



(51) International Patent Classification:

A61K 39/00 (2006.01) C12N 7/00 (2006.01)
A61K 39/12 (2006.01) C12N 7/01 (2006.01)

(21) International Application Number:

PCT/US2017/031067

(22) International Filing Date:

04 May 2017 (04.05.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/331,584 04 May 2016 (04.05.2016) US

(71) Applicant: UNIVERSITY OF MIAMI [US/US]; Office of Technology Transfer, 1951 NW 7th Avenue-3rd Floor-Suite 310, Miami, FL 33136 (US).

(72) Inventor: BARBER, Glen N.; 13520 S.W. 99th Court, Miami, FL 33176 (US).

(74) Agent: NEVILLE, Katherine L. et al.; Marshall, Gerstein & Borun LLP, 233 S. Wacker Drive, 6300 Willis Tower, Chicago, IL 60606-6357 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,

SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

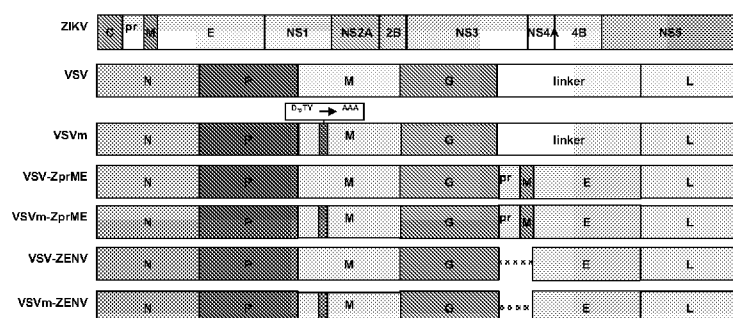
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: ZIKA VIRUS VECTOR FOR TREATING ZIKA VIRUS INFECTION

Figure 1A



(57) Abstract: The present disclosure relates, in general, to a vesicular stomatitis virus vector comprising a polynucleotide expressing a protein of a Zika virus, e.g., an envelope protein, for use as a therapeutic against Zika Virus infection.



ZIKA VIRUS VECTOR FOR TREATING ZIKA VIRUS INFECTION

FIELD OF THE INVENTION

[0001] The present disclosure is directed to a vesicular stomatitis virus vector comprising a polynucleotide expressing a protein of a Zika virus, e.g., an envelope protein, for use as a therapeutic against Zika Virus infection.

BACKGROUND

[0002] Zika virus (ZIKV) was first isolated in the Zika forest of Uganda in 1947. The virus belongs to the genus flavivirus and is related to Dengue Virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JAV) and West Nile Virus (WNV). The *Aedes* genus of mosquito is the major vector for ZENV and has been isolated as far away as Malaysia, as well as Africa and South America. ZIKV was not considered to be a major cause of human disease, being documented as triggering a mild and self-limiting disease characterized by rash, conjunctivitis and arthralgia, the symptoms of which are similar to those associated with DENV and Chikungunya (CHIKV). Nevertheless, the potential for the virus to infect the central nervous system of certain mammals was first described in 1971 (Weaver et al., Zika virus: History, emergence, biology, and prospects for control. Antiviral Res 130:69-80, 2016).

[0003] That ZIKV may be involved in causing more severe malaise was first reported in French Polynesia in 2013, where evidence indicated that this virus may be perinatally transmitted and potentially associated with Guillain-Barre syndrome, in which the immune system targets peripheral nerves (Focosi et al., Zika Virus: Implications for Public Health. Clin Infect Dis. 2016). However, a diagnosis to clearly implicate ZIKV was compounded due to concomitant outbreaks of DENV and CHICKV. In 2015, outbreaks of ZIKV were reported for the first time in Brazil and was associated with the cause of microcephaly as perceived in aborted fetuses and in infants born to ZIKV infected mothers. While such outbreaks similarly occurred in regions endemic with DENV and CHICKV, ZIKV was successfully retrieved from amniotic fluid and placental and brain tissue in affected fetuses (Rasmussen et al., Zika Virus and Birth Defects - Reviewing the Evidence for Causality. N Engl J Med, 2016). Of additional disturbance is that

ZIKV has also been documented as being sexually transmittable (Musso et al., Potential sexual transmission of Zika virus. *Emerg Infect Dis* 21:359-361, 2015). Brazil normally reports approximately 150 cases of microcephaly per year. However, in 2015 alone, approximately 3000 cases were documented, which manifests as a raise from 5.7 to 99.7 cases per 100,000 births. Instances of ZIKV have also now been reported in the USA. The possibility that ZIKV could become an epidemic worldwide lead the World Health Organization to declare ZIKV a global public health emergency (Focosi et al., 2016, *supra*).

[0004] There are presently no therapies or vaccines to treat or prevent ZIKV infection, respectively, and thus the development of such measures are naturally of paramount importance.

SUMMARY OF THE DISCLOSURE

[0005] The present disclosure describes the development of vaccines comprising Zika virus antigen comprising a nucleic acid expressing an envelope protein (ENV) or modified ENV protein of the Zika virus. Vaccines include naked DNA vaccines, plasmid DNA vaccines and viral vector vaccines. The present disclosure demonstrates that a vesicular stomatitis virus (VSV) vector comprising a nucleic acid expressing an envelope protein (ENV) of a Zika virus and shows that such a vector is immunogenic in a host animal, suggesting use of such a vector in the treatment or prevention of Zika virus infection in a subject.

[0006] In various embodiments, the disclosure provides a vaccine comprising a nucleic acid expressing an envelope protein (ENV) of a Zika virus. In various embodiments, the envelope protein is a full-length envelope protein. In various embodiments, the envelope protein lacks the transmembrane region (pr_mENV). In various embodiments, the vaccine is a naked DNA vaccine, a plasmid DNA vaccine or a viral vector vaccine. In various embodiments, the vaccine may comprise virus-like particles (VLPs). It is contemplated that the vaccine is a live viral vaccine, live attenuated viral vaccine, or inactivated or killed viral vaccine.

[0007] In various embodiments, the viral vector for the vaccine is a vesicular stomatitis virus (VSV), a lentivirus, an adenovirus, an adeno-associated virus, a vaccinia virus, or a modified vaccinia Ankara (MVA) virus.

[0008] In various embodiments, the disclosure provides a recombinant vesicular stomatitis virus (VSV) vector comprising a nucleic acid expressing an envelope protein (ENV) of a Zika virus. In various embodiments, the envelope protein is a full-length envelope protein. In various embodiments, the envelope protein lacks the transmembrane region (prmENV).

[0009] The amino acid and nucleotide sequence of the ZIKA virus genome useful in the vectors are set out in SEQ ID NOs: 1 and 2, respectively, and the location of the different viral proteins is described further in the Detailed Description.

[0010] In various embodiments, the VSV vector comprises a mutation in one or more of the VSV genes that allows for improved production of the virus. In various embodiments, the vector comprises a mutation in the VSV matrix protein (M). In one embodiment, the mutation is VSVM51.

[0011] Exemplary vectors are described in more detail in the Detailed Description and Examples. Such vectors contemplated herein include, but are not limited to, VSV- ZPRME (VSV-ZprME), VSV- ZENV, VSV- Δ M (VSVm), VSV- Δ M-ZPRME (VSVm-ZprME), VSV- Δ M- ZENV (VSVm-ZENV), pVAC-1- pRMEZIKA, pVAC-1-EnvZIKA, pCDNA3-ZPRME or pCDNA3-ZENV.

[0012] In various embodiments, the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L. It is contemplated that in various embodiments, the VSV, optionally, lacks G-protein function.

[0013] In various embodiments, the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).

[0014] Also contemplated herein is an immunogenic composition comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

[0015] The disclosure also provides a vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant.

[0016] The disclosure also provides a viral particles comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant.

[0017] It is contemplated for the immunogenic composition, vaccine and/or for the viral particles of the disclosure that the VSV vector of the immunogenic composition, vaccine and/or

the viral particle has the characteristics described in the paragraphs above and further in the Detailed Description.

[0018] In various embodiments, the disclosure provides a nucleic acid vaccine comprising a pharmaceutically acceptable carrier and a vesicular stomatitis virus (VSV) vector comprising at least one nucleic acid molecule encoding a Zika virus envelope protein, wherein the at least one nucleic acid molecule is expressed in a vaccine recipient, and wherein the expression product induces an immune response against Zika virus in the recipient.

[0019] Also contemplated herein is a method of immunizing a subject against Zika virus infection by administering to the subject a substance that is: a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

[0020] In various embodiments, the disclosure provides a method of diminishing a Zika virus infection in a subject, comprising administering to the subject a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

[0021] In various embodiments, the administration induces antibodies in the subject that neutralize Zika virus infection.

