized in the RNAVs of the present invention include any of those listed on the web-based vaccine adjuvant database, Vaxjo; http://www.violinet.org/vaxjo/and described in for example Sayers, et al., J. Biomedicine and Biotechnology, volume 2012 (2012), Article ID 831486, 13 pages, the content of which is incorporated herein by reference in its entirety.

Selection of appropriate adjuvants will be evident to one of ordinary skill in the art. Specific adjuvants may include, without limitation, cationic liposome-DNA complex JVRS-100, aluminum hydroxide vaccine adjuvant, aluminum phosphate vaccine adjuvant, aluminum potassium sulfate adjuvant, alhydrogel, ISCOM(s)™, Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, CpG DNA Vaccine Adjuvant, Cholera toxin, Cholera toxin B subunit, Liposomes, Saponin Vaccine Adjuvant, DDA Adjuvant, Squalene-based Adjuvants, Etx B subunit Adjuvant, IL-12 Vaccine Adjuvant, LTK63 Vaccine Mutant Adjuvant, TiterMax Gold Adjuvant, Ribi Vaccine Adjuvant, Montanide ISA 720 Adjuvant, *Corynebacterium*-derived P40 Vaccine Adjuvant, MPL™ Adjuvant, AS04, AS02, Lipopolysaccharide

Vaccine Adjuvant, Muramyl Dipeptide Adjuvant, CRL1005, Killed *Corynebacterium* parvum Vaccine Adjuvant, Montanide ISA 51, *Bordetella pertussis* component Vaccine Adjuvant, Cationic Liposomal Vaccine Adjuvant, Adamantylamide Dipeptide Vaccine Adjuvant, Arlacel A, VSA-3 Adjuvant, Aluminum vaccine adjuvant, Polygen Vaccine Adjuvant, Adjumer™, Algal Glucan, Bay R1005, Theramide®, Stearyl Tyrosine, Specol, Algammulin, Avridine®, Calcium Phosphate Gel, CTA1-DD gene fusion protein, DOC/Alum Complex, Gamma Inulin, Gerbu Adjuvant, GM-CSF, GMDP, Recombinant hIFN-gamma/Interferon-g, Interleukin-1β, Interleukin-2, Interleukin-7, Sclavo peptide, Rehydragel LV, Rehydragel HPA, Loxoribine, MF59, MTP-PE Liposomes, Murametide, Murapalmitine, D-Murapalmitine, NAGO, Non-Ionic Surfactant Vesicles, PMMA, Protein Cochleates, QS-21, SPT (Antigen Formulation), nanoemulsion vaccine adjuvant, AS03, Quil-A vaccine adjuvant, RC529 vaccine adjuvant, LTR192G Vaccine Adjuvant, *E. coli* heat-labile toxin, LT, amorphous aluminum hydroxyphosphate sulfate adjuvant, Calcium phosphate vaccine adjuvant, Montanide Incomplete Seppic Adjuvant, Imiquimod, Resiquimod, AF03, Flagellin, Poly(I:C), ISCOMATRIX®, Abisco-100 vaccine adjuvant, Albumin-heparin microparticles vaccine adjuvant, AS-2 vaccine adjuvant, B7-2 vaccine adjuvant, DHEA vaccine adjuvant, Immunoliposomes Containing Antibodies to

Costimulatory Molecules, SAF-1, Sendai Proteoliposomes, Sendai-containing Lipid Matrices, Threonyl muramyl dipeptide (TMDP), Ty Particles vaccine adjuvant, Bupivacaine vaccine adjuvant, DL-PGL (Polyester poly (DL-lactide-co-glycolide)) vaccine adjuvant, IL-15 vaccine adjuvant, LTK72 vaccine adjuvant, MPL-SE vaccine adjuvant, non-toxic mutant E112K of Cholera Toxin mCT-E112K, and/or Matrix-S.

Other adjuvants which may be co-administered with the NAVs of the invention include, but are not limited to interferons, TNF-alpha, TNF-beta, chemokines such as CCL21, eotaxin, HMGB1, SA100-8alpha, GCSF, GMCSF, granulysin, lactoferrin, ovalbumin, CD-40L, CD28 agonists, PD-1, soluble PD1, L1 or L2, or interleukins such as IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-21, IL-23, IL-15, IL-17, and IL-18. These may be administered with the NAV on the same encoded polynucleotide, e.g., polycistronic, or as separate mRNA encoding the adjuvant and antigen.

Valency

NAVs of the present invention may vary in their valency. Valency refers to the number of antigenic components in the NAV or NAV polynucleotide (e.g., RNA polynucleotide) or polypeptide. In some embodiments, the NAVs are monovalent. In some embodiments, the NAVs are divalent. In some embodments the NAVs are trivalent. In some embodiments the NAVs are multi-valent. Multivalent vaccines may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more antigens or antigenic moieties (e.g., antigenic peptides, etc.). The antigenic components of the NAVs may be on a single polynucleotide or on separate polynucleotides.

Therapeutics

The NAVs of the present invention can be used as therapeutic or prophylactic agents. They are provided for use in medicine and/or for the priming of immune effector cells, e.g., stimulate/transfect PBMCs ex vivo and re-infuse the activated cells. For example, a NAV described herein can be administered to a subject, wherein the polynucleotides is translated in vivo to produce an antigen. Provided are compositions, methods, kits, and reagents for diagnosis, treatment or prevention of a disease or condition in humans and other mammals. The active therapeutic agents of the invention include NAVs, cells containing NAVs or polypeptides translated from the polynucleotides contained in said NAVs.

Provided herein are methods of inducing translation of a polypeptide (e.g., antigen or immunogen) in a cell, tissue or organism using the polynucleotides of the NAVs described herein. Such translation can be in vivo, ex vivo, in culture, or in vitro. The cell, tissue or organism is contacted with an effective amount of a composition containing a NAV which contains a polynucletotide that has at least one a translatable region encoding the polypeptide of intereste (e.g., antigen or immunogen).

An "effective amount" of the NAV composition is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the polynucleotide (e.g., size, and extent of modified nucleosides) and other components of the NAV, and other determinants. In general, an effective amount of the NAV composition provides an induced or boosted immune response as a function of antigen production in the cell, preferably more efficient than a composition containing a corresponding unmodified polynucleotide encoding the same antigen. Increased antigen production may be demonstrated by increased cell transfection (i.e., the percentage of cells transfected with the NAV), increased protein translation from the polynucleotide, decreased nucleic acid degradation (as demonstrated, e.g., by increased duration of protein translation from a modified polynucleotide), or altered innate immune response of the host cell.

Aspects of the invention are directed to methods of inducing in vivo translation of a polypeptide antigen in a mammalian subject in need thereof. Therein, an effective amount of a NAV composition containing a polynucleotide that has at least one structural or chemical modification and a translatable region encoding the polypeptide (e.g., antigen or immunogen) is administered to the subject using the delivery methods described herein. The polynucleotide is provided in an amount and under other conditions such that the polynucleotide is translated in the cell. The cell in which the polynucleotide is localized, or the tissue in which the cell is present, may be targeted with one or more than one rounds of NAV administration.

In certain embodiments, the administered NAVs comprising polynucleotides directs production of one or more polypeptides that provide a functional immune system-related activity which is substantially absent in the cell, tissue or organism in which the polypeptide is translated. For example, the missing functional activity may be enzymatic, structural, or gene regulatory in nature. In related embodiments, the administered polynucleotides directs production of one or more polypeptides that increases (e.g., synergistically) a functional activity related to the immune system which is present but substantially deficient in the cell in which the polypeptide is translated.

Additionally, the polypeptide antagonizes, directly or indirectly, the activity of a biological moiety present in, on the surface of, or secreted from the cell. Examples of antagonized biological moieties include lipids (e.g., cholesterol), a lipoprotein (e.g., low density lipoprotein), a nucleic acid, a carbohydrate, a protein toxin such as shiga and tetanus toxins, or a small molecule toxin such as botulinum, cholera, and diphtheria toxins. Additionally, the antagonized biological molecule may be an endogenous protein that exhibits an undesirable activity, such as a cytotoxic or cytostatic activity.

The proteins described herein may be engineered for localization within the cell, potentially within a specific compartment such as the cytoplasms or nucleus, or are engineered for secretion from the cell or translocation to the plasma membrane of the cell.

