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(54) Title: LYME DISEASE VACCINES AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure relates, in part, to isolated nucleic acids encoding a recombinant virus comprising outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof, and recombinant viruses and/or vectors comprising the same. The present disclosure further relates to vaccines comprising the isolated nucleic acids, recombinant viruses, and/or vectors of the present disclosure, and methods of use thereof for generating immunity against infection by *Borrelia* bacteria, and/or for the treatment, prevention, and/or amelioration of Lyme disease.

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TITLE OF THE INVENTION

Lyme Disease Vaccines and Methods of Use Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 63/472,963, filed June 14, 2023, which is incorporated herein by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

The XML file named "205961_7101WO1_SequenceListing.xml" created on June 10, 2024, comprising 8.98 KB, is hereby incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Project Number 5R01AI154542-03 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Outer surface protein A (OspA) is a protein found on the outer surface of the bacterium *Borrelia burgdorferi*, which is the causative agent of Lyme disease. OspA is considered important in the transmission of the bacterium from the tick vector to humans. It is believed that when a tick feeds on an infected host, OspA is expressed on the surface of the bacterium, allowing the bacterium to adhere to the tick's midgut, with subsequent transmission of the bacterium as the tick feeds on a new host (*e.g.*, human).

There is a need in the art for effective compositions and methods suitable for generating immunity against infection by *Borrelia* bacteria, and/or for the treatment, prevention, and/or amelioration of diseases or disorders caused by or associated with infection by *Borrelia* bacteria such as, for example, Lyme disease. The present disclosure addresses these and other needs.

BRIEF SUMMARY

In one aspect, the disclosure provides an isolated nucleic acid encoding a recombinant virus comprising outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

In certain embodiments, the isolated nucleic acid comprises a nucleic acid sequence encoding at least a portion of the genome of a RABV, a fragment thereof, or a modified derivative thereof, and any combination thereof. In certain embodiments, the isolated nucleic acid comprises a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof. In certain embodiments, the at least a portion of the genome of the RABV comprises a nucleic acid sequence encoding a nucleoprotein (N) and a nucleic acid sequence encoding a phosphoprotein (P). In certain embodiments, the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, comprises a nucleic acid sequence encoding an OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof. In certain embodiments, the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, comprises one or more additional domains selected from the group consisting of a 51-residue ectodomain of rabies virus glycoprotein (RABV-G) (ED51), a RABV-G or henipavirus glycoprotein transmembrane domain (TM), a RABV-G or henipavirus glycoprotein cytoplasmic domain (CD), and a henipavirus glycoprotein stalk (S).

In another aspect, the disclosure provides a recombinant virus comprising a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

In another aspect, the disclosure provides a vector comprising the isolated nucleic acid of the disclosure.

In another aspect, the disclosure provides a vaccine comprising the isolated nucleic acid of the disclosure and a pharmaceutically acceptable carrier.

In another aspect, the disclosure provides a method for treating, preventing, and/or ameliorating Lyme disease in a subject, the method comprising administering to the subject an effective amount of the isolated nucleic acid of the disclosure, the recombinant virus of the disclosure, the vector of the disclosure, or the vaccine of the disclosure.

In another aspect, the disclosure provides a method for generating immunity in a subject against an infection, disease, or disorder caused by a bacterium of the *Borrelia* genus, the method comprising administering to the subject an effective amount of the isolated nucleic acid of the disclosure, the recombinant virus of the disclosure, the vector of the disclosure, or the vaccine of the disclosure.

In certain embodiments, the *Borrelia* bacterium is at least one selected from the group consisting of *B. burgdorferi*, *B. afzelii*, *B. garinii*, and *B. mayonii*. In certain embodiments, the infection, disease, or disorder is Lyme disease.

BRIEF DESCRIPTION OF THE FIGURES

The drawings illustrate generally, by way of example, but not by way of limitation, various embodiments of the present application.

FIG. 1 depicts exemplary vaccine vectors of the present disclosure, including BNSP333 vectors (*i.e.*, BNSP333-OspA-CT-RVG, BNSP333-OspA-HVG, and BNSP333-OspA-DS-HGV), wherein the BNSP333 vectors comprise a number of rabies virus (RABV) components, including nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (L). Each construct further comprises one or more additional domains, including OspA$^{138\text{-}273}$ (*i.e.*, C-terminal domain of OspA, comprising a polynucleotide encoding amino acid residues 138-237 of the chimeric OspA described herein), OspA (*i.e.*, chimeric OspA as described herein), a 51-residue ectodomain of rabies virus glycoprotein (ED51), rabies virus or henipavirus transmembrane protein (TM), a rabies virus or henipavirus cytoplasmic domain (CD), and a henipavirus glycoprotein stalk domain (S).

FIG. 2 depicts virus recovery of exemplary BNSP333 vector constructs of the present disclosure comprising plasmids with inserted OspA genes using reverse transcriptase methods, wherein anti-RABV-N is stained using fluorescein diacetate (FAD) stain, resulting in identification of recovered virus 14 days post-transfection on BSR cells. Imaged at 40X; scale bar = 300 μM.

FIG. 3 depicts staining of exemplary BNSP333 constructs comprising OspA recovered as described herein. Recovered constructs were surface stained for OspA (*i.e.*, LA-2 anti-OspA; orange) and RABV-G (*i.e.*, 4C12 human anti-RABV-G; green) at various exposure times. All constructs show proper incorporation of the OspA epitope.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to certain embodiments of the disclosed subject matter, examples of which are illustrated in part in the accompanying drawings. While the disclosed subject matter will be described in conjunction with the enumerated claims, it will be understood that the exemplified subject matter is not intended to limit the claims to the disclosed subject matter.

Throughout this document, values expressed in a range format should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. For example, a range of "about 0.1% to about 5%" or "about 0.1% to 5%" should be interpreted to include not just about 0.1% to about 5%, but also the individual values (*e.g.*, 1%, 2%, 3%, and 4%) and the sub-ranges (*e.g.*, 0.1% to 0.5%, 1.1% to 2.2%, 3.3% to 4.4%) within the indicated range. The statement "about X to Y" has the same meaning as "about X to about Y," unless indicated otherwise. Likewise, the statement "about X, Y, or about Z" has the same meaning as "about X, about Y, or about Z," unless indicated otherwise.

In this document, the terms "a," "an," or "the" are used to include one or more than one unless the context clearly dictates otherwise. The term "or" is used to refer to a nonexclusive "or" unless otherwise indicated. The statement "at least one of A and B" or "at least one of A or B" has the same meaning as "A, B, or A and B." In addition, it is to be understood that the phraseology or terminology employed herein, and not otherwise defined, is for the purpose of description only and not of limitation. Any use of section headings is intended to aid reading of the document and is not to be interpreted as limiting; information that is relevant to a section heading may occur within or outside of that particular section. All publications, patents, and patent documents referred to in this document are incorporated by reference herein in their entirety, as though individually incorporated by reference.

In the methods described herein, the acts can be carried out in any order, except when a temporal or operational sequence is explicitly recited. Furthermore, specified acts can be carried out concurrently unless explicit claim language recites that they be carried out separately. For example, a claimed act of doing X and a claimed act of doing Y can be conducted simultaneously within a single operation, and the resulting process will fall within the literal scope of the claimed process.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

The term "about" as used herein can allow for a degree of variability in a value or range, for example, within 10%, within 5%, or within 1% of a stated value or of a stated limit of a range, and includes the exact stated value or range.

The term “antibody” or “Ab” as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule, which specifically binds to a specific epitope on an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. The antibodies useful in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies (“intrabodies”), Fv, Fab and $F(ab)_2$, as well as single chain antibodies (scFv) and humanized antibodies (Harlow et al., 1998, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). An antibody may be derived from natural sources or from recombinant sources. Antibodies are typically tetramers of immunoglobulin molecules.

The term “ameliorating” or “treating” means that the clinical signs and/or the symptoms associated with a disease are lessened as a result of the actions performed. The signs or symptoms to be monitored will be well known to the skilled clinician.

The term “biological” or “biological sample” refers to a sample obtained from an organism or from components (e.g., cells) of an organism. The sample may be of any biological tissue or fluid. Frequently the sample will be a “clinical sample” which is a sample derived from a patient. Such samples include, but are not limited to, bone marrow, cardiac tissue, sputum, blood, lymphatic fluid, blood cells (e.g., white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

As used herein, the terms "control," or " reference" are used interchangeably and refer to a value that is used as a standard of comparison.

