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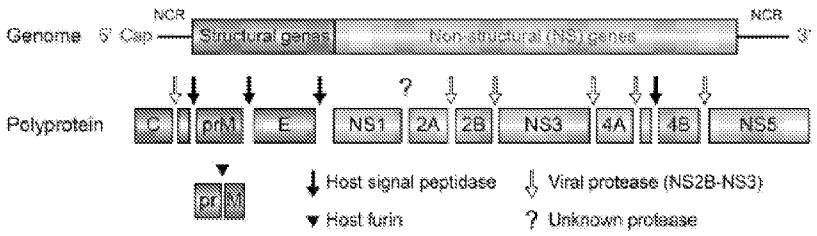
(54) Title: ZIKA VIRAL ANTIGEN CONSTRUCTS

FIG.1



(57) Abstract: Compounds useful as components of immunogenic compositions for the induction of an immunogenic response in a subject against viral infection, methods for their use in treatment, and processes for their manufacture are provided herein. The compounds comprise a nucleic acid construct comprising a sequence which encodes a Zika virus antigen. A particular embodiment is a nucleic acid-based vaccine construct encoding a polypeptide comprising a full-length Zika virus prME antigen. A praticular embodiment is a self-replicationg RNA molecule comprising a construct encoding a polypeptide comprising a full- length Zika virus prME antigen.

WO 2017/208191 A1

TITLE
ZIKA VIRAL ANTIGEN CONSTRUCTS

FIELD OF THE INVENTION

This invention is in the field of treating and preventing viral infections. In particular, the present invention relates to nucleic acid-based vaccine constructs encoding Zika viral antigens and the use of Zika viral antigens for treating and preventing Zika infections.

BACKGROUND TO THE INVENTION

Zika virus was first identified in Uganda in 1947 in rhesus monkeys through a monitoring network of sylvatic yellow fever. It was subsequently identified in humans in 1952 in Uganda and the United Republic of Tanzania. Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific. Zika virus belongs to the genus *flavivirus*. Its reservoir is unknown.

Zika virus is a plus-strand RNA virus belonging to the family *Flaviviridae*. Zika virus disease is caused by a virus transmitted primarily by *Aedes* mosquitoes. People with Zika virus disease can have symptoms that can include mild fever, skin rash, conjunctivitis, muscle and joint pain, malaise or headache. These symptoms normally last for 2-7 days.

The Zika virus is known to circulate in Africa, the Americas, Asia and the Pacific. Transmitted by *Aedes* mosquitos, the virus has been known to cause either asymptomatic infection (in the majority of people infected) or a self-limiting illness with descending rash, conjunctivitis and low grade fever. However, during the ongoing Zika virus outbreak in the Americas an alarming increase in the number of babies born with microcephaly, as well as an increase in the incidence of Guillain-Barré syndrome has been reported. In addition to microcephaly, other fetal malformations and neurological disorders have been described.

Dowd et al. (Science, Vol. 354 Issue 6309, pp. 237-40 (2016) recently reported that DNA vaccines expressing the premembrane and envelope proteins of Zika virus were immunogenic in mice and nonhuman primates when administered by electroporation or needle-free injection; and that protection against viremia after Zika virus challenge correlated with serum neutralizing activity.

Chahal et al. (Scientific Reports, 7:252, pp. 1-9 (2017)) describe an alphavirus RNA vector encoding Zika virus structural antigens. When formulated with a modified dendrimer nanomaterial and administered to mice intramuscularly, the vaccine was found to be immunogenic.

Richner et al. (Cell, 168, pp. 1–12, (2017) describe a modified mRNA vaccine encoding wild-type or mutant Zika structural proteins. When encapsulated in lipid nanoparticles and administered intramuscularly to mice, the mRNA vaccine elicited high neutralizing antibody titers and protection from viral challenge.

Given the concerning disease burden and the potential for rapid dissemination, there is an urgent need for the development of components for use in a Zika virus immunogenic or vaccine composition.

SUMMARY OF THE INVENTION

The present inventors provide constructs useful as components of immunogenic compositions for the induction of an immune response in a subject against Zika viral infection, methods for their use in treatment, and processes for their manufacture.

In some embodiments, a nucleic acid-based vaccine construct encoding a polypeptide comprising a full-length Zika virus pre-M-E antigen (prME), or an immunogenic fragment thereof is provided.

In some embodiments, a vector comprising the construct as described is provided.

In some embodiments, a self-replicating RNA molecule (also referred to herein as a self-amplifying mRNA, or SAM molecule) comprising the construct as described is provided.

In some embodiments, a composition comprising an immunologically effective amount of one or more of the constructs, vectors, or self-replicating RNA molecules as described above is provided.

In some embodiments, a composition as described above is provided wherein the composition comprises an RNA-based vaccine.

In some embodiments, a composition as described above is provided wherein the composition comprises one or more constructs, vectors, or self-replicating RNA

molecules as described above complexed with a particle of a cationic oil-in-water emulsion.

In some embodiments, a composition as described above for use in inducing an immune response against a Zika virus infection in a subject in need thereof is provided.

In some embodiments is provided a construct, a vector, a self-replicating RNA and/or molecule as described herein for use in therapy or medicine. In some embodiments, the compositions disclosed herein are for use in therapy or medicine. In preferred embodiment, the therapy is a vaccine therapy. Preferably the therapy is a vaccine to prevent Zika virus infection.

In some embodiments is provided a construct, a vector, a self-replicating RNA and/or molecule as described herein for use in preventing or treating Zika virus infection in a subject in need thereof.

In some embodiments, the compositions disclosed herein are for use in preventing or treating Zika virus infection in a subject in need thereof.

In some embodiments, a method is provided for inducing an immune response against a Zika virus infection in a subject in need thereof, which comprises administering to said subject an immunologically effective amount of a composition comprising one or more of the constructs, vectors, or self-replicating RNA molecules as described above.

In some embodiments, a method is provided for inducing an immune response sufficient to prevent or treat a Zika virus infection in a subject, which comprises administering to said subject a composition comprising one or more of the constructs, vectors, or self-replicating RNA molecules as described above in an amount sufficient to prevent or treat Zika virus infection.

In some embodiments, a method as described above is provided wherein the composition comprises one or more constructs, vectors, or self-replicating RNA molecules as described above complexed with a particle of a cationic oil-in-water emulsion.

In some embodiments, a process is provided for producing an RNA-based vaccine comprising a step of transcribing a vector or DNA molecule encoding a self-replicating RNA molecule described above to produce an RNA comprising a coding region for the antigen.

In some embodiments, a method of preparing a composition as described above is provided wherein the method comprises 1) preparing a cationic oil-in-water emulsion; 2) preparing one or more constructs, vectors, or self-replicating RNA molecules as described above; and 3) adding the one or more constructs, vectors, or self-replicating RNA molecules to the cationic oil-in-water emulsion so that the construct, vector, or self-replicating RNA molecule complexes with the emulsion.

In some embodiments, a composition produced by the process described above is provided.

In some embodiments, a use of the construct, vector, self-replicating RNA molecule, or composition described above for inducing an immune response against a Zika virus infection in a subject is provided.

In some embodiments, a use of the construct, vector, self-replicating RNA molecule, or composition described above in the manufacture of a medicament that induces an immune response against a Zika virus infection in a subject is provided.

DESCRIPTION OF DRAWINGS/FIGURES

FIG. 1: The organization of the *Flavivirus* genome, showing the polyprotein that is cleaved into structural and nonstructural proteins by a combination of viral and cellular proteases.

FIG. 2: Formation of *Flavivirus* virions and subviral particles. (1) In natural infections, *flavivirus* proteins are produced by the processing of a polyprotein translated from the viral genomic RNA and inserted co-translationally into the endoplasmic reticulum (ER) membrane. Horizontal arrows indicate polyprotein cleavages by signal peptidase and arrow heads indicate cleavage by the viral NS2B-3 protease. The open arrow indicates a signalase cleavage which is inefficient unless cytoplasmic capsid (C) cleavage has occurred. (2) The minimal requirement for production of subviral particles is the precursor membrane (prM) and envelope (E) proteins. (3) *Flavivirus* particles are formed by budding on the ER membrane driven by the prM and E proteins independent of the C protein or preformed nucleocapsids. Virus infection results predominantly in the formation of virions. (4) Nucleocapsid-free virus-like particles are efficiently produced by recombinant expression of the prM and E proteins and are a by-product of *flavivirus* infection. (5) Virions and virus-like

particles follow the exocytic pathway for secretion from infected/transfected cells. 'Cy' denotes the cytoplasmic side of the ER membrane.

FIG. 3A-D: CLUSTAL O(1.2.1) multiple sequence alignment of CprME proteins of Zika virus. See Sequences herein and SEQ ID NO:2 and SEQ ID NOS:15-23.

STRAIN	YEAR	GENBANK NUMBER
Uganda		NC_012532
Micronesia	2007	EU545988.1
Natal (Brazil)	2016	KU527068
Salvador (Brazil)	2016	KU707826.1
Sao Paulo (Brazil)	2016	KU321639
French Polynesia	2013	KJ776791

Table 1. Zika virus strains, year, and Genbank reference.

FIG.4A-C: CLUSTAL O(1.2.1) multiple sequence alignment of CprME proteins from Brazilian strains of Zika virus: Natal (SEQ ID NO:2); Salvador (SEQ ID NO:15); Genbank Accession No. KU365777 (SEQ ID NO:20); Genbank Accession No. KU365778 (SEQ ID NO:21); Genbank Accession No. KU365779 (SEQ ID NO:22); Genbank Accession No. KU365780 (SEQ ID NO:23); and Sao Paolo ("Sao") (SEQ ID NO:16). See Table 1 for Zika virus strains, year, and Genbank reference.

FIG.5: A SAM-Zika construct. The self-amplifying mRNA (SAM) background consists of VEE TC-83 replicon encoding the viral nonstructural proteins 1-4 (nsP1-4), followed by the subgenomic promoter, and either Zika prME or Zika CprME. The empty vector is shown in SEQ ID NO:24; the insert starts immediately after nucleotide 7561.

FIG.6. Design of a Zika-SAM construct encoding the viral prME proteins. The schematic shows the prM signal sequence region of the prM gene, which contains basic residues in the NH2-terminal region (n-region), a hydrophobic core uninterrupted by charged or polar residues (h-region), and a −3, −1 amino acid motif in the COOH-terminal cleavage region (c-region) suitable for signalase recognition.

The first construct is wild-type prME that has been codon-optimized for expression in mammalian cells. An uncharacteristic feature of the prM signal peptide of *flaviviruses* is the lack of polar residues in the c-region. Previously it has been shown that replacement of Gly, Phe, and Ala at positions −5, −4, and −2 with Pro, Gln, and Gln, respectively (PQAQA mutation), dramatically increases the extent of signalase cleavage of prM in vitro without requirement of prior cleavage of C. **Stocks, *et al.*** 1998. Signal peptidase cleavage at the *flavivirus* C-prM junction: dependence on the viral NS2B-3 protease for efficient processing requires determinants in C, the signal peptide, and prM. *J. Virol.* 72:2141-*2149.*

The second construct – ESS.1, has the PQAQA mutation. The third construct – ESS.2, has the native prM signal sequence replaced by the IgG signal peptide, which has been used previously for expression and secretion of IgG and Fab proteins from mammalian cells. Ciferri *et al.* (2015) "Antigenic Characterization of the HCMV gH/gL/gO and Pentamer Cell Entry Complexes Reveals Binding Sites for Potently Neutralizing Human Antibodies," *PLoS Pathog.* Oct 20;11(10):e1005230.

FIG. 7. Design of Zika-SAM constructs encoding the viral capsid and prME proteins. Zika capsid (C) protein is incorporated in constructs to test whether the presence of a cleavable capsid protein increases the efficiency of SVP generation. CprME.1 is a codon optimized nucleic acid sequence encoding the native Zika capsid, prM and E proteins, with the native NS2B-3 and signalase cleavage sites. CprME.2 is identical to CprME.1, but contains the -PQ-Q- mutation in the signal peptide C region. Finally, CprME.3 is identical to CprME.1, except that a P2A sequence is inserted at the NS2B-3 cleavage site.

FIG. 8. Analysis of expression and secretion of E protein from Zika-SAM constructs. Expression of E protein was detected by immunoblot in cell lysates for all Zika-SAM constructs tested (see Table 2): wild type prME ("WT"; construct #1), codon optimized prME ("CO", construct #2), enhanced -PQ-Q- signal sequence ("ESS.1", construct #3), IgG signal sequence ("ESS.2", construct #4), and the three capsid constructs: CprME.1 (construct #5), CprME.2 (construct #6) and CprME.3 (construct #7). As positive control, a SAM construct which expresses E protein of another *flavivirus*, Yellow Fever virus, was included ("SAM-YFV"). Negative controls included

a SAM-Respiratory Syncytial Virus ("SAM-RSV") construct and mock transfection ("Mock").

Secretion of E protein into cell supernatants was also detected by immunoblot for at least construct #1 ("WT"), construct #2 ("CO"), construct #4 (ESS.2), and construct #7 (CprME.3).

FIG 9. Immunoblots of wild type ("WT") and codon optimized ("CO") cell culture supernatants after 100 kDa cutoff concentration shows that secreted Zika E protein is present in a high molecular weight form that is retained in the column but not in the flow through. This result suggests that the secreted E protein forms higher order complexes than monomers or dimers, which is consistent with the hypothesis of SVPs.

FIG.10: Neutralizing Antibody Responses. Mice were found to have significant neutralizing Zika antibodies two weeks after a single vaccination with Zika-SAM constructs #1 or #2 (Co.prM-E and WT.prM-E, respectively)), or with positive control Zika DNA construct #5283, as measured by reporter virus particle (RVP) neutralization assay. Neutralizing antibody titers were further increased two weeks after a second vaccination with the same Zika-SAM construct or the positive control. A dose-response effect was observed for SAM constructs #1 and #2, with 15 ug of RNA eliciting more neutralizing antibodies than 1.5 ug RNA.

FIG.11: Protection from Zika Challenge: Mice vaccinated on days 0 and 21 were challenged with live Zika virus on day 49. Viral load was measured 3 days post-challenge. Vaccination with SAM constructs #1 and #2 (at 1.5 µg and 15 µg doses), as well as the positive control (Zika DNA #5283) were protective against Zika viremia, as compared to unvaccinated mice. Dotted line indicates the limit of quantification (LOQ) of the assay.

DETAILED DESCRIPTION OF THE INVENTION

Antigens; Variants; Fragments; and Constructs

The present inventors provide constructs useful as components of immunogenic compositions for the induction of an immune response in a subject against Zika viral infection constructs useful for the expression of antigens, methods

for their use in treatment, and processes for their manufacture. By "construct" is intended a nucleic acid that encodes polypeptide sequences described herein, and may comprise DNA, RNA, or non-naturally occurring nucleic acid monomers. The nucleic acid components of constructs are described more fully in the Nucleic Acids section herein.

In some embodiments, the constructs disclosed herein encode wild-type polypeptide sequences of a Zika virus, or a variant, or a fragment thereof. The constructs may further encode a polypeptide sequence heterologous to the polypeptide sequences of a Zika virus. In some embodiments, the constructs encode wild-type polypeptide sequences of a Brazilian strain Zika virus, or a variant, or a fragment thereof. By "Brazilian strain Zika virus" is intended any strain of Zika virus denoted as "Brazilian" in Table 1. Unless indicated otherwise, descriptions of the wild-type prME antigen are made by reference to the Natal strain (Brazil), GenBank number KU527068.1, as depicted in the SEQ ID NO:1 (nucleic acid) and SEQ ID NO:2 (polypeptide), and as depicted in FIG.3A-D, and FIG.4A-C.

A "variant" of a polypeptide sequence includes amino acid sequences having one or more amino acid substitutions, insertions and/or deletions when compared to the reference sequence. The variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:2. Alternatively, or in addition, a fragment of a polypeptide may comprise an immunogenic fragment (*i.e.* an epitope-containing fragment) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or more amino acids which is identical to a contiguous amino acid sequence of the full-length polypeptide.

A fragment of a polypeptide may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NO:2, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids.

As used herein, the term "antigen" refers to a molecule containing one or more epitopes (e.g., linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific immunological response (*i.e.* an immune response which specifically recognizes an antigen polypeptide). An "epitope" is that portion of an antigen that determines its immunological specificity.

T- and B-cell epitopes can be identified empirically (e.g. using PEPSCAN or similar methods). See the following references: Geysen *et al.* (1984) *PNAS USA* 81 :3998-4002; Carter (1994) *Methods Mol Biol* 36:207-23. They can be predicted (e.g. using the Jameson- Wolf antigenic index (see Jameson *et al.* (1988) *CABIOS* 4(1): 181-186), matrix-based approaches (see Raddrizzani & Hammer (2000) *Brief Bioinform* 1(2): 179-89), TEPITOPE (see De Lalla *et al.* (1999) *J. Immunol.* 163: 1725-29), neural networks (see Brusic *et al.* (1998) *Bioinformatics* 14(2): 121-30), OptiMer & EpiMer (see Meister *et al.* (1995) *Vaccine* 13(6):581-91; see Roberts *et al.* (1996) *AIDS Res Hum Retroviruses* 12(7):593-610), ADEPT (see Maksyutov & Zagrebelnaya (1993) *Comput Appl Biosci* 9(3):291-7), Tsites (see Feller & de la Cruz (1991) *Nature* 349(6311):720-1), hydrophilicity (see Hopp (1993) *Peptide Research* 6:183-190), antigenic index (see Welling *et al.* (1985) *FEBS Lett.* 188:215-218) or the methods disclosed in reference Davenport *et al.* (1995) *Immunogenetics* 42:392-297, etc.).

In some embodiments, the constructs herein encode a Zika virus prME antigen. By “Zika virus prME antigen” is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of a wild-type Zika virus structural protein prME, a variant, or a fragment thereof. FIG.3 and FIG.4 identify the amino acid sequence of several full-length wild-type Zika virus prME structural protein variants. The sequence identifier numbers for each are set forth in the Sequences section and Sequence Listing herein. See SEQ ID NOS:2 and 15-23.

Thus, where a Zika virus prME antigen is a variant of a wild-type prME polypeptide, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NOS:2 and 15-23. Alternatively, or in addition, a fragment of a polypeptide may comprise an immunogenic fragment (*i.e.* an epitope-containing fragment) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 8, at least 9, at least 10, at least 11, at

least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or more amino acids which is identical to a contiguous amino acid sequence of the full-length polypeptide.

