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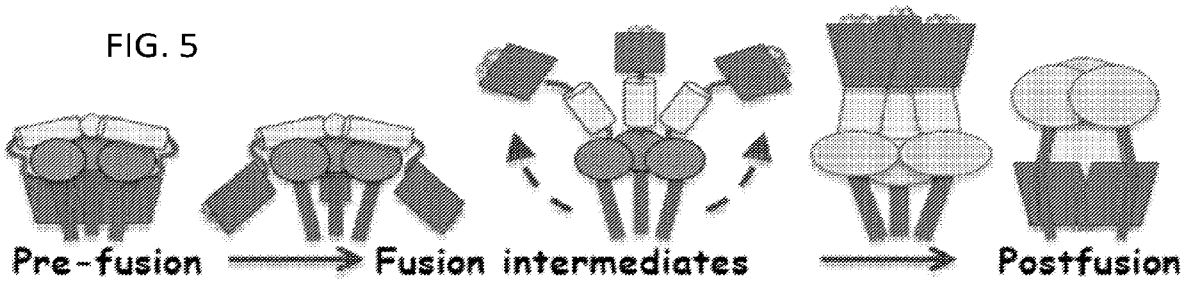
FIG. 5



(57) Abstract: The present disclosure provides pharmaceutical compositions for delivery of HSV antigens (e.g., an HSV vaccine) and related technologies (e.g., components thereof and/or methods relating thereto).

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PHARMACEUTICAL COMPOSITIONS FOR DELIVERY OF HERPES SIMPLEX VIRUS GLYCOPROTEIN B ANTIGENS AND RELATED METHODS

BACKGROUND

[0001] Herpes simplex viruses (HSV), commonly referred to only as herpes, are categorized into two types: herpes simplex virus, type 1 (HSV-1, or oral herpes) and herpes simplex virus, type 2 (HSV-2, or genital herpes). According to the World Health Organization, an estimated 3.7 billion people under age 50 (67% of global population) have HSV-1 infection globally. HSV-1 prevalence is understood as being highest in Africa and lowest in the Americas. An estimated 491 million people aged 15-49 (13% of global population) worldwide have HSV-2 infection. More women are infected with HSV-2 than men, because sexual transmission of HSV is more efficient from men to women than from women to men. Prevalence of HSV-2 infection was estimated to be highest in Africa, followed by the Americas. Prevalence of HSV-2 was also shown to increase with age, though the highest numbers of people newly-infected have historically been in adolescents. Both HSV-1 and HSV-2 infections are lifelong.

SUMMARY OF THE INVENTION

[0002] The present disclosure provides pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) for delivering particular herpes simplex virus (HSV) antigen constructs (e.g., HSV-1 antigen constructs, HSV-2 antigen constructs, or a combination thereof) to a subject (e.g., a patient) and related technologies (e.g., methods). In particular, the present disclosure provides HSV (e.g., HSV-1, HSV-2, or both) vaccine compositions and related technologies (e.g., methods).

[0003] The present disclosure also provides that HSV glycoprotein B (gB) antigens and antigenic fragments thereof can be useful in preventing or treating HSV, e.g., in HSV antigen constructs and/or HSV compositions (e.g., immunogenic compositions, e.g., vaccines) as further disclosed herein. The present disclosure further provides the recognition that stabilization of HSV gB or antigenic fragments thereof can be particularly advantageous for use, e.g., in preventing or treating HSV, e.g., in HSV antigen constructs and/or HSV compositions (e.g., immunogenic compositions, e.g., vaccines) as further disclosed herein. Accordingly, the present disclosure provides certain mutations that can stabilize HSV gB or antigenic fragments thereof.

[0004] The present disclosure provides a polyribonucleotide encoding a polypeptide. In some embodiments, a polypeptide comprises one or more HSV glycoprotein B (gB) antigens or antigenic fragments thereof.

[0005] In some embodiments, an HSV gB antigen or antigenic fragment thereof comprises one or more mutations that stabilize the HSV gB antigen or antigenic fragment thereof relative to a comparable HSV gB antigen or antigenic fragment thereof that does not comprise the one or more mutations. In some embodiments, one or more mutations are one or more amino acid substitutions. In some embodiments, one or more amino acid substitutions comprise 251C, 718C, and combinations thereof, wherein the numbering is with reference to SEQ ID NO: 1.

[0006] In some embodiments, a polypeptide comprises an HSV glycoprotein B (gB) antigen. In some embodiments, an HSV glycoprotein B (gB) antigen comprises one or more mutations that stabilize the HSV gB antigen or antigenic fragment thereof relative to a comparable HSV gB antigen or antigenic fragment thereof that does not comprise the one or more mutations. In some embodiments, one or more mutations are one or more amino acid substitutions. In some embodiments, one or more amino acid substitutions comprise 251C, 718C, and combinations thereof, wherein the numbering is with reference to SEQ ID NO: 1. In some embodiments, one or more mutations comprise: 251C and 718C; wherein the numbering is with reference to SEQ ID NO: 1.

[0007] In some embodiments, a polypeptide that comprises at least one HSV glycoprotein B (gB) antigen. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 1. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 2. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 3. In some embodiments, an

HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 4.

[0008] In some embodiments, an HSV gB antigen has an amino acid sequence that (i) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 1 and (ii) comprises one or more mutations, where the one or more mutations comprise 251C, 718C, and combinations thereof.

[0009] In some embodiments, an HSV gB antigen has an amino acid sequence that (i) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 1 and (ii) comprises one or more mutations, where the one or more mutations comprise: 251C and 718C, where the numbering is with reference to SEQ ID NO: 1.

[0010] In some embodiments, an HSV gB antigen or antigenic fragment thereof does not comprise an 516P mutation, where the numbering is with reference to SEQ ID NO: 1.

[0011] In some embodiments, a polypeptide comprises a secretory signal. In some embodiments, a secretory signal comprises or consists of a viral secretory signal. In some embodiments, a viral secretory signal comprises or consists of an HSV secretory signal. In some embodiments, an HSV secretory signal comprises or consists of an HSV-1 or HSV-2 secretory signal. In some embodiments, an HSV secretory signal comprises or consists of an HSV glycoprotein D (gD) secretory signal. In some embodiments, an HSV gD secretory signal consists of an amino acid sequence according to SEQ ID NO: 5. In some embodiments, an HSV secretory signal comprises or consists of an HSV glycoprotein B (gB) secretory signal. In some embodiments, an HSV secretory signal comprises or consists of an HSV-1 gB secretory signal. In some embodiments, an HSV secretory signal comprises or consists of an HSV-2 gB secretory signal. In some embodiments, an HSV gB secretory signal consists of an amino acid sequence according to SEQ ID NO: 63. In some embodiments, an HSV gB secretory signal consists of an amino acid sequence according to SEQ ID NO: 64. In some embodiments, an HSV gB secretory signal consists of an amino acid sequence according to SEQ ID NO: 65. In some embodiments, a secretory signal is located at the N-terminus of the polypeptide. In some embodiments, a polyribonucleotide comprises a nucleotide sequence according to SEQ ID NO: 66.

[0012] In some embodiments, a polypeptide comprises a transmembrane region. In some embodiments, a transmembrane region comprises or consists of a viral transmembrane region. In some embodiments, a transmembrane region comprises or consists of an HSV transmembrane region. In some embodiments, an HSV transmembrane region comprises or consists of an HSV-1 or HSV-2 transmembrane region. In some embodiments, an HSV transmembrane region comprises or consists of an HSV gD transmembrane region. In some embodiments, an HSV gD transmembrane region consists of an amino acid sequence according to SEQ ID NO: 42. In some embodiments, an HSV transmembrane region comprises or consists of an HSV-1 gB transmembrane region. In some embodiments, an HSV transmembrane region comprises or consists of an HSV-2 gB transmembrane region. In some embodiments, an HSV gB transmembrane region consists of an amino acid sequence according to SEQ ID NO: 67. In some embodiments, an HSV gB transmembrane region consists of an amino acid sequence according to SEQ ID NO: 68.

[0013] In some embodiments, a polypeptide does not comprise a transmembrane region.

[0014] In some embodiments, a polypeptide comprises a multimerization domain.

[0015] In some embodiments, a polypeptide comprises one or more linkers. In some embodiments, one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 45. In some embodiments, one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 47. In some embodiments, one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 48. In some embodiments, one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 52.

[0016] In some embodiments, a polyribonucleotide is an isolated polyribonucleotide.

[0017] In some embodiments, a polyribonucleotide is an engineered polyribonucleotide.

[0018] In some embodiments, a polyribonucleotide is a codon-optimized polyribonucleotide.

[0019] The present disclosure further provides an RNA construct comprises a polyribonucleotide as described herein.

[0020] In some embodiments, an RNA construct comprises a 5' UTR. In some embodiments, a 5' UTR comprises or consists of a modified human alpha-globin 5'-UTR. In some embodiments, a 5' UTR comprises or consists of a ribonucleic acid sequence according to SEQ ID NO: 57.

[0021] In some embodiments, an RNA construct comprises a 3' UTR. In some embodiments, a 3' UTR that comprises or consists of a first sequence from the amino terminal enhancer of split (AES) messenger RNA and a second sequence from the mitochondrial encoded 12S ribosomal RNA. In some embodiments, a 3' UTR comprises or consists of a ribonucleic acid sequence according to SEQ ID NO: 63.

[0022] In some embodiments, an RNA construct comprises a polyA tail sequence. In some embodiments, a polyA tail sequence is a split polyA tail sequence. In some embodiments, a split polyA tail sequence consists of a ribonucleic acid sequence according to SEQ ID NO: 60.

[0023] In some embodiments, an RNA construct comprises in 5' to 3' order: (i) a 5' UTR; (ii) a polyribonucleotide as described herein; (iii) a 3' UTR; and (iv) a polyA tail sequence.

[0024] In some embodiments, an RNA construct comprises a 5' cap. In some embodiments, an RNA construct comprises a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the polyribonucleotide.

[0025] In some embodiments, a 5' cap comprises or consists of m7(3'OMeG)(5')ppp(5')(2'OMeA_1)pG_2, wherein A_1 is position +1 of the polyribonucleotide, and G_2 is position +2 of the polyribonucleotide.

[0026] In some embodiments, a cap proximal sequence comprises A_1 and G_2 of the Cap1 structure, and a sequence comprising: $A_3A_4U_5$ (SEQ ID NO: 55) at positions +3, +4 and +5 respectively of the polyribonucleotide.

[0027] In some embodiments, a polyribonucleotide includes modified uridines in place of all uridines, optionally wherein modified uridines are each N1-methyl-pseudouridine.

[0028] The present disclosure also provides compositions. In some embodiments, a composition comprises one or more polyribonucleotides as described herein. In some embodiments, a composition comprising one or more RNA constructs as described herein.

[0029] In some embodiments, a composition comprises lipid nanoparticles, polyplexes (PLX), lipidated polyplexes (LPLX), or liposomes. In some embodiments, one or more polyribonucleotides are fully or partially encapsulated within lipid nanoparticles, polyplexes (PLX), lipidated polyplexes (LPLX), or liposomes.

[0030] In some embodiments, a composition comprises lipid nanoparticles. In some embodiments, one or more polyribonucleotides are encapsulated within lipid nanoparticles.

[0031] Among other things, the present disclosure provides pharmaceutical compositions. In some embodiments, a pharmaceutical composition comprises a composition as provided herein and at least one pharmaceutically acceptable excipient.

[0032] In some embodiments, a pharmaceutical composition comprises a cryoprotectant. In some embodiments, a cryoprotectant is sucrose.

[0033] In some embodiments, a pharmaceutical composition comprises an aqueous buffered solution. In some embodiments, an aqueous buffered solution comprises one or more of Tris base, Tris HCl, NaCl, KCl, Na_2HPO_4, and KH_2PO_4.

[0034] The present disclosure provides combinations of compositions. In some embodiments, a combination comprises a first pharmaceutical composition comprising a first polyribonucleotide, wherein the first polyribonucleotide encodes a polypeptide comprising one or more HSV gB antigens or antigenic portions thereof as described herein.

[0035] In some embodiments, a combination comprises a second pharmaceutical composition comprising a second polyribonucleotide, wherein the second polyribonucleotide encodes a second polypeptide, the second polypeptide comprises one or more HSV antigens, and wherein at least one of the HSV antigens is not gB. In some embodiments, one or more HSV antigens comprise: an HSV glycoprotein C, an HSV glycoprotein D, an HSV glycoprotein E, HSV glycoprotein B, or a combination thereof. In some embodiments, at least one of the HSV antigens is gB. In some embodiments, one or more HSV antigens comprise one or more HSV T cell antigens.

[0036] The present disclosure also provides methods. In some embodiments, a method comprises administering a polyribonucleotide described herein to a subject. In some embodiments, a method comprises administering an RNA construct described herein to a subject. In some embodiments, a method comprises administering a composition described herein to a subject. In some embodiments, a method comprises administering one or more doses of a pharmaceutical composition described herein to a subject.

[0037] In some embodiments, a method comprising administering a combination described herein to a subject. In some embodiments, a first pharmaceutical composition and a second

pharmaceutical composition are administered on the same day. In some embodiments, a first pharmaceutical composition and a second pharmaceutical composition are administered on different days. In some embodiments, a first pharmaceutical composition and a second pharmaceutical composition are administered to the subject at different locations on a subject's body.

[0038] In some embodiments, a method is a method of treating an HSV infection. In some embodiments, a method is a method of preventing an HSV infection.

[0039] In some embodiments, a subject has or is at risk of developing an HSV infection.

[0040] In some embodiments, a subject is a human.

[0041] In some embodiments, administration induces an anti-HSV immune response in the subject. In some embodiments, an anti-HSV immune response in the subject comprises an adaptive immune response. In some embodiments, an anti-HSV immune response in the subject comprises a T-cell response. In some embodiments, a T-cell response is or comprises a CD4+ T cell response. In some embodiments, a T-cell response is or comprises a CD8+ T cell response. In some embodiments, an anti-HSV immune system response comprises a B-cell response. In some embodiments, an anti-HSV immune system response comprises the production of antibodies directed against the one or more HSV gB antigens.

[0042] The present disclosure provides pharmaceutical composition described herein for use in the treatment of an HSV infection. In some embodiments, such a use comprises administering one or more doses of the pharmaceutical composition to a subject.

[0043] The present disclosure provides pharmaceutical composition described herein for use in the prevention of an HSV infection. In some embodiments, such a use comprises administering one or more doses of the pharmaceutical composition to a subject.

[0044] In some embodiments, a method or pharmaceutical composition for use as described herein comprise administering two or more doses of the pharmaceutical composition to a subject. In some embodiments, a method or pharmaceutical composition for use as described herein comprises administering three or more doses of the pharmaceutical composition to a subject.

[0045] In addition, the present disclosure provides uses of a pharmaceutical composition as provided herein in the treatment of an HSV infection. In addition, the present disclosure provides uses of

a pharmaceutical composition as provided herein in the prevention of an HSV infection. In addition, the present disclosure provides uses of a pharmaceutical composition as provided herein in inducing an anti-HSV immune response in a subject.

[0046] The present disclosure further provides polypeptides. In some embodiments, a polypeptide is encoded by a polyribonucleotide as provided herein or an RNA construct as provided herein.

[0047] Provided are also host cells. In some embodiments, a host cell comprises a polyribonucleotide as provided herein. In some embodiments, a host cell comprises an RNA construct as provided herein. In some embodiments, a host cell comprises a polypeptide as provided herein.

[0048] Still further, in many embodiments, provided compositions (e.g., pharmaceutical compositions, e.g., immunogenic compositions, e.g., vaccines) that include an RNA construct (e.g., as an active) are prepared, formulated, and/or utilized in particular LNP compositions, as described herein.

[0049] Among other things, the present disclosure provides technologies for rapid development of a pharmaceutical composition (e.g., immunogenic composition, e.g., HSV vaccine) for delivering particular HSV (e.g., HSV-1 and/or HSV-2) antigen constructs to a subject.

[0050] Additionally, the present disclosure provides, for example, nucleic acid constructs encoding HSV (e.g., HSV-1 and/or HSV-2) antigens as described herein, expressed HSV (e.g., HSV-1 and/or HSV-2) proteins, and various methods of production and/or use relating thereto, as well as compositions developed therewith and methods relating thereto.

[0051] For example, the present disclosure provides technologies for preventing, characterizing, treating, and/or monitoring HSV (e.g., HSV-1 and/or HSV-2) outbreaks and/or infections including, as noted, various nucleic acid constructs and encoded proteins, as well as agents (e.g., antibodies) that bind to such proteins, and compositions that comprise and/or deliver them.

[0052] In some aspects, provided herein are technologies (e.g., compositions and methods) for augmenting, inducing, promoting, enhancing and/or improving an immune response against HSV (e.g., HSV-1 and/or HSV-2) or a component thereof (e.g., a protein or fragment thereof). In some embodiments, technologies provided herein are designed to augment, induce, promote, enhance and/or improve immunological memory against HSV (e.g., HSV-1 and/or HSV-2) or a component thereof (e.g., a protein or fragment thereof). In some embodiments, technologies described herein are designed to act as an immunological boost to a primary vaccine, such as a vaccine directed to an epitope and/or epitopes of

HSV (e.g., HSV-1 and/or HSV-2). In some embodiments, compositions of the present disclosure comprise one or more polynucleotide constructs (e.g., one or more string constructs) that encode one or more epitopes from HSV (e.g., HSV-1 and/or HSV-2). In some embodiments, the present disclosure provides vaccines or other compositions comprising nucleic acids encoding such HSV (e.g., HSV-1 and/or HSV-2) epitopes; those skilled in the art will appreciate from context when reference to a particular polynucleotide (e.g., a DNA or RNA) as "encoding" such epitopes in fact is referencing a coding strand or its complement.

BRIEF DESCRIPTION OF THE DRAWING

[0053] The Drawing included herein, which is composed of the following Figures, is for illustration purposes only and not for limitation.

[0054] **Figure 1** is a schematic of an HSV particle.

[0055] **Figure 2** is a schematic overview of the HSV life cycle. Figure 2 has been modified from Ibanez, F.J., et al., "Experimental Dissection of the Lytic Replication Cycles of Herpes Simplex Virus in vitro," Front Microbiol. 2018; 9: 2406, which is incorporated herein by reference in its entirety.

[0056] **Figure 3** is a schematic of a model of HSV latent infection. Figure 3 has been modified from Knipe, D.M., et al., "Clues to mechanisms of herpesviral latent infection and potential cures," PNAS September 29, 2015 112 (39) 11993-11994, which is incorporated herein by reference in its entirety.

[0057] **Figure 4** is a summary table of clinical trial results with HSV vaccine candidates. The table has been modified from Aschner, C. B., & Herold, B. C. (2021), Alphaherpesvirus vaccines. Current Issues in Molecular Biology, 41, 469-508, which is incorporated herein by reference in its entirety.

[0059] **Figure 5** depicts conformational changes in HSV glycoprotein B (gB). Cell entry of HSV is achieved via viral proteins that mediates fusion with the host membrane by substantial structural rearrangements of viral proteins, including gB, from a metastable prefusion conformation to a stable postfusion conformation.

CERTAIN DEFINITIONS

[0060] In general, terminology used herein is in accordance with its understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context.

[0061] In order that the present invention may be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0062] ***About***: The term "about", when used herein in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by "about" in that context. For example, in some embodiments, the term "about" may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[0063] ***Agent***: As used herein, the term "agent", may refer to a physical entity or phenomenon. In some embodiments, an agent may be characterized by a particular feature and/or effect. In some embodiments, an agent may be a compound, molecule, or entity of any chemical class including, for example, a small molecule, polypeptide, nucleic acid, saccharide, lipid, metal, or a combination or complex thereof. In some embodiments, the term "agent" may refer to a compound, molecule, or entity that comprises a polymer. In some embodiments, the term may refer to a compound or entity that comprises one or more polymeric moieties. In some embodiments, the term "agent" may refer to a compound, molecule, or entity that is substantially free of a particular polymer or polymeric moiety. In some embodiments, the term may refer to a compound, molecule, or entity that lacks or is substantially free of any polymer or polymeric moiety.

[0064] ***Amino acid***: In its broadest sense, as used herein, the term "amino acid" refers to a compound and/or substance that can be, is, or has been incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In some embodiments, an amino acid is a naturally-occurring amino

acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (e.g., of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some embodiments, such modification may, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some embodiments, the term "amino acid" may be used to refer to a free amino acid; in some embodiments it may be used to refer to an amino acid residue of a polypeptide.

[0065] ***Antibody agent***: As used herein, the term "antibody agent" refers to an agent that specifically binds to a particular antigen. In some embodiments, the term encompasses a polypeptide or polypeptide complex that includes immunoglobulin structural elements sufficient to confer specific binding. For example, in some embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes one or more structural elements recognized by those skilled in the art as a complementarity determining region (CDR); in some embodiments an antibody agent is or comprises a polypeptide whose amino acid sequence includes at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR) that is substantially identical to one found in a reference antibody. In some embodiments an included CDR is substantially identical to a reference CDR in that it is either identical in sequence or contains between 1-5 amino acid substitutions as compared with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that it shows at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that it shows at least 96%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments an included CDR is substantially identical to a

reference CDR in that at least one amino acid within the included CDR is deleted, added, or substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that 1-5 amino acids within the included CDR are deleted, added, or substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical to the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that at least one amino acid within the included CDR is substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that 1-5 amino acids within the included CDR are deleted, added, or substituted as compared with the reference CDR but the included CDR has an amino acid sequence that is otherwise identical to the reference CDR. In some embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes structural elements recognized by those skilled in the art as an immunoglobulin variable domain. In some embodiments, an antibody agent in or comprises a polypeptide whose amino acid sequence includes structural elements recognized by those skilled in the art to correspond to CDRs1, 2, and 3 of an antibody variable domain; in some such embodiments, an antibody agent in or comprises a polypeptide or set of polypeptides whose amino acid sequence(s) together include structural elements recognized by those skilled in the art to correspond to both heavy chain and light chain variable region CDRs, e.g., heavy chain CDRs 1, 2, and/or 3 and light chain CDRs 1, 2, and/or 3. In some embodiments, an antibody agent is a polypeptide protein having a binding domain which is homologous or largely homologous to an immunoglobulin-binding domain. In some embodiments, an antibody agent may be or comprise a polyclonal antibody preparation. In some embodiments, an antibody agent may be or comprise a monoclonal antibody preparation. In some embodiments, an antibody agent may include one or more constant region sequences that are characteristic of a particular organism, such as a camel, human, mouse, primate, rabbit, rat; in many embodiments, an antibody agent may include one or more constant region sequences that are characteristic of a human. In some embodiments, an antibody agent may include one or more sequence elements that would be recognized by one skilled in the art as a humanized sequence, a primatized sequence, a chimeric sequence, etc. In some embodiments, an antibody agent may be a canonical antibody (e.g., may comprise two heavy chains and two light chains). In some embodiments, an antibody agent may be in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi- specific antibodies (e.g., Zybodies®, etc); antibody fragments such as Fab

fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPs™"); single chain or Tandem diabodies (TandAb®); VHHs; Anticalins®; Nanobodies® minibodies; BiTE®s; ankyrin repeat proteins or DARPINs®; Avimers®; DARTs; TCR-like antibodies;, Adnectins®; Affilins®; Trans-bodies®; Affibodies®; TrimerX®; MicroProteins; Fynomers®, Centyrins®; and KALBITOR®s. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload (e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc.), or other pendant group (e.g., poly-ethylene glycol, etc.)).

[0066] ***Antigen***: Those skilled in the art, reading the present specification, will appreciate that the term "antigen" refers to a molecule that is recognized by the immune system, e.g., in particular embodiments the adaptive immune system, such that it elicits an antigen-specific immune response. In some embodiments, an antigen-specific immune response may be or comprise generation of antibodies and/or antigen-specific T cells. In some embodiments, an antigen is a peptide or polypeptide that comprises at least one epitope against which an immune response can be generated. In one embodiment, an antigen is presented by cells of the immune system such as antigen presenting cells like dendritic cells or macrophages. In one embodiments, an antigen or a processed product thereof such as a T-cell epitope is bound by a T- or B-cell receptor, or by an immunoglobulin molecule such as an antibody. Accordingly, an antigen or a processed product thereof may react specifically with antibodies or T lymphocytes (T cells). In one embodiment, an antigen is a parasitic antigen. In accordance with the present disclosure, in some embodiments, an antigen may be delivered by RNA molecules as described herein. In some embodiments, a peptide or polypeptide antigen can be 2-100 amino acids, including for example, 5 amino acids, 10 amino acids, 15 amino acids, 20 amino acids, 25 amino acids, 30 amino acids, 35 amino acids, 40 amino acids, 45 amino acids, or 50 amino acids in length. In some embodiments, a peptide or polypeptide antigen can be greater than 50 amino acids. In some embodiments, a peptide or polypeptide antigen can be greater than 100 amino acids. In some embodiments, an antigen is recognized by an immune effector cell. In some embodiments, an antigen if recognized by an immune effector cell is able to induce in the presence of appropriate co-stimulatory signals, stimulation, priming and/or expansion of the immune effector cell carrying an antigen receptor recognizing the antigen. In the context of the

embodiments of the present disclosure, in some embodiments, an antigen can be presented or present on the surface of a cell, e.g., an antigen presenting cell. In one embodiment, an antigen is presented by a diseased cell such as a virus-infected cell. In one embodiment, an antigen receptor is a TCR which binds to an epitope of an antigen presented in the context of MHC. In one embodiment, binding of a TCR when expressed by T cells and/or present on T cells to an antigen presented by cells such as antigen presenting cells results in stimulation, priming and/or expansion of said T cells. In one embodiment, binding of a TCR when expressed by T cells and/or present on T cells to an antigen presented on diseased cells results in cytolysis and/or apoptosis of the diseased cells, wherein said T cells preferably release cytotoxic factors, e.g. perforins and granzymes.

[0067] ***Associated***: Two events or entities are "associated" with one another, as that term is used herein, if the presence, level, degree, type and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide, genetic signature, metabolite, microbe, etc) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of, susceptibility to, severity of, stage of, etc the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0068] ***Binding***: Those skilled in the art, reading the present specification, will appreciate that the term "binding" typically refers to a non-covalent association between or among entities or moieties. In some embodiments, binding data are expressed in terms of "IC50". As is understood in the art, IC50 is the concentration of an assessed agent in a binding assay at which 50% inhibition of binding of reference agent known to bind the relevant binding partner is observed. In some embodiments, assays are run under conditions in which the assays are run (*e.g.*, limiting binding target and reference concentrations), these values approximate K_D values. Assays for determining binding are well known in the art and are described in detail, for example, in PCT publications WO 94/20127 and WO 94/03205, and other publications such Sidney et al., Current Protocols in Immunology 18.3.1 (1998); Sidney, et al., J.

Immunol. 154:247 (1995); and Sette, et al., Mol. Immunol. 31:813 (1994). Alternatively, binding can be expressed relative to binding by a reference standard peptide. For example, can be based on its IC_{50}, relative to the IC_{50} of a reference standard peptide. Binding can also be determined using other assay systems including those using: live cells (e.g., Ceppellini et al., Nature 339:392 (1989); Christnick et al., Nature 352:67 (1991); Busch et al., Int. Immunol. 2:443 (1990); Hill et al., J. Immunol. 147:189 (1991); del Guercio et al., J. Immunol. 154:685 (1995)), cell free systems using detergent lysates (e.g., Cerundolo et al., J. Immunol 21:2069 (1991)), immobilized purified MHC (e.g., Hill et al., J. Immunol. 152, 2890 (1994); Marshall et al., J. Immunol. 152:4946 (1994)), ELISA systems (e.g., Reay et al., EMBO J. 11:2829 (1992)), surface plasmon resonance (e.g., Khilko et al., J. Biol. Chem. 268:15425 (1993)); high flux soluble phase assays (Hammer et al., J. Exp. Med. 180:2353 (1994)), and measurement of class I MHC stabilization or assembly (e.g., Ljunggren et al., Nature 346:476 (1990); Schumacher et al., Cell 62:563 (1990); Townsend et al., Cell 62:285 (1990); Parker et al., J. Immunol. 149:1896 (1992)).

[0069] ***Cap***: As used herein, the term "cap" refers to a structure comprising or essentially consisting of a nucleoside-5 '-triphosphate that is typically joined to a 5'-end of an uncapped RNA (*e.g.*, an uncapped RNA having a 5'- diphosphate). In some embodiments, a cap is or comprises a guanine nucleotide. In some embodiments, a cap is or comprises a naturally-occurring RNA 5' cap, including, *e.g.*, but not limited to a 7- methylguanosine cap, which has a structure designated as "m7G." In some embodiments, a cap is or comprises a synthetic cap analog that resembles an RNA cap structure and possesses the ability to stabilize RNA if attached thereto, including, *e.g.*, but not limited to anti-reverse cap analogs (ARCAs) known in the art). Those skilled in the art will appreciate that methods for joining a cap to a 5' end of an RNA are known in the art. For example, in some embodiments, a capped RNA may be obtained by *in vitro* capping of RNA that has a 5' triphosphate group or RNA that has a 5' diphosphate group with a capping enzyme system (including, *e.g.*, but not limited to vaccinia capping enzyme system or Saccharomyces cerevisiae capping enzyme system). Alternatively, a capped RNA can be obtained by *in vitro* transcription (IVT) of a single-stranded DNA template in the presence of a dinucleotide or trinucleotide cap analog.

[0070] ***Cell-mediated immunity***: "Cell-mediated immunity," "cellular immunity," "cellular immune response," or similar terms are meant to include a cellular response directed to cells characterized by expression of an antigen, in particular characterized by presentation of an antigen with class I or class II MHC. A cellular response relates to immune effector cells, in particular to T cells or T lymphocytes

which act as either "helpers" or "killers." The helper T cells (also termed $CD4^+$ T cells or CD4 T cells) play a central role by regulating the immune response and the killer cells (also termed cytotoxic T cells, cytolytic T cells, $CD8^+$ T cells, CD8 T cells, or CTLs) kill diseased cells such as virus-infected cells, preventing the production of more diseased cells.

[0071] ***Co-administration***: As used herein, the term "co-administration" refers to use of a pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) described herein and an additional therapeutic agent. The combined use of a pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) described herein and an additional therapeutic agent may be performed concurrently or separately (*e.g.*, sequentially in any order). In some embodiments, a pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) described herein and an additional therapeutic agent may be combined in one pharmaceutically-acceptable carrier, or they may be placed in separate carriers and delivered to a target cell or administered to a subject at different times. Each of these situations is contemplated as falling within the meaning of "co-administration" or "combination," provided that a pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) described herein and an additional therapeutic agent are delivered or administered sufficiently close in time that there is at least some temporal overlap in biological effect(s) generated by each on a target cell or a subject being treated.

[0072] ***Codon-optimized***: As used herein, the term "codon-optimized" refers to alteration of codons in a coding region of a nucleic acid molecule to reflect the typical codon usage of a host organism without preferably altering the amino acid sequence encoded by the nucleic acid molecule. Within the context of the present disclosure, in some embodiments coding regions are codon-optimized for optimal expression in a subject to be treated using the RNA molecules described herein. In some embodiments, codon-optimization may be performed such that codons for which frequently occurring tRNAs are available are inserted in place of "rare codons." In some embodiments, codon-optimization may include increasing guanosine/cytosine (G/C) content of a coding region of RNA described herein as compared to the G/C content of the corresponding coding sequence of a wild type RNA, wherein the amino acid sequence encoded by the RNA is preferably not modified compared to the amino acid sequence.

[0073] ***Combination therapy***: As used herein, the term "combination therapy" refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (e.g., two or more therapeutic agents). In some embodiments, the two or more regimens may be administered

simultaneously; in some embodiments, such regimens may be administered sequentially (e.g., all "doses" of a first regimen are administered prior to administration of any doses of a second regimen); in some embodiments, such agents are administered in overlapping dosing regimens. In some embodiments, "administration" of combination therapy may involve administration of one or more agent(s) or modality(ies) to a subject receiving the other agent(s) or modality(ies) in the combination. For clarity, combination therapy does not require that individual agents be administered together in a single composition (or even necessarily at the same time), although in some embodiments, two or more agents, or active moieties thereof, may be administered together in a combination composition.

[0074] ***Comparable:*** As used herein, the term "comparable" refers to two or more agents, entities, situations, sets of conditions, etc., that may not be identical to one another but that are sufficiently similar to permit comparison there between so that one skilled in the art will appreciate that conclusions may reasonably be drawn based on differences or similarities observed. In some embodiments, comparable sets of conditions, circumstances, individuals, or populations are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc to be considered comparable. For example, those of ordinary skill in the art will appreciate that sets of circumstances, individuals, or populations are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under or with different sets of circumstances, individuals, or populations are caused by or indicative of the variation in those features that are varied.

[0075] ***Corresponding to:*** As used herein, the term "corresponding to" refers to a relationship between two or more entities. For example, the term "corresponding to" may be used to designate the position/identity of a structural element in a compound or composition relative to another compound or composition (e.g., to an appropriate reference compound or composition). For example, in some embodiments, a monomeric residue in a polymer (e.g., an amino acid residue in a polypeptide or a nucleic acid residue in a polynucleotide) may be identified as "corresponding to" a residue in an appropriate reference polymer. For example, those of ordinary skill will appreciate that, for purposes of simplicity, residues in a polypeptide are often designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid "corresponding to" a residue at position 190, for example, need

not actually be the 190th amino acid in a particular amino acid chain but rather corresponds to the residue found at 190 in the reference polypeptide; those of ordinary skill in the art readily appreciate how to identify "corresponding" amino acids. For example, those skilled in the art will be aware of various sequence alignment strategies, including software programs such as, for example, BLAST, CS-BLAST, CUSASW++, DIAMOND, FASTA, GGSEARCH/GLSEARCH, Genoogle, HMMER, HHpred/HHsearch, IDF, Infernal, KLAST, USEARCH, parasail, PSI-BLAST, PSI-Search, ScalaBLAST, Sequilab, SAM, SSEARCH, SWAPHI, SWAPHI-LS, SWIMM, or SWIPE that can be utilized, for example, to identify "corresponding" residues in polypeptides and/or nucleic acids in accordance with the present disclosure. Those of skill in the art will also appreciate that, in some instances, the term "corresponding to" may be used to describe an event or entity that shares a relevant similarity with another event or entity (e.g., an appropriate reference event or entity). To give but one example, a gene or protein in one organism may be described as "corresponding to" a gene or protein from another organism in order to indicate, in some embodiments, that it plays an analogous role or performs an analogous function and/or that it shows a particular degree of sequence identity or homology, or shares a particular characteristic sequence element.

[0076] ***Derived***: In the context of an amino acid sequence (peptide or polypeptide) "derived from" a designated amino acid sequence (peptide or polypeptide), it refers to a structural analogue of a designated amino acid sequence. In some embodiments, an amino acid sequence which is derived from a particular amino acid sequence has an amino acid sequence that is identical, essentially identical or homologous to that particular sequence or a fragment thereof. Amino acid sequences derived from a particular amino acid sequence may be variants of that particular sequence or a fragment thereof. For example, it will be understood by one of ordinary skill in the art that the antigens suitable for use herein may be altered such that they vary in sequence from the naturally occurring or native sequences from which they were derived, while retaining the desirable activity of the native sequences.

[0077] ***Designed***: As used herein, the term "designed" refers to an agent (i) whose structure is or was selected by the hand of man; (ii) that is produced by a process requiring the hand of man; and/or (iii) that is distinct from natural substances and other known agents.

[0078] ***Dosing regimen***: Those skilled in the art will appreciate that the term "dosing regimen" may be used to refer to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a

recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, individual doses are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (*i.e.*, is a therapeutic dosing regimen).

[0079] ***Encode***: As used herein, the term "encode" or "encoding" refers to sequence information of a first molecule that guides production of a second molecule having a defined sequence of nucleotides (e.g., mRNA) or a defined sequence of amino acids. For example, a DNA molecule can encode an RNA molecule (e.g., by a transcription process that includes a DNA-dependent RNA polymerase enzyme). An RNA molecule can encode a polypeptide (e.g., by a translation process). Thus, a gene, a cDNA, or an RNA molecule (e.g., an mRNA) encodes a polypeptide if transcription and translation of mRNA corresponding to that gene produces the polypeptide in a cell or other biological system. In some embodiments, a coding region of an RNA molecule encoding a target antigen refers to a coding strand, the nucleotide sequence of which is identical to the mRNA sequence of such a target antigen. In some embodiments, a coding region of an RNA molecule encoding a target antigen refers to a non-coding strand of such a target antigen, which may be used as a template for transcription of a gene or cDNA.

[0080] ***Engineered***: In general, the term "engineered" refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be "engineered" when two or more sequences that are not linked together in that order in nature are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide and/or when a particular residue in a polynucleotide is non-naturally occurring and/or is caused through action of the hand of man to be linked with an entity or moiety with which it is not linked in nature.

[0081] ***Epitope***: As used herein, the term "epitope" refers to a moiety that is specifically recognized by an immunoglobulin (e.g., antibody or receptor) binding component. For example, an epitope may be recognized by a T cell, a B cell, or an antibody. In some embodiments, an epitope is comprised of a plurality of chemical atoms or groups on an antigen. In some embodiments, such chemical atoms or groups are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some embodiments, such chemical atoms or groups are physically near to each other in space when the antigen adopts such a conformation. In some embodiments, at least some such chemical atoms are groups are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized). Accordingly, in some embodiments, an epitope of an antigen may include a continuous or discontinuous portion of the antigen. In some embodiments, an epitope is or comprises a T cell epitope. In some embodiments, an epitope may have a length of about 5 to about 30 amino acids, or about 10 to about 25 amino acids, or about 5 to about 15 amino acids, or about 5 to 12 amino acids, or about 6 to about 9 amino acids.

[0082] ***Expression***: As used herein, the term "expression" of a nucleic acid sequence refers to the generation of a gene product from the nucleic acid sequence. In some embodiments, a gene product can be a transcript. In some embodiments, a gene product can be a polypeptide. In some embodiments, expression of a nucleic acid sequence involves one or more of the following: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, etc); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0083] ***Five prime untranslated region***: As used herein, the terms "five prime untranslated region" or "5' UTR" refer to a sequence of an mRNA molecule between a transcription start site and a start codon of a coding region of an RNA. In some embodiments, "5' UTR" refers to a sequence of an mRNA molecule that begins at a transcription start site and ends one nucleotide (nt) before a start codon (usually AUG) of a coding region of an RNA molecule, e.g., in its natural context.

[0084] ***Fragment***: The term "fragment" as used herein in the context of a nucleic acid sequence (e.g. RNA sequence) or an amino acid sequence may typically be a portion of a reference sequence. In some embodiments, a reference sequence is a full-length sequence of e.g. a nucleic acid sequence or an amino acid sequence. Accordingly, a fragment, typically, refers to a sequence that is identical to a corresponding stretch within a reference sequence. In some embodiments, a fragment comprises a

continuous stretch of nucleotides or amino acid residues that corresponds to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% of the total length of a reference sequence from which the fragment is derived. In some embodiments, the term "fragment", with reference to an amino acid sequence (peptide or polypeptide), relates to a part of an amino acid sequence, e.g., a sequence which represents the amino acid sequence shortened at the N-terminus and/or C-terminus. In some embodiments, a fragment of an amino acid sequence comprises at least 6, in particular at least 8, at least 12, at least 15, at least 20, at least 30, at least 50, or at least 100 consecutive amino acids from an amino acid sequence.

