The compositions and methods are described for generating an immune response to a flavivirus such as Zika virus. The compositions and methods described herein relate to a modified vaccinia Ankara (MVA) vector encoding one or more viral antigens for generating a protective immune response to a member of genus Flavivirus (such as a member of species Zika virus), in the subject to which the vector is administered. The compositions and methods of the present invention are useful both prophylactically and therapeutically and may be used to prevent and/or treat an infection caused by Flavivirus.

Specification includes a Sequence Listing.
FIG. 1

Positions are given in kilobase pairs in the MVA genome. For clarity and brevity, diagram is not to scale.
FIG. 3

pGeo-LSN1
6002 bp
FIG. 5

pGZ-02
7118 bp
FIG. 8

Expression of the Full-length ZIKV E and NS1 Proteins. 1. MVA-prME or MVA-NS1. 2. MVA

Western blots: DF1 chicken fibroblasts cells were co-transfected with MVA and plasmids expressing prME or NS1 genes of ZIKV strain Surniname 2015. Blots performed with anti-ZIKV-E-protein mouse monoclonal antibody (Jg51) from Abaxis, Catalog # AZ1176 and anti-ZIKV-NS1-protein mouse monoclonal antibody (Jg01) from Abaxis, Catalog # AZ1225.
FIG. 9

EM of MVA-Zika prME infected cells: Multi-nemellar Structures Containing Zika VLPs

MVA-Zika prME virus

Zika prME VLPs
COMPOSITIONS AND METHODS FOR GENERATING AN IMMUNE RESPONSE TO A FLAVIVIRUS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. 62/290, 744 filed Feb. 3, 2016, the contents of which are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] The present invention is directed to compositions, including vaccine compositions, for generating an immune response to a Flavivirus such as a Zika virus, as well as methods of manufacture and use thereof. More specifically, the compositions and methods described herein relate to a modified vaccinia Ankara (MVA) vector encoding one or more viral antigens for generating a protective immune response to a member of the Flaviviridae family in a subject to which the vector is administered. The compositions and methods of the present invention are useful both prophylactically and therapeutically.

BACKGROUND OF THE INVENTION

[0003] Zika virus disease ("Zika") is an emerging infectious disease. It was first isolated in 1947 in Uganda, but until 2007 was known only to cause small outbreaks of minor public health significance (Enfissi, A., et al., The Lancet 387, 2 (2016)). Large epidemics occurred in 2007 in Yap Island in Micronesia and in 2013 in French Micronesia, raising the profile of Zika as an emerging disease (Oehler, E., et al. Eurosurveillance 19, 3 (2014) Zanluca, C., et al. Mem Inst Oswaldo Cruz 110, 4 (2015)). Beginning in 2015, with the appearance of the infection in Brazil, it became clear that Zika is a serious threat potentially capable of causing a pandemic (Zanluca, C., et al. Mem Inst Oswaldo Cruz 110, 4 (2015); Fauci, A.S. & Morens, D. M. New England Journal of Medicine (2016)). Today, Zika continues to spread with multiple cases imported into the US by travelers from endemic regions. This rapid increase in transmission has prompted the world’s public health authorities to mobilize quickly to control the epidemic. However, there is currently no treatment or vaccine available to fight the epidemic (CDC. Zika virus. Vol. 2016 (US Department of Health and Human Services, CDC, Atlanta, Ga., 2016)).

[0004] The etiologic agent of Zika is Zika virus (ZIKV), a member of the Flaviviridae family which also includes dengue fever, yellow fever, Japanese encephalitis, tick-borne encephalitis and West Nile viruses (Zanluca, C., et al. Mem Inst Oswaldo Cruz 110, 4 (2015)). ZIKV is transmitted primarily through bites from infected Aedes mosquitoes, but human-to-human sexual transmission may also occur (CDC. Zika virus. Vol. 2016 (US Department of Health and Human Services, CDC, Atlanta, Ga., 2016) Musso, D., et al. Emerg Infect Dis 21, 3 (2015)). Phylogenetic analyses of ZIKV demonstrate that this Flavivirus consists of one serotype and 2 major lineages: African and Asian, which are >96% identical in amino acid sequences across the genome. The Asian lineage has been responsible for all ZIKV outbreaks in the Pacific and the Americas. A vaccine composed of sequences from either lineage should theoretically protect against all Zika viruses. Monkeys are believed to be the animal reservoir, and humans are occasional/accidental hosts (Faye, O., et al. PLoS neglected tropical diseases 8, 10 (2014)).

[0005] ZIKV infection is asymptomatic in approximately 80% of cases (Petersen, E. M., et al. MMWR Morb Mortal Wkly Rep 65, 4 (2016)). Symptoms are generally mild, usually last no more than a week, and may include fever, malaise, headache, dizziness, anorexia, rash, arthralgia, and conjunctivitis (Petersen, E. M., et al. MMWR Morb Mortal Wkly Rep 65, 4 (2016)). ZIKV infections complicated by Guillain-Barré syndrome have been reported since 2004 (Oehler, E., et al. Eurosurveillance 19, 3 (2014) Petersen, E. M., et al. MMWR Morb Mortal Wkly Rep 65, 4 (2016)). More recently, an alarming association between ZIKV infection and fetal brain abnormalities including microcephaly has emerged (Melo, A. S. O., et al. Ultrasound Obstet Gynecol 47, 2 (2016)). With no approved preventive or therapeutic products currently available to fight the ZIKV epidemic, public health officials have no specific medical products at their disposal and their recommendations are limited to avoiding exposure to ZIKV, delaying in becoming pregnant and following basic supportive care (fluids, rest, and ibuprofen) after infection (CDC. Zika virus. Vol. 2016 (US Department of Health and Human Services, CDC, Atlanta, Ga., 2016)). A vaccine is urgently needed to prevent a Zika pandemic.

[0006] Ab-dependent enhancement (ADE) of viral infection has been documented in vitro and in vivo as a significant risk with ZIKV E protein-directed vaccines when applied in dengue endemic areas (Dejnirattisai, W., et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. Nat Immunol 17, 1102-1108 (2016)). Kawiecki, A. B. & Christoferson, R. C. Zika Virus-Induced Antibody Response Enhances Dengue Virus Serotype 2 Replication In Vitro. J Infect Dis 214, 1357-1360 (2016). Smith, S. A., et al. Dengue Virus prM-Specific Human Monoclonal Antibodies with Virus Replication-Enhancing Properties Recognize a Single Immunodominant Antigenic Site. J Virol 90, 780-789 (2015); Stettler, K., et al. Specificity, cross-reactivity and function of antibodies elicited by Zika virus infection (Science 353, 823-826 (2016)). Large-scale studies have not yet been performed to rule out the threat of ADE (e.g. use of ZIKV vaccines in dengue endemic countries), and E protein-targeted zika vaccines could potentially present a risk to those vaccinated. Moreover, prM, a chaperon protein for E, induces limited neutralizing activities (Auskov et al., 1988; Beltramello et al., 2010; Kaufman et al., 1989; Vázquez et al., 2002). It has been suggested that these antibodies contribute to the pathogenesis of DENV virus infection (Dejnirattisai et al., 2010; Rodenhuis-Zybert et al., 2010). Given that ZIKV and DENV are co-endemic throughout their distributions, an alternative to prME proteins as a vaccine target is attractive.

[0007] Currently, there is no vaccine for humans against the Zika virus. What is therefore needed are vaccine compositions and methods of use to prevent and treat disease caused by Zika virus infection.

SUMMARY OF THE INVENTION

[0008] The compositions and methods of the invention described herein are useful for generating an immune response to at least one Flaviviridae virus in a subject in need thereof. Advantageously, the compositions and methods may be used prophylactically to immunize a subject...
against Zika virus infection, or used therapeutically to prevent, treat or ameliorate the onset and severity of disease.

[0009] In a first aspect, the present invention is a recombinant modified vaccinia Ankara (MVA) vector comprising at least one nucleic acid sequence encoding a Flavivirus protein, wherein the at least one nucleic acid sequence is inserted into the MVA vector under the control of at least one promoter compatible with poxvirus expression systems.

[0010] In one embodiment, the recombinant MVA vector comprises at least two nucleic acid sequences encoding Flavivirus proteins, wherein the at least two nucleic acid sequences are inserted into the MVA vector under the control of at least two promoters capable with poxvirus expression systems.

[0011] In one embodiment, the recombinant MVA vector comprises a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural (NS) protein, wherein both the first and second nucleic acid sequences are inserted into the MVA vector under the control of promoters compatible with poxvirus expression systems.

[0012] In one embodiment, the first Flavivirus structural protein is selected from PrM-E, soluble E without a transmembrane domain, E protein domain I, E protein domain II, or E protein domain III, PrM and fragments thereof.

[0013] In one embodiment, the first Flavivirus non-structural protein is selected from NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 and fragments thereof.

[0014] In one embodiment, the second Flavivirus non-structural protein is selected from NS1 non-structural protein sequence.

[0015] In one embodiment, the first and second nucleic acid sequences are inserted into one or more deletion sites of the MVA vector.

[0016] In one embodiment, the first and second nucleic acid sequences are inserted into the recombinant MVA vector in a natural deletion site, a modified natural deletion site, or between essential or non-essential MVA genes.

[0017] In another embodiment, the first and second nucleic acid sequences are inserted into the same natural deletion site, a modified natural deletion site, or between the same essential or non-essential MVA genes.

[0018] In another embodiment, the first nucleic acid sequence is inserted into a deletion site selected from I, II, III, IV, V or VI and the nonstructural protein sequence is inserted into a deletion site selected from I, II, III, IV, V or VI.

[0019] In another embodiment, the first and second nucleic acid sequences or fragments thereof are inserted into different natural deletion sites, modified deletion sites, or between different essential or non-essential MVA genes.

[0020] In another embodiment, the first nucleic acid sequence is inserted in a first deletion site and the second nucleic acid sequence is inserted into a second deletion site.

[0021] In a particular embodiment, the first nucleic acid sequence is inserted between two essential and highly conserved MVA genes and the second nucleic acid sequence is inserted into a restructured and modified deletion III.

[0022] In a particular embodiment, the non-structural protein is NS1.

[0023] In one embodiment, the deletion III is modified to remove non-essential sequences and the second nucleic acid sequence is inserted between essential genes.

[0024] In a particular embodiment, the first nucleic acid sequence is inserted between two essential and highly conserved MVA genes to limit the formation of viable deletion mutants.

[0025] In a particular embodiment, the first nucleic acid sequence is inserted between MVA genes, 18R and 56L.

[0026] In one embodiment, the promoter is selected from the group consisting of Pn2H5, Psyn II, and mH5 promoters or combinations thereof.

[0027] In one embodiment, the first nucleic acid sequence is optimized. In a particular embodiment, the first nucleic acid sequence is optimized by i) changing selected codons to other synonymous codons that are optimal for structural protein expression by MVA, ii) interrupting homopolymer stretches using silent mutations, iii) interrupting transcription terminator motifs using silent mutations, and iv) combinations thereof.

[0028] In one embodiment, the recombinant MVA vector expresses structural protein and non-structural proteins that assemble into VLPs.

[0029] In one embodiment, the structural protein and the non-structural protein sequence are from a Flavivirus species.

[0030] In a second aspect, the present invention is a pharmaceutical composition comprising the recombinant MVA vector of the present invention and a pharmaceutically acceptable carrier.

[0031] In one embodiment, the recombinant MVA vector is formulated for intraperitoneal, intramuscular, intradermal, epidermal, mucosal or intravenous administration.

[0032] In a third aspect, the present invention is a pharmaceutical composition comprising a first recombinant MVA vector and a second recombinant MVA vector, each comprising a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural protein, wherein (i) the first nucleic acid sequence of the first recombinant MVA vector is different than the first nucleic acid sequence of the second recombinant MVA vector and/or (ii) the second nucleic acid sequence of the first recombinant MVA vector is different than the second nucleic acid sequence of the second recombinant MVA vector.

[0033] In a particular embodiment, the first nucleic acid sequence encodes premembrane-E, and the first nucleic acid sequence of the first recombinant MVA vector is from a different species than the first nucleic acid sequence of the second recombinant MVA vector.

[0034] In another particular embodiment, the second nucleic acid sequence of the first recombinant MVA vector is from a different species than the second nucleic acid sequence of the second recombinant MVA vector.

[0035] In a particular embodiment, at least one of the species of Flavivirus is Zika virus.

[0036] In a particular embodiment, the non-structural protein sequence is NS1.

[0037] In a fourth aspect, the present invention is a pharmaceutical composition comprising three or more recombinant MVA vectors each comprising a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural protein sequence, wherein (i) the first nucleic acid sequence of the three or more recombinant MVA vectors are different and/or (ii) the second nucleic acid sequence of the three recombinant MVA vectors are different.
[0038] In a particular embodiment, the first nucleic acid sequence encodes premembrane-\(E\), and the first nucleic acid sequence of the first recombinant MVA vector is from a different species than the first nucleic acid sequence of the second recombinant MVA vector and is from a different species than the first nucleic acid sequence of the third recombinant MVA vector.

[0039] In a particular embodiment, the first nucleic acid sequence of each recombinant vector are from the same species.

[0040] In a particular embodiment, the first nucleic acid sequence of the three or more recombinant MVA vectors are from different species.

[0041] In a particular embodiment, at least one of the species of Flavivirus is Zika virus.

[0042] In a fifth aspect, the present invention is a method of inducing an immune response in a subject in need thereof, said method comprising administering the composition of the present invention to the subject in an amount sufficient to induce an immune response.

[0043] In one embodiment, the immune response is a humoral immune response, a cellular immune response or a combination thereof.

[0044] In a particular embodiment, the immune response comprises production of binding antibodies against the flavivirus.

[0045] In a particular embodiment, the immune response comprises production of neutralizing antibodies against the flavivirus.

[0046] In a particular embodiment, the immune response comprises production of non-neutralizing antibodies against the flavivirus.

[0047] In a particular embodiment, the immune response comprises production of a cell-mediated immune response against the flavivirus.

[0048] In a particular embodiment, the immune response comprises production of neutralizing and non-neutralizing antibodies against the flavivirus.

[0049] In a particular embodiment, the immune response comprises production of neutralizing antibodies and cell-mediated immunity against the flavivirus.

[0050] In a particular embodiment, the immune response comprises production of non-neutralizing antibodies and cell-mediated immunity against the flavivirus.

[0051] In a particular embodiment, the immune response comprises production of neutralizing antibodies, non-neutralizing antibodies, and cell-mediated immunity against the Flaviviridae virus.

[0052] In a particular embodiment, the immune response comprises production of neutralizing antibodies, non-neutralizing antibodies, and cell-mediated immunity against the Zika virus.

[0053] In a sixth aspect, the present invention is a method of preventing a Flaviviridae virus infection in a subject in need thereof, said method comprising administering the recombinant MVA vector of the present invention to the subject in a prophylactically effective amount.

[0054] In one embodiment, the viral infection is a Zika virus infection.

[0055] In a seventh aspect, the present invention is a method of inducing an immune response in a subject in need thereof, said method comprising administering the recombinant MVA vector of the present invention to the subject in a prophylactically effective amount.

[0056] In one embodiment, the immune response is considered a surrogate marker for protection.

[0057] In one embodiment, the method induces an immune response against a Zika virus.

[0058] In an eighth aspect, the present invention is a method of treating Flaviviridae virus infection in a subject in need thereof, said method comprising administering the recombinant MVA vector in a therapeutically effective amount to the subject.

[0059] In one embodiment, the Flaviviridae virus infection is caused by a Zika virus.

[0060] In one embodiment, the subject is exposed to Flaviviridae fever virus, but not yet symptomatic of Flaviviridae virus infection. In a particular embodiment, treatment results in prevention of a symptomatic infection.

[0061] In another embodiment, the subject is recently exposed but exhibits minimal symptoms of infections.

[0062] In another embodiment, the method results in amelioration of at least one symptom of infection.

[0063] In one embodiment, the symptom of infection is selected from mild headaches, maculopapular rash, fever, malaise, conjunctivitis, joint pains or a combination thereof.

[0064] In another embodiment, the method results in reduction or elimination of the subject’s ability to transmit the infection to an uninfected subject.

[0065] In one embodiment, the method prevents or ameliorates a Zika virus infection.

[0066] In a ninth aspect, the present invention is a method manufacturing a recombinant MAVector comprising inserting at least one nucleic acid sequence encoding premembrane-\(E\) and at least one nucleic acid sequence encoding a non-structural protein sequence into the recombinant MVA vector, wherein each nucleic acid sequence is operably linked to a promoter compatible with poxvirus expression systems.

[0067] In one embodiment, the non-structural sequence is NS1and the premembrane-\(E\) sequence is PrM-E.

[0068] In a particular embodiment, the NS1 sequence and the PrM-E sequence are from a Zika virus.

[0069] In one embodiment, the recombinant MVA viral vector expresses Zika virus premembrane-\(E\) and NS1 proteins that assemble into VLPs.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0070] FIG. 1 is a simple line drawing illustrating the design of the MVA vectors.

[0071] The numbering illustrates the positions (in kilobase pairs) of the various elements in the genome of the MVA vaccine vector. For clarity and brevity, the diagram is not to scale; pairs of diagonal lines indicate a section of the MVA genome that is not illustrated because its contents are not relevant to the invention. Arrows labeled “PrM-E” and “NS1” illustrate the positions of the genes encoding premembrane-\(E\) and NS1, respectively, for use with Zika virus sequences. Rectangles labeled “18R” and “GIL,” indicate the positions of the two MVA genetic elements flanking the gene encoding PrME. Rectangles labeled “ASOR” and “B1R” indicate the positions of the two MVA genetic elements flanking the gene encoding NS1.