[0022] In various embodiments, the contemplated herein is a method of reducing or alleviating one or more symptoms of Zika virus infection in a subject by administering to the subject a substance that is: a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus. Exemplary symptoms include, but are not limited to, fever, rash, joint pain, conjunctivitis, muscle pain, headache, symptoms of Guillain-Barre syndrome. Symptoms of Guillain-Barre syndrome include, but are not limited to,

pain, tingling and numbness in the spine, hands, feet, arms, or legs, progressive muscle weakness, co-ordination problems and unsteadiness, temporary paralysis of the legs, arms, and face, temporary paralysis of the respiratory muscles, blurred or double vision, difficulty speaking, difficulty chewing or swallowing (dysphagia), difficulty with digestion or bladder control, and fluctuations in heart rate or blood pressure.

[0023] It is understood that each feature or embodiment, or combination, described herein is a non-limiting, illustrative example of any of the aspects of the invention and, as such, is meant to be combinable with any other feature or embodiment, or combination, described herein. For example, where features are described with language such as “one embodiment”, “some embodiments”, “certain embodiments”, “further embodiment”, “specific exemplary embodiments”, and/or “another embodiment”, each of these types of embodiments is a non-limiting example of a feature that is intended to be combined with any other feature, or combination of features, described herein without having to list every possible combination. Such features or combinations of features apply to any of the aspects of the invention. Where examples of values falling within ranges are disclosed, any of these examples are contemplated as possible endpoints of a range, any and all numeric values between such endpoints are contemplated, and any and all combinations of upper and lower endpoints are envisioned.

[0024] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the disclosure, are given by way of illustration only, because various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **Figure 1A** is a diagram showing construction of recombinant VSV expressing Zika Envelope (ENV) or pre-Membrane-Envelope (PRME) proteins. **Figure 1B** shows that synthetic cDNA of Zika ENV and PRME was inserted into pcDNA3 vector attached with HA tag. 293T transfected with pcDNA3-ZprME or pcDNA3-ZENV plasmid overexpressed ZPRME or ZENV which can be detected by immunoblot using both HA antibody and ZIKA antibody 4G2. **Figure**

1C is an immunoblot showing expression of ZIKA PRME or ENV proteins in HeLa cells infected with various recombinant VSV-ZIKA viruses at M.O.I. 10 for 6 hours. **Figure 1D** is an immunofluorescent microscopy analysis of HeLa cells infected with various recombinant VSV-ZIKA viruses at M.O.I. 10 for 6 hours. HA antibody was used to stain Zika ENV proteins.

[0026] Figure 2 shows multi-cycle growth kinetics comparing different rVSVs. Data is shown as the average from three biological replicates titrated by plaque assay using BHK cells.

[0027] Figure 3 illustrates survival percentages of Balb/c mice injected I.M. with rVSVs at 5×10^7 PFU or 1×10^8 PFU in PBS. Time point is 28 days after VSV challenge.

[0028] **Figure 4A** is a diagram showing mouse a Balb/c vaccination and assay procedure.

(**Figure 4B**) Balb/c mice were inoculated intramuscularly following the procedure above. Anti-ZIKV ENV serum titer was analyzed by Elisa using recombinant ZIKV ENV protein and anti-Zika 4G2 antibody was used as standard. (**Figure 4C**) Balb/c mice were inoculated intravenously following the procedure as in (4a). Anti-Zika ENV serum titer was similarly analyzed as in (b).

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Student's t-test. Figure 4D shows serum Zika antibody neutralization effect from mice groups immunized with VSV Δ M-ZENV and VSV-ZENV ($n=3$ /group) were analyzed in Vero cells at 1/80 dilution and showing as percentage of neutralization. (**Figure 4E**) Serum ZIKV antibody neutralization effect from inoculated C57BL/6 mice same was analyzed by plaque reduction neutralization assay (PRNT) in Vero cells. Dilution factor that exhibit 50% neutralization effect (PRNT50) are shown. (**Figure 4F**) C57BL/6 mice were inoculated intravenously following the procedure described herein. Anti-ZIKV ENV serum titer was analyzed by ELISA using recombinant ZIKV ENV protein and anti-ZIKV 4G2 antibody was used as standard. **Figure 4G** shows IFN γ produced after stimulation of splenocytes with ZprME inoculated mice. **Figure 4H** shows flow cytometry analysis by intracellular staining in ZprME peptide-stimulated splenocytes, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Student's T test.

[0029] Figure 5 shows the effects of VSV or VSVm on wild type MEFs infected at M.O.I. 5. Culture medium was collect 8 or 24 hours post infection and measured for mouse interferon β using Elisa kit (PBL Assay Science). Error bars indicate s.d.

[0030] **Figures 6A and 6B** shows the effects of the constructs on immune response. (**Figure 6A**) IFN γ ELISA analysis of medium from ZprME peptide stimulated splenocytes isolated from

naïve or VSVm-prME inoculated C57BL/6 mice. (**Figure 6B**) Flow cytometry analysis of IFN γ intracellular staining in ZprME peptide stimulated splenocytes same as (a). Error bars indicate s.d. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Student's t-test.

[0031] Figure 7 shows real time PCR analysis of ZIKV in brain tissue of C57BL/6 wild type suckling mice infected with ZIKV-MR766 at 1×10^6 PFUs for 7 days post infection. Error bars indicate s.d.

[0032] **Figures 8A-8F** describe that offspring of VSVm-prME vaccinated female mice are protected from lethal ZIKV challenge. (**Figure 8A**) Diagram showing mouse vaccination, breeding and challenge procedure: female vaccinated C57BL/6 mice shown in Fig. 8A were breed with wild type male C57BL/6 mice. Offspring were challenged with 7×10^5 PFUs of ZIKV (MR766) intraperitoneally and monitored for survival rate and disease development. (**Figure 8B, Figure 8C**) The percentages of mice born from the Naïve group (**Figure 8B**) and the VSVm-prME group (**Figure 8C**) displaying the indicated disease signs are shown. (**Figure 8D**) Survival rate of ZIKV challenged newborn mice same as in (Figure 8B and C) are shown. **Figure 8E** shows ZIKV amplification in brain tissue of infant mice measured by plaque assay. **Figure 8F** shows ZIKV levels in brain tissue measured by RNA and real-time PCR.

[0033] **Figure 9** is a table illustrating the antibody titer and neutralization effect of C57BL/6 mice inoculated with rVSV constructs.

DETAILED DESCRIPTION

[0034] The present disclosure relates to a vesicular stomatitis virus vector comprising a polynucleotide encoding a Zika virus envelope protein for use in a therapeutic against Zika virus infection. Described herein are methods for generation of rVSVs (two types VSV and VSV Δ M) that express either the full-length Zika envelope protein (Zenv) incorporating the transmembrane (TM) region (VSV-ZprME) or a truncated version of the Zenv that lacks the TM (VSV-ZENV) and evaluation of their immunogenicity in murine models. Notably, ZENV does not significantly vary in strains isolated across the world, and has not changed significantly from the original isolate in 1947. Thus, the generation of neutralizing antibody to ZENV may suffice to protect against a variety, if not all, ZIKV variants isolated so far.

[0035] Two versions of ZENV (ZprME and ZENV, containing or lacking the ZENVTM domain, respectively) were successfully cloned into rVSV and were expressed efficiently when used to infect a variety of cells. Mice inoculated with rVSVs generated immunoglobulin to the ZENV protein which exhibited the ability to neutralize ZIKV infection when examined in vitro. Thus, rVSV based vectors may be a safe and effective way to provide protection against ZIKV infection and warrant the consideration of further assessment as a preventive measure against ZIKV infection.

[0036] Unless otherwise defined, 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 invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton, et al., *DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY* (2d ed. 1994); *THE CAMBRIDGE DICTIONARY OF SCIENCE AND TECHNOLOGY* (Walker ed., 1988); *THE GLOSSARY OF GENETICS*, 5TH ED., R. Rieger, et al. (eds.), Springer Verlag (1991); and Hale and Marham, *THE HARPER COLLINS DICTIONARY OF BIOLOGY* (1991).

[0037] Each publication, patent application, patent, and other reference cited herein is incorporated by reference in its entirety to the extent that it is not inconsistent with the present disclosure.

[0038] It is noted here that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

[0039] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

Definitions

[0040] A “DNA Vaccine” or “DNA vector” as used herein refers to a synthetic DNA structure that can be transcribed in target cells and can comprise a linear nucleic acid such as a purified DNA, a DNA incorporated in a plasmid vector, or a DNA incorporated into any other vector suitable for introducing DNA into a host cell. In various embodiments, the DNA vaccine can be naked DNA. Provided herein is a naked DNA vaccine, a plasmid DNA vaccine or a viral vector vaccine. It is contemplated that the vaccine is a live viral vaccine, live attenuated viral vaccine,

or inactivated or killed viral vaccine. In various embodiments, the vaccine may comprise virus-like particles (VLPs).