[0085] ***Homology:*** As used herein, the term "homology" or "homolog" refers to the overall relatedness between polynucleotide molecules (e.g., DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polynucleotide molecules (e.g., DNA molecules and/or RNA molecules) and/or polypeptide molecules are considered to be "homologous" to one another if their sequences are at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. In some embodiments, polynucleotide molecules (e.g., DNA molecules and/or RNA molecules) and/or polypeptide molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% similar (e.g., containing residues with related chemical properties at corresponding positions). For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as similar to one another as "hydrophobic" or "hydrophilic" amino acids, and/or as having "polar" or "non-polar" side chains. Substitution of one amino acid for another of the same type may often be considered a "homologous" substitution.

[0086] ***Humoral immunity:*** As used herein, the term "humoral immunity" or "humoral immune response" refers to antibody production and the accessory processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. It also refers to the effector functions of antibodies, which include pathogen neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination.

[0087] ***Identity:*** As used herein, the term "identity" refers to the overall relatedness between polynucleotide molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polynucleotide molecules (*e.g.*, DNA molecules and/or RNA

molecules) and/or between polypeptide molecules are considered to be "substantially identical" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (e.g., nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller, 1989, which has been incorporated into the ALIGN program (version 2.0). In some embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0088] ***Increased, Induced, or Reduced:*** As used herein, these terms or grammatically comparable comparative terms, indicate values that are relative to a comparable reference measurement. For example, in some embodiments, an assessed value achieved with a provided pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) may be "increased" relative to that obtained with a comparable reference pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine). Alternatively or additionally, in some embodiments, an assessed value achieved in a subject may be "increased" relative to that obtained in the same subject under different conditions (e.g., prior to or after an event; or presence or absence of an event such as administration of a pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) as described herein, or in a different, comparable subject (e.g.,

in a comparable subject that differs from the subject of interest in prior exposure to a condition, e.g., absence of administration of a pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) as described herein.). In some embodiments, comparative terms refer to statistically relevant differences (e.g., that are of a prevalence and/or magnitude sufficient to achieve statistical relevance). Those skilled in the art will be aware, or will readily be able to determine, in a given context, a degree and/or prevalence of difference that is required or sufficient to achieve such statistical significance. In some embodiments, the term "reduced" or equivalent terms refers to a reduction in the level of an assessed value by at least 5%, at least 10%, at least 20%, at least 50%, at least 75% or higher, as compared to a comparable reference. In some embodiments, the term "reduced" or equivalent terms refers to a complete or essentially complete inhibition, i.e., a reduction to zero or essentially to zero. In some embodiments, the term "increased" or "induced" refers to an increase in the level of an assessed value by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 80%, at least 100%, at least 200%, at least 500%, or higher, as compared to a comparable reference.

[0089] ***Ionizable***: The term "ionizable" refers to a compound or group or atom that is charged at a certain pH. In the context of an ionizable amino lipid, such a lipid or a function group or atom thereof bears a positive charge at a certain pH. In some embodiments, an ionizable amino lipid is positively charged at an acidic pH. In some embodiments, an ionizable amino lipid is predominately neutral at physiological pH values, e.g., in some embodiments about 7.0-7.4, but becomes positively charged at lower pH values. In some embodiments, an ionizable amino lipid may have a pKa within a range of about 5 to about 7.

[0090] ***Isolated:*** The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated", but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated". An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

[0091] ***Lipid***: As used herein, the terms "lipid" and "lipid-like material" are broadly defined as molecules which comprise one or more hydrophobic moieties or groups and optionally also one or more hydrophilic moieties or groups. Molecules comprising hydrophobic moieties and hydrophilic moieties are also typically denoted as amphiphiles.

[0092] ***RNA lipid nanoparticle***: As used herein, the term "RNA lipid nanoparticle" refers to a nanoparticle comprising at least one lipid and RNA molecule(s). In some embodiments, an RNA lipid nanoparticle comprises at least one ionizable amino lipid. In some embodiments, an RNA lipid nanoparticle comprises at least one ionizable amino lipid, at least one helper lipid, and at least one polymer-conjugated lipid (e.g., PEG-conjugated lipid). In various embodiments, RNA lipid nanoparticles as described herein can have an average size (*e.g.*, Z-average) of about 100 nm to 1000 nm, or about 200 nm to 900 nm, or about 200 nm to 800 nm, or about 250 nm to about 700 nm. In some embodiments of the present disclosure, RNA lipid nanoparticles can have a particle size (e.g., Z-average) of about 30 nm to about 200 nm, or about 30 nm to about 150 nm, about 40 nm to about 150 nm, about 50 nm to about 150 nm, about 60 nm to about 130 nm, about 70 nm to about 110 nm, about 70 nm to about 100 nm, about 80 nm to about 100 nm, about 90 nm to about 100 nm, about 70 to about 90 nm, about 80 nm to about 90 nm, or about 70 nm to about 80 nm. In some embodiments, an average size of lipid nanoparticles is determined by measuring the particle diameter. In some embodiments, RNA lipid nanoparticles may be prepared by mixing lipids with RNA molecules described herein.

[0093] ***Lipidoid***: As used herein, a "lipidoid" refers to a lipid-like molecule. In some embodiments, a lipoid is an amphiphilic molecule with one or more lipid-like physical properties. In the context of the present disclosure, the term lipid is considered to encompass lipidoids.

[0094] ***Nanoparticle***: As used herein, the term "nanoparticle" refers to a particle having an average size suitable for parenteral administration. In some embodiments, a nanoparticle has a longest dimension (*e.g.*, a diameter) of less than 1,000 nanometers (nm). In some embodiments, a nanoparticle may be characterized by a longest dimension (*e.g.*, a diameter) of less than 300 nm. In some embodiments, a nanoparticle may be characterized by a longest dimension (e.g., a diameter) of less than 100 nm. In many embodiments, a nanoparticle may be characterized by a longest dimension between about 1 nm and about 100 nm, or between about 1 μm and about 500 nm, or between about 1 nm and 1,000 nm. In many embodiments, a population of nanoparticles is characterized by an average size (e.g., longest dimension) that is below about 1,000 nm, about 500 nm, about 100 nm, about 50 nm, about 40 nm, about 30 nm, about 20 nm, or about 10 nm and often above about 1 nm. In many embodiments, a nanoparticle may be substantially spherical so that its longest dimension may be its diameter. In some embodiments, a nanoparticle has a diameter of less than 100 nm as defined by the National Institutes of Health.

[0095] ***Naturally occurring***: The term "naturally occurring" as used herein refers to an entity that can be found in nature. For example, a peptide or nucleic acid that is present in an organism (including viruses) and can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

[0096] ***Neutralization***: As used herein, the term "neutralization" refers to an event in which binding agents such as antibodies bind to a biological active site of a virus such as a receptor binding protein, thereby inhibiting the parasitic infection of cells. In some embodiments, the term "neutralization" refers to an event in which binding agents eliminate or significantly reduce ability of infecting cells.

[0097] ***Nucleic acid particle***: A "nucleic acid particle" can be used to deliver nucleic acid to a target site of interest (e.g., cell, tissue, organ, and the like). A nucleic acid particle may comprise at least one cationic or cationically ionizable lipid or lipid-like material, at least one cationic polymer such as protamine, or a mixture thereof and nucleic acid. In some embodiments, a nucleic acid particle is a lipid nanoparticle. In some embodiments, a nucleic acid particle is a lipoplex particle.

[0098] ***Nucleic acid/ Polynucleotide***: As used herein, the term "nucleic acid" refers to a polymer of at least 10 nucleotides or more. In some embodiments, a nucleic acid is or comprises DNA. In some embodiments, a nucleic acid is or comprises RNA. In some embodiments, a nucleic acid is or comprises peptide nucleic acid (PNA). In some embodiments, a nucleic acid is or comprises a single stranded nucleic acid. In some embodiments, a nucleic acid is or comprises a double-stranded nucleic acid. In some embodiments, a nucleic acid comprises both single and double-stranded portions. In some embodiments, a nucleic acid comprises a backbone that comprises one or more phosphodiester linkages. In some embodiments, a nucleic acid comprises a backbone that comprises both phosphodiester and non-phosphodiester linkages. For example, in some embodiments, a nucleic acid may comprise a backbone that comprises one or more phosphorothioate or 5'-N-phosphoramidite linkages and/or one or more peptide bonds, e.g., as in a "peptide nucleic acid". In some embodiments, a nucleic acid comprises one or more, or all, natural residues (e.g., adenine, cytosine, deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, guanine, thymine, uracil). In some embodiments, a nucleic acid comprises on or more, or all, non-natural residues. In some embodiments, a non-natural residue comprises a nucleoside analog (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3 -methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5 -propynyl-cytidine, C5-methylcytidine, 2-

aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 6-O-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a non-natural residue comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared to those in natural residues. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or polypeptide. In some embodiments, a nucleic acid has a nucleotide sequence that comprises one or more introns. In some embodiments, a nucleic acid may be prepared by isolation from a natural source, enzymatic synthesis (e.g., by polymerization based on a complementary template, *e.g.*, *in vivo* or *in vitro*, reproduction in a recombinant cell or system, or chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 1 10, 120, 130, 140, 150, 160, 170, 180, 190, 20, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, or 20,000 or more residues or nucleotides long.

[0099] ***Nucleotide:*** As used herein, the term "nucleotide" refers to its art-recognized meaning. When a number of nucleotides is used as an indication of size, e.g., of a polynucleotide, a certain number of nucleotides refers to the number of nucleotides on a single strand, e.g., of a polynucleotide.

[0100] ***Patient:*** As used herein, the term "patient" refers to any organism who is suffering or at risk of a disease or disorder or condition. Typical patients include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. In some embodiments, a patient is suffering from or susceptible to one or more diseases or disorders or conditions. In some embodiments, a patient displays one or more symptoms of a disease or disorder or condition. In some embodiments, a patient has been diagnosed with one or more diseases or disorders or conditions. In some embodiments, a disease or disorder or condition that is amenable to provided technologies is or includes an HSV infection. In some embodiments, a patient is receiving or has received certain therapy to diagnose and/or to treat a disease, disorder, or condition. In some embodiments, a patient is a patient suffering from or susceptible to an HSV infection.

[0101] ***PEG-conjugated lipid***: The term "PEG-conjugated lipid" refers to a molecule comprising a lipid portion and a polyethylene glycol portion.

[0102] ***Pharmaceutical composition***: As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for parenteral administration, for example, by subcutaneous, intramuscular, or intravenous injection as, for example, a sterile solution or suspension formulation.

[0103] ***Pharmaceutically effective amount***: The term "pharmaceutically effective amount" or "therapeutically effective amount" refers to the amount which achieves a desired reaction or a desired effect alone or together with further doses. In the case of the treatment of a particular disease, a desired reaction in some embodiments relates to inhibition of the course of the disease. In some embodiments, such inhibition may comprise slowing down the progress of a disease and/or interrupting or reversing the progress of the disease. In some embodiments, a desired reaction in a treatment of a disease may be or comprise delay or prevention of the onset of a disease or a condition. An effective amount of pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) described herein will depend, for example, on a disease or condition to be treated, the severity of such a disease or condition, individual parameters of the patient, including, e.g., age, physiological condition, size and weight, the duration of treatment, the type of an accompanying therapy (if present), the specific route of administration and similar factors. Accordingly, doses of pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) described herein may depend on various of such parameters. In the case that a reaction in a patient is insufficient with an initial dose, higher doses (or effectively higher doses achieved by a different, more localized route of administration) may be used.

[0104] ***Poly(A) sequence***: As used herein, the term "poly(A) sequence" or "poly-A tail" refers to an uninterrupted or interrupted sequence of adenylate residues which is typically located at the 3'-end of an RNA molecule. Poly(A) sequences are known to those of skill in the art and may follow the 3'-UTR in the RNAs described herein. An uninterrupted poly(A) sequence is characterized by consecutive adenylate residues. In nature, an uninterrupted poly(A) sequence is typical. RNAs disclosed herein can have a poly(A) sequence attached to the free 3'-end of the RNA by a template-independent RNA polymerase after transcription or a poly(A) sequence encoded by DNA and transcribed by a template-dependent RNA polymerase.

[0105] ***Polypeptide:*** As used herein, the term "polypeptide" refers to a polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some embodiments, a polypeptide may comprise or consist of only natural amino acids or only non-natural amino acids. In some embodiments, a polypeptide may comprise D-amino acids, L-amino acids, or both. In some embodiments, a polypeptide may comprise only D-amino acids. In some embodiments, a polypeptide may comprise only L-amino acids. In some embodiments, a polypeptide may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid side chains, at the polypeptide's N-terminus, at the polypeptide's C-terminus, or any combination thereof. In some embodiments, such pendant groups or modifications comprise acetylation, amidation, lipidation, methylation, pegylation, etc., including combinations thereof. In some embodiments, a polypeptide may be cyclic, and/or may comprise a cyclic portion. In some embodiments, a polypeptide is not cyclic and/or does not comprise any cyclic portion. In some embodiments, a polypeptide is linear. In some embodiments, a polypeptide may be or comprise a stapled polypeptide. In some embodiments, the term "polypeptide" may be appended to a name of a reference polypeptide, activity, or structure; in such instances it is used herein to refer to polypeptides that share the relevant activity or structure and thus can be considered to be members of the same class or family of polypeptides. For each such class, the present specification provides and/or those skilled in the art will be aware of exemplary polypeptides within the class whose amino acid sequences and/or functions are known; in some embodiments, such exemplary polypeptides are reference polypeptides for the polypeptide class or family. In some embodiments, a member of a polypeptide class or family shows significant sequence homology or identity with, shares a common sequence motif (e.g., a characteristic sequence element) with, and/or shares a common activity (in some embodiments at a comparable level or within a designated range) with a reference polypeptide of the class; in some embodiments with all polypeptides within the class). For example, in some embodiments, a member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (e.g., a conserved region that may in some embodiments be or comprise a characteristic sequence element) that

shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. In some embodiments, a relevant polypeptide may comprise or consist of a fragment of a parent polypeptide.

[0106] ***Prevent:*** As used herein, the term "prevent" or "prevention" when used in connection with the occurrence of a disease, disorder, and/or condition, refers to reducing the risk of developing the disease, disorder and/or condition and/or to delaying onset of one or more characteristics or symptoms of the disease, disorder or condition. Prevention may be considered complete when onset of a disease, disorder or condition has been delayed for a predefined period of time.

[0107] ***Recombinant:*** The term "recombinant" in the context of the present disclosure means "made through genetic engineering". In some embodiments, a "recombinant" entity such as a recombinant nucleic acid in the context of the present disclosure is not naturally occurring.

[0108] ***Reference:*** As used herein, the term "reference" describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent, animal, individual, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

[0109] ***Ribonucleic acid (RNA):*** As used herein, the term "RNA" or "polyribonucleotide" refers to a polymer of ribonucleotides. In some embodiments, an RNA is single stranded. In some embodiments, an RNA is double stranded. In some embodiments, an RNA comprises both single and double stranded portions. In some embodiments, an RNA can comprise a backbone structure as described in the definition of "Nucleic acid / Polynucleotide" above. An RNA can be a regulatory RNA (e.g., siRNA, microRNA, etc.), or a messenger RNA (mRNA). In some embodiments where an RNA is a mRNA. In some embodiments where an RNA is a mRNA, a RNA typically comprises at its 3' end a

poly(A) region. In some embodiments where an RNA is a mRNA, an RNA typically comprises at its 5' end an art-recognized cap structure, e.g., for recognizing and attachment of a mRNA to a ribosome to initiate translation. In some embodiments, a RNA is a synthetic RNA. Synthetic RNAs include RNAs that are synthesized in vitro (e.g., by enzymatic synthesis methods and/or by chemical synthesis methods).

[0110] ***Ribonucleotide:*** As used herein, the term "ribonucleotide" encompasses unmodified ribonucleotides and modified ribonucleotides. For example, unmodified ribonucleotides include the purine bases adenine (A) and guanine (G), and the pyrimidine bases cytosine (C) and uracil (U). Modified ribonucleotides may include one or more modifications including, but not limited to, for example, (a) end modifications, e.g., 5' end modifications (e.g., phosphorylation, dephosphorylation, conjugation, inverted linkages, etc.), 3' end modifications (e.g., conjugation, inverted linkages, etc.), (b) base modifications, e.g., replacement with modified bases, stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, or conjugated bases, (c) sugar modifications (e.g., at the 2' position or 4' position) or replacement of the sugar, and (d) internucleoside linkage modifications, including modification or replacement of the phosphodiester linkages. The term "ribonucleotide" also encompasses ribonucleotide triphosphates including modified and non-modified ribonucleotide triphosphates.

[0111] ***Risk:*** As will be understood from context, "risk" of a disease, disorder, and/or condition refers to a likelihood that a particular individual will develop the disease, disorder, and/or condition. In some embodiments, risk is expressed as a percentage. In some embodiments, risk is from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 up to 100%. In some embodiments risk is expressed as a risk relative to a risk associated with a reference sample or group of reference samples. In some embodiments, a reference sample or group of reference samples have a known risk of a disease, disorder, condition and/or event. In some embodiments a reference sample or group of reference samples are from individuals comparable to a particular individual. In some embodiments, relative risk is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. In some embodiments, risk may reflect one or more genetic attributes, e.g., which may predispose an individual toward development (or not) of a particular disease, disorder and/or condition. In some embodiments, risk may reflect one or more epigenetic events or attributes and/or one or more lifestyle or environmental events or attributes.

[0112] ***RNA lipoplex particle***: As used herein, the term "RNA lipoplex particle" refers to a complex comprising liposomes, in particular cationic liposomes, and RNA molecules. Without wishing to bound by a particular theory, electrostatic interactions between positively charged liposomes and

negatively charged RNA results in complexation and spontaneous formation of RNA lipoplex particles. In some embodiments, positively charged liposomes may comprise a cationic lipid, such as in some embodiments DOTMA, and additional lipids, such as in some embodiments DOPE. In one embodiment, a RNA lipoplex particle is a nanoparticle.

[0113] ***Selective or specific:*** The term "selective" or "specific", when used herein in reference to an agent having an activity, is understood by those skilled in the art to mean that the agent discriminates between potential target entities, states, or cells. For example, in some embodiments, an agent is said to bind "specifically" to its target if it binds preferentially with that target in the presence of one or more competing alternative targets. In many embodiments, specific interaction is dependent upon the presence of a particular structural feature of the target entity (e.g., an epitope, a cleft, a binding site). It is to be understood that specificity need not be absolute. In some embodiments, specificity may be evaluated relative to that of a target-binding moiety for one or more other potential target entities (e.g., competitors). In some embodiments, specificity is evaluated relative to that of a reference specific binding moiety. In some embodiments, specificity is evaluated relative to that of a reference non-specific binding moiety.

[0114] ***Subject:*** As used herein, the term "subject" refers to an organism to be administered with a composition described herein, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, domestic pets, etc.) and humans. In some embodiments, a subject is a human subject. In some embodiments, a subject is suffering from a disease, disorder, or condition (e.g., an HSV infection). In some embodiments, a subject is susceptible to a disease, disorder, or condition (e.g., an HSV infection). In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder, or condition (e.g., an HSV infection). In some embodiments, a subject displays one or more non-specific symptoms of a disease, disorder, or condition (e.g., an HSV infection). In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition (e.g., an HSV infection). In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition (e.g., an HSV infection). In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0115] ***Suffering from***: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[0116] ***Susceptible to***: An individual who is "susceptible to" a disease, disorder, and/or condition is one who has a higher risk of developing the disease, disorder, and/or condition than does a member of the general public. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may not have been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0117] ***Synthetic***: As used herein, the term "synthetic" refers to an entity that is artificial, or that is made with human intervention, or that results from synthesis rather than naturally occurring. For example, in some embodiments, a synthetic nucleic acid or polynucleotide refers to a nucleic acid molecule that is chemically synthesized, e.g., in some embodiments by solid-phase synthesis. In some embodiments, the term "synthetic" refers to an entity that is made outside of biological cells. For example, in some embodiments, a synthetic nucleic acid or polynucleotide refers to a nucleic acid molecule (e.g., an RNA) that is produced by *in vitro* transcription using a template.

[0118] ***Therapy***: The term "therapy" refers to an administration or delivery of an agent or intervention that has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect (e.g., has been demonstrated to be statistically likely to have such effect when administered to a relevant population). In some embodiments, a therapeutic agent or therapy is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. In some embodiments, a therapeutic agent or therapy is a medical intervention (e.g., surgery, radiation, phototherapy) that can be performed to alleviate, relieve, inhibit, present, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition.

[0119] ***Three prime untranslated region***: As used herein, the terms "three prime untranslated region" or "3' UTR" refer to a sequence of an mRNA molecule that begins following a stop codon of a coding region of an open reading frame sequence. In some embodiments, the 3' UTR begins immediately after a stop codon of a coding region of an open reading frame sequence, e.g., in its natural context. In other embodiments, the 3' UTR does not begin immediately after stop codon of the coding region of an open reading frame sequence, e.g., in its natural context.

[0120] ***Threshold level (e.g., acceptance criteria)***: As used herein, the term "threshold level" refers to a level that are used as a reference to attain information on and/or classify the results of a measurement, for example, the results of a measurement attained in an assay. For example, in some embodiments, a threshold level means a value measured in an assay that defines the dividing line between two subsets of a population (e.g. a batch that satisfy quality control criteria vs. a batch that does not satisfy quality control criteria). Thus, a value that is equal to or higher than the threshold level defines one subset of the population, and a value that is lower than the threshold level defines the other subset of the population. A threshold level can be determined based on one or more control samples or across a population of control samples. A threshold level can be determined prior to, concurrently with, or after the measurement of interest is taken. In some embodiments, a threshold level can be a range of values.

[0121] ***Treat***: As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits only early signs of the disease, disorder, and/or condition, for example for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject at a later-stage of disease, disorder, and/or condition.

[0122] ***Vaccination***: As used herein, the term "vaccination" refers to the administration of a composition intended to generate an immune response, for example to a disease-associated (e.g., disease-causing) agent. In some embodiments, vaccination can be administered before, during, and/or after exposure to a disease-associated agent, and in certain embodiments, before, during, and/or shortly after exposure to the agent. In some embodiments, vaccination includes multiple administrations, appropriately

spaced in time, of a vaccine composition. In some embodiments, vaccination generates an immune response to an infectious agent.

[0123] *Vaccine:* As used herein, the term "vaccine" refers to a composition that induces an immune response upon administration to a subject. In some embodiments, an induced immune response provides protective immunity.

[0124] *Variant:* As used herein in the context of molecules, e.g., nucleic acids, proteins, or small molecules, the term "variant" refers to a molecule that shows significant structural identity with a reference molecule but differs structurally from the reference molecule, e.g., in the presence or absence or in the level of one or more chemical moieties as compared to the reference entity. In some embodiments, a variant also differs functionally from its reference molecule. In general, whether a particular molecule is properly considered to be a "variant" of a reference molecule is based on its degree of structural identity with the reference molecule. As will be appreciated by those skilled in the art, any biological or chemical reference molecule has certain characteristic structural elements. A variant, by definition, is a distinct molecule that shares one or more such characteristic structural elements but differs in at least one aspect from the reference molecule. In some embodiments, a variant polypeptide or nucleic acid may differ from a reference polypeptide or nucleic acid as a result of one or more differences in amino acid or nucleotide sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, phosphate groups) that are covalently components of the polypeptide or nucleic acid (e.g., that are attached to the polypeptide or nucleic acid backbone). In some embodiments, a variant polypeptide or nucleic acid shows an overall sequence identity with a reference polypeptide or nucleic acid that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant polypeptide or nucleic acid does not share at least one characteristic sequence element with a reference polypeptide or nucleic acid. In some embodiments, a reference polypeptide or nucleic acid has one or more biological activities. In some embodiments, a variant polypeptide or nucleic acid shares one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid lacks one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid shows a reduced level of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a polypeptide or nucleic acid of interest is considered to be a "variant" of a reference polypeptide or nucleic acid if it has an amino acid or nucleotide sequence that is identical to that of the

reference but for a small number of sequence alterations at particular positions. Typically, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a variant are substituted, inserted, or deleted, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. Often, a variant polypeptide or nucleic acid comprises a very small number (e.g., fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional residues (i.e., residues that participate in a particular biological activity) relative to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises not more than about 5, about 4, about 3, about 2, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 additions or deletions as compared to the reference. In some embodiments, a reference polypeptide or nucleic acid is one found in nature.

[0125] *Vector*: as used herein, refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors." In some embodiments, known techniques may be used, for example, for generation or manipulation of recombinant DNA, for oligonucleotide synthesis, and for tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al.,

Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), which is incorporated herein by reference for any purpose.

[0126] All literature and similar material cited in this application, including, but not limited to, patents, patent applications, articles, books, treatises, and web pages, regardless of the format of such literature and similar materials, are expressly incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described in any way.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0127] As discussed above, the present disclosure provides pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) for delivering particular herpes simplex virus (HSV) antigen constructs (e.g., HSV-1 antigen constructs, HSV-2 antigen constructs, or a combination thereof) to a subject (e.g., a patient) and related technologies (e.g., methods). In particular, the present disclosure provides HSV (e.g., HSV-1, HSV-2, or both) vaccine compositions and related technologies (e.g., methods).

[0128] The present disclosure identifies and addresses, in part, a problem with current HSV vaccines, which have not been able to fully leverage antigenic potential of HSV gB (HSV-1 gB and/or HSV-2 gB). The present disclosure encompasses a recognition that stabilization of gB antigens or antigenic fragments thereof can be useful advantageous for eliciting an immune response. Accordingly, the present disclosure provides that HSV gB antigens and antigenic fragments thereof can be useful in preventing or treating HSV, e.g., in HSV antigen constructs and/or HSV compositions (e.g., immunogenic compositions, e.g., vaccines) as further disclosed herein. The present disclosure further provides the recognition that stabilization of HSV gB or antigenic fragments thereof can be particularly advantageous for use, e.g., in preventing or treating HSV, e.g., in HSV antigen constructs and/or HSV compositions (e.g., immunogenic compositions, e.g., vaccines) as further disclosed herein. Accordingly, the present disclosure provides certain mutations that can stabilize HSV gB or antigenic fragments thereof. The present disclosure also provides HSV gB antigens comprising certain mutations, e.g., 251C, 718C, and combinations thereof, wherein the numbering is with reference to SEQ ID NO: 1.

I. Herpes Simplex Virus (HSV)

[0129] Herpes simplex virus (HSV) belongs to the alpha subfamily of the human herpesvirus family and includes HSV-1 and HSV-2. The structure of HSV-1 and HSV-2 mainly include (from inside to outside) a DNA core, capsid, tegument and envelope. Each of HSV-1 and HSV-2 have a double stranded DNA genome of about 153kb, encoding at least 80 genes. The DNA core is enclosed by an icosapentahedral capsid composed of 162 capsomeres, 150 hexons and 12 pentons, made of six different viral proteins. The DNA is surrounded by at least 20 different viral tegument proteins that have structural and regulatory roles. Some of them participating in capsid transport to the nucleus and other organelles, viral DNA entry into the nucleus, activation of early genes transcription, suppression of cellular protein biosynthesis, and mRNA degradation. The viral envelope surrounding the tegument has at least 12 different glycoproteins (B-N) on their surface. The glycoproteins may exist as heterodimers (H/L and E/I) with most existing as monomers.

[0130] HSV-1 and HSV-2 are responsible for a number of minor, moderate and severe pathologies, including oral and genital ulceration, virally induced blindness, viral encephalitis and disseminated infection of neonates. HSV-1 and HSV-2 are usually transmitted by different routes and affect different areas of the body, but the signs and symptoms that they cause can overlap. Infections caused by HSV-1 represent one of the more widespread infections of the orofacial region and commonly causes herpes labialis, herpetic stomatitis, and keratitis. HSV-2 typically causes genital herpes and is transmitted primarily by direct sexual contact with lesions. Most genital HSV infections are caused by HSV-2, however, an increasing number of genital HSV infections have been attributed to HSV-1. Genital HSV-1 infections are typically less severe and less prone to occurrence than genital HSV-2 infections.

[0131] HSV infections are transmitted through contact with herpetic lesions, mucosal surfaces, genital secretions, or oral secretions. The average incubation period after exposure is typically 4 days, but may range between 2 and 12 days. HSV particles can infect neuronal prolongations enervating peripheral tissues and establish latency in these cells, namely in the trigeminal ganglia and dorsal root ganglia of the sacral area from where they can sporadically reactivate. Additionally, similar to other herpesviruses, HSV infections are lifelong and generally asymptomatic. Without wishing to be bound by any particular theory, it is understood that HSV particles can be shed from infected individuals independent of the occurrence of clinical manifestations.

[0132] HSV infections are rarely fatal, but are characterized by blisters that can rupture and become painful. There are few clear differences in clinical presentation based on the type of infecting virus. However, as discussed above, HSV-1 infections tend to be less severe than HSV-2 infections, and patients infected with HSV-2 generally have more outbreaks.

A. Lifecycle

[0133] As described herein, to initiate infection, an HSV (HSV-1 or HSV-2) particle binds to the cell surface using the viral glycoproteins and fuses its envelope with the plasma membrane (see, e.g., Figure 2, Step 1). After the fusion of membranes, the viral capsid and tegument proteins are internalized in the cytoplasm (see, e.g., Figure 2, Step 2). Once in the cytoplasm, the viral capsid accumulates in the nucleus and releases viral DNA into the nucleus (see, e.g., Figure 2, Step 3). HSV replicates by three rounds of transcription that yield: α (immediate early) proteins that mainly regulate viral replication; β (early) proteins that synthesise and package DNA; and γ (late) proteins, most of which are virion proteins (see, Whitley et.al., Lancet 2001 May 12;357(9267); Taylor et.al., Front Biosci. 2002 Mar 1;7:d752-64; and Ibáñez et.al., Front Microbiol. 2018 Oct 11;9:2406; each of which is incorporated herein by reference in its entirety) (see, e.g., Figure 2, Steps 4-6).

[0134] The HSV capsids are assembled within the nucleus of infected cells (see, e.g., Figure 2, Step 7). Once the assembly of viral capsids has been completed in the nucleus, these particles will continue their maturation process in this same compartment through the acquisition of tegument proteins. After leaving the nucleus, additional tegument proteins will be added to the capsids. Meanwhile, the glycoproteins are translated and glycosylated in the endoplasmic reticulum and processed in the trans-Golgi network (TGN) and then directed to multivesicular bodies (see, e.g., Figure 2, Step 8). Then, they are exported to the plasma membrane glycoproteins within early endosomes (see, e.g., Figure 2, Step 9). Viral capsids in the cytoplasm will then fuse with HSV-glycoprotein-containing endosomes to form infectious virions within vesicles (see, e.g., Figure 2, Steps 10-12).

[0135] HSV (HSV-1 or HSV-2) are able to establish a latent infection. After primary infection, HSV either replicates productively in epithelial cells or enters sensory neuron axons and moves to the neuronal cell nucleus. There, the viral DNA remains as circular, extra-chromosomal DNA, and does not possess any lytic gene expression; however, latency associated transcripts are expressed and then spliced to produce mRNA. This general transcriptional silence may allow the virus to remain hidden in the cell by avoiding immune surveillance. In some aspects, provided herein are technologies (e.g., compositions and

methods) for augmenting, inducing, promoting, enhancing and/or improving an immune response against HSV (e.g., HSV-1 and/or HSV-2) or a component thereof (e.g., a protein or fragment thereof). In some embodiments, technologies provided herein are designed to augment, induce, promote, enhance and/or improve immunological memory against HSV or a component thereof (e.g., a protein or fragment thereof). In some embodiments, technologies described herein are designed to act as an immunological boost to a primary vaccine, such as a vaccine directed to an epitope and/or epitopes of HSV (e.g., HSV-1 and/or HSV-2).

[0136] The virus remains in this state for the lifetime of the host, or until the proper signals reactivate the virus and new progeny are generated. Progeny virus then travel through the neuron axis to the site of the primary infection to re-initiate a lytic replication cycle.

B. HSV Genome

[0137] The genome of HSV-1 and the genome of HSV-2 are both approximately 150 kb long of double-stranded DNA, varying slightly between subtypes and strains. The genome encodes more than 80 genes and has high GC contents: 67 and 69% for HSV-1 and HSV-2, respectively (see, Whitley et.al., Lancet 2001 May 12;357(9267); Taylor et.al., Front Biosci. 2002 Mar 1;7:d752-64; and Jiao et.al., Microbiol Resour Announc. 2019 Sep; 8(39): e00993-19, which is incorporated herein by reference in its entirety).

[0138] The genome is organized as unique long region (UL) and a unique short region (US). The UL is typically bounded by terminal long (TRL) and internal long (IRL) repeats. The US is typically bounded by terminal short (IRS) and internal short (TRS) repeats. The genes found in the unique regions are present in the genome as a single copy, but genes that are encoded in the repeat regions are present in the genome in two copies (see, Whitley et.al., Lancet 2001 May 12;357(9267); Taylor et.al., Front Biosci. 2002 Mar 1;7:d752-64; and Jiao et.al., Microbiol Resour Announc. 2019 Sep; 8(39): e00993-19, which is incorporated herein by reference in its entirety).

[0139] HSV contains three origins of replication within the genome that are named depending upon their location in either the Long (oriL) or Short (oriS) region of the genome. OriL is found as a single copy in the UL segment, but oriS is located in the repeat region of the Short segment; thus, it is present in the genome in two copies. Both oriL and oriS are palindromic sequences consisting of an AT-rich center region flanked by inverted repeats that contain multiple binding sites of varying affinity for the

viral origin binding protein (UL9). Either oriL or one of the oriS sequences is sufficient for viral replication (see, Whitley et.al., Lancet 2001 May 12;357(9267); Taylor et.al., Front Biosci. 2002 Mar 1;7:d752-64; and Jiao et.al., Microbiol Resour Announc. 2019 Sep; 8(39): e00993-19, which is incorporated herein by reference in its entirety).

[0140] The viral genome also contains signals that orchestrate proper processing of the newly synthesized genomes for packaging into pre-formed capsids. Progeny genomes are generated in long concatemers that require cleavage into unit-length monomers. For this purpose, the viral genome contains two DNA sequence elements, pac1 and pac2, that ensure proper cleavage and packaging of unit-length progeny genomes. These elements are located within the direct repeats (DR) found within the inverted repeat regions at the ends of the viral genome (see, Whitley et.al., Lancet 2001 May 12;357(9267); Taylor et.al., Front Biosci. 2002 Mar 1;7:d752-64; and Jiao et.al., Microbiol Resour Announc. 2019 Sep; 8(39): e00993-19, which is incorporated herein by reference in its entirety).

C. HSV Vaccines

[0141] Several HSV vaccines, mainly targeting HSV-2 and primarily focused on the generation of neutralizing antibodies (nAbs) targeting the viral envelope glycoprotein D as the correlate of immune protection, have been developed and evaluated in human clinical trial, see **Table 1** below. Despite these vaccines exhibiting protection against HSV in preclinical studies and in some cases Phase 2 studies, none of these vaccines has demonstrated sufficient efficacy for further development or commercialization.

[0142] The present disclosure provides an insight that many prior strategies for developing pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) for treatment of and/or protection from HSV infection have focused primarily, or even almost exclusively, on development of neutralizing antibodies that target surface glycoproteins. The present disclosure identifies a problem with such strategies including, for example, that they may fail to appreciate value or even criticality of ensuring that an induced immune response includes significant T cell activity (in some embodiments, CD4 T cell activity, in some embodiments CD8 T cell activity, in some embodiments, both). In some embodiments, pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) that comprise or deliver CD4 and CD8 epitope(s) of one or more HSV antigens (e.g., HSV-1 antigens, HSV-2 antigens, or a combination thereof), e.g., in addition to one or more B cell antigens and/or epitopes may be used in treatment of and/or protection from HSV infection.

Table 1: Certain HSV Vaccines Under Clinical Development

Name	Platform	Antigens	Immune Response	Clinical results
gB/gD/MF59 (Chiron)	Subunit MF59 adjuvant	gD and gB	Neutralzing antibodies (Abs)	Phase 3 Prophylaxis 9% (95% CI: -29%, 36%)
gD/AS04 (GSK)	Subunit AS04 adjuvant	gD	Neutralizing Abs $CD4^+$ T cells	Phase 3 Prophylaxis 20% (95% CI: -29%, 50%)
gH-null (Cantab Pharmaceutical)	Single-cycle live	Multiple	Neutralizing Abs	Phase 3 therapeutic No difference in recurrences or shedding
HSV529 (Sanofi-Pasteur)	Replication-defective	Multiple	Neutralizing Abs $CD4^+$, $CD8^+$ T cells	Phase 1 Safe but immunogenic only in doubly seronegatives
Gen-003 (Genocea)	Subunit Saponin adjuvant	gD, ICP4	Neutralizing Abs Cytolytic T cells	Phase 2 therapeutic Dose variable decrease shedding
HerpV (Agenus)	Heat shock protein 70-HSV peptides QS-21 adjuvant	32 HSV peptides (gD, additional envelope, tegument, and other)	Cytotoxic T cells	Phase 2 therapeutic 17% reduction in shedding frequency
Vaxfectin (Vical)	DNA Vaxfectin adjuvant	gD, UL46/47	Neutralizing Abs	Phase 1/2 therapeutic Failed to reduce shedding
COR-1 (Admedus)	Codon optimized DNA	Codon optimized gD2 and ubiquitinfused truncated gD2 to target the antigen to the proteasome	gD-specific Abs Cytotoxic T cells	Phase 2 therapeutic No difference in recurrences

ΔNLS (Rational Vaccines)	Deleted in ICP0 Replication competent Attenuated for latency	Multiple targets	Neutralizing Abs gD-specific Abs	Phase 1 non-FDA Approved therapeutic study Self-reported reduction in recurrences

D. Anti-Viral Treatments for HSV

[0143] The present disclosure provides the recognition that constructs and/or compositions described herein may be administered as part of regimen with other therapeutic agents. The present disclosure also recognizes that subjects that are administered constructs and/or compositions described herein may have previously been administered other therapeutic agents.

[0144] In some embodiments, for example, a subject may be receiving or had previously received an anti-viral agent for HSV. In some embodiments, an anti-viral agent can be administered to treat HSV-1 or HSV-2 infection or recurrent episodes. In some embodiments, an anti-viral agent is or comprises acyclovir, valacyclovir, famciclovir, or a combination thereof. **Table 2** below provides certain information about select anti-viral agents.

Table 2: Antiviral Drugs for Treating HSV

Medication	Dosage	Most common adverse effects	Approximate price for complete dosing[1]
Oral formulations:			
acyclovir	200-400mg 5x/day; 5-365 days	malaise, headache, nausea, vomiting	35-800 mg tabs; $70
valacyclovir	1 g 3x/day; 7 days	headache, nausea, abdominal pain	21-1 g tabs; $220
famciclovir	250-1500 mg 2-3x/day; 1-30 days	headache, nausea, fatigue, diarrhea	21-500 mg tabs; $184
Intravenous formulations:			
acyclovir	10-15 mg/kg every 8h; 7 days	phlebitis, acute renal failure, nausea, vomiting, rash	
foscarnet	40-60 mg/kg/day, 14-21 days	fever, headache, renal dysfunction, electrolyte	

		abnormalities, nausea, vomiting diarrhea, anemia, granulocytopenia	
Topical application			
1-Docosanol (Treatment for HSV-1)	Apply to affected area on face or lips at the first sign of cold sore/fever blister .	Redness or swelling	

II. Glycoprotein B Constructs

A. Glycoprotein B

[0145] The present disclosure provides that HSV gB antigens and antigenic fragments thereof can be useful in preventing or treating HSV, e.g., in HSV antigen constructs and/or HSV compositions (e.g., immunogenic compositions, e.g., vaccines) as further disclosed herein.