[0072] FIG. 2 is a schematic for the shuttle vector for flavivirus PrM-E.

[0073] The ampicillin resistance marker, allowing the vector to replicate in bacteria, is illustrated with a block labeled “amp-R.” The two flanking sequences, allowing the vector
to recombine with the MVA genome, are illustrated with a block and a block labeled “Flank 1” and “Flank 2” respectively. The green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs, is illustrated with an arrow labeled “GFP.” The block labeled “DR” illustrates the location of a sequence homologous to part of Flank 1 of the MVA sequence. DR enables removal of the GFP sequence from the MVA vector after insertion of PrM-E into the MVA genome. The modified H5 (mH5) promoter, which enables transcription of the inserted heterologous gene, is illustrated with a triangle between the DR and PrM-E elements. The flavivirus PrM-E gene is illustrated with an arrow labeled “PrM-E”.

[0074] FIG. 3 is a schematic of the shuttle vector for flavivirus NS1.

[0075] The ampicillin resistance marker, allowing the vector to replicate in bacteria, is illustrated with a block labeled “amp-R.” The two flanking sequences, allowing the vector to recombine with the MVA genome, are illustrated with blocks labeled “Flank 1” and “Flank 2.” The green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs, is illustrated with an arrow labeled “GFP.” The block labeled “DR” illustrates the location of a sequence homologous to part of Flank 1 of the MVA sequence. DR enables removal of the GFP sequence from the MVA vector after insertion of NS1 into the MVA genome. The modified H5 (mH5) promoter, which enables transcription of the inserted heterologous gene, is illustrated with a triangle between the DR and NS1 elements. The flavivirus NS1 gene is illustrated with an arrow labeled “NS1.”

[0076] FIG. 4 is a schematic of the shuttle vector for flavivirus truncated NS1.

[0077] The ampicillin resistance marker, allowing the vector to replicate in bacteria, is illustrated with a block labeled “amp-R.” The two flanking sequences, allowing the vector to recombine with the MVA genome, are illustrated with blocks labeled “Flank 1” and “Flank 2.” The green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs, is illustrated with an arrow labeled “GFP.” The block labeled “DR” illustrates the location of a sequence homologous to part of Flank 1 of the MVA sequence. DR enables removal of the GFP sequence from the MVA vector after insertion of NS1 into the MVA genome. The modified H5 (mH5) promoter, which enables transcription of the inserted heterologous gene, is illustrated with a triangle between the DR and NS1 elements. The flavivirus TruncNS1 gene is illustrated with an arrow labeled “TruncNS1.”

[0078] FIG. 5 is a schematic for the flavivirus PCR substrate plasmid pGZ-02.

[0079] The ampicillin resistance marker, allowing the vector to replicate in bacteria, is illustrated with a block labeled “amp-R.” The two flanking sequences, allowing the vector to recombine with the MVA genome, are illustrated with blocks labeled “Flank 1” and “Flank 2.” The green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs, is illustrated with an arrow labeled “GFP.” The block labeled “DR” illustrates the location of a sequence homologous to part of Flank 1 of the MVA sequence. DR enables removal of the GFP sequence from the MVA vector after insertion of NS1 into the MVA genome. The modified H5 (mH5) promoter, which enables transcription of the inserted heterologous gene, is illustrated with a triangle between the DR and NS1 elements. The flavivirus PrM-E gene is illustrated with an arrow labeled “PrM” and “E”.

[0080] FIG. 6 is a schematic for the flavivirus vector pGZ-05.

[0081] The ampicillin resistance marker, allowing the vector to replicate in bacteria, is illustrated with a block labeled “amp-R.” The two flanking sequences, allowing the vector to recombine with the MVA genome, are illustrated with blocks labeled “Flank 1” and “Flank 2.” The green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs, is illustrated with an arrow labeled “GFP.” The block labeled “DR” illustrates the location of a sequence homologous to part of Flank 1 of the MVA sequence. DR enables removal of the GFP sequence from the MVA vector after insertion of NS1 into the MVA genome. The modified H5 (mH5) promoter, which enables transcription of the inserted heterologous gene, is illustrated with a triangle between the DR and NS1 elements. The flavivirus PrM and soluble E (sE) genes are illustrated with arrows labeled “PrM” and “sE”.

[0082] FIG. 7 is a schematic for the flavivirus vector pGZ-04.

[0083] The ampicillin resistance marker, allowing the vector to replicate in bacteria, is illustrated with a block labeled “amp-R.” The two flanking sequences, allowing the vector to recombine with the MVA genome, are illustrated with blocks labeled “Flank 1” and “Flank 2.” The green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs, is illustrated with an arrow labeled “GFP.” The block labeled “DR” illustrates the location of a sequence homologous to part of Flank 1 of the MVA sequence. DR enables removal of the GFP sequence from the MVA vector after insertion of NS1 into the MVA genome. The modified H5 (mH5) promoter, which enables transcription of the inserted heterologous gene, is illustrated with a triangle between the DR and NS1 elements. The flavivirus PrM and soluble E (sE) genes are illustrated with arrows labeled “PrM” and “sE”.

[0084] FIG. 8 provides a figure showing a Western blot showing expression of the Full-length ZIKV E and NS1 Proteins.

[0085] FIG. 9 provides a figure showing electron micrographs of MVA-Zika prME VLP infected cells.

[0086] FIG. 10 provides a figure showing the immunogenicity and survival of MVA-ZIKV-immunized mice. (A) Ag-specific Abs are evident by ELISA 14 days after boosting.

[0087] (B-C) Mice were challenged IC with 10^5 pfu ZIKV (MR766) 28 days after vaccination. Mice immunized by Prime Only (B) or Prime-Boost (C) regimen maintained weight (left) and were rescued from death (right).

DETAILED DESCRIPTION OF THE INVENTION

[0088] Compositions and methods are provided to produce an immune response to a flavivirus, such as a Zika virus, in a subject in need thereof. The compositions and methods of the present invention can be used to prevent infection in an unexposed person or to treat disease in a subject exposed to a flavivirus who is not yet symptomatic or has minimal symptoms. In one embodiment, treatment limits an infection and/or the severity of disease.
Ideal immunogenic compositions or vaccines are safe, effective, and provide sufficient scope of protection and longevity. However, compositions having fewer than all of these characteristics may still be useful in preventing viral infection or limiting symptoms or disease progression in an exposed subject treated prior to the development of symptoms. In an embodiment, the present invention provides a vaccine that permits at least partial, if not complete, protection after a single immunization.

In exemplary embodiments, the immune responses are long-lasting and durable so that repeated boosters are not required, but in one embodiment, one or more administrations of the compositions provided herein are provided to boost the initial primed immune response.

Where a term is provided in the singular, the inventors also contemplate aspects of the invention described by the plural of that term. As used in this specification and in the appended claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise, e.g., “a” peptide includes a plurality of peptides. Thus, for example, a reference to “a method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

The term “antigen” refers to a substance or molecule, such as a protein, or fragment thereof, that is capable of inducing an immune response.

The term “binding antibody” or “bAb” refers to an antibody which either is purified from, or is present in, a body fluid (e.g., serum or a mucosal secretion) and which recognizes a specific antigen. As used herein, the antibody can be a single antibody or a plurality of antibodies. Binding antibodies comprise neutralizing and non-neutralizing antibodies.

The term “cell-mediated immune response” refers to the immunological defense provided by lymphocytes, such as the defense provided by sensitized T cell lymphocytes when they directly lyse cells expressing foreign antigens and secrete cytokines (e.g., IFN-gamma), which can modulate macrophage and natural killer (NK) cell effector functions and augment T cell expansion and differentiation. The cellular immune response is the 2nd branch of the adaptive immune response.

The term “conservative amino acid substitution” refers to substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the size, polarity, charge, hydrophobicity, or hydrophilicity of the amino acid residue at that position, and without resulting in substantially altered immunogenicity. For example, these may be substitutions within the following groups: valine, glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Conservative amino acid modifications to the sequence of a polypeptide (and the corresponding modifications to the encoding nucleotides) may produce polypeptides having functional and chemical characteristics similar to those of a parental polypeptide.

The term “deletion” in the context of a polypeptide or protein refers to removal of codons for one or more amino acid residues from the polypeptide or protein sequence. The term deletion in the context of a nucleic acid refers to removal of one or more bases from a nucleic acid sequence.

As used herein, the term “E” refers to the flavivirus E protein or the gene or transcript encoding the flavivirus E protein.

The terms “gene”, “polynucleotide”, “nucleotide” and “nucleic acid” are used interchangeably herein.

The term “flavivirus” refers collectively to members of the Flaviviridae family of single stranded (-) RNA viruses including West Nile virus, dengue virus, tick-borne encephalitis virus, yellow fever virus, and Zika viruses.

The term “fragment” in the context of a proteinaceous agent refers to a peptide or polypeptide comprising an amino acid sequence of at least 2 contiguous amino acid residues, at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino acid residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues of the amino acid sequence of a peptide, polypeptide or protein. In one embodiment, a fragment of a full-length protein retains activity of the full-length protein. In another embodiment, the fragment of the full-length protein does not retain the activity of the full-length protein.

The term “fragment” in the context of a nucleic acid refers to a nucleic acid comprising an amino acid sequence of at least 2 contiguous nucleotides, at least 5 contiguous nucleotides, at least 10 contiguous nucleotides, at least 15 contiguous nucleotides, at least 20 contiguous nucleotides, at least 25 contiguous nucleotides, at least 30 contiguous nucleotides, at least 35 contiguous nucleotides, at least 40 contiguous nucleotides, at least 50 contiguous nucleotides, at least 60 contiguous nucleotides, at least 70 contiguous nucleotides, at least 80 nucleotides, at least 90 contiguous nucleotides, at least 100 contiguous nucleotides, at least 125 contiguous nucleotides, at least 150 contiguous nucleotides, at least 175 contiguous nucleotides, at least 200 contiguous nucleotides, at least 250 contiguous nucleotides, at least 300 contiguous nucleotides, at least 350 contiguous nucleotides, or at least 380 contiguous nucleotides of the nucleic acid sequence encoding a peptide, polypeptide or protein. In a preferred embodiment, a fragment of a nucleic acid encodes a peptide or polypeptide that retains activity of the full-length protein. In another embodiment, the fragment encodes a peptide or polypeptide that of the full-length protein does not retain the activity of the full-length protein.

As used herein, the phrase “heterologous sequence” refers to any nucleic acid, protein, polypeptide or peptide sequence which is not normally associated in nature with another nucleic acid or protein, polypeptide or peptide sequence of interest.

As used herein, the phrase “heterologous gene insert” refers to any nucleic acid sequence that has been, or is to be inserted into the recombinant vectors described herein. The heterologous gene insert may refer to only the gene product encoding sequence or may refer to a sequence comprising a promoter, a gene product encoding sequence
(such as GP, VP or Z), and any regulatory sequences associated or operably linked therewith.

The term “homopolymer stretch” refers to a sequence comprising at least four of the same nucleotides uninterrupted by any other nucleotide, e.g., GGGG or TTTTTT.

The term “humoral immune response” refers to the stimulation of Ab production. Humoral immune response also refers to the accessory proteins and events that accompany antibody production, including T helper cell activation and cytokine production, affinity maturation, and memory cell generation. The humoral immune response is one of two branches of the adaptive immune response.

The term “humoral immunity” refers to the immunological defense provided by antibody, such as neutralizing Ab that can directly block infection; or, binding Ab that identifies a virus or infected cell for killing by such innate immune responses as complement (C)-mediated lysis, phagocytosis, and natural killer cells.

The term “immune” or “immunity” refers to protection from disease (e.g., preventing or attenuating (e.g., suppression) of a sign, symptom or condition of the disease) upon exposure to a pathogen (e.g., a virus) capable of causing the disease.

The term “immune response” refers to any response to an antigen or antigenic determinant by the immune system of a subject (e.g., a human). Exemplary immune responses include humoral immune responses (e.g., production of antigen-specific antibodies) and cell-mediated immune responses (e.g., production of antigen-specific T cells).

The term “improved therapeutic outcome” relative to a subject diagnosed as infected with a particular virus (e.g., a flavivirus) refers to a slowing or diminution in the growth of virus, or viral load, or detectable symptoms associated with infection by that particular virus; or a reduction in the ability of the infected subject to transmit the infection to another, uninfected subject.

The term “inducing an immune response” means eliciting a humoral response (e.g., the production of antibodies) or a cellular response (e.g., the activation of T cells) directed against a virus (e.g., zika virus) in a subject to which the composition (e.g., a vaccine) has been administered.

The term “insertion” in the context of a polypeptide or protein refers to the addition of one or more non-native amino acid residues in the polypeptide or protein sequence. Typically, no more than about from 1 to 6 residues (e.g. 1 to 4 residues) are inserted at any one site within the polypeptide or protein molecule.

The term “modified vaccinia Ankara,” “modified vaccinia ankara,” “Modified Vaccinia Ankara,” or “MVA” refers to a highly attenuated strain of vaccinia virus developed by Dr. Anton Mayr by serial passage on chick embryo fibroblast cells; or variants or derivatives thereof. MVA is reviewed in (Mayr, A. et al. 1975 Infection 3:6-14; Swiss Patent No. 568,392).

The term “neutralizing antibody” or “NAb” refers to an antibody which is either purified from, or is present in, a body fluid (e.g., serum or a mucosal secretion) and which recognizes a specific antigen and inhibits the effect(s) of the antigen in the subject (e.g., a human). As used herein, the antibody can be a single antibody or a plurality of antibodies.

The term “non-neutralizing antibody” or “nnAb” refers to a binding antibody that is not a neutralizing antibody.

As used herein, the term “NS” refers to the flavivirus nonstructural protein or the gene or transcript encoding the flavivirus nonstructural protein. There are 7 nonstructural proteins in flavivirus denoted NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5.

The term “operably linked”, when used with reference to a promoter, refers to a configuration in which the promoter is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the promoter directs expression of the coding sequence. As used herein, the term “PrM” refers to the flavivirus premembrane protein or the gene or transcript encoding the flavivirus premembrane protein.

The term “prevent”, “preventing” and “prevention” refers to the inhibition of the development or onset of a condition (e.g., a flavivirus infection or a condition associated therewith), or the prevention of the recurrence, onset, or development of one or more symptoms of a condition in a subject resulting from the administration of a therapy or the administration of a combination of therapies.

The term “prophylactically effective amount” refers to the amount of a composition (e.g., the recombinant MVA vector or pharmaceutical composition) which is sufficient to result in the prevention of the development, recurrence, or onset of a condition or a symptom thereof (e.g., a flavivirus infection or a condition or symptom associated therewith) or to enhance or improve the prophylactic effect(s) of another therapy.

The term “recombinant” means a polynucleotide of semisynthetic, or synthetic origin that either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

The term “recombinant,” with respect to a viral vector, means a vector (e.g., a viral genome) that has been manipulated in vitro (e.g., using recombinant nucleic acid techniques) to express heterologous viral nucleic acid sequences.

The term “regulatory sequence” “regulatory sequences” refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites (“IRES”), enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence. Not all of these control sequences need always be present so long as the selected gene is capable of being transcribed and translated.

The term “shuttle vector” refers to a genetic vector (e.g., a DNA plasmid) that is useful for transferring genetic material from one host system into another. A shuttle vector can replicate alone (without the presence of any other vector) in at least one host (e.g., E. coli). In the context of MVA vector construction, shuttle vectors are usually DNA plasmids that can be manipulated in E. coli and then introduced into cultured cells infected with MVA vectors, resulting in the generation of new recombinant MVA vectors.

The term “silent mutation” means a change in a nucleotide sequence that does not cause a change in the primary structure of the protein encoded by the nucleotide sequence, e.g., a change from AAA (encoding lysine) to AAG (also encoding lysine).
The term “subject” means any mammal, including but not limited to, humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, rats, mice, guinea pigs and the like.

The term “surrogate endpoint” means a clinical measurement other than a measurement of clinical benefit that is used as a substitute for a measurement of clinical benefit.

The term “surrogate marker” means a laboratory measurement or physical sign that is used in a clinical or animal trial as a substitute for a clinically meaningful endpoint that is a direct measure of how a subject feels, functions, or survives and is expected to predict the effect of the therapy (Katz, R., NeuroRx 1:189-195 (2004); New drug, antibiotic, and biological drug product regulations; accelerated approval—FDA. Final rule: Fed Regist 57: 58042-58060, 1992.)

The term “surrogate marker for protection” means a surrogate marker that is used in a clinical or animal trial as a substitute for the clinically meaningful endpoint of prevention of flavivirus infection.

The term “synonymous codon” refers to the use of a codon with a different nucleic acid sequence to encode the same amino acid, e.g., AAA and AAG (both of which encode lysine). Codon optimization changes the codons for a protein to the synonymous codons that are most frequently used by a vector or a host cell.

The term “therapeutically effective amount” means the amount of the composition (e.g., the recombinant MVA vector or pharmaceutical composition) that, when administered to a mammal for treating an infection, is sufficient to effect treatment for the infection.