A fragment of a Zika virus prME polypeptide may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NOS:2 and 15-23, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids. In some embodiments, the Zika virus prME polypeptide comprises a fragment selected from the group consisting of amino acids 1 to 692 of SEQ ID NO:2 and amino acids 21 to 692 of SEQ ID NO:2.

In some embodiments, an immunogenic fragment of a prME antigen comprises the full-length of the Zika virus M antigen. By "Zika virus M antigen" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of SEQ ID NO:28. Where a Zika virus M antigen is a variant of a wild-type M polypeptide, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:28.

A fragment of a Zika virus M antigen may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NO:28, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids.

In some embodiments, an immunogenic fragment of a prME antigen comprises the full-length of the Zika virus E antigen. By "Zika virus E antigen" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of SEQ ID NO:29. Where a Zika virus E antigen is a variant of a wild-type E polypeptide, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:29.

A fragment of a Zika virus E antigen may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NO:29, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. In one embodiment, a Zika virus E antigen comprises amino acids 1 to 520 of SEQ ID NO:29. It may be specified that the deletions are of consecutive amino acids.

As noted elsewhere herein, the Zika virus RNA is translated as a polyprotein comprising a prM signal sequence. The prM signal sequence is located N-terminal to the prM antigen sequence. Cleavage occurs in the ER lumen by a cellular signal peptidase and generates the N terminus of prM. Where the polyprotein comprises a wild-type amino acid sequence, the polyprotein comprises a native prM signal sequence, SEQ ID NO:5. By "native prM signal sequence" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of a signal sequence of a wild-type viral prME, SEQ ID NO:5. FIG.3A-B and FIG.4A identify the amino acid sequence of several full-length native prM signal sequence variants from various Zika virus strains.

In some embodiments, the constructs encode a native prM signal sequence. Where the prM signal sequence is a variant of a native prM signal sequence, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the full-length polypeptide according to SEQ ID NO:5. Alternatively, or in addition, a fragment of a polypeptide may comprise a functional fragment (*i.e.*, containing the sequence recognized and cleaved by the protease) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17 amino acids of SEQ ID NO: 5 which is identical to a contiguous amino acid sequence of full-length polypeptide.

In some embodiments, the construct encodes a mutated prM signal sequence for enhanced prM cleavage. By "mutated prM signal sequence for enhanced prM cleavage" is intended a prM signal sequence in which the native amino acid sequence is modified such that residues are added, replaced or deleted to increase the extent of signalase cleavage. In one embodiment, the amino acid sequence of the native

prM signal sequence is altered by the replacement of Gly, Phe, and Ala at positions −5, −4, and −2 from the signalase cleavage site with Pro, Gln, and Gln, respectively. See FIG.6. The signalase cleavage site is located at the junction of the prM signal sequence and prME antigen. This is depicted in FIG.3A and FIG.4A for several Zika virus strains. In some embodiments, the mutated prM signal sequence for enhanced prM cleavage has the amino acid sequence set forth in FIG.6 (ESS.1) (SEQ ID NO:8), or may be a variant or fragment thereof. A variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:8. Alternatively, or in addition, a fragment of a polypeptide may comprise a functional fragment (*i.e.*, containing the sequence recognized and cleaved by the protease) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, which is identical to a contiguous amino acid sequence of the full-length polypeptide.

In some embodiments, the construct encodes a heterologous, non-Zika signal sequence. In some embodiments, the construct encodes an IgG signal sequence, variant, or fragment thereof, in place of the Zika prM signal sequence. By "IgG signal sequence" is intended the amino acid sequence as set forth in FIG.6 (ESS.2) (SEQ ID NO:10). A variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:10. Alternatively, or in addition, a fragment of a polypeptide may comprise a functional fragment (*i.e.*, containing the sequence recognized and cleaved by the protease) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, which is identical to a contiguous amino acid sequence of the full-length polypeptide.

In some embodiments, the construct encodes a polypeptide comprising a cleavable capsid protein. By "capsid sequence" is intended any of the amino acid sequences designated "capsid (C)" as set forth in FIG. 3 (for example, amino acids 1 to 104 of SEQ ID NO:12). A capsid sequence may further comprise the native C and

prM cleavage sites (corresponding to the NS2B-3 and signalase cleavage sites depicted in FIG.7). Where the capsid sequence is a variant of a wild-type capsid sequence, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type capsid polypeptide, for example, to amino acids 1 to 104 of SEQ ID NO:12. Alternatively, or in addition, a fragment of a polypeptide may comprise a functional fragment (*i.e.*, containing the sequence recognized and cleaved by the protease) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 25, at least 50 and at least 75 amino acids which is identical to a contiguous amino acid sequence of the full-length capsid polypeptide.

A fragment of a Zika virus capsid protein may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example amino acids 1 to 104 of SEQ ID NO:12, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence.

In some embodiments, the construct encodes a polypeptide comprising a porcine teschovirus-1 2A cleavage sequence. By "porcine teschovirus-1 2A cleavage sequence" is intended the amino acid sequence as set forth in FIG.7 (P2A sequence) (SEQ ID NO:14).

In some embodiments, the construct encodes a polypeptide comprising from the C-terminal portion to the N-terminal portion: a prME antigen, variant, or immunogenic fragment thereof; one or more components selected from the group consisting of a prM signal sequence, variant, or fragment thereof; a mutated prM signal sequence for enhanced prM cleavage, variant, or fragment thereof; an IgG signal sequence, variant, or fragment thereof; a porcine teschovirus-1 2A cleavage sequence variant, or fragment thereof; and a capsid sequence, variant, or fragment thereof.

In some embodiments, a construct encodes each component of the polypeptide, if present, juxtaposed immediately next to the adjacent component, *i.e.*, without any intervening amino acids. In some embodiments, a linker group of 1, 2, 3, 4, or 5 amino acids is present between one or more of the components.

In some embodiments, the construct encodes a polypeptide having a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:52, and SEQ ID NO:58. In some embodiments, the construct encodes a polypeptide which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:52, and SEQ ID NO:58. In some embodiments, the construct encodes a polypeptide which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:52, and SEQ ID NO:58, wherein the fragment comprises a contiguous stretch of the amino acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids shorter than the full-length sequence.

In some embodiments, the construct comprises a DNA sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO:35, SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:51, and SEQ ID NO:57. In some embodiments, the construct comprises a DNA sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO:35, SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:51, and SEQ ID NO:57. In some embodiments, the construct comprises a DNA sequence which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO:35, SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:51, and SEQ ID NO:57 wherein the fragment comprises a contiguous stretch of the DNA sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than the full-length sequence.

In some embodiments, the construct comprises a fragment of a full-length DNA sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO:35, SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:51, and SEQ ID NO:57, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids from the 5' end, the 3' end, or both the 5' and 3' ends of the full-length sequence. In some embodiments, the construct comprises nucleic acids 1 to 2076 of

a sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:30, SEQ ID NO:35, SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:51, and SEQ ID NO:57.

In some embodiments, the construct comprises an RNA sequence selected from the group consisting of SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, and SEQ ID NO:83. In some embodiments, the construct comprises an RNA sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, and SEQ ID NO:83. In some embodiments, the construct comprises an RNA sequence which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, and SEQ ID NO:83 wherein the fragment comprises a contiguous stretch of the RNA sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than the full-length sequence.

In some embodiments, the construct comprises a fragment of a full-length RNA sequence selected from the group consisting of SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids from the 5' end, the 3' end, or both the 5' and 3' ends of the full-length sequence. In some embodiments, the construct comprises nucleic acids 1 to 2076 of a sequence selected from the group consisting of SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83.

Polypeptides

In some embodiments, a polypeptide herein is in a non-naturally occurring form (e.g. a recombinant or modified form).

For example, polypeptides (e.g. antigens) disclosed herein can be prepared by chemical synthesis (in whole or in part), by digesting longer polypeptides using proteases, by translation from RNA, by purification from cell culture (e.g. from recombinant expression), from the organism itself, etc. An exemplary method for production of peptides <40 amino acids long involves in vitro chemical synthesis, see the following references: Bodanszky (1993) Principles of Peptide Synthesis (ISBN:

0387564314); and Fields *et al*. (1997) Meth Enzymol 289: Solid-Phase Peptide Synthesis. ISBN: 0121821900. Solid-phase peptide synthesis techniques, such as methods based on tBoc or Fmoc chemistry, are known in the art, see the following reference: Chan & White (2000) Fmoc Solid Phase Peptide Synthesis. ISBN: 0199637245. Enzymatic synthesis may also be used in part or in full, see the following reference: Kullmann (1987) Enzymatic Peptide Synthesis. ISBN: 0849368413. As an alternative to chemical synthesis, biological synthesis may be used e.g. the polypeptides may be produced by translation. This may be carried out in vitro or in vivo. Biological methods are in general restricted to the production of polypeptides based on L-amino acids, but manipulation of translation machinery (e.g. of aminoacyl tRNA molecules) can be used to allow the introduction of D-amino acids (or of other non- natural amino acids, such as iodotyrosine or methylphenylalanine, azidohomoalanine, etc.), see the following reference: Kullmann (1987) Enzymatic Peptide Synthesis. ISBN: 0849368413. Where D-amino acids are included, however, it is preferred to use chemical synthesis. Polypeptides of the disclosure may have covalent modifications at the C-terminus and/or N-terminus. They can also take various forms (e.g. native, fusions, glycosylated, non-glycosylated, lipidated, non-lipidated, phosphorylated, non-phosphorylated, myristoylated, non-myristoylated, monomeric, multimeric, particulate, denatured, etc.). The polypeptides can be naturally or non- naturally glycosylated (*i.e.* the polypeptide may have a glycosylation pattern that differs from the glycosylation pattern found in the corresponding naturally occurring polypeptide).

Non-naturally occurring forms of polypeptides herein may comprise one or more heterologous amino acid sequences (e.g. another antigen sequence, another signal sequence, a detectable tag, or the like) in addition to a Zika virus prME antigen sequence. For example, a polypeptide herein may be a fusion protein. Alternatively, or in addition, the amino acid sequence or chemical structure of the polypeptide may be modified (e.g. with one or more non-natural amino acids, by covalent modification, and/or or by having a different glycosylation pattern, for example, by the removal or addition of one or more glycosyl groups) compared to a naturally-occurring polypeptide sequence.

Polypeptides (e.g. antigens) disclosed herein are preferably provided in purified or substantially purified form *i.e.* substantially free from other polypeptides (e.g. free

from naturally-occurring polypeptides), particularly from other Zika virus or host cell polypeptides; for example, at least about 50% pure (by weight), at least about 60% pure (by weight), at least about 70% pure (by weight), at least about 80% pure (by weight), or at least about 90% pure, etc. Alternatively, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, or less than about 5% of a composition is made up of other expressed polypeptides.

Nucleic Acids

The present inventors disclose herein nucleic acid molecules comprising a sequence which encodes a Zika virus prME antigen. Nucleic acids as disclosed herein can take various forms (e.g. single-stranded, double-stranded, vectors etc.). Nucleic acids may be circular or branched, but will generally be linear.

The nucleic acids used herein are preferably provided in purified or substantially purified form *i.e.* substantially free from other nucleic acids (e.g. free from naturally-occurring nucleic acids), particularly from other Zika virus or host cell nucleic acids, generally being at least about 50% pure (by weight), at least about 60% pure (by weight), at least about 70% pure (by weight), at least about 80% pure (by weight), and usually at least about 90% pure.

Nucleic acids may be prepared in many ways e.g. by chemical synthesis (e.g. phosphoramidite synthesis of DNA) in whole or in part, by digesting longer nucleic acids using nucleases (e.g. restriction enzymes), by joining shorter nucleic acids or nucleotides (e.g. using ligases or polymerases), from genomic or cDNA libraries, etc.

The term "nucleic acid" in general means a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or their analogs. It includes DNA, RNA, DNA/RNA hybrids. It also includes DNA or RNA analogs, such as those containing modified backbones (e.g. peptide nucleic acids (PNAs) or phosphorothioates) or modified bases. Thus the nucleic acid of the disclosure includes mRNA, DNA, cDNA, recombinant nucleic acids, branched nucleic acids, plasmids, vectors, etc. Where the nucleic acid takes the form of RNA, it may or may not have a 5' cap.

The nucleic acids herein comprise a sequence which encodes at least one Zika virus prME antigen. Typically, the nucleic acids of the invention will be in recombinant form, i.e. a form which does not occur in nature. For example, the nucleic acid may

comprise one or more heterologous nucleic acid sequences (e.g. a sequence encoding another antigen and/or a control sequence such as a promoter or an internal ribosome entry site) in addition to the sequence encoding at least one Zika virus prME antigen. The nucleic acid may be part of a vector i.e. part of a nucleic acid construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, "expression vectors" which are designed for expression of a nucleotide sequence in a host cell, or "viral vectors" which are designed to result in the production of a recombinant virus or virus-like particle.

Alternatively, or in addition, the sequence or chemical structure of the nucleic acid may be modified compared to a naturally-occurring sequence which encodes a Zika virus prME antigen. The sequence of the nucleic acid molecule may be modified, e.g. to increase the efficacy of expression or replication of the nucleic acid, or to provide additional stability or resistance to degradation.

The nucleic acid encoding the polypeptides described above may be codon optimized. By “codon optimized” is intended modification with respect to codon usage that may increase translation efficacy and/or half- life of the nucleic acid. A poly A tail (e.g., of about 30 adenosine residues or more) may be attached to the 3' end of the RNA to increase its half-life. The 5' end of the RNA may be capped with a modified ribonucleotide with the structure m7G (5') ppp (5') N (cap 0 structure) or a derivative thereof, which can be incorporated during RNA synthesis or can be enzymatically engineered after RNA transcription (e.g., by using Vaccinia Virus Capping Enzyme (VCE) consisting of mRNA triphosphatase, guanylyl- transferase and guanine-7-methytransferase, which catalyzes the construction of N7-monomethylated cap 0 structures). Cap 0 structure plays an important role in maintaining the stability and translational efficacy of the RNA molecule. The 5' cap of the RNA molecule may be further modified by a 2 '-O-Methyltransferase which results in the generation of a cap 1 structure (m7Gppp [m2 '-O] N), which may further increases translation efficacy.

The nucleic acids may comprise one or more nucleotide analogs or modified nucleotides. As used herein, "nucleotide analog" or "modified nucleotide" refers to a nucleotide that contains one or more chemical modifications (e.g., substitutions) in or on the nitrogenous base of the nucleoside (e.g. , cytosine (C), thymine (T) or uracil (U)), adenine (A) or guanine (G)). A nucleotide analog can contain further chemical modifications in or on the sugar moiety of the nucleoside (e.g., ribose, deoxyribose,

modified ribose, modified deoxyribose, six-membered sugar analog, or open-chain sugar analog), or the phosphate. The preparation of nucleotides and modified nucleotides and nucleosides are well-known in the art, see the following references: US Patent Numbers 4373071, 4458066, 4500707, 4668777, 4973679, 5047524, 5132418, 5153319, 5262530, 5700642. Many modified nucleosides and modified nucleotides are commercially available.

Modified nucleobases which can be incorporated into modified nucleosides and nucleotides and be present in the RNA molecules include: m5C (5-methylcytidine), m5U (5-methyluridine), m6A (N6-methyladenosine), s2U (2-thiouridine), Um (2'-0-methyluridine), mlA (1-methyladenosine); m2A (2-methyladenosine); Am (2-1-O-methyladenosine); ms2m6A (2-methylthio-N6-methyladenosine); i6A (N6-isopentenyladenosine); ms2i6A (2-methylthio-N6-isopentenyladenosine); io6A (N6-(cis-hydroxyisopentenyl)adenosine); ms2io6A (2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine); g6A (N6-glycinylcarbamoyladenosine); t6A (N6-threonyl carbamoyladenosine); ms2t6A (2-methylthio-N6-threonyl carbamoyladenosine); m6t6A (N6-methyl-N6-threonylcarbamoyladenosine); hn6A(N6-hydroxynorvalylcarbamoyl adenosine); ms2hn6A (2-methylthio-N6-hydroxynorvalyl carbamoyladenosine); Ar(p) (2'-0-ribosyladenosine (phosphate)); I (inosine); mil (1-methylinosine); m'lm (l ,2'-0-dimethylinosine); m3C (3-methylcytidine); Cm (2T-0-methylcytidine); s2C (2-thiocytidine); ac4C (N4-acetylcytidine); 5FC (5-fonnylcytidine); m5Cm (5,2-O-dimethylcytidine); ac4Cm (N4acetyl2TOmethylcytidine); k2C (lysidine); mlG (1-methylguanosine); m2G (N2-methylguanosine); m7G (7-methylguanosine); Gm (2'-0-methylguanosine); m22G (N2,N2-dimethylguanosine); m2Gm (N2,2'-0-dimethylguanosine); m22Gm (N2,N2,2'-0-trimethylguanosine); Gr(p) (2'-0-ribosylguanosine (phosphate)); yW (wybutosine); o2yW (peroxywybutosine); OHyW (hydroxywybutosine); OHyW* (undermodified hydroxywybutosine); imG (wyosine); mimG (methylguanosine); Q (queuosine); oQ (epoxyqueuosine); galQ (galtactosyl-queuosine); manQ (mannosyl-queuosine); preQo (7-cyano-7-deazaguanosine); preQi (7-aminomethyl-7-deazaguanosine); G* (archaeosine); D (dihydrouridine); m5Um (5,2'-0-dimethyluridine); s4U (4-thiouridine); m5s2U (5-methyl-2-thiouridine); s2Um (2-thio-2'-0-methyluridine); acp3U (3-(3-amino-3-carboxypropyl)uridine); ho5U (5-hydroxyuridine); mo5U (5-methoxyuridine); cmo5U (uridine 5-oxyacetic acid); mcmo5U (uridine 5-oxyacetic acid methyl ester); chm5U (5-

(carboxyhydroxymethyl)uridine)); mchm5U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm5U (5-methoxycarbonyl methyluridine); mcm5Um (S-methoxycarbonylmethyl-2-O-methyluridine); mcm5s2U (5-methoxycarbonylmethyl-2-thiouridine); nm5s2U (5-aminomethyl-2-thiouridine); mnm5U (5-methylaminomethyluridine); mnm5s2U (5-methylaminomethyl-2-thiouridine); mnm5se2U (5-methylaminomethyl-2-selenouridine); ncm5U (5-carbamoylmethyl uridine); ncm5Um (5-carbamoylmethyl-2'-0-methyluridine); cmnm5U (5-carboxymethylaminomethyluridine); cnmm5Um (5-carboxymethy 1 aminomethyl-2-L-Omethyl uridine); cmnm5s2U (5-carboxymethylaminomethyl-2-thiouridine); m62A (N6,N6-dimethyladenosine); Tm (2'-0-methylinosine); m4C (N4-methylcytidine); m4Cm (N4,2-0-dimethylcytidine); hm5C (5-hydroxymethylcytidine); m3U (3-methyluridine); cm5U (5-carboxymethyluridine); m6Am (N6,T-0-dimethyladenosine); rn62Am (N6,N6,0-2-trimethyladenosine); m2'7G (N2,7-dimethylguanosine); m2'2'7G (N2,N2,7-trimethylguanosine); m3Um (3,2T-0-dimethyluridine); m5D (5-methyldihydrouridine); £5Cm (5-formyl-2'-0-methylcytidine); mlGm (l ,2'-0-dimethylguanosine); m'Am (1 ,2-O-dimethyl adenosine) irinomethyluridine); tm5s2U (S-taurinomethyl-2-thiouridine)); iniG-14 (4-demethyl guanosine); imG2 (isoguanosine); ac6A (N6-acetyladenosine), hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(Ci-Ce)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-Ce)-alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(Ci-C6)-alkylcytosine, 5-methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine, 5-bromocytosine, N2-dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4-diaminopurine, 2,6-diaminopurine, 8-azapurine, substituted 7-deazapurine, 7-deaza-7-substituted purine, 7-deaza-8-substituted purine, hydrogen (abasic residue), m5C, m5U, m6A, s2U, W, or 2'-0-methyl-U. Many of these modified nucleobases and their corresponding ribonucleosides are available from commercial suppliers.