[0041] "Vesicular stomatitis virus" or "VSV" as used herein refers to any strain of VSV or mutant forms of VSV, such as those described in WO 01/19380 or US20140088177. A VSV construct herein may be in any of several forms, including, but not limited to, genomic RNA, mRNA, cDNA, part or all of the VSV RNA encapsulated in the nucleocapsid core, VSV complexed with compounds such as PEG and VSV conjugated to a nonviral protein. VSV vectors useful herein encompass replication-competent and replication-defective VSV vectors, such as, VSV vectors lacking G glycoprotein.

[0042] As used herein, the term "vector" refers to a polynucleotide construct designed for transduction/transfection of one or more cell types. VSV vectors may be, for example, "cloning vectors" which are designed for isolation, propagation and replication of inserted nucleotides, "expression vectors" which are designed for expression of a nucleotide sequence in a host cell, or a "viral vector" which is designed to result in the production of a recombinant virus or virus-like particle, or "shuttle vectors", which comprise the attributes of more than one type of vector. The present invention encompasses viral vectors, such as vesicular stomatitis virus (VSV), a lentivirus, an adenovirus, an adeno-associated virus, a vaccinia virus, or a modified vaccinia Ankara (MVA) virus vectors that comprise nucleic acid encoding Zika virus proteins, such as an Env protein. It is contemplated that the vectors can comprises a polynucleotide encoding a Zika protein as well as a polynucleotide encoding another protein that may improve efficacy of the vector, such as cytokines, including but not limited to those cytokines described herein; chemokines, such as for example, Mip; co-stimulatory proteins, such as for example, B7-1 and B7-2; angiostatin; endostatin; and heat shock proteins, such as for example gp96.

[0043] The terms "polynucleotide" and "nucleic acid", used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. These terms include a single-, double- or triple-stranded DNA, genomic DNA, cDNA, genomic RNA, mRNA, DNA-RNA hybrid, or a polymer comprising purine and pyrimidine bases, or other natural, chemically, biochemically modified, non-natural or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively,

the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidates and thus can be a oligodeoxynucleoside phosphoramidate (P-NH₂) or a mixed phosphoramidate-phosphodiester oligomer. Peyrottes et al. (1996) *Nucleic Acids Res.* 24: 1841-8; Chaturvedi et al. (1996) *Nucleic Acids Res.* 24: 2318-23; Schultz et al. (1996) *Nucleic Acids Res.* 24: 2966-73. A phosphorothioate linkage can be used in place of a phosphodiester linkage. Braun et al. (1988) *J. Immunol.* 141: 2084-9; Latimer et al. (1995) *Molec. Immunol.* 32: 1057-1064. In addition, a double-stranded polynucleotide can be obtained from the single stranded polynucleotide product of chemical synthesis either by synthesizing the complementary strand and annealing the strands under appropriate conditions, or by synthesizing the complementary strand de novo using a DNA polymerase with an appropriate primer. Reference to a polynucleotide sequence (such as referring to a SEQ ID NO) also includes the complement sequence.

[0044] The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, genomic RNA, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars and linking groups such as fluororibose and thioate, and nucleotide branches. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides, or a solid support.

[0045] The phrase "substantially homologous" or "substantially identical" in the context of two nucleic acids or polypeptides, generally refers to two or more sequences or subsequences that have at least 40%, 60%, 80%, 90%, 95%, 96%, 97%, 98% or 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the

sequences are substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are substantially identical over the entire length of either or both comparison biopolymers. It is contemplated herein that the envelope protein of the Zika virus useful in the VSV vector and immunogenic composition, vaccine or viral particle can have 80%, 90%, 95%, 96%, 97%, 98% or 99% nucleotide or amino acid residue identity to a naturally-occurring Zika virus envelope protein.

[0046] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0047] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970, by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection. Alignment is also measured using such algorithms as PILEUP. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360, 1987. The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153, 1989. Another algorithm that is useful for generating multiple alignments of sequences is Clustal W (Thompson *et al.*, *Nucleic Acids Research* 22: 4673-4680, 1994). Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403-410, 1990. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[0048] "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of

nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[0049] "Under transcriptional control" is a term well understood in the art and indicates that transcription of a polynucleotide sequence depends on its being operably (operatively) linked to an element which contributes to the initiation of, or promotes, transcription. "Operably linked" refers to a juxtaposition wherein the elements are in an arrangement allowing them to function.

[0050] As used herein, in the context of the viral vectors, a "heterologous polynucleotide" or "heterologous gene" or "transgene" is any polynucleotide or gene that is not present in wild-type viral vector.

[0051] As used herein, in the context of the viral vectors, a "heterologous" promoter is one which is not associated with or derived from the viral vector itself.

[0052] A "host cell" includes an individual cell or cell culture which can be or has been a recipient of a VSV vector(s) described herein. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transfected, transformed or infected *in vivo* or *in vitro* with a vector herein.

[0053] "Replication" and "propagation" are used interchangeably and refer to the ability of a vector of the invention to reproduce or proliferate. These terms are well understood in the art. For purposes of this disclosure, replication involves production of viral proteins and is generally directed to reproduction of the viral vector. Replication can be measured using assays standard in the art. "Replication" and "propagation" include any activity directly or indirectly involved in

the process of virus manufacture, including, but not limited to, viral gene expression; production of viral proteins, nucleic acids or other components; packaging of viral components into complete viruses; and cell lysis.

[0054] As used herein, “antigenic composition” or “immunogenic composition” refers to a composition comprising material which stimulates the immune system and elicits an immune response in a host or subject. The term “elicit an immune response” refers to the stimulation of immune cells in vivo in response to a stimulus, such as an antigen. The immune response consists of both cellular immune response, e.g., T cell and macrophage stimulation, and humoral immune response, e.g., B cell and complement stimulation and antibody production. The cellular and humoral immune response are not mutually exclusive, and it is contemplated that one or both are stimulated by an antigenic composition, virus or vaccine as described herein. Immune response may be measured using techniques well-known in the art, including, but not limited to, antibody immunoassays, proliferation assays, and others described in greater detail in the Detailed Description.

[0055] As used herein, “vaccine” refers to a composition comprising a vector comprising a heterologous Zika virus protein as described herein, which is useful to establish immunity to the Zika virus in the subject. It is contemplated that the vaccine comprises a pharmaceutically acceptable carrier and/or an adjuvant. It is contemplated that vaccines are prophylactic or therapeutic. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology. The compounds of the invention may be given as a prophylactic treatment to reduce the likelihood of developing a pathology or to minimize the severity of the pathology, if developed. A “therapeutic” treatment is a treatment administered to a subject who exhibits signs or symptoms of pathology for the purpose of diminishing or eliminating those signs or symptoms. The signs or symptoms may be biochemical, cellular, histological, functional, subjective or objective.

[0056] As used herein, “isolated” refers to a virus or immunogenic composition that is removed from its native environment. Thus, an isolated biological material is free of some or all cellular components, i.e., components of the cells in which the native material occurs naturally (e.g., cytoplasmic or membrane component). In one aspect, a virus or antigenic composition is

deemed isolated if it is present in a cell extract or supernatant. In the case of nucleic acid molecules, an isolated nucleic acid includes a PCR product, an isolated mRNA, a cDNA, or a restriction fragment.

[0057] “Purified” as used herein refers to a virus or immunogenic composition that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, including endogenous materials from which the composition is obtained. By way of example, and without limitation, a purified virion is substantially free of host cell or culture components, including tissue culture or cell proteins and non-specific pathogens. In various embodiments, purified material substantially free of contaminants is at least 50% pure; at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or even at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, and other methods known in the art.

[0058] As used herein, “pharmaceutical composition” refers to a composition suitable for administration to a subject animal, including humans and mammals. A pharmaceutical composition comprises a pharmacologically effective amount of a virus or antigenic composition of the invention and also comprises a pharmaceutically acceptable carrier. A pharmaceutical composition encompasses a composition comprising the active ingredient(s), and the inert ingredient(s) that make up the pharmaceutically acceptable carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound or conjugate of the present invention and a pharmaceutically acceptable carrier.