The term “treatment” or “treat” refer to the eradication or control of a flavivirus, a reduction in the titer of the flavivirus, a reduction in the numbers of the flavivirus, the reduction or amelioration of the progression, severity, and/or duration of a condition or one or more symptoms caused by the flavivirus resulting from the administration of one or more therapies, or the reduction or elimination of the subject’s ability to transmit the infection to another, uninfected subject.

The term “vaccine” means material used to provoke an immune response and confer immunity after administration of the material to a subject. Such immunity may include a cellular or humoral immune response that occurs when the subject is exposed to the immunogen after vaccine administration.

The term “vaccine insert” refers to a nucleic acid sequence encoding a heterologous sequence that is operably linked to a promoter for expression when inserted into a recombinant vector. The heterologous sequence may encode a PrM-E or nonstructural protein described here.

The term “viral infection” means an infection by a viral pathogen (e.g., a member of genus Flavivirus) wherein there is clinical evidence of the infection based on symptoms or based on the demonstration of the presence of the viral pathogen in a biological sample from the subject.

The term “virus-like particles” or “VLP” refers to a structure which resembles the native virus antigenically and morphologically.

The term “Zika virus” which is synonymous with “zikavirus” and “ZIKV” refers to a member of the flavivirus family flaviviridae. Zika virus is enveloped and icosahedral and has a non-segmented, single-stranded, positive-sense RNA genome.

II. Flaviviruses

The compositions of the present invention are useful for inducing an immune response to a flavivirus.

Flavivirus is a genus of viruses in the family Flaviviridae. This genus includes the West Nile virus, dengue virus, tick-borne encephalitis virus, yellow fever virus, Zika virus and several other viruses which may cause encephalitis (Shi, P-Y. (editor) (2012). Molecular Virology and Control of Flaviviruses. Caister Academic Press).

Flaviviruses share several common aspects: common size (40-65 nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single-stranded RNA around 10,000-11,000 bases), and appearance in the electron microscope. Like all flaviviruses, ZIKV is a single-stranded RNA virus with a positive-polarity RNA genome of approximately 11 kb. Both termini of the genomic contain sequences that do not encode viral proteins, known as the 5’ and the 3’ untranslabeled region. The encoded polyprotein is translated and co- and posttranslationally processed by viral and cellular proteases into three structural (capsid [C], premembrane [prM] or membrane [M], and envelope [E]) and seven nonstructural (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) proteins.

Zika virus Species and Sequences

The term Zika virus (ZIKV) refers to a genus within the family Flavivirus. Like other Flaviviruses, species within the Zika virus genus consist of a single strand of positive sense RNA that is approximately 11 kb in length with two flanking non-coding regions (5’ and 3’ NCR) and a single long open reading frame encoding a polyprotein: 5’-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3’. That is cleaved into capsid (C), precursor of membrane (prM), envelope (E) and seven non-structural proteins (NS). The E protein (~53 kDa) is the major virion surface protein and is involved in various aspects of the viral cycle, mediating binding and membrane fusion. The 3NCR of the ZIKV genome contains about 428 nucleotides, including 27 folding patterns that may be involved in the recognition by cellular or viral factors, translation, genome stabilization, RNA packaging, or cyclization.

Zika fever is a mosquito-borne illness caused by a flavivirus. Human infections with ZIKV can cause fever, malaise, and cutaneous rash. Despite several reports since 1947, when it was first isolated at Zika forest in Uganda, molecular evolution of ZIKV is an emerging agent remains poorly understood. Moreover, despite several ZIKV reports from Africa and Asia, few human cases were notified until 2007 when an epidemic took place in Micronesia. In West Africa, surveillance programs have reported periodic circulation of the virus since 1968.

Using current methodology, Zika virus is detectable in blood only after onset of symptoms, which accompany the rise in circulating virus. It may take up to three days after symptoms start for the virus to reach detectable levels. Laboratory tests used in diagnosis include, for example, antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM ELISA, polymerase chain reaction (PCR), virus isolation, and later in the course of infection or recovery-detection of IgM and IgG antibodies.
No vaccine or therapeutic has been approved by the FDA for Zika virus, for either prophylactic or therapeutic use.

Ill. Recombinant Viral Vectors

In one aspect, the present invention is a recombinant viral vector comprising one or more genes of a flavivirus virus, such as Zika virus. In certain embodiments, the recombinant viral vector is a vaccinia virus vector, and more particularly, an MVA vector, comprising one or more genes of a flavivirus, such as Zika virus.

Vaccinia viruses have also been used to engineer viral vectors for recombinant gene expression and for the potential use as recombinant live vaccines (Mackett, M. et al. 1982 PNAS USA 79:7415-7419; Smith, G. L. et al. 1984 Biotech Genet Engin Rev 2:383-407). This entails DNA sequences (genes) which code for foreign antigens being introduced, with the aid of DNA recombination techniques, into the genome of the vaccinia viruses. If the gene is integrated at a site in the viral DNA which is non-essential for the life cycle of the virus, it is possible for the newly produced recombinant vaccinia virus to be infectious, that is to say able to infect foreign cells and thus to express the integrated DNA sequence (EP Patent Applications No. 83,286 and No. 110,385). The recombinant vaccinia viruses prepared in this way can be useful, on the one hand, as live vaccines for the prophylaxis of infectious diseases, on the other hand, for the preparation of heterologous proteins in eukaryotic cells.

Several such strains of vaccinia virus have been developed to avoid undesired side effects of smallpox vaccination. Thus, a modified vaccinia Ankara (MVA) has been generated by long-term serial passages of the Ankara strain of vaccinia virus (CVA) on chicken embryo fibroblasts (for review see Mayr, A. et al. 1975 Infection 3:6-14; Swiss Patent No. 568,392). The MVA virus is publicly available from American Type Culture Collection as ATCC No.: VR-1508. MVA is distinguished by its great attenuation, as demonstrated by diminished virulence and reduced ability to replicate in primate cells, while maintaining good immunogenicity. The MVA virus has been analyzed to determine alterations in the genome relative to the parental CVA strain. Six major deletions of genomic DNA (deletion I, II, III, IV, V, and VI) totaling 31,000 base pairs have been identified (Meyer, H. et al. 1991 J Gen Virol 72:1031-1038). The resulting MVA virus became severely host cell restricted to avian cells.

Furthermore, MVA is characterized by its extreme attenuation. When tested in a variety of animal models, MVA was proven to be avirulent even in immunosuppressed animals. More importantly, the excellent properties of the MVA strain have been demonstrated in extensive clinical trials (Mayr A. et al. 1978 Zentralbl Bakteriol [B] 167:375-390; Stickl et al. 1974 Dtsch Med Wschr 99:2386-2392). During these studies in over 120,000 humans, including high-risk patients, no side effects were associated with the use of MVA vaccine.

MVA replication in human cells was found to be blocked late in infection preventing the assembly to mature infectious virions. Nevertheless, MVA was able to express viral and recombinant genes at high levels even in non-permissive cells and was proposed to serve as an efficient and exceptionally safe gene expression vector (Sutter, G. and Moss, B. 1992 PNAS USA 89:10847-10851). Additionally, novel vaccinia vector vaccines were established based on MVA having foreign DNA sequences inserted at the site of deletion III within the MVA genome (Sutter, G. et al. 1994 Vaccine 12:1032-1040).

Recombinant MVA vaccinia viruses can be prepared as set out hereinafter. A DNA-construct which contains a DNA-sequence which codes for a foreign polypeptide flanked by MVA DNA sequences adjacent to a predetermined insertion site (e.g. between two conserved essential MVA genes such as 18R/11L; in restructured and modified deletion III; or at other non-essential sites within the MVA genome) is introduced into cells infected with MVA, to allow homologous recombination. Once the DNA-construct has been introduced into the eukaryotic cell and the foreign DNA has recombined with the viral DNA, it is possible to isolate the desired recombinant vaccinia virus in a manner known per se, preferably with the aid of a marker. The DNA-construct to be inserted can be linear or circular. A plasmid or polymerase chain reaction product is preferred. Such methods of making recombinant MVA vectors are described in PCT publication WO/2006/026667 incorporated by reference herein. The DNA-construct contains sequences flanking the left and the right side of a naturally occurring deletion. The foreign DNA sequence is inserted between the sequences flanking the naturally occurring deletion. For the expression of a DNA sequence or gene, it is necessary for regulatory sequences, which are required for the transcription of the gene, to be present on the DNA. Such regulatory sequences (called promoters) are known to those skilled in the art, and include for example those of the vaccinia 11 kDa gene as are described in EP-A-198,328, and those of the 7.5 kDa gene (EP-A-110,385). The DNA-construct can be introduced into the MVA infected cells by transfection, for example by means of calcium phosphate precipitation (Graham et al. 1973 Virol 52:456-467; Wglar et al. 1979 Cell 16:777-785), by means of electroporation (Neumann et al. 1982 EMBO J. 1:841-845), by microinjection (Gaussmann et al. 1983 Meth Enzymol 101:482-492), by means of liposomes (Staubinger et al. 1983 Meth Enzymol 101:512-527), by means of spheroplasts (Schaffler 1980 PNAS USA 77:2163-2167) or by other methods known to those skilled in the art.

The MVA vectors described and tested herein were unexpectedly found to be effective after a single prime or a homologous prime/boost regimen. Other MVA vector designs require a heterologous prime/boost regimen, while still other published studies have been unable to induce effective immune responses with MVA vectors. Conversely, the present MVA vector design and methods of manufacture are useful in producing effective MVA vaccine vectors for eliciting effective T-cell and antibody immune responses. Furthermore, the utility of an MVA vector capable of eliciting effective immune responses and antibody production after a single homologous prime boost is significant for considerations such as use, commercialization and transport of materials especially to affected third world locations.

In one embodiment, the present invention is a recombinant viral vector (e.g., an MVA vector) comprising one or more heterologous gene inserts of a flavivirus (e.g., a Zika virus). The viral vector (e.g., an MVA vector) may be constructed using conventional techniques known to one of skill in the art. The one or more heterologous gene inserts encode a polypeptide having desired immunogenicity, i.e., a polypeptide that can induce an immune reaction, cellular immunity and/or humoral immunity, in vivo by administra-
tion thereof. The gene region of the vector (e.g., an MVA vector) where the gene encoding a polypeptide having immunogenicity is introduced is flanked by regions that are indispensable. In the introduction of a gene encoding a polypeptide having immunogenicity, an appropriate promoter may be operatively linked upstream of the gene encoding a polypeptide having desired immunogenicity.

[0154] The one or more genes may be selected from any species of flavivirus. In one embodiment, the one more genes are selected from a Zika virus species. In exemplary embodiments, the gene encodes a polypeptide or protein capable of inducing an immune response in the subject to which it is administered, and more particularly, an immune response capable of providing a protective and/or therapeutic benefit to the subject. In one embodiment, the one or more genes encode the virus premembrane protein (PrM), the E protein (E), the or one or more nonstructural proteins (e.g. NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). The heterologous gene inserts are inserted into one or more deletion sites of the vector under the control of promoters compatible with poxvirus expression systems.

[0155] In one embodiment, the deletion III site is restructured and modified to remove non-essential flanking sequences.

[0156] In exemplary embodiments, the vaccine is constructed to express a Zika virus premembrane-E protein (PrM-E), which is inserted between two conserved essential MVA genes (I8R and G1L) using shuttle vector pGeo-PrM-E; and to express Zika virus NS1, which is inserted into deletion III using shuttle vector pGeo-NS1. pGeo-PrM-E and pGeo-NS1 are constructed with an ampicillin resistance marker, allowing the vector to replicate in bacteria; with two flanking sequences, allowing the vector to recombine with a specific location in the MVA genome; with a green fluorescent protein (GFP) selection marker, allowing the selection of recombinant MVAs; with a sequence homologous to part of Flank 1 of the MVA sequence, enabling removal of the GFP sequence from the MVA vector after insertion of NS1 into the MVA genome; with a modified I5 (mI5) promoter, which enables transcription of the inserted heterologous gene insert; and with a flavivirus gene. pGeo-PrM-E and pGeo-NS1 differ in that pGeo-PrM-E contains the PrM-E sequence, whereas pGeo-NS1 contains the NS1 sequence; and in that pGeo-PrM-E recombines with sequences of MVA I8R and G1L (two essential genes) and pGeo-NS1 recombines with regions flanking the restructured and modified Deletion III of MVA.

[0157] In exemplary embodiments, the present invention provides a recombinant MVA vector comprising a gene encoding the PrM-E gene and a gene encoding NS1, in each case, from a Zika virus.

[0158] In certain embodiments, the polypeptide, or the nucleic acid sequence encoding the polypeptide, may have a mutation or deletion (e.g., an internal deletion, truncation of the amino- or carboxy-terminus, or a point mutation).

[0159] The one or more genes introduced into the recombinant viral vector are under the control of regulatory sequences that direct its expression in a cell.

[0160] The nucleic acid material of the viral vector may be encapsulated, e.g., in a lipid membrane or by structural proteins (e.g., capsid proteins), that may include one or more viral polypeptides.

[0161] In exemplary embodiments, the present invention is a recombinant viral vector (e.g., a recombinant MVA vector) comprising one or more genes, or one or more polypeptides encoded by the gene or genes, from a Zika virus. The Zika virus gene may encode a polypeptide or protein capable of inducing an immune response in the subject to which it is administered, and more particularly, an immune response capable of providing a protective and/or therapeutic benefit to the subject, e.g., the Zika virus PrM-E.

The nucleic acid sequences of Zika virus premembrane and E proteins and nonstructural are published and are available from a variety of sources, including, e.g., GenBank and PubMed. Exemplary GenBank references including Zika virus sequences include those corresponding to accession numbers KU312312.

[0162] In certain embodiments, the one or more genes encodes a polypeptide, or fragment thereof, that is substantially identical (e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or even 100% identical) to the selected Zika virus PrM-E over at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 contiguous residues of the selected Zika virus PrM or E that retain immunogenic activity.

[0163] In certain embodiments, the one or more genes encodes a polypeptide, or fragment thereof, that is substantially identical (e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or even 100% identical) to the selected Zika virus E over at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 contiguous residues of the selected Zika virus E that retains immunogenic activity.

[0164] In exemplary embodiments, the recombinant viral vector may also include an Zika virus PrM-E present on its surface. The Zika virus PrM-E may be obtained by any suitable means, including, e.g., application of genetic engineering techniques to a viral source, chemical synthesis techniques, recombinant production, or any combination thereof.

[0165] In other embodiments, the present invention is a recombinant MVA vector comprising at least one heterologous gene insert from a Zika virus, wherein the gene is selected from the group encoding the premembrane protein (PrM), the E protein (E), the or one or more nonstructural proteins (e.g. NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5).

[0166] In a particular embodiment, the present invention is a recombinant MVA vector comprising a gene encoding PrM-E and a gene encoding NS1. In another embodiment, the present invention is a recombinant MVA vector comprising genes encoding PrM-E, and NS1. The heterologous gene inserts are inserted into one or more deletion sites of the MVA vector under the control of promoters compatible with poxvirus expression systems.

[0167] In one embodiment, the PrM-E is inserted into deletion site I, II, III, IV, V or VI of the MVA vector, and the NS1 is inserted into deletion site I, II, III, IV, V or VI of the MVA vector.

[0168] In one embodiment, the PrM-E is inserted between I8R and G1L of the MVA vector, or into restructured and modified deletion III of the MVA vector; and the NS1 is inserted between I8R and G1L of the MVA vector, or into restructured and modified deletion site III of the MVA vector.

[0169] In exemplary embodiments, the present invention is a recombinant MVA vector comprising at least one heterologous gene insert (e.g., one or more gene inserts) from a Zika virus which is under the control of regulatory sequences that direct its expression in a cell. The gene may
be, for example, under the control of a promoter selected from the group consisting of Pm2H5, Psyn II, or mH5 promoters.

[0170] In one embodiment, the recombinant MVA vaccine expresses proteins that assemble into virus-like particles (VLPs) comprising the PrM-E and NS1 proteins. While not wanting to be bound by any particular theory, it is believed that the PrM-E is provided to elicit a protective immune response and the NS1 (nonstructural protein) is provided to enable assembly of VLPs and as a target for T cell immune responses, thereby enhancing the protective immune response and providing cross-protection.

[0171] One or more genes may be optimized for use in the MVA vector. Optimization includes codon optimization, which employs silent mutations to change selected codons from the native sequences into synonymous codons that are optimally expressed by the host-vector system. Other types of optimization include the use of silent mutations to interrupt homopolymer stretches or transcription terminator motifs. Each of these optimization strategies can improve the stability of the gene, improve the stability of the transcript, or improve the level of protein expression from the gene. In exemplary embodiments, the number of homopolymer stretches in the PrM-E or NS1 sequence is reduced to stabilize the construct. A silent mutation may be provided for anything similar to a vaccinia termination signal.

[0172] In exemplary embodiments, optimization of genes may include interrupting homopolymer sequences (e.g., G/C and A/T) by silent mutations, adding a second TAA stop codon, or adding a Vaccinia Transcription Terminator Sequence at the end of the gene such as TTTTTAT.

[0173] In exemplary embodiments, the PrM-E and NS1 sequences are codon optimized for expression in MVA using a computer algorithm; PrM-E and NS1 sequences with runs of ≥5 deoxyguanosines, ≥5 deoxyctydines, ≥5 deoxyadenosines, and ≥5 deoxythymidines are interrupted by silent mutation to minimize loss of expression due to frame shift mutations; and the PrM-E sequence is modified through addition of an extra nucleotide to express the transmembrane, rather than the secreted, form of Zika virus PrM-E.