In some embodiments, polynucleotides of the NAVs and their encoded polypeptides in accordance with the present invention may be used for treatment of any of a variety of diseases, disorders, and/or conditions, including but not limited to viral infections (e.g., influenza, HIV, HCV, RSV), parasitic infentions or bacterial infections.

The subject to whom the therapeutic agent may be administered suffers from or may be at risk of developing a disease, disorder, or deleterious condition. Provided are methods of identifying, diagnosing, and classifying subjects on these bases, which may include clinical diagnosis, biomarker levels, genome-wide association studies (GWAS), and other methods known in the art.

The agents can be administered simultaneously, for example in a combined unit dose (e.g., providing simultaneous delivery of both agents). The agents can also be administered at a specified time interval, such as, but not limited to, an interval of minutes, hours, days or weeks. Generally, the agents may be concurrently bioavailable, e.g., detectable, in the subject. In some embodiments, the agents may be administered essentially simultaneously, for example two unit dosages administered at the same time, or a combined unit dosage of the two agents. In other embodiments, the agents may be delivered in separate unit dosages. The agents may be administered in any order, or as one or more preparations that includes two or more agents. In a preferred embodiment, at least one administration of one of the agents, e.g., the first agent, may be made within minutes, one, two, three, or four hours, or even within one or two days of the other agent, e.g., the second agent. In some embodiments, combinations can achieve synergistic results, e.g., greater than additive results, e.g., at least 25, 50, 75, 100, 200, 300, 400, or 500% greater than additive results.

Modulation of the Immune Response

Activation of the Immune Response

According to the present invention, the NAVs comprising the polynucleotides disclosed herein, e.g., comprising RNA polynucleotides, may act as a single composition as a vaccine. As used herein, a "vaccine" refers to a composition, for example, a substance or preparation that stimulates, induces, causes or improves immunity in an organism, e.g., an animal organism, for example, a mammalian organism (e.g., a human.) Preferably, a vaccine provides immunity against one or more diseases or disorders in the organism, including prophylactic and/or therapeutic immunity. Exemplary vaccines includes one or more agents that resembles an infectious agent, e.g., a disease-causing microorganism, and can be made, for example, from live, attenuated, modified, weakened or killed forms of disease-causing microorganisms, or antigens derived therefrom, including combinations of antigenic components. In exemplary embodiments, a vaccine stimulates, induces causes or improves immunity in an organism or causes or mimics infection in the organism without inducing any disease or disorder. A vaccine introduces an antigen into the tissues, extracellular space or cells of a subject and elicits an immune response, thereby protecting the subject from a particular disease or pathogen infection. The polynucleotides of the present invention may encode an antigen and when the polynucleotides are expressed in cells, a desired immune reponse is achieved.

NAVs 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.

The NAVs of the present invention may also be administered as a second line treatment after the standard first line treatments such as antibiotics and antivirals have failed to induce passive immunity. In this regard, the NAVs of the present invention are useful in settings where resistance to first line treatments has developed and disease persists and induces chronic disease.

NAVs may be administered as part of a treatment regimen for latent bacterial infections, such as MRSA or Clostridium infections. In this embodiment, one or more polynucleotides are administered which ultimately produce proteins which result in the removal or alterations of the protective shield surrounding a bacterium making the bacterium more susceptible to antibiotic treatement.

In one embodiment, a polynucleotide encoding one or several generic or patient-specific antibiotic resistance genes is administered to a patient, e.g. NDM-1. The polunucleotide is then translated to produce the enzyme in vivo. This production may raise an antibody-mediated immune response to the secreted and/or the intracellular enzyme that neutralized the antibiotic resistance and provides the bacteria susceptibal to the clearance by available antibiotics again. Given the broad range of mutantions and variants in antibiotic resistance genes, it would be possible to sequence the specific bacteria genes hosted by the patients and design the exact vaccine for the specific variant in the infected patient.

The use of RNA in or as a vaccine overcomes the disadvantages of conventional genetic vaccination involving incorporating DNA into cells in terms of safeness, feasibility, applicability, and effectiveness to generate immune responses. RNA molecules are considered to be significantly safer than DNA vaccines, as RNAs are more easily degraded. They are cleared quickly out of the organism and cannot integrate into the genome and influence the cell's gene expression in an uncontrollable manner. It is also less likely for RNA vaccines to cause severe side effects like the generation of autoimmune disease or anti-DNA antibodies (Bringmann A. et al., Journal of Biomedicine and Biotechnology (2010), vol. 2010, article ID623687). Transfetion with RNA requires only insertion into the cell's cytoplasm, which is easier to achieve than into the nucleus. Howerver, RNA is susceptible to RNase degradation and other natural decomposition in the cytoplasm of cells.

Various attempts to increase the stability and shelf life of RNA vaccines. US 2005/0032730 to Von Der Mulbe et al. discloses improving the stability of mRNA vaccine compositions by increasing G(guanosine)/C(cytosine) content of the mRNA molecules. U.S. Pat. No. 5,580,859 to Felgner et al. teaches incorporating polynucleotide sequences coding for regulatory proteins that binds to and regulates the stabilities of mRNA. While not wishing to be bound by theory, it is believed that the polynucleotides vaccines (NAVs) of the invention will result in improved stability and therapeutic efficacy due at least in part to the specificity, purity and selectivity of the construct designs.

Additionally, certain modified nucleosides, or combinations thereof, when introduced into the polynucleotides of the NAVs of the invention will activate the innate immune response. Such activating molecules are useful as adjuvants when combined with polypeptides and/or other vaccines. In certain embodiments, the activating molecules contain a translatable region which encodes for a polypeptide sequence useful as a vaccine, thus providing the ability to be a self-adjuvant.

In one embodiment, the polynucleotides of the NAVs of the present invention may be used in the prevention, treatment and diagnosis of diseases and physical disturbances caused by infectious agents. The polynucleotide of the present invention may encode at least one polypeptide of interest (antigen) and may be provided to an individual in order to stimulate the immune system to protect against the disease-causing agents. As a non-limiting example, the biological activity and/or effect from an antigen or infectious agent may be inhibited and/or abolished by providing one or more polynucleotides which have the ability to bind and neutralize the antigen and/or infectious agent.

As a non-limiting example, the polynucleotides encoding an immunogen may be delivered to cells to trigger multiple innate response pathways (see International Pub. No. WO2012006377 and US Patent Publication No. US20130177639; herein incorporated by reference in its entirety). As another non-limiting example, the polynucleotides of the NAVs of the present invention encoding an immunogen may be delivered to a vertebrate in a dose amount large enough to be immunogenic to the vertebrate (see International Pub. No. WO2012006372 and WO2012006369 and US Publication No. US20130149375 and US20130177640; the contents of each of which are herein incorporated by reference in their entirety).