Nucleic Acid-based Vaccines

The present inventors disclose compositions comprising a nucleic acid sequence which encodes a polypeptide comprising a Zika virus antigen, variant or fragment thereof. Such compositions may be a nucleic acid-based vaccine. A further composition comprising a nucleic acid sequence which encodes one or more additional (e.g., a second, third, fourth, fifth or sixth) Zika virus antigens may also be provided as a nucleic acid-based vaccine. In some embodiments, a composition comprises a nucleic acid sequence encoding a Zika virus prME antigen from a first Zika virus strain and an additional nucleic acid sequence encoding an additional Zika virus prME antigen from one or more other strains of Zika virus. In some embodiments, a composition comprises a nucleic acid sequence encoding a Zika virus prME antigen and one or more additional (e.g., a second, third, fourth, fifth or sixth) Zika virus antigens. Alternatively, one or more additional non-Zika virus antigens may be encoded.

The nucleic acid may, for example, be RNA (i.e. an RNA-based vaccine) or DNA (i.e. a DNA-based vaccine, such as a plasmid DNA vaccine). In certain embodiments, the nucleic acid-based vaccine is an RNA-based vaccine. In certain embodiments, the RNA-based vaccine comprises a self-replicating RNA molecule, also referred to herein as a self-amplifying mRNA (SAM) molecule. The self-replicating RNA molecule may be an alphavirus-derived RNA replicon.

Self-replicating RNA molecules are well known in the art and can be produced by using replication elements derived from, e.g., alphaviruses, and substituting the structural viral proteins with a nucleotide sequence encoding a protein of interest. A self-replicating RNA molecule is typically a +-strand molecule which can be directly translated after delivery to a cell, and this translation provides a RNA-dependent RNA polymerase which then produces both antisense and sense transcripts from the delivered RNA. Thus the delivered RNA leads to the production of multiple daughter RNAs. These daughter RNAs, as well as collinear subgenomic transcripts, may be translated themselves to provide in situ expression of an encoded antigen (*i.e.* a Zika virus prM-F antigen), or may be transcribed to provide further transcripts with the same sense as the delivered RNA which are translated to provide in situ expression of the antigen. The overall result of this sequence of transcriptions is a huge amplification in

the number of the introduced replicon RNAs and so the encoded antigen becomes a major polypeptide product of the cells.

One suitable system for achieving self-replication in this manner is to use an alphavirus-based replicon. These replicons are +-stranded (positive sense-stranded) RNAs which lead to translation of a replicase (or replicase-transcriptase) after delivery to a cell. The replicase is translated as a polyprotein which auto-cleaves to provide a replication complex which creates genomic-strand copies of the +-strand delivered RNA. These negative sense (- -strand) transcripts can themselves be transcribed to give further copies of the +-stranded parent RNA and also to give a subgenomic transcript which encodes the antigen. Translation of the subgenomic transcript thus leads to in situ expression of the antigen by the infected cell. Suitable alphavirus replicons can use a replicase from a Sindbis virus, a Semliki forest virus, an eastern equine encephalitis virus, a Venezuelan equine encephalitis virus, etc. Mutant or wild-type virus sequences can be used e.g. the attenuated TC83 mutant of VEEV has been used in replicons, see the following reference: WO2005/113782, the context of which is incorporated by reference.

In certain embodiments, the self-replicating RNA molecule described herein encodes (i) a RNA-dependent RNA polymerase which can transcribe RNA from the self-replicating RNA molecule and (ii) a Zika virus prME antigen. The polymerase can be an alphavirus replicase e.g. comprising one or more of alphavirus proteins nsPl, nsP2, nsP3 and nsP4.

Whereas natural alphavirus genomes encode structural virion proteins in addition to the non-structural replicase polyprotein, in certain embodiments, the self-replicating RNA molecules do not encode alphavirus structural proteins. Thus, the self-replicating RNA can lead to the production of genomic RNA copies of itself in a cell, but not to the production of RNA-containing virions. The inability to produce these virions means that, unlike a wild-type alphavirus, the self-replicating RNA molecule cannot perpetuate itself in infectious form. The alphavirus structural proteins which are necessary for perpetuation in wild- type viruses are absent from self-replicating RNAs of the present disclosure and their place is taken by gene(s) encoding the immunogen of interest, such that the subgenomic transcript encodes the immunogen rather than the structural alphavirus virion proteins.

Thus a self-replicating RNA molecule useful with the invention may have two open reading frames. The first (5') open reading frame encodes a replicase; the second (3') open reading frame encodes an antigen. In some embodiments the RNA may have additional (e.g. downstream) open reading frames e.g. to encode further antigens or to encode accessory polypeptides.

In certain embodiments, the self-replicating RNA molecule disclosed herein has a 5' cap (e.g. a 7-methylguanosine). This cap can enhance in vivo translation of the RNA. In some embodiments the 5' sequence of the self-replicating RNA molecule must be selected to ensure compatibility with the encoded replicase.

A self-replicating RNA molecule may have a 3' poly-A tail. It may also include a poly- A polymerase recognition sequence (e.g. AAUAAA) near its 3' end.

Self-replicating RNA molecules can have various lengths but they are typically 5000-25000 nucleotides long. Self-replicating RNA molecules will typically be single-stranded. Single-stranded RNAs can generally initiate an adjuvant effect by binding to TLR7, TLR8, RNA helicases and/or PKR. RNA delivered in double-stranded form (dsRNA) can bind to TLR3, and this receptor can also be triggered by dsRNA which is formed either during replication of a single-stranded RNA or within the secondary structure of a single-stranded RNA.

The self-replicating RNA can conveniently be prepared by in vitro transcription (IVT). IVT can use a (cDNA) template created and propagated in plasmid form in bacteria, or created synthetically (for example by gene synthesis and/or polymerase chain-reaction (PCR) engineering methods). For instance, a DNA-dependent RNA polymerase (such as the bacteriophage T7, T3 or SP6 RNA polymerases) can be used to transcribe the self-replicating RNA from a DNA template. Appropriate capping and poly-A addition reactions can be used as required (although the replicon's poly-A is usually encoded within the DNA template). These RNA polymerases can have stringent requirements for the transcribed 5' nucleotide(s) and in some embodiments these requirements must be matched with the requirements of the encoded replicase, to ensure that the IVT-transcribed RNA can function efficiently as a substrate for its self-encoded replicase.

A self-replicating RNA can include (in addition to any 5' cap structure) one or more nucleotides having a modified nucleobase. A RNA used with the invention ideally includes only phosphodiester linkages between nucleosides, but in some

embodiments it can contain phosphoramidate, phosphorothioate, and/or methylphosphonate linkages.

The self-replicating RNA molecule may encode a single heterologous polypeptide antigen (i.e. a Zika virus prME antigen) or, optionally, two or more heterologous polypeptide antigens linked together in a way that each of the sequences retains its identity (e.g., linked in series) when expressed as an amino acid sequence. The heterologous polypeptides generated from the self-replicating RNA may then be produced as a fusion polypeptide or engineered in such a manner to result in separate polypeptide or peptide sequences.

The self-replicating RNA molecules described herein may be engineered to express multiple nucleotide sequences, from two or more open reading frames, thereby allowing co-expression of proteins, such as one, two or more Zika virus antigens (e.g. one, two or more Zika virus prME antigens) together with cytokines or other immunomodulators, which can enhance the generation of an immune response. Such a self-replicating RNA molecule might be particularly useful, for example, in the production of various gene products (e.g., proteins) at the same time, for example, as a bivalent or multivalent vaccine.

If desired, the self-replicating RNA molecules can be screened or analyzed to confirm their therapeutic and prophylactic properties using various in vitro or in vivo testing methods that are known to those of skill in the art. For example, vaccines comprising self-replicating RNA molecule can be tested for their effect on induction of proliferation or effector function of the particular lymphocyte type of interest, e.g., B cells, T cells, T cell lines, and T cell clones. For example, spleen cells from immunized mice can be isolated and the capacity of cytotoxic T lymphocytes to lyse autologous target cells that contain a self-replicating RNA molecule that encodes a Zika virus prME antigen. In addition, T helper cell differentiation can be analyzed by measuring proliferation or production of TH1 (IL-2 and IFN-γ) and /or TH2 (IL-4 and IL-5) cytokines by ELISA or directly in CD4+ T cells by cytoplasmic cytokine staining and flow cytometry.

Self-replicating RNA molecules that encode a Zika virus prME antigen can also be tested for ability to induce humoral immune responses, as evidenced, for example, by induction of B cell production of antibodies specific for a Zika virus prME antigen of interest. These assays can be conducted using, for example, peripheral B

lymphocytes from immunized individuals. Such assay methods are known to those of skill in the art. Other assays that can be used to characterize the self-replicating RNA molecules can involve detecting expression of the encoded Zika virus prME antigen by the target cells. For example, FACS can be used to detect antigen expression on the cell surface or intracellularly. Another advantage of FACS selection is that one can sort for different levels of expression; sometimes-lower expression may be desired. Other suitable method for identifying cells which express a particular antigen involve panning using monoclonal antibodies on a plate or capture using magnetic beads coated with monoclonal antibodies.

In some embodiments, the self-replicating RNA molecules comprise a sequence selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:76. In some embodiments, the self-replicating RNA molecules comprise a sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:76. In some embodiments, the self-replicating RNA molecule comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:76 wherein the fragment comprises a contiguous stretch of the nucleic acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than full-length sequence.

In some embodiments, a DNA sequence encoding a self-replicating RNA molecule is provided, said DNA sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, and SEQ ID NO:69. In some embodiments, DNA sequence encoding a self-replicating RNA molecule comprises a sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, and SEQ ID NO:69. In some embodiments, the DNA sequence encoding a self-replicating RNA molecule comprises a fragment of a full-length

sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, and SEQ ID NO:69 wherein the fragment comprises a contiguous stretch of the nucleic acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than full-length sequence.

The nucleic acid-based vaccine may comprise a viral or a non-viral delivery system. The delivery system (also referred to herein as a delivery vehicle) may have adjuvant effects which enhance the immunogenicity of the encoded Zika virus prME antigen. For example, the nucleic acid molecule may be encapsulated in liposomes, non-toxic biodegradable polymeric microparticles or viral replicon particles (VRPs), or complexed with particles of a cationic oil-in-water emulsion. In some embodiments, the nucleic acid-based vaccine comprises a cationic nano-emulsion (CNE) delivery system or a lipid nanoparticle (LNP) delivery system. In some embodiments, the nucleic acid-based vaccine comprises a non-viral delivery system, *i.e.*, the nucleic acid-based vaccine is substantially free of viral capsid. Alternatively, the nucleic acid-based vaccine may comprise viral replicon particles. In other embodiments, the nucleic acid-based vaccine may comprise a naked nucleic acid, such as naked RNA (e.g. mRNA), but delivery via CNEs or LNPs is preferred.

In certain embodiments, the nucleic acid-based vaccine comprises a cationic nano-emulsion (CNE) delivery system. CNE delivery systems and methods for their preparation are described in the following reference: WO2012/006380. In a CNE delivery system, the nucleic acid molecule (e.g. RNA) which encodes the antigen is complexed with a particle of a cationic oil-in-water emulsion. Cationic oil-in-water emulsions can be used to deliver negatively charged molecules, such as an RNA molecule to cells. The emulsion particles comprise an oil core and a cationic lipid. The cationic lipid can interact with the negatively charged molecule thereby anchoring the molecule to the emulsion particles. Further details of useful CNEs can be found in the following references: WO2012/006380; WO2013/006834; and WO2013/006837 (the contents of each of which are incorporated herein in their entirety).

Thus, in a nucleic acid-based vaccine of the invention, an RNA molecule encoding a Zika virus prME antigen may be complexed with a particle of a cationic oil-in-water emulsion. The particles typically comprise an oil core (e.g. a plant oil or squalene) that is in liquid phase at 25°C, a cationic lipid (e.g. phospholipid) and,

optionally, a surfactant (e.g. sorbitan trioleate, polysorbate 80); polyethylene glycol can also be included. In some embodiments, the CNE comprises squalene and a cationic lipid, such as 1,2-dioleoyloxy-3-(trimethylammonio)propane (DOTAP). In some preferred embodiments, the delivery system is a non- viral delivery system, such as CNE, and the nucleic acid-based vaccine comprises a self-replicating RNA (mRNA). This may be particularly effective in eliciting humoral and cellular immune responses. Advantages also include the absence of a limiting anti- vector immune response and a lack of risk of genomic integration.

In some embodiments, an RNA molecule encoding a Zika virus prME antigen may be complexed with a submicron cationic oil-in-water emulsion. In some embodiments the cationic oil-in-water emulsion is characterized by an average particle size of from about 80 nm to 180 nm in diameter (or alternatively from about 80 to about 150 nm; from about 80 to 130 nm; or from about 100 nm). In some embodiments, the concentration of DOTAP in said emulsion, before RNA complexation, is at least about 2.5 mM, or from about 2.5 mM to about 8 mM. In a particular embodiment, the concentration of DOTAP in said emulsion is about 4 mg/ml (5.73 mM). The oil can be squalene or squalane.

In some embodiments, an RNA molecule encoding a Zika virus prME antigen is complexed to a cationic oil-in-water emulsion comprising DOTAP, squalene, sorbitan trioleate and polysorbate 80 in citrate buffer. Cationic oil-in-water emulsions suitable for delivery of an RNA molecule encoding a Zika virus prME antigen may contain about 2 mg/ml to 7 mg/ml DOTAP; about 3mg/ml to 6 mg/ml Span 85; about 3 mg/ml to 6 mg/ml Tween 80; and about 30 mg/ml to 50 mg/ml squalene. In certain embodiments, the cationic oil-in-water emulsion, before complexing with RNA, contains about 4.3% w/v squalene, 0.5% Tween 80, 0.5% SPAN85, and 4 mg/mL DOTAP.

Also provided is a method of preparing a composition comprising an RNA molecule encoding a Zika virus prME antigen complexed to a cationic oil-in-water emulsion, the method comprising: (i) providing an oil-in-water emulsion as described herein; (ii) providing an aqueous solution comprising the RNA molecule; and (iii) combining the aqueous solution of (ii) and the oil-in-water emulsion of (i), thereby preparing the composition. If desired, the aqueous solution comprising the RNA molecule may be a buffer. The buffer may comprise one or more salt, buffer,

saccharide, or polymer. In an preferred embodiment, the buffer comprises 560 mM sucrose, 20 mM NaCl, and 10 mM citrate, which can be mixed with a cationic oil in water emulsion described herein to produce a final aqueous phase that comprises 280 mM sucrose, 10 mM NaCl and 10 mM citrate.

LNP delivery systems and non-toxic biodegradable polymeric microparticles, and methods for their preparation are described in the following references: WO2012/006376 (LNP and microparticle delivery systems); Geall *et al*. (2012) PNAS USA. Sep 4; 109(36): 14604-9 (LNP delivery system); and WO2012/006359 (microparticle delivery systems). LNPs are non- virion liposome particles in which a nucleic acid molecule (e.g. RNA) can be encapsulated. The particles can include some external RNA (e.g. on the surface of the particles), but at least half of the RNA (and ideally all of it) is encapsulated. Liposomal particles can, for example, be formed of a mixture of zwitterionic, cationic and anionic lipids which can be saturated or unsaturated, for example; DSPC (zwitterionic, saturated), DlinDMA (cationic, unsaturated), and/or DMG (anionic, saturated). Preferred LNPs for use with the invention include an amphiphilic lipid which can form liposomes, optionally in combination with at least one cationic lipid (such as DOTAP, DSDMA, DODMA, DLinDMA, DLenDMA, etc.). A mixture of DSPC, DlinDMA, PEG-DMG and cholesterol is particularly effective. Other useful LNPs are described in the following references: WO2012/006376; WO2012/030901; WO2012/031046; WO2012/031043; WO2012/006378; WO2011/076807; WO2013/033563; WO2013/006825; WO2014/136086; WO2015/095340; WO2015/095346; WO2016/037053. In some embodiments, the LNPs are RV01 liposomes, see the following references: WO2012/006376 and Geall *et al*. (2012) PNAS USA. Sep 4; 109(36): 14604-9.

Pharmaceutical Compositions; Immunogenic Compositions

The disclosure provides compositions comprising a nucleic acid comprising a sequence which encodes a Zika virus polypeptide, for example a Zika virus prME antigen. The composition may be a pharmaceutical composition, e.g., an immunogenic composition or a vaccine composition. Accordingly, the composition may also comprise a pharmaceutically acceptable carrier. In some embodiments, the Zika virus is a Brazilian strain Zika virus.