[0174] The recombinant viral vectors of the present invention may be used alone or in combination. In one embodiment, two different recombinant viral vectors are used in combination, where the difference may refer to the one or more heterologous gene inserts or the other components of the recombinant viral vector or both. In exemplary embodiments, two or more recombinant viral vectors are used in combination in order to protect against infection by all versions of Flavivirus in humans.

[0175] The present invention also extends to host cells comprising the recombinant viral vector described above, as well as isolated virions prepared from host cells infected with the recombinant viral vector.

[0176] IV. Pharmaceutical Composition

[0177] The recombinant viral vectors of the present invention are readily formulated as pharmaceutical compositions for veterinary or human use, either alone or in combination. The pharmaceutical composition may comprise a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

[0178] In one embodiment, the present invention is a vaccine effective to protect and/or treat a Flavivirus (e.g., a Zika virus) comprising a recombinant MVA vector that expresses at least one Flavivirus polypeptide (e.g., a PrM-E) or an immunogenic fragment thereof. The vaccine composition may comprise one or more additional therapeutic agents.

[0179] The pharmaceutical composition may comprise 1, 2, 3, 4 or more than 4 different recombinant MVA vectors.

[0180] As used herein, the phrase “pharmaceutically acceptable carrier” encompasses any suitable pharmaceutical carrier, such as those suitable for parenteral administration, such as, for example, by intramuscular, intraarticular (in the joints), intravenous, intradermal, intraperitoneal, and subcutaneous routes. Examples of such formulations include aqueous and non-aqueous, isotonic sterile injection solutions, which contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. One exemplary pharmaceutically acceptable carrier is physiological saline.

[0181] Other physiologically acceptable diluents, excipients, carriers, or adjuvants and their formulations are known to those skilled in the art.

[0182] The compositions utilized in the methods described herein can be administered by a route any suitable method, e.g., parenteral, intramuscular, intraarticular, intravenous, intraperitoneal, subcutaneous, dermal, transdermal, ocular, inhalation, buccal, sublingual, perlingual, nasal, topical administration, and oral administration. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated). Formulations suitable for oral administration may consist of liquid solutions, such as an effective amount of the composition dissolved in a diluent (e.g., water, saline, or PEG-400), capsules, sachets or tablets, each containing a predetermined amount of the vaccine. The pharmaceutical composition may also be an aerosol formulation for inhalation, e.g., to the bronchial passageways. Aerosol formulations may be mixed with pressurized, pharmaceutically acceptable propellants (e.g., dichlorodifluoromethane, propane, or nitrogen).

[0183] For the purposes of this invention, pharmaceutical compositions suitable for delivering a therapeutic or biologically active agent can include, e.g., tablets, gels, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels, hydrogels, oral gels, pastes, eye drops, ointments, creams, plasters, drenches, delivery devices, microneedles, suppositories, enemas, injectables, implants, sprays, or aerosols. Any of these formulations can be prepared by well-known and accepted methods of art. See, for example, Remington: The Science and Practice of Pharmacy (21st sup. st ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2005, and Encyclopedia of Pharmaceutical Technology, ed. J. Swarbrick, Informa Healthcare, 2006, each of which is hereby incorporated by reference.

[0184] The immunogenicity of the composition (e.g., vaccine) may be significantly improved if the composition of the present invention is co-administered with an immunostimulatory agent or adjuvant. Suitable adjuvants well-known to those skilled in the art include, e.g., aluminum phosphate, aluminum hydroxide, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, glycolipid analogs, octodecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipo-
proteins, ISCOM-Matrix, DC-Chol, DDA, cytokines, and other adjuvants and derivatives thereof.

Pharmaceutical compositions according to the present invention may be formulated to release the composition immediately upon administration (e.g., targeted delivery) or at any predetermined time period after administration using controlled or extended release formulations. Administration of the pharmaceutical composition in controlled or extended release formulations is useful where the composition, either alone or in combination, has (i) a narrow therapeutic index (e.g., the difference between the plasma concentration leading to harmful side effects or toxic reactions and the plasma concentration leading to a therapeutic effect is small; generally, the therapeutic index, TI, is defined as the ratio of median lethal dose (LD₅₀) to median effective dose (ED₅₀); (ii) a narrow absorption window in the gastrointestinal tract; or (iii) a short biological half-life, so that frequent dosing during a day is required in order to sustain a therapeutic level.

Many strategies can be pursued to obtain controlled or extended release in which the rate of release outweighs the rate of metabolism of the pharmaceutical composition. For example, controlled release can be obtained by the appropriate selection of formulation parameters and ingredients, including, e.g., appropriate controlled release compositions and coatings. Suitable formulations are known to those skilled in the art. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, nanoparticles, patches, and liposomes.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the vaccine dissolved in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the vaccine, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; (d) suitable emulsions; and (e) polysaccharide polymers such as chitins. The vaccine, alone or in combination with other suitable components, may also be made into aerosol formulations to be administered via inhalation, e.g., to the bronchial passageways. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the vaccine with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the vaccine with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Pharmaceutical compositions comprising any of the nucleic acid molecules encoding flavivirus viral proteins of the present invention are useful to immunize a subject against disease caused by flavivirus infection. Thus, this invention further provides methods of immunizing a subject against disease caused by flavivirus infection, comprising administering to the subject an immunoeffective amount of a pharmaceutical composition of the invention. This subject may be an animal, for example a mammal, such as a primate or preferably a human.

The vaccines of the present invention may also be co-administered with cytokines to further enhance immunogenicity. The cytokines may be administered by methods known to those skilled in the art, e.g., as a nucleic acid molecule in plasmid form or as a protein or fusion protein.

Kits

This invention also provides kits comprising the vaccines of the present invention. For example, kits comprising a vaccine and instructions for use are within the scope of this invention.

V. Method of Use

The compositions of the invention can be used as vaccines for inducing an immune response to a flavivirus, such as a Zika virus.

In exemplary embodiments, the present invention provides a method of preventing a flavivirus (e.g., Zika virus) infection in a subject in need thereof (e.g., an unexposed subject), comprising administering the composition of the present invention to the subject in a prophylactically effective amount. The result of the method is that the subject is partially or completely immunized against the virus.

In exemplary embodiments, the present invention provides a method of treating a flavivirus (e.g., Zika virus) infection in a subject in need thereof (e.g., an exposed subject, such as a subject who has been recently exposed but is not yet symptomatic, or a subject who has been recently exposed and is only mildly symptomatic), comprising administering the composition of the present invention to the subject in a therapeutically effective amount. The result of treatment is a subject that has an improved therapeutic profile.

Typically, the vaccines will be in an admixture and administered simultaneously, but may also be administered separately.

A subject to be treated according to the methods described herein (e.g., a subject infected with a Zika virus) may be one who has been diagnosed by a medical practitioner as having such a condition. Diagnosis may be performed by any suitable means. A subject in whom the development of an infection is being prevented may or may not have received such a diagnosis. One skilled in the art will understand that a subject to be treated according to the present invention may have been identified using standard tests or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., exposure to Zika virus, etc.).

Prophylactic treatment may be administered, for example, to a subject not yet exposed to or infected by a flavivirus but who is susceptible to, or otherwise at risk of exposure or infection with an flavivirus.

Therapeutic treatment may be administered, for example, to a subject already exposed to or infected by a flavivirus who is not yet ill, or showing symptoms or infection, suffering from a disorder in order to improve or stabilize the subject’s condition (e.g., a patient already infected with a flavivirus). The result is an improved therapeutic profile. In some instances, as compared with an equivalent untreated control, treatment may ameliorate a disorder or a symptom thereof by, e.g., 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% as measured by any standard technique. In some instances, treatment can result in the inhibition of viral replication, a decrease in viral titer or viral load, eradication or clearing of the virus.

In other embodiments, treatment may result in amelioration of one or more symptoms of the infection, including any symptom identified above. According to this
In one specific embodiment, a 4-week interval is used between 2 administrations.

Dosage

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount, as will be therapeutically effective, immunogenic and protective. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the immune system of the individual to synthesize antibodies, and, if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and may be monitored on a patient-by-patient basis. However, suitable dosage ranges are readily determinable by one skilled in the art and generally range from about 5.0x10^3 TCID_{50} to about 5.0x10^6 TCID_{50}. The dosage may also depend, without limitation, on the route of administration, the patient’s state of health and weight, and the nature of the formulation.

The pharmaceutical compositions of the invention are administered in such an amount as will be therapeutically effective, immunogenic, and/or protective against a pathogenic species of Zika virus. The dosage administered depends on the subject to be treated (e.g., the manner of administration and the age, body weight, capacity of the immune system, and general health of the subject being treated). The composition is administered in an amount to provide a sufficient level of expression that elicits an immune response without undue adverse physiological effects. Preferably, the composition of the invention is a heterologous viral vector that includes one or more polypeptides of the flavivirus (e.g., the Zika virus PrM-E protein and NS1 protein), or a nucleic acid molecule encoding one or more genes of the flavivirus, and is administered at a dosage of, e.g., between 1.0x10^6 and 9.9x10^12 TCID_{50} of the viral vector, preferably between 1.0x10^6 TCID_{50} and 1.0x10^11 TCID_{50} pfu, more preferably between 1.0x10^6 and 1.0x10^10 TCID_{50} pfu, or most preferably between 5.0x10^6 and 5.0x10^9 TCID_{50}. The composition may include, e.g., at least 5.0x10^6 TCID_{50} of the viral vector (e.g., 1.0x10^6 TCID_{50} of the viral vector). A physician or researcher can decide the appropriate amount and dosage regimen.

The composition of the method may include, e.g., between 1.0x10^6 and 9.9x10^12 TCID_{50} of the viral vector, preferably between 1.0x10^6 TCID_{50} and 1.0x10^11 TCID_{50} pfu, more preferably between 1.0x10^6 and 1.0x10^10 TCID_{50} pfu, or most preferably between 5.0x10^6 and 5.0x10^9 TCID_{50}. The composition may include, e.g., at least 5.0x10^6 TCID_{50} of the viral vector (e.g., 1.0x10^6 TCID_{50} of the viral vector). The method may include, e.g., administering the composition to the subject two or more times.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. However, a suitable dosage range may be, for example, of the order of several hundred micrograms active ingredient per vaccination. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg body weight, about 5 microgram/kg body weight, about 10 microgram/kg body weight, about 50 microgram/kg body weight, about 100 microgram/kg body weight, about 200 microgram/kg body weight.
Similarly, FDA may allow approval of vaccines against *flavivirus* based on its Animal Rule. In this case, approval is achieved based on efficacy in animals. The value of the invention may lie in its ability to protect relevant animal species against infection with flaviviruses, thus providing adequate evidence to justify its approval.

The composition of the method may include, e.g., between $1.0 \times 10^7$ and $9.9 \times 10^2$ TCID$_{50}$ of the viral vector, preferably between $1.0 \times 10^5$ and $1.0 \times 10^3$ TCID$_{50}$ pfu, more preferably between $1.0 \times 10^4$ and $1.0 \times 10^3$ TCID$_{50}$ pfu, or most preferably between $5.0 \times 10^3$ and $5.0 \times 10^3$ TCID$_{50}$. The composition may include, e.g., at least $5.0 \times 10^6$ TCID$_{50}$ of the viral vector. The method may include, e.g., administering the composition two or more times.

In some instances it may be desirable to combine the *flavivirus* vaccines of the present invention with vaccines which induce protective responses to other agents, particularly other viruses. For example, the vaccine compositions of the present invention can be administered simultaneously, separately or sequentially with other genetic immunization vaccines such as those for influenza (Ulmer, J. B. et al., Science 259:1745-1749 (1993); Raz, E. et al., PNAS 1050 (USA) 91:9519-9523 (1994)), malaria (Doolan, D. L. et al., J. Exp. Med. 183:1739-1746 (1996); Sedegah, M. et al., PNAS (USA) 91:9866-9870 (1994)), and tuberculosis (Tascon, R. C. et al., Nat. Med. 2:888-892 (1996)).

As used herein, the term “administering” refers to a method of giving a dosage of a pharmaceutical composition of the invention to a subject. The compositions utilized in the methods described herein can be administered by a route selected from, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perlingual, nasal, rectal, topical administration, and oral administration. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intraarterial, intravascular, and intramuscular administration. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated).

Administration of the pharmaceutical compositions (e.g., vaccines) of the present invention can be by any of the routes known to one of skill in the art. Administration may be by, e.g., intramuscular. The compositions utilized in the methods described herein can also be administered by a route selected from, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perlingual, nasal, rectal, topical administration, and oral administration. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The preferred method of administration can vary depending on various factors, e.g., the components of the composition being administered and the severity of the condition being treated.

In addition, single or multiple administrations of the compositions of the present invention may be given to a subject. For example, subjects who are particularly susceptible to *flavivirus* infection may require multiple administrations to establish and/or maintain protection against the virus. Levels of induced immunity provided by the pharmaceutical compositions described herein can be monitored by, e.g., measuring amounts of neutralizing secretory and serum
antibodies. The dosages may then be adjusted or repeated as necessary to maintain desired levels of protection against viral infection.

[0225] The claimed invention is further described by way of the following non-limiting examples. Further aspects and embodiments of the present invention will be apparent to those of ordinary skill in the art, in view of the above disclosure and following experimental exemplification, included by way of illustration and not limitation, and with reference to the attached figures.

**EXAMPLES**

**Example 1**

**MVA Vaccine Vectors and Native Zika Virus Sequences**

[0226] This example provides information on exemplary MVA vaccine vectors. Table 1 lists five MVA vaccine vectors.

<table>
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<tr>
<th>Vaccine designation</th>
<th>PrM-E sequence</th>
<th>NS1 protein sequence</th>
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<tr>
<td>GEO-ZM01</td>
<td>PrM-E</td>
<td>none</td>
</tr>
<tr>
<td>GEO-ZM02</td>
<td>none</td>
<td>NS1 (Full length)</td>
</tr>
<tr>
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<td>none</td>
<td>NS1 (Soluble C)</td>
</tr>
<tr>
<td>GEO-ZM04</td>
<td>PrM-E</td>
<td>NS1 (Full length)</td>
</tr>
<tr>
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<td>PrM-E</td>
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<tr>
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</tr>
<tr>
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<td>E, full length</td>
<td>NS1 (Soluble C)</td>
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<tr>
<td>GEO-ZM11</td>
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</tr>
</tbody>
</table>

**[0227]** Native sequences are provided below which were used for development of viral inserts

```
SEQ ID 01: Native nucleotide sequence for Zika PrM-E, from GenBank (KU312312):
1 aacagttttta tttcgagattt gaaacgaga gttttcggtc atgatggaaac caaaaagaa
  61 atcggagaggg tgtcgagattc tcaataagct taaaagccgg gccagccgct gcagcctctt
  121 tggggcttgg aagagcgtgcc cagccgactc tctggtggttt gattyggccct cttgatgattt
  181 tctggagaggg tgtcgagattc tcaataagct taaaagccgg gccagccgct gcagcctctt
  241 tagctgtggt gcacgagcacc cagccgctgc gcagcctctt gattyggccct cttgatgattt
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  601 cagctgtgtg atcgctgtg gccacgtcag cagctgtgtg atcgctgtg gccacgtcag
  661 aagttgagacg cggtagctgtg gccatgtggt gctgtggtg gccatgtggt gccacgtcag
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  901 gcaccaacat cttggagatg ataatgcttgt tatataaagcc gcactgctgt gctgttgtgt
  961 cagctgtgtg atcgctgtg gccacgtcag cagctgtgtg atcgctgtg gccacgtcag
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541 KQTVVVLQGP QGAVTVHATL GALEAEMQGK QGRLSNHLK CRLNQKLRK KVSYSYLSTA
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661 NSMMLBDLP PGQDDSYTVIG VEGKTHHWH HREGSTGOKA FEATVRGAKR MAVLGDATAWD
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781 LQGVFILFLS AVSAQDSRCF DF53KETCQG TOPVYVNDVE AMNDRYXTHF DSPPRLEAAV
841 QKAWEDGQCG ISSVYRMENI MNRSVEGELN AIELEKNOVL TVVGSYVQNP MNGRGEQLPV
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1621 KKGDBGQAV ALDPAQSGSP ILDQGVRTIG QYGNQVHKN GSYVSATQOG RKEKETPVCB
1681 FPPSLMKKQ LTVLDLHPQA GTKREVFPLP VERAIKETLR TIVLAPFRVTV AAMAEBALSG
1741 LPVYRTMHTAV NUVGOSITEV CLMITEAFS RTLQFIQRVPVNYN VLMYMEDEH FTDPSIAAR
1801 GVISTVREIK EAAHPMTAT PGTEDADPD SNSPFMTEV EYFRABNESG FDNYTDNSQG
1861 TWDVFPSVRE GNHEAIACLT AGKVRQVLQR PEKTFERQKT HKBQWPDVFV TDIEMKJNHF
1921 KADVRISDR CLKEVILGDE RVLAPMNPV THASAQARG RIETPMHKPG DEFYQQOCGA
1981 ETDEHNAVLU ERMNLIQNQ LGQGIAGAG NERAVNYNEA GEGFKNLTSEQ KETFVENLKMR
2041 GQLLVWLVQ VGADGEHIIH SRWCPDOTTIN NTMMDYFPV EYFVRKGEXER VLKPFMNDAR
2101 VCSDAALAS PEKPAAKQGR AAPMVEALG TLHPQMERF QRAIDNLAVL MRAQGSRPY
2161 KAAALQPMTAT LETLMMGLL GTVQSHIFVQ LGHVEGQIKM GQFQMTLCGAS AMLWLRBE
2221 PARIACVLIV VPLLFFVLP EPEKQRPQQ MQMAIINMA VQGLLQILRT EAGLWKDSARS
2281 DLHSMOKRRE GAGIIPSLED ILDRPAASNA YVAATLFTP PAVQHAVVTS YNNISLAMA
2341 TQASVLFPSMG KNLIFVYADF QVLMLIQGC SLOQPLTLIV AIILLLAVMN YLIPGCQAAA
2401 ARAAQKCTAA GIMHNPFVVDG TTVDITDIT IDPQVXEGG QVLQQAVAVS SAILSTWAAG
2461 WSOAGALITA ATSTDIQEGS RKNYWSDTS LSCMIQFSGY LACAGLITYV TRHAGLVKRR
2521 GSGDGTGCL KWWKLQMOS ALEPSYKSS GITCVCREEA RRAKQGVEQ DGHAWVOSKA
2581 KLSNVLREVGY LQFIUKVVID LCOROQGWSY AATIRKQEG KETYKQOGP HTTPVLQVSYO
2641 NVWIRKLSQG DVFHMAMAEPC DTLCDQGHS SSSFRVEDAR TRLVLSSNVD KLEKRPAAAFC
2701 IKVLCQYTST MMEFLLQRG RYGGGLKVRP LSHESTHEH WVSQKSNHT EKVSTSGQLL
Example 2

Preparation of PrM-E Zika Sequences for Vaccine Development

This example shows the optimization of one PrM-E sequence. A vaccine construct is designed that encodes both the PrM gene and the E gene in the same construct. These two gene products are synthesized in the cell as a polyprotein and after translocation into the ER membrane are cleaved by signal peptidase into their individual forms.

According to the gene annotation of the French Polynesian isolate of Zika, ‘PrM’ is located at position 126-290 of the amino acid sequence and ‘E’ is located at position 291-794 of the amino acid sequence. The amino acid sequence from position 126-794 is the sequence that we will therefore use for vaccine development. This sequence is 669 amino acids in length.

This sequence is as follows, with PrM distinguished from E by underlining the sequence and the predicted transmembrane helix domains marked by Boldlettering:

```
(SEQ ID NO: 3)
1 TERGSAYTVY LGQMDAGKEl SFPTTLGMNK CVIQHMDLGK TCDATMSVY CPMLAEGVFP
61 CVICHCNMT TTVGYCTCHK KEQAAERSR AVTLDHSTR K/qtqfoTqlk BESYTHHL
121 RVEIVFARHP GFALAAAAl WLLGSSTFOK VITLVNILLI APAEBRCIO VSHNDFVBRM
181 SQOHTYDVL RKhGQCVTMA QDKPTVDIEL VTTVSINAE VEGSVEIGI SDMASDSCRP
241 TQCAAYLDEQ S DTOYQVCKRT LDVRGWGNCQ GLPQGHSLVTC RCPPCCKRM TQGSIQFENL
301 EVRIMLVSNG SQHMGMMVNG TQHOTTDEHRA KVEITPRHHR AEATLQOGPS LGMLCEPRTG
361 LDFSDLVLYLT MNISQMLWVHK EHPICIFLPIV HAGADTFQPH ONIKRSALVPEY KDAHARKEQTV
421 VVQOSQEOV Htalagalae endgakeyrls SGLKCLRGL DKLALGVSQ SLCTAAPFTP
481 KIPAELHTQ TTVQYQVQGAT DGCQVFSQAM VPDQLTLTPV GLSRITAPVQ TPLLEHRSMH
541 LELDPFGOOS YVIVGVXKK IQTHWHRSGS TIGKAFETAV ROAKRAVNVG DTAAPGFOSVG
601 GANLSLGKGI HQIPOQARAFKS LGQMSWFSIQ ILIGLTLWML GLNIAKINSIS LMCALGQGLVL
661 IPLSTAVSA
```
optimized for prM-E VLP production in other Flaviviruses. This JEV signal sequence is 24 amino acids long and the sequence is as follows:

(SEQ ID NO: 4)

**[0234]** Appending the JEV signal sequence (underlined) provides the following sequence:

(SEQ ID NO: 5)

Using this sequence, codon optimization for Vaccinia was performed using the IDT Codon Optimization Tool. This yielded the following codon-optimized DNA sequence of length 2079 bp:

(SEQ ID NO: 6)

**[0236]** Using this sequence, codon optimization for Vaccinia was performed using the IDT Codon Optimization Tool. This yielded the following codon-optimized DNA sequence of length 2079 bp:
The nucleotide sequence used to encode the full-length Zika virus NS1 protein is as follows:

```
AGAAATAGCTCTCTATACCTAGGCTCCCTGTAAAGCGGACGAGGATCACTATGT
```

Preparation of NS1 Sequences for Vaccine Development

According to the Zika annotation of the French Polynesian Zika isolate (GenBank Accession AHZ13508), NS1 is present as amino acids 796-1148 of the Zika polyprotein. This sequence is 353 nt long and is as follows:

```
AGAAATAGCTCTCTATACCTAGGCTCCCTGTAAAGCGGACGAGGATCACTATGT
```

A soluble C-terminal part of the protein can be isolated and used in place of the full length protein. This sequence is provided as follows:

```
AGAAATAGCTCTCTATACCTAGGCTCCCTGTAAAGCGGACGAGGATCACTATGT
```

Example 4

Construction of Zika Sequences for Vaccine Development: Derivation of Soluble E by PCR

A ZIKV soluble E (sE) gene was cloned into pLW-73. This method for production of sE required only a C-terminal truncation, so a second method is used to derive sE by PCR from the E sequences already in-house.

In short, pGZ-02 (the original pME shuttle vector in which the prME gene was cloned into pLW-73) was used as substrate for PCR. The PCR reaction incorporated (i) a forward primer that was placed upstream of the 5′ restriction site for pLW-73 cloning (Smal), and (ii) a reverse primer that truncated the E gene at the codon encoding the final luminal amino acid (just in front of the first amino acid of the first transmembrane domain of the E protein) using a primer with a tail that introduced two stop codons as well as the 3′ restriction site for pLW-73 cloning (SalI).

A map of the PCR substrate pGZ-02 is shown in FIG. 5.

This plasmid was designed to drive expression of the following ZIKV polyprotein behind the mH5 MVA promoter:

```
1-246 MGKSGASIN WLAALAVVIA CAGWHGIGA YMYLORDNE GEAISPPTTL GNNKCYIQIN
61 DLGHTCDAIM SYCOPMLEDQ VEPDDVDCWC NTTSTWVVYG TCHHKGSEAR RSRRAVTLPS
121 HETRELOTRS QTMLREBRTT EHILIPYNWI PANPQGALAA AAIAMILESS TSQVYTVLVM
181 IILIAPAYS RCIGVSNDRF VEGNOSGDYW DVLHELHGCY TVMAAQKPTY DIELAVTVVS
241 INHAVRSYCY KASIDNASD SRCPTQGAY LDKQSDTQYY CERLVDFRGN GNQUCPEKCI
301 SLVTCARFAC SKEHTQGQIK PENHETRIML SVNQSGQGMQ IVΝΤΗΘΙΟΕΔΕΗ ΕΝΚΡΑΝΕΙΤΡ
361 NSPFHATLIG GPWELGDLCE PETGLOPSDL YLTMBNKHWN LHKKSNFMDI LPWHSQMDT
421 QTHMNNDRK LUEPFEHHAK RQTVVVLGQQ GQAYHTALAQ ALREIMGQNA GRLSUGKLC
481 RLEWKLKILK GUVSYSTCAA FTFTKIPART LHSTTVTVEPV YAGTDOPCKY PAQGAVDMQT
541 LTFVGRLLTA NPVTETEN SKMKLELDPF PGQYTVIVG GKEKITHRTN RSGSTTIGKAF
601 ENTVRGAKRM AVLQUTWAVF GSVQALNSL GQEMIQFGA ANSFPGMPS WPSQILGTL
661 LNWLGQGLWN GSISLMLCLG GQVLILGSTA V2A
```

Sequence Annotation:
- **NMD** is in **boxed italic**
- prM sequence is **underlined**
- Predicted transmembrane domains are in **BOLD**
- E protein begins at residue 190 in this sequence numbering

(Grey box) is the proposed truncation site for creating sE

Tunneling down to the DNA sequence level, primers were designed to touch down on the substrate at the following locations (indicated by gray arrows, with partial 5′ to 3′ primer sequences visible inside the arrows):

The forward (F) and reverse (R) primer sequences used here for annealing to the substrate are as follows:
**P** primer final sequence:  
5'-GACTCACTCTAGAAGCGAGAAATAATCATTAATAAGCC-3'  
[SEQ ID NO: 16] 24 nt annealing, 16 nt tail: 40 nt total  
predicted Tm: 62.8°C (annealing), 73.8°C (final)  
38% CG content

**R** primer final sequence:  
5'-'TACTAGCCGGGATGATAGCGGGATGGGAAGACGATCAAGCGGGATCTATTAATGTGGCTATTACAGTGCGAG-3'  
[SEQ ID NO: 17] 24 nt annealing, 16 nt tail: 40 nt total  
predicted Tm: 60.1°C (annealing), 73.8°C (final)  
38% CG content

PCR of the pGZ-02 plasmid with these two primers yielded a PCR product 1989 nt in length, as follows with the Small site labeled as BOLD and the Sall site labeled as BOLD ITALIC in green and the start codon and two stop codons underlined:
Once digested with Smal and Sall, this sequence was ligated into similarly digested pLW-73. The resulting plasmid was named pGZ-05. This plasmid was slightly different from pGZ-04 (the SE shuttle plasmid constructed from the synthetic gene produced by Genscript) by (1) using stop codons of sequence ‘IAG’ (as necessitated by primer design) whereas pGZ-04 uses stop codons of sequence ‘TAA’, and (2) pGZ-05 is just smaller in size than pGZ-04 because the last nt of the second codon is also the first nt of the Sall site in pGZ-05, whereas these two are juxtaposed and non-overlapping in pGZ-04.

The map for pGZ-05 is shown in FIG. 6.

For completeness, it should be noted that this construct drives expression of the following gene product from the mH5 MVA promoter:

**Example 5**

Construction of Zika sequences for vaccine development: soluble E 160628

In addition to the prME-expressing vector, a soluble version was created wherein the transmembrane domains of E was obtained from ViPR Virus Pathogen Resource annotation of the gene (http://bit.ly/29m7yfi). This sequence is as follows, with prM distinguished from E by underlining the sequence and the predicted transmembrane helix domains marked by BOLD lettering:
[0262] It should be possible to create sE by truncating the sequence after amino acid 619 to yield the following sequence for prMsE:

```
(SEQ ID NO: 21)
1 TRGSAYMY LDNDAGEAI SFPTTLMK CYIQMIDHG TCDAEMTSEC PMLDGEVREP
61 DVDCCHRNTS TWYVTGTDH KEQAEARSSR AVTLPMSKTL KLQRTSQTWL EREYTLKLI
121 EVQNMIFNLP GALAALAIAL WLGGSSTQOK VIYVMILII APYSAIRCIG VSNRDVFSGEM
181 SGWTKVVDVL ENHGVTVMA QCKPTVDEI LVTTHSNNAE VRSYCYEASI SDMAESRSCP
241 TGEEAYLKKQ SNQTVYKCTL LVXRGWNGC GLFGKSKLVT CAFPKCSEM TOSKQFPHN
301 EYRILSVSH SQHSMOIVND TEGMTDERNA KEVITPMSPR ABATLQGSF LGLLCEPRTG
361 LDPSLLYLT MTNKHGWVHKE EMHDIPLPFW HACDSTTPH WNNKEALFYP KDANAEKQTV
421 VYLSQGRELH HTALAGALEA EMESAKRRLS SGLHKCRLEM DLKLKMGVSY SLCTAAPFTT
481 KIPAELTSLK VTVVEQYAGT DGPCKVPQAM AVQMDTLPFY GRLITANFVIT TESTENSEM
541 LELDFPPGDS YVIIVGVEKKE ITHSWHRSGS TIGKAFEAIV RGKKNMVVLG DITANDFGSVG
601 GALNLSLNGI HQIPGAAPFK LFQGMSWPSQ ILIGTTLLMW GLQAAAGGGIS LMCACLGGLVL
661 IFPLSTAVSA
```

[0263] The signal sequence for prM that directs the viral polyprotein to the ER translocon is located in the C-terminal end of the C gene. This signal sequence is complicated by the fact that under normal viral infection it is targeted by both the signal peptidase (in the ER lumen) and the viral protease NS3 (in the cytoplasm). This construct was chosen to utilize a Japanese Encephalitis Virus signal sequence that has been optimized for prM-E VLP production in other Flaviviruses. This JEV signal sequence is 24 amino acids long and the sequence is as follows:

```
(SEQ ID NO: 22)
1 MGERSAGSIM WLASLAVVIA CAGA
```

[0264] Appending this signal sequence to the N-terminal of prMsE results in the following sequence:

```
(SEQ ID NO: 23)
1 MGERSAGSIM WLASLAVVIA CAGAATRGSQA YYMILDENDA GEAISFPITL GMKCYIQIM
61 DLGHICDATM SYCBMMDGE VEPDDVDCWC NTTRVTVVYQG TCHHRKGEAR RSRAATLPS
121 HSTKLQSTRS QTWLECLRTY KHLIPVHI FRRPFGHALAA AIAMWLLGSS TSQVYVLYM
181 ILLIAAPAYSI RCIGVSNRFY VGMSQGCTGW DVLVEHSGCG TVMADQKPTV DLYLTTTVTS
241 NMAEVRSYCY EASISMDASD SRCPQGEAY LDQQSDTQTV CERLVURGW GNQGGLFQKG
```
Using this sequence, codon optimization for Vaccinia was performed using the IDT Codon Optimization Tool. This yielded the following codon-optimized DNA sequence of length 1929 bp:

```
[SEQ ID NO: 24]
ATGGGAAACGATGACGCGGACATCTACATTAGGCTTCCGACTTAGGCTCT
GTATATCTGCTTGCGGGACGGGAGGATTAGAAGGAAATGAGCTAAGCT
ACCCGCGATGTGCTCTCCATGTTAGGACGATGAGATACACACACACAT
CGGAGATGGTGCTGAGACGAGCGGATGAGTGGTGGTTGCGGACCCCAAG
GGGCTGAGCAGCGAGCGGATGAGTGGTGGTTGCGGACCCCAAG
```

The preparation of the sequence used the following protocol:

1. Start with the natural sequence
2. Codon optimize DNA sequence for vaccinia virus
3. Research the sequence for homopolymer stretches of >4 nt and ≥G, ≥C, ≥T and ≥A
4. If any, interrupt all homopolymer sequences by silent mutations
5. Research sequence for vaccinia virus transcription terminator: T1,NT (UUUUUNU)
6. If any, interrupt all transcription terminator motifs by silent mutation
7. Add a second stop codon (TAA)
8. Add restriction enzymes for cloning of the Zika genes into MVA-shuttle plasmids
9. Choose the site and location of the gene into MVA virus
10. Choose the appropriate MVA-shuttle

No homopolymer stretches of >4 nt were found in the sequence for bases G, C, or T after steps 1-2. One A<sub>3</sub> sequence was found at sites 1446-1450. The AAAAA was changed to AAAGA, a silent mutation for lysine.
The sequence was scanned for internal SmaI or SalI restriction sites. A Sall restriction site (GTCGAC) was found at 688-693. This sequence was changed to GTAGAC, a silent mutation for valine.

A SmaI site (CCCGGG) was added to the 5' end of the construct. A SalI (GTCGAC) site was added to the 3' end of the construct. This yielded a final sequence of length 1947 nt as follows:

```
1  CCCCCGGATGG GAAACAGGAC AGCCGGGATCT ATAAAATGGCC TGGCGAACTT GAGCTTTGGTT
61  ATAGCTGTGT CGGAGAAGCC GCGTGAAGGA TCCGCGTATT ATATGATCT AGATCTGAA
121  GAGCGGGGAG AGGGCTATTCT ATCCCTCTAG CTCCTGTTAGT TGATTAAAGT GCTCTTTAC
181  ATGCTGGACT TAGGACGAC ACGTGATGG CCGATGTCC CAGATCTAGAT CAGATCTAGAT
241  GCTGGAGTAC AACCCAGATGA CTTCTCTGG TGGCTGAACT CGATCTCCCA ATGCTTTGT
301  TCTGAATCT ACATCCTACA GAAGCTGCAA GCTCTCTAGT ATCTGACGTG CTGCTCTCTT
361  CCCAGTCTATT CCAACAGAAA ACTTCAACGG GTGCTCTCAA CTTGCTTACA AGATCTGAA
421  TACAGAAGAC ATTTAATCTG TATAGAAAGAC GATCTCTTTC GCTACCACAG TCTACCTCTT
481  GCGGCCGCG CGATACCTTG GTAATGGTGC ATCAGACAT CCGAAAGGCT CATTTAATCT
541  GTTGAATACC TTTCTCACGG CCGCGGCGAT TCTCTACTG ATCTGCTCGT ATCGAAATCA
601  GACATGGTGG AAGAGGTGTG CGACGGAAGC TGGGTTTTAG TATGCCTCTG AGCTGGTG
661  TGGCTCAGAC ATGCTGGCGA GGATATAAAGC AGATCTGACA TCAATGTTTG TACGACACA
721  GTCTGAATACG TCCCTGCGAG ATAGCTGATG ATGTTGAAAG CATCAATATCG TAGCATCCGG
781  TCGAATGAC GATGCGCTCT ACAGAGGCTGA CAGTACTGAT ATATAGAGAC TGCTCAGCAC
841  TACATGCTGA AGAAGAAGCT ATGCTGATTG CGGCGCGATC GCCTCTCTAGA AGAAGATAGC GGAAAAGCTA
901  ATCCACCGCG AGAATCTTGG TAATCCTATG ATGTTACGAG TCCACGATC TCCGACCTCA
961  GAGATGTAGT TAAACGACAC TGACATGGAG AGCAGAGAGA AGAAGACCAA GGTGGAATTCT
1021  AGCGCAATGT CACCTGCAGT AGAGCAAGTG CTGGTGGACT TGAGACTGAGT AGGATCTTAG
1081  TCGGAGTACG AGAAGATCTAT CAATTTTCTG GTATTCTTTA ATCTTAAATAG TAAAGAACA
1141  CATGGCTTGT TCCATAGGGA ATGTTCTCTC GTATCTCCCC CTCCTCCTGA TGGAGGAGCT
1201  GATACCGGGA CACCTCTATTG GAAACAAAGG AGAACACTTG TGCAATTATA AGAAGACGAT
1261  GGCGAGCGAG AAACCCGATT TGGTCTGGTT TCCCAAGAAG GTCTGCGTCA CCAAGCCTTT
1321  GCGGAGGGCG TTGAAGCCAG AAAGGAGTTA GACTATCTTG CGCAGACCCT
1381  CAGGATGCGG GGGAATGGCA CAAAGAGGTA CAAAGTAGTTA AACACTTACA
1441  AAATGCTGAA TAAAGTGGTA CAAACCTCGA CTAAAAGGGA TAGTATATA TGAGATCAG
1501  GCCTGATATT CTCTTACCA AATACGAACGC GAGCTGTGAC AGCGATGCTG TACGCTGGAG
1561  GTGATCGTGG CGCTGATAGA TGCTCGCGC AAGGCGCGCC CCAAAATTGC AGTTGACATG
1621  CAGACTTTGG CGCCCCGGAT TGCTTTGATC AGCGGAAACC CGGTCTACCA AGATCCTCAG
1681  GAAAACCTCA AGATGCTTGT AGAAGCTAGAC CCTCCATCGC GTGACTCTGTA CATAGTCTT
1741  GGTGTTGAGG AGAAAGATAC TACGACATAG TTGCGACAGT CGGAGATGAA CATTGCTGAG
1801  GGTGTTGAGG CGAACGCTCC AGCTAGGCC AACATGCCG TGCTTTGTTA TACGCGCTAG
1861  GATTCTGGAT CCGCGCGCGT TCTCTGAAAT AGCTTTGGGA AAGTTTACCA CGAGATTTT
1921  GGAGCGGCGG TAAATTTAAAA GTACGAC
```
This construct was submitted for synthesis and cloning into pLW-73, driven by the mH5 promoter, with the P11 promoter driving GFP expression in the same plasmid and all CDS flanked 5' by B (ISSR) and 3' by G1 (G1L). This plasmid will be named pGZ-04. A map is shown in FIG. 7:

Preclinical Evaluation of MVA-Zika Vaccine Compositions

Western Blots and Electron Microscopy.

The expression of full-length prME and NS1 was confirmed by western blot (WB). MVA-Zika plaques were stained with Zika specific antibodies directed to the E proteins (FIG. 8). VLP formation was evaluated with thin section electron micrographs performed at the Emory University Apekarian Integrated Electron Microscopy Core (FIG. 9). The native conformation of the E and NS1 proteins expressed on MVA-VLPs was assessed by immunostaining using ZIKV-specific E and NS1 antibodies (Aalto Bio Reagents) and flavivirus group reactive E-specific monoclonal antibody (mAb) 4G2.

Efficacy Testing of MVA-ZIKA Vaccine Candidates in Mice.

Pre-clinical testing of MVA-ZIKV-NS1 and MVA-ZIKV-prME in mice demonstrated outstanding protection for the MVA-ZIKV-NS1 vaccine (FIG. 10). We have developed a rigorous challenge model system using high-dose intracranial/intracerebral (IC) inoculation of CD1 mice with a heterologous ZIKV strain (MR766). Protection in this context represents a very high bar for immunity and no other vaccine has yet been shown to confer protection with this challenge model. Vaccination with $10^7$ TCID$_{50}$ MVA-ZIKV-NS1 or MVA-ZIKV-prME was well tolerated by recipient mice with no overt signs of illness or weight loss prior to challenge. ELISA data demonstrated abundant Abs generated to ZIKV NS1 and E proteins, respectively (FIG. 3A). Upon challenge, MVA-ZIKV-NS1-immunized mice were completely (100%) protected after both prime only and prime-boost immunizations, whereas MVA-ZIKV-prME-immunized mice showed 60-80% protection. No significant symptoms or weight loss were observed with any vaccinated animals. In contrast, most sham-immunized animals lost weight and were euthanized according to the approved animal protocols at CDC.

Due to its structure, NS1 is expressed by cells as either a dimer that non-covalently binds to the surface of cells or as a hexamer that is secreted. As an immunogen, NS1 provides two potential targets for protective immune responses: (i) intracellular NS1 as a target for CD8+ T cell responses, and (ii) cell-associated extracellular dimeric NS1 is present as repetitive epitopes on the surface of infected cells as a target for Fc-mediated Ab killing. Expression of NS1 alone does not lead to VLP formation. Our data indicate that cells infected with the MVA-ZIKV-NS1 vaccine express both the cell-associated NS1 and secreted NS1 into the supernatant (FIG. 8).

MVA-ZIKV-prME drives excellent production of E protein in infected cells (FIG. 8) and produces VLPs that bud into the secretory pathway of cells and are secreted into supernatant (FIG. 9). This vaccination approach provides two pools of Ag to stimulate the immune system: (i) intracellular E protein, and (ii) E protein displayed on the plasma membrane surface of secreted VLPs, thereby strongly stimulating both the cellular and humoral arms of the immune system.

Using the MVA platform, two vaccines have been constructed: (1) an MVA vaccine that expresses ZIKV NS1 in host cells, leading to both endogenous expression and secretion of NS1 (MVA-ZIKV-NS1); and (2) an MVA vaccine that drives expression of ZIKV prME in host cells and has the additional feature of budding virus-like particles (VLPs) that display E protein in its native form from the same cells (MVA-ZIKV-prME). The sequences used in these vaccine constructs were derived from the Asian Surname isolate Z1106033 of the 2015 ZIKV epidemic. The Asian and American strains have maintained >96% amino acid homologies with their African ancestors (Lanciotti, R. S., Albert, A. J., Holmes, M., Saavedra, S. & del Carmen Castillo, L. Phylogeney of Zika Virus in Western Hemisphere, 2015. Emerging Infectious Disease journal 22(2016); Staut, C. B., Gorbatseychy, O., Cello, J., Wimmer, E. & Futcher, B. Comparison of African, Asian, and American Zika Viruses in Swiss Webster mice: Virulence, neutralizing antibodies, and serotypes. bioRxiv (2016) (e.g. MR766 used in our challenge studies) and immunity to one strain has been shown to confer immunity to other strains (Dowd, K. A., et al. Broadly Neutralizing Activity of Zika Virus-Immune Sera Identifies a Single Viral Serotype. Cell Rep 16, 1485-1491 (2016). Both African and Asian lineages shown to induce microcephaly in mice. NS1 and prME-sequences in our vaccine maintain 96-99% identity with their Asian and African strains indicating that our vaccines will likely be effective for all circulating strains.}

The ideal ZIKV vaccine is safe for women of child-bearing age, cost effective to manufacture, and induces protective levels of long-lasting antibody and T cell responses after a single dose. Given that ZIKV is currently circulating predominantly in developing countries, the MVA-NS1 vaccine is attractive for accelerated development of a ZIKV vaccine because it provides the potential for single-dose elicitation of durable immune responses (Marzi et al, in preparation) and cost effective manufacturability. MVA vaccines are replication competent in avian cells used for vaccine production, yet replication deficient in mammalian cells making them safe for humans, including immuno-compromised individuals. MVA has been shown to be safe in >120,000 individuals, including HIV-infected individuals, and has shown no reproductive toxicity in studies in pregnant rats (CHIMP), C. I.M.P.F.H.U., Assessment report. IMVANEX. Common name: Modified Vaccinia Ankara virus, Procedure No. EMEA/H/C/002596. (ed. (CHIMP), C. I.M.P.F.H.U.) (European Medicines Agency, London, UK, 2013). Cosma, A., et al. Therapeutic vaccination with MVA-HIV nef elicits Nef-specific T-helper cell responses in chronically HIV-infected individuals. Vaccine 22, 21-29 (2003). Carroll, M. W. & Moss, B. Host range and cytopathogenicity of the highly attenuated MVA strain of vaccinia virus: propagation and generation of recombinant viruses in a nonhuman mammalian cell line. Virology 238, 198-211 (1997). For ZIKV vaccines, WHO recommended non-live/inactivated approaches for vaccination of women of child-bearing age (WHO and experts prioritize vaccines, diagnostics and innovative vector control tools for Zika RD). (2016). Our approach is in line with this recommendation as MVA-ZIKV vaccines match the excellent safety profile of non-live/inactivated vaccines without the need for an adjuvant, and additionally offer potential for high levels of immunogenicity and efficacy after a single dose. Moreover, the NS1 vaccine poses no potential risk of induction of ADE in vaccinated subjects living in dengue endemic areas

The very high bar of intracranial inoculation with heterologous virus, MVA-ZIKV-prME vaccine showed good protection (Fig. 10) and will be tested side-by-side with MVA-ZIKV-NS1 in NHP before selection of the final candidate to be advanced into clinic. A high level of protection against ZIKV infection is expected through at least 3 different mechanisms: (1) Fe-mediated non-neutralizing antibodies that bind virus or virus infected cells displaying E or NS1 proteins and kill through such mechanisms as binding complement, initiating antibody-dependent cellular cytotoxicity and phagocytosis; (2) Ag-specific T cells targeting prME or NS1 epitopes in infected cells; and (3) neutralizing antibodies that target viral epitopes on E that are critical for virus entry (e.g. receptor binding and fusion peptides).

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

All references cited herein are incorporated by reference in their entirety.

**SEQUENCE LISTING**

```sql
"NUMBER OF SEQ ID NOS: 25"
"SEQ ID NO 1"
"LENGTH: 10374"
"TYPE: DNA"
"ORGANISM: Zika virus"
"SEQUENCE:"
"acaggtttta tttgggattt ggaaacggga gttcttggtc atgaaaaacc caaaaagaag 60"
"atcgggagga ttcgggattt tcaatagctg aaaaacgaga gtaacccgtgc tggaccccctt 120"
"tggggtggtt aagaggtgtgc cagccggatt ctctctgggt gatggggccca ttcgggatgt 180"
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"tcctggcctc atcgggaggga taataagtgc taggggaagg aagaaagagc agggccgaga 360"
"tacagtgggc gggatgttggt gcctctggct gacacagct atggcagcgg aggttctag 420"
"agcggggtt gcataacta tgaacttggga cagaagaagct gttcggagg ccatactggtt 480"
"tcaaccaca ttcgggatga taatgtttta tatacagacc atggatctgg gacaccagct 540"
"tgatgcgacc atgaggctag aatggccctat gctggatgag ggggtggaac cagatgacgt 600"
"cgatgttgg tggaaacgagc agacatcttg gcctgtgctc ggaacgtgcct ccatacaca 660"
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Val Glu Gly Glu Leu Asn Ala Ile Leu Glu Glu Asn Gly Val Gin Leu
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910 915 920 925
Arg Leu Pro Val Pro Asn Glu Leu Pro His Gly Trp Lys Ala Trp
930 935 940 945
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950 955 960 965
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970 975 980 985
Asn Ser Phe Leu Val Glu Asp His Gly Phe Val Gly Val Phe His Thr Ser
990 995 1000 1005
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Lys Ser Leu Ala Gly Pro Leu Ser His His Asn Thr Arg Glu Gly
1110 1115 1120 1125
Tyr Arg Thr Gin Met Lys Gly Pro Trp His Ser Glu Glu Leu Glu
1130 1135 1140 1145
Ile Arg  Phe Glu Glu Cys Pro  Gly Thr Lys Val His  Val Glu Glu  1070  1075  1080  
Thr Cys  Gly Thr Arg Gly Pro  Ser Leu Arg Ser Thr  Thr Ala Ser  1085  1090  1095  
Gly Arg  Val Ile Glu Glu Trp  Cys Cys Arg Glu Cys  Thr Met Pro  1100  1105  1110  
Pro Leu  Ser Phe Arg Ala Lys  Asp Gly Cys Trp Tyr  Gly Met Glu  1115  1120  1125  
Ile Arg  Pro Arg Lys Glu Pro  Glu Ser Asn Leu Val Arg Ser Met  1130  1135  1140  
Val Thr  Ala Gly Ser Thr Asp His Met Asp His Phe Ser Leu Gly  1145  1150  1155  
Val Leu  Val Ile Leu Leu Met  Val Glu Glu Gly Leu Lys Lys Arg  1160  1165  1170  
Met Thr  Thr Lys Ile Ile Ile Ser Thr Ser Met Ala Val Leu Val  1175  1180  1185  
Ala Met  Ile Leu Gly Gly Phe Ser Met Ser Asp Leu Ala Lys Leu  1190  1195  1200  
Ala Ile  Leu Met Gly Ala Thr Phe Ala Glu Met Asn Thr Gly Gly  1205  1210  1215  
Asp Val  Ala His Leu Ala Leu Ile Ala Ala Phe Lys Val Arg Pro  1220  1225  1230  
Ala Leu  Leu Val Ser Phe Ile Phe Arg Ala Asn Trp Thr Pro Arg  1235  1240  1245  
Glu Ser  Met Leu Leu Ala Leu Ala Ser Cys Leu Leu Gln Thr Ala  1250  1255  1260  
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Ala Leu  Ala Trp Leu Ala Ile Arg Ala Val Val Pro Arg Thr  1280  1285  1290  
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Gly Gly  Phe Met Leu Leu Ser Leu Lys Gly Gly Lys Gly Ser Val Lys  1325  1330  1335  
Lys Asn  Leu Pro Phe Val Met Ala Leu Gly Leu Thr Ala Val Arg  1340  1345  1350  
Leu Val  Asp Pro Ile Asn Val Val Gly Leu Leu Leu Leu Thr Arg  1355  1360  1365  
Ser Gly  Lys Arg Ser Trp Pro Pro Ser Glu Val Leu Thr Ala Val  1370  1375  1380  
Gly Leu  Ile Cys Ala Leu Ala Gly Gly Phe Ala Lys Ala Asp Ile  1385  1390  1395  
Glu Met  Ala Gly Pro Met Ala Ala Val Gly Leu Ile Val Ser  1400  1405  1410  
Tyr Val  Val Ser Gly Lys Ser Val Asp Met Tyr Ile Glu Arg Ala  1415  1420  1425  
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Val Thr Thr Asp Ile Ser Glu Met Gly Ala Asn Phe Lys Ala Asp

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Arg Gly Ala Ala Phe Gly Val Met Glu Ala Leu Gly Thr Leu Pro

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Ala Ala Gin Leu Pro Glu Thr Leu Glu Thr Ile Met Leu Leu Gly

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 Gly Arg Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp Thr Arg Ile

 Ser Arg Phe Asp Leu Glu Asn Glu Ala Leu Ile Thr Asn Gln Met

 Glu Lys Gly His Arg Ala Leu Ala Leu Ala Ile Lys Tyr Thr

 Tyr Glu Asn Lys Val Val Lys Val Leu Arg Pro Ala Glu Lys Gly

 Lys Thr Val Met Asp Ile Ile Ser Arg Gln Asp Gln Arg Gly Ser

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 Val Glu Leu Ile Arg Asn Met Glu Ala Glu Glu Val Leu Glu Met

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 Leu Gln Ser Asn Gly Trp Asp Arg Leu Lys Arg Met Ala Val Ser

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 Ala Leu Arg Phe Leu Asn Asp Met Gly Lys Val Arg Lys Asp Thr

 Glu Trp Lys Pro Ser Thr Gly Trp Asp Asn Trp Glu Glu Val

 Pro Phe Cys Ser His His Phe Asn Lys Leu His Leu Lys Asp Gly

 Arg Ser Ile Val Val Pro Cys Arg His Gln Asp Glu Leu Ile Gly

 Arg Ala Arg Val Ser Pro Gly Ala Gly Trp Ser Ile Arg Glu Thr

 Ala Cys Leu Ala Lys Ser Tyr Ala Gln Met Trp Gln Leu Leu Tyr

 Phe His Arg Arg Asp Leu Arg Leu Met Ala Asn Ala Ile Cys Ser

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<212> TYPE: PRT
<213> ORGANISM: Zika virus