[0146] HSV gB is the most highly conserved of all surface glycoproteins and acts primarily as a fusion protein. Glycoprotein B is involved in the viral cell entry of HSV. Herpesviruses have a lipid bilayer, called the envelope, which contains twelve surface glycoproteins. For infectivity to be attained, the double stranded DNA genome of HSV must enter the host cell through means of fusion of its envelope with the cellular membrane or via endocytosis. HSV gC, gB, gD, gH, and gL are involved in the process of viral cell entry, but only gC, gB, gD, and gH are required for the fusion of HSV's envelope with a cellular membrane.

[0147] gB is a type-1 transmembrane protein with a signal sequence at its N terminus. HSV gB has five structural domains (I-V). Domain I contains two internal fusion loops, which may insert into a cellular membrane during virus-cell fusion. Domain II may interact with gH/gL during the fusion process. Domain III includes a structurally important elongated alpha helix. Domain IV is hypothesized to interact with cellular receptors. Domain V acts in conjunction with domain I during protein-lipid interactions.

[0148] Example amino acid sequences of certain HSV gB proteins is provided in **Table 3** below.

Table 3: HSV Glycoprotein B Amino Acid Sequences

SEQ ID NO	Antigen / ORF	Strain	Amino Acid Sequence
1	HSV-1 gB / UL27		MHQGAPSWGRRWFVVWALLGLTLGVLVASAAPTSPGTPGVAAA TQAANGGPATPAPPPLGAAPTGDPKPKKNKKPKNPTPPRPAGDN ATVAAGHATLREHLRDIKAENTDANFYVCPPPTGATVVQFEQPR RCPTRPEGQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWF GHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNLET TAFHRDDHETDMELKPANAATRTSRGWHTTDLKYNPSRVEAFHR YGTTVNCIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGSH TEHTTYAADRFKQVDGFYARDLTTKARATAPTTRNLLTTPKFTV AWDWVPKRPSVCTMTKWQEVDEMLRSEYGGSFRFSSDAISTTFT TNLTEYPLSRVDLGDCIGKDARDAMDRIFARRYNATHIKVGQPQ YYQANGGFLIAYQPLLSNTLAELYVREHLREQSRKPPNPTPPPPGA SANASVERIKTTSSIEFARLQFTYNHIQRHVNDMLGRVAIAWCEL QNHELTLWNEARKLNPNAIASVTVGRRVSARMLGDVMAVSTCV PVAADNVIVQNSMRISSRPGACYSRPLVSFRYEDQGPLVEGQLGE NNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYSHQLSRADI TTVSTFIDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQ LHDLRFADIDTVIHADANAAMFAGLGAFFEGMGDLGRAVGKVV MGIVGGVVSAVSGVSSFMSNPFGALAVGLLVLAGLAAAFFAFRY VMRLQSNPMKALYPLTTKELKNPTNPDASGEGEEGGDFDEAKLA EAREMIRYMALVSAMERTEHKAKKKGTSALLSAKVTDMVMRKR RNTNYTQVPNKDGDADEDDL
2	HSV-2 gB / UL27	HG52	MRGGGLICALVVGALVAAVASAAPAAPAAPRASGGVAATVAAN GGPASRPPPVPSPATTKARKRKTKKPPKRPEATPPPDANATVAAG HATLRAHLREIKVENADAQFYVCPPPTGATVVQFEQPRRCPTRPE GQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQF MGIFEDRAPVPFEEVIDKINTKGVCRSTAKYVRNNMETTAFHRDD HETDMELKPAKVATRTSRGWHTTDLKYNPSRVEAFHRYGTTVN CIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGSHTEHTSYA ADRFKQVDGFYARDLTTKARATSPTTRNLLTTPKFTVAWDWVPK RPAVCTMTKWQEVDEMLRAEYGGSFRFSSDAISTTFTTNLTEYSL SRVDLGDCIGRDAREAIDRMFARKYNATHIKVGQPQYYLATGGF LIAYQPLLSNTLAELYVREYMREQDRKPRNATPAPLREAPSANAS VERIKTTSSIEFARLQFTYNHIQRHVNDMLGRIAVAWCELQNHEL TLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAPD NVIVQNSMRVSSRPGTCYSRPLVSFRYEDQGPLIEGQLGENNELRL TRDALEPCTVGHRRYFIFGGGYVYFEEYAYSHQLSRADVTTVSTF IDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRF ADIDTVIRADANAAMFAGLCAFFEGMGDLGRAVGKVVMGVVGG VVSAVSGVSSFMSNPFGALAVGLLVLAGLVAAFFAFRYVLQLQR

			NPMKALYPLTTKELKTSDPGGVGGEGEEGAEGGGFDEAKLAEAR EMIRYMALVSAMERTEHKARKKGTSALLSSKVTNMVLRKRNKA RYSPLHNEDEAGDEDEL
3	HSV-2 gB / UL27	333	MRGGGLICALVVGALVAAVASAAPAAPAAPRASGGVAATVAAN GGPASRPPPVPSPATTKARKRKTKKPPKRPEATPPPDANATVAAG HATLRAHLREIKVENADAQFYVCPPPTGATVVQFEQPRRCPTRPE GQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQF MGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNMETTAFHRDD HETDMELKPAKVATRTSRGWHTTDLKYNPSRVEAFHRYGTTVN CIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGSHTEHTSYA ADRFKQVDGFYARDLTTKARATSPTTRNLLTTPKFTVAWDWVPK RPAVCTMTKWQEVDEMLRAEYGGSFRFSSDAISTTFTTNLTQYSL SRVDLGDCIGRDAREAIDRMFARKYNATHIKVGQPQYYLATGGF LIAYQPLLSNTLAELYVREYMREQDRKPRNATPAPLREAPSANAS VERIKTTSSIEFARLQFTYNHIQRHVNDMLGRIAVAWCELQNHEL TLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAPD NVIVQNSMRVSSRPGTCYSRPLVSFRYEDQGPLIEGQLGENNELRL TRDALEPCTVGHRRYFIFGGGYVYFEEYAYSHQLSRADVTTVSTF IDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRF ADIDTVIRADANAAMFAGLCAFFEGMGDLGRAVGKVVMGVVGG VVSAVSGVSSFMSNPFGALAVGLLVLAGLVAAFFAFRYVLQLQR NPMKALYPLTTKELKTSDPGGVGGEGEEGAEGGGFDEAKLAEAR EMIRYMALVSAMERTEHKARKKGTSALLSSKVTNMVLRKRNKA RYSPLHNEDEAGDEDEL
4	HSV-2 gB / UL27	MS	MRGGGLICALVVGALVAAVASAAPAAPRASGGVAATVAANGGP APQPPPVPSPATTKARKRKTKKPPKRPEATPPPDANATVAAGHAT LRAHLREIKVENADAQFYVCPPPTGATVVQFEQPRRCPTRPEGQN YTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQFMGI FEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNMETTAFHRDDHET DMELKPAKVATRTSRGWHTTDLKYNPSRVEAFHRYGTTVNCIVE EVDARSVYPYDEFVLATGDFVYMSPFYGYREGSHTEHTSYAADR FKQVDGFYARDLTTKAQATSPTTRNLLTTPKFTVAWDWVPKRPA VCTMTKWQEVDEMLRAEYGGSFRFSSDAISTTFTTNLTEYSLSRV DLGDCIGRDAREAIDRMFARKYNATHIKVGQPQYYLATGGFLIAY QPLLSNTLAELYVREYMREQDRKPRNATPAPLREAPSANASVERI KTTSSIEFARLQFTYNHIQRHVNDMLGRIAVAWCELQNHELTLW NEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAPDNVIV QNSMRVSSRPGTCYSRPLVSFRYEDQGPLIEGQLGENNELRLTRD ALEPCTVGHRRYFIFGGGYVYFEEYAYSHQLSRADVTTVSTFIDL NITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRFAD IDTVIRADANAAMFAGLCAFFEGMGDLGRAVGKVVMGVVGGVV SAVSGVSSFMSNPFGALAVGLLVLAGLVAAFFAFRYVLQLQRNP MKALYPLTTKELKTSDPGGVGGEGEEGAEGGGFDEAKLAEARQ MIRYMALVSAMERTEHKARKKGTSALLSSKVTNMVLRKRNKAR YSPLHNEDEAGDEDEL

90	HSV-1 gB / UL27	KOS	MHQGAPSWGRRWFVVWALLGLTLGVLVASAAPSSPGTPGVAAA TQAANGGPATPAPPALGAAPTGDPKPKKNKKPKNPTPPRPAGDN ATVAAGHATLREHLRDIKAENTDANFYVCPPPTGATVVQFEQPR RCPTRPEGQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWF GHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNLET TAFHRDDHETDMELKPANAATRTSRGWHTTDLKYNPSRVEAFHR YGTTVNCIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGSH TEHTSYAADRFKQVDGFYARDLTTKARATAPTTRNLLTTPKFTV AWDWVPKRPSVCTMTKWQEVDEMLRSEYGGSFRFSSDAISTTFT TNLTEYPLSRVDLGDCIGKDARDAMDRIFARRYNATHIKVGQPQ YYLANGGFLIAYQPLLSNTLAELYVREHLREQSRKPPNPTPPPPGA SANASVERIKTTSSIEFARLQFTYNHIQRHVNDMLGRVAIAWCEL QNHELTLWNEARKLNPNAIASVTVGRRVSARMLGDVMAVSTCV PVAADNVIVQNSMRISSRPGACYSRPLVSFRYEDQGPLVEGQLGE NNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYSHQLSRADI TTVSTFIDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQ LHDLRFADIDTVIHADANAAMFAGLGAFFEGMGDLGRAVGKVV MGIVGGVVSAVSGVSSFMSNPFGALAVGLLVLAGLAAAFFAFRY VMRLQSNPMKALYPLTTKELKNPTNPDASGEGEEGGDFDEAKLA EAREMIRYMALVSAMERTEHKAKKKGTSALLSAKVTDMVMRKR RNTNYTQVPNKDGDADEDDL
91	HSV-2 gB / UL27	HG52	MRGGGLICALVVGALVAAVASAAPAAPAAPRASGGVAATVAAN GGPASRPPPVPSPATTKARKRKTKKPPKRPEATPPPDANATVAAG HATLRAHLREIKVENADAQFYVCPPPTGATVVQFEQPRRCPTRPE GQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQF MGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNMETTAFHRDD HETDMELKPAKVATRTSRGWHTTDLKYNPSRVEAFHRYGTTVN CIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGSHTEHTSYA ADRFKQVDGFYARDLTTKARATSPTTRNLLTTPKFTVAWDWVPK RPAVCTMTKWQEVDEMLRAEYGGSFRFSSDAISTTFTTNLTEYSL SRVDLGDCIGRDAREAIDRMFARKYNATHIKVGQPQYYLATGGF LIAYQPLLSNTLAELYVREYMREQDRKPRNATPAPLREAPSANAS VERIKTTSSIEFARLQFTYNHIQRHVNDMLGRIAVAWCELQNHEL TLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAPD NVIVQNSMRVSSRPGTCYSRPLVSFRYEDQGPLIEGQLGENNELRL TRDALEPCTVGHRRYFIFGGGYVYFEEYAYSHQLSRADVTTVSTF IDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRF ADIDTVIRADANAAMFAGLCAFFEGMGDLGRAVGKVVMGVVGG VVSAVSGVSSFMSNPFGALAVGLLVLAGLVAAFFAFRYVLQLQR NPMKALYPLTTKELKTSDPGGVGGEGEEGAEGGGFDEAKLAEAR EMIRYMALVSAMERTEHKARKKGTSALLSSKVTNMVLRKRNKA RYSPLHNEDEAGDEDEL

[0149] Provided herein is a polyribonucleotide encoding a polypeptide. In some embodiments, a polypeptide comprises one or more HSV glycoprotein B (gB) antigens or antigenic fragments thereof.

[0150] In some embodiments, an HSV gB antigen has an amino acid sequence that is identical to the amino acid of SEQ ID NO: 1. In some embodiments, an HSV gB antigen has an amino acid sequence that is identical to the amino acid of SEQ ID NO: 2. In some embodiments, an HSV gB antigen has an amino acid sequence that is identical to the amino acid of SEQ ID NO: 3. In some embodiments, an HSV gB antigen has an amino acid sequence that is identical to the amino acid of SEQ ID NO: 4. In some embodiments, an HSV gB antigen has an amino acid sequence that is identical to the amino acid of SEQ ID NO: 90. In some embodiments, an HSV gB antigen has an amino acid sequence that is identical to the amino acid of SEQ ID NO: 91.

[0151] Further, as discussed throughout the present disclosure, an issue with current HSV vaccines is that they have not been able to fully leverage antigenic potential of HSV gB (HSV-1 gB, HSV-2 gB or both). One reason for these results is that HSV gB can be unstable. In particular, cell entry of enveloped viruses requires specialized viral proteins that mediate fusion with the host membrane. During this process, the viral proteins, including gB, undergo substantial structural rearrangements from a metastable prefusion conformation to a stable postfusion conformation (Figure 5). This metastability renders the herpes simplex virus (e.g., HSV-1 and/or HSV-2) fusion gB highly unstable.

[0152] As used herein, the term "stable", when applied to glycoprotein B, means that glycoprotein B maintains one of more aspects of a physical structure (e.g., maintains a specific conformation) and/or activity for a specific period of time. In some embodiments, a stable glycoprotein B has been modified (e.g., certain mutations) so that its structure is stabilized. In some embodiments, a stable glycoprotein B structure is maintained for a specific period of time. In some embodiments, a stable glycoprotein B is in a stable prefusion conformation. In some embodiments, a stable glycoprotein B is in a stable postfusion conformation. In some embodiments, a stable glycoprotein B maintains a biological relevant activity (e.g., antigenic potential). In some embodiments, a stable glycoprotein B is in a stable prefusion conformation and maintains antigenic potential.

[0153] The present disclosure encompasses a recognition that stabilization of gB antigens or antigenic fragments thereof can be useful or advantageous for eliciting an immune response. The present disclosure further provides the recognition that stabilization of HSV gB or antigenic fragments thereof can be particularly advantageous for use, e.g., in preventing or treating HSV, e.g., in HSV antigen constructs and/or HSV compositions (e.g., immunogenic compositions, e.g., vaccines) as further

disclosed herein. Accordingly, the present disclosure provides certain mutations that can stabilize HSV gB or antigenic fragments thereof.

[0154] Provided herein is a polyribonucleotide encoding a polypeptide that comprises one or more HSV glycoprotein B (gB) antigens or antigenic fragments thereof. In some embodiments, an HSV gB antigen or antigenic fragment thereof comprises one or more mutations that stabilize the HSV gB antigen or antigenic fragment thereof relative to a comparable HSV gB antigen or antigenic fragment thereof that does not comprise the one or more mutations. In some embodiments, one or more mutations are one or more amino acid substitutions. In some embodiments, one or more amino acid substitutions comprise 251C, 718C, and combinations thereof, wherein the numbering is with reference to SEQ ID NO: 1.

[0155] In some embodiments, a polypeptide comprises at least one HSV glycoprotein B (gB) antigen or antigenic portion thereof. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 1. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 2. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 3. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 4. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 90. In some embodiments, an HSV gB antigen has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 91.

[0156] In some embodiments, an HSV gB antigen comprises an amino acid sequence according to SEQ ID NO: 1 or antigenic fragment thereof, except that the HSV gB antigen or antigenic fragment thereof comprises one or more mutations comprising 251C, 718C, and combinations thereof.

[0157] In some embodiments, an HSV gB antigen has an amino acid sequence that (i) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 1 and (ii) comprises one or more mutations, where the one or more mutations comprise 251C, 718C, and combinations thereof.

[0158] In some embodiments, an HSV gB antigen has an amino acid sequence that comprises one or more mutations, where the one or more mutations comprise 251C and 718C, where the numbering is with reference to SEQ ID NO: 1.

[0159] In some embodiments, an HSV gB antigen has an amino acid sequence that (i) is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid SEQ ID NO: 1 and (ii) comprises one or more mutations, where the one or more mutations comprise 251C and 718C, where the numbering is with reference to SEQ ID NO: 1.

[0160] In some embodiments, an HSV gB antigen or antigenic fragment thereof does not comprise an 516P mutation, where the numbering is with reference to SEQ ID NO: 1.

B. Secretory Signals

[0161] In some embodiments, an HSV-2 gB construct described herein includes a secretory signal, *e.g.*, that is functional in mammalian cells. In some embodiments, a utilized secretory signal is a heterologous secretory signal. In some embodiments, a heterologous secretory signal comprises or consists of a non-human secretory signal. In some embodiments, a heterologous secretory signal comprises or consists of a viral secretory signal. In some embodiments, a viral secretory signal comprises or consists of an HSV secretory signal (e.g., an HSV-1 or HSV-2 secretory signal). In some embodiments, an HSV secretory signal comprises or consists of an HSV glycoprotein D (gD) secretory signal. In some embodiments, an HSV secretory signal comprises or consists of an HSV glycoprotein B (gB) secretory signal (e.g., an HSV-1 gB or HSV-2 gB). In some embodiments, an HSV secretory signal comprises or consists of a secrecon secretory signal.

[0162] In some embodiments, a secretory signal comprises or consists of an Ebola virus secretory signal. In some embodiments, an Ebola virus secretory signal comprises or consists of an Ebola virus spike glycoprotein (SGP) secretory signal.

[0163] In some embodiments, a secretory signal is characterized by a length of about 15 to 30 amino acids.

[0164] In many embodiments, a secretory signal is positioned at the N-terminus of an HSV-2 gB construct described herein. In some embodiments, a secretory signal preferably allows transport of an HSV-2 gB construct with which it is associated into a defined cellular compartment, preferably a cell surface, endoplasmic reticulum (ER) or endosomal-lysosomal compartment.

[0165] In some embodiments, a secretory signal is selected from an S1S2 secretory signal (aa 1-19), an immunoglobulin secretory signal (aa 1-22), a human SPARC secretory signal, a human insulin isoform 1 secretory signal, a human albumin secretory signal, *etc.* Those skilled in the art will be aware of other secretory signal such as, for example, as disclosed in WO2017/081082, which is incorporated herein by reference in its entirety (e.g., SEQ ID NOs: 1-1115 and 1728, or fragments or variants thereof). In some embodiments, an HSV-2 gB construct described herein does not comprise a secretory signal.

[0166] In some embodiments, a secretory signal is one listed in **Table 4**, or a secretory signal having 1, 2, 3, 4, or 5 amino acid differences relative thereto. In some embodiments, a signal sequence is selected from those included in the **Table 4** below and/or those encoded by the sequences in **Tables 5 and 6** below.

Table 4: Example secretory signals

SEQ ID NO:	Secretory Signal	Sequence (Amino Acid)
5	HSV-1 gD + KY	MGGAAARLGAVILFVVIVGLHGVRGKY
6	HSV-2 gD	MGRLTSGVGTAALLVVAVGLRVVCA
7	HSV-2 gD +KYA	MGRLTSGVGTAALLVVAVGLRVVCAKYA
8	Csp (isolate 3D7)	MMRKLAILSVSSFLFVEA
9	HSV-1 gD	MGGAAARLGAVILFVVIVGLHGVRG
10	Ebola spike glycoprotein GP	MGVTGILQLPRDRFKRTSFFLWVIILFQRTFS

11	SARS-CoV-2-S	MFVFLVLLPLVSSQCVNLT
12	human Ig heavy chain signal peptide (huSec)	MDWIWRILFLVGAATGAHSQM
13	HuIgGk signal peptide	METPAQLLFLLLLWLPDTTG
14	IgE heavy chain epsilon-1signal peptide	MDWTWILFLVAAATRVHS
15	Japanese encephalitis PRM signal sequence	MLGSNSGQRVVFTILLLLVAPAYS
16	VSVg protein signal sequence	MKCLLYLAFLFIGVNCA
17	TRIO	MCRGLSAVLILLVSLSAQLHVVVG
18	human Ig heavy chain signal peptide 1	MELGLSWIFLLAILKGVQC
19	human Ig heavy chain signal peptide 2	MELGLRWVFLVAILEGVQC
20	human Ig heavy chain signal peptide 3	MKHLWFFLLLVAAPRWVLS
21	human Ig heavy chain signal peptide 4	MDWTWRILFLVAAATGAHS
22	human Ig heavy chain signal peptide 5	MDWTWRFLFVVAAATGVQS
23	human Ig heavy chain signal peptide 6	MEFGLSWLFLVAILKGVQC
24	human Ig heavy chain signal peptide 7	MEFGLSWVFLVALFRGVQC
25	human Ig heavy chain signal peptide 8	MDLLHKNMKHLWFFLLLVAAPRWVLS
26	human Ig kappa chain signal peptide 1	MDMRVPAQLLGLLLLWLSGARC
27	human Ig kappa chain signal peptide 2	MKYLLPTAAAGLLLLAAQPAMA
63	HSV-1 gB + AP	MHQGAPSWGRRWFVVWALLGLTLGVLVASAAP

69	HSV-1 gB + A	MHQGAPSWGRRWFVVWALLGLTLGVLVASAA
64	HSV-1 gB	MHQGAPSWGRRWFVVWALLGLTLGVLVASA
65	HSV-2 gB	MRGGGLICALVVGALVAAVASA
70	Secrecon	MWWRLWWLLLLLLLLWPMVWAAA

Table 5: Example polynucleotide sequences encoding secretory signals

SEQ ID NO:	Signal	Sequence (Ribonucleotide)
28	HSV-1 gD	AUGGGGGGGGCUGCCGCCAGGUUGGGGGCCGUGAU UUUGUUUGUCGUCAUAGUGGGCCUCCAUGGGGUCC GCAGCAAAUAU
29	HSV-1 gD –Version 3	AUGGGAGGAGCCGCCGCCAGACUGGGAGCCGUGAU CCUGUUCGUGGUGAUCGUGGGACUGCAUGGAGUG AGAAGCAAGUAC
30	SARS-CoV-2-S	AUGUUUGUGUUUCUUGUGCUGCUGCCUCUUGUGUC UUCUCAGUGUGUGAAUUUGACA
31	human Ig heavy chain signal peptide (huSec)	AUGGAUUGGAUUUGGAGAAUCCUGUUCCUCGUGG GAGCCGCUACAGGAGCCCACUCCCAGAUG
32	human Ig heavy chain signal peptide 1	AUGGAGUUGGGACUGAGCUGGAUUUUCCUUUUGG CUAUUUUAAAAGGUGUCCAGUGU
33	human Ig heavy chain signal peptide 2	AUGGAACUGGGGCUCCGCUGGGUUUUCCUUGUUGC UAUUUUAGAAGGUGUCCAGUGU
34	human Ig heavy chain signal peptide 3	AUGAAACACCUGUGGUUCUUCCUCCUGCUGGUGGC AGCUCCCAGAUGGGUCCUGUCC
35	human Ig heavy chain signal peptide 4	AUGGACUGGACCUGGAGGAUCCUCUUCUUGGUGGC AGCAGCAACAGGUGCCCACUCG
36	human Ig heavy chain signal peptide 5	AUGGACUGGACCUGGAGGUUCCUCUUUGUGGUGGC AGCAGCUACAGGUGUCCAGUCC
37	human Ig heavy chain signal peptide 6	AUGGAGUUUGGGCUGAGCUGGCUUUUUCUUGUGG CGAUUCUAAAAGGUGUCCAGUGU

38	human Ig heavy chain signal peptide 7	AUGGAGUUUGGGCUGAGCUGGGUUUUCCUCGUUG CUCUUUUUAGAGGUGUCCAGUGU
39	human Ig heavy chain signal peptide 8	AUGGACCUCCUGCACAAGAACAUGAAACACCUGUG GUUCUUCCUCCUCCUGGUGGCAGCUCCCAGAUGGG UGCUGUCC
40	human Ig kappa chain signal peptide 1	AUGGACAUGAGGGUCCCUGCUCAGCUCCUGGGGCU CCUGCUGCUCUGGCUCUCAGGUGCCAGAUGU
41	human Ig kappa chain signal peptide 2	AUGAAAUACCUAUUGCCUACGGCAGCCGCUGGAUU GUUAUUACUCGCGGCCCAGCCGGCCAUGGCC
66	HSV-1 gB	AUGCAUCAAGGUGCUCCAUCUUGGGGUAGACGUUG GUUCGUUGUGUGGGCCCUGCUGGGACUGACACUGG GAGUUCUUGUUGCCAGCGCU
71	HSV-1 gB + A	AUGCAUCAAGGUGCUCCAUCUUGGGGUAGACGUUG GUUCGUUGUGUGGGCCCUGCUGGGACUGACACUGG GAGUUCUUGUUGCCAGCGCUGCU
72	Secrecon	AUGUGGUGGCGACUGUGGUGGCUGCUGUUGCUGC UUCUGCUGCUGUGGCCUAUGGUUUGGGCCGCUGCU

Table 6: Example polynucleotide sequences encoding secretory signals

SEQ ID NO:	Signal	Sequence (Deoxyribonucleotide)
73	HSV-1 gD SP wild-type	ATGGGGGGGGCTGCCGCCAGGTTGGGGGCCGTGATT TTGTTTGTCGTCATAGTGGGCCTCCATGGGGTCCGCA GCAAATAT
74	HSV-1 gD SP Variant 3	ATGGGAGGAGCCGCCGCCAGACTGGGAGCCGTGAT CCTGTTCGTGGTGATCGTGGGACTGCATGGAGTGAG AAGCAAGTAC
75	SARS-CoV-2-S	ATGTTTGTGTTTCTTGTGCTGCTGCCTCTTGTGTCTTC TCAGTGTGTGAATTTGACA
76	human Ig heavy chain signal peptide (huSec)	ATGGATTGGATTTGGAGAATCCTGTTCCTCGTGGGA GCCGCTACAGGAGCCCACTCCCAGATG
77	human Ig heavy chain signal peptide 1	ATGGAGTTGGGACTGAGCTGGATTTTCCTTTTGGCTA TTTTAAAAGGTGTCCAGTGT

78	human Ig heavy chain signal peptide 2	ATGGAACTGGGGCTCCGCTGGGTTTTCCTTGTTGCTA TTTTAGAAGGTGTCCAGTGT
79	human Ig heavy chain signal peptide 3	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCA GCTCCCAGATGGGTCCTGTCC
80	human Ig heavy chain signal peptide 4	ATGGACTGGACCTGGAGGATCCTCTTCTTGGTGGCA GCAGCAACAGGTGCCCACTCG
81	human Ig heavy chain signal peptide 5	ATGGACTGGACCTGGAGGTTCCTCTTTGTGGTGGCA GCAGCTACAGGTGTCCAGTCC
82	human Ig heavy chain signal peptide 6	ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCGA TTCTAAAAGGTGTCCAGTGT
83	human Ig heavy chain signal peptide 7	ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTC TTTTTAGAGGTGTCCAGTGT
84	human Ig heavy chain signal peptide 8	ATGGACCTCCTGCACAAGAACATGAAACACCTGTGG TTCTTCCTCCTCCTGGTGGCAGCTCCCAGATGGGTGC TGTCC
85	human Ig kappa chain signal peptide 1	ATGGACATGAGGGTCCCTGCTCAGCTCCTGGGGCTC CTGCTGCTCTGGCTCTCAGGTGCCAGATGT
86	human Ig kappa chain signal peptide 2	ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTG TTATTACTCGCGGCCCAGCCGGCCATGGCC
87	HSV-1 gB	ATGCATCAAGGTGCTCCATCTTGGGGTAGACGTTGG TTCGTTGTGTGGGCCCTGCTGGGACTGACACTGGGA GTTCTTGTTGCCAGCGCT
88	HSV-1 gB	ATGCATCAAGGTGCTCCATCTTGGGGTAGACGTTGG TTCGTTGTGTGGGCCCTGCTGGGACTGACACTGGGA GTTCTTGTTGCCAGCGCTGCT
89	Secrecon	ATGTGGTGGCGACTGTGGTGGCTGCTGTTGCTGCTTC TGCTGCTGTGGCCTATGGTTTGGGCCGCTGCT

C. Transmembrane Regions

[0167] In some embodiments, an HSV-2 gB construct described herein includes a transmembrane region. In some embodiments, a transmembrane region is located at the N-terminus of an HSV-2 gB construct. In some embodiments, a transmembrane region is located at the C-terminus of an HSV-2 gB construct. In some embodiments, a transmembrane region is not located at the N-terminus or C-terminus of an HSV-2 gB construct.

[0168] Transmembrane regions are known in the art, any of which can be utilized in an HSV-2 gB construct described herein. In some embodiments, a transmembrane region comprises or is a transmembrane domain of Hemagglutinin (HA) of Influenza virus, Env of HIV-1, equine infectious anaemia virus (EIAV), murine leukaemia virus (MLV), mouse mammary tumor virus, G protein of vesicular stomatitis virus (VSV), Rabies virus, or a seven transmembrane domain receptor.

[0169] In some embodiments, a heterologous transmembrane region does not comprise a hemagglutin transmembrane region. In some embodiments, a heterologous transmembrane region comprises or consists of a non-human transmembrane region. In some embodiments, a heterologous transmembrane region comprises or consists of a viral transmembrane region. In some embodiments, a heterologous transmembrane region comprises or consists of an HSV transmembrane region, e.g., an HSV-1 or HSV-2 transmembrane region. In some embodiments, an HSV transmembrane region comprises or consists of an HSV gD transmembrane region, e.g., comprising or consisting of an amino acid sequence of GLIAGAVGGSLLAALVICGIVYWMRRHTQKAPKRIRLPHIR (SEQ ID NO: 42).

[0170] In some embodiments, a heterologous transmembrane region comprises or consists of a human transmembrane region. In some embodiments, a human transmembrane region comprises or consists of a human decay accelerating factor glycosylphosphatidylinositol (hDAF-GPI) anchor region. In some embodiments, an hDAF-GPI anchor region comprises or consists of an amino acid sequence of PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT (SEQ ID NO: 43).

[0171] In some embodiments, an HSV transmembrane region comprises or consists of an HSV gB transmembrane region, e.g., comprising or consisting of an amino acid sequence of MSNPFGALAVGLLVLAGLAAAFFAFRYVMRL (SEQ ID NO: 67) or MSNPFGALAVGLLVLAGLVAAFFAFRYVLQL (SEQ ID NO: 68).

[0172] In some embodiments, a utilized transmembrane region is a heterologous transmembrane region.

[0173] In some embodiments, an HSV-2 gB construct described herein does not comprise a transmembrane region.

[0174] Example transmembrane are provided in the following **Table 7**:

Table 7: Example transmembrane regions

SEQ ID NO:	Transmembrane Region	Sequence (Amino Acid)
42	HSV-1 gD	GLIAGAVGGSLLAALVICGIVYWMRRHTQKAPKRIRLPHIR
43	hDAF-GPI anchor region	PNKGSGTTSGTTRLLSGHTCFTLTGLLGTLVTMGLLT
67	HSV-1 gB	MSNPFGALAVGLLVLAGLAAAFFAFRYVMRL
68	HSV-2 gB	MSNPFGALAVGLLVLAGLVAAFFAFRYVLQL

D. Multimerization Regions

[0175] In some embodiments, an HSV-2 gB construct described herein includes one or more multimerization regions (e.g., a heterologous multimerization region). In some embodiments, a heterologous multimerization region comprises a dimerization, trimerization or tetramerization region.

[0176] In some embodiments, a multimerization region is one described in WO2017/081082, which is incorporated herein by reference in its entirety (*e.g.*, SEQ ID NOs: 1116-1167, or fragments or variants thereof). Example trimerization and tetramerization regions include, but are not limited to, engineered leucine zippers, fibritin foldon domain from enterobacteria phage T4, GCN4pII, GCN4-pII, and p53.

[0177] In some embodiments, a provided HSV-2 gB construct described herein is able to form a trimeric complex. For example, a provided HSV-2 gB construct may comprise a multimerization region allowing formation of a multimeric complex, such as for example a trimeric complex of an HSV-2 gB construct described herein. In some embodiments, a multimerization region allowing formation of a multimeric complex comprises a trimerization region, for example, a trimerization region described herein. In some embodiments, an HSV-2 gB construct includes a T4-fibritin-derived "foldon" trimerization region, for example, to increase its immunogenicity. In some embodiments, an HSV-2 gB construct includes a multimerization region comprising or consisting of the amino acid sequence GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 44).

E. Linkers

[0178] In some embodiments, an HSV-2 gB construct described herein includes one or more linkers. In some embodiments, a linker is or comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids. In some embodiments, a linker is or comprises no more than about 30, 25, 20, 15, 10 or fewer amino acids.

A linker can include any amino acid sequence and is not limited to any particular amino acids. In some embodiments, a linker comprises one or more glycine (G) amino acids. In some embodiments, a linker comprises one or more serine (S) amino acids. In some embodiments, a linker comprises a glycine-serine linker. A "glycine-serine linker" as used herein refers to a linker that comprises predominantly (e.g., 80% or more) glycine and serine amino acids. In some embodiments, a linker includes amino acids selected based on a cleavage predictor to generate highly-cleavable linkers.

[0179] In some embodiments, a linker is or comprises S-G_4-S-G_4-S. In some embodiments, a linker is or comprises GSPGSGSGS (SEQ ID NO: 46). In some embodiments, a linker is or comprises GGSGGGGSGG (SEQ ID NO: 47). In some embodiments, a linker is one presented in **Table 8**. In some embodiments, a linker is or comprises a sequence as set forth in WO2017/081082, which is incorporated herein by reference in its entirety (see SEQ ID NOs: 1509-1565, or a fragment or variant thereof).

[0180] In some embodiments, an HSV-2 gB construct described herein comprises a linker between a C-terminal region or fragment thereof and a transmembrane region. In some embodiments, an HSV-2 gB construct described herein comprises a linker after a minor repeat sequence.

[0181] Example linkers are provided in the following **Table 8**:

Table 8: Example linkers

SEQ ID NO:	Sequence (Amino Acid)
45	SGGGGSGGGGS
46	GSPGSGSGS
47	GGSGGGGSGG
48	GGS
49	GGGS
50	GGGGSGGGGSGGGGS
51	AGNRVRRSVG
52	GSGSGS
53	GGSLGGGGSG
54	SGG

III. Polyribonucleotides

A. Example Polyribonucleotides Features

[0182] Polyribonucleotides described herein encode one or more HSV-2 gB constructs described herein. In some embodiments, polyribonucleotides described herein can comprise a nucleotide sequence that encodes a 5'UTR of interest and/or a 3' UTR of interest. In some embodiments, polynucleotides described herein can comprise a nucleotide sequence that encodes a polyA tail. In some embodiments, polyribonucleotides described herein may comprise a 5' cap, which may be incorporated during transcription, or joined to a polyribonucleotide post-transcription.

1. 5' Cap

[0183] A structural feature of mRNAs is cap structure at five-prime end (5'). Natural eukaryotic mRNA comprises a 7-methylguanosine cap linked to the mRNA via a 5′ to 5′-triphosphate bridge resulting in cap0 structure (m7GpppN). In most eukaryotic mRNA and some viral mRNA, further modifications can occur at the 2'-hydroxy-group (2'-OH) (*e.g.*, the 2'-hydroxyl group may be methylated to form 2'-O-Me) of the first and subsequent nucleotides producing "cap1" and "cap2" five-prime ends, respectively). Diamond, et al., (2014) *Cytokine & growth Factor Reviews*, 25:543–550, which is incorporated herein by reference in its entirety, reported that cap0-mRNA cannot be translated as efficiently as cap1-mRNA in which the role of 2'-O-Me in the penultimate position at the mRNA 5' end is determinant. Lack of the 2'-O-met has been shown to trigger innate immunity and activate IFN response. Daffis, et al. (2010) *Nature*, 468:452-456; and Züst et al. (2011) *Nature Immunology*, 12:137-143, each of which is incorporated herein by reference in its entirety.

[0184] RNA capping is well researched and is described, e.g., in Decroly E et al. (2012) Nature Reviews 10: 51-65; and in Ramanathan A. et al., (2016) Nucleic Acids Res; 44(16): 7511–7526, the entire contents of each of which is hereby incorporated by reference. For example, in some embodiments, a 5'-cap structure which may be suitable in the context of the present invention is a cap0 (methylation of the first nucleobase, e.g., m7GpppN), cap1 (additional methylation of the ribose of the adjacent nucleotide of m7GpppN), cap2 (additional methylation of the ribose of the 2nd nucleotide downstream of the m7GpppN), cap3 (additional methylation of the ribose of the 3rd nucleotide downstream of the m7GpppN), cap4 (additional methylation of the ribose of the 4th nucleotide downstream of the m7GpppN), ARCA ("anti-reverse cap analogue"), modified ARCA (e.g. phosphothioate modified

ARCA), inosine, N1 -methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0185] The term "5'-cap" as used herein refers to a structure found on the 5'-end of an RNA, *e.g.*, mRNA, and generally includes a guanosine nucleotide connected to an RNA, *e.g.*, mRNA, via a 5'- to 5'-triphosphate linkage (also referred to as Gppp or G(5')ppp(5')). In some embodiments, a guanosine nucleoside included in a 5' cap may be modified, for example, by methylation at one or more positions (*e.g.*, at the 7-position) on a base (guanine), and/or by methylation at one or more positions of a ribose. In some embodiments, a guanosine nucleoside included in a 5' cap comprises a 3'O methylation at a ribose (3'OMeG). In some embodiments, a guanosine nucleoside included in a 5' cap comprises methylation at the 7-position of guanine (m7G). In some embodiments, a guanosine nucleoside included in a 5' cap comprises methylation at the 7-position of guanine and a 3' O methylation at a ribose (m7(3'OMeG)). It will be understood that the notation used in the above paragraph, *e.g.*, "($m_2^{7,3'-O}$)G" or "m7(3'OMeG)", applies to other structures described herein.

[0186] In some embodiments, providing an RNA with a 5'-cap disclosed herein may be achieved by *in vitro* transcription, in which a 5'-cap is co-transcriptionally expressed into an RNA strand, or may be attached to an RNA post-transcriptionally using capping enzymes. In some embodiments, co-transcriptional capping with a cap disclosed improves the capping efficiency of an RNA compared to co-transcriptional capping with an appropriate reference comparator. In some embodiments, improving capping efficiency can increase a translation efficiency and/or translation rate of an RNA, and/or increase expression of an encoded polypeptide. In some embodiments, alterations to polynucleotides generates a non-hydrolyzable cap structure which can, for example, prevent decapping and increase RNA half-life.

[0187] In some embodiments, a utilized 5' caps is a cap0, a cap1, or cap2 structure. *See, e.g.*, Figure 1 of Ramanathan A *et al.*, and Figure 1 of Decroly E *et al.*, each of which is incorporated herein by reference in its entirety. *See, e.g.*, Figure 1 of Ramanathan A et al., and Figure 1 of Decroly E *et al.*, each of which is incorporated herein by reference in its entirety. In some embodiments, an RNA described herein comprises a cap1 structure. In some embodiments, an RNA described herein comprises a cap2.