A non-limiting list of infectious diseases that the polynucleotide vaccines may treat includes, viral infectious diseases such as AIDS (HIV), HIV resulting in mycobacterial infection, AIDS related Cacheixa, AIDS related Cytomegalovirus infection, HIV-associated nephropathy, Lipodystrophy, AID related cryptococcal meningitis, AIDS related neutropaenia, Pneumocysitis jiroveci (Pneumocystis carinii) infections, AID related toxoplasmosis, hepatitis A, B, C, D or E, herpes, herpes zoster (chicken pox), German measles (rubella virus), yellow fever, dengue fever etc. (flavi viruses), flu (influenza viruses), haemorrhagic infectious diseases (Marburg or Ebola viruses), bacterial infectious diseases such as Legionnaires' disease (Legionella), gastric ulcer (Helicobacter), cholera (Vibrio), *E. coli* infections, staphylococcal infections, salmonella infections or streptococcal infections, tetanus (*Clostridium tetani*), protozoan infectious diseases (malaria, sleeping sickness, leishmaniasis, toxoplasmosis, i.e. infections caused by plasmodium, trypanosomes, leishmania and toxoplasma), diphtheria, leprosy, measles, pertussis, rabies, tetanus, tuberculosis, typhoid, varicella, diarrheal infections such as Amoebiasis, *Clostridium difficile*-associated diarrhea (CDAD), Cryptosporidiosis, Giardiasis, Cyclosporiasis and Rotaviral gastroenteritis, encephalitis such as Japanese encephalitis, Wester equine encephalitis and Tick-borne encephalitis (TBE), fungal skin diseases such as candidiasis, onychomycosis, Tinea captis/scal ringworm, Tinea corporis/body ringworm, Tinea cruris/jock itch, sporotrichosis and Tinea pedis/Athlete's foot, Meningitis such as Haemophilus influenza type b (Hib), Meningitis, viral, meningococcal infections and pneumococcal infection, neglected tropical diseases such as Argentine haemorrhagic fever, Leishmaniasis, Nematode/roundworm infections, Ross river virus infection and West Nile virus (WNV) disease, Non-HIV STDs such as Trichomoniasis, Human papillomavirus (HPV) infections, sexually transmitted chlamydial diseases, Chancroid and Syphilis, Non-septic bacterial infections such as cellulitis, lyme disease, MRSA infection, pseudomonas, staphylococcal infections, Boutonneuse fever, Leptospirosis, Rheumatic fever, Botulism, Rickettsial disease and Mastoiditis, parasitic infections such as Cysticercosis, Echinococcosis, Trematode/Fluke infections, Trichinellosis, Babesiosis, Hypodermyiasis, Diphyllobothriasis and Trypanosomiasis, respiratory infections such as adenovirus infection, aspergillosis infections, avian (H5N1) influenza, influenza, RSV infections, severe acute respiratory syndrome (SARS), sinusitis, Legionellosis, Coccidioidomycosis and swine (H1N1) influenza, sepsis such as bacteraemia, sepsis/septic shock, sepsis in premature infants, urinary tract infection such as vaginal infections (bacterial), vaginal infections (fungal) and gonococcal infection, viral skin diseases such as B19 parvovirus infections, warts, genital herpes, orofacial herpes, shingles, inner ear infections, fetal cytomegalovirus syndrome, foodborn illnesses such as brucellosis (*Brucella* species), *Clostridium perfringens* (Epsilon toxin), *E. Coli* O157:H7 (*Escherichia coli*), Salmonellosis (*Salmonella* species), Shingellosis (*Shingella*), Vibriosis and Listeriosis, bioterrorism and potential epidemic diseases such as Ebola haemorrhagic fever, Lassa fever, Marburg haemorrhagic fever, plague, Anthrax Nipah virus disease, Hanta virus, Smallpox, Glanders (*Burkholderia mallei*), Melioidosis (*Burkholderia pseudomallei*), Psittacosis (*Chlamydia psittaci*), Q fever (*Coxiella burnetii*), Tularemia (*Fancisella tularensis*), rubella, mumps and polio.

NAVs of the present invention 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 NAVs of the present invention may be utilized to treat and/or prevent influenza infection, i.e. diseases and conditions related to influenza virus infection (seasonal and pandemic).

Symptoms of the influenza infection include dry cough, fever, chills, myalgias progressing to respiratory failure and the risk of secondary bacterial infections (e.g., MRSA). Seasonal influenza is ubiquitous and consists of three principal strains (A [H1N1], A [H3N2], and B), which are covered by the annual vaccine. Pandemic flu occurs because the viruses' unique reassortment ability allowing antigenic shift as well as transfer between avian and swine flu strains. One emerging concern in Southeast Asia is the pandemic potential of several new strains. Such pandemic outbreaks have a high mortality rate with few available treatments. Anti-virals only provide symptomatic relief and must be given in the first 48 hours.

The NAVs of the present invention have superior properties in that they produce much larger antibody titers, produce responses early than commercially available antivirals and may be administered after the critical 48 hour period while retaining efficacy.

While not wishing to be bound by theory, the inventors hypothesize that the NAVs of the invention, as mRNA polynucleotides, are better designed to produce the appropriate protein conformation on translation as the NAVs co-opt natural cellular machinery. Unlike traditional vaccines which are manufactured ex vivo and may trigger unwanted cellular responses, the NAVs are presented to the cellular system in a more native fashion. Adding to the superior effects may also involve the formulations utilized which may neither serve to shield nor traffic the NAVs. According to the present invention, NAVs represent a tailored active vaccine that not only can prevent infection but can limit transmission of influenza.

In some embodiments, the NAVs may be used to prevent pandemic influenza by reacting to emerging new strains with the very rapid NAV-based vaccine production process. In some embodiments, new NAV for treating or prophylactically preventing influenza outbreaks, including for emerging

strains (e.g., H10N8), may be produced in less than six weeks, from the time of antigen identification to available vaccine.

In some embodiments a single injection of a single antigen encoding NAV polynucleotide may provide protection for an entire flu season.

The polynucleotides of the NAVs of the invention are not self-replicating RNA. Self-replicating RNA have been described, for instance in US Pub. No. US20110300205 and International Pub. No. WO2011005799 and WO2013055905, the contents of each of which are herein incorporated by reference in their entirety.

In one embodiment, the polynucleotides of the NAVs of the invention may encode amphipathic and/or immunogenic amphipathic peptides.

In on embodiment, a formulation of the polynucleotides of the NAVs of the invention may further comprise an amphipathic and/or immunogenic amphipathic peptide. As a non-limiting example, the polynucleotides comprising an amphipathic and/or immunogenic amphipathic peptide may be formulated as described in US. Pub. No. US20110250237 and International Pub. Nos. WO2010009277 and WO2010009065; each of which is herein incorporated by reference in their entirety.

In one embodiment, the polynucleotides of the NAVs of the invention may be immunostimultory. As a non-limiting example, the polynucleotides may encode all or a part of a positive-sense or a negative-sense stranded RNA virus genome (see International Pub No. WO2012092569 and US Pub No. US20120177701, each of which is herein incorporated by reference in their entirety). In another non-limiting example, the immunostimultory polynucleotides of the present invention may be formulated with an excipient for administration as described herein and/or known in the art (see International Pub No. WO2012068295 and US Pub No. US20120213812, each of which is herein incorporated by reference in their entirety). The polynucleotides may further comprise a sequence region encoding a cytokine that promotes the immune response, such as a monokine, lymphokine, interleukin or chemokine, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, INF-α, INF-γ, GM-CFS, LT-α, or growth factors such as hGH.

In one embodiment, the response of the vaccine formulated by the methods described herein may be enhanced by the addition of various compounds to induce the therapeutic effect. As a non-limiting example, the vaccine formulation may include a MHC II binding peptide or a peptide having a similar sequence to a MHC II binding peptide (see International Pub Nos. WO2012027365, WO2011031298 and US Pub No. US20120070493, US20110110965, each of which is herein incorporated by reference in their entirety). As another example, the vaccine formulations may comprise modified nicotinic compounds which may generate an antibody response to nicotine residue in a subject (see International Pub No. WO2012061717 and US Pub No. US20120114677, each of which is herein incorporated by reference in their entirety).

In one embodiment, the effective amount of the polynucleotides of the NAVs of the invention provided to a cell, a tissue or a subject may be enough for immune prophylaxis.

In one embodiment, the polynucleotides of the NAVs of the invention may be administrated with other prophylactic or therapeutic compounds. As a non-limiting example, the 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 composition. A booster (or booster vaccine) may be given after an earlier administration of the prophylactic composition. The time of administration between the intial 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 one embodiment, the polynucleotides of the NAVs of the invention may be administered intranasally similar to the administration of live vaccines. In another aspect the polynucleotide may be administered intramuscularly or intradermally similarly to the administration of inactivated vaccines known in the art.

In one embodiment, the NAVs of the invention may be used to protect against and/or prevent the transmission of an emerging or engineered threat which may be known or unknown.

In another embodiment, the NAVs may be formulated by the methods described herein. In one aspect, the formulation may comprise a NAV or polynucleotide which can can have a therapetutic and/or prophylactic effect on more than one disease, disorder or condition. As a non-limiting example, the formulation may comprise polynucleotides encoding an antigen, including but not limited to a protein from an infectious agent such as a viral protein, a parasite protein or a bacterial protein.

In addition, the NAV antibodies of the present invention may be used for research in many applications, such as, but not limited to, identifying and locating intracellular and extracellular proteins, protein interaction, signal pathways and cell biology.

In another embodiment, the NAV may be used in to reduce the risk or inhibit the infection of influenza viruses such as, but not limited to, the highly pathogenic avian influenza virus (such as, but not limited to, H5N1 subtype) infection and human influenza virs (such as, but not limited to, H1N1 subtype and H3N2 subtype) infection. The polynucleotide described herein which may encode any of the protein sequences described in U.S. Pat. No. 8,470,771, the contents of which are herein incorporated by reference in its entirety, may be used in the treatment or to reduce the risk of an influenza infection.