A "pharmaceutically acceptable carrier" includes any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The compositions may also contain a pharmaceutically acceptable diluent, such as water, saline, glycerol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier.

Pharmaceutical compositions may include the constructs, nucleic acid sequences, and/or polypeptide sequences described elsewhere herein in plain water (e.g. “w.f.i.”) or in a buffer e.g. a phosphate buffer, a Tris buffer, a borate buffer, a succinate buffer, a histidine buffer, or a citrate buffer. Buffer salts will typically be included in the 5-20mM range. Pharmaceutical compositions may have a pH between 5.0 and 9.5 e.g. between 6.0 and 8.0. Compositions may include sodium salts (e.g. sodium chloride) to give tonicity. A concentration of 10±2 mg/mL NaCl is typical, e.g. about 9 mg/mL. Compositions may include metal ion chelators. These can prolong RNA stability by removing ions which can accelerate phosphodiester hydrolysis. Thus a composition may include one or more of EDTA, EGTA, BAPTA, pentetic acid, etc.. Such chelators are typically present at between 10-500 μM e.g. 0.1 mM. A citrate salt, such as sodium citrate, can also act as a chelator, while advantageously also providing buffering activity. Pharmaceutical compositions may have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, e.g. between 240-360 mOsm/kg, or between 290-310 mOsm/kg. Pharmaceutical compositions may include one or more preservatives, such as thiomersal or 2-phenoxyethanol. Mercury-free compositions are preferred, and preservative-free vaccines can be prepared. Pharmaceutical compositions may be aseptic or sterile. Pharmaceutical compositions may be non-pyrogenic e.g. containing <1 EU (endotoxin unit, a standard measure) per dose, and preferably <0.1 EU per dose. Pharmaceutical compositions may be gluten free. Pharmaceutical compositions may be prepared in unit dose form. In some embodiments a unit dose may have a volume of between 0.1 -1.0 mL e.g. about 0.5mL.

In some embodiments, the compositions disclosed herein are immunogenic composition that, when administered to a subject, induce a humoral and/or cellular antigen-specific immune response (*i.e.* an immune response which specifically recognizes a naturally occurring Zika virus polypeptide). For example, an immunogenic composition may induce a memory T and/or B cell population relative to an untreated subject following Zika virus infection, particularly in those embodiments where the composition comprises a nucleic acid comprising a sequence which encodes a Zika virus prME antigen or comprises a Zika virus antigen. In some embodiments, the subject is a vertebrate, such as a mammal e.g. a human or a veterinary mammal.

The compositions of the invention can be formulated as vaccine compositions. The vaccine will comprise an immunologically effective amount of antigen. By "an immunologically effective amount" is intended that the administration of that amount to a subject, either in a single dose or as part of a series, is effective for inducing a measurable immune response against Zika virus in the subject. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (e.g. human, non-human primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the composition or vaccine, the treating doctor's assessment of the medical situation, the severity of the disease, the potency of the compound administered, the mode of administration, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. In one embodiment, an immunologically effective amount of a Zika virus antigen is an amount sufficient to prevent or treat Zika virus infection. Vaccines as disclosed herein may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. In some embodiments, the vaccine compositions disclosed herein may induce an effective immune response against a Zika virus infection, *i.e.*, a response sufficient for treatment or prevention of a Zika virus infection.

In some embodiments, the composition further comprises an additional antigen. In some embodiments, the composition is administered to a subject in combination with a further composition which comprises an additional antigen.

A composition of the present disclosure may also comprise, or be administered in conjunction with, one or more adjuvants (e.g. vaccine adjuvants), in particular where the composition comprises an immunologically effective amount of a nucleic acid encoding a Zika virus prME antigen or a Zika virus prME antigen. By "adjuvant" is intended that is capable of increasing an immune response against an antigen compared to administration of said antigen alone. In some aspects, adjuvant compositions as disclosed herein further comprise one or more immunostimulants, for example, a saponin such as QS21.

Adjuvants which may be used in compositions of the invention include, but are not limited to: (A) Mineral- containing compositions, for example aluminum and calcium salts, such as aluminum phosphates. (B) Oil emulsions, for example squalene-in-water emulsions, such as MF59 or AS03. Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IF A) may also be used. (C) Saponin formulations. (D) Virosomes and virus-like particles (VLPs). (E) Bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof. (F) Human immunomodulators, for example cytokines, such as interleukins, interferons, macrophage colony stimulating factor, and tumor necrosis factor. (G) Bioadhesives and mucoadhesives, such as esterified hyaluronic acid microspheres, cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. (H) Microparticles, for example particles of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10 μm in diameter) formed from materials that are biodegradable and non-toxic (e.g. a poly(a-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, etc.), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (e.g. with SDS) or a positively-charged surface (e.g. with a cationic detergent, such as CTAB). (I) Liposomes. (J) Polyoxyethylene ether and polyoxyethylene ester formulations. (K) Polyphosphazene (PCPP). (L) Muramyl peptides. (M) Imidazoquinolone compounds, for example Imiquamod and its homologues.

Combinations of one or more of the adjuvants identified above may also be used with the invention.

Methods of Use/Uses

In some embodiments are provided methods for inducing an immune response against a Zika virus infection in a subject in need thereof comprising a step of administering an immunologically effective amount of a construct or composition as disclosed herein. In some embodiments are provided the use of the constructs or compositions disclosed herein for inducing an immune response to a Zika virus prME antigen in a subject in need thereof. In some embodiments are provided the use of the constructs or compositions disclosed herein for inducing an immune response against a Zika virus infection in a subject. In some embodiments are provided use of the construct or composition as disclosed herein in the manufacture of a medicament that induces an immune response to a Zika virus infection in a subject. By "subject" is intended a vertebrate, such as a mammal e.g. a human or a veterinary mammal. In some embodiments the subject is human. By "immune response" is intended a humoral and/or cellular antigen-specific immunological response (*i.e.* an immune response which specifically recognizes an antigen polypeptide) that can be demonstrated to neutralize Zika virus *in vitro* or control/reduce/eliminate Zika virus infection *in vivo*.

In some embodiments, the immune response is characterized by immunological memory against the Zika virus and/or an effective Zika virus-responsive memory T cell population.

In some embodiments the composition comprises an RNA molecule encoding a polypeptide selected from the group consisting of SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58. In some embodiments, the composition comprises an RNA molecule encoding a polypeptide which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58. In some embodiments, the composition comprises an RNA molecule encoding a polypeptide which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:36, SEQ ID NO:41, SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58, wherein the fragment comprises a

contiguous stretch of the amino acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids shorter than full-length sequence.

In some embodiments is provided a construct, a vector, a self-replicating RNA and/or molecule as described herein for use in therapy or medicine. In some embodiments, the compositions disclosed herein are for use in therapy or medicine. In preferred embodiment, the therapy is a vaccine therapy. Preferably the therapy is a vaccine to prevent Zika virus infection.

In some embodiments is provided a construct, a vector, a self-replicating RNA and/or molecule as described herein for use in preventing or treating Zika virus infection in a subject in need thereof.

In some embodiments, the compositions disclosed herein are for use in preventing or treating Zika virus infection in a subject in need thereof.

In some embodiments, the compositions disclosed herein are for use in inducing an immune response against a Zika virus infection in a subject in need thereof.

In some embodiments is provided a construct, a vector, a self-replicating RNA molecule, and/or a composition as described herein for use in a method of inducing an immune response to a Zika virus infection in a subject in need thereof.

In some embodiments, methods are provided for preventing or shortening Zika virus infection and/or reducing or preventing the clinical symptoms upon Zika virus infection in a subject in need thereof, which comprises administering to said subject an immunologically effective amount of an immunogenic composition as provided herein.

In some embodiments is provided use of a construct or composition disclosed herein in the manufacture of an immunogenic composition for preventing or shortening Zika virus infection in a subject and/or reducing or prevent the clinical symptoms upon Zika virus infection in a subject.

In some embodiments, methods are provided for preventing or reducing transmission of a Zika virus infection from one subject to another. In specific embodiments, methods are provided for preventing or reducing transmission of a Zika virus infection to a fetus across the placental barrier. In some embodiments, a composition as described herein is administered to a woman in an amount effective to prevent transmission of a Zika virus infection across the placental barrier.

In some embodiments, methods are provided for inducing an immune response sufficient to prevent or treat a Zika virus infection in a subject, which comprises administering to said subject a composition comprising one or more of the constructs, vectors, or self-replicating RNA molecules as described above in an amount sufficient to prevent or treat Zika virus infection.In some embodiments is provided use of a construct or composition disclosed herein in the manufacture of an immunogenic composition for preventing or reducing transmission of a Zika virus infection to a fetus across the placental barrier.

In some embodiments is provided a construct, a vector, a self-replicating RNA molecule, and/or a composition as described herein for use in a method of preventing or reducing transmission of a Zika virus infection to a fetus across the placental barrier.

In some embodiments, the subject is a human subject. In specific embodiments, the human subject has been exposed, or is at risk of being exposed, to a Zika virus infection.

Routes of Administration/Dosages

Compositions disclosed herein will generally be administered directly to a subject. Direct delivery may be accomplished by parenteral injection (e.g. subcutaneously, intraperitoneally, intravenously, intramuscularly, intradermally, or to the interstitial space of a tissue). Alternative delivery routes include rectal, oral (e.g. tablet, spray), buccal, sublingual, vaginal, topical, transdermal or transcutaneous, intranasal, ocular, aural, pulmonary or other mucosal administration. Intradermal and intramuscular administration are two preferred routes. Injection may be via a needle (e.g. a hypodermic needle), but needle-free injection may alternatively be used. A typical human intramuscular dose volume is 0.5 ml.

A dose of a nucleic acid (e.g. a nucleic acid-based vaccine, such as a Zika SAM vaccine) may have about 50 μg to about 100 μg nucleic acid. In one embodiment, a Zika SAM vaccine dose contains 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 μg RNA. In other embodiments, a dose of a Zika SAM vaccine may have <10μg nucleic acid; e.g. from 1-10μg, such as about 1μg, 2.5μg, 5μg, 7.5μg or 10μg, but expression can be seen at much lower levels; e.g. using <1μg/dose, <100ng/dose, <10ng/dose, <1ng/dose, etc. Similarly, a dose of a protein antigen may have <10μg protein; e.g. from 1-10μg, such as about 1μg, 2.5μg, 5μg, 7.5μg or 10μg.

In preferred embodiments, a Zika SAM vaccine or vaccine composition is administered to a subject at an effective dose, meaning a dose sufficient to achieve a desired immune response, such as induction of neutralizing antibodies to Zika virus and/or protection against Zika virus infection.

In some embodiments, a Zika SAM vaccine described herein has an effective dose that is less than or equal to 50%, 40%, 30%, 20% or 10% of the effective dose of a DNA vaccine or vaccine composition encoding the same antigen. In some embodiments, a Zika SAM vaccine described herein has an effective dose that is one third or less of the effective dose of a DNA vaccine or vaccine composition encoding the same antigen.

Processes of Manufacture/Formulation

Processes for the manufacture of self-replicating RNA are provided herein. In some embodiments, the process of manufacturing a self-replicating RNA comprises a step of in vitro transcription (IVT) as described elsewhere herein. In some embodiments, the process of manufacturing a self-replicating RNA comprises a step of IVT to produce a RNA, and further comprises a step of combining the RNA with a non-viral delivery system as described elsewhere herein. In some embodiments, the process of manufacturing a self-replicating RNA comprises a step of IVT to produce a RNA, and further comprises a step of combining the RNA with a CNE delivery system as described elsewhere herein.

Sequence Identity

Identity or homology with respect to an amino acid sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the reference amino acid sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Identity or homology with respect to a nucleic acid sequence is defined herein as the percentage of nucleotides in the candidate sequence that are identical with the

reference nucleic acid sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff *et al.*, in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the shorter sequences in order to align the two sequences. The same methods used to compare polypeptides can also be used to calculate the percent identity of two polynucleotide sequences.

Where the present disclosure refers to a sequence by reference to a UniProt or Genbank accession code, the sequence referred to is the current version at the filing date of the present application.

General

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "plurality" refers to two or more. Additionally, numerical limitations given with respect to concentrations or levels of a substance, such as solution component concentrations or ratios thereof, and reaction conditions such as temperatures, pressures and cycle times are intended to be approximate. The term “about” used herein is intended to mean the amount ±10%.

The term “comprises” means “includes.” Thus, unless the context requires otherwise, the word “comprises,” and variations such as “comprise” and “comprising”

will be understood to imply the inclusion of a stated compound or composition (e.g., nucleic acid, polypeptide, antigen) or step, or group of compounds or steps, but not to the exclusion of any other compounds, composition, steps, or groups thereof. Embodiments described as comprising certain components are intended to include embodiments consisting of the indicated components.

The abbreviation, "e.g." is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

The invention will be further described by reference to the following, non-limiting, figures and examples.

EXAMPLES

Example 1. Project Summary

The present inventors initiated work on a Zika vaccine using the SAM platform - synthetic, self-amplifying mRNA (SAM) derived from the alphavirus genome, expressing antigens of interest. The SAM constructs are evaluated for robust antigen production and antigenicity and further tested for their immunogenicity and efficacy using in vivo models.

Methods

The SAM vector VEE TC-83 was used as the background construct for cloning in the Examples. See SEQ ID NO:24.

Example 2. Selection of Antigen

The *Flavivirus* genome consists of capped single-stranded RNA of positive polarity of approximately 11.3 kb in length (Fig. 1). The 5′ proximal quarter of the genome encodes the structural proteins capsid (C), pre-membrane (prM), and envelope (E). The nonstructural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 are involved in viral RNA replication. The coding region is flanked by 5′ and 3′ untranslated regions (5′ and 3′ UTRs) of approximately 100 and 600 nucleotides in length, respectively. Translation of the viral genome yields a single polypeptide that is processed into the individual proteins by a combination of cellular proteases and a viral protease consisting of a catalytic subunit, NS3, and its cofactor, NS2B.

The structural proteins prM and E are cotranslationally inserted into the endoplasmic reticulum (ER) membrane and processed by signal peptidases, producing proteins that encapsidate C together with the viral RNA, by budding into the ER lumen (Fig. 2). At a later step in viral maturation, prM on these particles is cleaved into mature M protein by a cellular furin protease prior to release from the cell. This prM cleavage is required for infectivity of the released virions. In addition to infectious virions, *flavivirus*-infected cells release sub-viral particles (SVPs) (Fig. 2). These particles are smaller than virions, but contain the antigenically important E protein and the prM/M protein, which is essential for correct folding and incorporation of the E protein into SVPs and viral particles. However, unlike virions, SVPs do not contain either the C protein or the viral genome, and are thus non-infectious. SVPs can be produced in a variety of systems by co-expression of the prM and E proteins, and SVPs share properties with wild-type viruses, such as fusogenic activity and induction of a neutralizing immune response and have repeatedly been shown to stimulate protective immune responses against a number of *flavivirus* diseases. The present inventors selected the structural proteins of Zika virus, namely- prM and E, and in some cases, C, for further experimentation.

Example 3. Strain Selection

The amino acid sequences of the C-prME proteins from Zika virus strains (available from NCBI/Genbank) from Zika outbreaks around the world from 2007 onwards were aligned to look for similarities and differences (Fig. 3). These included the original African lineage strain from Uganda, Micronesia (2007), French Polynesia (2013), and the Brazilian strains from 2016 (Fig.3). In addition seven strains of Zika virus from various regions in Brazil, were also compared for amino acid differences in the C-prME region (Fig. 4). A high conservation was observed across the strains from different outbreaks, with the Brazilian strains almost identical in the CprME region. The Natal, Bahia strain (KU527068) was chosen as the representative strain. KU527068 was one of the first strains to be isolated from the brain of a fetus showing microcephaly.

Example 4. Design of Constructs

The design of Zika-SAM constructs of Fig. 5 includes cloning the sequence encoding the Zika virus (Natal, Brazil strain) structural pre-membrane (prM) and envelope (E) proteins [with or without the Capsid (C)], under the subgenomic promoter in a SAM vector. A series of modifications to the SAM-prME constructs were made (Table 1, Fig. 6 and Fig. 7). These include:

i. Codon optimization of the coding sequence for the antigen (CO-prME or CO-CprME).

ii. Genetic modifications in the native prM signal peptide (Fig. 6). In addition to proteolytic processing by signal peptidases, the viral NS3/NS2B protease is also involved in maturation of the structural proteins. The junction of the C and prM region undergoes two proteolytic cleavage events during maturation. One cleavage liberates C from its trans-membrane anchor sequence and is dependent on NS2B/NS3 activity. A second cleavage occurs at the end of the C-anchor sequence in the ER lumen by a cellular signal peptidase and generates the N terminus of prM. Previous studies conclude that processing by the viral protease, regardless of the presence of the signal peptidase cleavage site, is required for efficient secretion of viral particles. However, in some *flaviviruses*, when this obligatory sequence of cleavages was uncoupled in a mutant virus, there is greatly reduced incorporation of virions into budding membranes and augmented release of subviral particles (Lobigs *et al* (2004) "Inefficient signalase cleavage promotes efficient nucleocapsid incorporation into budding *flavivirus* membranes," *J Virol*. 2004 Jan;78(1):178-86).

iii. Replacement of the native prM signal peptide with a heterogeneous signal peptide to enhance SVP generation. The present inventors used the signal peptide of IgG1 used previously for cleavage and secretion of IgG or Fab proteins (Ciferri *et al*. (2015) "Antigenic Characterization of the HCMV gH/gL/gO and Pentamer Cell Entry Complexes Reveals Binding Sites for Potently Neutralizing Human Antibodies," *PLoS Pathog*. Oct 20; 11(10):e1005230.).

iv. Zika capsid (C) protein is incorporated in other constructs to test whether the presence of a cleavable capsid protein increases the efficiency of SVP generation. (Fig.7). This includes the porcine teschovirus-1 2A (P2A)-mediated cleavage of C protein in the absence of the viral protease.