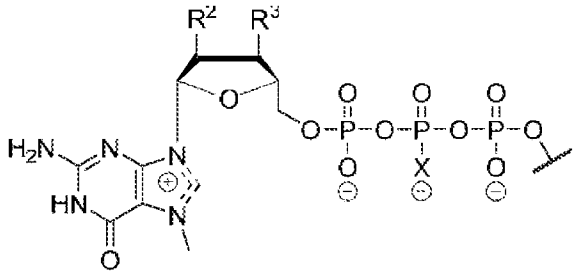
[0188] In some embodiments, an RNA described herein comprises a cap0 structure. In some embodiments, a cap0 structure comprises a guanosine nucleoside methylated at the 7-position of guanine ((m^7)G). In some embodiments, such a cap0 structure is connected to an RNA via a 5'- to 5'-triphosphate linkage and is also referred to herein as (m^7)Gppp. In some embodiments, a cap0 structure comprises a

guanosine nucleoside methylated at the 2'-position of the ribose of guanosine. In some embodiments, a cap0 structure comprises a guanosine nucleoside methylated at the 3'-position of the ribose of guanosine. In some embodiments, a guanosine nucleoside included in a 5' cap comprises methylation at the 7-position of guanine and at the 2'-position of the ribose ($(m_2^{7,2'\text{-O}})G$). In some embodiments, a guanosine nucleoside included in a 5' cap comprises methylation at the 7-position of guanine and at the 2'-position of the ribose ($(m_2^{7,3'\text{-O}})G$).

[0189] In some embodiments, a cap1 structure comprises a guanosine nucleoside methylated at the 7-position of guanine ($(m^7)G$) and optionally methylated at the 2' or 3' position pf the ribose, and a 2'O methylated first nucleotide in an RNA ($(m^{2'\text{-O}})N_1$). In some embodiments, a cap1 structure comprises a guanosine nucleoside methylated at the 7-position of guanine ($(m^7)G$) and the 3' position of the ribose, and a 2'O methylated first nucleotide in an RNA ($(m^{2'\text{-O}})N_1$). In some embodiments, a cap1 structure is connected to an RNA via a 5'- to 5'-triphosphate linkage and is also referred to herein as, e.g., $((m^7)Gppp(^{2'\text{-O}})N_1)$ or $(m_2^{7,3'\text{-O}})Gppp(^{2'\text{-O}})N_1)$, wherein N_1 is as defined and described herein. In some embodiments, a cap1 structure comprises a second nucleotide, N_2, which is at position 2 and is chosen from A, G, C, or U, e.g., $(m^7)Gppp(^{2'\text{-O}})N_1pN_2$ or $(m_2^{7,3'\text{-O}})Gppp(^{2'\text{-O}})N_1pN_2$, wherein each of N_1 and N_2 is as defined and described herein.

[0190] In some embodiments, a cap2 structure comprises a guanosine nucleoside methylated at the 7-position of guanine ($(m^7)G$) and optionally methylated at the 2' or 3' position of the ribose, and a 2'O methylated first and second nucleotides in an RNA ($(m^{2'\text{-O}})N_1p(m^{2'\text{-O}})N_2$). In some embodiments, a cap2 structure comprises a guanosine nucleoside methylated at the 7-position of guanine ($(m^7)G$) and the 3' position of the ribose, and a 2'O methylated first and second nucleotide in an RNA. In some embodiments, a cap2 structure is connected to an RNA via a 5'- to 5'-triphosphate linkage and is also referred to herein as, e.g., $((m^7)Gppp(^{2'\text{-O}})N_1p(^{2'\text{-O}})N_2)$ or $(m_2^{7,3'\text{-O}})Gppp(^{2'\text{-O}})N_1p(^{2'\text{-O}})N_2)$, wherein each of N_1 and N_2 is as defined and described herein.

[0191] In some embodiments, the 5' cap is a dinucleotide cap structure. In some embodiments, the 5' cap is a dinucleotide cap structure comprising N_1, wherein N_1 is as defined and described herein. In some embodiments, the 5' cap is a dinucleotide cap G^*N_1, wherein N_1 is as defined above and herein, and G* comprises a structure of formula (I):



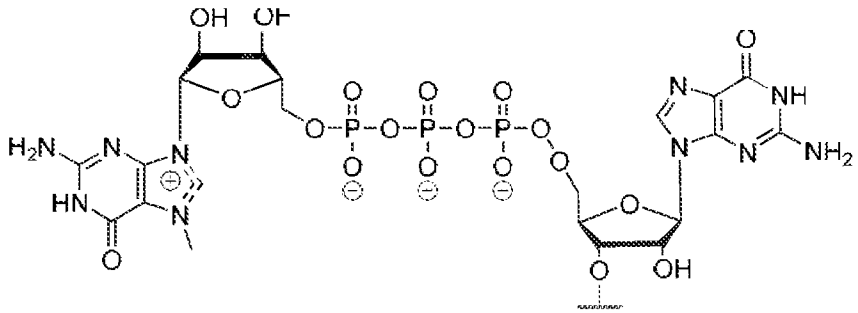
(I)

or a salt thereof, wherein each R^2 and R^3 is -OH or -OCH_3; and X is O or S.

[0192] In some embodiments, R^2 is -OH. In some embodiments, R^2 is -OCH_3. In some embodiments, R^3 is -OH. In some embodiments, R^3 is -OCH_3. In some embodiments, R^2 is -OH and R^3 is -OH. In some embodiments, R^2 is -OH and R^3 is -CH_3. In some embodiments, R^2 is -CH_3 and R^3 is -OH. In some embodiments, R^2 is -CH_3 and R^3 is -CH_3.

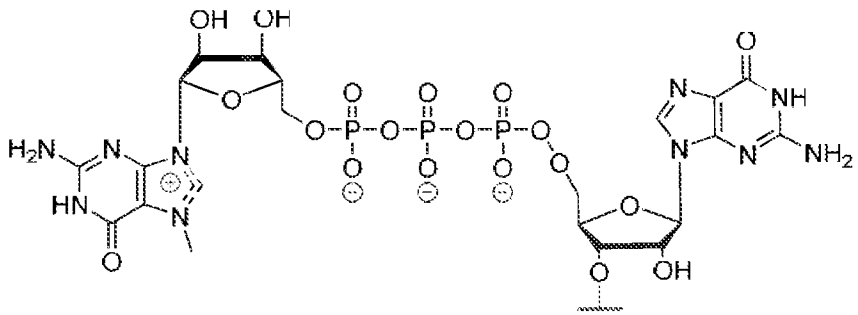
[0193] In some embodiments, X is O. In some embodiments, X is S.

[0194] In some embodiments, the 5' cap is a dinucleotide cap0 structure (*e.g.*, (m^7)GpppN_1, ($m_2^{7,2'-O}$)GpppN_1, ($m_2^{7,3'-O}$)GpppN_1, (m^7)GppSpN_1, ($m_2^{7,2'-O}$)GppSpN_1, or ($m_2^{7,3'-O}$)GppSpN_1), wherein N_1 is as defined and described herein. In some embodiments, the 5' cap is a dinucleotide cap0 structure (*e.g.*, (m^7)GpppN_1, ($m_2^{7,2'-O}$)GpppN_1, ($m_2^{7,3'-O}$)GpppN_1, (m^7)GppSpN_1, ($m_2^{7,2'-O}$)GppSpN_1, or($m_2^{7,3'-O}$)GppSpN_1), wherein N_1 is G. In some embodiments, the 5' cap is a dinucleotide cap0 structure (*e.g.*, (m^7)GpppN_1, ($m_2^{7,2'-O}$)GpppN_1, ($m_2^{7,3'-O}$)GpppN_1, (m^7)GppSpN_1, ($m_2^{7,2'-O}$)GppSpN_1, or ($m_2^{7,3'-O}$)GppSpN_1), wherein N_1 is A, U, or C. In some embodiments, the 5' cap is a dinucleotide cap1 structure (*e.g.*, (m^7)Gppp($m^{2'-O}$)N_1, ($m_2^{7,2'-O}$)Gppp($m^{2'-O}$)N_1, ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)N_1, (m^7)GppSp($m^{2'-O}$)N_1, ($m_2^{7,2'-O}$)GppSp($m^{2'-O}$)N_1, or ($m_2^{7,3'-O}$)GppSp($m^{2'-O}$)N_1), wherein N_1 is as defined and described herein. In some embodiments, the 5' cap is selected from the group consisting of (m^7)GpppG ("Ecap0"), (m^7)Gppp($m^{2'-O}$)G ("Ecap1"), ($m_2^{7,3'-O}$)GpppG ("ARCA" or "D1"), and ($m_2^{7,2'-O}$)GppSpG ("beta-S-ARCA"). In some embodiments, the 5' cap is (m^7)GpppG ("Ecap0"), having a structure:



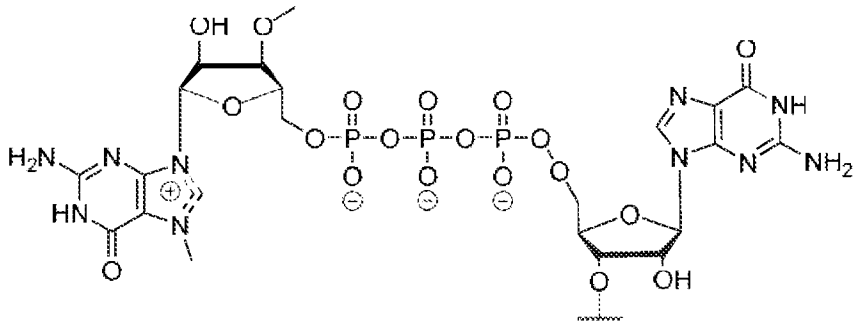
or a salt thereof.

[0195] In some embodiments, the 5' cap is (m^7)Gppp($m^{2'-O}$)G ("Ecap1"), having a structure:



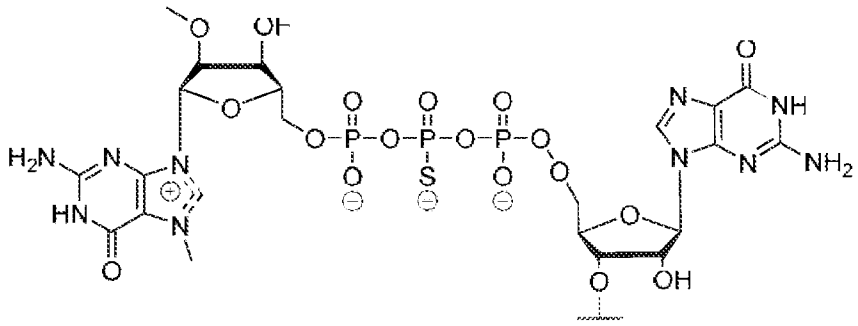
or a salt thereof.

[0196] In some embodiments, the 5' cap is ($m_2^{7,3'-O}$)GpppG ("ARCA" or "D1"), having a structure:



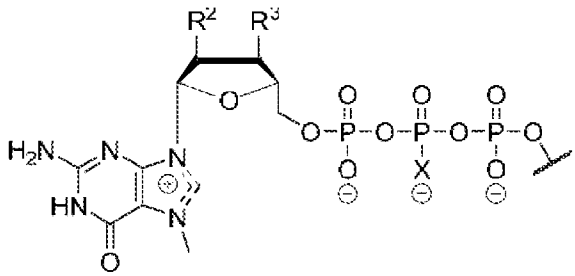
or a salt thereof.

[0197] In some embodiments, the 5' cap is ($m_2^{7,2'-O}$)GppSpG ("beta-S-ARCA"), having a structure:



or a salt thereof.

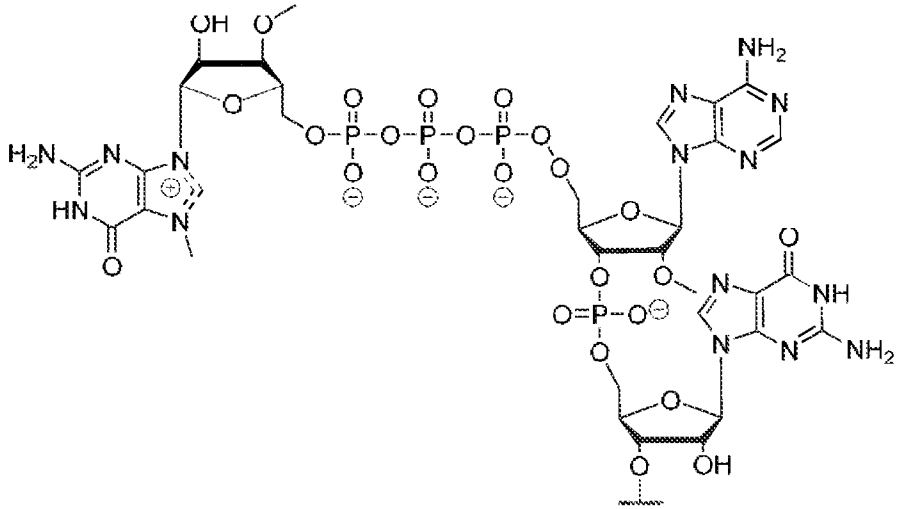
[0198] In some embodiments, the 5' cap is a trinucleotide cap structure. In some embodiments, the 5' cap is a trinucleotide cap structure comprising N_1pN_2, wherein N_1 and N_2 are as defined and described herein. In some embodiments, the 5' cap is a dinucleotide cap $G^*N_1pN_2$, wherein N_1 and N_2 are as defined above and herein, and G* comprises a structure of formula (I):



or a salt thereof, wherein R^2, R^3, and X are as defined and described herein.

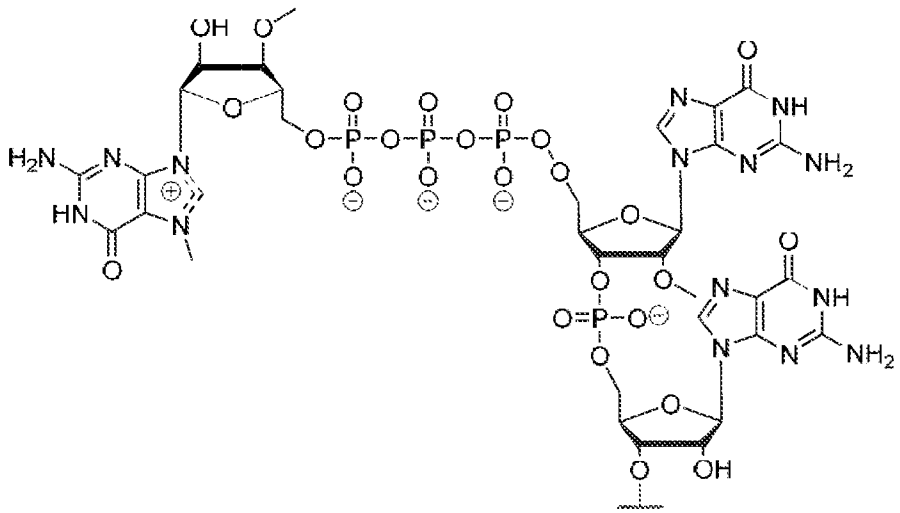
[0199] In some embodiments, the 5' cap is a trinucleotide cap0 structure (*e.g.* (m^7)GpppN$_1$pN$_2$, ($m_2^{7,2'-O}$)GpppN$_1$pN$_2$, or ($m_2^{7,3'-O}$)GpppN$_1$pN$_2$), wherein N_1 and N_2 are as defined and described herein). In some embodiments, the 5' cap is a trinucleotide cap1 structure (*e.g.*, (m^7)Gppp($m^{2'-O}$)N$_1$pN$_2$, ($m_2^{7,2'-O}$)Gppp($m^{2'-O}$)N$_1$pN$_2$, ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)N$_1$pN$_2$), wherein N_1 and N_2 are as defined and described herein. In some embodiments, the 5' cap is a trinucleotide cap2 structure (*e.g.*, (m^7)Gppp($m^{2'-O}$)N$_1$p($m^{2'-O}$)N$_2$, ($m_2^{7,2'-O}$)Gppp($m^{2'-O}$)N$_1$p($m^{2'-O}$)N$_2$, ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)N$_1$p($m^{2'-O}$)N$_2$), wherein N_1 and N_2 are as defined and described herein. In some embodiments, the 5' cap is selected from the group consisting of ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)ApG ("CleanCap AG", "CC413"), ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)GpG ("CleanCap GG"), (m^7)Gppp($m^{2'-O}$)ApG, (m^7)Gppp($m^{2'-O}$)GpG, ($m_2^{7,3'-O}$)Gppp($m_2^{6,2'-O}$)ApG, and (m^7)Gppp($m^{2'-O}$)ApU.

[0200] In some embodiments, a 5' cap is ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)ApG ("CleanCap AG", "CC413"), having a structure:



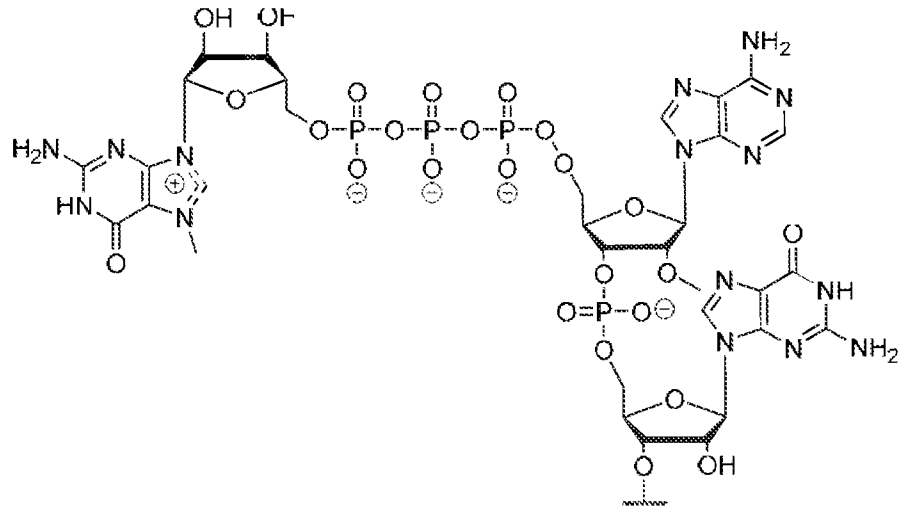
or a salt thereof.

[0201] In some embodiments, the 5' cap is ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)GpG ("CleanCap GG"), having a structure:



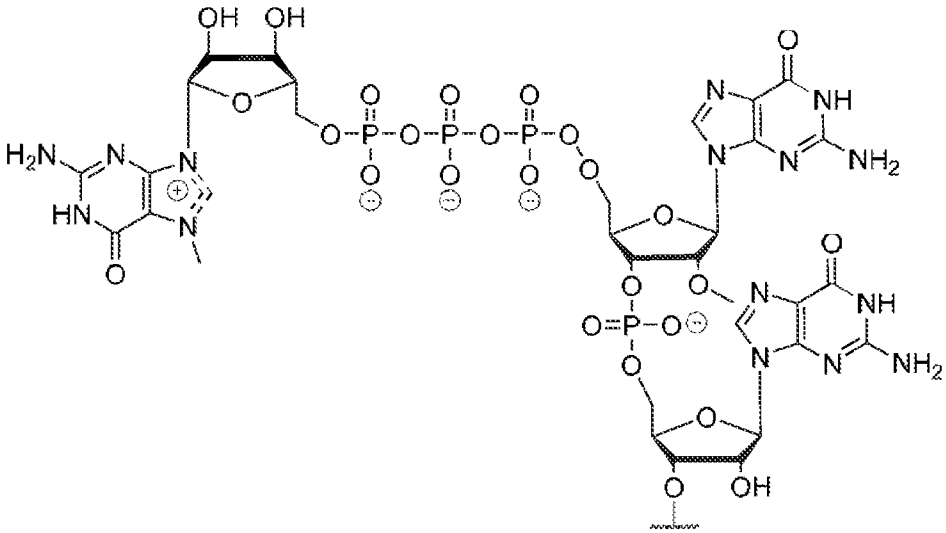
or a salt thereof.

[0202] In some embodiments, the 5' cap is (m^7)Gppp($m^{2'-O}$)ApG, having a structure:



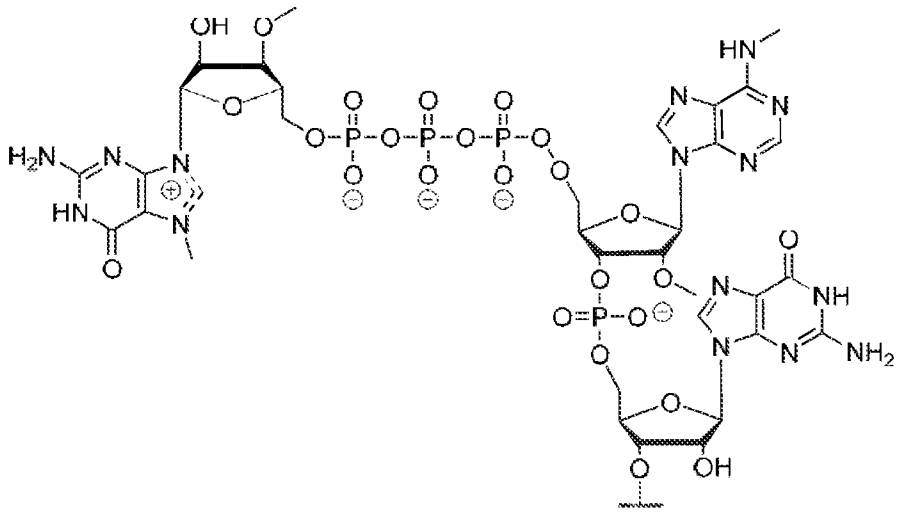
or a salt thereof.

[0203] In some embodiments, a 5' cap is (m^7)Gppp($m^{2'-O}$)GpG, having a structure:



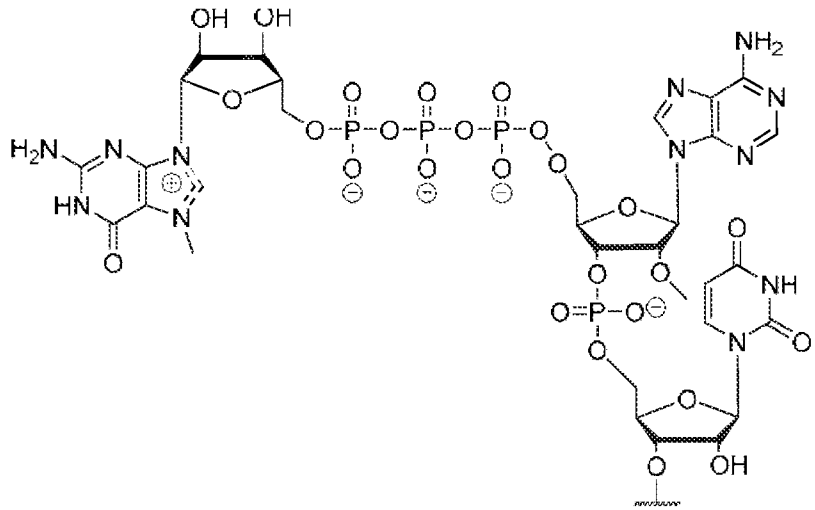
or a salt thereof.

[0204] In some embodiments, a 5' cap is ($m_2^{7,3'-O}$)Gppp($m_2^{6,2'-O}$)ApG, having a structure:



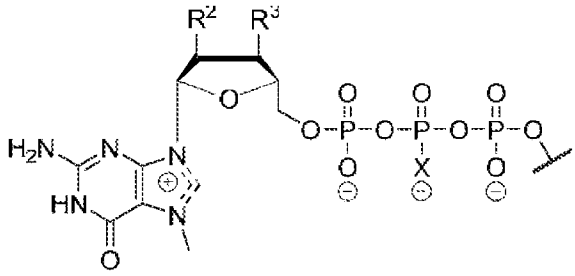
or a salt thereof.

[0205] In some embodiments, the 5' cap is (m^7)Gppp($m^{2'-O}$)ApU, having a structure:



or a salt thereof.

[0206] In some embodiments, the 5' cap is a tetranucleotide cap structure. In some embodiments, the 5' cap is a tetranucleotide cap structure comprising $N_1pN_2pN_3$, wherein N_1, N_2, and N_3 are as defined and described herein. In some embodiments, the 5' cap is a tetranucleotide cap $G^*N_1pN_2pN_3$, wherein N_1, N_2, and N_3 are as defined above and herein, and G* comprises a structure of formula (I):

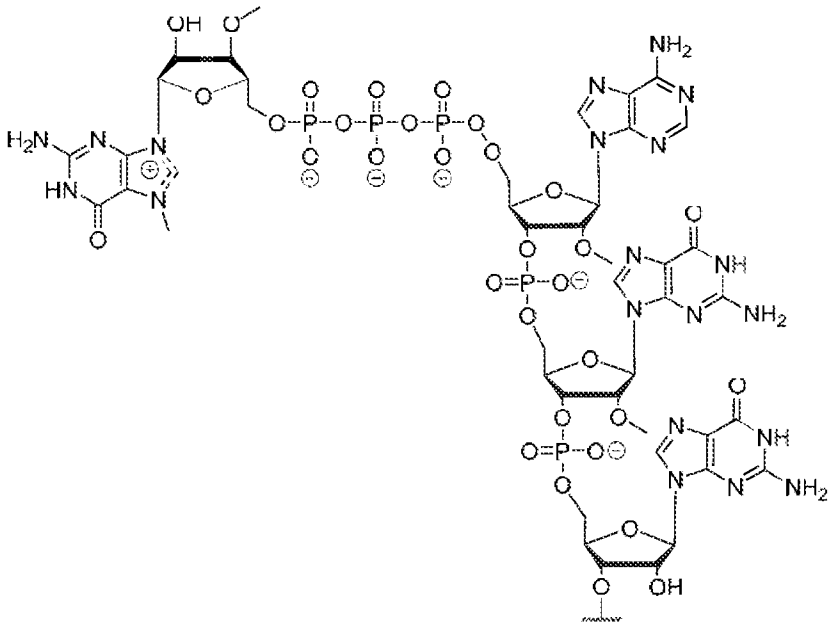


(I)

or a salt thereof, wherein R^2, R^3, and X are as defined and described herein.

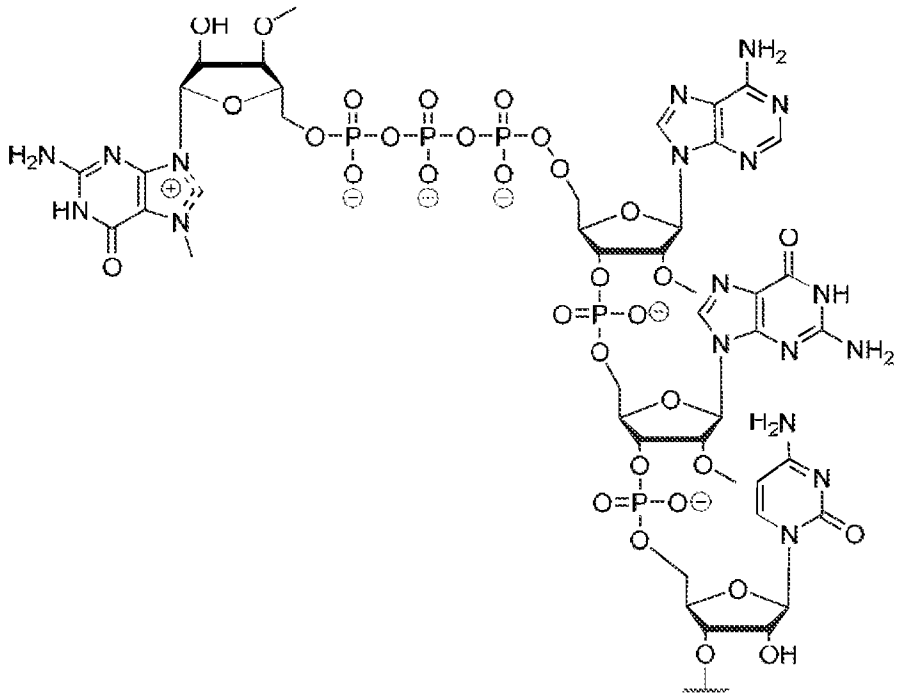
[0207] In some embodiments, the 5' cap is a tetranucleotide cap0 structure (*e.g.* (m^7)GpppN$_1$pN$_2$pN$_3$, ($m_2^{7,2'\text{-O}}$)GpppN$_1$pN$_2$pN$_3$, or ($m_2^{7,3'\text{-O}}$)GpppN$_1$N$_2$pN$_3$), wherein N$_1$, N$_2$, and N$_3$ are as defined and described herein). In some embodiments, the 5' cap is a tetranucleotide Cap1 structure (*e.g.*, (m^7)Gppp($m^{2'\text{-O}}$)N$_1$pN$_2$pN$_3$, ($m_2^{7,2'\text{-O}}$)Gppp($m^{2'\text{-O}}$)N$_1$pN$_2$pN$_3$, ($m_2^{7,3'\text{-O}}$)Gppp($m^{2'\text{-O}}$)N$_1$pN$_2$N$_3$), wherein N$_1$, N$_2$, and N$_3$ are as defined and described herein. In some embodiments, the 5' cap is a tetranucleotide Cap2 structure (*e.g.*, (m^7)Gppp($m^{2'\text{-O}}$)N$_1$p($m^{2'\text{-O}}$)N$_2$pN$_3$, ($m_2^{7,2'\text{-O}}$)Gppp($m^{2'\text{-O}}$)N$_1$p($m^{2'\text{-O}}$)N$_2$pN$_3$, ($m_2^{7,3'\text{-O}}$)Gppp($m^{2'\text{-O}}$)N$_1$p($m^{2'\text{-O}}$)N$_2$pN$_3$), wherein N$_1$, N$_2$, and N$_3$ are as defined and described herein. In some embodiments, the 5' cap is selected from the group consisting of ($m_2^{7,3'\text{-O}}$)Gppp($m^{2'\text{-O}}$)Ap($m^{2'\text{-O}}$)GpG, ($m_2^{7,3'\text{-O}}$)Gppp($m^{2'\text{-O}}$)Gp($m^{2'\text{-O}}$)GpC, (m^7)Gppp($m^{2'\text{-O}}$)Ap($m^{2'\text{-O}}$)UpA, and (m^7)Gppp($m^{2'\text{-O}}$)Ap($m^{2'\text{-O}}$)GpG.

[0208] In some embodiments, a 5' cap is ($m_2^{7,3'\text{-O}}$)Gppp($m^{2'\text{-O}}$)Ap($m^{2'\text{-O}}$)GpG, having a structure:



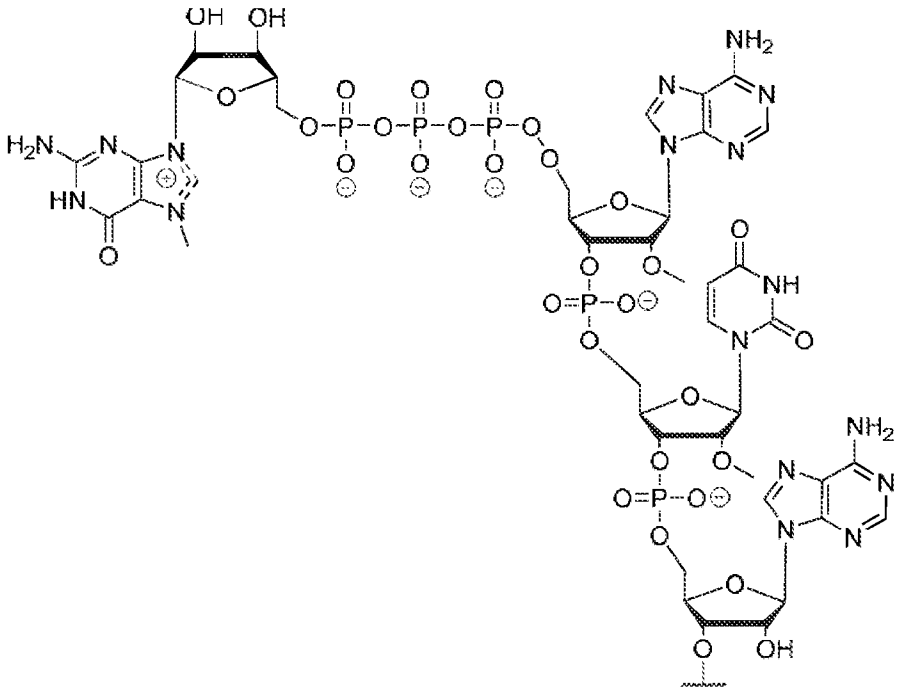
or a salt thereof.

[0209] In some embodiments, a 5' cap is ($m_2^{7,3'-O}$)Gppp($m^{2'-O}$)Gp($m^{2'-O}$)GpC, having a structure:



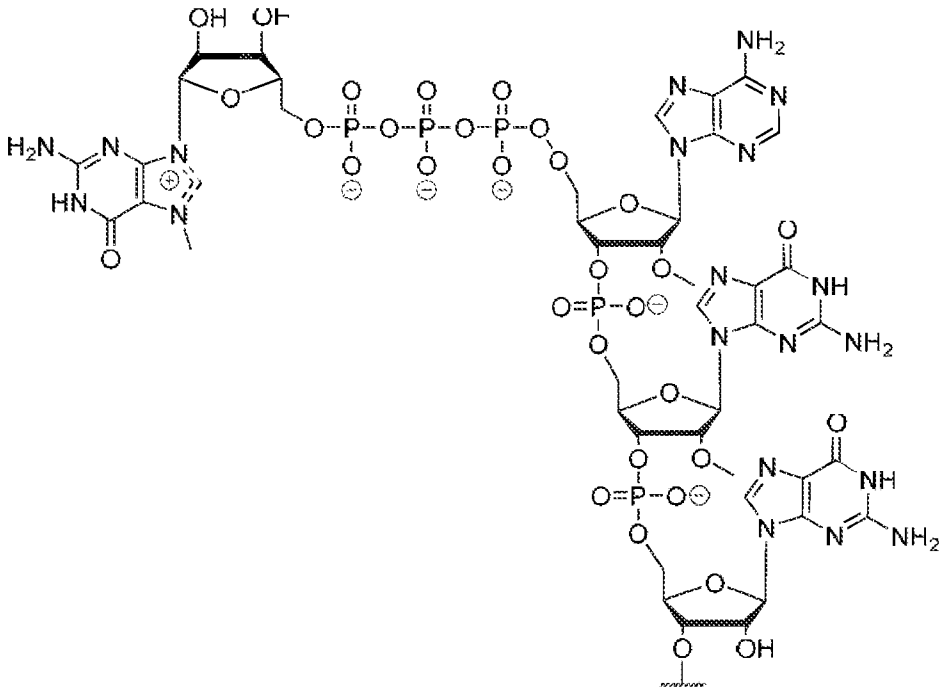
or a salt thereof.

[0210] In some embodiments, a 5' cap is (m^7)Gppp($m^{2'-O}$)Ap($m^{2'-O}$)UpA, having a structure:



or a salt thereof.

[0211] In some embodiments, a 5' cap is (m^7)Gppp($m^{2'-O}$)Ap($m^{2'-O}$)GpG, having a structure:



or a salt thereof.

2. Cap Proximal Sequences

[0212] In some embodiments, a 5' UTR utilized in accordance with the present disclosure comprises a cap proximal sequence, *e.g.*, as disclosed herein. In some embodiments, a cap proximal sequence comprises a sequence adjacent to a 5' cap. In some embodiments, a cap proximal sequence comprises nucleotides in positions +1, +2, +3, +4, and/or +5 of an RNA polynucleotide.

[0213] In some embodiments, a cap structure comprises one or more polynucleotides of a cap proximal sequence. In some embodiments, a cap structure comprises an m^7 Guanosine cap and nucleotide +1 (N_1) of an RNA polynucleotide. In some embodiments, a cap structure comprises an m^7 Guanosine cap and nucleotide +2 (N_2) of an RNA polynucleotide. In some embodiments, a cap structure comprises an m^7 Guanosine cap and nucleotides +1 and +2 (N_1 and N_2) of an RNA polynucleotide. In some embodiments, a cap structure comprises an m^7 Guanosine cap and nucleotides +1, +2, and +3 (N_1, N_2, and N_3) of an RNA polynucleotide.

[0214] Those skilled in the art, reading the present disclosure, will appreciate that, in some embodiments, one or more residues of a cap proximal sequence (*e.g.*, one or more of residues +1, +2, +3,

+4, and/or +5) may be included in an RNA by virtue of having been included in a cap entity (*e.g.*, a cap1 or cap2 structure, etc.); alternatively, in some embodiments, at least some of the residues in a cap proximal sequence may be enzymatically added (*e.g.*, by a polymerase such as a T7 polymerase). For example, in certain exemplified embodiments where a $m_2^{7,3'-O}$Gppp($m_1^{2'-O}$)ApG cap is utilized, +1 (i.e., N_1) and +2 (i.e. N_2) are the ($m_1^{2'-O}$)A and G residues of the cap, and +3, +4, and +5 are added by polymerase (*e.g.*, T7 polymerase).

[0215] In some embodiments, the 5' Cap is a dinucleotide cap structure, wherein the cap proximal sequence comprises N_1 of the 5' Cap, where N_1 is any nucleotide, *e.g.*, A, C, G or U. In some embodiments, the 5' Cap is a trinucleotide cap structure (*e.g.*, the trinucleotide cap structures described above and herein), wherein the cap proximal sequence comprises N_1 and N_2 of the 5' Cap, wherein N_1 and N_2 are independently any nucleotide, *e.g.*, A, C, G or U. In some embodiments, the 5' Cap is a tetranucleotide cap structure (*e.g.*, the trinucleotide cap structures described above and herein), wherein the cap proximal sequence comprises N_1, N_2, and N_3 of the 5' Cap, wherein N_1, N_2, and N_3 are any nucleotide, *e.g.*, A, C, G or U.

[0216] In some embodiments, *e.g.*, where the 5' Cap is a dinucleotide cap structure, a cap proximal sequence comprises N_1 of a the 5' Cap, and N_2, N_3, N_4 and N_5, wherein N_1 to N_5 correspond to positions +1, +2, +3, +4, and/or +5 of an RNA polynucleotide. In some embodiments, *e.g.*, where the 5' Cap is a trinucleotide cap structure, a cap proximal sequence comprises N_1 and N_2 of a the 5' Cap, and N_3, N_4 and N_5, wherein N_1 to N_5 correspond to positions +1, +2, +3, +4, and/or +5 of an RNA polynucleotide. In some embodiments, *e.g.*, where the 5' Cap is a tetranucleotide cap structure, a cap proximal sequence comprises N_1, N_2, and N_3 of a the 5' cap, and N_4 and N_5, wherein N_1 to N_5 correspond to positions +1, +2, +3, +4, and/or +5 of an RNA polynucleotide.

[0217] In some embodiments, N_1 is A. In some embodiments, N_1 is C. In some embodiments, N_1 is G. In some embodiments, N_1 is U. In some embodiments, N_2 is A. In some embodiments, N_2 is C. In some embodiments, N_2 is G. In some embodiments, N_2 is U. In some embodiments, N_3 is A. In some embodiments, N_3 is C. In some embodiments, N_3 is G. In some embodiments, N_3 is U. In some embodiments, N_4 is A. In some embodiments, N_4 is C. In some embodiments, N_4 is G. In some embodiments, N_4 is U. In some embodiments, N_5 is A. In some embodiments, N_5 is C. In some embodiments, N_5 is G. In some embodiments, N_5 is U. It will be understood that, each of the

embodiments described above and herein (e.g., for N_1 through N_5) may be taken singly or in combination and/or may be combined with other embodiments of variables described above and herein (e.g., 5' caps).