In one embodiment, the NAV may be used to as a vaccine or modulating the immune response against a protein produced by a parasite. Bergmann-Leitner et al. in U.S. Pat. No. 8,470,560, the contents of which are herein incorporated by reference in its entirety, describe a DNA vaccine against the circumsporozoite protein (CSP) of malaria parasites. As a non-limiting example, the polynucleotide may encode the CR2 binding motif of C3d and may be used a vaccine or therapeutic to modulate the immune system against the CSP of malaria parasites.

In one embodiment, the NAV may be used as a vaccine and may further comprise an adjuvant which may enable the vaccine to elicit a higher immune response. As a non-limiting example, the adjuvant could be a sub-micron oil-in-water emulsion which can elicit a higher immune response in human pediatric populations (see e.g., the adjuvanted vaccines described in US Patent Publication No. US20120027813 and U.S. Pat. No. 8,506,966, the contents of each of which are herein incorporated by reference in its entirety).

II. Infectious Agents and Antigens

NAVs of the present invention may be used to protect, treat or cure infection arising from contact with an infectious agent, e.g., microorganism. Infectious agents include bacteria, viruses, fungi, protozoa and parasites.

A. Managing Infection

In one embodiment, provided are methods for treating or preventing a microbial infection (e.g., a bacterial infection) and/or a disease, disorder, or condition associated with a microbial or viral infection, or a symptom thereof, in a subject, by administering a NAV comprising one or more polynucleotide encoding an anti-microbial polypeptide. The administration may be in combination with an anti-microbial agent (e.g., an anti-bacterial agent), e.g., an anti-microbial polypeptide or a small molecule anti-microbial compound described herein. The anti-microbial agents include, but are not limited to, anti-bacterial agents, anti-viral agents, anti-fungal agents, anti-protozoal agents, anti-parasitic agents, and anti-prion agents.

Conditions Associated with Bacterial Infection

Diseases, disorders, or conditions which may be associated with bacterial infections which may be treated using the NAVs of the invention include, but are not limited to one or more of the following: abscesses, actinomycosis, acute prostatitis, *Aeromonas hydrophila*, annual ryegrass toxicity, anthrax, bacillary peliosis, bacteremia, bacterial gastroenteritis, bacterial meningitis, bacterial pneumonia, bacterial vaginosis, bacterium-related cutaneous conditions, bartonellosis, BCG-oma, botryomycosis, botulism, Brazilian purpuric fever, Brodie abscess, brucellosis, Buruli ulcer, campylobacteriosis, caries, Carrion's disease, cat scratch disease, cellulitis, chlamydia infection, cholera, chronic bacterial pro statitis, chronic recurrent multifocal osteomyelitis, clostridial necrotizing enteritis, combined periodontic-endodontic lesions, contagious bovine pleuropneumonia, diphtheria, diphtheritic stomatitis, ehrlichiosis, erysipelas, piglottitis, erysipelas, Fitz-Hugh-urtis syndrome, flea-borne spotted fever, foot rot (infectious pododermatitis), Garre's sclerosing osteomyelitis, Gonorrhea, Granuloma inguinale, human granulocytic anaplasmosis, human monocytotropic ehrlichiosis, hundred days' cough, impetigo, late congenital syphilitic oculopathy, legionellosis, Lemierre's syndrome, leprosy (Hansen's Disease), leptospirosis, listeriosis, Lyme disease, lymphadenitis, melioidosis, meningococcal disease, meningococcal septicaemia, methicillin-resistant Staphylococcus aureus (MRSA) infection, *Mycobacterium avium*-intracellulare (MAI), mycoplasma pneumonia, necrotizing fasciitis, nocardiosis, noma (cancrum oris or gangrenous stomatitis), omphalitis, orbital cellulitis, osteomyelitis, overwhelming post-splenectomy infection (OPSI), ovine brucellosis, pasteurellosis, periorbital cellulitis, pertussis (whooping cough), plague, pneumococcal pneumonia, Pott disease, proctitis, pseudomonas infection, psittacosis, pyaemia, pyomyositis, Q fever, relapsing fever (typhinia), rheumatic fever, Rocky Mountain spotted fever (RMSF), rickettsiosis, salmonellosis, scarlet fever, sepsis, serratia infection, shigellosis, southern tick-associated rash illness, staphylococcal scalded skin syndrome, streptococcal pharyngitis, swimming pool granuloma, swine brucellosis, syphilis, syphilitic aortitis, tetanus, toxic shock syndrome (TSS), trachoma, trench fever, tropical ulcer, tuberculosis, tularemia, typhoid fever, typhus, urogenital tuberculosis, urinary tract infections, vancomycin-resistant Staphylococcus aureus infection, Waterhouse-Friderichsen syndrome, pseudotuberculosis (Yersinia) disease, and yersiniosis

Bacterial Pathogens

The bacterium described herein can be a Gram-positive bacterium or a Gram-negative bacterium. Bacterial pathogens include, but are not limited to, *Acinetobacter baumannii, Bacillus anthracis, Bacillus subtilis, Bordetella pertussis, Borrelia burgdorferi, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydophila psittaci, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, coagulase Negative Staphylococcus, Corynebacterium diphtheria, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, enterotoxigenic Escherichia coli* (ETEC), enteropathogenic *E. coli, E. coli* O157:H7, *Enterobacter sp., Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumoniae, Legionella pneumophila, Leptospira interrogans, Listeria monocytogenes, Moraxella catarralis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitides, Preteus mirabilis, Proteus sps., Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella typhi, Salmonella typhimurium, Serratia marcesens, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Vibrio cholerae*, and *Yersinia pestis.*

Bacterial pathogens may also include bacteria that cause resistant bacterial infections, for example, clindamycin-resistant *Clostridium difficile*, fluoroquinolon-resistant *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Enterococcus faecalis*, multidrug-resistant *Enterococcus faecium*, multidrug-resistance *Pseudomonas aeruginosa*, multidrug-resistant *Acinetobacter baumannii*, and vancomycin-resistant *Staphylococcus aureus* (VRSA).

Antibiotic Combinations

In one embodiment, the NAVs of the present invention, e.g., NAVs comprising one or more antigen-encoding polynucleotides of the present invention, may be administered in conjunction with one or more antibiotics.

Antibacterial Agents

Anti-bacterial agents include, but are not limited to, aminoglycosides (e.g., amikacin (AMIKIN®), gentamicin (GARAMYCIN®), kanamycin (KANTREX®), neomycin (MYCIFRADIN®), netilmicin (NETROMYCIN®), tobramycin (NEBCIN®), Paromomycin (HUMATIN®)), ansamycins (e.g., geldanamycin, herbimycin), carbacephem (e.g., loracarbef (LORABID®), Carbapenems (e.g., ertapenem (INVANZ®), doripenem (DORIBAX®), imipenem/cilastatin (PRIMAXIN®), meropenem (MEREM®), cephalosporins (first generation) (e.g., cefadroxil (DURICEF®), cefazolin (ANCEF®), cefalotin or cefalothin (KEFLIN®), cefalexin (KEFLEX®), cephalosporins (second generation) (e.g., cefaclor (CECLOR®), cefamandole (MANDOL®), cefoxitin (MEFOXIN®), cefprozil (CEFZIL®), cefuroxime (CEFTIN®, ZINNAT®)), cepha-