No.	SAM insert	Description
1	WT-prME	Wildtype prM & E sequences
2	CO-prME	Codon optimized prM & E sequences
3	CO-prME-ESS.1	Same as CO-prME but with an enhanced signal sequence (ESS) - PQAQA mutation in prM signal peptide c region to promote VLP
4	CO-prME-ESS.2	Same as CO-prME but with an ESS – prM signal peptide sequence is replaced with the IgG signal peptide sequence to promote VLP
5	CO-CprME.1	Same as CO-prME but also expressing Zika capsid protein with the native C and prM cleavage sites
6	CO-CprME.2	Same as CO-prME but also expressing Zika capsid protein with the signal peptide c region mutation PQAQA
7	CO-CprME.3	Same as CO-prME but also expressing Zika capsid protein with a P2A site inserted after the native NS2B-3 cleavage site

Table 2: SAM Zika constructs. Key: WT- wild-type; CO-Codon-optimized. Constructs are in the SAM vector described elsewhere. Zika sequences are derived from the Natal strain (Brazil) – KU527068.1, unless noted otherwise.

Evaluation/Study design

The constructs are evaluated in mammalian cells following electroporation of Zika-SAM RNA into BHK cells using the following methods:

a. SAM RNA replication- potency of the SAM-Zika constructs is tested by using antibodies against dsRNA and FACS.

b. Antigen expression is determined by immunoblots and immunofluorescence assays, to investigate cleaved prM and E protein in cell lysates and cell supernatant.

c. The production of SVPs is tested in mammalian cells by using established procedures for SVP isolation from cell supernatant.

Following identification of the most efficient candidate constructs formulation into LNP/CNE based-delivery systems is carried out and testing for antigenicity and immunogenicity is carried out in vivo.

Example 5. Expression and Secretion of Zika-SAM Constructs

The ability of cells to express and secrete Zika E protein from the Zika-SAM constructs described above was evaluated according to the following methods.

On Day 0, BHK cells were plated at $8x10^6$ in T225 flasks in Growth Media. For trypsinization, media was removed and cells were washed with 5 mls of PBS. The PBS wash was removed, and 5mls of pre-warmed trypsin was added and spread thoroughly across the plate. Trypsin was removed and plates were kept at 37 deg C for 1-2 mins. Cells were then resuspended in 10mls of growth media (5% FBS). Cells were counted and plated at required concentration into a new flask. The cells were then incubated at 37 deg C, 5% CO_2 for about 20 hours.

On Day 1, plates were prepared by adding 2ml DMEM + 1% FBS + P/S (outgrowth media) to each well of a 6-well plate (one well per electroporation). Plates were kept warm in a 37 deg C incubator. The electroporator was prepared to deliver 120V, 25ms pulse, .0 pulse interval, 1 pulse for a 2mm cuvette. Cuvettes were labeled and kept on ice. Cells in growth phase were harvested as normal into BHK media (growth) and counted using a hemocytometer. Cells were trypsinized following the same trypsinization protocol as above. Standards and negative control electroporations were also prepared.

Cells were centrifuged at 1500 rpm (462x g) for 5 mins. Media was aspirated, and cells were washed once with 20ml cold Opti-MEM media. Cells were again centrifuged at 1500rpm (462x g) for 5 mins. Media was aspirated, and the cells were resuspended in Opti-MEM media to 0.25ml per electroporation.

For each sample, 4000ng of RNA was mixed with 250ul cells, and the mixture was pipetted gently 4-5 times. The cells and RNA mixture were transferred to 2mm cuvettes and subjected to one pulse of electroporation using the parameters described above. Cells were allowed to rest at room temperature for 10 mins. Cells from one cuvette were added to one well of a pre-warmed 6-well plate, and the plate was tipped front and back and then side to side at a 45° angle to distribute cells evenly.

On Day 2 (30h post-electroporation), the supernatant was collected. An aliquot of 75ul was removed for Western blot, 25ul 4X NuPAGE buffer was added to the aliquot (no reducing agent), and the aliquot was stored at -20 deg C. The rest of the supernatant was stored at -80 deg C.

Cells were washed once with ice cold PBS, and then scraped into 200ul of RIPA buffer containing protease inhibitor cocktail (1 tablet in 10 ml) while keeping the plate on ice. The buffer containing cells was collected in microcentrifuge tubes, and subjected to two rounds of freeze thawing on dry ice. Samples were vortexed briefly, and pelleted at 8000rpm for 5 min. Pellets were discarded and the supernatants were retained. 25ul 4X NuPAGE buffer was added to a 75ul aliquot of the lysates for Western blotting. Aliquots were stored at -20 deg C. The rest of the lysates were stored at -80 deg C.

Concentration and filtration of Zika E protein species from cell supernatant

Cell supernatant from single transfections (1 million cells, 4000 ng RNA, 30h post-electroporation) were loaded, 500 µl each, into the upper chamber of the Amicon Ultra-0.5 Centrifugal Filter Devices, 100K (100,000 NMWL). The Centrifugal Filter Devices were centrifuged at 14000 x g for 10 minutes each. After each spin, the flow through was collected in a separate tube. After centrifugation, the samples remaining in the upper chamber were further washed by mixing with 500 µl of buffer HEPES 20mM, pH=7.4, and centrifuging again at 14000 x g for 10 minutes each.

The final samples remaining in the upper chamber, about 35-40 µl, were centrifuged into a fresh Eppendorf tube according to the manufacturer's instructions. Five µl of this sample was mixed with 5 µl of 4XNuPAGE buffer for immunoblotting, and the remaining samples were stored at -80 deg C. Also, 75 µl of the flowthrough was mixed with 25 µl of the 4X NuPAGE buffer for immunoblotting, and the remaining flow through was saved at -80 deg C.

Immunoblotting

15µl of the cell culture supernatants and 15 µl of the cell lysates (or 10µl of the concentrated supernatants and 15µl flow through) were run on a 4-12% SDS PAGE gel (Bis-Tris) in 1X MOPS running buffer. The separated samples were transferred onto nitrocellulose membranes. Membranes were blocked for 2-3 hours in PBS-Tween 20 +5% milk. The *flavivirus* 4G2 primary antibody was added at 1:120 dilution in PBS-T-Milk and membranes were incubated overnight at 4 deg C. Membranes were then washed 3 times for 10 minutes each in PBS-T. Anti-mouse secondary (Odyssey® anti-Mouse 800CW-green (LI-COR, Inc., Lincoln, NE) at 1:5000) in LI-

COR blocking buffer was then added, and the membranes were incubated for 1 hour. Membranes were washed three times for 2 minutes each, and then scanned on LI-COR Odyssey® imager (LI-COR, Inc., Lincoln, NE) at 800 channel, medium intensity.

Results

Zika E protein expression was detectable by immunoblot in all lysates from cells electroporated with Zika-SAM constructs or with positive control SAM-YFV (FIG. 8). Expression of Zika E protein was not detected in the SAM-RSV or Mock negative controls. However, Zika E protein secretion was detectable by immunoblot only in the supernatants of Construct #1 (wild type, WT), Construct #2 (codon optimized, CO), Construct #4 (codon optimized with IgG signal peptide, CO-prME-ESS.2), and Construct #7 (codon optimized with Zika capsid protein and P2A site, CO-CprME.3).

The filtration of supernatant through 100kD cutoff filters and immunoblotting showed that almost all of the E protein was retained in the filter and there was no detectable E in the flow through (FIG. 9). This indicated that the E protein detected in the supernatant may be a part of a higher molecular weight structure, presumably SVPs.

Example 6: Cationic Oil-in-Water Emulsions

Cationic nanoemulsions (CNEs) were prepared essentially according to the methods described in Brito et al., Molecular Therapy, Vol. 22, No. 12, pp. 2118-29 (2014) and International Patent Publication WO2013006834.

Briefly, squalene (Sigma, St. Louis, MO) was heated to 37°C, and DOTAP (Lipoid, Ludwigshafen Germany) was dissolved directly in squalene in the presence of sorbitan trioleate (SPAN 85; Sigma, St. Louis, MO). The resulting oil phase was then combined with the aqueous phase (Tween 80; Sigma, St. Louis, MO, in citrate buffer) and immediately homogenized for 2 min using an T25 homogenizer (IKA, Wilmington, NC) at 24K RPM to produce a primary emulsion. The primary emulsions were passed three to five times through a M-110S Microfluidizer or a M-110P Microfluidizer (Microfluidics, Newton, MA) with an ice bath cooling coil at a homogenization pressure of approximately 15K-20K PSI. The batch samples were removed from the unit and stored at 4ºC. The CNE formulation used in the present

examples contains 4 mg/ml DOTAP; 4.7 mg/ml Span 85; 4.7 mg/ml Tween 80; and 39 mg/ml squalene.

Example 7. Preparation of RNA-CNE Complexes

1. RNA synthesis

Zika SAM constructs contain a bacteriophage T7 promoter located upstream of the alphavirus cDNA to facilitate the synthesis of the replicon RNA in vitro. SAM-Zika RNA for construct #1 (encoding wild type Zika prM & E sequences (WT-prME)) and construct #2 (encoding codon-optimized prM & E sequences (CO-prME)), were synthesized using standard molecular biology techniques. Briefly, plasmid DNA encoding Zika-SAM constructs were linearized by endonuclease digestion a unique site located at the 3' end of the replicon sequence. The linearized DNA was then transcribed into RNA by *in vitro* synthesis using a T7 RNA polymerase in the presence of the template DNA and nucleoside triphosphates (ATP, CTP, GTP and UTP). Following transcription, DNA template was digested with DNase, and the RNA transcripts were purified by LiCl precipitation and reconstituted in nuclease-free water. RNA was then capped using the Vaccinia Capping System (New England BioLabs, Ipswich, MA) and purified by LiCl precipitation. RNA concentration in each reaction was determined by spectrophotometry. Prior to RNA complexation, RNA was diluted to a concentration of 300 µg/ml in citrate buffer (10 mM citrate pH 6.2, 20 mM NaCl, 560 mM sucrose).

2. RNA complexation

Zika SAM RNA was complexed with cationic nanoemulsion (CNE) particles essentially as described in Brito et al., Molecular Therapy, Vol. 22, No. 12, pp. 2118-29 (2014). Briefly, Zika SAM RNA (300 µg/ml in citrate buffer) was added to an equal volume of the CNE produced in Example 6, mixed, and allowed to complex on ice for 30 minutes to 2 hours. The final concentration of CNE-complexed RNA was 150 µg/ml.

The ratio of RNA to cationic lipid can be expressed as an N/P ratio, defined as the amount (moles) of protonatable nitrogen (N) atoms in the cationic lipid divided by

the amount (moles) of phosphates (P) on the RNA. DOTAP for example has one nitrogen that can be protonated per molecule. The RNA concentration was used to calculate the amount of phosphate in solution using an estimate of 3 nmols of phosphate per microgram of RNA. The CNE formulations described above have an N/P ratio of 6.3:1.

Example 8. In Vivo Immunogenicity and Protection of Zika SAM CNE Formulations

Female BALB/c mice (6-12 weeks old; The Jackson Laboratory), were housed and bred in the animal facility of the Vaccine Research Center, NIAID, NIH, Bethesda, MD. All animal experiments were reviewed and approved by the Animal Care and Use Committee of the VRC, NIAID, NIH. All animals were housed and cared for in accordance with local, state, federal, and institutional policies in an American Association for Accreditation of Laboratory Animal Care-accredited facility at the NIH.

Mice were immunized twice according to the study design shown in Table 2. Briefly, groups of 10 mice each were administered CNE formulations containing the Zika SAM RNA constructs #1 or #2. As a positive control, another group of mice received 50 µg of a Zika DNA vaccine (construct #5283, as described in Dowd et al., Science, Vol. 354 Issue 6309, pp. 237-40 (2016)) by intramuscular electroporation. All mice were challenged by intraperitoneal (i.p.) injection of live Zika virus on day 49.

Table 2: Mouse study design

Group	n	Delivery	Construct	Immunization Day 0	Immunization Day 21	Challenge Day 49
1	10	CNE56 / RNA	CO.prME	15 µg	15 µg	100 PFU, IP
2	10	CNE56 / RNA	CO.prME	1.5 µg	1.5 µg	100 PFU, IP
3	10	CNE56 / RNA	WT.prME	15 µg	15 µg	100 PFU, IP
4	10	CNE56 / RNA	WT.prME	1.5 µg	1.5 µg	100 PFU, IP
5	10	Electroporation/ DNA	5283	50 µg	50 µg	100 PFU, IP

Blood sera were collected on day 0, as well as 2 weeks after the first immunization, 2 weeks after the second immunization, and three days after the Zika virus challenge.

Zika neutralizing antibody titers were measured by reporter virus particle (RVP) neutralization assay according to methods described in Dowd, KA et al. Cell Rep. 16(6):1485-9 (2016). Results are shown in FIG. 10. Two weeks after the first immunization with Zika SAM constructs #1 or #2, or the positive control Zika DNA construct, there were significant levels of Zika neutralizing antibodies detected in the sera of immunized mice. Zika neutralizing antibody levels were even higher two weeks after the second immunization with the same Zika-SAM construct or the positive control.

A dose-dependent effect was observed, with the 15 μg dose of Zika SAM constructs #1 and #2 producing higher levels of neutralizing antibodies than the 1.5 μg dose. Notably, the 15 μg dose of Zika SAM constructs #1 and #2 produced a neutralizing antibody response that was comparable to the 50 μg dose of the Zika DNA vaccine construct (DNA #5283). These results indicate that Zika SAM constructs #1 and #2 are capable of inducing a significant neutralizing antibody response to Zika virus.

On day 49 of the study, mice were challenged with intraperitoneal injections of live Zika virus (strain PRVABC57) at a dose of 100 plaque forming units (PFU). Serum samples were taken three days after challenge, and viral loads were determined by real time quantitative PCR (qPCR) of the Zika virus capsid gene.

As shown in FIG. 11, mice vaccinated with Zika SAM constructs #1 or #2 (1.5 or 15 μg doses) or the positive control construct (DNA #5283) showed markedly reduced Zika virus detected in the serum as compared to unvaccinated animals. These results indicate that Zika SAM constructs #1 and #2 are capable of generating a protective immune response against Zika virus infection.

WE CLAIM:

1. A nucleic acid-based vaccine construct encoding a polypeptide comprising a full-length Zika virus prME antigen, or an immunogenic fragment or variant thereof.

2. The construct of claim 1, wherein said nucleic acid is an RNA comprising the coding region for the antigen.

3. The construct of any of claims 1-2, wherein the construct is codon optimized.

4. The construct of any of claims 1-3 comprising a prM signal sequence.

5. The construct of any of claims 1-4, further comprising a mutated prM signal sequence for enhanced prM cleavage.

6. The construct of any of claims 1-5, further comprising an IgG signal sequence.

7. The construct of any of claims 1-6, further comprising a codon optimized capsid sequence.

8. The construct of any of claims 1-7, further comprising a porcine teschovirus-1 2A cleavage sequence.

9. The construct of any of claims 1-8, wherein said construct comprises a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:26; SEQ ID NO:31; SEQ ID NO:36; SEQ ID NO:41; SEQ ID NO:46; SEQ ID NO:52; and SEQ ID NO:58;

(b) a nucleic acid sequence comprising the DNA sequence of SEQ ID NO:25; SEQ ID NO:30; SEQ ID NO:35; SEQ ID NO:40; SEQ ID NO:45; SEQ ID NO:51; and SEQ ID NO:57;

(c) a nucleic acid sequence comprising the RNA sequence corresponding to the DNA sequence of SEQ ID NO:77; SEQ ID NO:78; SEQ ID NO:79; SEQ ID NO:80; SEQ ID NO:81; SEQ ID NO:82; and SEQ ID NO:83; and

(d) a variant or fragment of (a)-(c).

10. A vector comprising the construct of any of claims 1-9.

11. A self-replicating RNA molecule comprising the construct of any of claims 1-9.

12. A self-replicating RNA molecule encoding an antigen comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:76.

13. A DNA molecule encoding the self-replicating RNA molecule of claims 11-12 comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, and SEQ ID NO:69.

14. A composition comprising an immunologically effective amount of one or more of the constructs of any of claims 1-9; the vector of claim 10; or the self-replicating RNA molecule of claims 11-12.

15. The composition according to claim 14 comprising a RNA-based vaccine.

16. The composition according to claim 15 comprising a self-replicating RNA molecule.

17. The composition according to claim 16, wherein the self-replicating RNA molecule comprises a sequence selected from the group consisting of SEQ ID NO:70,

SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:76.

18. The composition according to any of claims 14-17, wherein the composition comprises a non-viral delivery material, such as a submicron cationic oil-in-water emulsion; a liposome; or a biodegradable polymeric microparticle delivery system.

19. The composition according to claim 18, wherein the submicron cationic oil-in-water emulsion comprises an oil core, a cationic lipid, and a surfactant.

20. The composition according to any of claims 14-19 wherein the composition further comprises one or more nucleic acid sequences which encode one or more additional antigens and/or the composition further comprises one or more additional antigens.

21. The composition according to any of claims 14-20 wherein the composition is pharmaceutically acceptable for administration to a subject in combination with a further composition which comprises a nucleic acid comprising a sequence which encodes an additional antigen; and/or the composition is pharmaceutically acceptable for administration to the subject in combination with a further composition which comprises an additional antigen.

22. The composition according to any of claims 14-21 wherein the composition comprises one or more adjuvants.

23. A method of inducing an immune response against a Zika virus infection in a subject in need thereof, which comprises administering to said subject an immunologically effective amount of a composition comprising one or more of a construct of claims 1-9; the vector of claim 10; the self-replicating RNA molecule of claims 11-12; or a composition of any one of claims 14-22.

24. The method according to claim 23 wherein the immune response is characterized by immunological memory against the Zika virus and/or an effective Zika virus-responsive memory T cell population.

25. The method according to any of claims 23-24 wherein the subject is human.

26. A process for producing an RNA-based vaccine comprising a step of transcribing the vector of claim 10 or the DNA of claim 13 to produce an RNA comprising a coding region for the antigen.

27. The process of claim 26, wherein said transcription is in vitro.

28. The process of claims 26, wherein said transcription is in vivo.

29. The process of any claim 26-28, further comprising a step of formulating the RNA comprising the coding region for the antigen with a delivery system.

30. The process of claim 29, wherein the delivery system is a non-viral delivery material.

31. The process of claim 30, wherein the delivery system is selected from the group consisting of: a submicron cationic oil-in-water emulsion; a liposome; and a biodegradable polymeric microparticle delivery system.

32. The process of any of claims 26-31, further comprising a step of combining the RNA comprising the coding region for the antigen with an additional composition comprising an adjuvant.

33. The process of claim 32, wherein said adjuvant comprises an immunostimulant.

34. A composition produced by the process of any of claims 26-33.

35. Use of the construct of claims 1-9; the vector of claim 10; the self-replicating RNA molecule of claims 11-12; or a composition of any one of claims 14-22 for inducing an immune response to a Zika virus infection in a subject.