[0218] In some embodiments, a cap proximal sequence comprises A_1 and G_2 of the Cap1 structure, and a sequence comprising: $A_3A_4U_5$ (SEQ ID NO: 55) at positions +3, +4 and +5 respectively of the polyribonucleotide.

3. 5' UTR

[0219] In some embodiments, a nucleic acid (*e.g.*, DNA, RNA) utilized in accordance with the present disclosure comprises a 5'-UTR. In some embodiments, 5'-UTR may comprise a plurality of distinct sequence elements; in some embodiments, such plurality may be or comprise multiple copies of one or more particular sequence elements (*e.g.*, as may be from a particular source or otherwise known as a functional or characteristic sequence element). In some embodiments, a 5' UTR comprises multiple different sequence elements.

[0220] The term "untranslated region" or "UTR" is commonly used in the art to a region in a DNA molecule which is transcribed but is not translated into an amino acid sequence, or to the corresponding region in an RNA polynucleotide, such as an mRNA molecule. An untranslated region (UTR) can be present 5' (upstream) of an open reading frame (5'-UTR) and/or 3' (downstream) of an open reading frame (3'-UTR). As used herein, the terms "five prime untranslated region" or "5' UTR" refer to a sequence of a polyribonucleotide between the 5' end of the polyribonucleotide (e.g., a transcription start site) and a start codon of a coding region of the polyribonucleotide. In some embodiments, "5' UTR" refers to a sequence of a polyribonucleotide that begins at the 5' end of the polyribonucleotide (e.g., a transcription start site) and ends one nucleotide (nt) before a start codon (usually AUG) of a coding region of the polyribonucleotide, e.g., in its natural context. In some embodiments, a 5' UTR comprises a Kozak sequence. A 5'-UTR is downstream of the 5'-cap (if present), *e.g.*, directly adjacent to the 5'-cap. In some embodiments, a 5' UTR disclosed herein comprises a cap proximal sequence, *e.g.*, as defined and described herein. In some embodiments, a cap proximal sequence comprises a sequence adjacent to a 5' cap.

[0221] Exemplary 5' UTRs include a human alpha globin (hAg) 5'UTR or a fragment thereof, a TEV 5' UTR or a fragment thereof, a HSP70 5' UTR or a fragment thereof, or a c-Jun 5' UTR or a fragment thereof.

[0222] In some embodiments, an RNA disclosed herein comprises a hAg 5' UTR or a fragment thereof.

[0223] In some embodiments, an RNA disclosed herein comprises a 5' UTR having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a 5' UTR with the sequence AGAATAAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAACCCGCCACC (SEQ ID NO: 56). In some embodiments, an RNA disclosed herein comprises a 5' UTR having the sequence AGAATAAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAACCCGCCACC (SEQ ID NO: 56).

[0224] In some embodiments, an RNA disclosed herein comprises a 5' UTR having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a 5' UTR with the sequence AACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACC (SEQ IDNO: 57)(hAg-Kozak/5'UTR). In some embodiments, an RNA disclosed herein comprises a 5' UTR having the sequence AACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACC (SEQ ID NO: 57)(hAg-Kozak/5'UTR).

4. PolyA Tail

[0225] In some embodiments, a polynucleotide (*e.g.*, DNA, RNA) disclosed herein comprises a polyadenylate (polyA) sequence, *e.g.*, as described herein. In some embodiments, a polyA sequence is situated downstream of a 3'-UTR, *e.g.*, adjacent to a 3'-UTR.

[0226] As used herein, the term "poly(A) sequence" or "poly-A tail" refers to an uninterrupted or interrupted sequence of adenylate residues which is typically located at the 3'-end of an RNA polynucleotide. Poly(A) sequences are known to those of skill in the art and may follow the 3'-UTR in the RNAs described herein. An uninterrupted poly(A) sequence is characterized by consecutive adenylate residues. In nature, an uninterrupted poly(A) sequence is typical. In some embodiments, polynucleotides disclosed herein comprise an uninterrupted Poly(A) sequence. In some embodiments, polynucleotides disclosed herein comprise interrupted Poly(A) sequence. In some embodiments, RNAs disclosed herein can have a poly(A) sequence attached to the free 3'-end of the RNA by a template-independent RNA

polymerase after transcription or a poly(A) sequence encoded by DNA and transcribed by a template-dependent RNA polymerase.

[0227] It has been demonstrated that a poly(A) sequence of about 120 A nucleotides has a beneficial influence on the levels of RNA in transfected eukaryotic cells, as well as on the levels of protein that is translated from an open reading frame that is present upstream (5') of the poly(A) sequence (Holtkamp *et al.*, 2006, Blood, vol. 108, pp. 4009-4017, which is herein incorporated by reference).

[0228] In some embodiments, a poly(A) sequence in accordance with the present disclosure is not limited to a particular length; in some embodiments, a poly(A) sequence is any length. In some embodiments, a poly(A) sequence comprises, essentially consists of, or consists of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 A nucleotides, and, in particular, about 120 A nucleotides. In this context, "essentially consists of" means that most nucleotides in the poly(A) sequence, typically at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% by number of nucleotides in the poly(A) sequence are A nucleotides, but permits that remaining nucleotides are nucleotides other than A nucleotides, such as U nucleotides (uridylate), G nucleotides (guanylate), or C nucleotides (cytidylate). In this context, "consists of" means that all nucleotides in the poly(A) sequence, i.e., 100% by number of nucleotides in the poly(A) sequence, are A nucleotides. The term "A nucleotide" or "A" refers to adenylate.

[0229] In some embodiments, a poly(A) sequence is attached during RNA transcription, e.g., during preparation of *in vitro* transcribed RNA, based on a DNA template comprising repeated dT nucleotides (deoxythymidylate) in the strand complementary to the coding strand. The DNA sequence encoding a poly(A) sequence (coding strand) is referred to as poly(A) cassette.

[0230] In some embodiments, the poly(A) cassette present in the coding strand of DNA essentially consists of dA nucleotides, but is interrupted by a random sequence of the four nucleotides (dA, dC, dG, and dT). Such random sequence may be 5 to 50, 10 to 30, or 10 to 20 nucleotides in length. Such a cassette is disclosed in WO 2016/005324 A1, hereby incorporated by reference. Any poly(A) cassette disclosed in WO 2016/005324 A1, which is incorporated herein by reference in its entirety, may be used in accordance with the present disclosure. A poly(A) cassette that essentially consists of dA nucleotides, but is interrupted by a random sequence having an equal distribution of the four nucleotides (dA, dC, dG, dT) and having a length of e.g., 5 to 50 nucleotides shows, on DNA level, constant

propagation of plasmid DNA in *E. coli* and is still associated, on RNA level, with the beneficial properties with respect to supporting RNA stability and translational efficiency is encompassed. In some embodiments, the poly(A) sequence contained in an RNA polynucleotide described herein essentially consists of A nucleotides, but is interrupted by a random sequence of the four nucleotides (A, C, G, U). Such random sequence may be 5 to 50, 10 to 30, or 10 to 20 nucleotides in length.

[0231] In some embodiments, no nucleotides other than A nucleotides flank a poly(A) sequence at its 3'-end, i.e., the poly(A) sequence is not masked or followed at its 3'-end by a nucleotide other than A.

[0232] In some embodiments, the poly(A) sequence may comprise at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 nucleotides. In some embodiments, the poly(A) sequence may essentially consist of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 nucleotides. In some embodiments, the poly(A) sequence may consist of at least 20, at least 30, at least 40, at least 80, or at least 100 and up to 500, up to 400, up to 300, up to 200, or up to 150 nucleotides. In some embodiments, the poly(A) sequence comprises at least 100 nucleotides. In some embodiments, the poly(A) sequence comprises about 150 nucleotides. In some embodiments, the poly(A) sequence comprises about 120 nucleotides.

[0233] In some embodiments, a poly A tail comprises a specific number of Adenosines, such as about 50 or more, about 60 or more, about 70 or more, about 80 or more, about 90 or more, about 100 or more, about 120, or about 150 or about 200. In some embodiments a poly A tail of a string construct may comprise 200 A residues or less. In some embodiments, a poly A tail of a string construct may comprise about 200 A residues. In some embodiments, a poly A tail of a string construct may comprise 180 A residues or less. In some embodiments, a poly A tail of a string construct may comprise about 180 A residues. In some embodiments, a poly A tail may comprise 150 residues or less.

[0234] In some embodiments, RNA comprises a poly(A) sequence comprising the nucleotide sequence of AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCATATGACTAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 58), or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCATATGACTAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 58). In some embodiments, a poly(A) tail comprises a plurality of A residues interrupted by a linker. In some embodiments, a linker comprises the nucleotide sequence GCATATGAC (SEQ ID NO: 59).

[0235] In some embodiments, RNA comprises a poly(A) sequence comprising the nucleotide sequence of AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCAUAUGACUAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 60), or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCAUAUGACUAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 60). In some embodiments, a poly(A) tail comprises a plurality of A residues interrupted by a linker. In some embodiments, a linker comprises the nucleotide sequence GCAUAUGAC (SEQ ID NO: 61).

5. 3' UTR

[0236] In some embodiments, an RNA utilized in accordance with the present disclosure comprises a 3'-UTR. As used herein, the terms "three prime untranslated region," "3' untranslated region," or "3' UTR" refer to a sequence of an mRNA molecule that begins following a stop codon of a coding region of an open reading frame sequence. In some embodiments, the 3' UTR begins immediately after a stop codon of a coding region of an open reading frame sequence, e.g., in its natural context. In other embodiments, the 3' UTR does not begin immediately after stop codon of the coding region of an open reading frame sequence, e.g., in its natural context. The term "3'-UTR" does preferably not include the poly(A) sequence. Thus, the 3'-UTR is upstream of the poly(A) sequence (if present), e.g. directly adjacent to the poly(A) sequence.

[0237] In some embodiments, an RNA disclosed herein comprises a 3' UTR comprising an F element and/or an I element. In some embodiments, a 3' UTR or a proximal sequence thereto comprises a restriction site. In some embodiments, a restriction site is a *BamHI* site. In some embodiments, a restriction site is a *XhoI* site.

[0238] In some embodiments, an RNA construct comprises an F element. In some embodiments, a F element sequence is a 3'-UTR of amino-terminal enhancer of split (AES).

[0239] In some embodiments, an RNA disclosed herein comprises a 3' UTR having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a 3' UTR with the sequence of CTGGTACTGCATGCACGCAATGCTAGCTGCCCCTTTCCCGTCCTGGGTACCCCGAGTCTCCCC CGACCTCGGGTCCCAGGTATGCTCCCACCTCCACCTGCCCCACTCACCACCTCTGCTAGTTCC AGACACCTCCCAAGCACGCAGCAATGCAGCTCAAAACGCTTAGCCTAGCCACACCCCCACG GGAAACAGCAGTGATTAACCTTTAGCAATAAACGAAAGTTTAACTAAGCTATACTAACCCCA GGGTTGGTCAATTTCGTGCCAGCCACACC (SEQ ID NO: 62). In some embodiments, an RNA disclosed herein comprises a 3' UTR with the sequence of CTGGTACTGCATGCACGCAATGCTAGCTGCCCCTTTCCCGTCCTGGGTACCCCGAGTCTCCCC CGACCTCGGGTCCCAGGTATGCTCCCACCTCCACCTGCCCCACTCACCACCTCTGCTAGTTCC AGACACCTCCCAAGCACGCAGCAATGCAGCTCAAAACGCTTAGCCTAGCCACACCCCCACG GGAAACAGCAGTGATTAACCTTTAGCAATAAACGAAAGTTTAACTAAGCTATACTAACCCCA GGGTTGGTCAATTTCGTGCCAGCCACACC (SEQ ID NO: 62).

[0240] In some embodiments, an RNA disclosed herein comprises a 3' UTR having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a 3' UTR with the sequence of CUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCUUUCCCGUCCUGGGUACCCCGAGUCUC CCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGCCCCACUCACCACCUCUGCUA GUUCCAGACACCUCCCAAGCACGCAGCAAUGCAGCUCAAAACGCUUAGCCUAGCCACACC CCCACGGGAAACAGCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAAGCUAUA CUAACCCCAGGGUUGGUCAAUUUCGUGCCAGCCACACC (SEQ ID NO: 63). In some embodiments, an RNA disclosed herein comprises a 3' UTR with the sequence of CUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCUUUCCCGUCCUGGGUACCCCGAGUCUC CCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGCCCCACUCACCACCUCUGCUA GUUCCAGACACCUCCCAAGCACGCAGCAAUGCAGCUCAAAACGCUUAGCCUAGCCACACC CCCACGGGAAACAGCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAAGCUAUA CUAACCCCAGGGUUGGUCAAUUUCGUGCCAGCCACACC (SEQ ID NO: 63).

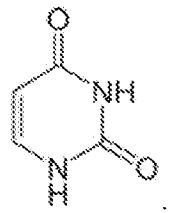
[0241] In some embodiments, a 3'UTR is an FI element as described in WO2017/060314, which is herein incorporated by reference in its entirety.

B. RNA Formats

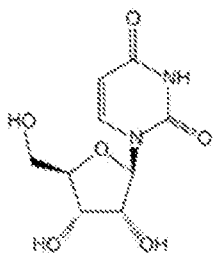
[0242] At least three distinct formats useful for RNA compositions (*e.g.*, pharmaceutical compositions) have been developed, namely non-modified uridine containing mRNA (uRNA), nucleoside-modified mRNA (modRNA), and self-amplifying mRNA (saRNA). Each of these platforms displays unique features. In general, in all three formats, RNA is capped, contains open reading frames (ORFs) flanked by untranslated regions (UTR), and have a polyA-tail at the 3' end. An ORF of an uRNA and modRNA vectors encode an antibody agent or fragment thereof. An saRNA has multiple ORFs.

[0243] In some embodiments, the RNA described herein may have modified nucleosides. In some embodiments, the RNA comprises a modified nucleoside in place of at least one (e.g., every) uridine.

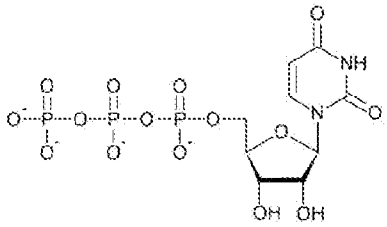
[0244] The term "uracil," as used herein, describes one of the nucleobases that can occur in the nucleic acid of RNA. The structure of uracil is:



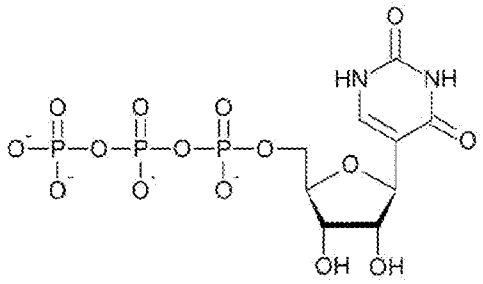
[0245] The term "uridine," as used herein, describes one of the nucleosides that can occur in RNA. The structure of uridine is:



[0246] UTP (uridine 5'-triphosphate) has the following structure:

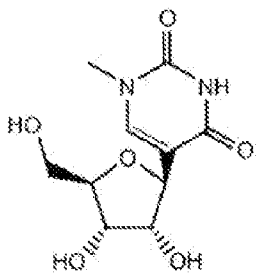


[0247] Pseudo-UTP (pseudouridine 5'-triphosphate) has the following structure:

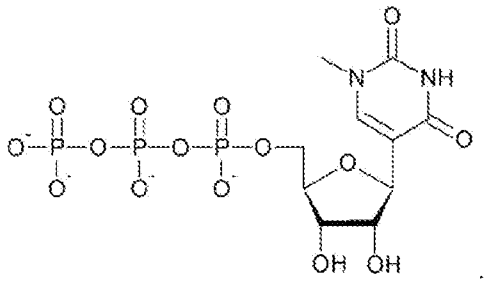


[0248] "Pseudouridine" is one example of a modified nucleoside that is an isomer of uridine, where the uracil is attached to the pentose ring via a carbon-carbon bond instead of a nitrogen-carbon glycosidic bond.

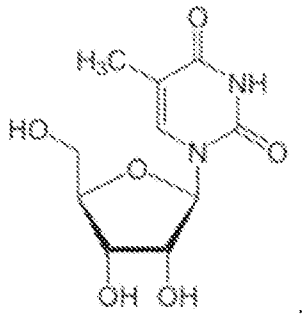
[0249] Another exemplary modified nucleoside is N1-methyl-pseudouridine (m1Ψ), which has the structure:



[0250] N1-methyl-pseudo-UTP has the following structure:



[0251] Another exemplary modified nucleoside is 5-methyl-uridine (m5U), which has the structure:



[0252] In some embodiments, one or more uridine in an RNA described herein is replaced by a modified nucleoside. In some embodiments, the modified nucleoside is a modified uridine.

[0253] In some embodiments, RNA comprises a modified nucleoside in place of at least one uridine. In some embodiments, RNA comprises a modified nucleoside in place of each uridine.

[0254] In some embodiments, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U). In some embodiments, the modified nucleoside comprises pseudouridine (ψ). In some embodiments, the modified nucleoside comprises N1-methyl-pseudouridine (m1ψ). In some embodiments, the modified nucleoside comprises 5-methyl-uridine (m5U). In some embodiments, RNA may comprise more than one type of modified nucleoside, and the modified nucleosides are independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U). In some embodiments, the modified nucleosides comprise pseudouridine (ψ) and N1-methyl-pseudouridine (m1ψ). In some embodiments, the modified nucleosides comprise pseudouridine (ψ) and 5-methyl-uridine (m5U). In some embodiments, the modified nucleosides comprise N1-methyl-pseudouridine (m1ψ) and 5-methyl-uridine (m5U). In some

embodiments, the modified nucleosides comprise pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

[0255] In some embodiments, the modified nucleoside replacing one or more, e.g., all, uridine in the RNA may be any one or more of 3-methyl-uridine (m3U), 5-methoxy-uridine (mo5U), 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s2U), 4-thio-uridine (s4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridine or 5-bromo-uridine), uridine 5-oxyacetic acid (cmo5U), uridine 5-oxyacetic acid methyl ester (mcmo5U), 5-carboxymethyl-uridine (cm5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm5U), 5-carboxyhydroxymethyl-uridine methyl ester (mchm5U), 5-methoxycarbonylmethyl-uridine (mcm5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm5s2U), 5-aminomethyl-2-thio-uridine (nm5s2U), 5-methylaminomethyl-uridine (mnm5U), 1-ethyl-pseudouridine, 5-methylaminomethyl-2-thio-uridine (mnm5s2U), 5-methylaminomethyl-2-seleno-uridine (mnm5se2U), 5-carbamoylmethyl-uridine (ncm5U), 5-carboxymethylaminomethyl-uridine (cmnm5U), 5-carboxymethylaminomethyl-2-thio-uridine (cmnm5s2U), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm5U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine(τm5s2U), 1-taurinomethyl-4-thio-pseudouridine), 5-methyl-2-thio-uridine (m5s2U), 1-methyl-4-thio-pseudouridine (m1s4ψ), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine (m3ψ), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m5D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp3U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp3 ψ), 5-(isopentenylaminomethyl)uridine (inm5U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm5s2U), α-thio-uridine, 2′-O-methyl-uridine (Um), 5,2′-O-dimethyl-uridine (m5Um), 2′-O-methyl-pseudouridine (ψm), 2-thio-2′-O-methyl-uridine (s2Um), 5-methoxycarbonylmethyl-2′-O-methyl-uridine (mcm5Um), 5-carbamoylmethyl-2′-O-methyl-uridine (ncm5Um), 5-carboxymethylaminomethyl-2′-O-methyl-uridine (cmnm5Um), 3,2′-O-dimethyl-uridine (m3Um), 5-(isopentenylaminomethyl)-2′-O-methyl-uridine (inm5Um), 1-thio-uridine, deoxythymidine, 2′-F-ara-uridine, 2′-F-uridine, 2′-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, 5-[3-(1-E-propenylamino)uridine, or any other modified uridine known in the art.

[0256] In some embodiments, the RNA comprises other modified nucleosides or comprises further modified nucleosides, e.g., modified cytidine. For example, in some embodiments, in the RNA 5-methylcytidine is substituted partially or completely, preferably completely, for cytidine. In some embodiments, the RNA comprises 5-methylcytidine and one or more selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U). In some embodiments, the RNA comprises 5-methylcytidine and N1-methyl-pseudouridine (m1ψ). In some embodiments, the RNA comprises 5-methylcytidine in place of each cytidine and N1-methyl-pseudouridine (m1ψ) in place of each uridine.

[0257] In some embodiments of the present disclosure, the RNA is "replicon RNA" or simply a "replicon," in particular "self-replicating RNA" or "self-amplifying RNA." In one particularly preferred embodiment, the replicon or self-replicating RNA is derived from or comprises elements derived from a single-stranded (ss) RNA virus, in particular a positive-stranded ssRNA virus, such as an alphavirus. Alphaviruses are typical representatives of positive-stranded RNA viruses. Alphaviruses replicate in the cytoplasm of infected cells (for review of the alphaviral life cycle see José et al., Future Microbiol., 2009, vol. 4, pp. 837–856, which is incorporated herein by reference in its entirety). The total genome length of many alphaviruses typically ranges between 11,000 and 12,000 nucleotides, and the genomic RNA typically has a 5'-cap, and a 3' poly(A) tail. The genome of alphaviruses encodes non-structural proteins (involved in transcription, modification and replication of viral RNA and in protein modification) and structural proteins (forming the virus particle). There are typically two open reading frames (ORFs) in the genome. The four non-structural proteins (nsP1–nsP4) are typically encoded together by a first ORF beginning near the 5′ terminus of the genome, while alphavirus structural proteins are encoded together by a second ORF which is found downstream of the first ORF and extends near the 3' terminus of the genome. Typically, the first ORF is larger than the second ORF, the ratio being roughly 2:1. In cells infected by an alphavirus, only the nucleic acid sequence encoding non-structural proteins is translated from the genomic RNA, while the genetic information encoding structural proteins is translatable from a subgenomic transcript, which is an RNA molecule that resembles eukaryotic messenger RNA (mRNA; Gould et al., 2010, Antiviral Res., vol. 87 pp. 111–124, which is incorporated herein by reference in its entirety). Following infection, i.e. at early stages of the viral life cycle, the (+) stranded genomic RNA directly acts like a messenger RNA for the translation of the open reading frame encoding the non-structural poly-protein (nsP1234).

[0258] Alphavirus-derived vectors have been proposed for delivery of foreign genetic information into target cells or target organisms. In simple approaches, a first ORF encodes an alphavirus-derived RNA-dependent RNA polymerase (replicase), which upon translation mediates self-amplification of the RNA. A second ORF encoding alphaviral structural proteins is replaced by an open reading frame encoding an HSV-2 gB construct described herein. Alphavirus-based trans-replication systems rely on alphavirus nucleotide sequence elements on two separate nucleic acid molecules: one nucleic acid molecule encodes a viral replicase, and the other nucleic acid molecule is capable of being replicated by said replicase in trans (hence the designation trans-replication system). Trans-replication requires the presence of both these nucleic acid molecules in a given host cell. The nucleic acid molecule capable of being replicated by the replicase in trans must comprise certain alphaviral sequence elements to allow recognition and RNA synthesis by the alphaviral replicase.

[0259] Features of a non-modified uridine platform may include, for example, one or more of intrinsic adjuvant effect, as well as good tolerability and safety. Features of modified uridine (*e.g.*, pseudouridine) platform may include reduced adjuvant effect, blunted immune innate immune sensor activating capacity and thus good tolerability and safety. Features of self-amplifying platform may include, for example, long duration of protein expression, good tolerability and safety, higher likelihood for efficacy with very low vaccine dose.

[0260] The present disclosure provides particular RNA constructs optimized, for example, for improved manufacturability, encapsulation, expression level (and/or timing), etc. Certain components are discussed below, and certain preferred embodiments are exemplified herein.

C. Codon Optimization and GC Enrichment

[0261] As used herein, the term "codon-optimized" refers to alteration of codons in a coding region of a nucleic acid molecule (e.g., a polyribonucleotide) to reflect the typical codon usage of a host organism (e.g., a subject receiving a nucleic acid molecule (e.g., a polyribonucleotide)) without preferably altering the amino acid sequence encoded by the nucleic acid molecule. Within the context of the present disclosure, in some embodiments, coding regions are codon-optimized for optimal expression in a subject to be treated using the RNA molecules described herein. In some embodiments, codon-optimization may be performed such that codons for which frequently occurring tRNAs are available are inserted in place of "rare codons." In some embodiments, codon-optimization may include increasing guanosine/cytosine (G/C) content of a coding region of RNA described herein as compared to the G/C content of the

corresponding coding sequence of a wild type RNA, wherein the amino acid sequence encoded by the RNA is preferably not modified compared to the amino acid sequence.

[0262] In some embodiments, a coding sequence (also referred to as a "coding region") is codon optimized for expression in the subject to whom a composition (e.g., a pharmaceutical composition) is to be administered (*e.g.*, a human). Thus, in some embodiments, sequences in such a polynucleotide (*e.g.*, a polyribonucleotide) may differ from wild type sequences encoding the relevant antigen or fragment or epitope thereof, even when the amino acid sequence of the antigen or fragment or epitope thereof is wild type.

[0263] In some embodiments, strategies for codon optimization for expression in a relevant subject (*e.g.*, a human), and even, in some cases, for expression in a particular cell or tissue.

[0264] Various species exhibit particular bias for certain codons of a particular amino acid. Without wishing to be bound by any one theory, codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell may generally be a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes may be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are available, for example, at the "Codon Usage Database" available at www.kazusa.orjp/codon/ and these tables may be adapted in a number of ways. Computer algorithms for codon optimizing a particular sequence for expression in a particular subject or its cells are also available, such as Gene Forge (Aptagen; Jacobus, PA), are also available.

[0265] In some embodiments, a polynucleotide (*e.g.*, a polyribonucleotide) of the present disclosure is codon optimized, wherein the codons in the polynucleotide (*e.g.*, the polyribonucleotide) are adapted to human codon usage (herein referred to as "human codon optimized polynucleotide"). Codons encoding the same amino acid occur at different frequencies in a subject, *e.g.*, a human. Accordingly, in some embodiments, the coding sequence of a polynucleotide of the present disclosure is modified such that the frequency of the codons encoding the same amino acid corresponds to the naturally occurring frequency of that codon according to the human codon usage, e.g., as shown in **Table 9**. For example, in the case of the amino acid Ala, the wild type coding sequence is preferably adapted in a way that the codon "GCC" is used with a frequency of 0.40, the codon "GCT" is used with a frequency of 0.28, the

codon "GCA" is used with a frequency of 0.22 and the codon "GCG" is used with 30 a frequency of 0.10 etc. (see **Table 9**). Accordingly, in some embodiments, such a procedure (as exemplified for Ala) is applied for each amino acid encoded by the coding sequence of a polynucleotide to obtain sequences adapted to human codon usage.

Table 9: Human codon usage with frequencies indicated for each amino acid.

Amino acid	codon	frequency		Amino acid	codon	frequency
Ala	GCG	0.10		Pro	CCG	0.11
Ala	GCA	0.22		Pro	CCA	0.27
Ala	GCT	0.28		Pro	CCT	0.29
Ala	GCC*	0.40		Pro	CCC*	0.33
Cys	TGT	0.42		Gin	CAG*	0.73
Cys	TGC*	0.58		Gin	CAA	0.27
Asp	GAT	0.44		Arg	AGG	0.22
Asp	GAC*	0.56		Arg	AGA*	0.21
Glu	GAG*	0.59		Arg	CGG	0.19
Glu	GAA	0.41		Arg	CGA	0.10
Phe	TTT	0.43		Arg	CGT	0.09
Phe	TTC*	0.57		Arg	CGC	0.19
Gly	GGG	0.23		Ser	AGT	0.14
Gly	GGA	0.26		Ser	AGC*	0.25
Gly	GGT	0.18		Ser	TCG	0.06
Gly	GGC*	0.33		Ser	TCA	0.15
His	CAT	0.41		Ser	TCT	0.18
His	CAC*	0.59		Ser	TCC	0.23
Lle	ATA	0.14		Thr	ACG	0.12
Lle	ATT	0.35		Thr	ACA	0.27

Ile	ATC*	0.52		Thr	ACT	0.23
Lys	AAG*	0.60		Tor	ACC*	0.38
Lys	AAA	0.40		Val	GTG*	0.48
Leu	TTG	0.12		Val	GTA	0.10
Leu	TTA	0.06		Val	GTT	0.17
Leu	CTG*	0.43		Val	GTC	0.25
Leu	CTA	0.07		Trp	TGG*	1
Leu	CTT	0.12		Tyr	TAT	0 42
Lou	CTC	0.20		Tyr	TAC*	0.58
Met	ATG*	1		Stop	TGA*	0 61
Asn	AAT	0.44		Stop	TAG	0.17
Asn	AAC*	0.56		Stop	TAA	0.22

[0266] Certain strategies for codon optimization and/or G/C enrichment for human expression are described in WO2002/098443, which is incorporated by reference herein in its entirety. In some embodiments, a coding sequence may be optimized using a multiparametric optimization strategy. In some embodiments, optimization parameters may include parameters that influence protein expression, which can be, for example, impacted on a transcription level, an mRNA level, and/or a translational level. In some embodiments, optimization parameters include, but are not limited to transcription-level parameters (including, e.g., GC content, consensus splice sites, cryptic splice sites, SD sequences, TATA boxes, termination signals, artificial recombination sites, and combinations thereof); mRNA-level parameters (including, e.g., RNA instability motifs, ribosomal entry sites, repetitive sequences, and combinations thereof); translation-level parameters (including, e.g., codon usage, premature poly(A) sites, ribosomal entry sites, secondary structures, and combinations thereof); or combinations thereof. In some embodiments, a coding sequence may be optimized by a GeneOptimizer algorithm as described in Fath et al. "Multiparameter RNA and Codon Optimization: A Standardized Tool to Assess and Enhance Autologous Mammalian Gene Expression" PLoS ONE 6(3): e17596; Rabb et al., which is incorporated herein by reference in its entirety, "The GeneOptimizer Algorithm: using a sliding window approach to cope with the vast sequence space in multiparameter DNA sequence optimization" Systems and Synthetic

Biology (2010) 4:215-225; and Graft et al. "Codon-optimized genes that enable increased heterologous expression in mammalian cells and elicit efficient immune responses in mice after vaccination of naked DNA" Methods Mol Med (2004) 94:197-210, the entire content of each of which is incorporated herein for the purposes described herein. In some embodiments, a coding sequence may be optimized by Eurofins' adaption and optimization algorithm "GENEius" as described in Eurofins' Application Notes: Eurofins' adaption and optimization software "GENEius" in comparison to other optimization algorithms, the entire content of which is incorporated by reference for the purposes described herein.

[0267] In some embodiments, a coding sequence utilized in accordance with the present disclosure has G/C content that is increased compared to a coding sequence for an HSV gB (or fragment thereof) construct described herein. In some embodiments, guanosine/cytidine (G/C) content of a coding region is modified relative to a comparable coding sequence for an HSV gB (or fragment thereof) construct described herein, but the amino acid sequence encoded by the polyribonucleotide not modified.

[0268] Without wishing to be bound by any particular theory, it is proposed that GC enrichment may improve translation of a payload sequence. Typically, sequences having an increased G (guanosine)/C (cytidine) content are more stable than sequences having an increased A (adenosine)/U (uridine) content. In respect to the fact that several codons code for one and the same amino acid (so-called degeneration of the genetic code), the most favorable codons for the stability can be determined (so-called alternative codon usage). Depending on the amino acid to be encoded by a polyribonucleotide, there are various possibilities for modification of the ribonucleic acid sequence, compared to its wild type sequence. In particular, codons which contain A and/or U nucleosides can be modified by substituting these codons by other codons, which code for the same amino acids but contain no A and/or U or contain a lower content of A and/or U nucleosides.

[0269] In some embodiments, G/C content of a coding region of a polyribonucleotide described herein is increased by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, or even more compared to the G/C content of the coding region prior to codon optimization, e.g., of the wild type RNA. In some embodiments, G/C content of a coding region of a polyribonucleotide described herein is decreased by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, or even more compared to the G/C content of the coding region prior to codon optimization, e.g., of the wild type RNA.

[0270] In some embodiments, stability and translation efficiency of a polyribonucleotide may incorporate one or more elements established to contribute to stability and/or translation efficiency of the

polyribonucleotide; exemplary such elements are described, for example, in PCT/EP2006/009448 incorporated herein by reference. In some embodiments, to increase expression of a polyribonucleotide used according to the present disclosure, a polyribonucleotide may be modified within the coding region, i.e., the sequence encoding the expressed peptide or protein, without altering the sequence of the expressed peptide or protein, for example so as to increase the GC-content to increase mRNA stability and/or to perform a codon optimization and, thus, enhance translation in cells.

IV. RNA Delivery Technologies

[0271] Provided polyribonucleotides may be delivered for therapeutic applications described herein using any appropriate methods known in the art, including, *e.g.*, delivery as naked RNAs, or delivery mediated by viral and/or non-viral vectors, polymer-based vectors, lipid compositions, nanoparticles (*e.g.*, lipid nanoparticles, polymeric nanoparticles, lipid-polymer hybrid nanoparticles, *etc.*), and/or peptide-based vectors. See, e.g., Wadhwa *et al.* "Opportunities and Challenges in the Delivery of mRNA-Based Vaccines" *Pharmaceutics* (2020) 102 (27 pages), the content of which is incorporated herein by reference, for information on various approaches that may be useful for delivery polyribonucleotides described herein.

[0272] In some embodiments, one or more polyribonucleotides can be formulated with lipid nanoparticles for delivery (*e.g.*, administration).

[0273] In some embodiments, lipid nanoparticles can be designed to protect polyribonucleotides from extracellular RNases and/or engineered for systemic delivery of the RNA to target cells. In some embodiments, such lipid nanoparticles may be particularly useful to deliver polyribonucleotides when polyribonucleotides are intravenously or intramuscularly administered to a subject.

A. Lipid Compositions

1. Lipids and Lipid-Like Materials

[0274] The terms "lipid" and "lipid-like material" are broadly defined herein as molecules which comprise one or more hydrophobic moieties or groups and optionally also one or more hydrophilic moieties or groups. Molecules comprising hydrophobic moieties and hydrophilic moieties are also frequently denoted as amphiphiles. Lipids are usually poorly soluble in water. In an aqueous environment, the amphiphilic nature allows the molecules to self-assemble into organized structures and different phases. One of those phases consists of lipid bilayers, as they are present in vesicles,

multilamellar/unilamellar liposomes, or membranes in an aqueous environment. Hydrophobicity can be conferred by the inclusion of polar groups that include, but are not limited to, long-chain saturated and unsaturated aliphatic hydrocarbon groups and such groups substituted by one or more aromatic, cycloaliphatic, or heterocyclic group(s). The hydrophilic groups may comprise polar and/or charged groups and include carbohydrates, phosphate, carboxylic, sulfate, amino, sulfhydryl, nitro, hydroxyl, and other like groups.

[0275] Often, an amphiphilic compound has a polar head attached to a long hydrophobic tail. In some embodiments, the polar portion is soluble in water, while the non-polar portion is insoluble in water. In addition, the polar portion may have either a formal positive charge, or a formal negative charge. Alternatively, the polar portion may have both a formal positive and a negative charge, and be a zwitterion or inner salt. For purposes of the disclosure, the amphiphilic compound can be, but is not limited to, one or a plurality of natural or non-natural lipids and lipid-like compounds.

[0276] A "lipid-like material" is a substance that is structurally and/or functionally related to a lipid but may not be considered a lipid in a strict sense. For example, the term includes compounds that are able to form amphiphilic layers as they are present in vesicles, multilamellar/unilamellar liposomes, or membranes in an aqueous environment and includes surfactants, or synthesized compounds with both hydrophilic and hydrophobic moieties. Generally speaking, the term refers to molecules, which comprise hydrophilic and hydrophobic moieties with different structural organization, which may or may not be similar to that of lipids.

[0277] Specific examples of amphiphilic compounds that may be included in an amphiphilic layer include, but are not limited to, phospholipids, aminolipids and sphingolipids.

[0278] Generally, lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides (derived from condensation of ketoacyl subunits), sterols and prenol lipids (derived from condensation of isoprene subunits). Although the term "lipid" is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as sterol-containing metabolites such as cholesterol.

[0279] Fatty acids are a diverse group of molecules made of a hydrocarbon chain that terminates with a carboxylic acid group; this arrangement confers the molecule with a polar, hydrophilic end, and a

nonpolar, hydrophobic end that is insoluble in water. The carbon chain, typically between four and 24 carbons long, may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen, and sulfur. If a fatty acid contains a double bond, there is the possibility of either a cis or trans geometric isomerism, which significantly affects the molecule's configuration. Cis-double bonds cause the fatty acid chain to bend, an effect that is compounded with more double bonds in the chain. Other major lipid classes in the fatty acid category are the fatty esters and fatty amides.

[0280] Glycerolipids are composed of mono-, di-, and tri-substituted glycerols, the best-known being the fatty acid triesters of glycerol, called triglycerides. The word "triacylglycerol" is sometimes used synonymously with "triglyceride". In these compounds, the three hydroxyl groups of glycerol are each esterified, typically by different fatty acids. Additional subclasses of glycerolipids are represented by glycosylglycerols, which are characterized by the presence of one or more sugar residues attached to glycerol via a glycosidic linkage.

[0281] Glycerophospholipids are amphipathic molecules (containing both hydrophobic and hydrophilic regions) that contain a glycerol core linked to two fatty acid-derived "tails" by ester linkages and to one "head" group by a phosphate ester linkage. Examples of glycerophospholipids, usually referred to as phospholipids (though sphingomyelins are also classified as phospholipids) are phosphatidylcholine (also known as PC, GPCho or lecithin), phosphatidylethanolamine (PE or GPEtn) and phosphatidylserine (PS or GPSer).

[0282] Sphingolipids are members of a complex family of compounds that share a common structural feature, a sphingoid base backbone. The major sphingoid base in mammals is commonly referred to as sphingosine. Ceramides (N-acyl-sphingoid bases) are a major subclass of sphingoid base derivatives with an amide-linked fatty acid. The fatty acids are typically saturated or mono-unsaturated with chain lengths from 16 to 26 carbon atoms. The major phosphosphingolipids of mammals are sphingomyelins (ceramide phosphocholines), whereas insects contain mainly ceramide phosphoethanolamines and fungi have phytoceramide phosphoinositols and mannose-containing headgroups. The glycosphingolipids are a diverse family of molecules composed of one or more sugar residues linked via a glycosidic bond to the sphingoid base. Examples of these are the simple and complex glycosphingolipids such as cerebrosides and gangliosides.

[0283] Sterols, such as cholesterol and its derivatives, or tocopherol and its derivatives, are important components of membrane lipids, along with the glycerophospholipids and sphingomyelins.

[0284] Saccharolipids are compounds in which fatty acids are linked directly to a sugar backbone, forming structures that are compatible with membrane bilayers. In the saccharolipids, a monosaccharide substitutes for the glycerol backbone present in glycerolipids and glycerophospholipids. The most familiar saccharolipids are the acylated glucosamine precursors of the Lipid A component of the lipopolysaccharides in Gram-negative bacteria. Typical lipid A molecules are disaccharides of glucosamine, which are derivatized with as many as seven fatty-acyl chains. The minimal lipopolysaccharide required for growth in E. coli is Kdo2-Lipid A, a hexa-acylated disaccharide of glucosamine that is glycosylated with two 3-deoxy-D-manno-octulosonic acid (Kdo) residues.