The term " immunogenicity" as used herein, refers to the innate ability of an antigen or organism to elicit an immune response in an animal when the antigen or organism is administered to the animal. Thus, "enhancing the immunogenicity" refers to increasing the ability of an antigen or organism to elicit an immune response in an animal when the antigen or organism is administered to an animal. The increased ability of an antigen or organism to elicit an immune response can be measured by, among other things, a greater number of antibodies that bind to an antigen or organism, a greater diversity of antibodies to an antigen or organism, a greater number of T-cells specific for an antigen or organism, a greater cytotoxic or helper T-cell response to an antigen or organism, a greater expression of cytokines in response to an antigen, and the like.

As used herein, the terms "eliciting an immune response" or "immunizing" refer to the process of generating a B cell and/or a T cell response against a heterologous protein.

The term "antigen" or "Ag" as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full-length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

The term “specifically binds”, “selectively binds” or “binding specificity” refers to the ability of the humanized antibodies or binding compounds of the invention to bind to a target epitope with a greater affinity than that which results when bound to a non-target epitope. In certain embodiments, specific binding refers to binding to a target with an affinity that is at least 10, 50, 100, 250, 500, or 1000 times greater than the affinity for a non-target epitope.

As used herein, by “combination therapy” is meant that a first agent is administered in conjunction with another agent. “in combination with” or “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in combination with” refers to administration of one treatment modality before, during, or after delivery of the other treatment modality to the individual. Such combinations are considered to be part of a single treatment regimen or regime.

“Humoral immunity” or “humoral immune response” both refer to B-cell mediated immunity and are mediated by highly specific antibodies, produced and secreted by B-lymphocytes (B-cells).

“Prevention” refers to the use of a pharmaceutical compositions for the vaccination against a disorder.

“Adjuvant” refers to a substance that is capable of potentiating the immunogenicity of an antigen. Adjuvants can be one substance or a mixture of substances and function by acting directly on the immune system or by providing a slow release of an antigen. Examples of adjuvants are aluminum salts, polyanions, bacterial glycopeptides and slow release agents as Freund's incomplete.

The term “expression” as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

As used herein, the term “expression cassette” means a nucleic acid sequence capable of directing the transcription and/or translation of a heterologous coding sequence. In some embodiments, the expression cassette comprises a promoter sequence operably linked to a sequence encoding a heterologous protein. In some embodiments, the expression cassette further comprises at least one regulatory sequence operably linked to the sequence encoding the heterologous protein.

The terms “incorporated into” or “encapsulated in” as used herein refer to an antigenic peptide that is within a delivery vehicle, such as microparticles, bacterial ghosts, attenuated bacteria, virus like particles, attenuated viruses, ISCOMs, liposomes and preferably virosomes.

As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that may comprise a protein or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

The term “RNA” as used herein is defined as ribonucleic acid.

"Transform", "transforming", and "transformation "is used herein to refer to a process of introducing an isolated nucleic acid into the interior of an organism.

The term “treatment” as used within the context of the present invention is meant to include therapeutic treatment as well as prophylactic, or suppressive measures for the disease or disorder. As used herein, the term “treatment” and associated terms such as “treat” and “treating” means the reduction of the progression, severity and/or duration of a disease condition or at least one symptom thereof. The term ‘treatment’ therefore refers to any regimen that can benefit a subject. The treatment may be in respect of an existing condition or may be prophylactic (preventative treatment). Treatment may include curative, alleviative or prophylactic effects. References herein to “therapeutic” and “prophylactic” treatments are to be considered in their broadest context. The term “therapeutic” does not necessarily imply that a subject is treated until total recovery. Similarly, “prophylactic” does not necessarily mean that the subject will not eventually contract a disease condition. Thus, for example, the term treatment includes the administration of an agent prior to or following the onset of a disease or disorder thereby preventing or removing all signs of the disease or disorder. As another example, administration of the agent after clinical manifestation of the disease to combat the symptoms of the disease comprises “treatment” of the disease.

The term “equivalent,” when used in reference to nucleotide sequences, is understood to refer to nucleotide sequences encoding functionally equivalent polypeptides. Equivalent nucleotide sequences will include sequences that differ by one or more nucleotide substitutions, additions- or deletions, such as allelic variants; and will, therefore, include sequences that differ from the nucleotide sequence of the nucleic acids described herein due to the degeneracy of the genetic code.

The term “isolated” as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively that are present in the natural source of the macromolecule. The term isolated as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is meant to include nucleic acid fragments, which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polypeptides, which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. An “isolated cell” or “isolated population of cells” is a cell or population of cells that is not present in its natural environment.

“Identity” as used herein refers to the subunit sequence identity between two polymeric molecules particularly between two amino acid molecules, such as, between two polypeptide molecules. When two amino acid sequences have the same residues at the same positions; *e.g.*, if a position in each of two polypeptide molecules is occupied by an Arginine, then they are identical at that position. The identity or extent to which two amino acid sequences have the same residues at the same positions in an alignment is often expressed as a percentage. The identity between two amino acid sequences is a direct function of the number of matching or identical positions; *e.g.*, if half (*e.g.*, five positions in a polymer ten amino acids in length) of the positions in two sequences are identical, the two sequences are 50% identical; if 90% of the positions (*e.g.*, 9 of 10), are matched or identical, the two amino acids sequences are 90% identical.

A “mutation” as used therein is a change in a DNA sequence resulting in an alteration from its natural state. The mutation can comprise a deletion and/or insertion and/or duplication and/or substitution of at least one deoxyribonucleic acid base such as a purine (adenine and/or thymine) and/or a pyrimidine (guanine and/or cytosine).
Mutations may or may not produce discernible changes in the observable characteristics (phenotype) of an organism.

As used herein, the term “nucleic acid” refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides. ESTs, chromosomes, cDNAs, mRNAs, and rRNAs are representative examples of molecules that may be referred to as nucleic acids. As used herein, nucleic acids include but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a viral genome, using ordinary cloning technology and PCR™, and the like, and by synthetic means.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. There are numerous expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art that may be used in the compositions of the invention. “Operably linked” should be construed to include RNA expression and control sequences in addition to DNA expression and control sequences.

As used herein, the term “pharmaceutical composition” refers to a mixture of at least one compound useful within the invention with other chemical components, such as carriers, stabilizers, diluents, adjuvants, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to: intravenous, oral, aerosol, parenteral, ophthalmic, pulmonary and topical administration.

The language “pharmaceutically acceptable carrier” includes a pharmaceutically acceptable salt, pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it may perform its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each salt or carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, and not injurious to the subject. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; diluent; granulating agent; lubricant; binder; disintegrating agent; wetting agent; emulsifier; coloring agent; release agent; coating agent; sweetening agent; flavoring agent; perfuming agent; preservative; antioxidant; plasticizer; gelling agent; thickener; hardener; setting agent; suspending agent; surfactant; humectant; carrier; stabilizer; and other non-toxic compatible substances employed in pharmaceutical formulations, or any combination thereof. As used herein, “pharmaceutically acceptable carrier” also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound, and are physiologically acceptable to the subject. Supplementary active compounds may also be incorporated into the compositions.

As used herein, the term “effective amount” or “therapeutically effective amount” means the amount of the virus like particle generated from vector of the invention which is required to prevent the particular disease condition, or which reduces the severity of and/or ameliorates the disease condition or at least one symptom thereof or condition associated therewith.

A “subject” or “patient,” as used therein, may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is human.

"Titers" are numerical measures of the concentration of a virus or viral vector compared to a reference sample, where the concentration is determined either by the activity of the virus, or by measuring the number of viruses in a unit volume of buffer. The titer of viral stocks are determined, e.g., by measuring the infectivity of a solution or solutions (typically serial dilutions) of the viruses, e.g., on HeLa cells using the soft agar method (see, Graham & Van Der eb (1973) Virology 52:456-467) or by monitoring resistance conferred to cells, e.g., G418 resistance encoded by the virus or vector, or by quantitating the viruses by UV spectrophotometry (see, Chardonnet & Dales (1970) Virology 40:462-477).

“Vaccination” refers to the process of inoculating a subject with an antigen to elicit an immune response in the subject, that helps to prevent or treat the disease or disorder the antigen is connected with. The term “immunization” is used interchangeably herein with vaccination.