36. Use of the construct of claims 1-9; the vector of claim 10; the self-replicating RNA molecule of claims 11-12; or a composition of any one of claims 14-22 in the manufacture of a medicament inducing an immune response against a Zika virus infection in a subject.

37. The construct of claims 1-9; the vector of claim 10; the self-replicating RNA molecule of claims 11-12; or a composition of any one of claims 14-22 or 34 for use in therapy or medicine.

38. The construct of claims 1-9; the vector of claim 10; the self-replicating RNA molecule of claims 11-12; or a composition of any one of claims 14-22 or 34 for use in vaccine therapy.

39. The construct of claims 1-9; the vector of claim 10; the self-replicating RNA molecule of claims 11-12; or a composition of any one of claims 14-22 or 34 for use in preventing or treating Zika virus infection in a subject in need thereof.

FIG.1

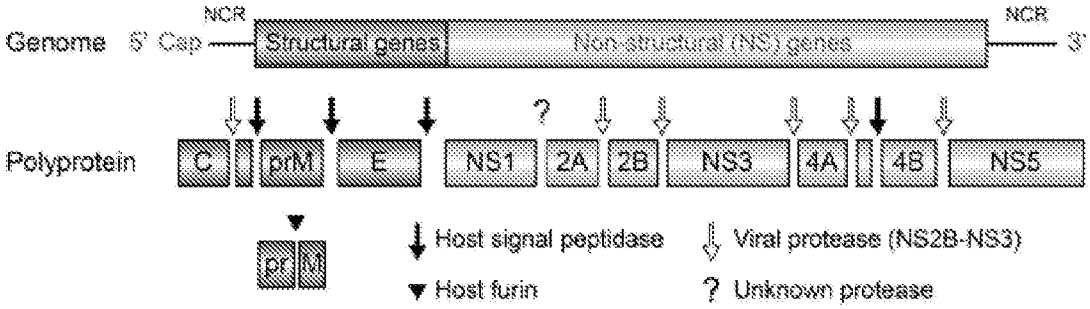

Genome
5' Cap
NCR
Structural genes
Non-structural (NS) genes
NCR
3'
Polyprotein
C
prM
E
NS1
2A
2B
NS3
4A
4B
NS5
pr
M
Host signal peptidase
Host furin
Viral protease (NS2B-NS3)
? Unknown protease

FIG.2

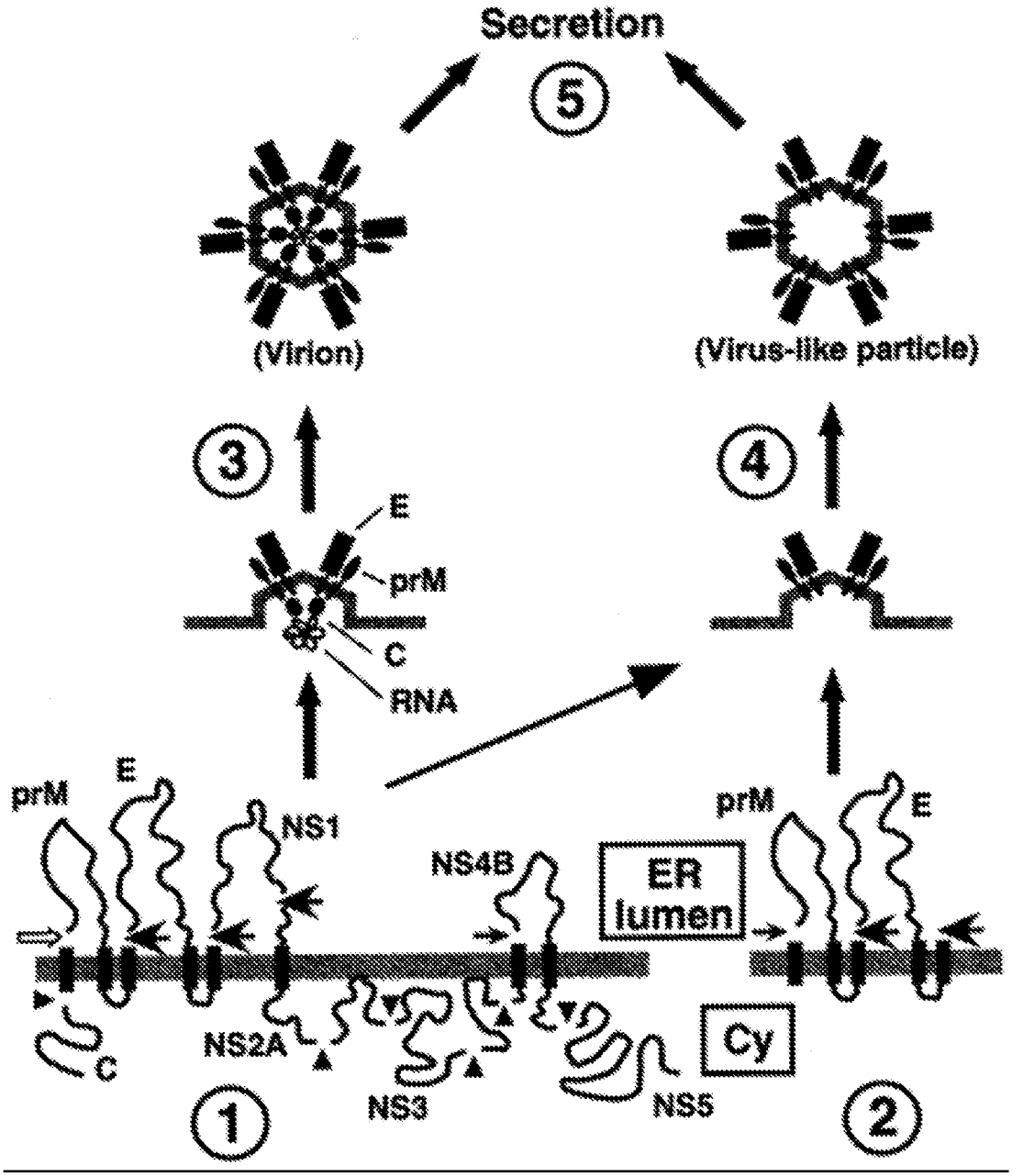
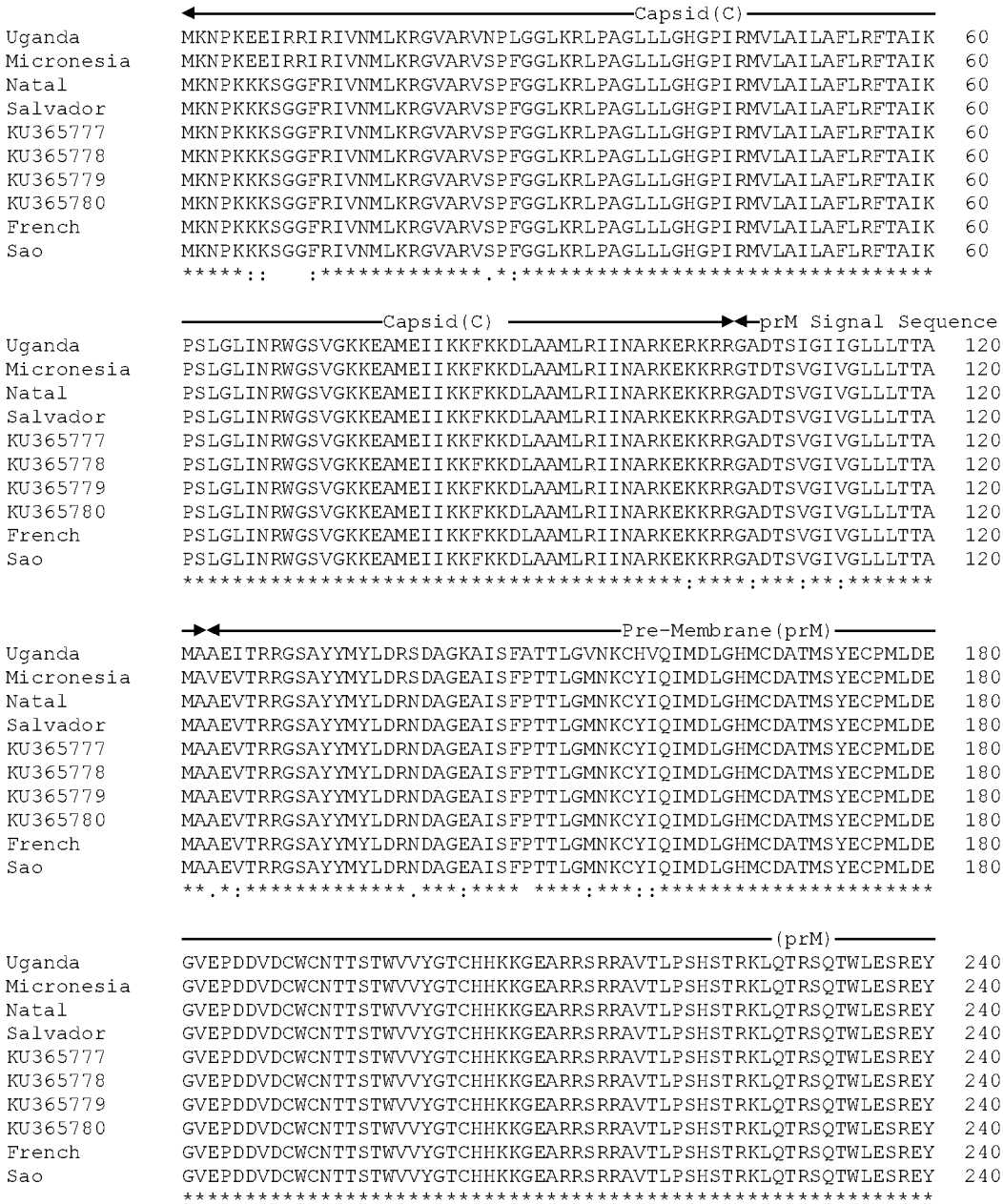

Secretion
5
(Virion)
(Virus-like particle)
3
4
E
prM
C
RNA
prM
E
NS1
NS4B
ER lumen
prM
E
NS2A
NS3
NS5
Cy
C
1
2