[0059] As used herein, “pharmaceutically acceptable carrier” include any and all clinically useful solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, and excipients, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose or mannitol, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co., Easton, 1995). Pharmaceutical carriers useful for the composition depend upon the intended mode of administration of the active agent. Typical modes of administration

include, but are not limited to, enteral (*e.g.*, oral) or parenteral (*e.g.*, subcutaneous, intramuscular, intravenous or intraperitoneal injection; or topical, transdermal, or transmucosal administration). A “pharmaceutically acceptable salt” is a salt that can be formulated into a compound or conjugate for pharmaceutical use including, *e.g.*, metal salts (sodium, potassium, magnesium, calcium, *etc.*) and salts of ammonia or organic amines.

[0060] As used herein, “pharmaceutically acceptable” or “pharmacologically acceptable” refers to a material which is not biologically or otherwise undesirable, *i.e.*, the material may be administered to an individual without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained, or when administered using routes well-known in the art, as described below.

Vesicular stomatitis virus (VSV)

[0061] Vesicular stomatitis virus (VSV) is a nonsegmented, negative-strand RNA virus that belongs to the family of rhabdoviridae (Barber, G., *Oncogene*. 24(52):7710–9, 2005) widely used as a vaccine platform as well as an anticancer therapeutic. VSV comprises approximately an 11 kilobase genome that encodes for five proteins referred to as the nucleocapsid (N), polymerase proteins large (L) and (P) (formerly termed NS, originally indicating nonstructural), surface glycoprotein (G) and a peripheral matrix protein (M). The virus particles contain a helical, nucleocapsid core composed of the genomic RNA and protein. The genome is tightly encased in nucleocapsid protein and also comprises the polymerase proteins L and P. An additional matrix (M) protein lies within the membrane envelope, perhaps interacting both with the membrane and the nucleocapsid core. A single glycoprotein (G) species spans the membrane and forms the spikes on the surface of the virus particle. Glycoprotein G is responsible for binding to cells and membrane fusion.

[0062] The VSV genome is the negative sense (*i.e.*, complementary to the RNA sequence (positive sense) that functions as mRNA to directly produce encoded protein), and rhabdoviruses must encode and package an RNA-dependent RNA polymerase in the virion (Baltimore et al., 1970, *Proc. Natl. Acad. Sci. USA* 66: 572-576), composed of the P and L proteins. This enzyme transcribes genomic RNA to make subgenomic mRNAs encoding the 5-6 viral proteins and also replicates full-length positive and negative sense RNAs. The genes are transcribed sequentially, starting at the 3' end of the genomes.

[0063] The sequences of the VSV mRNAs and genome is described in Gallione et al. 1981, J. Virol. 39:529-535; Rose and Gallione, 1981, J. Virol. 39:519-528; Rose and Schubert, 1987, Rhabdovirus genomes and their products, p. 129-166, in R. R. Wagner (ed.), The Rhabdoviruses. Plenum Publishing Corp., NY; Schubert et al., 1985, Proc. Natl. Acad. Sci. USA 82:7984-7988. WO 96/34625 published Nov. 7, 1996, disclose methods for the production and recovery of replicable vesiculovirus. U.S. Pat. No. 6,168,943, issued Jan. 2, 2001, describes methods for making recombinant vesiculoviruses.

[0064] VSV is predominantly a pathogen of livestock (Letchworth et al., Vet. J. 157:239-260, 1999) and usually produces a self-limiting disease in livestock. It is essentially non-pathogenic in humans (Balachandran and Barber (2000, IUBMB Life 50: 135-8), but does, however, have a very broad species tropism. The cellular tropism of VSV is determined predominantly at postentry steps, since the G glycoprotein of the virus mediates entry into most tissues in nearly all animal species (Carneiro et al., J. Virol. 76:3756-3764, 2002). Though viral entry can take place in nearly all cell types (Kelly et al., J Virol. 84(3): 1550–1562, 2010), in vivo models of VSV infection have revealed that the virus is highly sensitive to the innate immune response, limiting its pathogenesis (Barber, G. N. Oncogene 24:7710-7719, 2005). VSV is intensively responsive to type I interferon (IFN), as the double-stranded RNA (dsRNA)-dependent PKR (Balachandran, S., and G. N. Barber. Cancer Cell 5:51-65, 2004), the downstream effector of pattern recognition receptors MyD88 (Lang et al., Eur. J. Immunol. 37:2434-2440, 2007), and other molecules mediate shutdown of viral translation and allow the adaptive immune response to clear the virus.

[0065] VSV induces potent in vitro and in vivo tumor cytotoxic effects, and its efficacy has been tested in a number of xenograft and syngeneic models. VSV-induced neurotoxicity, however is dose limiting (Clarke et al., Springer Semin Immunopathol. 2006;28(3):239–53, 2006; Johnson et al., Virology. 2007;360(1):36–49), and can limit clinical development efforts of this agent (Kurisetty et al., Head Neck. 36(11):1619–1627, 2014).

[0066] A table of various VSV strains is shown in "Fundamental Virology", second edition, supra, at page 490. WO 01/19380 and U.S. Pat. No. 6,168,943 disclose that strains of VSV include Indiana, New Jersey, Piry, Colorado, Coccal, Chandipura and San Juan. The complete nucleotide and deduced protein sequence of a VSV genome is known and is available as

Genbank VSVCG, accession number JO2428; NCBI Seq ID 335873; and is published in Rose and Schubert, 1987, in *The Viruses: The Rhabdoviruses*, Plenum Press, NY. pp. 129-166. A complete sequence of a VSV strain is shown in U.S. Pat. No. 6,168,943. VSV New Jersey strain is available from the American Type Culture Collection (ATCC) and has ATCC accession number VR-159. VSV Indiana strain is available from the ATCC and has ATCC accession number VR-1421.

[0067] The present disclosure provides recombinant vesicular stomatitis virus (VSV) vectors comprising nucleic acid encoding a Zika virus protein, for example, the envelope protein, wherein said recombinant VSV vector expresses the Zika virus protein and is useful for therapy against a Zika virus infection. It is contemplated that the Zika envelope protein is a full length envelope protein or an E protein lacking the transmembrane region (prMEV). In various embodiments, the vector comprises a mutation in the VSV matrix protein (M), optionally wherein the mutation is VSVM51.

[0068] The present disclosure contemplates VSV vectors comprising nucleic acid encoding more than one biologically active protein, such as for example, a VSV vector comprising nucleic acid encoding a Zika virus protein and cytokines, such as for example, an interferon and an interleukin; two interferons; or two interleukins. In other examples, the VSV vector is replication-competent. In additional examples, the VSV vector is replication-defective. In yet other examples, the VSV vector lacks a protein function essential for replication, such as G-protein function or M and/or N protein function. The VSV vector may lack several protein functions essential for replication. In further embodiments, the subject or patient is an animal, preferably a mammal, such as a human. The present disclosure also provides viral particles comprising a VSV vector, such as a VSV vector comprising nucleic acid encoding a Zika virus envelope protein. The present disclosure also contemplates isolated nucleic acid encoding a recombinant VSV vector herein as well as host cells comprising a recombinant VSV vector of described herein.

[0069] In various embodiments, the VSV vector further comprises one or more deletions or mutations in one or more VSV nucleic acid sequences. A wild-type VSV genome has the following gene order: 3'-NPMGL-5'. So in one embodiment, the VSV vector may lack a G protein sequence or it may have one or more mutations which result in a VSV vector lacking G-

protein function or express a mutated or truncated G-protein. In another embodiment, the VSV vector has mutations or deletions of M sequences, producing VSV vectors which do not express M protein or lack M protein function or express a mutated or truncated M protein. In one embodiment, a VSV vector of the invention comprises one or more mutations in its genome. For example, a of the invention includes, but is not limited to, a VSV temperature-sensitive N gene mutation, a temperature-sensitive L gene mutation, a point mutation, a G-stem mutation, a non-cytopathic M gene mutation, a gene shuffling or rearrangement mutation, a truncated G gene mutation, an ambisense RNA mutation, a G gene insertion mutation, a gene deletion mutation and the like. Thus the term, a "mutation" includes mutations known in the art as insertions, deletions, substitutions, gene rearrangement or shuffling modifications.

[0070] In various embodiments, for the VSV vectors described herein, a polynucleotide sequence may also encode one or more heterologous (or foreign) polynucleotide sequences or open reading frames (ORFs). The foreign polynucleotide sequences can vary as desired, and include, but are not limited to Zika virus proteins, a co-factor, a cytokine (such as an interferon or interleukin) or other protein of interest. In another embodiment, the heterologous polynucleotide sequence further encodes a cytokine, such as interferon, which are selected to improve the prophylactic or therapeutic characteristics of the recombinant VSV. In preferred embodiments, a foreign nucleic acid can be inserted into regions of VSV encoding for G-protein, M-protein or combinations thereof.