[0285] Polyketides are synthesized by polymerization of acetyl and propionyl subunits by classic enzymes as well as iterative and multimodular enzymes that share mechanistic features with the fatty acid synthases. They comprise a large number of secondary metabolites and natural products from animal, plant, bacterial, fungal and marine sources, and have great structural diversity. Many polyketides are cyclic molecules whose backbones are often further modified by glycosylation, methylation, hydroxylation, oxidation, or other processes.

[0286] Lipids and lipid-like materials may be cationic, anionic or neutral. Neutral lipids or lipid-like materials exist in an uncharged or neutral zwitterionic form at a selected pH.

[0287] In some embodiments, suitable lipids or lipid-like materials for use in the present disclosure include those described in WO2020/128031 and US20200163878, the entire contents of each of which are incorporated herein by reference for the purposes described herein.

2. Cationic or cationically ionizable lipids or lipid-like materials

[0288] In some embodiments cationic or cationically ionizable lipids or lipid-like materials contemplated for use herein include any cationic or cationically ionizable lipids or lipid-like materials which are able to electrostatically bind nucleic acid. In one embodiment, cationic or cationically ionizable lipids or lipid-like materials contemplated for use herein can be associated with nucleic acid, e.g. by forming complexes with the nucleic acid or forming vesicles in which the nucleic acid is enclosed or encapsulated.

[0289] Cationic lipids or lipid-like materials are characterized in that they have a net positive charge (e.g., at a relevant pH). Cationic lipids or lipid-like materials bind negatively charged nucleic acid

by electrostatic interaction. Generally, cationic lipids possess a lipophilic moiety, such as a sterol, an acyl chain, a diacyl or more acyl chains, and the head group of the lipid typically carries the positive charge.

[0290] In certain embodiments, a cationic lipid or lipid-like material has a net positive charge only at certain pH, in particular acidic pH, while it has preferably no net positive charge, preferably has no charge, i.e., it is neutral, at a different, preferably higher pH such as physiological pH. This ionizable behavior is thought to enhance efficacy through helping with endosomal escape and reducing toxicity as compared with particles that remain cationic at physiological pH.

[0291] In some embodiments, a cationic or cationically ionizable lipid or lipid-like material comprises a head group which includes at least one nitrogen atom (N) which is positive charged or capable of being protonated.

[0292] Examples of cationic lipids include, but are not limited to 1,2-dioleoyl-3-trimethylammonium propane (DOTAP); N,N-dimethyl-2,3-dioleyloxypropylamine (DODMA), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), 3-(N—(N′,N′-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol), dimethyldioctadecylammonium (DDAB); 1,2-dioleoyl-3-dimethylammonium-propane (DODAP); 1,2-diacyloxy-3-dimethylammonium propanes; 1,2-dialkyloxy-3-dimethylammonium propanes; dioctadecyldimethyl ammonium chloride (DODAC), 1,2-distearyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 2,3-di(tetradecoxy)propyl-(2-hydroxyethyl)-dimethylazanium (DMRIE), 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC), 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), and 2,3-dioleoyloxy- N-[2(spermine carboxamide)ethyl]-N,N-dimethyl-l-propanamium trifluoroacetate (DOSPA), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), dioctadecylamidoglycyl spermine (DOGS), 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-oc-tadecadienoxy)propane (CLinDMA), 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethyl-1-(cis,cis-9′,12′-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N′-dioleylcarbamyl-3-dimethylaminopropane (DOcarbDAP), 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (DLinDAP), 1,2-N,N′-Dilinoleylcarbamyl-3-dimethylaminopropane (DLincarbDAP), 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane (DLinCDAP), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-K-XTC2-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), heptatriaconta-

6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), N-(2-Hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (DMRIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(cis-9-tetradecenyloxy)-1-propanaminium bromide (GAP-DMORIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide (GAP-DLRIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (GAP-DMRIE), N-(2-Aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (βAE-DMRIE), N-(4-carboxybenzyl)-N,N-dimethyl-2,3-bis(oleoyloxy)propan-1-aminium (DOBAQ), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), 1,2-dimyristoyl-3-dimethylammonium-propane (DMDAP), 1,2-dipalmitoyl-3-dimethylammonium-propane (DPDAP), N1-[2-((1S)-1-[(3-aminopropyl)amino]-4-[di(3-amino-propyl)amino]butylcarboxamido)ethyl]-3,4-di[oleyloxy]-benzamide (MVL5), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOEPC), 2,3-bis(dodecyloxy)-N-(2-hydroxyethyl)-N,N-dimethylpropan-1-amonium bromide (DLRIE), N-(2-aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)propan-1-aminium bromide (DMORIE), di((Z)-non-2-en-1-yl) 8,8'-((((2(dimethylamino)ethyl)thio)carbonyl)azanediyl)dioctanoate (ATX), N,N-dimethyl-2,3-bis(dodecyloxy)propan-1-amine (DLDMA), N,N-dimethyl-2,3-bis(tetradecyloxy)propan-1-amine (DMDMA), Di((Z)-non-2-en-1-yl)-9-((4-(dimethylaminobutanoyl)oxy)heptadecanedioate (L319), N-Dodecyl-3-((2-dodecylcarbamoyl-ethyl)-{2-[(2-dodecylcarbamoyl-ethyl)-2-{(2-dodecylcarbamoyl-ethyl)- [2-(2-dodecylcarbamoyl-ethylamino)-ethyl]-amino}-ethylamino)propionamide (lipidoid 98N12-5), 1-[2-[bis(2-hydroxydodecyl)amino]ethyl-[2-[4-[2-[bis(2 hydroxydodecyl)amino]ethyl]piperazin-1-yl]ethyl]amino]dodecan-2-ol (lipidoid C12-200), LIPOFECTIN® (commercially available cationic liposomes comprising DOTMA and 1 ,2-dioleoyl-sn-3phosphoethanolamine (DOPE), from GIBCO/BRL, Grand Island, N.Y.); LIPOFECTAMINE® (commercially available cationic liposomes comprising N-(1 -(2,3dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA) and (DOPE), from GIBCO/BRL); and TRANSFECTAM® (commercially available cationic lipids comprising dioctadecylamidoglycyl carboxyspermine (DOGS) in ethanol from Promega Corp., Madison, Wis.) or any combination of any of the foregoing. Further suitable cationic lipids for use in the present disclosure include those described in WO2020/128031 and US20200163878, the entire contents of each of which are incorporated herein by reference for the purposes described herein. Further suitable cationic lipids for use in the present disclosure include those described in WO2010/053572 (including Cl 2-200 described at paragraph [00225]) and WO2012/170930, both of which are incorporated herein by

reference for the purposes described herein. Additional suitable cationic lipids for use in the present disclosure include HGT4003, HGT5000, HGTS001, HGT5001, HGT5002 (see US20150140070A1, which is incorporated herein by reference in its entirety).

[0293] In some embodiments, formulations that are useful for pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) compositions as described herein can comprise at least one cationic lipid. Representative cationic lipids include, but are not limited to, 1 ,2-dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1 ,2-dilinoleyoxy-3morpholinopropane (DLin-MA), 1,2-dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1 ,2-dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1 -linoleoyl-2-linoleyloxy-3dimethylaminopropane (DLin-2-DMAP), 1 ,2-dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1 ,2-dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1 ,2-dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), 3-(N,Ndilinoleylamino)-1 ,2-propanediol (DLinAP), 3-(N,N-dioleylamino)-1 ,2-propanediol (DOAP), 1 ,2-dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), and 2,2-dilinoleyl-4-dimethylaminomethyl-[1 ,3]-dioxolane (DLin-K-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1 ,3]-dioxolane (DLin-KC2-DMA); dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA); MC3 (US20100324120, which is incorporated herein by reference in its entirety).

[0294] In some embodiments, amino or cationic lipids useful in accordance with the present disclosure have at least one protonatable or deprotonatable group, such that the lipid is positively charged at a pH at or below physiological pH (e.g. pH 7.4), and neutral at a second pH, preferably at or above physiological pH. It will, of course, be understood that the addition or removal of protons as a function of pH is an equilibrium process, and that the reference to a charged or a neutral lipid refers to the nature of the predominant species and does not require that all of lipids have to be present in the charged or neutral form. Lipids having more than one protonatable or deprotonatable group, or which are zwitterionic, are not excluded and may likewise suitable in the context of the present invention.

[0295] In some embodiments, a protonatable lipid has a pKa of the protonatable group in the range of about 4 to about 11, e.g., a pKa of about 5 to about 7.

[0296] In some embodiments, a cationic lipid may comprise from about 10 mol % to about 100 mol %, about 20 mol % to about 100 mol %, about 30 mol % to about 100 mol %, about 40 mol % to about 100 mol %, or about 50 mol % to about 100 mol % of total lipid present in a lipid composition utilized in accordance with the present disclosure.

3. Additional lipids or lipid-like materials

[0297] In some embodiments, formulations utilized in accordance with the present disclosure may comprise lipids or lipid-like materials other than cationic or cationically ionizable lipids or lipid-like materials, i.e., non-cationic lipids or lipid-like materials (including non-cationically ionizable lipids or lipid-like materials). Collectively, anionic and neutral lipids or lipid-like materials are referred to herein as non-cationic lipids or lipid-like materials. In some embodiments, optimizing a formulation of nucleic acid particles by addition of other hydrophobic moieties, such as cholesterol and lipids, in addition to an ionizable/cationic lipid or lipid-like material may, for example, enhance particle stability and efficacy of nucleic acid delivery.

[0298] In some embodiments, a lipid or lipid-like material may be incorporated which may or may not affect the overall charge of particles. In certain embodiments, such lipid or lipid-like material is a non-cationic lipid or lipid-like material.

[0299] In some embodiments, a non-cationic lipid may comprise, e.g., one or more anionic lipids and/or neutral lipids. An "anionic lipid" is negatively charged (e.g., at a selected pH).

[0300] A "neutral lipid" exists either in an uncharged or neutral zwitterionic form (e.g., at a selected pH). In some embodiments, a formulation comprises one of the following neutral lipid components: (1) a phospholipid, (2) cholesterol or a derivative thereof; or (3) a mixture of a phospholipid and cholesterol or a derivative thereof. Examples of cholesterol derivatives include, but are not limited to, cholestanol, cholestanone, cholestenone, coprostanol, cholesteryl-2'-hydroxyethyl ether, cholesteryl-4'-hydroxybutyl ether, tocopherol and derivatives thereof, and mixtures thereof.

[0301] Specific exemplary phospholipids that can be used include, but are not limited to, phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidic acids, phosphatidylserines or sphingomyelin. Such phospholipids include in particular diacylphosphatidylcholines, such as distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), dipentadecanoylphosphatidylcholine, dilauroylphosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), diarachidoylphosphatidylcholine (DAPC), dibehenoylphosphatidylcholine (DBPC), ditricosanoylphosphatidylcholine (DTPC), dilignoceroylphatidylcholine (DLPC), palmitoyloleoyl-phosphatidylcholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-

glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC) and phosphatidylethanolamines, in particular diacylphosphatidylethanolamines, such as dioleoylphosphatidylethanolamine (DOPE), distearoyl-phosphatidylethanolamine (DSPE), dipalmitoyl-phosphatidylethanolamine (DPPE), dimyristoyl-phosphatidylethanolamine (DMPE), dilauroyl-phosphatidylethanolamine (DLPE), diphytanoyl-phosphatidylethanolamine (DPyPE), and further phosphatidylethanolamine lipids with different hydrophobic chains.

[0302] In certain embodiments, a formulation utilized in accordance with the present disclosure includes DSPC or DSPC and cholesterol.

[0303] In certain embodiments, formulations utilized in accordance with the present disclosure include both a cationic lipid and an additional (non-cationic) lipid.

[0304] In some embodiments, formulations herein include a polymer conjugated lipid such as a pegylated lipid. "Pegylated lipids" comprise both a lipid portion and a polyethylene glycol portion. Pegylated lipids are known in the art.

[0305] Without wishing to be bound by theory, the amount of (total) cationic lipid compared to the amount of other lipid(s) in formulation may affect important characteristics, such as charge, particle size, stability, tissue selectivity, and bioactivity of the nucleic acid. In some embodiments, the molar ratio of the at least one cationic lipid to the at least one additional lipid is from about 10:0 to about 1:9, about 4:1 to about 1:2, or about 3:1 to about 1:1.

[0306] In some embodiments, a non-cationic lipid, in particular a neutral lipid, (e.g., one or more phospholipids and/or cholesterol) may comprise from about 0 mol % to about 90 mol %, from about 0 mol % to about 80 mol %, from about 0 mol % to about 70 mol %, from about 0 mol % to about 60 mol %, or from about 0 mol % to about 50 mol %, of the total lipid present in a formulation.

4. Lipoplex Particles

[0307] In certain embodiments of the present disclosure, the RNA described herein may be present in RNA lipoplex particles.

[0308] An "RNA lipoplex particle" contains lipid, in particular cationic lipid, and RNA. Electrostatic interactions between positively charged liposomes and negatively charged RNA results in complexation and spontaneous formation of RNA lipoplex particles. Positively charged liposomes may be

generally synthesized using a cationic lipid, such as DOTMA, and additional lipids, such as DOPE. In one embodiment, a RNA lipoplex particle is a nanoparticle.

[0309] In certain embodiments, RNA lipoplex particles include both a cationic lipid and an additional lipid. In some embodiments, the cationic lipid is DOTMA and the additional lipid is DOPE.

[0310] In some embodiments, the molar ratio of the at least one cationic lipid to the at least one additional lipid is from about 10:0 to about 1:9, about 4:1 to about 1:2, or about 3:1 to about 1:1. In specific embodiments, the molar ratio may be about 3:1, about 2.75:1, about 2.5:1, about 2.25:1, about 2:1, about 1.75:1, about 1.5:1, about 1.25:1, or about 1:1. In some embodiments, the molar ratio of the at least one cationic lipid to the at least one additional lipid is about 2:1.

[0311] In some embodiments, RNA lipoplex particles have an average diameter that in one embodiment ranges from about 200 nm to about 1000 nm, from about 200 nm to about 800 nm, from about 250 to about 700 nm, from about 400 to about 600 nm, from about 300 nm to about 500 nm, or from about 350 nm to about 400 nm. In specific embodiments, the RNA lipoplex particles have an average diameter of about 200 nm, about 225 nm, about 250 nm, about 275 nm, about 300 nm, about 325 nm, about 350 nm, about 375 nm, about 400 nm, about 425 nm, about 450 nm, about 475 nm, about 500 nm, about 525 nm, about 550 nm, about 575 nm, about 600 nm, about 625 nm, about 650 nm, about 700 nm, about 725 nm, about 750 nm, about 775 nm, about 800 nm, about 825 nm, about 850 nm, about 875 nm, about 900 nm, about 925 nm, about 950 nm, about 975 nm, or about 1000 nm. In an embodiment, the RNA lipoplex particles have an average diameter that ranges from about 250 nm to about 700 nm. In another embodiment, the RNA lipoplex particles have an average diameter that ranges from about 300 nm to about 500 nm. In some embodiments, RNA lipoplex particles have an average diameter of about 400 nm.

[0312] RNA lipoplex particles and compositions comprising RNA lipoplex particles described herein are useful for delivery of RNA to a target tissue after parenteral administration, in particular after intravenous administration. The RNA lipoplex particles may be prepared using liposomes that may be obtained by injecting a solution of the lipids in ethanol into water or a suitable aqueous phase. In one embodiment, the aqueous phase has an acidic pH. In one embodiment, the aqueous phase comprises acetic acid, e.g., in an amount of about 5 mM. Liposomes may be used for preparing RNA lipoplex particles by mixing the liposomes with RNA. In one embodiment, the liposomes and RNA lipoplex particles comprise at least one cationic lipid and at least one additional lipid. In one embodiment, the at

least one cationic lipid comprises 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and/or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP). In one embodiment, the at least one additional lipid comprises 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol (Chol) and/or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). In one embodiment, the at least one cationic lipid comprises 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and the at least one additional lipid comprises 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE). In one embodiment, the liposomes and RNA lipoplex particles comprise 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE).

[0313] Spleen targeting RNA lipoplex particles are described in WO 2013/143683, herein incorporated by reference. It has been found that RNA lipoplex particles having a net negative charge may be used to preferentially target spleen tissue or spleen cells such as antigen-presenting cells, in particular dendritic cells. Accordingly, following administration of the RNA lipoplex particles, RNA accumulation and/or RNA expression in the spleen occurs. Thus, RNA lipoplex particles of the disclosure may be used for expressing RNA in the spleen. In an embodiment, after administration of the RNA lipoplex particles, no or essentially no RNA accumulation and/or RNA expression in the lung and/or liver occurs. In one embodiment, after administration of the RNA lipoplex particles, RNA accumulation and/or RNA expression in antigen presenting cells, such as professional antigen presenting cells in the spleen occurs. Thus, RNA lipoplex particles of the disclosure may be used for expressing RNA in such antigen presenting cells. In one embodiment, the antigen presenting cells are dendritic cells and/or macrophages.

5. Lipid Nanoparticles (LNPs)

[0314] In some embodiments, nucleic acid such as RNA described herein is administered in the form of lipid nanoparticles (LNPs). In some embodiments, LNPs may comprise any lipid capable of forming a particle to which the one or more nucleic acid molecules are attached, or in which the one or more nucleic acid molecules are encapsulated.

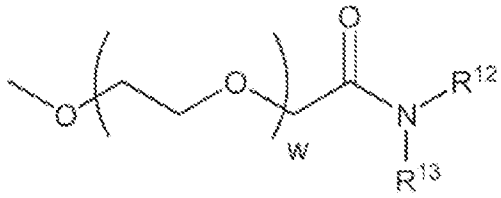
[0315] In some embodiments, an LNP comprises one or more cationic lipids, and one or more stabilizing lipids. Stabilizing lipids include neutral lipids and pegylated lipids.

[0316] In some embodiments, an LNP comprises a cationic lipid, a neutral lipid, a sterol, a polymer conjugated lipid; and an RNA, encapsulated within or associated with the lipid nanoparticle.

[0317] In some embodiments, a neutral lipid is selected from the group consisting of DSPC, DPPC, DMPC, DOPC, POPC, DOPE, DOPG, DPPG, POPE, DPPE, DMPE, DSPE, and SM. In some embodiments, the neutral lipid is selected from the group consisting of DSPC, DPPC, DMPC, DOPC, POPC, DOPE and SM. In some embodiments, the neutral lipid is DSPC.

[0318] In some embodiments, a sterol is cholesterol.

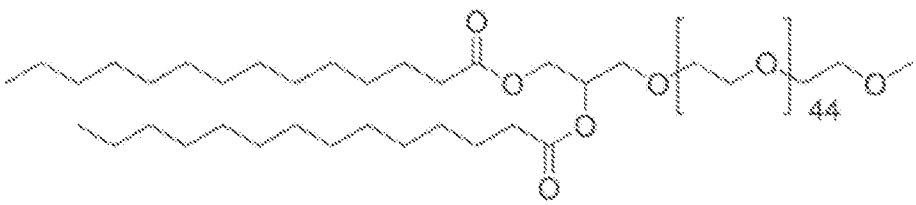
[0319] In some embodiments, a polymer conjugated lipid is a pegylated lipid. In some embodiments, a pegylated lipid has the following structure:



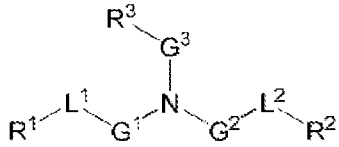
or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, wherein:

R^{12} and R^{13} are each independently a straight or branched, saturated or unsaturated alkyl chain containing from 10 to 30 carbon atoms, wherein the alkyl chain is optionally interrupted by one or more ester bonds; and w has a mean value ranging from 30 to 60. In some embodiments, R^{12} and R^{13} are each independently straight, saturated alkyl chains containing from 12 to 16 carbon atoms. In some embodiments, w has a mean value ranging from 40 to 55. In some embodiments, the average w is about 45. In some embodiments, R^{12} and R^{13} are each independently a straight, saturated alkyl chain containing about 14 carbon atoms, and w has a mean value of about 45.

[0320] In some embodiments, a pegylated lipid is DMG-PEG 2000, e.g., having the following structure:



[0321] In some embodiments, a cationic lipid component of LNPs has the structure of Formula (III):



(III)

or a pharmaceutically acceptable salt, tautomer, prodrug or stereoisomer thereof, wherein:

one of L^1 or L^2 is –O(C=O)-, -(C=O)O-, -C(=O)-, -O-, -$S(O)_x$-, -S-S-, -C(=O)S-, SC(=O)-, -$NR^aC(=O)$-, -$C(=O)NR^a$-, $NR^aC(=O)NR^a$-, -$OC(=O)NR^a$- or -$NR^aC(=O)O$-, and the other of L^1 or L^2 is –O(C=O)-, -(C=O)O-, -C(=O)-, -O-, -$S(O)_x$-, -S-S-, -C(=O)S-, SC(=O)-, -$NR^aC(=O)$-, -$C(=O)NR^a$-, $NR^aC(=O)NR^a$-, -$OC(=O)NR^a$- or -$NR^aC(=O)O$- or a direct bond;

G^1 and G^2 are each independently unsubstituted C_1-C_{12} alkylene or C_1-C_{12} alkenylene;

G^3 is C_1-C_{24} alkylene, C_1-C_{24} alkenylene, C_3-C_8 cycloalkylene, C_3-C_8 cycloalkenylene;

R^a is H or C_1-C_{12} alkyl;

R^1 and R^2 are each independently C_6-C_{24} alkyl or C_6-C_{24} alkenyl;

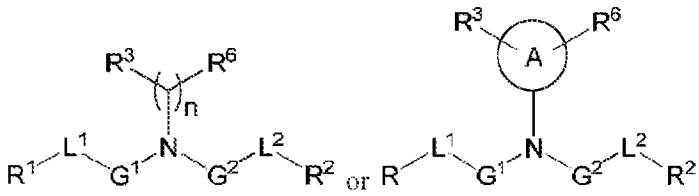
R^3 is H, OR^5, CN, -C(=O)OR^4, -OC(=O)R^4 or –$NR^5C(=O)R^4$;

R^4 is C_1-C_{12} alkyl;

R^5 is H or C_1-C_6 alkyl; and

x is 0, 1 or 2.

[0322] In some of the foregoing embodiments of Formula (III), the lipid has one of the following structures (IIIA) or (IIIB):



(IIIA) (IIIB)

wherein:

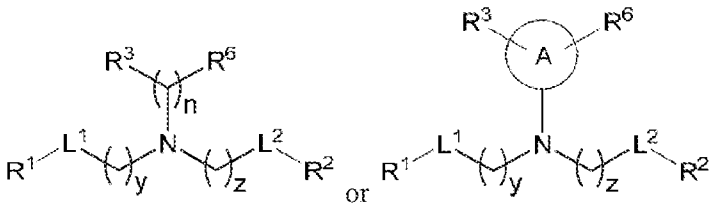
A is a 3 to 8-membered cycloalkyl or cycloalkylene ring;

R^6 is, at each occurrence, independently H, OH or C_1-C_{24} alkyl; and

n is an integer ranging from 1 to 15.

[0323] In some of the foregoing embodiments of Formula (III), the lipid has structure (IIIA), and in other embodiments, the lipid has structure (IIIB).

[0324] In other embodiments of Formula (III), the lipid has one of the following structures (IIIC) or (IIID):

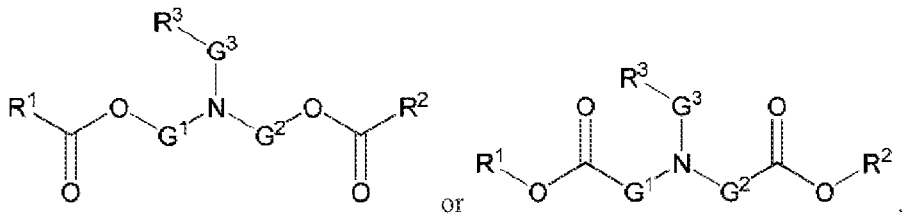


(IIIC) (IIID)

wherein y and z are each independently integers ranging from 1 to 12.

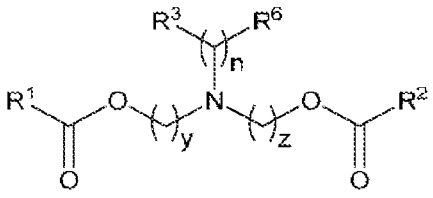
[0325] In any of the foregoing embodiments of Formula (III), one of L^1 or L^2 is -O(C=O)-. For example, in some embodiments each of L^1 and L^2 are -O(C=O)-. In some different embodiments of any of the foregoing, L^1 and L^2 are each independently -(C=O)O- or -O(C=O)-. For example, in some embodiments each of L^1 and L^2 is -(C=O)O-.

[0326] In some different embodiments of Formula (III), the lipid has one of the following structures (IIIE) or (IIIF):

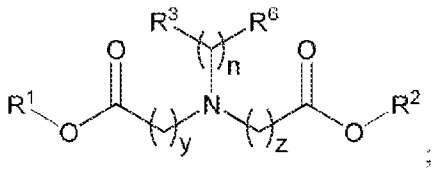


(IIIE) (IIIF)

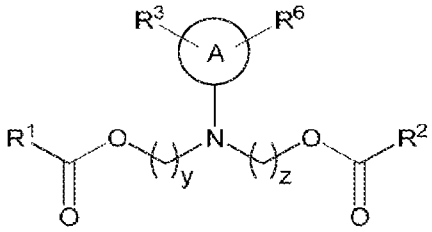
[0327] In some of the foregoing embodiments of Formula (III), the lipid has one of the following structures (IIIG), (IIIH), (IIII), or (IIIJ):



(IIIG)

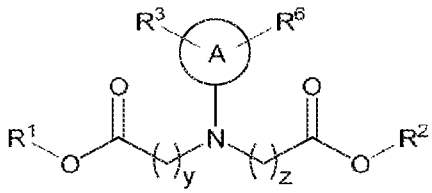


(IIIH)



(IIII)

or



(IIIJ)

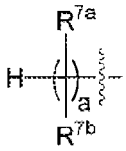
[0328] In some of the foregoing embodiments of Formula (III), n is an integer ranging from 2 to 12, for example from 2 to 8 or from 2 to 4. For example, in some embodiments, n is 3, 4, 5 or 6. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6.

[0329] In some other of the foregoing embodiments of Formula (III), y and z are each independently an integer ranging from 2 to 10. For example, in some embodiments, y and z are each independently an integer ranging from 4 to 9 or from 4 to 6.

[0330] In some of the foregoing embodiments of Formula (III), R^6 is H. In other of the foregoing embodiments, R^6 is C_1-C_{24} alkyl. In other embodiments, R^6 is OH.

[0331] In some embodiments of Formula (III), G^3 is unsubstituted. In other embodiments, G3 is substituted. In various different embodiments, G^3 is linear C_1-C_{24} alkylene or linear C_1-C_{24} alkenylene.

[0332] In some other foregoing embodiments of Formula (III), R^1 or R^2, or both, is C_6-C_{24} alkenyl. For example, in some embodiments, R^1 and R^2 each, independently have the following structure:



,

wherein:

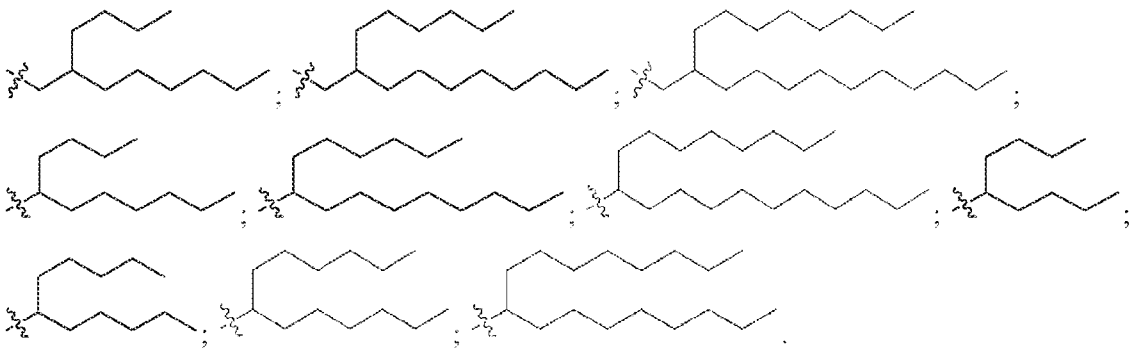
R^{7a} and R^{7b} are, at each occurrence, independently H or C_1-C_{12} alkyl; and

a is an integer from 2 to 12, and

wherein R^{7a}, R^{7b} and a are each selected such that R^1 and R^2 each independently comprise from 6 to 20 carbon atoms. For example, in some embodiments a is an integer ranging from 5 to 9 or from 8 to 12.

[0333] In some of the foregoing embodiments of Formula (III), at least one occurrence of R^{7a} is H. For example, in some embodiments, R^{7a} is H at each occurrence. In other different embodiments of the foregoing, at least one occurrence of R^{7b} is C_1-C_8 alkyl. For example, in some embodiments, C_1-C_8 alkyl is methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-hexyl or n-octyl.

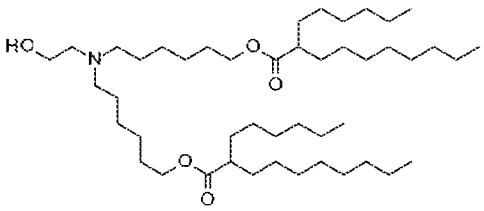
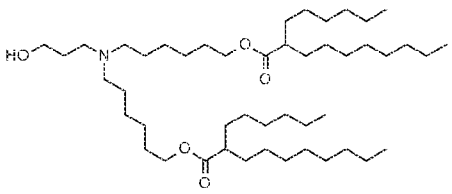
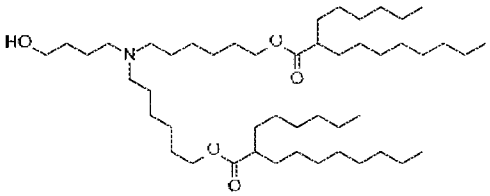
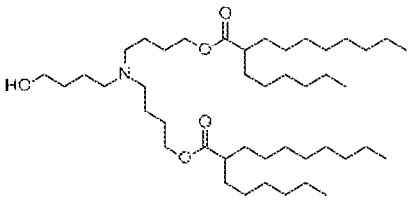
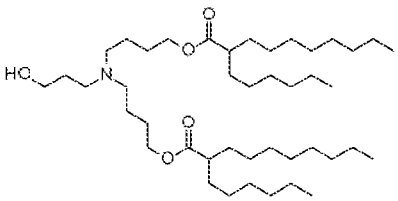
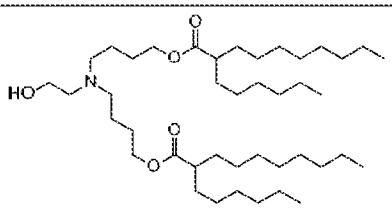
[0334] In different embodiments of Formula (III), R^1 or R^2, or both, has one of the following structures:

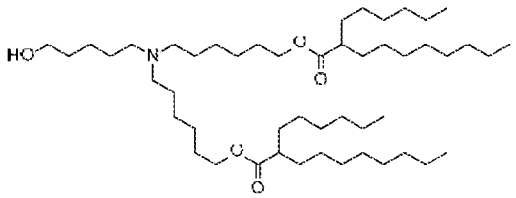
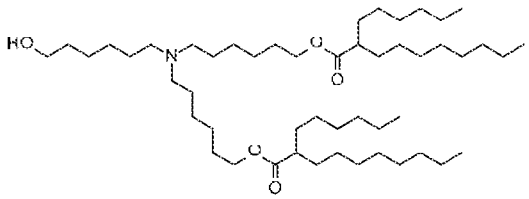
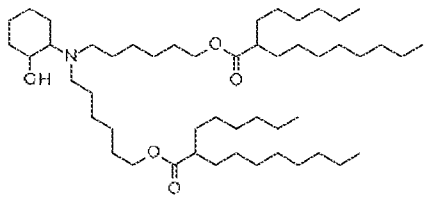
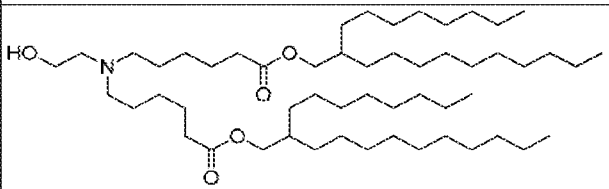
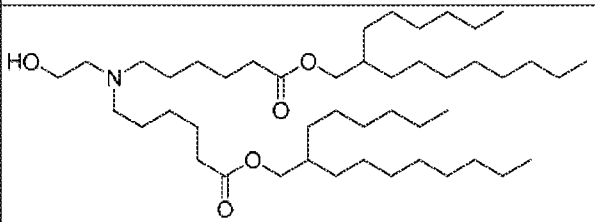
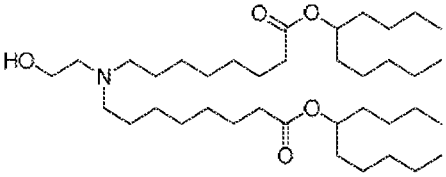


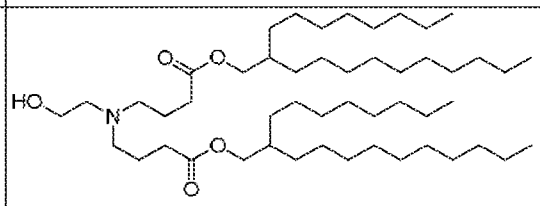
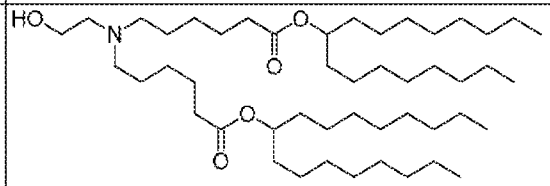
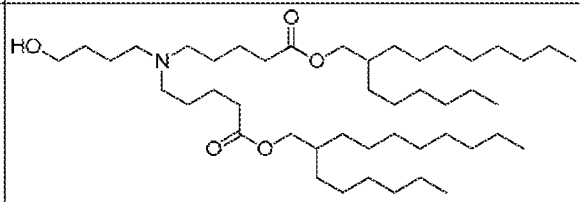
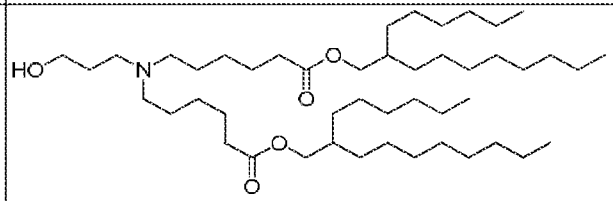
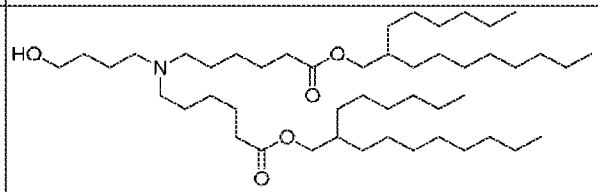
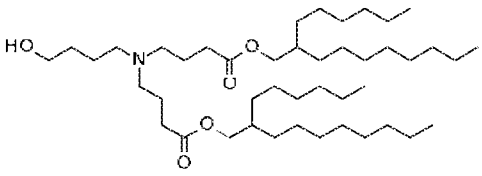
[0335] In some of the foregoing embodiments of Formula (III), R^3 is OH, CN, -C(=O)OR^4, -OC(=O)R^4 or –NHC(=O)R^4. In some embodiments, R^4 is methyl or ethyl.

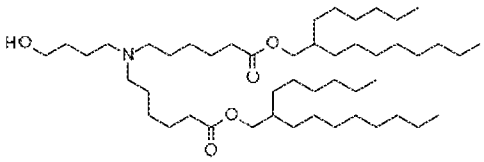
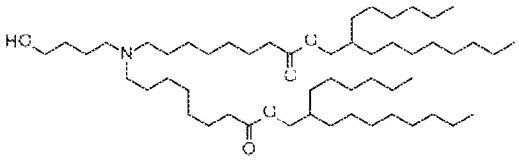
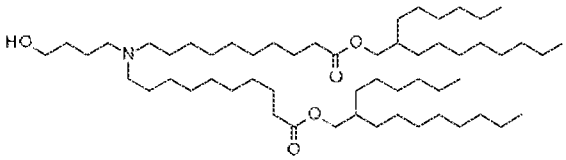
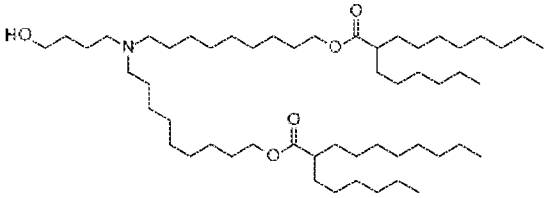
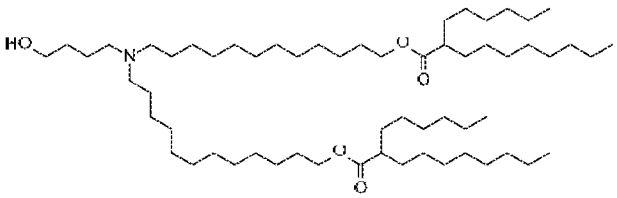
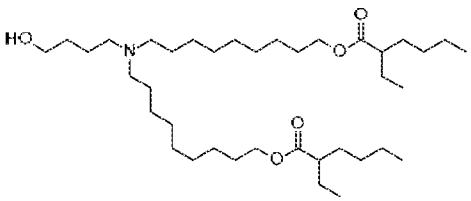
[0336] In various different embodiments, the cationic lipid of Formula (III) has one of the structures set forth in in **Table 10** below.

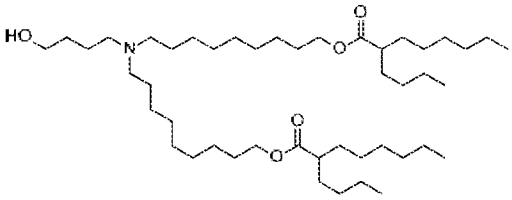
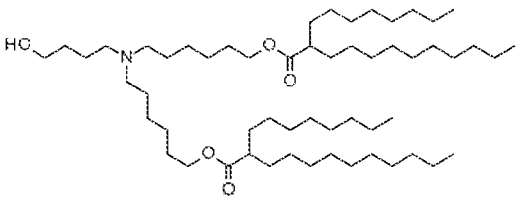
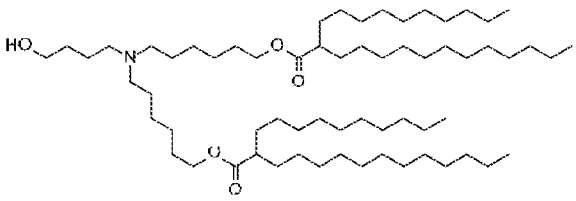
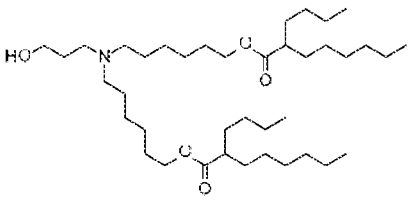
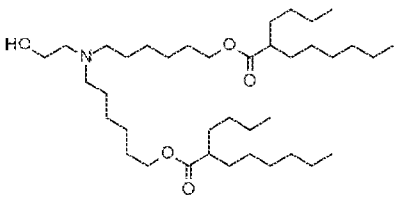
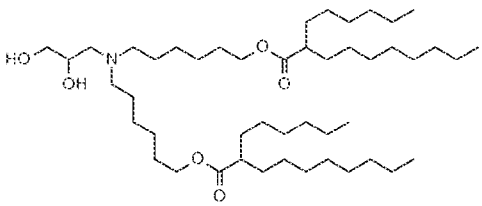
Table 10: Example Compounds of Formula (III).