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Glu Cys Pro Met Leu Asp Glu Val Glu Pro Asp Asp Val Asp Cys 50   55   60
Trp Cys Asn Thr Thr Ser Thr Trp Val Val Tyr Glu Thr Cys His His 65   70   75   80
Lys Lys Gly Glu Ala Arg Arg Ser Arg Arg Ala Val Thr Leu Pro Ser 85   90   95
His Ser Thr Arg Lys Leu Gin Thr Arg Ser Gin Thr Trp Leu Glu Ser 100  105  110
Arg Glu Tyr Thr Lys His Leu Ile Arg Val Glu Asn Trp Ile Phe Arg 115  120  125
Asn Pro Gly Phe Ala Leu Ala Ala Ala Ala Ile Ala Trp Leu Leu Gly 130  135  140
Ser Ser Thr Ser Glu Val Lys Tyr Leu Leu Val Met Ile Leu Leu Ile 145  150  155  160
Ala Pro Ala Tyr Ser Ile Arg Cys Ile Gly Val Ser Asn Asp Asp Phe 165  170  175
Val Glu Gly Met Ser Gly Glu Thr Trp Val Asp Val Val Leu Glu His 180  185  190
Gly Gly Cys Val Thr Val Met Ala Gin Asp Lys Pro Thr Val Asp Ile 195  200  205
Glu Leu Val Thr Thr Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr 210  215  220
Cys Tyr Glu Ala Ser Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro 225  230  235  240
Thr Glu Gly Glu Ala Tyr Leu Asp Lys Gin Ser Asp Thr Gin Tyr Val 245  250  255
Cys Lys Arg Thr Leu Val Asp Arg Gly Thr Gly Asn Gly Cys Gly Leu 265  265  270
Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys
Lys Met Thr Gly Lys Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile

Met Leu Ser Val His Gly Ser Gln His Ser Gly Met Ile Val Asn Asp

Thr Gly His Glu Thr Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro

Asn Ser Pro Arg Ala Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly

Leu Asp Cys Glu Pro Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr

Leu Thr Met Asn Asn Lys His Trp Leu Val His Lys Glu Trp Phe His

Asp Ile Pro Leu Pro Trp His Ala Gly Ala Asp Thr Gly Thr Pro His

Trp Asn Asn Lys Glu Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys

Arg Glu Thr Val Val Leu Gly Ser Gln Glu Gly Ala Val His Thr

Glu Ala Leu Ala Leu Ala Leu Ala Glu Met Asp Gly Ala Lys Gly Arg

Leu Ser Ser Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg

Leu Lys Gly Val Ser Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr

Lys Ile Pro Ala Glu Thr His Gly Thr Val Thr Val Glu Val Gln

Tyr Ala Gly Thr Asp Gly Pro Cys Lys Val Pro Ala Gln Met Ala Val

Asp Met Gln Thr Leu Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro

Val Ile Thr Glu Ser Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp

Pro Pro Phe Gly Asp Ser Tyr Ile Val Ile Gly Val Gly Gly Lys Lys

Ile Thr His His Trp His Ser Gly Ser Thr Ile Gly Lys Ala Phe

Glu Ala Thr Val Arg Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr

Ala Trp Asp Phe Gly Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys

Gly Ile His Gly Ile Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly

Met Ser Trp Phe Ser Gln Ile Leu Ile Gly Thr Leu Leu Met Trp Leu

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<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Zika virus

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<211> LENGTH: 693
<212> TYPE: PRT
<213> ORGANISM: Zika virus

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Met Tyr Leu Asp Arg Asn Asp Ala Gly Ala Ile Ser Phe Pro Thr
35     40     45

Thr Leu Gly Met Asn Lys Cys Tyr Ile Gln Ile Met Asp Leu Gly His
50     55     60

Thr Cys Asp Ala Thr Met Ser Tyr Glu Cys Pro Met Leu Asp Glu Gly
65     70     75     80

Val Glu Pro Asp Asp Val Asp Cys Trp Cys Asn Thr Thr Ser Thr Trp
95     100    105    110

Val Val Tyr Gly Thr Cys His His Lys Lys Gly Glu Ala Arg Arg Ser
120    125

Arg Arg Ala Val Thr Leu Pro Ser His Ser Thr Arg Lys Leu Gln Thr
115    120

Arg Ser Gln Thr Trp Leu Glu Ser Arg Glu Tyr Thr Lys His Leu Ile
135    140

Arg Val Glu Asn Trp Ile Phe Arg Asn Pro Gly Phe Ala Leu Ala Ala
145    150    155    160

Ala Ala Ile Ala Trp Leu Leu Gly Ser Ser Thr Ser Gin Lys Val Ile
165    170    175

Tyr Leu Val Met Ile Leu Leu Ile Ala Pro Ala Tyr Ser Ile Arg Cys
190    195    200

Ile Gly Val Ser Asn Arg Phe Val Glu Gly Met Ser Gly Gly Thr
196    200    205

Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr Val Met Ala
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Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr Thr Val Ser
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Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser Ile Ser Asp
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Met Ala Ser Asp Ser Arg Cys Pro Thr Gln Gly Glu Ala Tyr Leu Asp
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Lys Gln Ser Asp Thr Gln Tyr Val Cys Lye Arg Thr Leu Val Asp Arg
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**LENGTH:** 693
**TYPE:** PRT
**ORGANISM:** Zika virus

**SEQUENCE:** 6

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Thr Leu Gly Met Asp Gln Tyr Ile Gln Ile Met Asp Leu Gly His
50    55    60
Thr Cys Asp Ala Thr Met Ser Tyr Glu Cys Pro Met Leu Asp Glu Gly
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Val Glu Pro Asp Asp Val Asp Trp Cys Asn Thr Thr Ser Thr Trp
85    90    95
Val Val Tyr Gly Thr Cys His His Lys Lys Gly Glu Ala Arg Arg Ser
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Arg Arg Ala Val Thr Leu Pro Ser His Ser Thr Arg Lys Leu Gln Thr
115   120   125
Arg Ser Gln Thr Trp Leu Glu Ser Arg Glu Tyr Thr Tyr His Leu Ile
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Arg Val Glu Asn Trp Ile Phe Arg Asn Pro Gly Phe Ala Leu Ala Ala
145   150   155   160
Ala Ala Ile Ala Trp Leu Leu Gly Ser Ser Thr Ser Gin Lys Val Ile
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Tyr Leu Val Met Ile Leu Leu Ala Pro Ala Tyr Ser Ile Arg Cys
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Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr Val Met Ala
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Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr Thr Thr Val Ser
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Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser Ile Ser Asp
245   250   255
Met Ala Ser Asp Ser Arg Cys Pro Thr Gin Gly Glu Ala Tyr Leu Asp
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Cys Ala Lys Phe Ala Cys Ser Lys Met Thr Gly Lys Ser Ile Gln
305   310   315
Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His Gly Ser Gin
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His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr Asp Glu Asn
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Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala Glu Ala Thr
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Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg Gly Ala Lys
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Val Gly Cys Ser Val Asp Phe Ser Lys Lye Glu Thr Arg Cys Gly Thr
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Gly Val Phe Val Tyr Asn Asp Val Glu Ala Trp Arg Asp Arg Tyr Lys
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Tyr His Pro Asp Ser Pro Arg Arg Leu Ala Ala Ala Val Lys Gln Ala
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Trp Glu Asp Gly Ile Cys Gly Ile Ser Ser Val Ser Arg Met Glu Asn
  50  55  60

Ile Met Trp Arg Ser Val Glu Gly Glu Leu Asn Ala Ile Leu Glu Glu
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Asn Gly Val Gln Leu Thr Val Val Val Gly Ser Val Lys Asn Pro Met
  85  90  95

Trp Arg Gly Pro Gln Arg Leu Pro Val Pro Val Asn Glu Leu Pro His
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Gly Trp Lys Ala Trp Gly Lys Ser Tyr Phe Val Arg Ala Ala Ala Lys Thr
 115 120 125

Asn Asn Ser Phe Val Asp Gly Asp Thr Leu Lys Gly Cys Pro Leu
 130 135 140

Lys His Arg Ala Trp Asn Ser Phe Leu Val Glu Asp His Gly Phe Gly
 145 150 155 160

Val Phe His Thr Ser Val Trp Lys Val Arg Glu Asp Tyr Ser Leu
 165 170 175

Glu Cys Asp Pro Ala Val Ile Gly Thr Ala Val Lys Gly Lys Glu Ala
 180 185 190

Val His Ser Asp Leu Gly Tyr Trp Ile Glu Ser Gly Lys Asn Asp Thr
 195 200 205

Trp Arg Leu Lys Arg Ala His Leu Ile Glu Met Lys Thr Cys Glu Trp
 210 215 220

Pro Lys Ser His Thr Leu Trp Thr Asp Gly Ile Glu Glu Ser Asp Leu
 225 230 235 240

Ile Ile Pro Lys Ser Leu Ala Gly Pro Leu Ser His His Asn Thr Arg
 245 250 255

Glu Gly Tyr Arg Thr Glu Met Lys Gly Pro Trp His Ser Glu Glu Leu
 260 265 270

Glu Ile Arg Phe Glu Glu Cys Pro Gly Thr Lys Val His Val Glu Glu
 275 280 285

Thr Cys Gly Thr Arg Gly Pro Ser Leu Arg Ser Thr Thr Ala Ser Gly
 290 295 300

Arg Val Ile Glu Glu Trp Cys Cys Arg Glu Cys Thr Met Pro Pro Leu
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**ORGANISM: Zika virus**

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Arg Arg Ala Val Thr Leu Pro Ser His Ser Thr Arg Lys Leu Gln Thr
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Arg Ser Glu Thr Trp Leu Glu Ser Arg Glu Tyr Thr Lys His Leu Ile
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Leu Ala Ile Ala Trp Leu Leu Gly Ser Ser Thr Ser Glu Lys Val Ile
165 170 175
Tyr Leu Val Met Ile Leu Leu Ile Ala Pro Ala Tyr Ser Ile Arg Cys
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Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr Thr Val Ser
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260 265 270
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ataaaccctcg gagcaacact gatgattttgt ccagcagccact cagttatagt gcagaggtga 780
gctgattgt ttcagaaacc ctccccctgt ccagcggcgact cggccctcgg 840
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ttgacaggg atggggtgcc gttgtgtgtaa aaggaagctca gttgacgtgct 960
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acagtatct gttataagtcc agcaaggagtc agcaaggtcg aatgactgta aacgacagtg
agca aaaccgtcg ttgctgagct ggtaccttac ccctcttcctg ggtaccttac ccctcttcctg
acaaacaagag cacactttggt cagttttaaag atgcgcagtc gaacgagcaca acgctaggg
1020
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1080
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1200
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1260
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1380
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1440
cgctcacttg gcacagatgg gatcaacataa cttttgctgc gctggagaggg ccccttgggctc
1500
gttgtaaagag atgcgcagtc cctgccttaa gctggactgtg cctgccttaa gctggactgtg
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cgactagta
1680
<210> SEQ ID NO 19
<211> LENGTH: 643
<212> TYPE: PRT
<213> ORGANISM: Zika virus
<400> SEQUENCE: 19

Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser Leu Ala
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Val Val Ile Ala Cys Ala Gly Ala Thr Arg Arg Gly Ser Ala Tyr Tyr
20 25 30
Met Tyr Leu Arg Arg Asp Ala Gly Glu Ala Ile Ser Phe Pro Thr
35 40 45
Thr Leu Gly Met Asp Ala Gly Lys Tyr Ile Gly Ile Met Asp Leu Gly His
50 55 60
Thr Cys Asp Ala Thr Met Ser Tyr Glu Cys Pro Met Leu Asp Glu Gly
65 70 75 80
Val Glu Pro Asp Arg Val Asp Cys Thr Asn Thr Ser Thr Thr Trp
85 90 95
Val Val Tyr Gly Thr Cys His His Lys Gly Gly Ala Arg Arg Ser
100 105 110
Arg Arg Ala Val Thr Leu Pro Ser His Ser Thr Arg Lys Leu Gln Thr
115 120 125
Arg Ser Gln Thr Trp Leu Glu Ser Arg Glu Tyr Thr Lys His Leu Ile
130 135 140
Arg Val Gly Asn Thr Leu Leu Gly Ser Ser Thr Ser Gln Lys Val Ile
145 150 155 160
Ala Ala Ile Ala Trp Leu Leu Gly Ser Ser Thr Ser Gln Lys Val Ile
165 170 175
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Tyr Leu Val Met Ile Leu Leu Ile Ala Pro Ala Tyr Ser Ile Arg Cys
180 185 190
Ile Gly Val Ser Asn Arg Asp Phe Val Gly Met Ser Gly Gly Thr
195 200 205
Trp Val Asp Val Val Leu His Gly Cys Val Thr Val Met Ala
210 215 220
Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr Thr Ser
225 230 235 240
Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser Ile Ser Asp
245 250 255
Met Ala Ser Asp Ser Arg Cys Pro Thr Gin Gly Glu Ala Tyr Leu Asp
260 265 270
Lys Gln Ser Asp Thr Gin Tyr Val Cys Arg Thr Leu Val Asp Arg
275 280 285
Gly Trp Gly Asn Gly Cys Leu Phe Gly Lys Gly Ser Leu Val Thr
290 295 300
Cys Ala Lys Phe Ala Cys Ser Lys Met Thr Gly Lys Ser Ile Gln
305 310 315 320
Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His Gly Ser Gin
325 330 335
His Ser Gly Met Ile Val Asn Gin Thr Gin His Thr Asp Glu Asn
340 345 350
Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala Glu Ala Thr
355 360 365
Leu Gly Phe Gly Ser Leu Gly Leu Asp Cys Pro Arg Thr Gly
370 375 380
Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Gin Lys His Trp
385 390 395 400
Leu Val His Lys Glu Trp Phe His Asp Ile Pro Leu Pro Trp His Ala
405 410 415
Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu Ala Leu Val
420 425 430
Glu Phe Lys Asp Ala His Ala Lys Arg Gin Thr Val Val Leu Gly
435 440 445
Ser Gin Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala Leu Glu Ala
460 465 470 475 480
Glu Met Asp Gly Ala Lys Gly Arg Ser Ser Gly His Leu Lys Cys
485 490 495
Arg Leu Lys Met Asp Lys Leu Arg Leu Gly Val Ser Tyr Ser Leu
500 505 510
Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu Thr Leu His
520 525
Gly Thr Val Thr Val Glu Val Gin Tyr Ala Gly Thr Asp Gly Pro Cys
530 535 540
Lys Val Pro Ala Gln Met Ala Val Asp Met Gin Thr Leu Thr Pro Val
545 550 555 560
Gly Arg Leu Ile Thr Ala Asn Pro Val Ile Thr Glu Ser Thr Glu Asn
570 575
Val Ile Gly Val Gly Glu Lys Lys Ile Thr His His Trp His Arg Ser
580 585 590

Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg Gly Ala Lys
595 600 605

Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly
610 615 620

Gly Ala Leu Asn Ser Leu Gly Lys Gly Ile His Gln Ile Phe Gly Ala
625 630 635 640

 Ala Phe Lys

<210> SEQ ID NO: 20
<211> LENGTH: 669
<212> TYPE: PRT
<213> ORGANISM: Zika virus

<400> SEQUENCE: 20
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1  5   10  15

Gly Glu Ala Ile Ser Phe Pro Thr Thr Leu Gly Met Asn Lys Cys Tyr
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Ile Gln Ile Met Asp Leu Gly His Thr Cys Asp Ala Thr Met Ser Tyr
35 40 45

Glu Cys Pro Met Leu Asp Glu Val Glu Pro Asp Asp Val Asp Cys
50 55 60

Trp Cys Asn Thr Thr Ser Thr Trp Val Val Tyr Gly Thr Cys His His
65 70 75 80

Lys Lys Gly Glu Ala Arg Arg Ser Arg Arg Ala Val Thr Leu Pro Ser
85 90 95

His Ser Thr Arg Lys Leu Gln Thr Arg Ser Gln Thr Trp Leu Glu Ser
100 105 110

Arg Glu Tyr Thr Lys His Leu Ile Arg Val Glu Asn Trp Ile Phe Arg
115 120 125

Asn Pro Gly Phe Ala Leu Ala Ala Ala Ile Ala Trp Leu Leu Gly
130 135 140

Ser Ser Thr Ser Gln Lys Val Ile Tyr Leu Val Met Ile Leu Ile
145 150 155 160

 Ala Pro Ala Tyr Ser Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe
165 170 175

Val Glu Gly Met Ser Gly Thr Trp Val Asp Val Val Leu Glu His
180 185 190

Gly Gly Cys Val Thr Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile
195 200 205

Glu Leu Val Thr Thr Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr
210 215 220

Cys Tyr Glu Ala Ser Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro
225 230 235 240

Thr Glu Gly Glu Ala Tyr Leu Asp Lys Gln Ser Thr Gln Tyr Val
245 250 255

Cys Lys Arg Thr Leu Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu
260 265 270

Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys
275 280 285
Lys Met Thr Gly Lys Ser Ile Glu Pro Glu Asn Leu Glu Tyr Arg Ile
290 295 300
Met Leu Ser Val His Gly Ser Gln His Ser Gly Met Ile Val Asn Asp
305 310 315 320
Thr Gly His Glu Thr Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro
325 330 335
Asn Ser Pro Arg Ala Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly
340 345 350
Leu Asp Cys Glu Pro Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr
355 360 365
Leu Thr Met Asn Asn Lys His Trp Leu Val His Lys Glu Trp Phe His
370 375 380
Asp Ile Pro Leu Pro Trp His Ala Gly Ala Asp Thr Gly Thr Pro His
385 390 395 400
Trp Asn Asn Lys Glu Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys
405 410 415
Arg Glu Thr Val Val Val Leu Gly Ser Gin Glu Gly Ala Val His Thr
420 425 430
Ala Leu Ala Gly Ala Leu Glu Ala Glu Met Asp Gly Ala Lys Gly Arg
435 440 445
Leu Ser Gly His His Lys Cys Arg Leu Lys Met Asp Lys Leu Arg
450 455 460
Leu Lys Gly Val Ser Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr
465 470 475 480
Lys Ile Pro Ala Glu Thr Leu His Gly Thr Val Thr Val Glu Val Gin
485 490 495
Tyr Ala Gly Thr Asp Gly Pro Cys Lys Val Pro Ala Gin Met Ala Val
500 505 510
Asp Met Gin Thr Leu Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro
515 520 525
Val Ile Thr Glu Ser Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp
530 535 540
Pro Pro Phe Gly Asp Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys
545 550 555 560
Ile Thr His His His Thr His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe
565 570 575
Glu Ala Thr Val Arg Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr
580 585 590
Ala Trp Asp Phe Gly Ser Val Gly Gly Ala Leu Asn Ser Leu Gly Lys
595 600 605
Gly Ile His Gin Ile Phe Gly Ala Ala Phe Lys Ser Leu Phe Gly Gly
610 615 620
Met Ser Trp Phe Ser Gin Ile Leu Ile Gly Thr Leu Leu Met Trp Leu
625 630 635 640
Gly Leu Asn Ala Lys Asn Gly Ser Ile Ser Met Cys Leu Ala Leu
645 650 655
Gly Gly Val Leu Ile Phe Leu Ser Thr Ala Val Ser Ala
660 665
ORGANISM: Zika virus

SEQUENCE: 60

Thr Arg Arg Gly Ser Ala Tyr Tyr Met Tyr Leu Asp Arg Asn Asp Ala
Gly Glu Ala Ile Ser Phe Pro Thr Thr Leu Gly Met Asn Lys Cys Tyr
Ile Gln Ile Met Asp Leu Gly His Thr Cys Asp Ala Thr Met Ser Tyr
Glu Cys Pro Met Leu Asp Glu Gly Val Glu Pro Asp Asp Val Asp Cys
Trp Cys Asn Thr Thr Ser Thr Trp Val Tyr Gly Thr Cys His His
Lys Lys Gly Glu Ala Arg Arg Arg Arg Ala Val Thr Leu Pro Ser
His Ser Thr Arg Lys Leu Gln Thr Arg Ser Gln Thr Trp Leu Glu Ser
Arg Glu Tyr Thr Lys His Leu Ile Arg Val Glu Asn Trp Ile Phe Arg
Asn Pro Gly Phe Ala Leu Ala Ala Ala Ile Ala Ala Ile Ala Trp Leu Leu Gly
Ser Ser Thr Ser Gln Lys Val Ile Tyr Leu Val Met Ile Leu Leu Ile
Ala Pro Ala Tyr Ser Ile Arg Cys Ile Gly Val Ser Asn Arg Asp Phe
Val Glu Gly Met Ser Gly Thr Trp Val Asp Val Val Leu Glu His
Gly Gly Cys Val Thr Val Met Ala Gln Asp Lys Pro Thr Val Asp Ile
Glu Leu Val Thr Thr Thr Val Ser Asn Met Ala Glu Val Arg Ser Tyr
Cys Tyr Glu Ala Ser Ile Ser Asp Met Ala Ser Asp Ser Arg Cys Pro
Thr Glu Gly Glu Ala Tyr Leu Asp Lys Gln Ser Asp Thr Gln Tyr Val
Cys Lys Arg Thr Leu Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu
Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Ala Cys Ser Lys
Lys Met Thr Gly Lys Ser Ile Gln Pro Glu Asn Leu Glu Tyr Arg Ile
Met Leu Ser Val His Gly Ser Gln His Ser Gly Met Ile Val Asn Asp
Thr Gly His Glu Thr Asp Glu Asn Arg Ala Lys Val Glu Ile Thr Pro
Asn Ser Pro Arg Ala Glu Ala Thr Leu Gly Gly Phe Gly Ser Leu Gly
Leu Asp Cys Glu Pro Arg Thr Gly Leu Asp Phe Ser Asp Leu Tyr Tyr
Leu Thr Met Asn Asn Lys His Trp Leu Val His Lys Glu Trp Phe His
Asp Ile Pro Leu Pro Trp His Ala Gly Ala Asp Thr Gly Thr Pro His 385 390 395 400
Trp Asn Asn Lys Glu Ala Leu Val Glu Phe Lys Asp Ala His Ala Lys 405 410 415
Arg Gln Thr Val Val Val Gly Ser Gin Glu Gly Ala Val His Thr 420 425 430 435
Ala Leu Ala Gly Ala Leu Gly Ala Glu Met Asp Gly Ala Lys Gly Arg 440 445
Leu Ser Ser Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Arg 450 455 460
Leu Lys Gly Val Ser Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr 465 470 475 480
Lys Ile Pro Ala Glu Thr Leu His Gly Thr Val Thr Val Glu Val Gin 485 490 495 500
Tyr Ala Gly Thr Asp Gly Pro Cys Lys Val Pro Ala Gin Met Ala Val 505 510
Asp Met Gin Thr Leu Thr Pro Val Gly Arg Leu Ile Thr Ala Asn Pro 515 520 525
Val Ile Thr Glu Ser Thr Glu Asn Ser Lys Met Met Leu Glu Leu Asp 530 535 540
Pro Pro Phe Gly Asp Ser Tyr Ile Val Ile Gly Val Gly Glu Lys Lys 545 550 555 560
Ile Thr His His Trp His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe 565 570 575
Glu Ala Thr Val Arg Gly Ala Lys Arg Met Ala Val Leu Gly Asp Thr 580 585 590
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Gly Ile His Gin Ile Phe Gly Ala Ala Phe Lys 610 615

<210> SEQ ID NO 22
<211> LENGTH: 643
<212> TYPE: PRT
<213> ORGANISM: Zika virus

<400> SEQUENCE: 22

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Val Val Ile Ala Cys Ala Gly Ala 20

<210> SEQ ID NO 23
<211> LENGTH: 643
<212> TYPE: PRT
<213> ORGANISM: Zika virus

<400> SEQUENCE: 23

Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser Leu Ala 1 5 10 15
Val Val Ile Ala Cys Ala Gly Ala Thr Arg Arg Gly Ser Ala Tyr Tyr 20 25 30
Met Tyr Leu Asp Arg Asn Asp Ala Gly Glu Ala Ile Ser Phe Pro Thr 35 40 45
Thr Leu Gly Met Asn Lys Cys Tyr Ile Gln Ile Met Asp Leu Gly His
50 55 60

Thr Cys Asp Ala Thr Met Ser Tyr Glu Cys Pro Met Leu Asp Glu Gly
65 70 75 80

Val Glu Pro Asp Asp Val Asp Cys Trp Cys Asn Thr Thr Ser Thr Trp
90 90 95

Val Val Tyr Gly Thr Cys His His Lys Lys Gly Glu Ala Arg Arg Ser
100 105 110

Arg Arg Ala Val Thr Leu Pro Ser His Ser Thr Arg Lys Leu Glu Thr
115 120 125

Arg Ser Gln Thr Trp Leu Glu Ser Arg Glu Tyr Thr Lys His Leu Ile
130 135 140

Arg Val Glu Asn Trp Ile Phe Arg Asn Pro Gly Phe Ala Leu Ala Ala
145 150 155 160

Ala Ala Ile Ala Trp Leu Leu Gly Ser Ser Thr Ser Gln Lys Val Ile
165 170 175

Tyr Leu Val Met Ile Leu Leu Ile Ala Pro Ala Tyr Ser Ile Arg Cys
180 185 190

Ile Gly Val Ser Asn Arg Asp Phe Val Glu Gly Met Ser Gly Gly Thr
195 200 205

Trp Val Asp Val Val Leu Glu His Gly Gly Cys Val Thr Val Met Ala
210 215 220

Gln Asp Lys Pro Thr Val Asp Ile Glu Leu Val Thr Thr Val Ser
225 230 235 240

Asn Met Ala Glu Val Arg Ser Tyr Cys Tyr Glu Ala Ser Ile Ser Asp
245 250 255

Met Ala Ser Asp Ser Arg Asp Pro Thr Gln Gly Glu Ala Tyr Leu Asp
260 265 270

Lys Gln Ser Asp Thr Gln Tyr Val Cys Lys Arg Thr Leu Val Asp Arg
275 280 285

Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser Leu Val Thr
290 295 300

Cys Ala Lys Phe Ala Cys Ser Lys Lys Met Thr Gly Lys Ser Ile Gln
305 310 315 320

Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His Gly Ser Gln
325 330 335

His Ser Gly Met Ile Val Asn Asp Thr Gly His Glu Thr Asp Glu Asn
340 345 350

Arg Ala Lys Val Glu Ile Thr Pro Asn Ser Pro Arg Ala Glu Ala Thr
355 360 365

Leu Gly Gly Phe Gly Ser Leu Gly Leu Asp Cys Glu Pro Arg Thr Gly
370 375 380

Leu Asp Phe Ser Asp Leu Tyr Tyr Leu Thr Met Asn Asn Lys His Trp
385 390 395 400

Leu Val His Lys Gly Trp Phe Asp Ile Pro Leu Pro Trp His Ala
405 410 415

Gly Ala Asp Thr Gly Thr Pro His Trp Asn Asn Lys Glu Ala Leu Val
420 425 430

Glu Phe Lys Asp Ala His Ala Lys Arg Gln Thr Val Val Val Leu Gly
435 440 445

Ser Gln Glu Gly Ala Val His Thr Ala Leu Ala Gly Ala Leu Glu Ala

-continued
Glu Met Asp Gly Ala Lys Gly Arg Leu Ser Ser Gly His Leu Lys Cys
450 470 475 480
Arg Leu Lys Met Asp Lys Leu Arg Leu Lys Gly Val Ser Tyr Ser Leu
485 490 495
Cys Thr Ala Ala Phe Thr Phe Thr Lys Ile Pro Ala Glu Thr Leu His
500 505 510
Gly Thr Val Thr Val Glu Val Gln Tyr Ala Gly Thr Asp Gly Pro Cys
515 520 525
Lys Val Pro Ala Gln Met Ala Val Asp Met Gln Thr Leu Thr Pro Val
530 535 540
Gly Arg Leu Ile Thr Ala Asn Pro Ile Thr Glu Ser Thr Glu Asn
545 550 555 560
Ser Lys Met Met Leu Glu Leu Asp Pro Pro Phe Gly Asp Ser Tyr Ile
565 570 575
Val Ile Gly Val Gly Lys Lys Ile Thr His His Trp His Arg Ser
580 585 590
Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg Gly Ala Lys
595 600 605
Arg Met Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly
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625 630 635 640
Ala Phe Lys

<210> SEQ ID NO 24
<211> LENGTH: 1929
<212> TYPE: DNA
<213> ORGANISM: Zika virus
<400> SEQUENCE: 24
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ggagagcag tttcattccc tctacttttg ggtatgata agtctacat ctagactag 180
gacctaggac aacactttga tggcacagtg atctagatg gtccatgggtatatggat 240
gtagaaccag atagcactga tggctggtgc aatacagct ccaacaggg tggcttggt 300
acctgtgcc acaagaaggg tgaagctctg agatcactac gttggctgac tcttcccag 360
cattgaca caaagcttca aacacttggc tgaagaagtt ctaactcag 420
aagcatttaa ttctgtgatg gaactggctg ttctgtaacc cagttttggc ttactgcggc 480
ggctcctag cttgcttatt ggtgccatca acctcccaaa aaggtcattta ctgctagctcgt 540
attttctta tgtcctg gctctttata ctgctgtactg gtctagcagc tggagactctt 600
gtgcctggag tcgctgggag aacctgggtc atgtagctgc ctagactagtg tggatgtgc 660
aacgctcag caccagggct acactgggct gactctgact gttccctgcat aacctggct 720
aatgtgggag agtttaagtc gtatgtggca ggaacatcctttgcctagct gccgtgtgg 780
tctggctgc ctacccagggt tgaagcatct ctagataaac agatgataac aacagctgtg 840
tgtgagagga ccgtctgtga cagaggttgc ggctagggcg cggattgcct ggttaagaga 900
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**<210> SEQ ID NO 25**

**<211> LENGTH: 1947**

**<212> TYPE: DNA**

**<213> ORGANISM: Zika virus**

**<400> SEQUENCE: 25**

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ggatctgtcg gaggtgctct aaatagtctt ggaaaaggta tccaccagat atttggagca 1929
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tccagatga tgtgagatg agaaccctcaa ttcgtcgcttg acaatagtctt ggaaaaggta tccaccagat atttggagca 1920
gcgtttaaa 1929
```
1. A recombinant modified vaccinia Ankara (MVA) vector comprising at least one nucleic acid sequence encoding at least one Flavivirus protein, wherein the at least one nucleic acid sequence is inserted into the MVA vector under the control of at least one promoter compatible with poxvirus expression systems.

2. The recombinant MVA vector of claim 1 wherein the recombinant modified vaccinia Ankara (MVA) vector comprises a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural (NS) protein, wherein both the first nucleic acid sequence and the second nucleic acid sequence are inserted into the MVA vector under the control of promoters compatible with poxvirus expression systems.

3. The recombinant MVA vector of claim 2, wherein the Flavivirus structural protein is expressed from a promoter-E (PrM-E), soluble E without a transmembrane domain, E protein domain I, E protein domain II, or E protein domain, PrM, and fragments thereof.

4. The recombinant MVA vector of claim 2, wherein the Flavivirus non-structural protein is expressed from NS1, NS2A, NS2B, NS3, NS4A, NS4B or NS5.

5. The recombinant MVA vector of claim 2, wherein the Flavivirus non-structural protein is NS1.

6. The recombinant MVA vector of claim 2, wherein the Flavivirus structural protein is expressed from a deletion site selected from I, II, III, IV, V or VI, and wherein the first nucleic acid sequence is inserted into the deletion site selected from I, II, III, IV, V or VI.

7. The recombinant MVA vector of claim 2, wherein the Flavivirus structural protein is expressed from a deletion site selected from III, IV, V or VI, and wherein the first nucleic acid sequence is inserted between two essential and highly conserved MVA genes; and wherein the second nucleic acid sequence is inserted into a restructured and modified deletion III.

8. The recombinant MVA vector of claim 2, wherein the Flavivirus structural protein is expressed from a deletion site selected from III, IV, V or VI, and wherein the first nucleic acid sequence is inserted into a restructured and modified deletion III.

9. The recombinant MVA vector of claim 2, wherein the Flavivirus structural protein is expressed from a deletion site selected from III, IV, V or VI, and wherein the first nucleic acid sequence is inserted into a restructured and modified deletion III.

10. The recombinant MVA vector of claim 2, wherein the Flavivirus structural protein is expressed from a deletion site selected from III, IV, V or VI, and wherein the first nucleic acid sequence is inserted into a restructured and modified deletion III.

11. A pharmaceutical composition comprising a recombinant MVA vector comprising a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural (NS) protein, wherein both the first and second nucleic acid sequences are inserted into the MVA vector under the control of promoters compatible with poxvirus expression systems.

12. The pharmaceutical composition of claim 10, wherein the recombinant MVA vector is formulated for intraperitoneal, intramuscular, intradermal, epididymal, mucosal or intravenous administration.

13. A method of inducing an immune response in a subject in need thereof, said method comprising administering to the subject an amount of a recombinant MVA vector effective to induce an immune response, wherein the recombinant MVA vector comprises a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural (NS) protein, wherein both the first and second nucleic acid sequences are inserted into the MVA vector under the control of promoters compatible with poxvirus expression systems.

14. The method of claim 13, wherein the recombinant MVA vector is formulated for intraperitoneal, intramuscular, intradermal, epididymal, mucosal or intravenous administration.
Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus nonstructural (NS) protein, and

wherein both the first and second nucleic acid sequences are inserted into the MVA vector under the control of promoters compatible with poxvirus expression systems.

14. The method of claim 12, wherein the immune response is a humoral immune response, a cellular immune response or a combination thereof.

15. The method of claim 12, wherein the immune response comprises production of binding antibodies against the flavivirus.

16. The method of claim 12, wherein the immune response comprises production of neutralizing antibodies against the flavivirus.

17. The method of claim 12, wherein the immune response comprises production of a cell-mediated immune response against the flavivirus.

18. A method of treating a Flaviviridae virus infection in a subject in need thereof, said method comprising administering to the subject an amount of a recombinant MVA vector effective to induce an immune response, wherein the recombinant MVA vector comprises a first nucleic acid sequence encoding a Flavivirus structural protein and a second nucleic acid sequence encoding a Flavivirus non-structural (NS) protein sequence, and

wherein both the first and second nucleic acid sequences are inserted into the MVA vector under the control of promoters compatible with poxvirus expression systems.

19. The method of claim 17, wherein the Flaviviridae virus infection is caused by a Zika virus.

20. The method of claim 17, wherein the subject is exposed to Flaviviridae virus, but not yet symptomatic of Flaviviridae virus infection.

21. (canceled)