[0071] In other embodiments, a composition comprises an attenuated vesicular stomatitis (VSV) vector expressing a one or more oligonucleotides which modulate expression or function of target molecules. In various embodiments, the oligonucleotides comprises: dsRNA, siRNA, antisense RNA, RNA, enzymatic RNA or microRNA.

[0072] Also contemplated herein is an immunogenic composition comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus. In various embodiments, in the immunogenic composition, the envelope protein is a full-length envelope protein. In various embodiments, the envelope protein lacks the transmembrane region (prmENV).

[0073] In various embodiments, in the immunogenic composition the VSV vector comprises a mutation in one or more of the VSV genes that allows for improved production of the virus. In

various embodiments, the vector comprises a mutation in the VSV matrix protein (M). In one embodiment, the mutation is VSVM51.

[0074] In various embodiments, in the immunogenic composition the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L. It is contemplated that in various embodiments, the VSV, optionally, lacks G-protein function. In various embodiments, the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).

[0075] The disclosure also provides a vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant. In various embodiments, in the vaccine, the envelope protein is a full-length envelope protein. In various embodiments, the envelope protein lacks the transmembrane region (prmENV).

[0076] In various embodiments, in the vaccine the VSV vector comprises a mutation in one or more of the VSV genes that allows for improved production of the virus. In various embodiments, the vector comprises a mutation in the VSV matrix protein (M). In one embodiment, the mutation is VSVM51.

[0077] In various embodiments, in the vaccine the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L. It is contemplated that in various embodiments, the VSV, optionally, lacks G-protein function. In various embodiments, the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).

[0078] The disclosure also provides viral particles comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant. In various embodiments, in the viral particles, the envelope protein is a full-length envelope protein. In various embodiments, the envelope protein lacks the transmembrane region (prmENV).

[0079] In various embodiments, in the viral particles the VSV vector comprises a mutation in one or more of the VSV genes that allows for improved production of the virus. In various embodiments, the vector comprises a mutation in the VSV matrix protein (M). In one embodiment, the mutation is VSVM51.

[0080] In various embodiments, in the viral particles the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L. It is

contemplated that in various embodiments, the VSV, optionally, lacks G-protein function. In various embodiments, the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).

Other Viral Vectors

[0081] It is further contemplated that the viral vector for the vaccine is a retrovirus, including a lentivirus, an adenovirus, an adeno-associated virus, a vaccinia virus, or a modified vaccinia Ankara (MVA) virus. Constructs as described above with respect to the Zika virus protein can also be made in these other viral vectors. For example, the present disclosure provides recombinant vectors comprising nucleic acid encoding a Zika virus protein, for example, the envelope protein, wherein said recombinant vector expresses the Zika virus protein and is useful for therapy against a Zika virus infection. It is contemplated that the Zika envelope protein is a full length envelope protein or an E protein lacking the transmembrane region (prmENV). Also contemplated is an immunogenic composition comprising a viral vector comprising an envelope protein (ENV) of a Zika virus. In various embodiments, in the immunogenic composition, the envelope protein is a full-length envelope protein. In various embodiments, the envelope protein lacks the transmembrane region (prmENV). The Env protein in the vector may be used as described herein.

[0082] Retroviruses are enveloped RNA viruses that are capable of infecting animal cells, and that utilize the enzyme reverse transcriptase in the early stages of infection to generate a DNA copy from their RNA genome, which is then typically integrated into the host genome. Examples of retroviral vectors Moloney murine leukemia virus (MLV)-derived vectors, retroviral vectors based on a Murine Stem Cell Virus, which provides long-term stable expression in target cells such as hematopoietic precursor cells and their differentiated progeny (see, e.g., Hawley et al., PNAS USA 93:10297-10302, 1996; Keller et al., Blood 92:877-887, 1998), hybrid vectors (see, e.g., Choi, et al., Stem Cells 19:236-246, 2001), and complex retrovirus-derived vectors, such as lentiviral vectors.

[0083] Examples of lentiviruses include HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2), visna-maedi, the caprine arthritis-encephalitis virus, equine infectious anemia virus, feline immunodeficiency virus (FIV), bovine immune deficiency virus (BIV), and simian immunodeficiency virus (SIV). Lentiviral vectors can be derived from any one or more

of these lentiviruses (see, e.g., Evans et al., Hum Gene Ther. 10:1479-1489, 1999; Case et al., PNAS USA 96:2988-2993, 1999; Uchida et al., PNAS USA 95:11939-11944, 1998; Miyoshi et al., Science 283:682-686, 1999; Sutton et al., J Virol 72:5781-5788, 1998; and Frecha et al., Blood. 112:4843-52, 2008).

[0084] Adenoviral vectors, methods for construction thereof and methods for propagating thereof, are well known in the art and are described in, for example, U.S. Patent Nos. 9,125,870, 5,559,099, 5,837,511, 5,846,782, 5,851,806, 5,994,106, 5,994,128, 5,965,541, 5,981,225, 6,040,174, 6,020,191, and 6,113,913, and Thomas Shenk, "Adenoviridae and their Replication," M. S. Horwitz, "Adenoviruses," Chapters 67 and 68, respectively, in Virology, B. N. Fields et al., eds., 3d ed., Raven Press, Ltd., New York (1996).

[0085] Adeno-associated viral vectors, methods for construction thereof and methods for propagating thereof, are well known in the art and are described in, for example in U.S. Patents 6,448,074, 8,318,687, and 8,394,386.

[0086] Vaccinia viruses have been used for decades as vectors for foreign antigens (Smith et al., Biotechnology and Genetic Engineering Reviews 2. 383-407 [1984]). Methods of inserting foreign DNA into vaccinia virus is well-known to those in the field of vaccine development and protein engineering.

[0087] Modified Vaccinia Ankara (MVA) virus is related to vaccinia virus. MVA was engineered for use as a viral vector for recombinant gene expression or as a recombinant vaccine (Sutter, G. et al. [1994], Vaccine 12: 1032-40). Modified MVA for use as vaccines or other viral vector are described in U.S. Patent Nos. 6,913,752, 6,960,345, 9,133,478 and 9,463,238.

[0088] Construction of viral vectors involves the use of standard molecular biological techniques, such as those described in, for example, Sambrook et al., Molecular Cloning, a Laboratory Manual, 2d ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), Watson et al., Recombinant DNA, 2d ed., Scientific American Books (1992), and Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, NY (1995), and other references mentioned herein.

[0089] Virus Like Particles typically comprise a viral polypeptide(s) derived from a structural protein(s) of a virus. As described in U.S. Patent 9,051,359, methods for producing and

characterizing recombinantly produced VLPs have been described based on several viruses, including influenza virus (Bright et al. (2007) *Vaccine*. 25:3871), human papilloma virus type 1 (Hagnese et al. (1991) *J. Virol.* 67:315), human papilloma virus type 16 (Kirnbauer et al. *Proc. Natl. Acad. Sci.* (1992) 89:12180), HIV-1 (Haffer et al., (1990) *J. Virol.* 64:2653), and hepatitis A (Winokur (1991) 65:5029), and can be adapted to the viral strain of interest.

Zika Virus

[0090] The Zika virus (ZIKV) genome consists of a single-stranded positive sense RNA molecule with 10794 kb of length with 2 flanking non-coding regions (5' and 3' NCR) and a single long open reading frame encoding a polyprotein: 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3', that is cleaved into capsid (C), precursor of membrane (prM), envelope (E or env) and seven non-structural proteins (NS) (Faye et al., *PLoS Negl Trop Dis.* 8(1): e2636, 2014; Chambers et al., *Annu Rev Microbiol* 44:649–688, 1990; Kuno G, Chang G-JJ., *Arch Virol* 152: 687–696, 2007). The envelope protein, E, is approximately 53 kDa and is the major virion surface protein, involved in various aspects of the viral cycle. E protein also mediates binding and membrane fusion (Lindenbach BD, Rice CM *Adv Virus Res* 59: 23–61, 2003).

[0091] Faye et al. (*supra*) carried out a phylogenetic analysis of 43 isolates of Zika virus, including analysis of glycosylation sites within the E protein. Faye identified possible N-linked glycosylation site in the residue Asn-154, and a probable mucin-type O-linked glycosylated site at residue Thr-170 in E protein from all ZIKV strains, and other mucin sites at residues Thr-245 and Thr-381 in some isolates. Probable O-GlcNAc attachment sites were identified at residues Ser-142, Ser-227, Thr-231, Ser-304, Thr-366 and Thr-381 in E from some strains. Glycosylation sites that may have infective or adaptive value were previously described in Lanciotti et al. (*Emerg Infect Dis* 14: 1232–1239, 2008); Kuno et al., (*Arch Virol* 152: 687–696, 2007) and Haddow et al., (*PLoS Negl Trop Dis* 6: e1477, 2012). These sites may be relevant for modification for making a VSV vector comprising E protein with modifications in the glycosylation sites.