A “vector” is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. In the present disclosure, the term “vector” includes an autonomously replicating virus.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

Lyme disease is an infectious disease caused by the bacterium *Borrelia burgdorferi*. Symptoms of Lyme disease can include fever, headache, fatigue, and a characteristic skin rash called "erythema migrans". If left untreated, the infection can spread to the joints, heart, and nervous system, causing more severe symptoms such as arthritis, facial palsy, and meningitis. Lyme disease is the most common vector-borne disease in the United States and Europe, with tens of thousands of cases reported each year, and there are currently no approved vaccines for Lyme disease prophylaxis.

As described elsewhere herein, outer surface protein A (OspA) is a protein found on the outer surface of the bacterium *Borrelia burgdorferi*, which is the causative agent of Lyme disease. OspA plays a critical role in the transmission of *B. burgdorferi* from the tick vector to the new host.

The protective and/or immunogenic epitopes on OspA are located in the C-terminal domain of the protein (*i.e.*, amino acids 138-273 of OspA).

In one aspect, the present disclosure relates to vectors and/or vaccine compositions comprising a polynucleotide encoding OspA. In certain embodiments, the vaccine compositions comprise a rabies virus (RABV) vector comprising OspA, a fragment thereof, or a modified derivative thereof, or any combination thereof.

Isolated Nucleic Acids

In one aspect, the present disclosure provides an isolated nucleic acid encoding a recombinant virus comprising outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

In certain embodiments, the virus is a rhabdovirus. In certain embodiments, the virus is a paramyxovirus. In certain embodiments, the virus is a rabies virus (RABV). In certain embodiments, the virus is a henipavirus.

In certain embodiments, the isolated nucleic acid comprises a nucleic acid sequence encoding at least a portion of the genome of the virus.

In certain embodiments, the isolated nucleic acid comprises:

(a) a nucleic acid sequence encoding at least a portion of the genome of a RABV; and

(b) a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

In certain embodiments, the at least a portion of the genome of the RABV comprises a nucleic acid sequence encoding a nucleoprotein (N) and a nucleic acid sequence encoding a phosphoprotein (P).

In certain embodiments, the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, is inserted into a position between the nucleic acid sequence encoding the nucleoprotein (N) and the nucleic acid sequence encoding the phosphoprotein (P).

In certain embodiments, the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, comprises:

(a) a nucleic acid sequence encoding an OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof; and

(b) one or more additional domains selected from the group consisting of a 51-residue ectodomain of rabies virus glycoprotein (RABV-G) (ED51), a RABV-G or henipavirus glycoprotein transmembrane domain (TM), a RABV-G or henipavirus glycoprotein cytoplasmic domain (CD), and a henipavirus glycoprotein stalk (S).

In certain embodiments, the nucleic acid sequence encoding an OspA has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with SEQ ID NO:1. In certain embodiments, the nucleic acid sequence encoding an OspA has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with SEQ ID NO:2. In certain embodiments, the nucleic acid sequence encoding an OspA has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with SEQ ID NO:3.

In certain embodiments, the nucleic acid sequences comprising the ED51, TM, CD, and S, if present, are each inserted into a position between the nucleic acid encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3 and the nucleic acid sequence encoding the phosphoprotein (P).

In certain embodiments, the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a matrix protein (M).

In certain embodiments, the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a glycoprotein (G). In certain embodiments, the glycoprotein (G) comprises an attenuating mutation. In certain embodiments, the mutation is R333E.

In certain embodiments, the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a RNA-dependent RNA polymerase (L).

In certain embodiments, the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3’ to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5’-N and 3’-OspA). In certain embodiments, the nucleic acid sequence encoding the ED51 is positioned immediately 3’ to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5’-OspA and 3’-ED51). In certain embodiments, the nucleic acid sequence encoding the TM positioned immediately 3’ to the nucleic acid sequence encoding the ED51 (*i.e.*, 5’-ED51 and 3’-TM). In certain embodiments, the nucleic acid sequence encoding the CD positioned immediately 3’ to the nucleic acid sequence encoding the TM (*i.e.*, 5’-TM and 3’-CD). In certain embodiments, the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3’ to the nucleic acid sequence encoding the CD (*i.e.*, 5’-CD and 3’-P). In certain embodiments, the nucleic acid sequence encoding the matrix protein (M) is immediately 3’ to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5’-P and 3’-M). In certain embodiments, the nucleic acid sequence encoding the glycoprotein (G) is immediately 3’ to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5’-M and 3’-G). In certain embodiments, the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3’ to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5’-G and 3’-L).

In certain embodiments, the transmembrane domain (TM) is a RABV-G TM. In certain embodiments, cytoplasmic domain (CD) is a RABV-G CD.

In certain embodiments, the nucleic acid sequence encoding the OspA has at least 85% sequence homology with SEQ ID NO:3, or a fragment thereof. In certain embodiments, the fragment thereof comprises amino acid residues 138-273 of SEQ ID NO:3.

In certain embodiments, the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA). In certain embodiments, the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) is positioned immediately 3' to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-S). In certain embodiments, the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) (*i.e.*, 5'-S and 3'-TM). In certain embodiments, the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD). In certain embodiments, the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P). In certain embodiments, the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M). In certain embodiments, the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G). In certain embodiments, the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

In certain embodiments, the transmembrane domain (TM) is a henipavirus TM. In certain embodiments, the cytoplasmic domain (CD) is a henipavirus CD.

In certain embodiments, the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA). In certain embodiments, the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-TM). In certain embodiments, the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD). In certain embodiments, the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P). In certain embodiments, the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M). In certain embodiments, the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G). In certain embodiments, the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

In certain embodiments, the transmembrane domain (TM) is a henipavirus TM. In certain embodiments, the cytoplasmic domain (CD) is a henipavirus CD.

In certain embodiments, the nucleic acid encoding the recombinant virus is codon optimized for expression in a host cell, optionally wherein the host cell is a mammalian cell.

Vaccines and Vectors

In one aspect, the present disclosure provides a recombinant virus comprising a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

In certain embodiments, the virus is a rhabdovirus. In certain embodiments, the virus is a paramyxovirus. In certain embodiments, the virus is a rabies virus (RABV). In certain embodiments, the virus is a henipavirus.

In certain embodiments, the isolated nucleic acid comprises a nucleic acid sequence encoding at least a portion of the genome of the virus.

In certain embodiments, the isolated nucleic acid comprises:

(a) a nucleic acid sequence encoding at least a portion of the genome of a RABV; and

(b) a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

In certain embodiments, the at least a portion of the genome of the RABV comprises a nucleic acid sequence encoding a nucleoprotein (N) and a nucleic acid sequence encoding a phosphoprotein (P).

In certain embodiments, the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, is inserted into a position between the nucleic acid sequence encoding the nucleoprotein (N) and the nucleic acid sequence encoding the phosphoprotein (P).

In certain embodiments, the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, comprises:

(a) a nucleic acid sequence encoding an OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof; and

(b) one or more additional domains selected from the group consisting of a 51-residue ectodomain of rabies virus glycoprotein (RABV-G) (ED51), a RABV-G or henipavirus glycoprotein transmembrane domain (TM), a RABV-G or henipavirus glycoprotein cytoplasmic domain (CD), and a henipavirus glycoprotein stalk (S).

In certain embodiments, the nucleic acid sequence encoding an OspA has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with SEQ ID NO:1. In certain embodiments, the nucleic acid sequence encoding an OspA has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with SEQ ID NO:2. In certain embodiments, the nucleic acid sequence encoding an OspA has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence homology with SEQ ID NO:3.

In certain embodiments, the nucleic acid sequences comprising the ED51, TM, CD, and S, if present, are each inserted into a position between the nucleic acid encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3 and the nucleic acid sequence encoding the phosphoprotein (P).

In certain embodiments, the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a matrix protein (M).

In certain embodiments, the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a glycoprotein (G). In certain embodiments, the glycoprotein (G) comprises an attenuating mutation. In certain embodiments, the mutation is R333E.

In certain embodiments, the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a RNA-dependent RNA polymerase (L).

In certain embodiments, the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA). In certain embodiments, the nucleic acid sequence encoding the ED51 is positioned immediately 3' to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-ED51). In certain embodiments, the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the ED51 (*i.e.*, 5'-ED51 and 3'-TM). In certain embodiments, the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD). In certain embodiments, the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P). In certain embodiments, the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M). In certain embodiments, the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G). In certain embodiments, the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

In certain embodiments, the transmembrane domain (TM) is a RABV-G TM. In certain embodiments, cytoplasmic domain (CD) is a RABV-G CD.