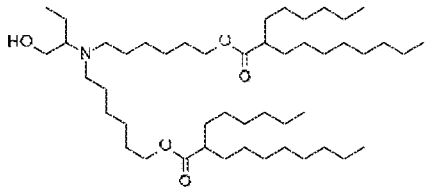
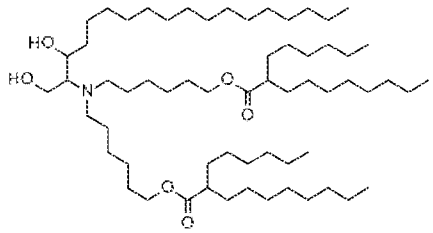
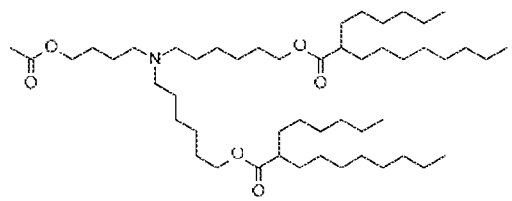
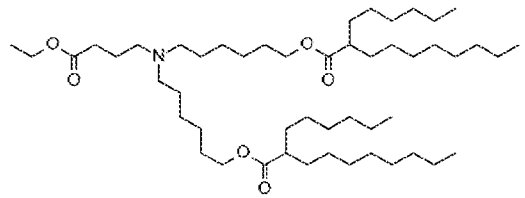
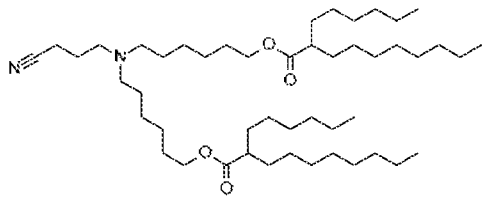
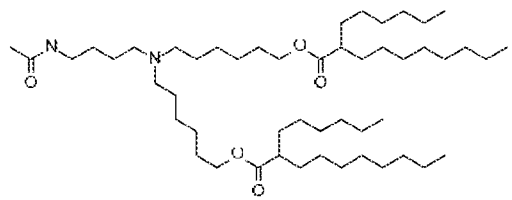
No.	Structure
III-1	
III-2	
III-3	
III-4	
III-5	
III-6	

No.	Structure
III-7	
III-8	
III-9	
III-10	
III-11	
III-12	

No.	Structure
III-13	
III-14	
III-15	
III-16	
III-17	
III-18	

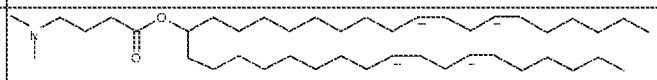
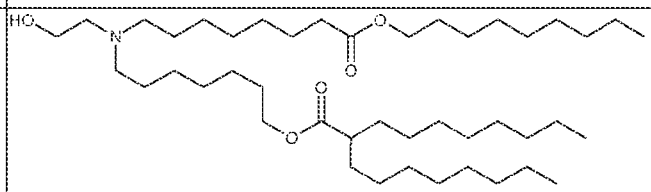
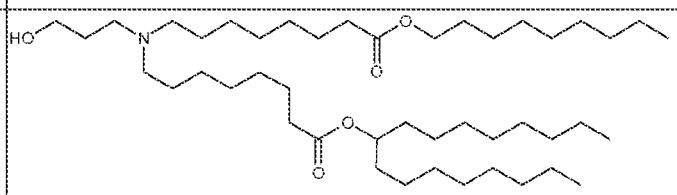
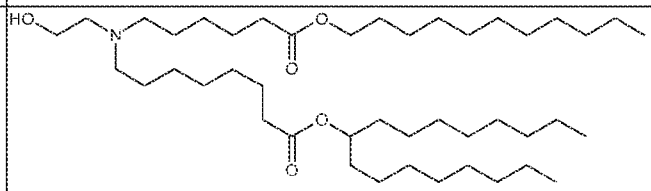
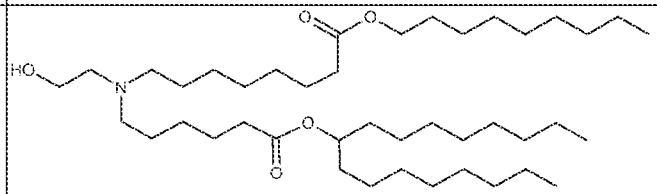
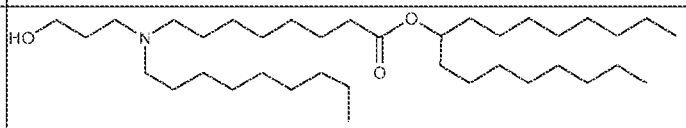
No.	Structure
III-19	
III-20	
III-21	
III-22	
III-23	
III-24	

No.	Structure
III-25	
III-26	
III-27	
III-28	
III-29	
III-30	

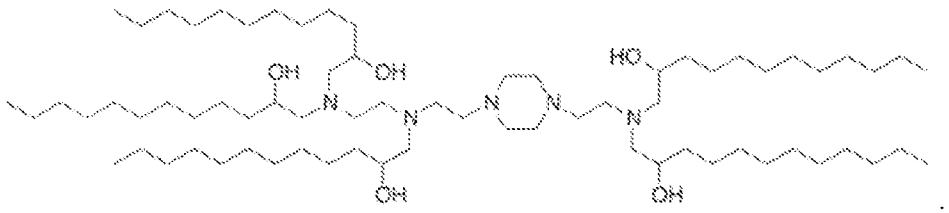
No.	Structure
III-31	
III-32	
III-33	
III-34	
III-35	
III-36	

[0337] In various different embodiments, a cationic lipid has one of the structures set forth in **Table 11** below.

Table 11: Example Cationic Lipid Structures

No.	Structure
A	
B	
C	
D	
E	
F	

[0338] In some embodiments, an LNP comprises a cationic lipid that is an ionizable lipid-like material (lipidoid). In some embodiments, a cationic lipid has the following structure:



[0339] In some embodiments, lipid nanoparticles can have an average size (*e.g.*, mean diameter) of about 30 nm to about 150 nm, about 40 nm to about 150 nm, about 50 nm to about 150 nm, about 60 nm to about 130 nm, about 70 nm to about 110 nm, about 70 nm to about 100 nm, about 70 to about 90 nm, or about 70 nm to about 80 nm. In some embodiments, lipid nanoparticles in accordance with the present disclosure can have an average size (*e.g.*, mean diameter) of about 50 nm to about 100 nm. In some embodiments, lipid nanoparticles may have an average size (*e.g.*, mean diameter) of about 50 nm to about 150 nm. In some embodiments, lipid nanoparticles may have an average size (*e.g.*, mean diameter) of about 60 nm to about 120 nm. In some embodiments, lipid nanoparticles in accordance with the present disclosure can have an average size (*e.g.*, mean diameter) of about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm. The term "average diameter" or "mean diameter" refers to the mean hydrodynamic diameter of particles as measured by dynamic laser light scattering (DLS) with data analysis using the so-called cumulant algorithm, which provides as results the so-called Z-average with the dimension of a length, and the polydispersity index (PI), which is dimensionless (Koppel, D., J. Chem. Phys. 57, 1972, pp 4814-4820, ISO 13321, which is herein incorporated by reference). Here "average diameter," "mean diameter," "diameter," or "size" for particles is used synonymously with this value of the Z-average.

[0340] In some embodiments, lipid nanoparticles described herein may exhibit a polydispersity index less than about 0.5, less than about 0.4, less than about 0.3, or about 0.2 or less. By way of example, lipid nanoparticles can exhibit a polydispersity index in a range of about 0.1 to about 0.3 or about 0.2 to about 0.3. The "polydispersity index" is preferably calculated based on dynamic light scattering measurements by the so-called cumulant analysis as mentioned in the definition of the "average diameter." Under certain prerequisites, it can be taken as a measure of the size distribution of an ensemble of ribonucleic acid nanoparticles (e.g., ribonucleic acid nanoparticles).

[0341] Lipid nanoparticles described herein can be characterized by an "N/P ratio," which is the molar ratio of cationic (nitrogen) groups (the "N" in N/P) in the cationic polymer to the anionic (phosphate) groups (the "P" in N/P) in RNA. It is understood that a cationic group is one that is either in cationic form (*e.g.*, N^+), or one that is ionizable to become cationic. Use of a single number in an N/P ratio (*e.g.*, an N/P ratio of about 5) is intended to refer to that number over 1, e.g., an N/P ratio of about 5 is intended to mean 5:1. In some embodiments, a lipid nanoparticle described herein has an N/P ratio greater than or equal to 5. In some embodiments, a lipid nanoparticle described herein has an N/P ratio that is about 5, 6, 7, 8, 9, or 10. In some embodiments, an N/P ratio for a lipid nanoparticle described herein is from about 10 to about 50. In some embodiments, an N/P ratio for a lipid nanoparticle described herein is from about 10 to about 70. In some embodiments, an N/P ratio for a lipid nanoparticle described herein is from about 10 to about 120.

B. Example Methods of Making Lipid Nanoparticles

[0342] Lipids and lipid nanoparticles comprising nucleic acids and their method of preparation are known in the art, including, *e.g.*, as described in U.S. Patent Nos. 8,569,256, 5,965,542 and U.S. Patent Publication Nos. 2016/0199485, 2016/0009637, 2015/0273068, 2015/0265708, 2015/0203446, 2015/0005363, 2014/0308304, 2014/0200257, 2013/086373, 2013/0338210,2013/0323269, 2013/0245107, 2013/0195920, 2013/0123338, 2013/0022649, 2013/0017223, 2012/0295832, 2012/0183581, 2012/0172411, 2012/0027803, 2012/0058188, 2011/0311583, 2011/0311582, 2011/0262527, 2011/0216622, 2011/0117125, 2011/0091525, 2011/0076335, 2011/0060032, 2010/0130588, 2007/0042031, 2006/0240093, 2006/0083780, 2006/0008910, 2005/0175682, 2005/017054, 2005/0118253, 2005/0064595, 2004/0142025, 2007/0042031, 1999/009076 and PCT Pub. Nos. WO 99/39741, WO 2018/081480, WO 2017/004143, WO 2017/075531, WO 2015/199952, WO 2014/008334, WO 2013/086373, WO 2013/086322, WO 2013/016058, WO 2013/086373, W02011/141705, and WO 2001/07548, the full disclosures each of which are herein incorporated by reference in their entirety for the purposes described herein.

[0343] For example, in some embodiments, cationic lipids, neutral lipids (*e.g.*, DSPC, and/or cholesterol) and polymer-conjugated lipids can be solubilized in ethanol at a pre-determined molar ratio (*e.g.*, ones described herein). In some embodiments, lipid nanoparticles (lipid nanoparticle) are prepared at a total lipid to polyribonucleotides weight ratio of approximately 10: 1 to 30: 1. In some embodiments, such polyribonucleotides can be diluted to 0.2 mg/mL in acetate buffer.

[0344] In some embodiments, using an ethanol injection technique, a colloidal lipid dispersion comprising polyribonucleotides can be formed as follows: an ethanol solution comprising lipids, such as cationic lipids, neutral lipids, and polymer-conjugated lipids, is injected into an aqueous solution comprising polyribonucleotides (*e.g.*, ones described herein).

[0345] In some embodiments, lipid and polyribonucleotide solutions can be mixed at room temperature by pumping each solution at controlled flow rates into a mixing unit, for example, using piston pumps. In some embodiments, the flow rates of a lipid solution and a RNA solution into a mixing unit are maintained at a ratio of 1:3. Upon mixing, nucleic acid-lipid particles are formed as the ethanolic lipid solution is diluted with aqueous polyribonucleotides. The lipid solubility is decreased, while cationic lipids bearing a positive charge interact with the negatively charged RNA.

[0346] In some embodiments, a solution comprising RNA-encapsulated lipid nanoparticles can be processed by one or more of concentration adjustment, buffer exchange, formulation, and/or filtration.

[0347] In some embodiments, RNA-encapsulated lipid nanoparticles can be processed through filtration.

[0348] In some embodiments, particle size and/or internal structure of lipid nanoparticles (with or without RNAs) may be monitored by appropriate techniques such as, *e.g.*, small-angle X-ray scattering (SAXS) and/or transmission electron cryomicroscopy (CryoTEM).

V. Pharmaceutical Compositions

[0349] The present disclosure provides compositions, e.g., pharmaceutical compositions comprising one or more polyribonucleotides described herein. Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or

otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this disclosure.

[0350] In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by the United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0351] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical formulations. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

[0352] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

[0353] In some embodiments, pharmaceutical compositions provided herein may be formulated with one or more pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

[0354] Pharmaceutical compositions described herein can be administered by appropriate methods known in the art. As will be appreciated by a skilled artisan, the route and/or mode of administration may depend on a number of factors, including, *e.g.*, but not limited to stability and/or pharmacokinetics and/or pharmacodynamics of pharmaceutical compositions described herein.

[0355] In some embodiments, pharmaceutical compositions described herein are formulated for parenteral administration, which includes modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular,

intraarterial, intradermal, subcutaneous, subcuticular, or intraarticular injection and infusion. In preferred embodiments, pharmaceutical compositions described herein are formulated for intravenous, intramuscular, or subcutaneous administration. In particularly preferred embodiments, pharmaceutical compositions described herein are formulated for intramuscular administration.

[0356] In some embodiments, pharmaceutical compositions described herein are formulated for intravenous administration. In some embodiments, pharmaceutically acceptable excipients that may be useful for intravenous administration include sterile aqueous solutions or dispersions and sterile powders for preparation of sterile injectable solutions or dispersions.

[0357] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, lipid nanoparticles, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. In some embodiments, prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0358] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization and/or microfiltration. In some embodiments, pharmaceutical compositions can be prepared as described herein and/or methods known in the art. In some embodiments, a pharmaceutical composition includes ALC-0315; ALC-0159; DSPC; Cholesterol; Sucrose; NaCl; KCl; Na_2HPO_4; KH_2PO_4; Water for injection. In some embodiments, normal saline (isotonic 0.9% NaCl) is used as diluent.

[0359] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the presence of microorganisms may be ensured both by sterilization procedures, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into pharmaceutical compositions described

herein. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0360] Formulations of pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing active ingredient(s) into association with a diluent or another excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0361] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of at least one RNA product produced using a system and/or method described herein.

[0362] Relative amounts of polyribonucleotides encapsulated in lipid nanoparticles, a pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition can vary, depending upon the subject to be treated, target cells, diseases or disorders, and may also further depend upon the route by which the composition is to be administered.

[0363] In some embodiments, pharmaceutical compositions described herein are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Actual dosage levels of the active ingredients (e.g., polyribonucleotides encapsulated in lipid nanoparticles) in the pharmaceutical compositions described herein may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0364] A physician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician could start doses of active ingredients (e.g., polyribonucleotides encapsulated in lipid nanoparticles) employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0365] In some embodiments, a pharmaceutical composition is formulated (e.g., but not limited to, for intravenous, intramuscular, or subcutaneous administration) to deliver a dose of about 5 mg RNA/kg.

[0366] In some embodiments, a pharmaceutical composition described herein may further comprise one or more additives, for example, in some embodiments that may enhance stability of such a composition under certain conditions. Examples of additives may include but are not limited to salts, buffer substances, preservatives, and carriers. For example, in some embodiments, a pharmaceutical composition may further comprise a cryoprotectant (e.g., sucrose) and/or an aqueous buffered solution, which may in some embodiments include one or more salts, including, e.g., alkali metal salts or alkaline earth metal salts such as, e.g., sodium salts, potassium salts, and/or calcium salts.

[0367] In some embodiments, a pharmaceutical composition provided herein is a preservative-free, sterile RNA-lipid nanoparticle dispersion in an aqueous buffer for intravenous or intramuscular administration.

[0368] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions that are suitable for administration to humans, it will be 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/or perform such modification with merely ordinary, if any, experimentation.

VI. Patient Populations

[0369] In some aspects, technologies of the present disclosure are used for therapeutic and/or prophylactic purposes. In some embodiments, technologies of the present disclosure are used in the

treatment and/or prophylactic of an HSV infection. Prophylactic purposes of the present disclosure comprise pre-exposure prophylaxis and/or post-exposure prophylaxis.

[0370] In some embodiments, technologies of the present disclosure are used in the treatment and/or prophylaxis of a disorder related to such an HSV (e.g., HSV-1 and/or HSV-2) infection. A disorder related to such an HSV (e.g., HSV-1 and/or HSV-2) infection comprises, for example, a typical symptom and/or a complication of an HSV (e.g., HSV-1 and/or HSV-2) infection.

[0371] In some embodiments, provided compositions (*e.g.*, that are or comprise HSV antigens, HSV gB antigens, e.g., HSV-1 gB antigens) may be useful to detect and/or characterize one or more features of an anti-HSV (e.g., anti-HSV-1 and/or anti-HSV-2) immune response (*e.g.*, by detecting binding to a provided antigen by serum from an infected subject).

[0372] In some embodiments, provided compositions (*e.g.*, that are or comprise HSV antigens, HSV gB antigens, e.g., HSV-1 gB antigens) are useful to raise antibodies to one or more epitopes included therein; such antibodies may themselves be useful, for example for detection or treatment of an HSV infection.

[0373] The present disclosure provides use of encoding nucleic acids (*e.g.*, DNA or RNA) to produce encoded antigens and/or use of DNA constructs to produce RNA.

[0374] In some embodiments, technologies of the present disclosure are utilized in a non-limited subject population; in some embodiments, technologies of the present disclosure are utilized in particular subject populations.

[0375] In some embodiments, a subject population comprises an adult population. In some embodiments, an adult population comprises subjects between the ages of about 19 years and about 60 years of age (*e.g.*, about 20, 25, 30, 35, 40, 45, 50, 55, or 60 years of age).

[0376] In some embodiments, a subject population comprises an elderly population. In some embodiments, an elderly population comprises subjects of about 60 years of age, about 70 years of age, or older (*e.g.*, about 65, 70, 75, 80, 85, 90, 95, or 100 years of age).

[0377] In some embodiments, a subject population comprises a pediatric population. In some embodiments, a pediatric population comprises subjects approximately 18 years old or younger. In some

such embodiments, a pediatric population comprises subjects between the ages of about 1 year and about 18 years (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 years of age).

[0378] In some embodiments, a subject population comprises a newborn population. In some embodiments, a newborn population comprises subjects about 12 months or younger (*e.g.*, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 months or younger). In some embodiments, subject populations to be treated with technologies described herein include infants (*e.g.*, about 12 months or younger) whose mothers did not receive such technologies described herein during pregnancy. In some embodiments, subject populations to be treated with technologies described herein may include pregnant women; in some embodiments, infants whose mothers were treated with disclosed technologies during pregnancy (*e.g.*, who received at least one dose, or alternatively only who received both doses), are not vaccinated during the first weeks, months, or even years (e.g., 1, 2, 3, 4, 5, 6, 7, 8 weeks or more, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, or 1, 2, 3, 4, 5 years or more) post-birth. Alternatively or additionally, in some embodiments, infants whose mothers were treated with disclosed technologies during pregnancy (*e.g.*, who received at least one dose, or alternatively only who received both doses), receive reduced treated with disclosed technologies (*e.g.*, lower doses and/or smaller numbers of administrations – *e.g.*, boosters – and/or lower total exposure over a given period of time) after birth, for example during the first weeks, months, or even years (e.g., 1, 2, 3, 4, 5, 6, 7, 8 weeks or more, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, or 1, 2, 3, 4, 5 years or more) post-birth or may need reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters – over a given period of time), In some embodiments, compositions as provided herein are administered to subject populations that do not include pregnant women.

[0379] In some embodiments, a subject population is or comprises children aged 6 weeks to up to 17 months of age.

[0380] In some embodiments, a provided pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) may be administered in combination with (i.e., so that subject(s) are simultaneously exposed to both) another pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) or therapeutic intervention, e.g., to treat or prevent an HSV infection, or another disease, disorder, or condition.

[0381] In some embodiments, a provided pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) may be administered with a protein vaccine, a DNA vaccine, an RNA vaccine, a cellular vaccine, a conjugate vaccine, etc. In some embodiments, one or more doses of a provided pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) may be administered together with (e.g., in a single visit) another vaccine or other therapy.

[0382] In some embodiments, a provided pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) may be administered to subjects who have been exposed, or expect they have been exposed, to HSV (e.g., HSV-1 and/or HSV-2). In some embodiments, a provided pharmaceutical composition (e.g., immunogenic composition, e.g., vaccine) may be administered to subjects who do not have symptoms of an HSV (e.g., HSV-1 and/or HSV-2).

VII. Treatment Methods

[0383] In some embodiments, technologies of the present disclosure may be administered to subjects according to a particular dosing regimen. In some embodiments, a dosing regimen may involve a single administration; in some embodiments, a dosing regimen may comprise one or more "booster" administrations after the initial administration. In some embodiments, initial and boost doses are the same amount; in some embodiments they differ. In some embodiments, two or more booster doses are administered. In some embodiments, a plurality of doses are administered at regular intervals. In some embodiments, periods of time between doses become longer. In some embodiments, one or more subsequent doses is administered if a particular clinical (*e.g.*, reduction in neutralizing antibody levels) or situational (*e.g.*, local development of a new strain) event arises or is detected.

[0384] In some embodiments, administered pharmaceutical compositions (e.g., immunogenic compositions, e.g., vaccines) comprising RNA constructs that encode HSV-2 gB constructs are administered in RNA doses of from about 0.1 μg to about 300 μg, about 0.5 μg to about 200 μg, or about 1 μg to about 100 μg, such as about 1 μg, about 3 μg, about 10 μg, about 30 μg, about 50 μg, or about 100 μg. In some embodiments, an saRNA construct is administered at a lower dose (*e.g.*, 2, 4, 5, 10 fold or more lower) than a modRNA or uRNA construct.

[0385] In some embodiments, a first booster dose is administered within about six months of the initial dose, and preferably within about 5, 4, 3, 2, or 1 months. In some embodiments, a first booster dose is administered in a time period that begins about 1, 2, 3, or 4 weeks after the first dose, and ends about 2,

3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after the first dose (*e.g.*, between about 1 and about 12 weeks after the first dose, or between about 2 or 3 weeks and about 5 and 6 weeks after the first dose, or about 3 weeks or about 4 weeks after the first dose).

[0386] In some embodiments, a plurality of booster doses (*e.g.*, 2, 3, or 4) doses are administered within 6 months of the first dose, or within 12 months of the first dose.

[0387] In some embodiments, 3 doses or fewer are required to achieve effective vaccination (*e.g.*, greater than 60%, and in some embodiments greater than about 70%, about 75%, about 80%, about 85%, about 90% or more) reduction in risk of infection, or of serious disease. In some embodiments, not more than two doses are required. In some embodiments, a single dose is sufficient. In some embodiments, an RNA dose is about 60 μg or lower, 50 μg or lower, 40 μg or lower, 30 μg or lower, 20 μg or lower, 10 μg or lower, 5 μg or lower, 2.5 μg or lower, or 1 μg or lower. In some embodiments, an RNA dose is about 0.25 μg, at least 0.5 μg, at least 1 μg, at least 2 μg, at least 3 μg, at least 4 μg, at least 5 μg, at least 10 μg, at least 20 μg, at least 30 μg, or at least 40 μg. In some embodiments, an RNA dose is about 0.25 μg to 60 μg, 0.5 μg to 55 μg, 1 μg to 50 μg, 5 μg to 40 μg, or 10 μg to 30 μg may be administered per dose. In some embodiments, an RNA dose is about 30 μg. In some embodiments, at least two such doses are administered. For example, a second dose may be administered about 21 days following administration of the first dose. In some embodiments, a first booster dose is administered about one month after an initial dose. In some such embodiments, at least one further booster is administered at one-month interval(s). In some embodiments, after 2 or 3 boosters, a longer interval is introduced and no further booster is administered for at least 6, 9, 12, 18, 24, or more months. In some embodiments, a single further booster is administered after about 18 months. In some embodiments, no further booster is required unless, for example, a material change in clinical or environmental situation is observed.

VIII. Methods of Manufacture

[0388] Individual polyribonucleotides can be produced by methods known in the art. For example, in some embodiments, polyribonucleotides can be produced by *in vitro* transcription, for example, using a DNA template. A plasmid DNA used as a template for *in vitro* transcription to generate a polyribonucleotide described herein is also within the scope of the present disclosure.

[0389] A DNA template is used for *in vitro* RNA synthesis in the presence of an appropriate RNA polymerase (*e.g.*, a recombinant RNA-polymerase such as a T7 RNA-polymerase) with ribonucleotide triphosphates (*e.g.*, ATP, CTP, GTP, UTP). In some embodiments, polyribonucleotides (*e.g.*, ones described herein) can be synthesized in the presence of modified ribonucleotide triphosphates. By way of example only, in some embodiments, pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), or 5-methyl-uridine (m5U) can be used to replace uridine triphosphate (UTP). In some embodiments, pseudouridine (ψ) can be used to replace uridine triphosphate (UTP). In some embodiments, N1-methyl-pseudouridine (m1ψ) can be used to replace uridine triphosphate (UTP). In some embodiments, 5-methyl-uridine (m5U) can be used to replace uridine triphosphate (UTP).

[0390] As will be clear to those skilled in the art, during *in vitro* transcription, an RNA polymerase (*e.g.*, as described and/or utilized herein) typically traverses at least a portion of a single-stranded DNA template in the 3'→ 5' direction to produce a single-stranded complementary RNA in the 5'→ 3' direction.

[0391] In some embodiments where a polyribonucleotide comprises a polyA tail, one of those skill in the art will appreciate that such a polyA tail may be encoded in a DNA template, *e.g.*, by using an appropriately tailed PCR primer, or it can be added to a polyribonucleotide after *in vitro* transcription, *e.g.*, by enzymatic treatment (*e.g.*, using a poly(A) polymerase such as an *E. coli* Poly(A) polymerase). Suitable poly(A) tails are described herein above. For example, in some embodiments, a poly(A) tail comprises a nucleotide sequence of AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCATATGACTAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 58). In some embodiments, a poly(A) tail comprises a plurality of A residues interrupted by a linker. In some embodiments, a linker comprises the nucleotide sequence GCATATGAC (SEQ ID NO: 59).

[0392] In some embodiments, those skilled in the art will appreciate that addition of a 5' cap to an RNA (*e.g.*, mRNA) can facilitate recognition and attachment of the RNA to a ribosome to initiate translation and enhances translation efficiency. Those skilled in the art will also appreciate that a 5' cap can also protect an RNA product from 5' exonuclease mediated degradation and thus increases half-life. Methods for capping are known in the art; one of ordinary skill in the art will appreciate that in some embodiments, capping may be performed after *in vitro* transcription in the presence of a capping system (*e.g.*, an enzyme-based capping system such as, *e.g.*, capping enzymes of vaccinia virus). In some

embodiments, a cap may be introduced during *in vitro* transcription, along with a plurality of ribonucleotide triphosphates such that a cap is incorporated into a polyribonucleotide during transcription (also known as co-transcriptional capping). In some embodiments, a GTP fed-batch procedure with multiple additions in the course of the reaction may be used to maintain a low concentration of GTP in order to effectively cap the RNA. Suitable 5' cap are described herein above. For example, in some embodiments, a 5' cap comprises m7(3'OMeG)(5')ppp(5')(2'OMeA)pG.

[0393] Following RNA transcription, a DNA template is digested. In some embodiments, digestion can be achieved with the use of DNase I under appropriate conditions.

[0394] In some embodiments, in-vitro transcribed polyribonucleotides may be provided in a buffered solution, for example, in a buffer such as HEPES, a phosphate buffer solution, a citrate buffer solution, an acetate buffer solution; in some embodiments, such solution may be buffered to a pH within a range of, for example, about 6.5 to about 7.5; in some embodiments approximately 7.0. In some embodiments, production of polyribonucleotides may further include one or more of the following steps: purification, mixing, filtration, and/or filling.

[0395] In some embodiments, polyribonucleotides can be purified (*e.g.*, in some embodiments after *in vitro* transcription reaction), for example, to remove components utilized or formed in the course of the production, like, *e.g.*, proteins, DNA fragments, and/or nucleotides. Various nucleic acid purifications that are known in the art can be used in accordance with the present disclosure. Certain purification steps may be or include, for example, one or more of precipitation, column chromatography (including, *e.g.*, but not limited to anionic, cationic, hydrophobic interaction chromatography (HIC)), solid substrate-based purification (*e.g.*, magnetic bead-based purification). In some embodiments, polyribonucleotides may be purified using magnetic bead-based purification, which in some embodiments may be or comprise magnetic bead-based chromatography. In some embodiments, polyribonucleotides may be purified using hydrophobic interaction chromatography (HIC) and/or diafiltration. In some embodiments, polyribonucleotides may be purified using HIC followed by diafiltration.

[0396] In some embodiments, dsRNA may be obtained as a side product during *in vitro* transcription. In some such embodiments, a second purification step may be performed to remove dsRNA contamination. For example, in some embodiments, cellulose materials (*e.g.*, microcrystalline cellulose) may be used to remove dsRNA contamination, for examples in some embodiments in a chromatographic format. In some embodiments, cellulose materials (*e.g.*, microcrystalline cellulose) can be pretreated to

inactivate potential RNase contamination, for example in some embodiments by autoclaving followed by incubation with aqueous basic solution, *e.g.*, NaOH. In some embodiments, cellulose materials may be used to purify polyribonucleotides according to methods described in WO 2017/182524, the entire content of which is incorporated herein by reference.

[0397] In some embodiments, a batch of polyribonucleotides may be further processed by one or more steps of filtration and/or concentration. For example, in some embodiments, polyribonucleotide(s), for example, after removal of dsRNA contamination, may be further subject to diafiltration (*e.g.*, in some embodiments by tangential flow filtration), for example, to adjust the concentration of polyribonucleotides to a desirable RNA concentration and/or to exchange buffer to a drug substance buffer.

[0398] In some embodiments, polyribonucleotides may be processed through 0.2 μm filtration before they are filled into appropriate containers.

[0399] In some embodiments, polyribonucleotides and compositions thereof may be manufactured in accordance with a process as described herein, or as otherwise known in the art.

[0400] In some embodiments, polyribonucleotides and compositions thereof may be manufactured at a large scale. For example, in some embodiments, a batch of polyribonucleotides can be manufactured at a scale of greater than 1 g, greater than 2 g, greater than 3 g, greater than 4 g, greater than 5 g, greater than 6 g, greater than 7 g, greater than 8 g, greater than 9 g, greater than 10 g, greater than 15 g, greater than 20 g, or higher.

[0401] In some embodiments, RNA quality control may be performed and/or monitored at any time during production process of polyribonucleotides and/or compositions comprising the same. For example, in some embodiments, RNA quality control parameters, including one or more of RNA identity (*e.g.*, sequence, length, and/or RNA natures), RNA integrity, RNA concentration, residual DNA template, and residual dsRNA, may be assessed and/or monitored after each or certain steps of a polyribonucleotide manufacturing process, *e.g.*, after *in vitro* transcription, and/or each purification step.

[0402] In some embodiments, the stability of polyribonucleotides (*e.g.*, produced by *in vitro* transcription) and/or compositions comprising polyribonucleotides can be assessed under various test storage conditions, for example, at room temperatures vs. fridge or sub-zero temperatures over a period of time (*e.g.*, at least 3 months, at least 6 months, at least 9 months, at least 12 months, or longer). In some

embodiments, polyribonucleotides (*e.g.*, ones described herein) and/or compositions thereof may be stored stable at a fridge temperature (*e.g.*, about 4°C to about 10°C) for at least 1 month or longer including, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, or at least 12 months or longer. In some embodiments, polyribonucleotides (*e.g.*, ones described herein) and/or compositions thereof may be stored stable at a sub-zero temperature (*e.g.*, -20°C or below) for at least 1 month or longer including, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, or at least 12 months or longer. In some embodiments, polyribonucleotides (*e.g.*, ones described herein) and/or compositions thereof may be stored stable at room temperature (*e.g.*, at about 25°C) for at least 1 month or longer.

[0403] In some embodiments, one or more assessments may be utilized during manufacture, or other preparation or use of polyribonucleotides (*e.g.*, as a release test).

[0404] In some embodiments, one or more quality control parameters may be assessed to determine whether polyribonucleotides described herein meet or exceed acceptance criteria (*e.g.*, for subsequent formulation and/or release for distribution). In some embodiments, such quality control parameters may include, but are not limited to RNA integrity, RNA concentration, residual DNA template and/or residual dsRNA. Certain methods for assessing RNA quality are known in the art; for example, one of skill in the art will recognize that in some embodiments, one or more analytical tests can be used for RNA quality assessment. Examples of such certain analytical tests may include but are not limited to gel electrophoresis, UV absorption, and/or PCR assay.

[0405] In some embodiments, a batch of polyribonucleotides may be assessed for one or more features as described herein to determine next action step(s). For example, a batch of polyribonucleotides can be designated for one or more further steps of manufacturing and/or formulation and/or distribution if RNA quality assessment indicates that such a batch of polyribonucleotides meet or exceed the relevant acceptance criteria. Otherwise, an alternative action can be taken (*e.g.*, discarding the batch) if such a batch of polyribonucleotides does not meet or exceed the acceptance criteria.

[0406] In some embodiments, a batch of polyribonucleotides that satisfy assessment results can be utilized for one or more further steps of manufacturing and/or formulation and/or distribution.

IX. DNA Constructs

[0407] Among other things, the present disclosure provides DNA constructs, for example that may encode one or more antibody agents as described herein, or components thereof. In some embodiments, DNA constructs provided by and/or utilized in accordance with the present disclosure are comprised in a vector.

[0408] Non-limiting examples of a vector include plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as retroviral, adenoviral or baculoviral vectors, or artificial chromosome vectors such as bacterial artificial chromosomes (BAC), yeast artificial chromosomes (YAC), or P1 artificial chromosomes (PAC). In some embodiments, a vector is an expression vector. In some embodiments, a vector is a cloning vector. In general, a vector is a nucleic acid construct that can receive or otherwise become linked to a nucleic acid element of interest (*e.g.*, a construct that is or encodes a payload, or that imparts a particular functionality, *etc.*).

[0409] Expression vectors, which may be plasmid or viral or other vectors, typically include an expressible sequence of interest (*e.g.*, a coding sequence) that is functionally linked with one or more control elements (*e.g.*, promoters, enhancers, transcription terminators, etc.). Typically, such control elements are selected for expression in a system of interest. In some embodiments, a system is *ex vivo* (*e.g.*, an *in vitro* transcription system); in some embodiments, a system is *in vivo* (*e.g.*, a bacterial, yeast, plant, insect, fish, vertebrate, mammalian cell or tissue, etc.).

[0410] Cloning vectors are generally used to modify, engineer, and/or duplicate (*e.g.*, by replication *in vivo,* for example in a simple system such as bacteria or yeast, or *in vitro*, such as by amplification such as polymerase chain reaction or other amplification process). In some embodiments, a cloning vector may lack expression signals.

[0411] In many embodiments, a vector may include replication elements such as primer binding site(s) and/or origin(s) of replication. In many embodiments, a vector may include insertion or modification sites such as restriction endonuclease recognition sites and/or guide RNA binding sites, etc.

[0412] In some embodiments, a vector is a viral vector (*e.g.*, an AAV vector). In some embodiments, a vector is a non-viral vector. In some embodiments, a vector is a plasmid.

[0413] Those skilled in the art are aware of a variety of technologies useful for the production of recombinant polynucleotides (*e.g.*, DNA or RNA) as described herein. For example, restriction digestion,

reverse transcription, amplification (*e.g.*, by polymerase chain reaction), Gibson assembly, etc., are well established and useful tools and technologies. Alternatively or additionally, certain nucleic acids may be prepared or assembled by chemical and/or enzymatic synthesis. In some embodiments, a combination of known methods is utilized to prepare a recombinant polynucleotide.

[0414] In some embodiments, polynucleotide(s) of the present disclosure are included in a DNA construct (*e.g.*, a vector) amenable to transcription and/or translation.

[0415] In some embodiments, an expression vector comprises a polynucleotide that encodes proteins and/or polypeptides of the present disclosure operatively linked to a sequence or sequences that control expression (*e.g.*, promoters, start signals, stop signals, polyadenylation signals, activators, repressors, *etc.*). In some embodiments, a sequence or sequences that control expression are selected to achieve a desired level of expression. In some embodiments, more than one sequence that controls expression (*e.g.*, promoters) are utilized. In some embodiments, more than one sequence that controls expression (*e.g.*, promoters) are utilized to achieve a desired level of expression of a plurality of polynucleotides that encode a plurality proteins and/or polypeptides. In some embodiments, a plurality of recombinant proteins and/or polypeptides are expressed from the same vector (*e.g.*, a bi-cistronic vector, a tri-cistronic vector, multi-cistronic). In some embodiments, a plurality of polypeptides are expressed, each of which is expressed from a separate vector.

[0416] In some embodiments, an expression vector comprising a polynucleotide of the present disclosure is used to produce a RNA and/or protein and/or polypeptide in a host cell. In some embodiments, a host cell may be *in vitro* (*e.g.*, a cell line) – for example a cell or cell line (*e.g.*, Human Embryonic Kidney (HEK cells), Chinese Hamster Ovary cells, *etc.*) suitable for producing polynucleotides of the present disclosure and proteins and/or polypeptides encoded by said polynucleotides.

[0417] In some embodiments, an expression vector is an RNA expression vector. In some embodiments, an RNA expression vector comprises a polynucleotide template used to produce a RNA in cell-free enzymatic mix. In some embodiments, an RNA expression vector comprising a polynucleotide template is enzymatically linearized prior to in vitro transcription. In some embodiments, a polynucleotide template is generated through PCR as a linear polynucleotide template. In some embodiments, a linearized polynucleotide is mixed with enzymes suitable for RNA synthesis, RNA

capping and/or purification. In some embodiments, the resulting RNA is suitable for producing proteins encoded by the RNA.

[0418] A variety of methods are known in the art to introduce an expression vector into host cells. In some embodiments, a vector may be introduced into host cells using transfection. In some embodiments, transfection is completed, for example, using calcium phosphate transfection, lipofection, or polyethylenimine-mediated transfection. In some embodiments, a vector may be introduced into a host cell using transduction.

[0419] In some embodiments, transformed host cells are cultured following introduction of a vector into a host cell to allow for expression of said recombinant polynucleotides. In some embodiments, a transformed host cells are cultured for at least 12 hours, 16 hours, 20 hours, 24 hours, 28 hours, 32 hours, 36 hours 40 hours, 44 hours, 48 hours, 52 hours, 56 hours, 60 hours, 64 hours, 68 hours, 72 hours or longer. Transformed host cells are cultured in growth conditions (e.g., temperature, carbon-dioxide levels, growth medium) in accordance with the requirements of a host cell selected. A skilled artisan would recognize culture conditions for host cells selected are well known in the art.

EXAMPLES

[0420] The disclosure is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the disclosure in any way.