[0092] The Zika virus genome is set out in Genbank Accession No. KU321639. The database predicts that the different Zika proteins are encoded by the nucleotide sequence as follows: capsid, nucleotides 106-480; propeptide, nucleotides 478-750; membrane protein, nucleotides 751-975; envelope protein, nucleotides 976-2490; NS1, nucleotides 2491-3576; NS2A,

nucleotides 3577-4230; NS2B, nucleotides 4231-4662; NS3, nucleotides 4663-6471; NS4A, nucleotides 6472-6912; NS4B, nucleotides 6913-8418; NS5, nucleotides 8419-10374. The protein sequence is disclosed in Genbank Accession No. ALU33341. The membrane protein is set out in amino acids 216-290 and the envelope protein is set out in amino acids 291-795 of the encoded genome. The amino acid and nucleotide sequence of the viral genome is set out in SEQ ID NOs: 1 and 2, respectively.

[0093] Also contemplated is modified envelope protein that may comprise all or part of the full length protein amino acid sequences or may have an amino acid substitution, deletion or other modification that allows for immunogenicity of the protein but may improve expression of the protein in the vector, or some other characteristic.

[0094] A “fragment” of a polypeptide refers to any portion of the polypeptide smaller than the full-length polypeptide or protein expression product. Fragments are, in one aspect, deletion variants of the full-length polypeptide wherein one or more amino acid residues have been removed from the amino terminus and/or the carboxy terminus of the full-length polypeptide.

[0095] In one aspect, an variant exhibits about 70% sequence similarity but less than 100% sequence similarity with the wild-type or naturally-occurring sequence, *e.g.*, a peptide. For example, the variant can have 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to a naturally-occurring E protein. Such variants, are, in one aspect, comprised of non-naturally occurring amino acid residues, including by way of example and not limitation, homoarginine, ornithine, penicillamine, and norvaline, as well as naturally occurring amino acid residues. Such variants are, in another aspect, composed of one or a plurality of D-amino acid residues, or contain non-peptide interlinkages between two or more amino acid residues. In one embodiment, the variant may be a fragment of a polypeptide, wherein the fragment is substantially homologous (*i.e.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% homologous) over a length of at least 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 amino acids of the wild-type polypeptide.

[0096] Substitutions are conservative or non-conservative based on the physico-chemical or functional relatedness of the amino acid that is being replaced and the amino acid replacing it. Substitutions of this type are well known in the art. Alternatively, the invention embraces

substitutions that are also non-conservative. Exemplary conservative substitutions are described in Lehninger, [Biochemistry, 2nd Edition; Worth Publishers, Inc., New York (1975), pp.71-77].

Methods of Making Vector

[0097] The present disclosure also provides methods for making a recombinant vector described herein comprising growing a cell comprising said vector under conditions whereby the modified ZIKA protein is produced; and optionally isolating said vector.

[0098] In various embodiments, the vector is a VSV vector, optionally a replication defective VSV and the host cells comprising the VSV protein function essential for VSV replication such that said VSV vector is capable of replication in said host cell. In some embodiments, the VSV vector comprises nucleic acid encoding a Zika virus protein, such as an envelope protein, or other biological protein, such as a heat shock protein, such as for example, gp96, and endostatin and angiostatin.

[0099] Methods of making VSV vectors are described in U.S. Patent Publication 20140088177, incorporated herein by reference. Briefly, VSV mRNA can be synthesized in vitro, and cDNA prepared by standard methods, followed by insertion into cloning vectors (see, e.g., Rose and Gallione, 1981, J. Virol. 39(2):519-528). VSV or portions of VSV can be prepared using oligonucleotide restriction enzymes). Polynucleotides used for making VSV vectors herein may be obtained using standard methods in the art, such as chemical synthesis, recombinant methods and/or obtained from biological sources. Individual cDNA clones of VSV RNA can be joined by use of small DNA fragments covering the gene junctions, generated by use of reverse transcription and polymerase chain reaction (RT-PCR) from VSV genomic RNA.

[0100] VSV may be genetically modified in order to alter its properties for use in vivo. Methods for the genetic modification of VSV are well established within the art. For example, a reverse genetic system has been established for VSV (Roberts et al., Virology, 1998, 247: 1-6) allowing for modifications of the genetic properties of the VSV. Standard techniques well-known to one of skill in the art may be used to genetically modify VSV and introduce desired genes within the VSV genome to produce recombinant VSVs (see for example, Sambrooke et al., 1989, A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press). For insertion of nucleotide sequences into VSV vectors, for example nucleotide sequences encoding a Zika virus protein, or for VSV gene sequences inserted into vectors, such as for the production helper

cell lines, specific initiation signals are required for efficient translation of inserted protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire VSV gene, such as G-protein including its own initiation codon and adjacent sequences are inserted into the appropriate vectors, no additional translational control signals may be needed. However, in cases where only a portion of the gene sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. The initiation codon must furthermore be in phase with the reading frame of the protein coding sequences to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic.

[0101] Following infection of a host cell, recombinant VSV shuts down host cell protein synthesis and expresses not only its own five gene products, but also heterologous proteins encoded within its genome. Successful expression of heterologous nucleic acid from VSV recombinants requires only the addition of the heterologous nucleic acid sequence into the full-length cDNA along with the minimal conserved sequence found at each VSV gene junction. This sequence consists of the polyadenylation/transcription stop signal (3' AUACU7) followed by an intergenic dinucleotide (GA or CA) and a transcription start sequence (3'-UUGUCNNUAG) complementary to the 5' ends of all VSV mRNAs. Ball et al. 1999, J. Virol. 73:4705-4712; Lawson et al. 1995, P.N.A.S. USA 92:4477-4481; Whelan et al. 1995, P.N.A.S. USA 92:8388-8392. Additionally, restriction sites, preferably unique, (e.g., in a polylinker) are introduced into the VSV cDNA, for example in intergenic regions, to facilitate insertion of heterologous nucleic acid, such as nucleic acid encoding an interleukin or interferon.

[0102] In other examples, the VSV cDNA is constructed so as to have a promoter operatively linked thereto. The promoter should be capable of initiating transcription of the cDNA in an animal or insect cell in which it is desired to produce the recombinant VSV vector. Promoters which may be used include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); heat shock promoters (e.g., hsp70 for use in Drosophila S2 cells); the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the

following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Omitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol Cell Biol.* 5:1639-1648; Hammer et al. 1987, *Science* 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 161-171), beta-globin gene control region which is active in myeloid cells (Mogam et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712); and myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286). Optionally, the promoter is an MA polymerase promoter, preferably a bacteriophage or viral or insect RNA polymerase promoter, including but not limited to the promoters for T7 RNA polymerase, SP6 RNA polymerase, and T3 RNA polymerase. If an RNA polymerase promoter is used in which the RNA is not endogenously produced by the host cell in which it is desired to produce the recombinant VSV, a recombinant source of the RNA polymerase must also be provided in the host cell. Such RNA polymerase are known in the art.

[0103] The VSV cDNA can be operably linked to a promoter before or after insertion of nucleic acid encoding a heterologous protein, such as a Zika virus protein, including all or part of a Zika envelope protein. In some examples, a transcriptional terminator is situated downstream of the VSV cDNA. In other examples, a DNA sequence that can be transcribed to produce a ribozyme sequence is situated at the immediate 3' end of the VSV cDNA, prior to the transcriptional termination signal, so that upon transcription a self-cleaving ribozyme sequence is produced at the 3' end of the antigenomic RNA, which ribozyme sequence will autolytically cleave (after a U) this fusion transcript to release the exact 3' end of the VSV antigenomic

(+)RNA. Any ribozyme sequence known in the art may be used, as long as the correct sequence is recognized and cleaved. (It is noted that hammerhead ribozyme is probably not suitable for use.)