In certain embodiments, the nucleic acid sequence encoding the OspA has at least 85% sequence homology with SEQ ID NO:3, or a fragment thereof. In certain embodiments, the fragment thereof comprises amino acid residues 138-273 of SEQ ID NO:3.

In certain embodiments, the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA). In certain embodiments, the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) is positioned immediately 3' to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-S). In certain embodiments, the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) (*i.e.*, 5'-S and 3'-TM). In certain embodiments, the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD). In certain embodiments, the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P). In certain embodiments, the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M). In certain embodiments, the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G). In certain embodiments, the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

In certain embodiments, the transmembrane domain (TM) is a henipavirus TM. In certain embodiments, the cytoplasmic domain (CD) is a henipavirus CD.

In certain embodiments, the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA). In certain embodiments, the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-TM). In certain embodiments, the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD). In certain embodiments, the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P). In certain embodiments, the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M). In certain embodiments, the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G). In certain embodiments, the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

In certain embodiments, the transmembrane domain (TM) is a henipavirus TM. In certain embodiments, the cytoplasmic domain (CD) is a henipavirus CD.

In certain embodiments, the nucleic acid encoding the recombinant virus is codon optimized for expression in a host cell, optionally wherein the host cell is a mammalian cell.

In another aspect, the present disclosure provides a vaccine comprising the isolated nucleic acid of the present disclosure or the recombinant virus of the present disclosure and a pharmaceutically acceptable carrier.

In certain embodiments, the vaccine further comprises an adjuvant.

Methods

In one aspect, the present disclosure provides a method for treating, preventing, and/or ameliorating Lyme disease in a subject. In certain embodiments, the method comprises administering to the subject an effective amount of the isolated nucleic acid of the present disclosure. In certain embodiments, the method comprises administering to the subject an effective amount of the recombinant virus of the present disclosure. In certain embodiments, the method comprises administering to the subject an effective amount of the vector of the present disclosure. In certain embodiments, the method comprises administering to the subject an effective amount of the vaccine of the present disclosure.

In another aspect, the present disclosure provides a method for generating immunity in a subject against an infection, disease, or disorder caused by a bacterium of the *Borrelia* genus. In certain embodiments, the method comprises administering to the subject an effective amount of the isolated nucleic acid of the present disclosure. In certain embodiments, the method comprises administering to the subject an effective amount of the recombinant virus of the present disclosure. In certain embodiments, the method comprises administering to the subject an effective amount of the vector of the present disclosure. In certain embodiments, the method comprises administering to the subject an effective amount of the vaccine of the present disclosure.

In certain embodiments, the *Borrelia* bacterium is *B. burgdorferi*. In certain embodiments, the *Borrelia* bacterium is *B. afzelii*. In certain embodiments, the *Borrelia* bacterium is *B. garinii*. In certain embodiments, the *Borrelia* bacterium is *B. mayonii*.

In certain embodiments, the infection, disease, or disorder is Lyme disease.

Pharmaceutical Compositions and Formulations

The vaccine of the invention may be formulated as a pharmaceutical composition. In some embodiments, the vaccine contains a live virus. In some embodiments, the vaccine contains deactivated viral particles. In some embodiments, the virus is a recombinant virus encoded by any one of the nucleic acid constructs as described herein.

Such a pharmaceutical composition may be in a form suitable for administration to a subject (*i.e.*, mammal), or the pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The various components of the pharmaceutical composition may be present in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

In one embodiment, the pharmaceutical compositions useful for practicing the method of the invention comprises an adjuvant. Non-limiting examples of suitable adjuvants are Freund's complete adjuvant, Freund's incomplete adjuvant, Quil A, Detox, ISCOMs, squalene, MPLA, and CpG or other activators of TLR or inflammasome. The pharmaceutical composition or vaccine composition can comprise any one or more of the adjuvants described herein.

Pharmaceutical compositions that are useful in the methods of the invention may be suitably developed for inhalation, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intravenous or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration is readily apparent to the skilled artisan and depends upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions suitable for ethical administration to humans, it is understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation.

In other embodiments, the composition of the invention comprises a preservative from about 0.005% to 2.0% by total weight of the composition. In one embodiment, the preservative prevents spoilage e.g., in the case of exposure to contaminants in the environment.

Administration/Dosing

The regimen of administration may affect what constitutes an effective amount. For example, the nucleic acid of the invention may be administered to the subject (*i.e.*, mammal) in a single dose, in several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a subject, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat the disease in the subject. An effective amount of the composition necessary to achieve the intended result will vary and will depend on factors such as the disease to be treated or prevented, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors well-known in the medical arts. In particular embodiments, it is especially advantageous to formulate the composition in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the composition and the heterologous protein to be expressed, and the particular therapeutic effect to be achieved.

Routes of Administration

One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Routes of administration of any of the compositions of the invention include inhalation, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (e.g., sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (e.g., trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, electroporation and topical administration.

Kits

In some embodiments a kit is provided for treating, preventing, or ameliorating a given disease, disorder or condition, or a symptom thereof, as described herein wherein the kit comprises: a) compositions as described herein; and optionally b) an additional agent or therapy as described herein. The kit can further include instructions or a label for using the kit to treat, prevent, or ameliorate the disease, disorder or condition. In yet other embodiments, the invention extends to kits assays for a given disease, disorder or condition, or a symptom thereof, as described herein. Such kits may, for example, contain the reagents from PCR or other nucleic acid hybridization technology (microarrays) or reagents for immunologically based detection techniques (e.g., ELISpot, ELISA).

Virus production

In yet another aspect, the present disclosure includes a method for increasing expression of a recombinant virus in a host cell. In one embodiment, the recombinant virus is a rabies virus. In some embodiments, the method comprises expressing in the host cell a nucleic acid sequence described herein.

The recombinant virus can be produced in a host cell using methods known in the art, e.g., as described in Fisher et al., Cell Reports 32, 107920, July 21, 2020. In some embodiments, the host cell is a mammalian cell. In one embodiment, the host cell is a human cell. In one embodiment, the host cell is a primate cell. In some embodiments, the host cell is a BSR cell (a derivative of baby hamster kidney cell line BHK-21).

EXAMPLES

Various embodiments of the present application can be better understood by reference to the following Examples which are offered by way of illustration. The scope of the present application is not limited to the Examples given herein.

Example 1: Design and evaluation of an exemplary tick vaccine

In one aspect, the present disclosure relates to an anti-tick vaccine composition comprising a recombinant rabies virus (RABV) vector expressing outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

The OspA gene sequence used to generate the recombinant vector described herein is based off of the OspA sequence from *Borrelia burgdorferi* strain B31 (SEQ ID NO:1). It has been hypothesized that the lymphocyte function-associated antigen (LFA-1) epitope of *B. burgdorferi* strain B31 may trigger and/or promote development of autoantibodies when immunized against LFA-1 in humans. Thus, in one aspect, the present disclosure relates to vaccine compositions comprising a modified OspA sequence. It was reasoned that the chimeric OspA sequence would not change the folding of the antigen.

In certain embodiments, the LFA-1 epitope present in OspA *B. burgdorferi* serotype 1 (SEQ ID NO:1) comprises an amino acid fragment YVLEGTLTA. In certain embodiments, the LFA-1 epitope present in OspA *Borrelia garinii* serotype 3 (SEQ ID NO:2) comprises an amino acid fragment FALEGTLTD.

In certain embodiments, the isolated nucleic acid sequence of the present disclosure, which comprising the vector and/or vaccine compositions described herein, may encode a modified OspA having at least 85% sequence homology with SEQ ID NO:3. In certain embodiments, the modified OspA gene comprises a substitution of the second A with T in the LFA-1 epitope in *B. garinii* serotype 3 (*e.g.*, for optimal folding). In certain embodiments, the modified OspA gene comprises a substitution of the middle T with A in the LFA-1 epitope in *B. garinii* serotype 3. In certain embodiments, the modified OspA gene comprises a substitution of D with A in the LFA-1 epitope in *B. garinii* serotype 3 (*e.g.*, for optimal folding). In certain embodiments, the LFA-1 epitope thereof comprises an amino acid fragment FTLEGALTA (SEQ ID NO:8). In some embodiments, the LFA-1 epitope thereof is encoded by a nucleotide sequence comprising TTTACTCTTGAAGGAGCTCTAACTGCT (SEQ ID NO:4).