losporins (third generation) (e.g., cefixime (SUPRAX®), cefdinir (OMNICEF®, CEFDIEL®), cefditoren (SPECTRACEF®), cefoperazone (CEFOBID®), cefotaxime (CLAFORAN®), cefpodoxime (VANTIN®), ceftazidime (FORTAZ®), ceftibuten (CEDAX®), ceftizoxime (CEFIZOX®), ceftriaxone (ROCEPHIN®)), cephalosporins (fourth generation) (e.g., cefepime (MAXIPIME®)), cephalosporins (fifth generation) (e.g., ceftobiprole (ZEFTERA®)), glycopeptides (e.g., teicoplanin (TARGOCID®), vancomycin (VANCOCIN®), telavancin (VIBATIV®)), lincosamides (e.g., clindamycin (CLEOCIN®), lincomycin (LINCOCIN®)), lipopeptide (e.g., daptomycin (CUBICIN®)), macrolides (e.g., azithromycin (ZITHROMAX®, SUMAMED®, ZITROCIN®), clarithromycin (BIAXIN®), dirithromycin (DYNABAC®), erythromycin (ERYTHOCIN®, ERYTHROPED®), roxithromycin, troleandomycin (TAO®), telithromycin (KETEK®), spectinomycin (TROBICIN®)), monobactams (e.g., aztreonam (AZACTAM®)), nitrofurans (e.g., furazolidone (FUROXONE®), nitrofurantoin (MACRODANTIN®, MACROBID®)), penicillins (e.g., amoxicillin (NOVAMOX®, AMOXIL®), ampicillin (PRINCIPEN®), azlocillin, carbenicillin (GEOCILLIN®), cloxacillin (TEGOPEN®), dicloxacillin (DYNAPEN®), flucloxacillin (FLOXAPEN®), mezlocillin (MEZLIN®), methicillin (STAPHCILLIN®), nafcillin (UNIPEN®), oxacillin (PROSTAPHLIN®), penicillin G (PENTIDS®), penicillin V (PEN-VEE-K®), piperacillin (PIPRACIL®), temocillin (NEGABAN®), ticarcillin (TICAR®)), penicillin combinations (e.g., amoxicillin/clavulanate (AUGMENTIN®), ampicillin/sulbactam (UNASYN®), piperacillin/tazobactam (ZOSYN®), ticarcillin/clavulanate (TIMENTIN®)), polypeptides (e.g., bacitracin, colistin (COLYMYCIN-S®), polymyxin B, quinolones (e.g., ciprofloxacin (CIPRO®, CIPROXIN®, CIPROBAY®), enoxacin (PENETREX®), gatifloxacin (TEQUIN®), levofloxacin (LEVAQUIN®), lomefloxacin (MAXAQUIN®), moxifloxacin (AVELOX®), nalidixic acid (NEGGRAM®), norfloxacin (NOROXIN®), ofloxacin (FLOXIN®, OCUFLOX®), trovafloxacin (TROVAN®), grepafloxacin (RAXAR®), sparfloxacin (ZAGAM®), temafloxacin (OMNIFLOX®)), sulfonamides (e.g., mafenide (SULFAMYLON®), sulfonamidochrysoidine (PRONTOSIL®), sulfacetamide (SULAMYD®, BLEPH-10®), sulfadiazine (MICRO-SULFON®), silver sulfadiazine (SILVADENE®), sulfamethizole (THIOSULFIL FORTE®), sulfamethoxazole (GANTANOL®), sulfanilimide, sulfasalazine (AZULFIDINE®), sulfisoxazole (GANTRISIN®), trimethoprim (PROLOPRIM®), TRIMPEX®), trimethoprim-sulfamethoxazole (co-trimoxazole) (TMP-SMX) (BACTRIM®, SEPTRA®)), tetracyclines (e.g., demeclocycline (DECLOMYCIN®), doxycycline (VIBRAMYCIN®), minocycline (MINOCIN®), oxytetracycline (TERRAMYCIN®), tetracycline (SUMYCIN®, ACHROMYCIN® V, STECLIN®)), drugs against mycobacteria (e.g., clofazimine (LAMPRENE®), dapsone (AVLOSULFON®), capreomycin (CAPASTAT®), cycloserine (SEROMYCIN®), ethambutol (MYAMBUTOL®), ethionamide (TRECATOR®), isoniazid (I.N.H.®), pyrazinamide (ALDINAMIDE®), rifampin (RIFADIN®, RIMACTANE®), rifabutin (MYCOBUTIN®), rifapentine (PRIFTIN®), streptomycin), and others (e.g., arsphenamine (SALVARSAN®), chloramphenicol (CHLOROMYCETIN®), fosfomycin (MONUROL®), fusidic acid (FUCIDIN®), linezolid (ZYVOX®), metronidazole (FLAGYL®), mupirocin (BACTROBAN®), platensimycin, quinupristin/dalfopristin (SYNERCID®), rifaximin (XIFAXAN®), thiamphenicol, tigecycline (TIGACYL®), tinidazole (TINDAMAX®, FASIGYN®)).

Conditions Associated with Viral Infection

In another embodiment, provided are methods for treating or preventing a viral infection and/or a disease, disorder, or condition associated with a viral infection, or a symptom thereof, in a subject, by administering aRNAV comprising one or more polynucleotides encoding an anti-viral polypeptide, e.g., an anti-viral polypeptide described herein in combination with an anti-viral agent, e.g., an anti-viral polypeptide or a small molecule anti-viral agent described herein.

Diseases, disorders, or conditions associated with viral infections which may be treated using the NAVs of the invention include, but are not limited to, acute febrile pharyngitis, pharyngoconjunctival fever, epidemic keratoconjunctivitis, infantile gastroenteritis, Coxsackie infections, infectious mononucleosis, Burkitt lymphoma, acute hepatitis, chronic hepatitis, hepatic cirrhosis, hepatocellular carcinoma, primary HSV-1 infection (e.g., gingivostomatitis in children, tonsillitis and pharyngitis in adults, keratoconjunctivitis), latent HSV-1 infection (e.g., herpes labialis and cold sores), primary HSV-2 infection, latent HSV-2 infection, aseptic meningitis, infectious mononucleosis, Cytomegalic inclusion disease, Kaposi sarcoma, multicentric Castleman disease, primary effusion lymphoma, AIDS, influenza, Reye syndrome, measles, postinfectious encephalomyelitis, Mumps, hyperplastic epithelial lesions (e.g., common, flat, plantar and anogenital warts, laryngeal papillomas, epidermodysplasia verruciformis), cervical carcinoma, squamous cell carcinomas, croup, pneumonia, bronchiolitis, common cold, Poliomyelitis, Rabies, bronchiolitis, pneumonia, influenza-like syndrome, severe bronchiolitis with pneumonia, German measles, congenital rubella, Varicella, and herpes zoster.

Viral Pathogens

Examples of viral infectious agents include, but are not limited to, adenovirus; Herpes simplex, type 1; Herpes simplex, type 2; encephalitis virus, papillomavirus, Varicella-zoster virus; Epstein-barr virus; Human cytomegalovirus; Human herpesvirus, type 8; Human papillomavirus; BK virus; JC virus; Smallpox; polio virus, Hepatitis B virus; Human bocavirus; Parvovirus B19; Human astrovirus; Norwalk virus; coxsackievirus; hepatitis A virus; poliovirus; rhinovirus; Severe acute respiratory syndrome virus; Hepatitis C virus; yellow fever virus; dengue virus; West Nile virus; Rubella virus; Hepatitis E virus; Human immunodeficiency virus (HIV); Influenza virus, type A or B; Guanarito virus; Junin virus; Lassa virus; Machupo virus; Sabia virus; Crimean-Congo hemorrhagic fever virus; Ebola virus; Marburg virus; Measles virus; Mumps virus; Parainfluenza virus; Respiratory syncytial virus; Human metapneumovirus; Hendra virus; Nipah virus; Rabies virus; Hepatitis D; Rotavirus; Orbivirus; Coltivirus; Hantavirus, Middle East Respiratory Coronavirus; Chikungunya virus or Banna virus.

Viral pathogens may also include viruses that cause resistant viral infections.