[0104] The present disclosure provides for expression systems comprising a VSV vector comprising one or more heterologous nucleotide sequence(s), such as, a nucleotide sequence encoding a Zika virus protein, such as for example, E protein, inserted within a region of the VSV essential for replication, such as the G glycoprotein region, or other region essential for replication, such that the VSV lacks the essential function and is replication-defective. The VSV vector may have a mutation, such as a point mutation or deletion of part or all, of any region of the VSV genome, including the G, M, N, L or P region. If the mutation is in a region essential for replication, the VSV will be grown in a helper cell line that provides the essential region function. The VSV may also comprise a mutation, such as for example, a point mutation or deletion of part or all of a nucleotide sequence essential for replication, and optionally, with the heterologous nucleotide sequence inserted in the site of the deleted nucleotide sequence. The heterologous nucleotide sequence may be operably linked to a transcriptional regulatory sequence. Following infection of a target malignant or tumor cell, progeny viruses will lack essential protein function and cannot disseminate to infect surrounding tissue. In additional embodiments, the VSV vector is mutated in nucleic acid, such as by point mutation, substitution or addition of nucleic acid, or deletion of part or all, of nucleic acid encoding other VSV protein function such as, M protein and/or N protein function. VSV may be targeted to a desired site in vitro to increase viral efficiency. For example, modification of VSV G protein (or other VSV proteins) to produce fusion proteins that target specific sites may be used to enhance VSV efficiency in vivo. Such fusion proteins may comprise, for example, but not limited to single chain Fv fragments that have specificity for tumor antigens. (Lorimer et al., P.N.A.S. U.S.A., 1996. 93: 14815-20).

[0105] A VSV vector lacking a gene(s) essential for viral replication can be grown in an appropriate complementary cell line. Accordingly, the present invention provides recombinant helper cell lines or helper cells that provide a VSV protein function essential for replication of a replication-deficient VSV construct. In some examples, the protein function is G-protein function. For example, a VSV vector comprising nucleic acid encoding a cytokine and lacking G-protein function can be grown in a cell line, i.e., a helper cell line, for example, a mammalian

cells line such as CHO cell line, permissive for VSV replication, wherein said cell line expresses an appropriate G-protein function, such that said VSV is capable of replicating in the cell line. These complementing or helper cell lines are capable of allowing a replication-defective VSV to replicate and express one or more foreign genes or fragments thereof encoded by the heterologous nucleotide sequence. In some embodiments, the VSV vector lacks a protein host cell line comprises nucleic acid encoding the protein function essential for replication, such as for example, VSV G-protein function. Complementing cell lines can provide VSV viral function through, for example, co-infection with a helper virus, or by integration or otherwise maintaining in stable form part or all of a viral genome encoding a particular viral function. In other examples, additional VSV non-essential proteins can be deleted or heterologous nucleotide sequences inserted into nucleotide regions encoding non-essential VSV, such as for example, the M and N proteins. The heterologous nucleotide sequence can be inserted into a region non-essential for replication wherein the VSV is replication competent. Heterologous nucleotide sequences can be inserted in non-essential regions of the VSV genome, without necessitating the use of a helper cell line for growth of the VSV vector.

[0106] The recombinant VSV of the disclosure are produced for example, by providing in an appropriate host cell VSV cDNA wherein said cDNA comprises nucleotide sequence encoding a heterologous protein, such as for example, a Zika virus protein, including an E protein. The nucleic acid encoding a heterologous protein can be inserted in a region non-essential for replication, or a region essential for replication, in which case the VSV is grown in the presence of an appropriate helper cell line. The production of recombinant VSV vector is carried out in vitro, in cell culture, or in cells permissive for growth of the VSV. Standard recombinant techniques can be used to construct expression vectors containing DNA encoding VSV proteins. Expression of such proteins may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of VSV proteins can be constitutive or inducible.

Host Cells

[0107] The present invention also provides host cells comprising (i.e., transformed, transfected or infected with) the vectors or particles described herein. Both prokaryotic and eukaryotic host cells, including insect cells, can be used as long as sequences requisite for maintenance in that

host, such as appropriate replication origin(s), are present. For convenience, selectable markers are also provided. Host systems are known in the art and need not be described in detail herein. Prokaryotic host cells include bacterial cells, for example, *E. coli*., *B. subtilis*, and mycobacteria. Among eukaryotic host cells are yeast, insect, avian, plant, *C. elegans* (or nematode) and mammalian host cells. Examples of fungi (including yeast) host cells are *S. cerevisiae*. *Kluyveromyces lactis* (*K. lactis*), species of *Candida* including *C. albicans* and *C. glabrata*. *Aspergillus nidulans*. *Schizosaccharomyces pombe* (*S. pombe*), *Pichia pastoris*, and *Yarrowia lipolytica*. Examples of mammalian cells are COS cells, mouse L cells, LNCaP cells, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK) cells and African green monkey cells. *Xenopus laevis* oocytes, or other cells of amphibian origin, may also be used.

[0108] The present disclosure also includes compositions, including pharmaceutical compositions, containing the vector(s), immunogenic compositions, vaccines or viral particles described herein. Such compositions are useful for administration in vivo, for example, when measuring the degree of transduction and/or effectiveness of viral infectivity of a Zika virus. Compositions can comprise a vector(s) described herein and a suitable solvent, such as a physiologically acceptable buffer. These are well known in the art. In other embodiments, these compositions further comprise a pharmaceutically acceptable excipient. These compositions, which can comprise an effective amount of a vector in a pharmaceutically acceptable excipient, are suitable for systemic or local administration to individuals in unit dosage forms, sterile parenteral solutions or suspensions, sterile non-parenteral solutions or oral solutions or suspensions, oil in water or water in oil emulsions and the like. Formulations for parenteral and nonparenteral drug delivery are known in the art and are set forth in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing (1995). Compositions also include lyophilized and/or reconstituted forms of the vectors (including those packaged as a virus) of the invention.

[0109] The present disclosure also contemplates kits containing vector(s), immunogenic compositions, vaccines or viral particles described herein. These kits can be used for example for producing proteins for screening, assays and biological uses, such as a vaccine therapeutic. Procedures using these kits can be performed by clinical laboratories, experimental laboratories, medical practitioners, or private individuals.

[0110] The kits comprise a vector described herein in suitable packaging. The kit may optionally provide additional components that are useful in the procedure, including, but not limited to, buffers, developing reagents, labels, reacting surfaces, means for detection, control samples, instructions, and interpretive information. The kit may include instructions for administration of a VSV vector or vaccine composition.

Methods of Use

[0111] The disclosure provides methods of immunizing an individual or a method of diminishing a Zika virus infection in an individual, e.g., a patient or subject, against Zika virus infection by administering to the subject a substance that is: a) an immunogenic composition comprising vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vector comprising an envelope protein (ENV) of a Zika virus.

[0112] In various embodiments, the disclosure provides methods of immunizing an individual, e.g., a patient or subject, against Zika virus infection by administering to the subject a substance that is: a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

[0113] Also provided is a method of diminishing a Zika virus infection in an individual, e.g., a patient or subject, comprising administering to the subject a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

[0114] In various embodiments, the administration induces antibodies in the subject that neutralize Zika virus infection. In various embodiments, administration of the VSV vector, immunogenic compositions, vaccines or viral particles reduces one or more symptoms or side effects of Zika virus infection, including, but not limited to, fever, rash, joint pain, conjunctivitis, muscle pain, headache, symptoms of Guillain-Barre syndrome. Symptoms of Guillain-Barre

syndrome include, but are not limited to, pain, tingling and numbness in the spine, hands, feet, arms, or legs, progressive muscle weakness, co-ordination problems and unsteadiness, temporary paralysis of the legs, arms, and face, temporary paralysis of the respiratory muscles, blurred or double vision, difficulty speaking, difficulty chewing or swallowing (dysphagia), difficulty with digestion or bladder control, and fluctuations in heart rate or blood pressure.

[0115] It is contemplated that the individual, subject or patient is a human. In various embodiments, the subject is an adult. In various embodiments, the subject is pregnant. In various embodiments, the subject is an infant or child.

Assays

[0116] Various techniques are known in the art for detecting immunospecific binding of an antibody to an antigen which are useful to detect the antigenicity and induction of an immune response of a VSV vector, immunogenic compositions, vaccines or viral particles, of the present disclosure. Methods of detecting interaction between an antigen and an antibody involved detection and analysis of the complex by precipitation in gels and ELISA assays. A further method of detecting an antigen-antibody binding pair includes the use of radioiodinated detector antibodies or a radioiodinated protein which is reactive with IgG, such as Protein A. These early methods are well known to persons skilled in the art, as reviewed in *Methods in Enzymology* 70:166-198, 1980. Additional assays are described in more detail in the Examples.

Methods of Administration

[0117] Many methods may be used to administer or introduce the vectors, immunogenic compositions, vaccines or viral particles into individuals (i.e., including subjects or patients), including but not limited to, oral, intradermal, intramuscular, intraperitoneal, intravenous, intratumor, subcutaneous, and intranasal routes. The individual to which a vector or viral particle is administered is a primate, or in other examples, a mammal, or in other examples, a human, but can also be a non-human mammal including but not limited to cows, horses, sheep, pigs, fowl, cats, dogs, hamsters, mice and rats. In the use of a vector, immunogenic compositions, vaccines or viral particles, the individual can be any animal in which a vector or virus is capable introducing the Zika virus protein and results in an immunological response.