Through molecular cloning, the chimeric OspA genes were inserted into rabies virus (RABV) vector (BNSP333) plasmids. Three different OspA constructs were prepared (*i.e.*, exemplary constructs 1-3), each comprising a LFA-1 epitope described herein, or a fragment thereof.

For proper presentation of the protective C-terminal epitopes, a first exemplary construct was prepared comprising the C-terminal domain of OspA (*i.e.*, amino acids 138-273), with the addition of the final 51 amino acids of the ectodomain of RABV glycoprotein (ED51), the transmembrane domain (TM) and cytoplasmic domain (CD). The fragment additional fragment comprising RABV ED51, TM, and CD is referred to herein as the “RVG tail”, and the first construct is referred to herein as “BNSP333-OspA-CT-RVG”.

A second exemplary construct was prepared comprising the full modified OspA sequence. The second construct differs from the first construct in that the RVG tail is substituted for a henipavirus tail (*i.e.*, "HVG tail") comprising a cytoplasmic domain (CD), transmembrane domain (TM), and henipavirus glycoprotein stalk (S). Without wishing to be bound by any theory, this change allows the protein's C-terminal domain to be the outer most domain when situated in the RABV virion. The second construct is referred to herein as "BNSP333-OspA-HVG".

A third exemplary construct was prepared, also comprising the full modified OspA sequence. The third construct differs from the first construct in that the henipavirus glycoprotein stalk (S) is absent from the HVG tail thereof. The third construct is referred to herein as "BNSP333-OspA-DS-HVG".

Each of the three exemplary constructs described herein were configured on DNA Star, and proper cloning of all constructs was confirmed by Sanger sequencing. Next, BNSP333-OspA-CT-RVG, BNSP333-OspA-HVG, and BNSP333-OspA-DS-HVG plasmids were used to recover infectious RABV with addition of each respective antigen. The viruses were successfully recovered (FIG. 2). Viruses were stained for RABV nucleoprotein (N) in addition to RABV-G and OspA (FIGs. 2-3). Surface and intracellular staining determined if the antigen was successfully incorporated into the RABV virion, a necessity to develop a successful immune response against the inserted *Borrelial* antigens.

Sequence Listing

SEQ ID NO:1 (OspA *Borrelia burgdorferi* serotype 1)

MKKYLLGIGL ILALIACKQN VSSLDEKNSV SVDLPGEMKV LVSKEKNKDG
KYDLIATVDK LELKGTSDKN NGSGVLEGVK ADKSKVKLTI SDDLGQTTLE
VFKEDGKTLV SKKVTSKDKS STEEKFNEKG EVSEKIITRA DGTRLEYTGI
KSDGSGKAKE VLKGYVLEGT LTAEKTTLVV KEGTVTLSKN ISKSGEVSVE
LNDTDSSAAT KKTAAWNSGT STLTITVNSK KTKDLVFTKE NTITVQQYDS
NGTKLEGSAV EITKLDEIKN ALK

SEQ ID NO:2 (OspA *Borrelia garinii* protein sequence serotype 3)

MKKYLLGIGL ILALIACKQN VSSLDEKNSV SVDLPGGMKV LVSKEKDKDG

KYSLMATVEK LELKGTSDKS NGSGVLEGEK ADKSKAKLTI SQDLNQTTFE
IFKEDGKTLV SRKVNSKDKS STEEKFNDKG KLSEKVVTRA NGTRLEYTEI
KNDGSGKAKE VLKGFALEGT LTDGGETKLT VTEGTVTLSK NISKSGEITV
ALNDTETTPA DKKTGEWKSD TSTLTISKNS QKTKQLVFTK ENTITVQNYN
RAGNALEGSP AEIKDLAELK AALK

SEQ ID NO:3 (Exemplary OspA)

MKKYLLGIGL ILALIACKQN VSSLDEKNSV SVDLPGGMKV LVSKEKDKDG
KYSLMATVEK LELKGTSDKS NGSGVLEGEK ADKSKAKLTI SQDLNQTTFE
IFKEDGKTLV SRKVNSKDKS STEEKFNDKG KLSEKVVTRA NGTRLEYTEI
KNDGSGKAKE VLKGFTLEGA LTAGGETKLT VTEGTVTLSK NISKSGEITV
ALNDTETTPA DKKTGEWKSD TSTLTISKNS QKTKQLVFTK ENTITVQNYN
RAGNALEGSP AEIKDLAELK AALK

SEQ ID NO:4 (LFA-1 epitope nucleotide)

tttactcttgaaggagctctaactgct

SEQ ID NO:5 (RABV-G ED51)

gagagctctgttatcccactggtgcatcctttggctgacccatcaactgtatttaaagatggag
atgaagcagaggactttgtggaggtacatctgcctgatgtgcacaaccaggtcagcggcgtgga
cctgggcctacccaactggggcaag

SEQ ID NO:6 (RABV-G TM)

tacgtgcttctcagtgctggggcgttgacagccctgatgctgatcattttcctcatgacctgct
gc

SEQ ID NO:7 (RABV-G CD)

aggcgcgtcaatagatcagagcccacccagcacaatttaagaggtacaggccgggaagtttcag
tcacgccccagtctggaaaaattatatcgtcctgggagtcccataaaagtggtggggaaactcg
tttatga

SEQ ID NO:8 (LFA-1 epitope amino acid)

FTLEGALTA

Enumerated Embodiments

The following exemplary embodiments are provided, the numbering of which is not to be construed as designating levels of importance:

Embodiment 1 provides an isolated nucleic acid encoding a recombinant virus comprising outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

Embodiment 2 provides the isolated nucleic acid of Embodiment 1, wherein the virus is a rhabdovirus or paramyxovirus.

Embodiment 3 provides the isolated nucleic acid of Embodiment 1 or 2, wherein the virus is selected from the group consisting of a rabies virus (RABV) and a henipavirus.

Embodiment 4 provides the isolated nucleic acid of any one of Embodiments 1-3, wherein the isolated nucleic acid comprises a nucleic acid sequence encoding at least a portion of the genome of the virus.

Embodiment 5 provides the isolated nucleic acid of any one of Embodiments 1-4, wherein the isolated nucleic acid comprises:

(a) a nucleic acid sequence encoding at least a portion of the genome of a RABV; and

(b) a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

Embodiment 6 provides the isolated nucleic acid of Embodiment 5, wherein the at least a portion of the genome of the RABV comprises a nucleic acid sequence encoding a nucleoprotein (N) and a nucleic acid sequence encoding a phosphoprotein (P).

Embodiment 7 provides the isolated nucleic acid of Embodiment 6, wherein the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, is inserted into a position between the nucleic acid sequence encoding the nucleoprotein (N) and the nucleic acid sequence encoding the phosphoprotein (P).

Embodiment 8 provides the isolated nucleic acid of Embodiment 7, wherein the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, comprises:

(a) a nucleic acid sequence encoding an OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof; and

(b) one or more additional domains selected from the group consisting of a 51-residue ectodomain of rabies virus glycoprotein (RABV-G) (ED51), a RABV-G or henipavirus glycoprotein transmembrane domain (TM), a RABV-G or henipavirus glycoprotein cytoplasmic domain (CD), and a henipavirus glycoprotein stalk (S).

Embodiment 9 provides the isolated nucleic acid of Embodiment 8, wherein the nucleic acid sequences comprising the ED51, TM, CD, and S, if present, are each inserted into a position between the nucleic acid encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3 and the nucleic acid sequence encoding the phosphoprotein (P).

Embodiment 10 provides the nucleic acid of any one of Embodiments 5-9, wherein the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a matrix protein (M).

Embodiment 11 provides the isolated nucleic acid of any one of Embodiments 5-10, wherein the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a glycoprotein (G).

Embodiment 12 provides the isolated nucleic acid of Embodiment 11, wherein the glycoprotein (G) comprises an attenuating mutation.

Embodiment 13 provides the isolated nucleic acid of Embodiment 12, wherein the mutation is R333E.

Embodiment 14 provides the isolated nucleic acid of any one of Embodiments 5-13, wherein the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a RNA-dependent RNA polymerase (L).