FIG.3A


Capsid(C)
Uganda MKNPKEEIRRIRIVNMLKRGVARVNPLGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Micronesia MKNPKEEIRRIRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Natal MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Salvador MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
KU365777 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
KU365778 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
KU365779 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
KU365780 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
French MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Sao MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Capsid(C)
prM Signal Sequence
Uganda PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKERKRRGADTSIGIIGLLLTTA 120
Micronesia PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGTDTSVGIVGLLLTTA 120
Natal PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
Salvador PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
KU365777 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
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KU365780 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
French PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
Sao PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
Pre-Membrane(prM)
Uganda MAAEITRRGSAYYMYLDRSDAGKAISFATTLGVNKCHVQIMDLGHMCDATMSYECPMLDE 180
Micronesia MAVEVTRRGSAYYMYLDRSDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
Natal MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
Salvador MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
KU365777 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
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Sao MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
(prM)
Uganda GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Micronesia GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Natal GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Salvador GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
KU365777 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
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French GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
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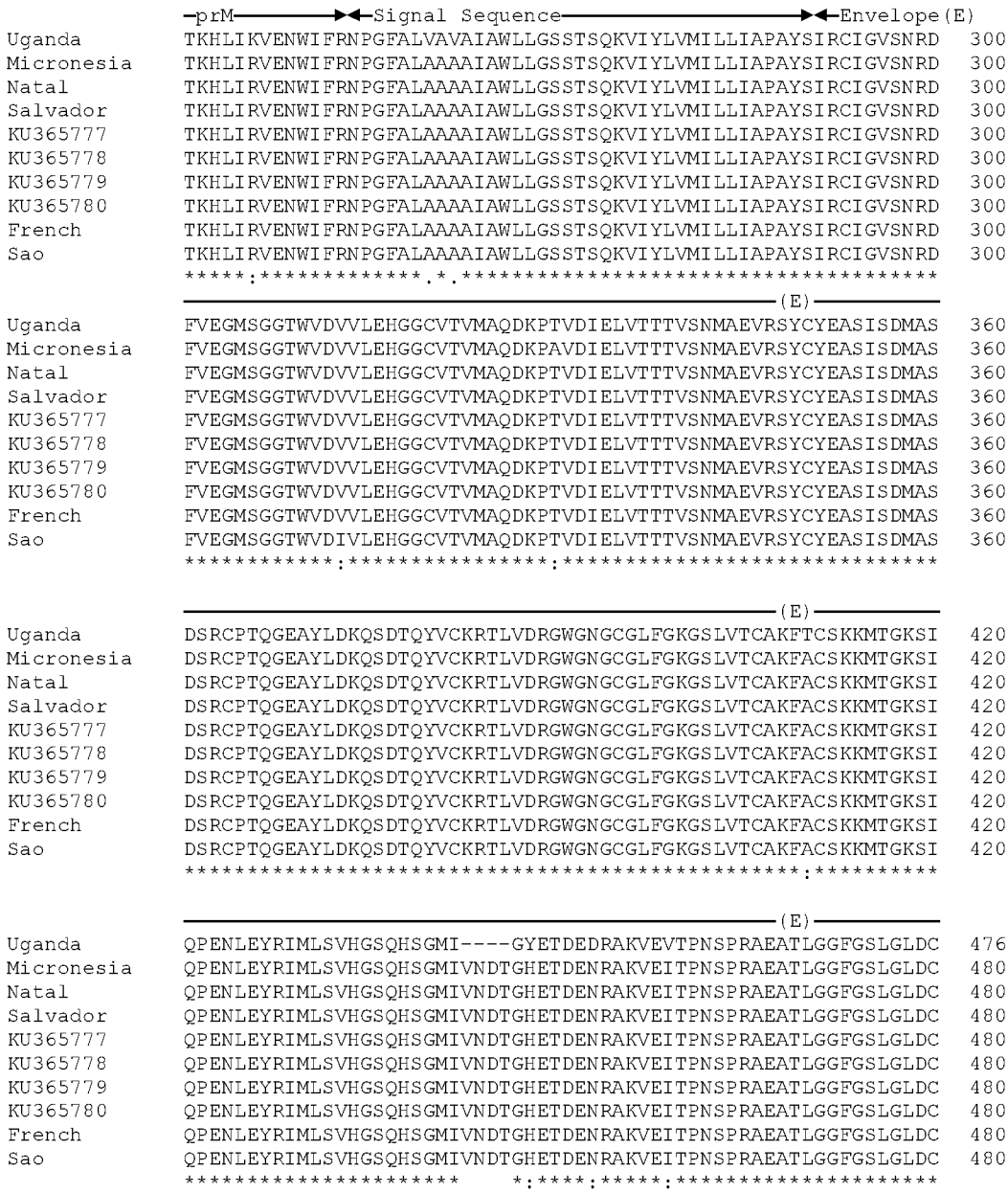
FIG.3B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Salvador TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
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French TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
Sao TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
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Micronesia FVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Natal FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Salvador FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
KU365777 FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
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French FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Sao FVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
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Micronesia DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
Natal DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
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French DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
Sao DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
(E)
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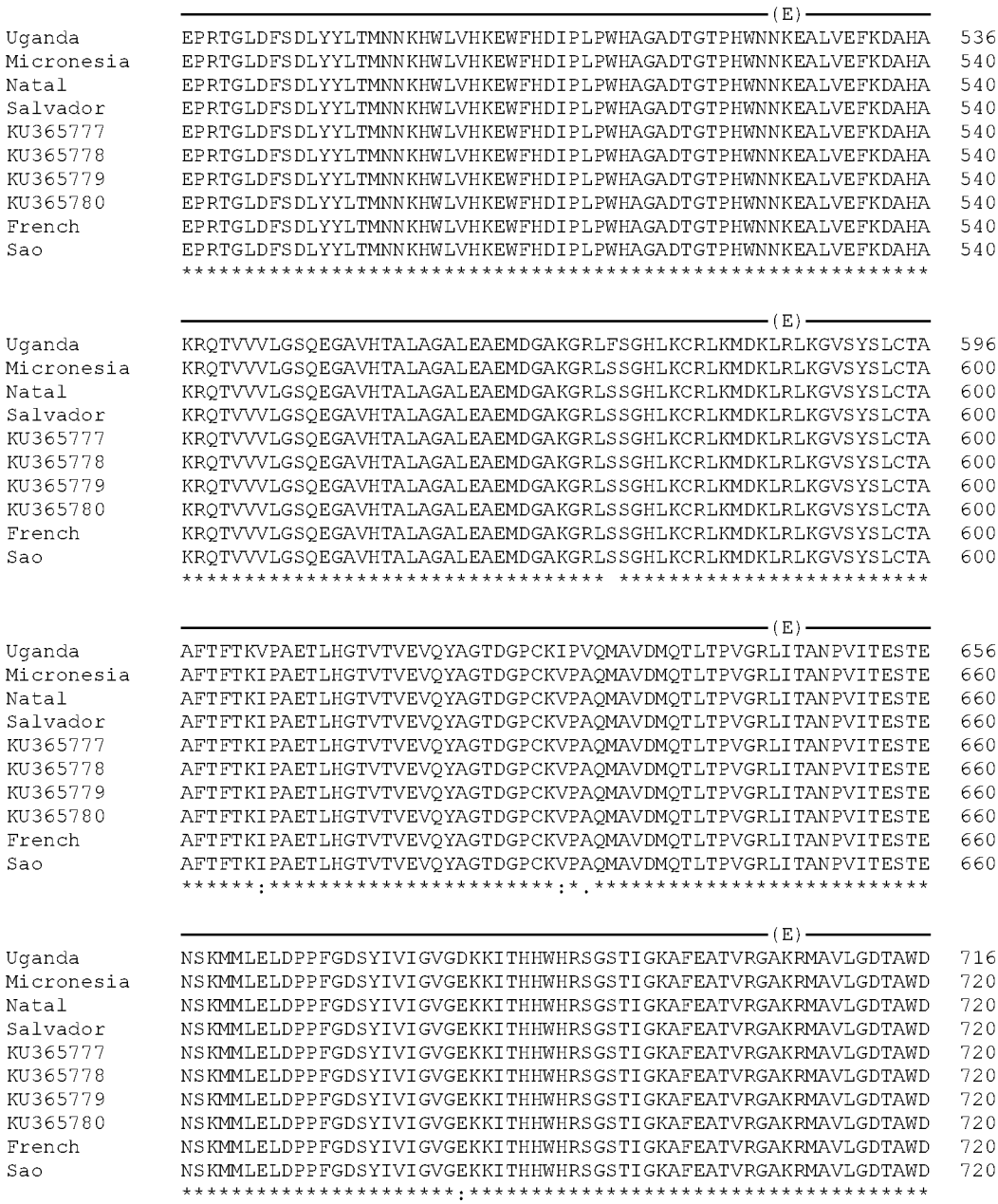
FIG.3C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French EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA 540
Sao EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA 540
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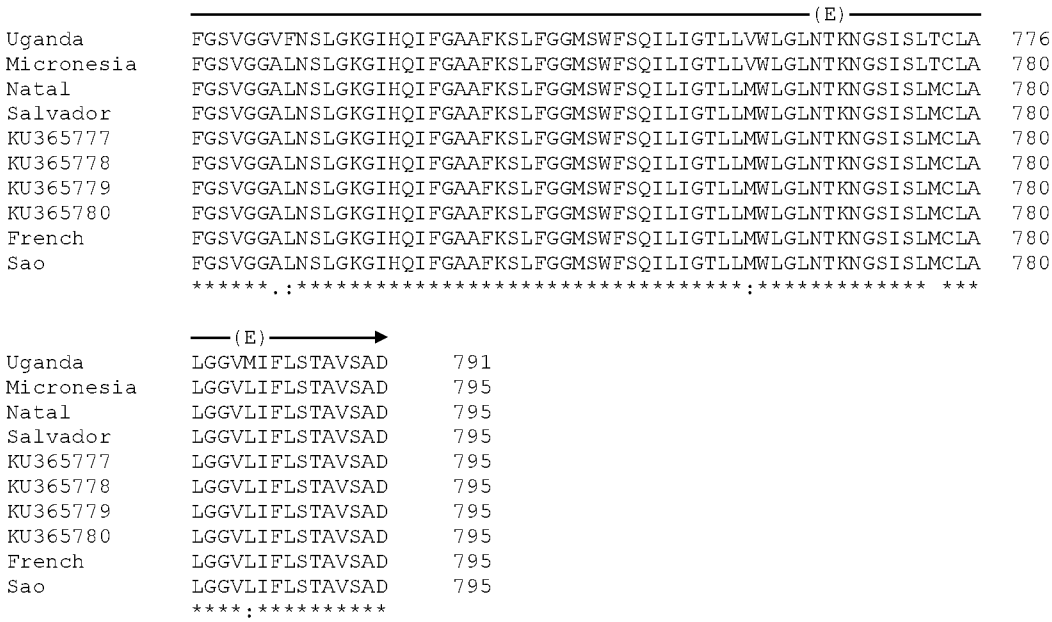
FIG.3D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Micronesia FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLA 780
Natal FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA 780
Salvador FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA 780
KU365777 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA 780
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French FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA 780
Sao FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA 780
(E)
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Micronesia LGGVLIFLSTAVSAD 795
Natal LGGVLIFLSTAVSAD 795
Salvador LGGVLIFLSTAVSAD 795
KU365777 LGGVLIFLSTAVSAD 795
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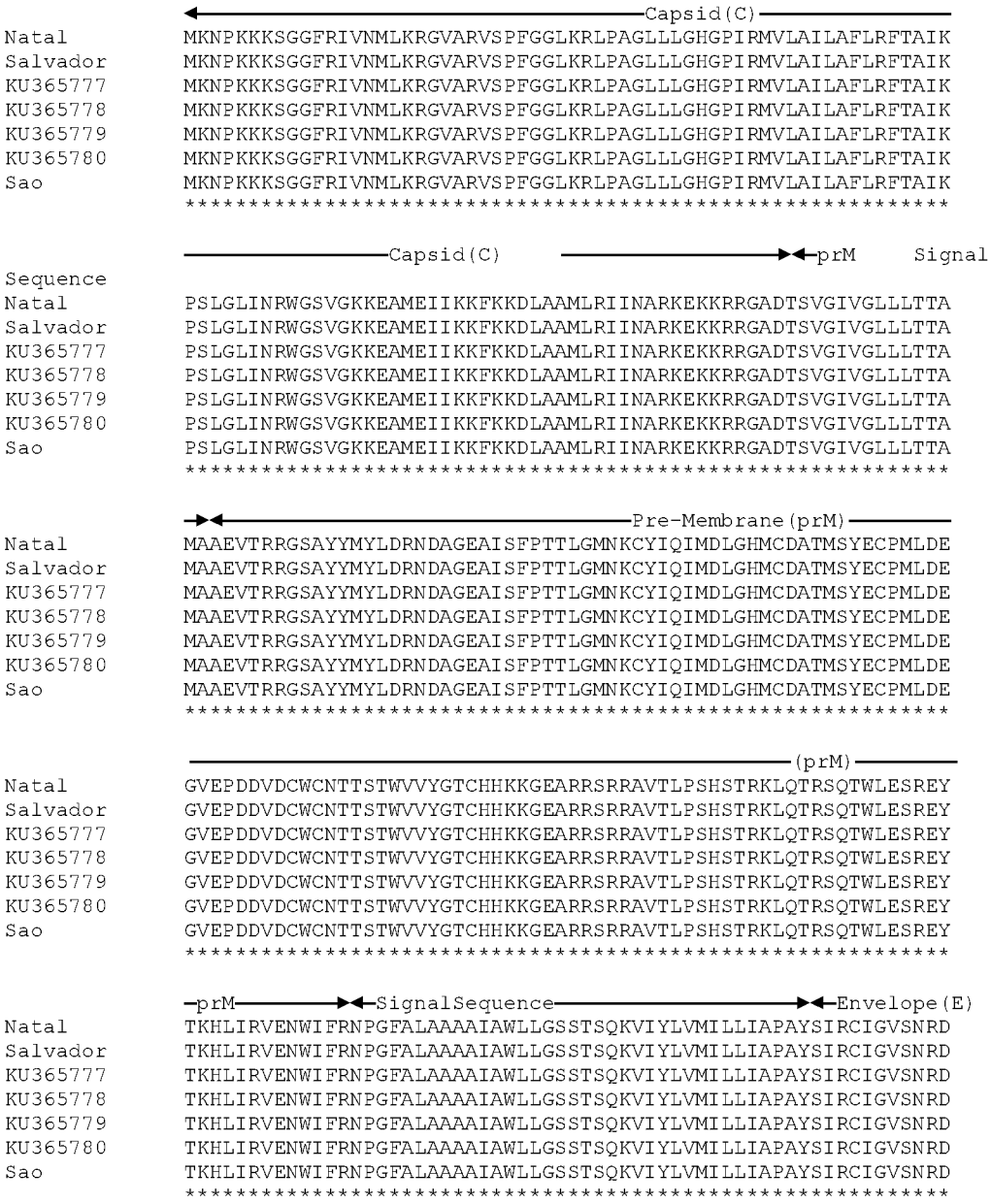
FIG.4A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Sao MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
(prM)
Natal GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
Salvador GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
KU365777 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
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KU365780 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
Sao GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
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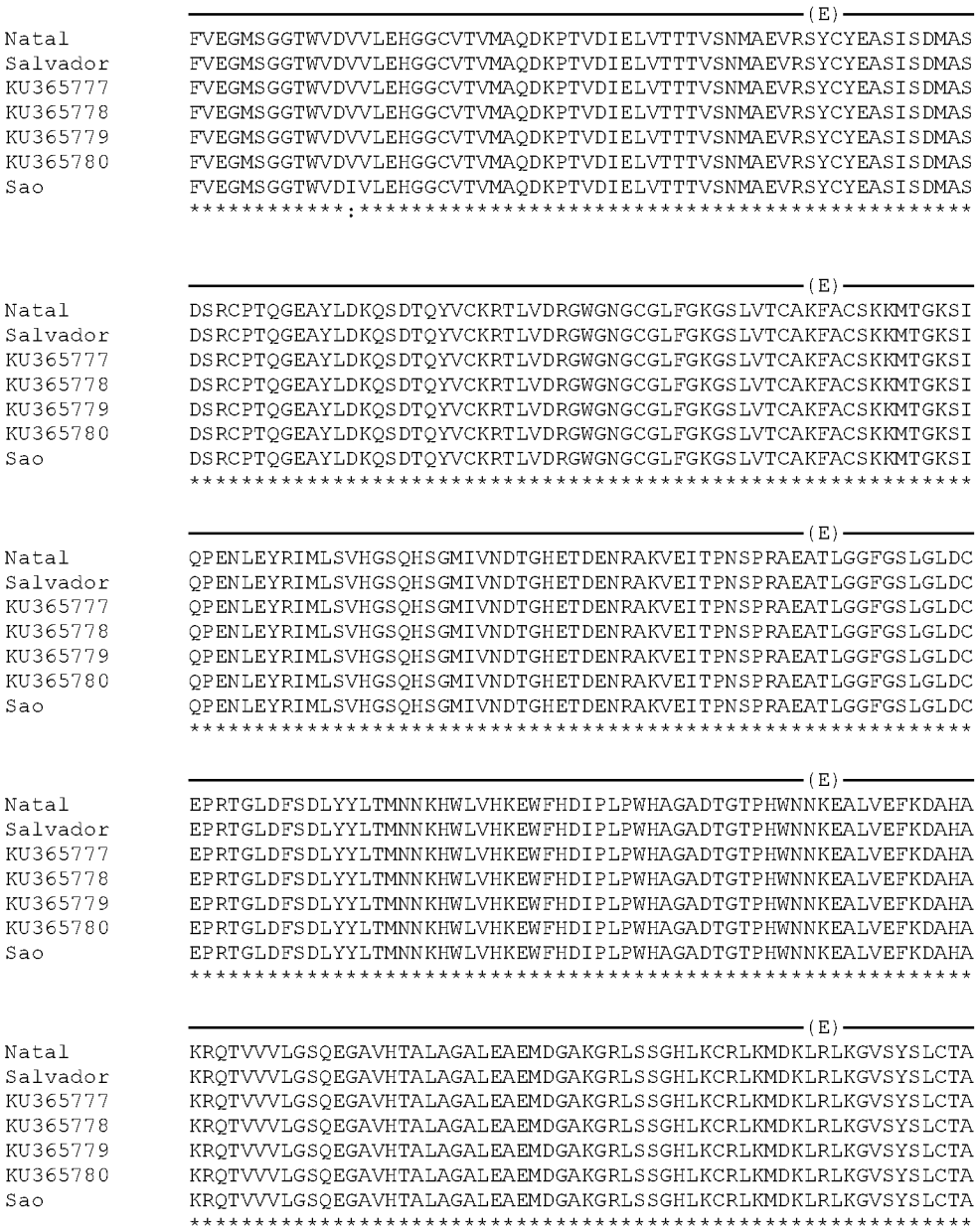
FIG.4B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Sao EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
(E)
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Salvador KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
KU365777 KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
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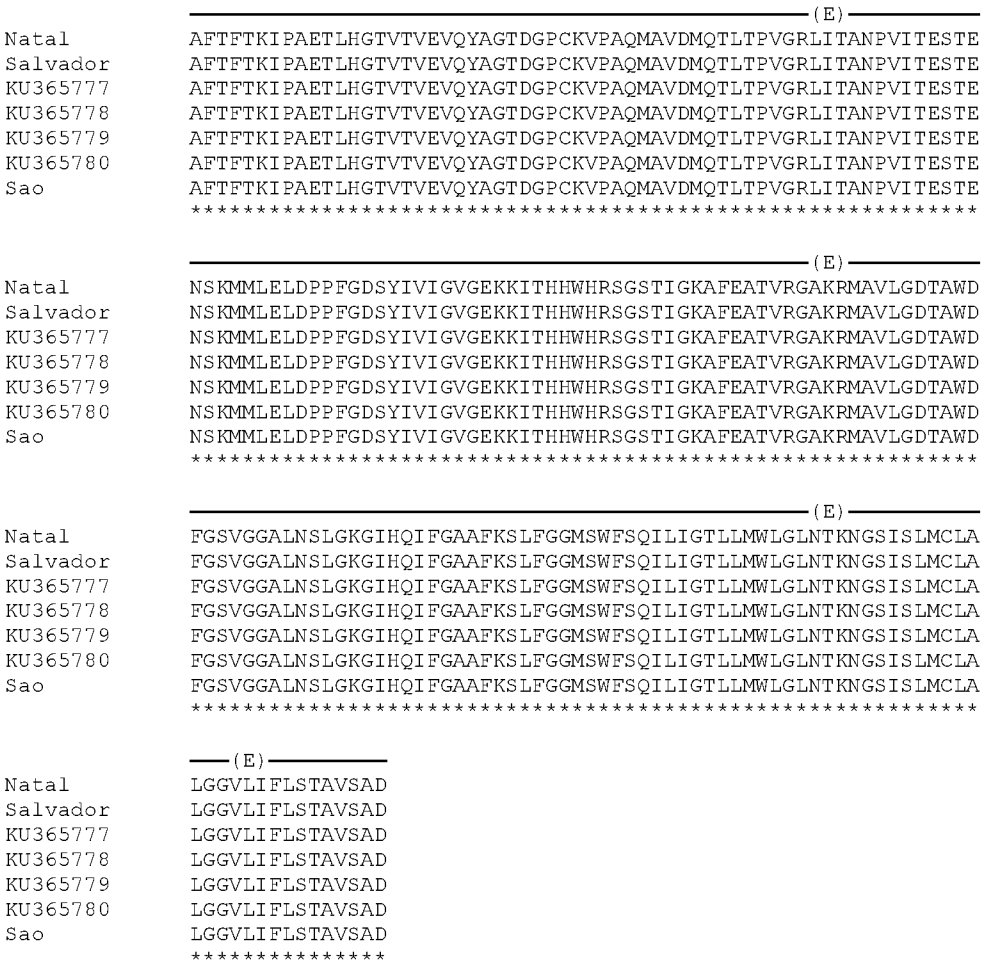
FIG.4C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(E)
Natal NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
Salvador NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365777 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365778 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365779 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365780 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
Sao NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
(E)
Natal FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
Salvador FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365777 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365778 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365779 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365780 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
Sao FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
(E)
Natal LGGVLIFLSTAVSAD
Salvador LGGVLIFLSTAVSAD
KU365777 LGGVLIFLSTAVSAD
KU365778 LGGVLIFLSTAVSAD
KU365779 LGGVLIFLSTAVSAD
KU365780 LGGVLIFLSTAVSAD
Sao LGGVLIFLSTAVSAD

FIG.5

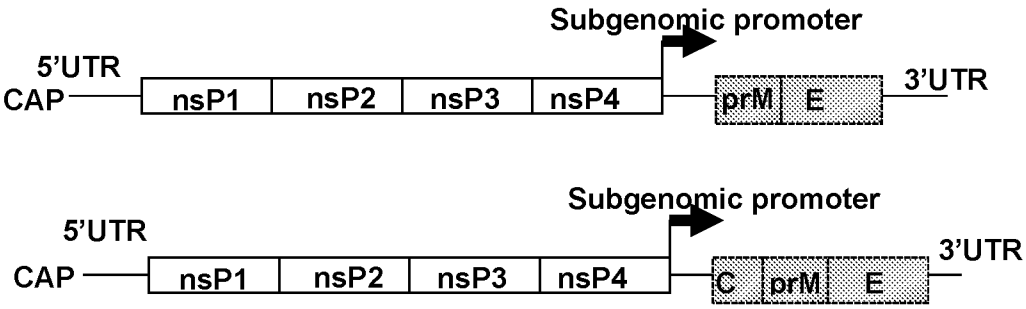

Subgenomic promoter
5'UTR
CAP
nsP1
nsP2
nsP3
nsP4
prM
E
3'UTR
Subgenomic promoter
5'UTR
CAP
nsP1
nsP2
nsP3
nsP4
C
prM
E
3'UTR

FIG.6

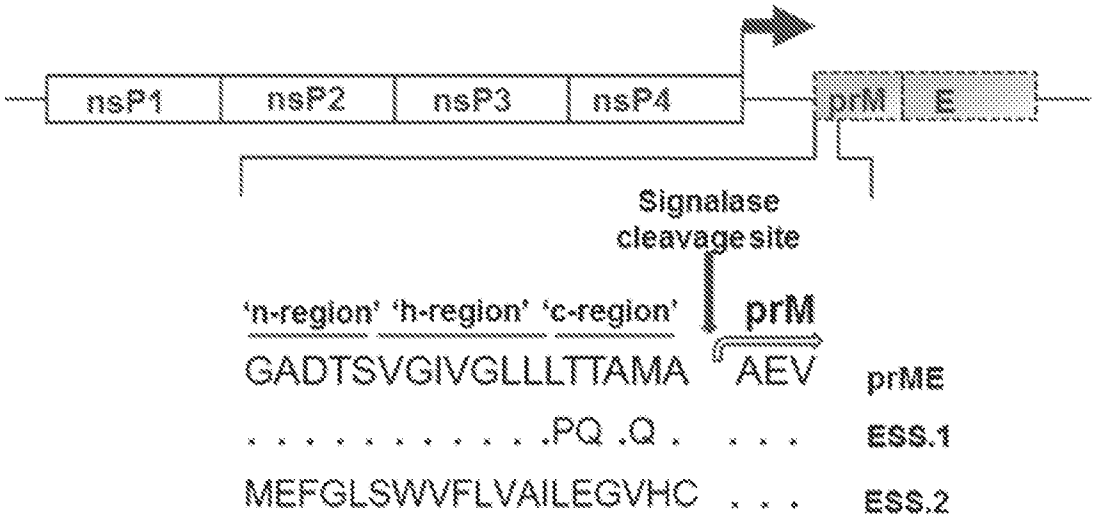

nsP1
nsP2
nsP3
nsP4
prM
E
Signalase cleavage site
'n-region' 'h-region' 'c-region'
prM
GADTSVGIVGLLLTTAMA AEV
prME
. . . . . .PQ .Q . . . .
ESS.1
MEFGLSWVFLVAILEGVHC . . .
ESS.2