Antiviral Agents

Exemplary anti-viral agents include, but are not limited to, abacavir (ZIAGEN®), abacavir/lamivudine/zidovudine (Trizivir®), aciclovir or acyclovir (CYCLOVIR®, HERPEX®, ACIVIR®, ACIVIRAX®, ZOVIRAX®, ZOVIR®), adefovir (Preveon®, Hepsera®), amantadine (SYMMETREL®), amprenavir (AGENERASE®), ampligen, arbidol, atazanavir (REYATAZ®), boceprevir, cidofovir, darunavir (PREZISTA®), delavirdine (RESCRIPTOR®), didanosine (VIDEX®), docosanol (ABREVA®), edoxudine, efavirenz (SUSTIVA®, STOCRIN®), emtricitabine (EMTRIVA®),

emtricitabine/tenofovir/efavirenz (ATRIPLA®), enfuvirtide (FUZEON®), entecavir (BARACLUDE®, ENNAVIR®), famciclovir (FAMVIR®), fomivirsen (VITRAVENE®), fosamprenavir (LEXIVA®, TELZIR®), foscarnet (FOSCAVIR®), fosfonet, ganciclovir (CYTOVENE®, CYMEVENE®, VITRASERT®), GS 9137 (ELVITEGRAVIR®), imiquimod (ALDARA®, ZYCLARA®, BESELNA®), indinavir (CRIXIVAN®), inosine, inosine pranobex (IMUNOVIR®), interferon type I, interferon type II, interferon type III, kutapressin (NEXAVIR®), lamivudine (ZEFFIX®, HEPTOVIR®, EPIVIR®), lamivudine/zidovudine (COMBIVIR®), lopinavir, loviride, maraviroc (SELZENTRY®, CELSENTRI®), methisazone, MK-2048, moroxydine, nelfinavir (VIRACEPT®), nevirapine (VIRAMUNE®), oseltamivir (TAMIFLU®), peginterferon alfa-2a (PEGASYS®), penciclovir (DENAVIR®), peramivir, pleconaril, podophyllotoxin (CONDYLOX®), raltegravir (ISENTRESS®), ribavirin (COPEGUs®, REBETOL®, RIBASPHERE®, VILONA® AND VIRAZOLE®), rimantadine (FLUMADINE®), ritonavir (NORVIR®), pyramidine, saquinavir (INVIRASE®, FORTOVASE®), stavudine, tea tree oil (melaleuca oil), tenofovir (VIREAD®), tenofovir/emtricitabine (TRUVADA®), tipranavir (APTIVUS®), trifluridine (VIROPTIC®), tromantadine (VIRUMERZ®), valaciclovir (VALTREX®), valganciclovir (VALCYTE®), vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir (RELENZA®), and zidovudine (azidothymidine (AZT), RETROVIR®, RETROVIS®).

Conditions Associated with Fungal Infections

Diseases, disorders, or conditions associated with fungal infections which may be treated using the NAVs of the invention include, but are not limited to, aspergilloses, blastomycosis, candidasis, coccidioidomycosis, cryptococcosis, histoplasmosis, mycetomas, paracoccidioidomycosis, and tinea pedis. Furthermore, persons with immuno-deficiencies are particularly susceptible to disease by fungal genera such as Aspergillus, Candida, Cryptoccocus, Histoplasma, and Pneumocystis. Other fungi can attack eyes, nails, hair, and especially skin, the so-called dermatophytic fungi and keratinophilic fungi, and cause a variety of conditions, of which ringworms such as athlete's foot are common. Fungal spores are also a major cause of allergies, and a wide range of fungi from different taxonomic groups can evoke allergic reactions in some people.

Fungal Pathogens

Fungal pathogens include, but are not limited to, Ascomycota (e.g., *Fusarium oxysporum, Pneumocystis jirovecii, Aspergillus* spp., *Coccidioides immitis/posadasii, Candida albicans*), Basidiomycota (e.g., *Filobasidiella neoformans, Trichosporon*), Microsporidia (e.g., *Encephalitozoon cuniculi, Enterocytozoon bieneusi*), and Mucoromycotina (e.g., *Mucor circinelloides, Rhizopus oryzae, Lichtheimia corymbifera*).

Anti Fungal Agents

Exemplary anti-fungal agents include, but are not limited to, polyene antifungals (e.g., natamycin, rimocidin, filipin, nystatin, amphotericin B, candicin, hamycin), imidazole antifungals (e.g., miconazole (MICATIN®, DAKTARIN®), ketoconazole (NIZORAL®, FUNGORAL®, SEBIZOLE®), clotrimazole (LOTRIMIN®, LOTRIMIN® AF, CANESTEN®), econazole, omoconazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole (ERTACZO®), sulconazole, tioconazole), triazole antifungals (e.g., albaconazole fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole), thiazole antifungals (e.g., abafungin), allylamines (e.g., terbinafine (LAMISIL®), naftifine (NAFTIN®), butenafine (LOTRIMIN® Ultra)), echinocandins (e.g., anidulafungin, caspofungin, micafungin), and others (e.g., polygodial, benzoic acid, ciclopirox, tolnaftate (TINACTIN®, DESENEX®, AFTATE®), undecylenic acid, flucytosine or 5-fluorocytosine, griseofulvin, haloprogin, sodium bicarbonate, allicin).

Conditions Associated with Protozoal Infection

Diseases, disorders, or conditions associated with protozoal infections which may be treated using the NAVs of the invention include, but are not limited to, amoebiasis, giardiasis, trichomoniasis, African Sleeping Sickness, American Sleeping Sickness, leishmaniasis (Kala-Azar), balantidiasis, toxoplasmosis, malaria, acanthamoeba keratitis, and babesiosis.

Protozoan Pathogens

Protozoal pathogens include, but are not limited to, *Entamoeba histolytica, Giardia lambila, Trichomonas vaginalis, Trypanosoma brucei, T. cruzi, Leishmania donovani, Balantidium coli, Toxoplasma gondii, Plasmodium* spp., and *Babesia microti.*

Anti-Protozoan Agents

Exemplary anti-protozoal agents include, but are not limited to, eflornithine, furazolidone (FUROXONE®, DEPENDAL-M®), melarsoprol, metronidazole (FLAGYL®), ornidazole, paromomycin sulfate (HUMATIN®), pentamidine, pyrimethamine (DARAPRIM®), and tinidazole (TINDAMAX®, FASIGYN®).

Conditions Associated with Parasitic Infection

Diseases, disorders, or conditions associated with parasitic infections which may be treated using the NAVs of the invention include, but are not limited to, acanthamoeba keratitis, amoebiasis, ascariasis, babesiosis, balantidiasis, baylisascariasis, chagas disease, clonorchiasis, cochliomyia, cryptosporidiosis, diphyllobothriasis, dracunculiasis, echinococcosis, elephantiasis, enterobiasis, fascioliasis, fasciolopsiasis, filariasis, giardiasis, gnathostomiasis, hymenolepiasis, isosporiasis, katayama fever, leishmaniasis, lyme disease, malaria, metagonimiasis, myiasis, onchocerciasis, pediculosis, scabies, schistosomiasis, sleeping sickness, strongyloidiasis, taeniasis, toxocariasis, toxoplasmosis, trichinosis, and trichuriasis.

Parasitic Pathogens

Parasitic pathogens include, but are not limited to, *Acanthamoeba, Anisakis, Ascaris lumbricoides*, botfly, *Balantidium coli*, bedbug, *Cestoda*, chiggers, *Cochliomyia hominivorax, Entamoeba histolytica, Fasciola hepatica, Giardia lamblia, hookworm, Leishmania, Linguatula serrata*, liver fluke, Loa boa, *Paragonimus*, pinworm, *Plasmodium falciparum, Schistosoma, Strongyloides stercoralis*, mite, tapeworm, *Toxoplasma gondii, Trypanosoma*, whipworm, *Wuchereria bancrofti.*

Anti-Parasitic Agents

Exemplary anti-parasitic agents include, but are not limited to, antinematodes (e.g., mebendazole, pyrantel pamoate, thiabendazole, diethylcarbamazine, ivermectin), anticestodes (e.g., niclosamide, praziquantel, albendazole), antitrematodes (e.g., praziquantel), antiamoebics (e.g., rifampin, amphotericin B), and antiprotozoals (e.g., melarsoprol, eflornithine, metronidazole, tinidazole).

B. Therapeutic Settings and/or Situations

NAVs of the present invention may be utilized in various settings depending on the prevalence of the infection or the degree or level of unmet medical need. Some applications of the NAVs of the invention are outlined in Table 1.

TABLE 1

Infectious Agents by prevalence and Medical Need

	Unmet Need (Infectious Agent Target)		
Prevalence	Short term benign Long term sequelae	Short term morbidity but some treatment	Short term mortality; no treatment available
Ubiquitous	HPV, HCV, UTRIs	Dengue, Chikungunya, ETEC and GI bacteria and *S. Pneumo* PNA	seasonal and pandemic influenza, MRSA and TB
At risk populations	VZV, Lyme and *Chlamydia*, *N. gonorrhea* and HSV	Noroviruses, HEV, CMV, HIV and *N. meningitis*	*Klebsiella*, *Pseudomonas*, Rabies and *C. difficile*
Rare disease	—	VEV	toxin-mediated diseases, hantavirus, arborviruses such as JE, WNV and EEE

Certain abbreviations include: HPV—Human Papillomavirus; HCV—Hepatitis C Virus; HEV—Human Enterovirus; MERS-CoV: Middle East Respiratory Syndrom Corona Virus; VZV—Varicella-zoster Virus; MRSA—Methicillin-resistant *Staph areus*; TB—tuberculosis; WNV—West Nile Virus; VEV—vesicular exanthema virus; EEE—Eastern equine encephalitis, JE—Japanese encephalitis, ETEC—Enterotoxigenic *E. coli*.