Embodiment 15 provides the isolated nucleic acid of any one of Embodiments 8-14, wherein at least one of the following applies:

(a) the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3’ to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5’-N and 3’-OspA);

(b) the nucleic acid sequence encoding the ED51 is positioned immediately 3’ to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-ED51);

(c) the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the ED51 (*i.e.*, 5'-ED51 and 3'-TM);

(d) the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD);

(e) the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P);

(f) the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M);

(g) the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G); and

(h) the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

Embodiment 16 provides the isolated nucleic acid of Embodiment 15, wherein the nucleic acid sequence encoding the OspA has at least 85% sequence homology with SEQ ID NO:3, or a fragment thereof, optionally wherein the fragment thereof comprises amino acid residues 138-273 of SEQ ID NO:3.

Embodiment 17 provides the isolated nucleic acid of any one of Embodiments 8-14, wherein at least one of the following applies:

(a) the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA);

(b) the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) is positioned immediately 3' to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-S);

(c) the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) (*i.e.*, 5'-S and 3'-TM);

(d) the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD);

(e) the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P);

(f) the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M);

(g) the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G); and

(h) the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

Embodiment 18 provides the isolated nucleic acid of any one of Embodiments 8-14, wherein at least one of the following applies:

(a) the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA);

(b) the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-TM);

(c) the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD);

(d) the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P);

(e) the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M);

(f) the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G); and

(g) the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

Embodiment 19 provides the isolated nucleic acid of Embodiment 15 or 16, wherein at least one of the following applies:

(a) the transmembrane domain (TM) is a RABV-G TM; and

(b) the cytoplasmic domain (CD) is a RABV-G CD.

Embodiment 20 provides the isolated nucleic acid of Embodiment 17 or 18, wherein at least one of the following applies:

(a) the transmembrane domain (TM) is a henipavirus TM; and

(b) the cytoplasmic domain (CD) is a henipavirus CD.

Embodiment 21 provides the isolated nucleic acid of any one of Embodiments 1-20, wherein the nucleic acid encoding the recombinant virus is codon optimized for expression in a host cell, optionally wherein the host cell is a mammalian cell.

Embodiment 22 provides a recombinant virus comprising a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

Embodiment 23 provides the recombinant virus of Embodiment 22, wherein the virus is a rhabdovirus or paramyxovirus.

Embodiment 24 provides the recombinant virus of Embodiment 22 or 23, wherein the virus is selected from the group consisting of a rabies virus (RABV) and a henipavirus.

Embodiment 25 provides the recombinant virus of any one of Embodiments 22-24, wherein the isolated nucleic acid comprises a nucleic acid sequence encoding at least a portion of the genome of the virus.

Embodiment 26 provide sthe recombinant virus of any one of Embodiments 22-25, wherein the isolated nucleic acid comprises:

(a) a nucleic acid sequence encoding at least a portion of the genome of a RABV;

(b) a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

Embodiment 27 provides a vector comprising the isolated nucleic acid of any one of Embodiments 1-21.

Embodiment 28 provides a vaccine comprising the isolated nucleic acid of any one of Embodiments 1-21 and a pharmaceutically acceptable carrier.

Embodiment 29 provides the vaccine of Embodiment 28, further comprising an adjuvant.

Embodiment 30 provides a method for treating, preventing, and/or ameliorating Lyme disease in a subject, the method comprising administering to the subject an effective amount of the isolated nucleic acid of any one of Embodiments 1-21, the recombinant virus of any one of Embodiments 22-26, the vector of Embodiment 27, or the vaccine of Embodiments 28 or 29.

Embodiment 31 provides a method for generating immunity in a subject against an infection, disease, or disorder caused by a bacterium of the *Borrelia* genus, the method comprising administering to the subject an effective amount of the isolated nucleic acid of any one of Embodiments 1-21, the recombinant virus of any one of Embodiments 22-26, the vector of Embodiment 27, or the vaccine of Embodiments 28 or 29.

Embodiment 32 provides the method of Embodiment 31, wherein the *Borrelia* bacterium is at least one selected from the group consisting of *B. burgdorferi*, *B. afzelii*, *B. garinii*, and *B. mayonii*.

Embodiment 33 provides the method of Embodiment 31 or 32, wherein the infection, disease, or disorder is Lyme disease.

The terms and expressions employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the embodiments of the present application. Thus, it should be understood that although the present application describes specific embodiments and optional features, modification and variation of the compositions, methods, and concepts herein disclosed may be resorted to by those of ordinary skill in the art, and that such modifications and variations are considered to be within the scope of embodiments of the present application.

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a recombinant virus comprising outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

2. The isolated nucleic acid of claim 1, wherein the virus is a rhabdovirus or paramyxovirus.

3. The isolated nucleic acid of claim 1 or 2, wherein the virus is selected from the group consisting of a rabies virus (RABV) and a henipavirus.

4. The isolated nucleic acid of any one of claims 1-3, wherein the isolated nucleic acid comprises a nucleic acid sequence encoding at least a portion of the genome of the virus.

5. The isolated nucleic acid of any one of claims 1-4, wherein the isolated nucleic acid comprises:

(a) a nucleic acid sequence encoding at least a portion of the genome of a RABV; and

(b) a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

6. The isolated nucleic acid of claim 5, wherein the at least a portion of the genome of the RABV comprises a nucleic acid sequence encoding a nucleoprotein (N) and a nucleic acid sequence encoding a phosphoprotein (P).

7. The isolated nucleic acid of claim 6, wherein the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, is inserted into a position between the nucleic acid sequence encoding the nucleoprotein (N) and the nucleic acid sequence encoding the phosphoprotein (P).

8. The isolated nucleic acid of claim 7, wherein the nucleic acid sequence encoding the OspA, a fragment thereof, or a modified derivative thereof, and any combination thereof, comprises:

(a) a nucleic acid sequence encoding an OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof; and

(b) one or more additional domains selected from the group consisting of a 51-residue ectodomain of rabies virus glycoprotein (RABV-G) (ED51), a RABV-G or henipavirus glycoprotein transmembrane domain (TM), a RABV-G or henipavirus glycoprotein cytoplasmic domain (CD), and a henipavirus glycoprotein stalk (S).

9. The isolated nucleic acid of claim 8, wherein the nucleic acid sequences comprising the ED51, TM, CD, and S, if present, are each inserted into a position between the nucleic acid encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3 and the nucleic acid sequence encoding the phosphoprotein (P).

10. The isolated nucleic acid of any one of claims 5-9, wherein the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a matrix protein (M).

11. The isolated nucleic acid of any one of claims 5-10, wherein the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a glycoprotein (G).

12. The isolated nucleic acid of claim 11, wherein the glycoprotein (G) comprises an attenuating mutation.

13. The isolated nucleic acid of claim 12, wherein the mutation is R333E.

14. The isolated nucleic acid of any one of claims 5-13, wherein the at least a portion of the genome of the rabies virus comprises a nucleic acid sequence encoding a RNA-dependent RNA polymerase (L).

15. The isolated nucleic acid of any one of claims 8-14, wherein at least one of the following applies:

(a) the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA);

(b) the nucleic acid sequence encoding the ED51 is positioned immediately 3' to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-ED51);

(c) the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the ED51 (*i.e.*, 5'-ED51 and 3'-TM);

(d) the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD);

(e) the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P);

(f) the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M);

(g) the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G); and

(h) the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

16. The isolated nucleic acid of claim 15, wherein the nucleic acid sequence encoding the OspA has at least 85% sequence homology with SEQ ID NO:3, or a fragment thereof, optionally wherein the fragment thereof comprises amino acid residues 138-273 of SEQ ID NO:3.