Example 1: In-vitro Expression of Example Polyribonucleotides Encoding HSV gB Antigen Constructs

[0421] The present Example demonstrates that exemplary polyribonucleotides encoding different HSV gB antigen constructs, as described herein, can be assessed for *in-vitro* expression (e.g., intracellular, surface) in mammalian cells (HEK293T cells).

[0422] *In vitro* expression assays are used to assess expression and localization of different HSV gB antigen constructs. Polynucleotides encoding various HSV gB antigen constructs provided herein are generated. Assays are initially performed with non-formulated DNA constructs to determine functionality. HEK293T cells are transfected with DNA constructs. HEK293T cells transfected with DNA constructs are assessed for protein expression by antibody staining and FACS. Expression from the DNA constructs is also assessed by Western blot and/or mass spectrometry. Antigenicity of polypeptides

expressed from DNA constructs is assessed by antibody binding assays. Purified protein is also used to assess antigenicity.

[0423] Based on results from assays using DNA constructs, corresponding RNA constructs are selected for further assessment. *In vitro* expression assays are used to assess expression and localization of polyribonucleotides encoding HSV gB antigen constructs. Polyribonucleotides encoding various HSV gB antigen constructs provided herein are generated. Assays are initially performed with non-formulated RNA constructs to determine functionality. Formulated RNA constructs are also assessed. HEK293T cells are transfected with (i) RNA constructs or (ii) LNP formulated RNA constructs. HEK293T cells transfected with RNA constructs or with formulated RNA constructs are assessed for protein expression by antibody staining and FACS. A transfection rate is determined by measuring percentage of positive cells, and total expression is determined by measuring median fluorescence of the total HEK population. HEK293T cells transfected with formulated RNA constructs are assessed for protein secretion by detecting protein in the culture supernatant of transfected cells.

Example 2: Immunogenicity Studies of Example Polyribonucleotides Encoding HSV gB antigen Constructs

[0424] The present Example describes the ability of certain polyribonucleotides encoding HSV gB antigen constructs, provided by the present disclosure, to induce immune responses, as assessed in mice.

[0425] C57BL6 female mice (10-12-weeks old) are immunized intramuscularly (IM) twice, generally on days 0 and 21, with a formulated RNA construct (described in Example 1) or injected with phosphate buffer saline (vehicle). Blood samples are collected pre-immunization (day 0) and after the first dose (on days 7, 14, 21, 28 and 35) to generate serum samples at various time points. At the end of the experiment (e.g., day 35), splenocytes are harvested and cryopreserved. Animals are divided into multiple groups receiving treatment as indicated in **Table 12** below:

Table 12 – Study plan for certain example immunogenicity studies with RNA constructs.

Group	Treatment	Example Dose	D0	D7,14,21	D21	D28	D35
1	Buffer (NaCl)	-		BC	IM	BC	

2	F1	1 µg	Blood collection (BC)* Intramuscular (IM) injection (20 µL in one leg)				Final Blood/Spleen Collection (FBC)
3	F2	1 µg					
4	F3	1 µg					
5	F4	1 µg					

F1, F2, F3, F4 = LNP formulations comprising RNA construct with modified nucleotides

BC = Blood collection / Serum generation (amount of blood that can be collected: 14 days interval between collections: 150µl/20g mouse; 7 days interval between collections: 110µl/20g mouse)

* pre-immune serum: blood sample is collected from 10% of all animals = 4 animals.

FBC = Final blood collection/ Serum generation; Spleen, serum or cell collection (for, e.g., Fluorospot analysis)

[0426] Serum samples obtained from each group of immunized animals are analyzed by one or more of the following method(s): (1) Enzyme-linked Immunosorbent Assay (ELISA), (2) multiplex assay, and/or (3) Fluorospot assay.

(1) Enzyme-linked Immunosorbent Assay (ELISA)

[0427] Provided polyribonucleotides can be assessed for their ability to induce production of antibodies that may bind to an HSV gB antigen or antigenic fragment thereof. In some embodiments, a provided polyribonucleotide is determined to induce a useful immune response if serum from a subject (e.g., a mouse) immunized with such construct is shown to bind an HSV gB antigen or antigenic fragment thereof.

[0428] RNA constructs (as described in Example 1) are assessed for their ability to induce production of antibodies that bind to an HSV gB antigen or antigenic fragment thereof using an ELISA assay.

[0429] Briefly, MaxiSorp 96-well plates are coated with 100 ng/well an HSV gB antigen or antigenic fragment thereof in coating buffer (50mM sodium carbonate, pH 9.6) and incubated overnight at 4°C, or 250 ng/well of an HSV gB antigen overlapping peptides in PBS and incubated 1h at 37°C. Plates are then blocked with 1% BSA in PBS for 1h at 37°C (for an HSV gB antigen or antigenic fragment

thereof) or overnight at 4°C (for an HSV gB antigen or antigenic fragment thereof overlapping peptides). Bound IgG is detected using horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG. Signal is detected after adding the substrate 3,3',5,5'-Tetramethylbenzidine (TMB) and 25% sulfuric acid to stop the reaction. Optical densities (OD) are read at 450 nm.

[0430] Reciprocal end titers at day 35, after 2 immunizations, are used as a representative as they showed the highest antibody response.

(2) Multiplex Assay

[0431] Provided polyribonucleotides can be assessed for their ability to induce production of antibodies that bind to specific HSV gB antigens or antigenic fragments thereof. In some embodiments, a provided polyribonucleotide is determined to induce a useful immune response if serum from a subject (e.g., a mouse) immunized with such construct is shown to target peptides from an HSV gB antigen or antigenic fragment thereof in a multiplex assay, as described herein.

[0432] RNA constructs (as described in Example 1) are assessed for their ability to induce production of antibodies that bind to specific HSV gB antigens or antigenic fragments thereof by performing a multiplex analysis (Meso Scale Discovery) according to the manufacturer's instructions. Briefly, HSV gB antigens or antigenic fragments thereof are conjugated with bovine serum albumin (BSA) and then bound to the wells of a 96-well plate, in a specific spot on the well. After incubation with serum from immunized mice, antibodies bound to each specific peptide are detected with a "Sulfo-Tag" conjugated secondary antibody. A multiplex reader instrument (MESO QuickPlex SQ 120) is used to quantify the light emitted from the Sulfo-Tag.

(3) FluoroSpot Assay

[0433] Provided polyribonucleotides can be assessed for their ability to induce production of antibodies responsive to an HSV gB antigen or antigenic fragment thereof. In some embodiments, a polyribonucleotide is determined to induce a useful immune response if splenocytes from a subject (e.g., a mouse) immunized with such construct, following incubation with peptide(s) as described herein, exhibit T-cell secretion of one or more pro-inflammatory cytokines (e.g., IFN-γ, TNF-α, or IL-2) in a FluoroSpot Assay, as described herein. FluoroSpot assays are performed with mouse IFN-γ/IL-2/TNF-α FluoroSpot^{PLUS} kit according to the manufacturer's instructions (Mabtech).

Example 3: Protection Studies of Example Polyribonucleotides Encoding HSV gB Antigen Constructs

[0434] The present Example documents the ability of certain polyribonucleotides, provided by the present disclosure, to induce immune responses, as assessed in mice.

[0435] Provided polyribonucleotides can be assessed for their ability to protect a subject from HSV challenge. In some embodiments, a provided polyribonucleotide is determined to induce a useful immune response if a subject (e.g., a mouse) immunized with a polyribonucleotide and exposed to HSV demonstrate a reduced level of infection in a challenge assay as described herein.

[0436] A challenge assay is performed in which C57BL/6 female mice (7-week-old, ~20 g at day 0) were immunized intramuscularly (IM) twice, on days 0 and 21, with 1 μg of a RNA construct (as described in Example 1) or injected with vehicle (n=7 mice/group). Blood samples are collected on days 7, 14, 20, 28, 35, 42, and 49 for analysis of antibody titers and functionality in serum. HSV challenge was performed at day 50. Protection against infection was assessed at experimental day 60 (day 10 after challenge).

[0437] Antibody titers are assessed by ELISA. Pre-boost and pre-challenge samples (days 20 and 49) are also used in functionality assays.

EQUIVALENTS

[0438] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A polyribonucleotide encoding a polypeptide, wherein the polypeptide comprises one or more HSV glycoprotein B (gB) antigens or antigenic fragments thereof.

2. The polyribonucleotide of claim 1, wherein the HSV gB antigen or antigenic fragment thereof comprises one or more mutations that stabilize the HSV gB antigen or antigenic fragment thereof relative to a comparable HSV gB antigen or antigenic fragment thereof that does not comprise the one or more mutations.

3. The polyribonucleotide of claim 2, wherein the one or more mutations are one or more amino acid substitutions.

4. The polyribonucleotide of claim 3, wherein the one or more amino acid substitutions comprise 251C, 718C, and combinations thereof, wherein the numbering is with reference to SEQ ID NO: 1.

5. The polyribonucleotide of claim 1, wherein the polypeptide comprises an HSV glycoprotein B (gB) antigen.

6. The polyribonucleotide of claim 5, wherein the HSV glycoprotein B (gB) antigen comprises one or more mutations that stabilize the HSV gB antigen relative to a comparable HSV gB antigen that does not comprise the one or more mutations.

7. The polyribonucleotide of claim 6, wherein the one or more mutations are one or more amino acid substitutions.

8. The polyribonucleotide of claim 7, wherein the one or more amino acid substitutions comprise 251C, 718C, and combinations thereof, wherein the numbering is with reference to SEQ ID NO: 1.

9. The polyribonucleotide of any one of claims 6-8, wherein the one or more mutations comprise 251C and 718C, wherein the numbering is with reference to SEQ ID NO:1.

10. The polyribonucleotide of any one of claims 1-9, wherein the polypeptide comprises a secretory signal.

11. The polyribonucleotide of claim 10, wherein the secretory signal comprises or consists of a viral secretory signal.

12. The polyribonucleotide of claim 11, wherein the viral secretory signal comprises or consists of an HSV secretory signal.

13. The polyribonucleotide of claim 12, wherein the HSV secretory signal comprises or consists of an HSV-1 or HSV-2 secretory signal.

14. The polyribonucleotide of claim 13, wherein the HSV secretory signal comprises or consists of an HSV glycoprotein B (gB) secretory signal.

15. The polyribonucleotide of claim 14, wherein the HSV gB secretory signal comprises or consists of an amino acid sequence according to SEQ ID NO: 63.

16. The polyribonucleotide of claim 14, wherein the HSV gB secretory signal comprises or consists of an amino acid sequence according to SEQ ID NO: 64.

17. The polyribonucleotide of claim 14, wherein the HSV gB secretory signal comprises or consists of an amino acid sequence according to SEQ ID NO: 65.

18. The polyribonucleotide of claim 14, wherein the HSV gB secretory signal comprises or consists of an amino acid sequence according to SEQ ID NO: 69.

19. The polyribonucleotide of claim 10, wherein the secretory signal comprises or consists of an amino acid sequence according to SEQ ID NO: 70.

20. The polyribonucleotide of claim 14, wherein the polyribonucleotide comprises a nucleotide sequence according to SEQ ID NO: 66.

21. The polyribonucleotide of claim 14, wherein the polyribonucleotide comprises a nucleotide sequence according to SEQ ID NO: 71.

22. The polyribonucleotide of claim 10, wherein the polyribonucleotide comprises a nucleotide sequence according to SEQ ID NO: 72.

23. The polyribonucleotide of any one of claims 10-22, wherein the secretory signal is located at the N-terminus of the polypeptide.

24. The polyribonucleotide of any one of claims 1-23, wherein the polypeptide comprises a transmembrane region.

25. The polyribonucleotide of claim 24, wherein the transmembrane region comprises or consists of a viral transmembrane region.

26. The polyribonucleotide of claim 25, wherein the transmembrane region comprises or consists of an HSV transmembrane region.

27. The polyribonucleotide of claim 26, wherein the HSV transmembrane region comprises or consists of an HSV-1 or HSV-2 transmembrane region.

28. The polyribonucleotide of claim 27, wherein the HSV transmembrane region comprises or consists of an HSV gB transmembrane region.

29. The polyribonucleotide of claim 28, wherein the HSV gB transmembrane region consists of an amino acid sequence according to SEQ ID NO: 67.

30. The polyribonucleotide of claim 28, wherein the HSV gB transmembrane region consists of an amino acid sequence according to SEQ ID NO: 68.

31. The polyribonucleotide of any one of claims 1-23, wherein the polypeptide does not comprise a transmembrane region.

32. The polyribonucleotide of any one of claims 1-31, wherein the polypeptide comprises a multimerization domain.

33. The polyribonucleotide of any one of claims 1-32, wherein the polypeptide comprises one or more linkers.

34. The polyribonucleotide of claim 33, wherein the one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 45.

35. The polyribonucleotide of claim 33, wherein the one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 47.

36. The polyribonucleotide of claim 33, wherein the one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 48.

37. The polyribonucleotide of claim 33, wherein the one or more linkers comprise or consist of an amino acid sequence according to SEQ ID NO: 52.

38. The polyribonucleotide of any one of claims 1-37, wherein the polyribonucleotide is an isolated polyribonucleotide.

39. The polyribonucleotide of any one of claims 1-37, wherein the polyribonucleotide is an engineered polyribonucleotide.

40. The polyribonucleotide of any one of claims 1-37, wherein the polyribonucleotide is a codon-optimized polyribonucleotide.

41. An RNA construct comprising in 5' to 3' order:

(i) a 5' UTR;

(ii) a polyribonucleotide of any one of claims 1-40;

(iii) a 3' UTR; and

(iv) a polyA tail sequence.

42. The RNA construct of claim 41, wherein

(i) the 5' UTR comprises or consists of a modified human alpha-globin 5'-UTR; and/or

(ii) the 3' UTR that comprises or consists of a first sequence from the amino terminal enhancer of split (AES) messenger RNA and a second sequence from the mitochondrial encoded 12S ribosomal RNA.

43. The RNA construct of claim 41 or 42, wherein the 5' UTR consists of a ribonucleic acid sequence according to SEQ ID NO: 57.

44. The RNA construct of any one of claims 41-43, wherein the 3' UTR consists of a ribonucleic acid sequence according to SEQ ID NO: 63.

45. The RNA construct of any one of claims 41-44, wherein the polyA tail sequence is a split polyA tail sequence.

46 The RNA construct of claim 45, wherein the split polyA tail sequence consists of a ribonucleic acid sequence according to SEQ ID NO: 60.

47. The RNA construct of any one of claims 41-46, further comprising a 5' cap.

48. The RNA construct of claim 47, further comprising a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the polyribonucleotide.

49. The RNA construct of claim 47 or 48, wherein the 5' cap comprises or consists of m7(3'OMeG)(5')ppp(5')(2'OMeA_1)pG_2, wherein A_1 is position +1 of the polyribonucleotide, and G_2 is position +2 of the polyribonucleotide.

50. The RNA construct of claim 48 or 49, wherein the cap proximal sequence comprises A_1 and G_2 of the Cap1 structure, and a sequence comprising: $A_3A_4U_5$ (SEQ ID NO: 55) at positions +3, +4 and +5 respectively of the polyribonucleotide.

51. The RNA construct of any one of claims 41-50, wherein the polyribonucleotide includes modified uridines in place of all uridines, optionally wherein modified uridines are each N1-methyl-pseudouridine.

52. A composition comprising one or more polyribonucleotides of any one of claims 1-40.

53. A composition comprising one or more RNA constructs of any one of claims 41-51.

54. The composition of claim 52 or 53, wherein the composition further comprises lipid nanoparticles, polyplexes (PLX), lipidated polyplexes (LPLX), or liposomes,

wherein the one or more polyribonucleotides are fully or partially encapsulated within the lipid nanoparticles, polyplexes (PLX), lipidated polyplexes (LPLX), or liposomes.

55. The composition of any one of claims 52-54, wherein the composition further comprises lipid nanoparticles,

wherein the one or more polyribonucleotides are encapsulated within the lipid nanoparticles.

56. A pharmaceutical composition comprising the composition of any one of claims 52-55 and at least one pharmaceutically acceptable excipient.

57. The pharmaceutical composition of claim 56, wherein the pharmaceutical comprises a cryoprotectant, optionally wherein the cryoprotectant is sucrose.

58. The pharmaceutical composition of claim 56 or 57, wherein the pharmaceutical comprises an aqueous buffered solution, optionally wherein the aqueous buffered solution comprises one or more of Tris base, Tris HCl, NaCl, KCl, Na_2HPO_4, and KH_2PO_4.

59. A combination comprising:

(i) a first pharmaceutical composition comprising a first polyribonucleotide, wherein the first polyribonucleotide encodes a polypeptide of any one of claims 1-40; and

(ii) a second pharmaceutical composition comprising a second polyribonucleotide, wherein the second polyribonucleotide encodes a second polypeptide, the second polypeptide comprises one or more HSV antigens, and wherein at least one of the HSV antigens is not gB.

60. The combination of claim 59, wherein the one or more HSV antigens comprise:

(i) an HSV glycoprotein C,

(ii) an HSV glycoprotein D,

(iii) an HSV glycoprotein E,

(iv) an HSV glycoprotein B, or

(v) a combination thereof.

61. The combination of claim 59 or 60, wherein at least one of the HSV antigens is gB.

62. The combination of any one of claims 59-61, wherein the one or more HSV antigens comprise one or more HSV T cell antigens.

63. A method comprising administering a polyribonucleotide according to any one of claims 1-40 to a subject.

64. A method comprising administering an RNA construct according to any one of claims 41-51 to a subject.

65. A method comprising administering a composition according to any one of claims 52-55 to a subject.

66. A method comprising administering one or more doses of the pharmaceutical composition of any one of claims 56-58 to a subject.

67. The pharmaceutical composition of any one of claims 56-58 for use in the treatment of an HSV infection comprising administering one or more doses of the pharmaceutical composition to a subject.

68. The pharmaceutical composition of any one of claims 56-58 for use in the prevention of an HSV infection comprising administering one or more doses of the pharmaceutical composition to a subject.

69. The method of any one of claims 59-62 or the pharmaceutical composition for use of claim 67 or 68, comprising administering two or more doses of the pharmaceutical composition to a subject.

70. The method of any one of claims 63-66 or the pharmaceutical composition for use of claim 63 or 64, comprising administering three or more doses of the pharmaceutical composition to a subject.

71. A method comprising administering a combination of any one of claims 59-62 to a subject.

72. The method of claim 71, wherein the first pharmaceutical composition and the second pharmaceutical composition are administered on the same day.

73. The method of claim 71 or 72, wherein the first pharmaceutical composition and the second pharmaceutical composition are administered on different days.

74. The method of any one of claims 71-73, wherein the first pharmaceutical composition and the second pharmaceutical composition are administered to the subject at different locations on the subject's body.

75. The method of any one of claims 63-66 and 69-74, wherein the method is a method of treating an HSV infection.

76. The method of any one of claims 63-66 and 69-74, wherein the method is a method of preventing an HSV infection.

77. The method of any one of claims 63-66 and 69-73, wherein the subject has or is at risk of developing an HSV infection.

78. The method of any one of claims 63-66 and 69-77, wherein the subject is a human.

79. The method of any one of claims 63-66 and 69-78, wherein administration induces an anti-HSV immune response in the subject.

80. The method of claim 79, wherein the anti-HSV immune response in the subject comprises an adaptive immune response.

81. The method of claim 79 or 80, wherein the anti-HSV immune response in the subject comprises a T-cell response.

82. The method of claim 81, wherein the T-cell response is or comprises a CD4+ T cell response.

83. The method of claim 81, wherein the T-cell response is or comprises a CD8+ T cell response.

84. The method of any one of claims 79-83, wherein the anti-HSV immune system response comprises a B-cell response.

85. The method of any one of claims 79-84, wherein the anti-HSV immune system response comprises the production of antibodies directed against the one or more HSV gB antigens.

86. Use of the pharmaceutical composition of any one of claims 56-58 in the treatment of an HSV infection.

87. Use of the pharmaceutical composition of any one of claims 56-58 in the prevention of an HSV infection.

88. Use of the pharmaceutical composition of any one of claims 56-58 in inducing an anti-HSV immune response in a subject.

89. A polypeptide encoded by a polyribonucleotide of any one of claims 1-40.

90. A polypeptide encoded by an RNA construct of any one of claims 41-51.

91. A host cell comprising a polyribonucleotide of any one of claims 1-40.

92. A host cell comprising an RNA construct of any one of claims 41-51.

93. A host cell comprising a polypeptide of claim 89 or 90.

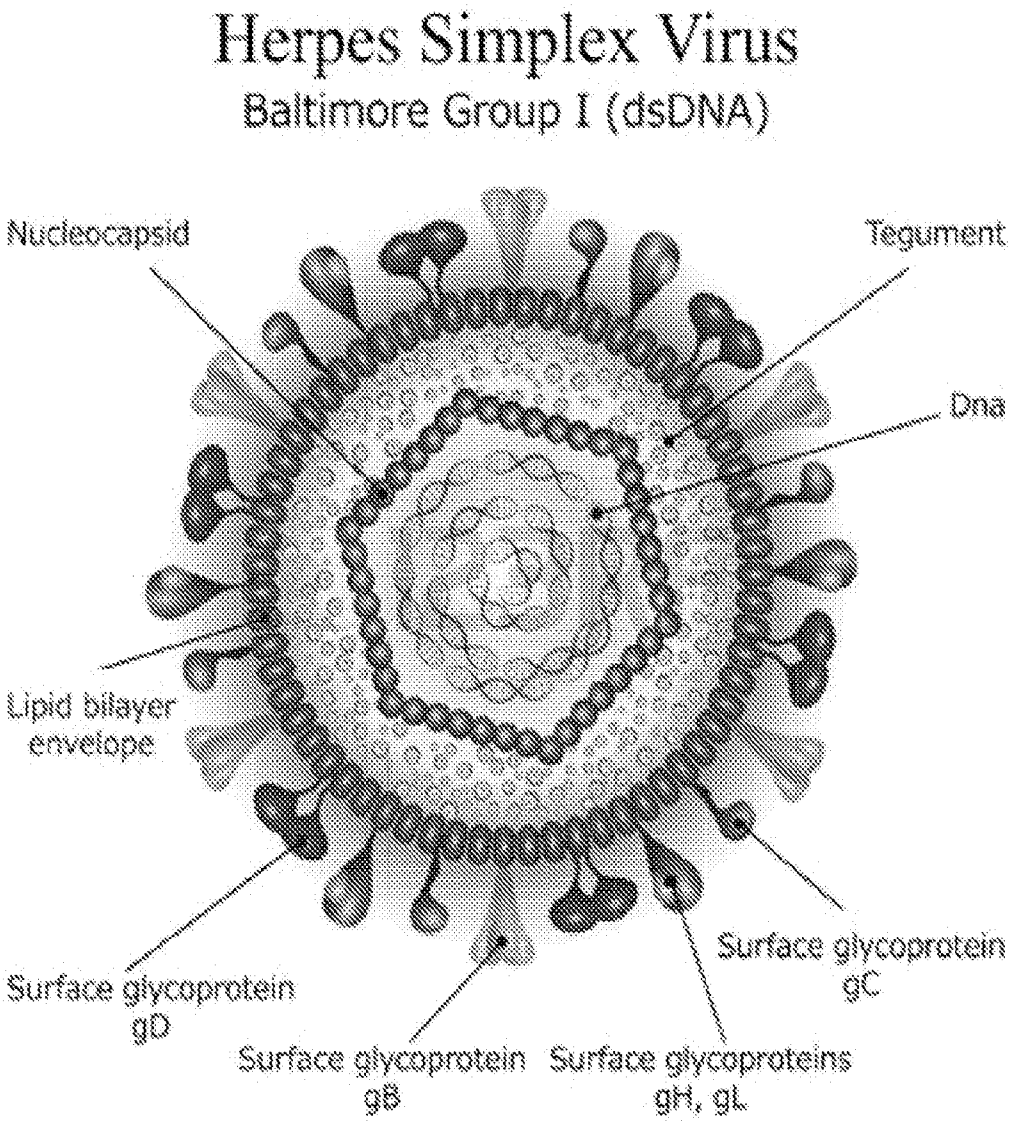

Herpes Simplex Virus
Baltimore Group I (dsDNA)
Nucleocapsid
Tegument
Dna
Lipid bilayer envelope
Surface glycoprotein gD
Surface glycoprotein gB
Surface glycoproteins gH, gL
Surface glycoprotein gC

FIG. 1

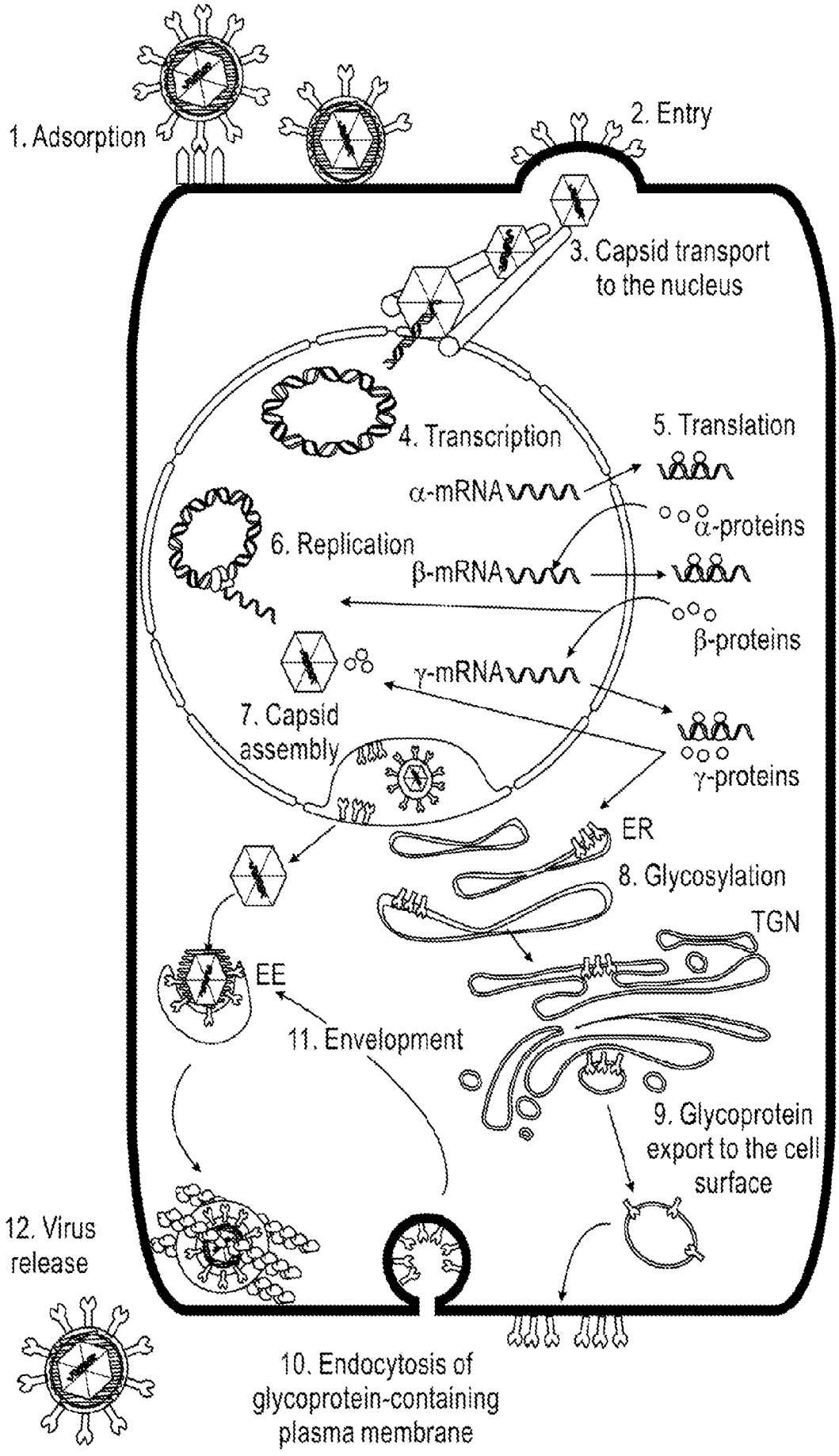

1. Adsorption
2. Entry
3. Capsid transport to the nucleus
4. Transcription
5. Translation
α-mRNA
α-proteins
6. Replication
β-mRNA
β-proteins
γ-mRNA
7. Capsid assembly
γ-proteins
ER
8. Glycosylation
TGN
EE
11. Envelopment
9. Glycoprotein export to the cell surface
12. Virus release
10. Endocytosis of glycoprotein-containing plasma membrane

FIG. 2

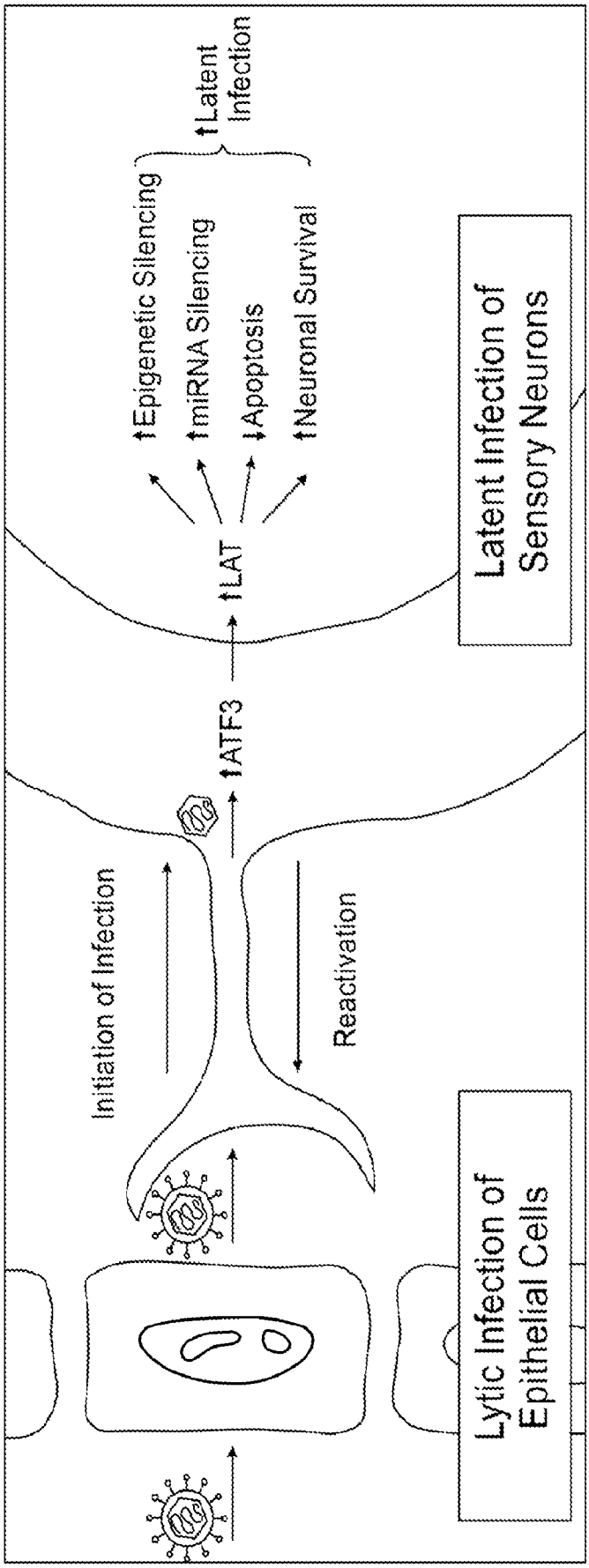

Lytic Infection of Epithelial Cells
Initiation of Infection
Reactivation
↑ATF3
↑LAT
Latent Infection of Sensory Neurons
↑Epigenetic Silencing
↑miRNA Silencing
↓Apoptosis
↑Neuronal Survival
↑Latent Infection

FIG. 3

Name	Platform	Antigens	Immune Response	Clinical results
gB/gD/MF59 (Chiron)	Subunit MF59 adjuvant	gD and gB	**Neutralizing antibodies (Abs)**	Phase 3 Prophylaxis **9% (95% CI: -29%, 36%)**
gD/AS04 (GSK)	Subunit AS04 adjuvant	gD	**Neutralizing Abs** $CD4^+$ T cells	Phase 3 Prophylaxis **20% (95% CI: -29%, 50%)**
gH-null (Cantab Pharmaceutical)	Single-cycle live	Multiple	Neutralizing Abs	Phase 3 therapeutic No difference in recurrences or shedding
HSV529 (Sanofi-Pasteur)	Replication-defective	Multiple	Neutralizing Abs $CD4^+$, $CD4^+$ T cells	Phase 1 Safe but immunogenic only in doubly seronegatives
Gen-003 (Genocea)	Subunit Saponin adjuvant	gD, ICP4	Neutralizing Abs Cytolytic T cells	Phase 2 therapeutic Dose variable decrease shedding
HerpV (Agenus)	Heat shock protein 70-HSV peptides QS-21 adjuvant	32 HSV peptides (gD, additional envelope, tegument, and other)	Cytotoxic T cells	Phase 2 therapeutic 17% reduction in shedding frequency
Vaxfectin (Vical)	DNA Vaxfectin adjuvant	gD, UL46/47	Neutralizing Abs	Phase 1/2 therapeutic Failed to reduce shedding
COR-1 (Admedus)	Codon optimized DNA	Codon optimized gD2 and ubiquitinfused truncated gD2 to target the antigen to the proteasome	gD-specific Abs Cytotoxic T cells	Phase 2 therapeutic No difference in recurrences
ΔNLS (Rational Vaccines)	Deleted in ICP0 Replication competent Attenuated for latency	Multiple targets	Neutralizing Abs gD-specific Abs	Phase 1 non-FDA Approved therapeutic study Self-reported reduction in recurrences

Aschner CB and Herold B, Curr. Issues Mol. Bio.2021

FIG. 4

Name	Platform	Antigens	Immune Response	Clinical results
THV	Trivalent subunit	gD, gC, gE	Neutralizing antibodies (Abs)	Prophylactic in mice and guinea pigs Decreased shedding in guinea pigs
ΔgD-2	gD null Single cycle	Multiple targets excluding gD	FcγR-activating Abs $CD4^+$, $CD4^+$ T cell responses Passive protection	100% protection against HSV-1 and HSV-2 clinical isolates in male and female mice with multiple challenge routes; Prevents establishment of latency Passive protection
gD27	gD-nectin binding impaired; Live attenuated	Multiple	Neutralizing Abs	Improved protection compared to gD-2 subunit
VC2	Deleted in gK and UL20 Defective in establishing latency; replication competent	Multiple	Neutralizing Abs T cell memory (gB, gD) T_{FH} and Th17 responses	100 % protection in mice intravaginally challenged with HSV-1(McKrae) or HSV-2(G) Prevents latency

Aschner CB and Herold B, Curr. Issues Mol. Bio.2021

FIG. 4 (continued)

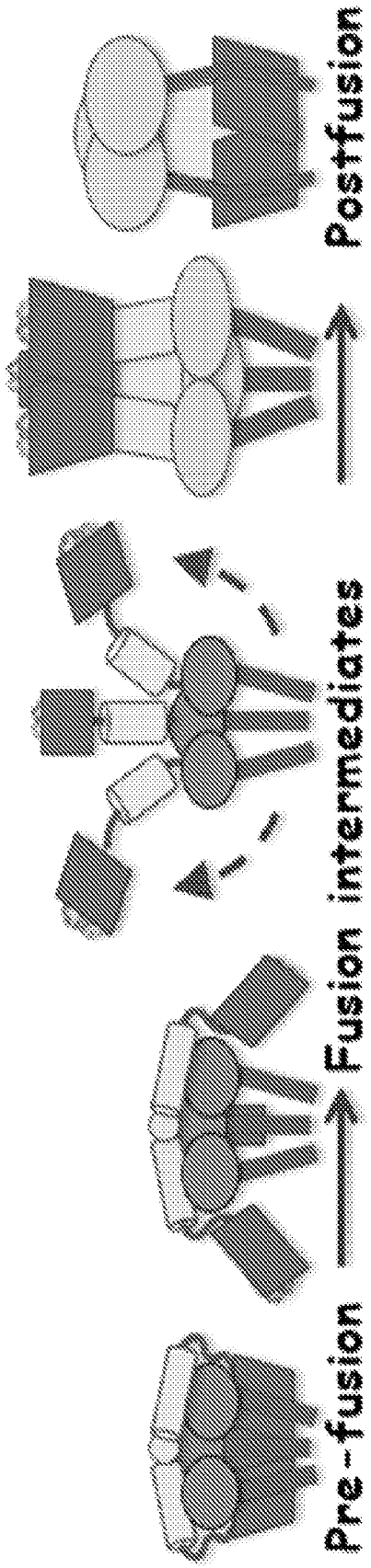


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/040844

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 A61P31/22
ADD.

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)
A61K C07K A61P C12N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VOLLMER B. ET AL: "The prefusion structure of herpes simplex virus glycoprotein B", SCIENCE ADVANCES, vol. 6, no. 39, 25 September 2020 (2020-09-25), XP093223159, US ISSN: 2375-2548, DOI: 10.1126/sciadv.abc1726	1-3,5-7, 10-14, 23-28, 32,33, 38-41, 52,53, 56-58, 89-93
Y	*** page 3 left col., Figures 3, 4 ***	4,8,9, 15-22, 29-31, 34-37, 42-51, 54,55, 59-88

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

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 November 2024	28/11/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Heder, Andreas

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHONG LING ET AL: "Targeting herpesvirus entry complex and fusogen glycoproteins with prophylactic and therapeutic agents", TRENDS IN MICROBIOLOGY, ELSEVIER SCIENCE LTD., KIDLINGTON, GB, vol. 31, no. 8, 24 March 2023 (2023-03-24) , pages 788-804, XP087356265, ISSN: 0966-842X, DOI: 10.1016/J.TIM.2023.03.001 [retrieved on 2023-03-24]	1-3,5-7, 10-14, 23-28, 32,33, 38-41, 52,53, 56-58, 89-93
Y	*** Figure 3A, page 795 4th para., ref. 69 ***	4,8,9, 15-22, 29-31, 34-37, 42-51, 54,55, 59-88

A	M. MAGRO ET AL: "Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer unique possibilities for clinical intervention", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 109, no. 8, 21 February 2012 (2012-02-21), pages 3089-3094, XP055067859, ISSN: 0027-8424, DOI: 10.1073/pnas.1115941109 *** page 3091 - 3092 left col., page 3094 left col., Figs. 4, 5 ***	1-93

A	NORAIS N ET AL: "Disulfide bonds of herpes simplex virus type 2 glycoprotein gB", JOURNAL OF VIROLOGY, vol. 70, no. 11, 1 November 1996 (1996-11-01), pages 7379-7387, XP093222873, US ISSN: 0022-538X, DOI: 10.1128/jvi.70.11.7379-7387.1996	1-93

Y	WO 2018/115527 A2 (CUREVAC AG [DE]) 28 June 2018 (2018-06-28) *** para. 148-150, 166, 227, 343, 351, 372-374, 382, 407-456, 584-586, Table XI ***	4,8,9, 15-22, 29-31, 34-37, 42-51, 54,55, 59-88

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/040844

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018115527 A2	28-06-2018	EP 3558356 A2	30-10-2019
		US 2019351048 A1	21-11-2019
		US 2021401971 A1	30-12-2021
		US 2024091344 A1	21-03-2024
		WO 2018115527 A2	28-06-2018