Influenza (Seasonal and Pandemic)

Symptoms of the flu include dry cough, fever, chills, myalgias progressing to respiratory failure and the risk of secondary bacterial infections (e.g., MRSA). Seasonal influenza is ubiquitous and consists of three principal strains (A [H1N1], A [H3N2], and B), which are covered by the annual vaccine. Pandemic flu occurs because the viruses' unique reassortment ability allowing antigenic shift as well as transfer between avian and swine flu strains. One emerging concern in Southeast Asia is the pandemic potential of several new strains. Such pandemic outbreaks have a high mortality rate with few available treatments. Anti-virals only provide symptomatic relief and must be given in the first 48 hours.

The NAVs of the present invention have superior properties in that they produce much larger antibody titers, produce responses early than commercially available anti-virals and may be administered after the critical 48 hour period while retaining efficacy.

While not wishing to be bound by theory, the inventors hypothesize that the NAVs of the invention, as mRNA polynucleotides, are better designed to produce the appropriate protein conformation on translation as the NAVs co-opt natural cellular machinery. Unlike tranditional vaccines which are manufactured ex vivo and may trigger unwanted cellular responses, the NAVs are presented to the cellular system in amore native fashion. Adding to the superior effects may also involve the formulations utilized which may enither serve to shield or traffic the NAVs.

According to the present invention, NAVs represent a tailored active vaccine that not only can prevent infection but can limit transmission of influenza.

In some embodiments, the NAVs may be used to prevent pandemic influenza by reacting to emerging new strains with the very rapid NAV-based vaccine production process. In some embodiments, new NAV for treating or prophylactically preventing influenza outbreaks, including for emerging strains (e.g., H7N9 and H10N8), may be produced in less than six weeks, from the time of antigen identification to available vaccine.

In some embodiments a single injection of a single antigen encoding NAV polynucleotide may provide protection for an entire flu season.

Influenza: Maintenance of Antigenic Memory

The NAV compositions of the present invention may also be used to maintain or restore antigenic memory in a subject or population as part of a vaccination plan.

With the speed and versatility of the NAV technology of the present invention, it is now possible to create a vaccination plan that spans both temporal and viral strain space.

In one embodiment, NAV compositions may be created which include polynucleotides that encode one or more flu year antigens. As used herein a flu year antigen is an antigen which is selected from a strain of influenza used as a component of a flu vaccine from a particular year. For example, the influenza A strain, A/Port Chalmers/1/1973 (H3N2)-like virus, represents one strain component of the Northern Hemisphere vaccine from 1974-1975.

According to the present invention, a vaccination scheme or plan is developed which allows for not only ongoing vaccination in the current year but antigenic memory booster vaccinations across years, strains, or groups thereof to establish and maintain antigenic memory in a population. In this manner, a population is less likely to succumb to any pandemic or outbreak involving recurrence of older strains or the appearance of antigens from older strains.

Any combination of prior vaccine component strains utilized to create or design an antigenic memory booster vaccine is referred to here as a reference set.

In one embodiment, NAVs which are antigenic memory booster vaccines are administered to boost antigenic memory across a time period of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50 or more years.

In one embodiment, NAVs which are antigenic memory booster vaccines are administered to boost antigenic memory for alternating historic years including every other year from the past vaccine component strains relative to a current year. In some embodiments the selection of the vaccine components can be from every 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th} or more years.

In one embodiment, NAVs which are antigenic memory booster vaccines are administered to boost antigenic memory over ten year periods.

In some embodiments NAVs which are antigenic memory booster vaccines are administered to boost antigenic memory and are selected from a number of influenza type A strains as a first selection combined with a selection from a number of influenza type B strains or other strains listed

herein. The number of selections of type A or type B may be independently, 1, 2, 1, 4, 5, 6, 7, 8, 9, 10 or more.

In all cases, the antigenic memory booster vaccine strains for antigen encoding in the NAVs may be selected from either the Northern or Southern hemisphere vaccine components independently.

In some embodiments, the NAV booster vaccine may be used in a population either once or periodically to create herd immunity. Such immunity is present when greater than 30% of a population is protected.

The components or strains of influenza which may be utilized in the antigenic memory booster vaccines include, but are not limited to, those in Tables 2-5.

TABLE 2

Influenza vaccine components by year

Northern hemisphere	H1N1	H3N2	B-strain	additional B-strain for QIV
1974-1975	N/A	A/Port Chalmers/1/ 1973(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1975-1976	A/Scotland/840/ 74-like virus H1N1	A/Port Chalmers/1/ 1973(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1976-1977	N/A	A/Victoria/3/ 75(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1977-1978	N/A	A/Victoria/3/ 75(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1978-1979	A/USSR/90/ 77(H1N1)-like virus	A/Texas/1/ 77(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1979-1980	A/USSR/90/ 77(H1N1)-like virus	A/Texas/1/ 77(H3N2)-like virus	N/A	N/A
1980-1981	A/Brazil/11/ 78(H1N1)-like virus	A/Bangkok/01/1979 (H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1981-1982	A/Brazil/11/ 78(H1N1)-like virus	A/Bangkok/01/1979 (H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1982-1983	A/Brazil/11/ 78(H1N1)-like virus	A/Bangkok/01/1979 (H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1983-1984	A/Brazil/11/ 78(H1N1)-like virus	A/Philippines/2/ 82(H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1984-1985	A/Chile/1/ 83(H1N1)-like virus	A/Philippines/2/ 82(H3N2)-like virus	B/USSR/100/ 83-like virus	N/A
1985-1986	A/Chile/1/ 83(H1N1)-like virus	A/Philippines/2/ 82(H3N2)-like virus	B/USSR/100/ 83-like virus	N/A
1986-1987	A/Chile/1/ 83(H1N1)-like virus	A/Christchurch/4/ 1985(H3N2)-like virus and A/Mississippi/1/ 85(H3N2)-like virus	B/Ann Arbor/1/ 86-like virus	N/A
1987-1988	A/Singapore/6/1986 (H1N1)-like virus	A/Leningrad/360/ 1986(H3N2)-like strain	N/A	N/A
1988-1989	A/Singapore/6/1986 (H1N1)-like virus	A/Sichuan/02/ 87(H3N2)-like virus	B/Beijing/1/ 87-like rivus	N/A
1989-1990	A/Singapore/6/1986 (H1N1)-like virus	A/Shanghai/11/ 87(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1990-1991	A/Singapore/6/1986 (H1N1)-like virus	A/Guizhou/54/ 89(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1991-1992	A/Singapore/6/1986 (H1N1)-like virus	A/Beijing/353/ 89(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1992-1993	N/A	A/Beijing/353/ 89(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1993-1994	A/Singapore/6/1986 (H1N1)-like virus	A/Beijing/32/ 92(H3N2)-like virus	B/Panama/45/ 90-like virus	N/A
1994-1995	A/Singapore/6/1986 (H1N1)-like virus	A/Shangdong/9/ 93(H3N2)-like virus	B/Panama/45/ 90-like virus	N/A
1995-1996	A/Singapore/6/1986 (H1N1)-like virus	A/Johannesburg/33/ 94(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A
1996-1997	A/Singapore/6/1986 (H1N1)-like virus	A/Wuhan/359/ 95(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A
1997-1998	A/Bayern/7/ 95(H1N1)-like virus	A/Wuhan/359/ 95(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A