17. The isolated nucleic acid of any one of claims 8-14, wherein at least one of the following applies:

(a) the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA);

(b) the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) is positioned immediately 3' to the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-S);

(c) the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the henipavirus glycoprotein stalk (S) (*i.e.*, 5'-S and 3'-TM);

(d) the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD);

(e) the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P);

(f) the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M);

(g) the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G); and

(h) the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

18. The isolated nucleic acid of any one of claims 8-14, wherein at least one of the following applies:

(a) the nucleic acid sequence encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof, is positioned immediately 3' to the nucleic acid sequence encoding the nucleoprotein (N) (*i.e.*, 5'-N and 3'-OspA);

(b) the nucleic acid sequence encoding the TM positioned immediately 3' to the nucleic acid sequence encoding the encoding the OspA having at least 85% sequence homology with any one of SEQ ID NOs:1-3, or a fragment thereof (*i.e.*, 5'-OspA and 3'-TM);

(c) the nucleic acid sequence encoding the CD positioned immediately 3' to the nucleic acid sequence encoding the TM (*i.e.*, 5'-TM and 3'-CD);

(d) the nucleic acid sequence encoding the phosphoprotein (P) is immediately 3' to the nucleic acid sequence encoding the CD (*i.e.*, 5'-CD and 3'-P);

(e) the nucleic acid sequence encoding the matrix protein (M) is immediately 3' to the nucleic acid sequence encoding the phosphoprotein (P) (*i.e.*, 5'-P and 3'-M);

(f) the nucleic acid sequence encoding the glycoprotein (G) is immediately 3' to the nucleic acid sequence encoding the matrix protein (M) (*i.e.*, 5'-M and 3'-G); and

(g) the nucleic acid sequence encoding the RNA-dependent RNA polymerase (L) is immediately 3' to the nucleic acid sequence encoding the glycoprotein (G) (*i.e.*, 5'-G and 3'-L).

19. The isolated nucleic acid of claim 15 or 16, wherein at least one of the following applies:

(a) the transmembrane domain (TM) is a RABV-G TM; and

(b) the cytoplasmic domain (CD) is a RABV-G CD.

20. The isolated nucleic acid of claim 17 or 18, wherein at least one of the following applies:

(a) the transmembrane domain (TM) is a henipavirus TM; and

(b) the cytoplasmic domain (CD) is a henipavirus CD.

21. The isolated nucleic acid of any one of claims 1-20, wherein the nucleic acid encoding the recombinant virus is codon optimized for expression in a host cell, optionally wherein the host cell is a mammalian cell.

22. A recombinant virus comprising a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

23. The recombinant virus of claim 22, wherein the virus is a rhabdovirus or paramyxovirus.

24. The recombinant virus of claim 22 or 23, wherein the virus is selected from the group consisting of a rabies virus (RABV) and a henipavirus.

25. The recombinant virus of any one of claims 22-24, wherein the isolated nucleic acid

comprises a nucleic acid sequence encoding at least a portion of the genome of the virus.

26. The recombinant virus of any one of claims 22-25, wherein the isolated nucleic acid comprises:

(a) a nucleic acid sequence encoding at least a portion of the genome of a RABV;

(b) a nucleic acid sequence encoding outer surface protein A (OspA), a fragment thereof, or a modified derivative thereof, and any combination thereof.

27. A vector comprising the isolated nucleic acid of any one of claims 1-21.

28. A vaccine comprising the isolated nucleic acid of any one of claims 1-21 and a pharmaceutically acceptable carrier.

29. The vaccine of claim 28, further comprising an adjuvant.

30. A method for treating, preventing, and/or ameliorating Lyme disease in a subject, the method comprising administering to the subject an effective amount of the isolated nucleic acid of any one of claims 1-21, the recombinant virus of any one of claims 22-26, the vector of claim 27, or the vaccine of claims 28 or 29.

31. A method for generating immunity in a subject against an infection, disease, or disorder caused by a bacterium of the *Borrelia* genus, the method comprising administering to the subject an effective amount of the isolated nucleic acid of any one of claims 1-21, the recombinant virus of any one of claims 22-26, the vector of claim 27, or the vaccine of claims 28 or 29.

32. The method of claim 31, wherein the *Borrelia* bacterium is at least one selected from the group consisting of *B. burgdorferi*, *B. afzelii*, *B. garinii*, and *B. mayonii*.