FIG.7

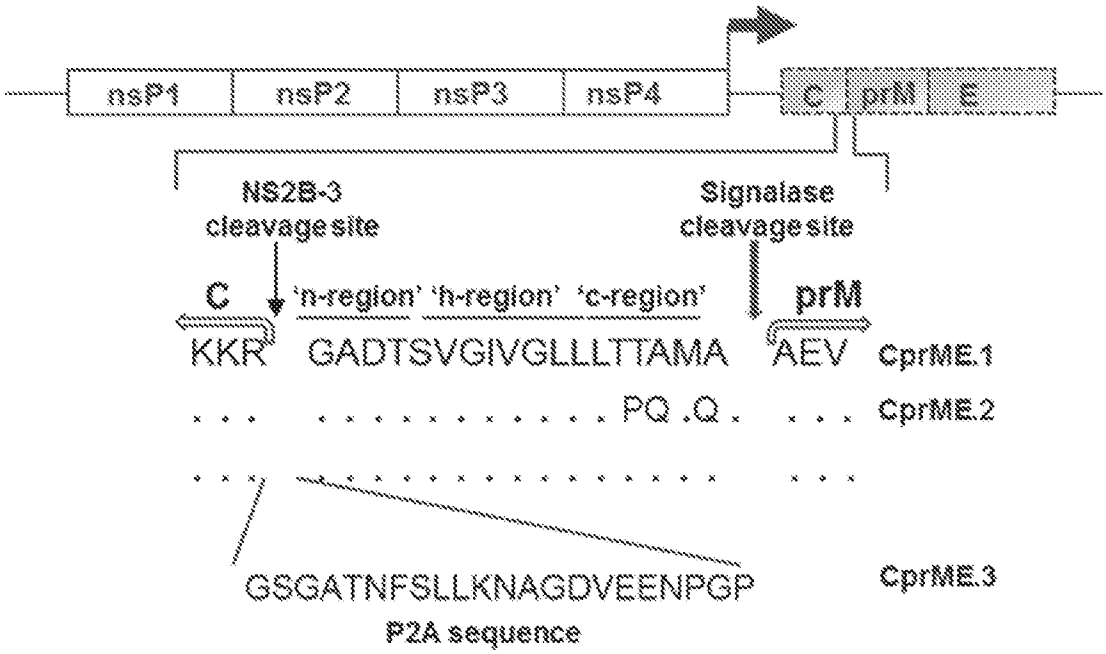

nsP1
nsP2
nsP3
nsP4
C
prM
E
NS2B-3
cleavage site
Signalase
cleavage site
C
'n-region'
'h-region'
'c-region'
prM
KKR
GADTSVGIVGLLLTTAMA
AEV
CprME.1
PQ .Q .
CprME.2
CprME.3
GSGATNFSLLKNAGDVEENPGP
P2A sequence

FIG.8

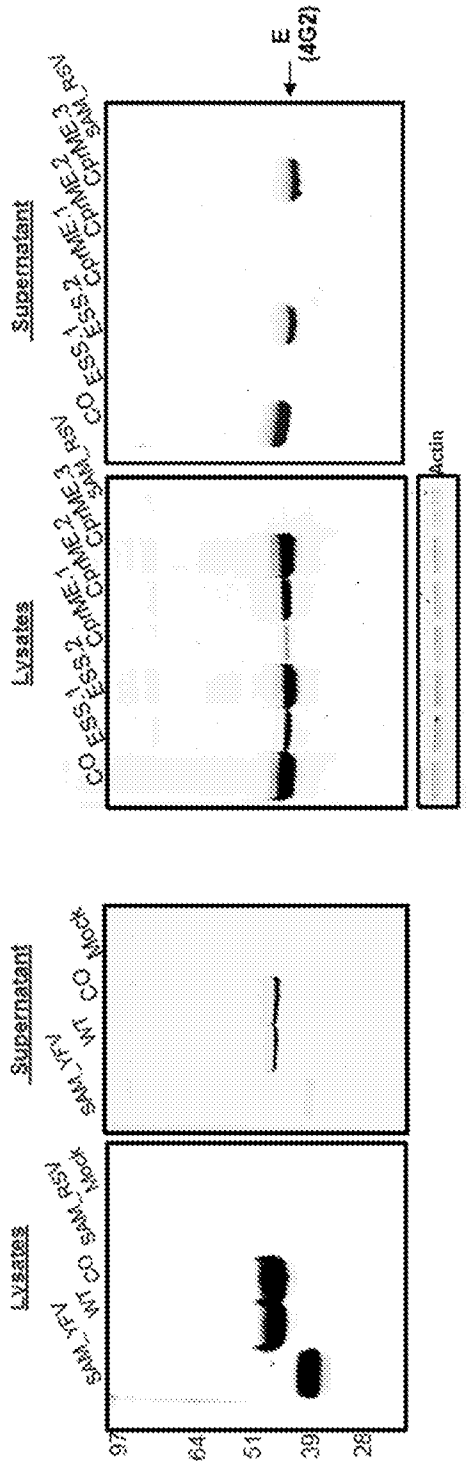

Lysates
Supernatant
Lysates
Supernatant
E (4G2)
β-actin
97
64
51
39
28

FIG.9

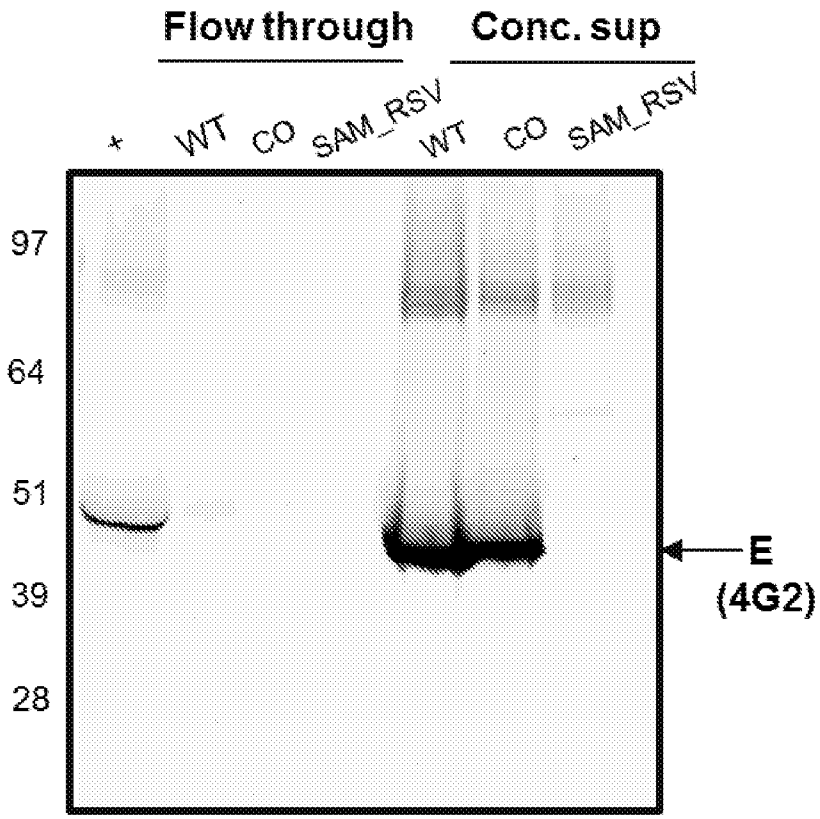

Flow through
Conc. sup
+
WT
CO
SAM_RSV
WT
CO
SAM_RSV
97
64
51
39
28
E
(4G2)

FIG.10

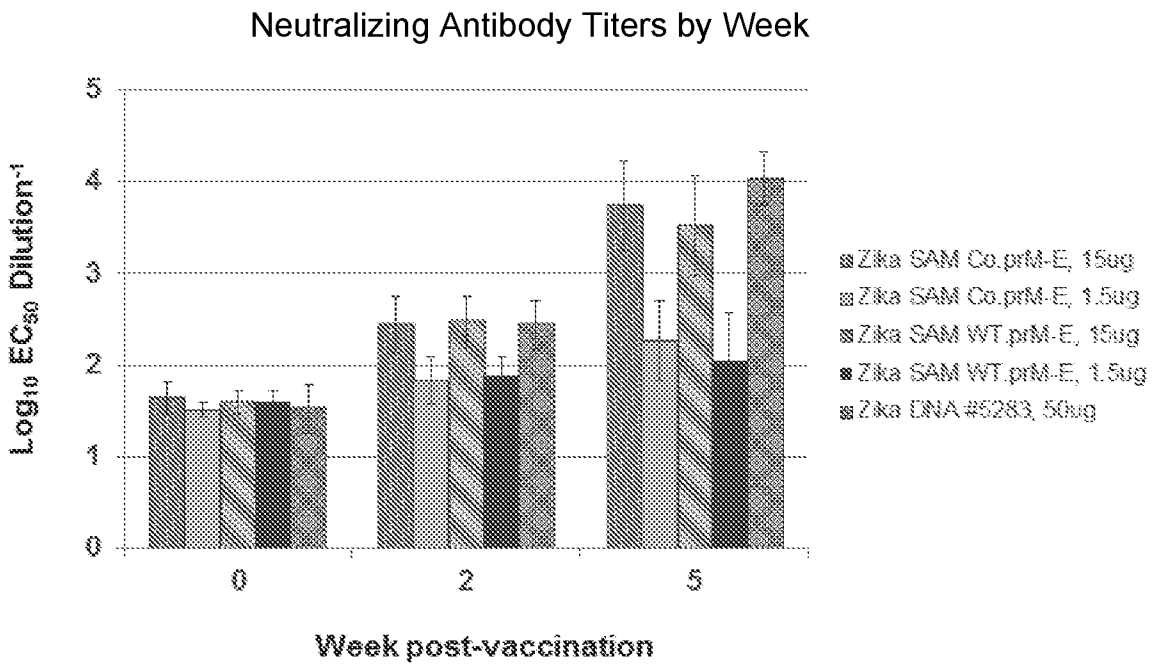
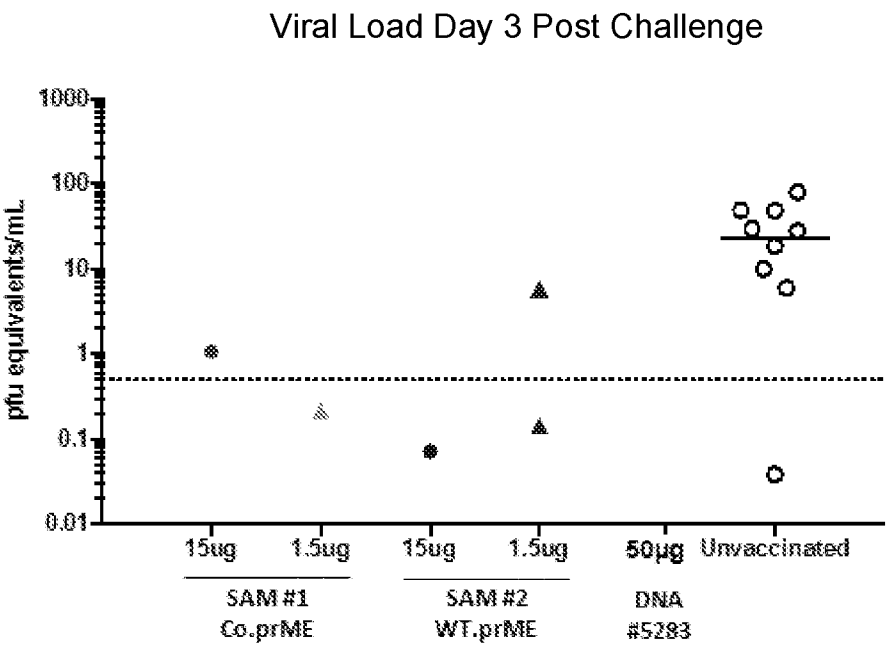

Neutralizing Antibody Titers by Week
Log10 EC50 Dilution-1
0
1
2
3
4
5
0
2
5
Week post-vaccination
Zika SAM Co.prM-E, 15ug
Zika SAM Co.prM-E, 1.5ug
Zika SAM WT.prM-E, 15ug
Zika SAM WT.prM-E, 1.5ug
Zika DNA #5283, 50ug

FIG.11


Viral Load Day 3 Post Challenge
pfu equivalents/mL
1000
100
10
1
0.1
0.01
15ug
1.5ug
15ug
1.5ug
50µg
Unvaccinated
SAM #1
Co.prME
SAM #2
WT.prME
DNA
#5283

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/053242

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 A61K39/39 C07K14/18 C12N15/86 A61K39/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, WPI Data, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REGINA M. KOFLER ET AL: "Mimicking live flavivirus immunization with a noninfectious RNA vaccine", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, vol. 101, no. 7, 17 February 2004 (2004-02-17), pages 1951-1956, XP55361243, US ISSN: 0027-8424, DOI: 10.1073/pnas.0307145101	1,2, 9-11, 14-16, 18,20, 23,26, 27,29, 30,34, 37,38
Y	See e.g. page 1951, right column, second paragraph; page 1952, left column; page 1954, left column, last paragraph.	1-39

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2017	11/08/2017

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

1

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 691 961 B1 (PUFFER BRIDGET [US] ET AL) 8 April 2014 (2014-04-08) See e.g. column 4, 3rd and 4th paragraphs; columns 7 and 8	1-4,9,10
X	EP 1 985 709 A1 (UNIV WIEN MED [AT]) 29 October 2008 (2008-10-29) Se e.g. the sentemce bridging pages 2 and 3; see paragraph [0057]	1,2,4,9, 10
Y	DATABASE UniProt [Online] 16 March 2016 (2016-03-16), "RecName: Full=Genome polyprotein {ECO:0000256¦SAAS:SAAS00368684};", XP002772523, retrieved from EBI accession no. UNIPROT:A0A0X1LH65 Database accession no. A0A0X1LH65 See sequence; sequence	1-39
Y	DATABASE UniProt [Online] 11 May 2016 (2016-05-11), "RecName: Full=Genome polyprotein {ECO:0000256¦SAAS:SAAS00368684};", XP002772524, retrieved from EBI accession no. UNIPROT:A0A140FB25 Database accession no. A0A140FB25 See sequence; sequence	1-39
Y	SANDRA LAZZARO ET AL: "CD8 T-cell priming upon mRNA vaccination is restricted to bone-marrow-derived antigen-presenting cells and may involve antigen transfer from myocytes", IMMUNOLOGY, vol. 146, no. 2, 28 October 2015 (2015-10-28), pages 312-326, XP055392907, GB ISSN: 0019-2805, DOI: 10.1111/imm.12505 See e.g. the abstract	1-39

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LUIS A BRITO ET AL: "A Cationic Nanoemulsion for the Delivery of Next-generation RNA Vaccines", MOLECULAR THERAPY, vol. 22, no. 12, 16 July 2014 (2014-07-16) , pages 2118-2129, XP55180488, ISSN: 1525-0016, DOI: 10.1038/mt.2014.133 cited in the application See e.g. the abstract	1-39
Y	WO 2013/006837 A1 (NOVARTIS AG [CH]; BRITO LUIS [US]; CHAN MICHELLE [US]; GEALL ANDREW [U) 10 January 2013 (2013-01-10) cited in the application See e.g. the asbstract or SEQ ID NO: 9	1-39
X	J. COHEN: "The race for a Zika vaccine is on", SCIENCE, vol. 351, no. 6273, 5 February 2016 (2016-02-05), pages 543-544, XP55360676, ISSN: 0036-8075, DOI: 10.1126/science.351.6273.543	1,9,10, 14
Y	See the whole document, but see in particular, page 544, left column, beginning of the second paragraph	1-39
X	ISHIKAWA T ET AL: "Construction and evaluation of a chimeric pseudoinfectious virus vaccine to prevent Japanese encephalitis", VACCINE, ELSEVIER, AMSTERDAM, NL, vol. 26, no. 22, 23 May 2008 (2008-05-23), pages 2772-2781, XP022649804, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2008.03.010 [retrieved on 2008-03-31] See e.g. the section bridging pages 2775 and 2776	1,2,4,5, 9,10,14, 37,38
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X,P	RAFAEL A. LAROCCA ET AL: "Vaccine protection against Zika virus from Brazil", NATURE, vol. 536, no. 7617, 26 June 2016 (2016-06-26), pages 474-478, XP55314483, ISSN: 0028-0836, DOI: 10.1038/nature18952	1-4,9, 10,14, 15,18-39
Y,P	the whole document	1-39

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	P. ABBINK ET AL: "Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys", SCIENCE, vol. 353, no. 6304, 9 September 2016 (2016-09-09), pages 1129-1132, XP55314484, ISSN: 0036-8075, DOI: 10.1126/science.aah6157	1-4,9, 10,14, 15,18-39
Y,P	the whole document	1-39

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Y	the whole document	1-39

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Y,P	the whole document	1-39

X,P	GUIRAKHOO F ET AL: "Development of a Zika vaccine using a novel MVA-VLP platform", INTERNATIONAL JOURNAL OF INFECTIOUS DISEASES, vol. 53, 1 December 2016 (2016-12-01), pages 16-17, XP029860956, ISSN: 1201-9712, DOI: 10.1016/J.IJID.2016.11.046	1-39
Y,P	the whole document	1-4,9, 10,14, 15,18-39

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	JUSTIN M. RICHNER ET AL: "Modified mRNA Vaccines Protect against Zika Virus Infection", CELL, vol. 168, no. 6, 1 March 2017 (2017-03-01), pages 1114-1125.e10, XP55370676, US ISSN: 0092-8674, DOI: 10.1016/j.cell.2017.02.017	1-4,9, 10,14, 15,18-39
Y,P	the whole document	1-39

X,P	JASDAVE S. CHAHAL ET AL: "An RNA nanoparticle vaccine against Zika virus elicits antibody and CD8+ T cell responses in a mouse model", SCIENTIFIC REPORTS, vol. 7, no. 1, 1 January 2017 (2017-01-01), XP55392682, DOI: 10.1038/s41598-017-00193-w	1-4,9, 10,14, 15,18-39
Y,P	the whole document	1-39

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Y,P	The whole document, but see e.g. the claims	1-39

X,P	WO 2017/009873 A1 (BHARAT BIOTECH INT LTD [IN]) 19 January 2017 (2017-01-19)	1-4,9, 10,14, 15,18-39
Y,P	The whole document, but see e.g. the claims	1-39

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Y,P	The whole document, but see e.g. the claims	1-39

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Y,P	The whole document, but see e.g. the claims	1-39

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2017/053242

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP 1985709 A1	29-10-2008	NONE	
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		WO 2017009873 A1	19-01-2017
WO 2017015463 A2	26-01-2017	NONE	
WO 2017070624 A1	27-04-2017	NONE	