TABLE 3

Influenza vaccine components by year-Southern Hemisphere

Southern Hemisphere	H1N1	H3N2	B-strain	additional B-strain for QIV
1975	N/A	A/Port Chalmers/1/ 1973(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1976	A/Scotland/840/ 74-like virus (H1N1)	A/Port Chalmers/1/ 1973(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1977	N/A	A/Victoria/3/ 75(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1978	N/A	A/Victoria/3/ 75(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1979	A/USSR/90/ 77(H1N1)-like virus	A/Texas/1/ 77(H3N2)-like virus	B/HongKong/05/ 1972-like virus	N/A
1980	A/USSR/90/ 77(H1N1)-like virus	A/Texas/1/ 77(H3N2)-like virus	N/A	N/A
1981	A/Brazil/11/ 78(H1N1)-like virus	A/Bangkok/01/1979 (H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1982	A/Brazil/11/ 78(H1N1)-like virus	A/Bangkok/01/1979 (H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1983	A/Brazil/11/ 78(H1N1)-like virus	A/Bangkok/01/1979 (H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1984	A/Brazil/11/ 78(H1N1)-like virus	A/Philippines/2/ 82(H3N2)-like virus	B/Singapore/222/ 79-like virus	N/A
1985	A/Chile/1/ 83(H1N1)-like virus	A/Philippines/2/ 82(H3N2)-like virus	B/USSR/100/ 83-like virus	N/A
1986	A/Chile/1/ 83(H1N1)-like virus	A/Philippines/2/ 82(H3N2)-like virus	B/USSR/100/ 83-like virus	N/A
1987	A/Chile/1/ 83(H1N1)-like virus	A/Christchurch/4/ 1985(H3N2)-like virus and A/Mississippi/1/ 85(H3N2)-like virus	B/Ann Arbor/1/ 86-like virus	N/A
1988	A/Singapore/6/1986 (H1N1)-like virus	A/Leningrad/360/ 1986(H3N2)-like virus	N/A	N/A
1989	A/Singapore/6/1986 (H1N1)-like virus	A/Sichuan/02/ 87(H3N2)-like virus	B/Beijing/1/ 87-like virus	N/A
1990	A/Singapore/6/1986 (H1N1)-like virus	A/Shanghai/11/ 87(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1991	A/Singapore/6/1986 (H1N1)-like virus	A/Guizhou/54/ 89(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1992	A/Singapore/6/1986 (H1N1)-like virus	A/Beijing/353/ 89(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1993	A/Singapore/6/1986 (H1N1)-like virus	A/Beijing/353/ 89(H3N2)-like virus	B/Yamagata/16/ 88-like virus	N/A
1994	A/Singapore/6/1986 (H1N1)-like virus	A/Beijing/32/ 92(H3N2)-like virus	B/Panama/45/ 90-like virus	N/A
1995	A/Singapore/6/1986 (H1N1)-like virus	A/Shangdong/9/ 93(H3N2)-like virus	B/Panama/45/ 90-like virus	N/A
1996	A/Singapore/6/1986 (H1N1)-like virus	A/Johannesburg/33/ 94(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A
1997	A/Singapore/6/1986 (H1N1)-like virus	A/Wuhan/359/ 95(H3N2)-like virus	B/Beijing/184/ 93-like strain	N/A
1998	A/Bayern/7/ 95(H1N1)-like virus	A/Wuhan/359/ 95(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A
1999	A/Beijing/262/ 95(H1N1)-like virus	A/Sydney/5/ 97(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A

TABLE 4

Influenza Vaccine components by year-Northern Hemisphere

Northern hemisphere	H1N1	H3N2	B-strain	additional B-strain for QIV
November 1998-April 1999	A/Beijing/262/ 95(H1N1)-like virus	A/Sydney/5/ 97(H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A
November 1999-April 2000	A/Beijing/262/95 (H1N1)-like virus	A/Sydney/5/97 (H3N2)-like virus	B/Beijing/184/ 93-like virus or B/Shangdong/7/ 97-like virus	N/A
2000-2001	A/New Caledonia/20/ 99(H1N1)-like virus	A/Moscow/10/99 (H3N2)-like virus	B/Beijing/184/ 93-like virus	N/A

TABLE 4-continued

Influenza Vaccine components by year-Northern Hemisphere

Northern hemisphere	H1N1	H3N2	B-strain	additional B-strain for QIV
2001-2002	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Sichuan/379/99-like virus	N/A
2002-2003	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Hong Kong/330/2001-like virus	N/A
2003-2004	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Hong Kong/330/2001-like virus	N/A
2004-2005	A/New Caledonia/20/99(H1N1)-like virus	A/Fujian/411/2002(H3N2)-like virus	B/Shanghai/361/2002-like virus	N/A
2005-2006	A/New Caledonia/20/99(H1N1)-like virus	A/California/7/2004(H3N2)-like virus	B/Shanghai/361/2002-like virus	N/A
2006-2007	A/New Caledonia/20/99(H1N1)-like	A/Wisconsin/67/2005(H3N2)-like virus	B/Malaysia/2506/2004-like virus	N/A
2007-2008	A/Solomon Islands/3/2006(H1N1)-like virus	A/Wisconsin/67/2005(H3N2)-like virus	B/Malaysia/2506/2004-like virus	N/A
2008-2009	A/Brisbane/59/2007(H1N1)-like virus	A/Brisbane/10/2007(H3N2)-like virus	B/Florida/4/2006-like virus	N/A
2009-2010	A/Brisbane/59/2007(H1N1)-like virus	A/Brisbane/10/2007(H3N2)-like virus	B/Brisbane/60/2008-like virus	N/A
2010-2011	A/California/7/2009(H1N1)-like virus	A/Perth/16/2009(H3N2)-like virus	B/Brisbane/60/2008-like virus	N/A
2011-2012	A/California/7/2009(H1N1)-like virus	A/Perth/16/2009(H3N2)-like virus	B/Brisbane/60/2008-like virus	N/A
2012-2013	A/California/7/2009(H1N1)pdm09-like virus	A/Victoria/361/2011(H3N2)-like virus	B/Wisconsin/1/2010-like virus	B/Brisbane/60/2008-like virus
2013-2014	A/California/7/2009(H1N1)pdm09-like virus	A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011	B/Massachusetts/2/2012-like virus	B/Brisbane/60/2008-like virus
2014-2015	A/California/7/2009(H1N1)pdm09-like virus	A/Texas/50/2012(H3N2)-like virus	B/Massachusetts/2/2012-like virus	B/Brisbane/60/2008-like virus

TABLE 5

Influenza Vaccine components by year-Southern Hemisphere

Southern hemisphere	H1N1	H3N2	B-strain	additional B-strain for QIV
1999	A/Beijing/262/95(H1N1)-like virus	A/Sydney/5/97(H3N2)-like virus	B/Beijing/184/93-like virus	N/A
May-October 2000	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Beijing/184/93-like virus or B/Shangdong/7/97-like virus	N/A
May-October 2001	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Sichuan/379/99-like virus	N/A
2002	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Sichuan/379/99-like virus	N/A
2003	A/New Caledonia/20/99(H1N1)-like virus	A/Moscow/10/99(H3N2)-like virus	B/Hong Kong/330/2001-like virus	N/A
2004	A/New Caledonia/20/99(H1N1)-like virus	A/Fujian/411/2002(H3N2)-like virus	B/Hong Kong/330/2001-like virus	N/A
2005	A/New Caledonia/20/99(H1N1)-like virus	A/Wellington/1/2004(H3N2)-like virus	B/Shanghai/361/2002-like virus	N/A
2006	A/New Caledonia/20/99(H1N1)-like virus	A/California/7/2004(H3N2)-like virus	B/Malaysia/2506/2004-like virus	N/A
2007	A/New Caledonia/20/99(H1N1)-like	A/Wisconsin/67/2005 (H3N2)-like virus	B/Malaysia/2506/2004-like virus	N/A
2008	A/Solomon Islands/3/2006(H1N1)-like virus	A/Brisbane/10/2007(H3N2)-like virus	B/Florida/4/2006-like virus	N/A
2009	A/Brisbane/59/2007(H1N1)-like virus	A/Brisbane/10/2007(H3N2)-like virus	B/Florida/4/2006-like virus	N/A
2010	A/California/7/2009(H1N1)-like virus	A/Perth/16/2009(H3N2)-like virus	B/Brisbane/60/2008-like virus	N/A
2011	A/California/7/2009(H1N1)-like virus	A/Perth/16/2009(H3N2)-like virus	B/Brisbane/60/2008-like virus	N/A
2013	A/California/7/2009(H1N1)pdm09 like virus	A/Perth/16/2009(H3N2)-like virus	B/Brisbane/60/2008-like virus	N/A