33. The method of claim 31 or 32, wherein the infection, disease, or disorder is Lyme disease.

FIG. 1

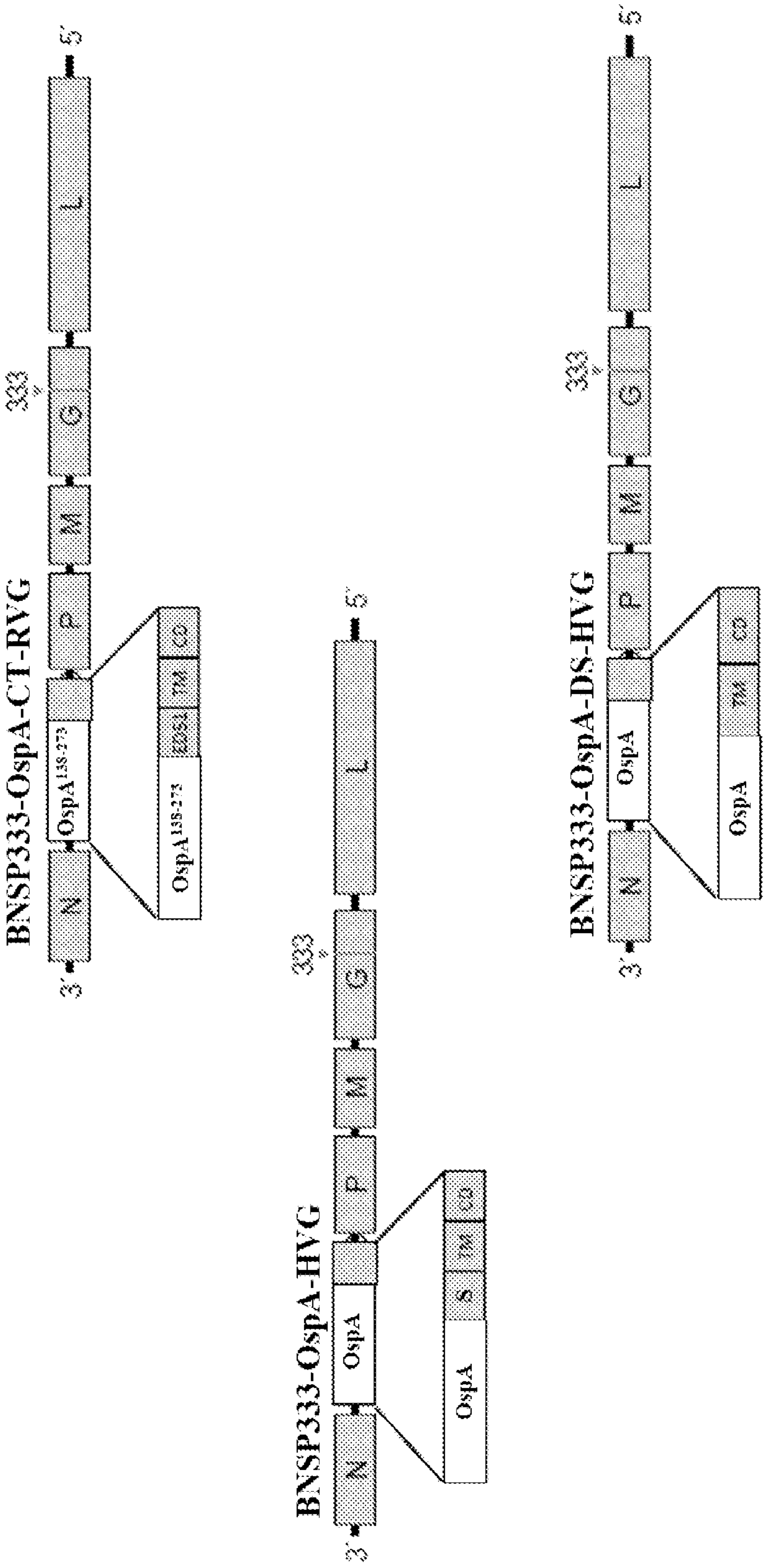

BNSP333-OspA-CT-RVG
BNSP333-OspA-HVG
BNSP333-OspA-DS-HVG
3'
5'
N
P
M
G
L
333
OspA
OspA^{138-273}
TM
CD
S

FIG. 2

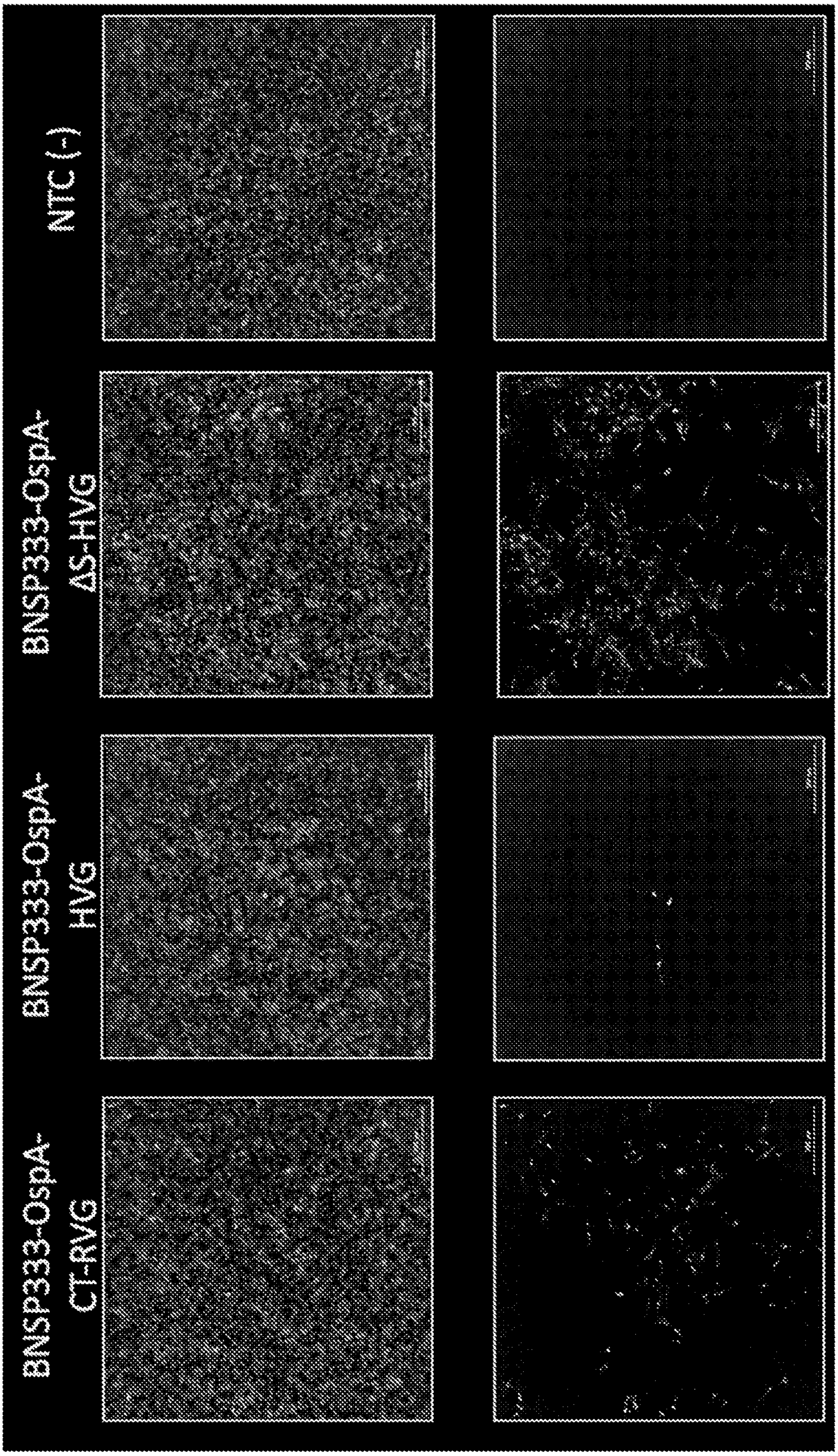
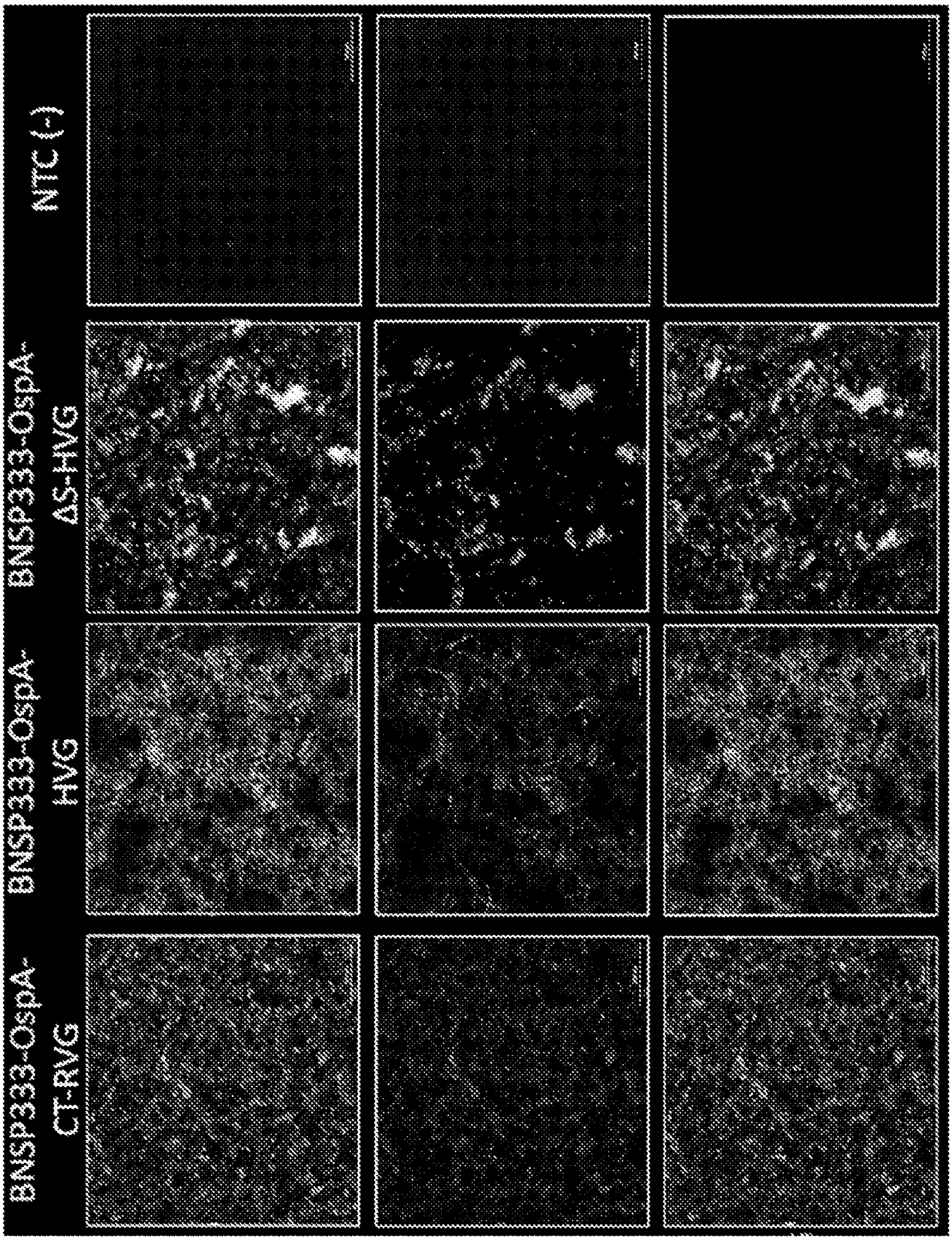


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033625

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12N 15/86*** (2024.01); ***A61K 39/02*** (2024.01); ***A61P 31/04*** (2024.01); ***C07K 14/20*** (2024.01)

CPC: ***C12N 15/86; A61K 39/0225; A61P 31/04; C07K 14/20;*** *C12N 2760/18243; C12N 2760/20143*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	US 2021/0046130 A1 (VIROGIN BIOTECH CANADA LTD) 18 February 2021 (18.02.2021)	
X	entire document	1, 2, 22, 23
Y	entire document	3, 24
	US 2018/0326046 A1 (VON MESSLING et al.) 15 November 2018 (15.11.2018)	
Y	entire document	3, 24
	US 2011/0236468 A1 (LORIN et al.) 29 September 2011 (29.09.2011)	
A	entire document	1-3, 22-24
P, X	GINGERICH et al., Intranasal vaccine for Lyme disease provides protection against tick transmitted Borrelia burgdorferi beyond one year, NPJ Vaccines, Vol. 9, No. 33, 15 February 2024, Pgs. 1-12, [retrieved 05 August 2024]. Retrieved from the Internet <URL: https://www.nature.com/articles/s41541-023-00802-y#:~:text=These%20findings%20indicate%20that%20immunization,a%20parenteral%20recombinant%20OspA%20vaccine.>. entire document	1-3, 22-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 August 2024 (08.08.2024)	**20 August 2024 (20.08.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033625

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

3. ☑ Claims Nos.: **4-21, 25-33**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).