[0118] The present invention encompasses compositions comprising vectors, including VSV vectors, immunogenic compositions, vaccines or viral particles wherein said compositions can further comprise a pharmaceutically acceptable carrier. The amount of vector(s) to be administered will depend on several factors, such as route of administration, the condition of the individual, the degree of aggressiveness of the malignancy, and the particular vector employed. Also, the vector may be used in conjunction with other treatment modalities.

[0119] If administered as a VSV vector(s), immunogenic compositions, vaccines or viral particles from about 10^2 up to about 10^7 p.f.u., in other examples, from about 10^3 up to about 10^6 p.f.u., and in other examples, from about 10^4 up to about 10^5 p.f.u. is administered. If administered as a polynucleotide construct (i.e., not packaged as a virus), about 0.01 μ g to about 100 μ g of a VSV construct of the present invention can be administered, in other examples, 0.1 μ g to about 500 μ g, and in other examples, about 0.5 μ g to about 200 μ g can be administered. More than one VSV vector can be administered, either simultaneously or sequentially. Administrations are typically given periodically, while monitoring any response. Administration can be given, for example, intramuscularly, intravenously or intraperitoneally.

[0120] It is contemplated that an effective amount of the vector(s), immunogenic compositions, vaccines or viral particles is administered. An “effective amount” is an amount sufficient to achieve a desired biological effect such as to induce enough humoral or cellular immunity. This may be dependent upon the type of vaccine, the age, sex, health, and weight of the recipient. Examples of desired biological effects include, but are not limited to, production of no symptoms, reduction in symptoms, reduction in virus titer in tissues or mucosal secretions, complete protection against infection by Zika virus, and partial protection against infection by Zika virus.

[0121] A vaccine or composition of the present invention is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient that enhances at least one primary or secondary humoral or cellular immune response against at least one strain of an infectious Zika virus. The vaccine composition is administered to protect against viral infection. The “protection” need not be absolute, i.e., the Zika infection need not be totally prevented or eradicated, if there is a statistically significant improvement compared with a

control population or set of patients. Protection may be limited to reducing the severity or timing of onset of symptoms of the Zika virus infection.

[0122] In one embodiment, a vector, immunogenic composition, vaccine or viral particle composition of the present invention is provided either before the onset of infection (so as to prevent or attenuate an anticipated infection) or after the initiation of an actual infection, and thereby protects against viral infection.

[0123] Pharmaceutically acceptable carriers are well known in the art and include but are not limited to saline, buffered saline, dextrose, water, glycerol, sterile isotonic aqueous buffer, and combinations thereof. One example of such an acceptable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc. The carrier is preferably sterile. The formulation should suit the mode of administration.

[0124] The composition, if desired, can also contain minor amounts of wetting or emulsifying agents or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

[0125] Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

[0126] In a specific embodiment, a lyophilized recombinant VSV herein is provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (e.g., 0.005% brilliant green).

[0127] The precise dose of vector, immunogenic composition, vaccine or viral particle to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. The exact amount of vector or

virus utilized in a given preparation is not critical provided that the minimum amount of virus necessary to produce immunologic activity is given. A dosage range of as little as about 10 mg, up to amount a milligram or more, is contemplated.

[0128] Effective doses of the vector or viral particle of the invention may also be extrapolated from dose-response curves derived from animal model test systems.

[0129] A vaccine is prepared using standard adjuvants and vaccine preparations known in the art. Adjuvants include, but are not limited to, saponin, non-ionic detergents, vegetable oil, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, and potentially useful human adjuvants such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-s- n-glycero-3-hydroxyphosphoryloxy)-ethylamine, BCG (bacille Calmette-Guerin) *Corynebacterium parvum*, ISCOMs, nano-beads, squalene, and block copolymers, which are contemplated for use alone or in combination.

[0130] ISCOM is an acronym for Immune Stimulating Complex, described initially in Morein et al. (Nature 308:457-460,1984). ISCOM's are a novel vaccine delivery system and are unlike conventional adjuvants. An ISCOM is formed in two ways. In some embodiments, the antigen is physically incorporated in the structure during its formulation. In other embodiments, an ISCOM-matrix (as supplied by, for example, Isconova) does not contain antigen but is mixed with the antigen of choice by the end-user prior to immunization. After mixing, the antigens are present in solution with the ISCOM-matrix but are not physically incorporated into the structure.

[0131] In one embodiment, the adjuvant is an oil in water emulsion. Oil in water emulsions are well known in the art, and have been suggested to be useful as adjuvant compositions (EP 399843; WO 95/17210, U.S. Patent Publication No. 20080014217). In one embodiment, the metabolizable oil is present in an amount of 0.5% to 20% (final concentration) of the total volume of the antigenic composition or isolated virus, at an amount of 1.0% to 10% of the total volume, or in an amount of 2.0% to 6.0% of the total volume.

[0132] In some embodiments, oil-in-water emulsion systems useful as adjuvant have a small oil droplet size. In certain embodiments, the droplet sizes will be in the range 120 to 750 nm, or from 120 to 600 nm in diameter.

[0133] In order for any oil in water composition to be suitable for human administration, the oil phase of the emulsion system comprises a metabolizable oil. The oil may be any vegetable oil, fish oil, animal oil or synthetic oil, which is not toxic to the recipient and is capable of being transformed by metabolism. Nuts, seeds, and grains are common sources of vegetable oils. Synthetic oils are also part of this invention and can include commercially available oils such as NEOBEE® and others. A particularly suitable metabolizable oil is squalene. Squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene) is an unsaturated oil which is found in large quantities in shark-liver oil, and in lower quantities in olive oil, wheat germ oil, rice bran oil, and yeast, and is a particularly suitable oil for use in this invention. Squalene is a metabolizable oil by virtue of the fact that it is an intermediate in the biosynthesis of cholesterol (Merck index, 10th Edition, entry no. 8619). Exemplary oils useful for an oil in water emulsion, include, but are not limited to, sterols, tocopherols, and alpha-tocopherol.

[0134] In additional embodiments, immune system stimulants are added to the vaccine and/or pharmaceutical composition. Immune stimulants include: cytokines, growth factors, chemokines, supernatants from cell cultures of lymphocytes, monocytes, or cells from lymphoid organs, cell preparations and/or extracts from plants, cell preparation and, or extracts from bacteria (e.g., BCG, mycobacterium, Corynebacterium), parasites, or mitogens, and novel nucleic acids derived from other viruses, or other sources (e.g. double stranded RNA, CpG) block co-polymers, nano-beads, or other compounds known in the art, used alone or in combination.

[0135] Particular examples of adjuvants and other immune stimulants include, but are not limited to, lysolecithin; glycosides (e.g., saponin and saponin derivatives such as Quil A (QS7 and QS21) or GPI-0100); cationic surfactants (e.g. DDA); quaternary hydrocarbon ammonium halogenides; pluronic polyols; polyanions and polyatomic ions; polyacrylic acids, non-ionic block polymers (e.g., Pluronic F-127); and 3D-MPL (3 de-O-acylated monophosphoryl lipid A). See e.g., U.S. Patent Publication Nos. 20080187546 and 20080014217.

EXAMPLES

Example 1

Production of VSV-Zika Vector and Neutralizing Antibodies Against Zika Virus

[0136] The following experiments were carried out using the exemplary protocols below.

[0137] Cells. 293T, Hela, and BHK-21-WI (generously provided by M.A. Whitt) cells were maintained in Dulbecco modified Eagle medium (DMEM; Gibco/Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 5% penicillin-streptomycin. Vero cells were maintained in Medium 199 (Sigma) supplemented with 10% fetal bovine serum (FBS) and 5% penicillin-streptomycin.

[0138] Generation of rVSVs expressing Zika antigens. Plasmid clones that contain the Zika (ZIKV) genes for the premembrane and envelope proteins were ordered from GenScript. The sequences contain the premembrane and envelope region together (ZPRME, ZprME) or pRMEZIKA) and the envelope region alone (ZENV or EnvZIKA) and were constructed using Zika virus strain ZikaSPH2015 (Genbank Accession KU321639) as a reference. The sequences were made with restriction sites XhoI and NheI flanking the gene and an HA tag towards the C-terminal region of the gene. The generation of VSV and VSVΔM (VSVm) vectors containing either the ZprME or ZENV proteins was done using restriction digest with XhoI and NheI (NEB) to create compatible ends to ligate into the VSV and VSVΔM (VSVm) cDNA plasmids using quick T4 DNA ligation (NEB). The ligated product was then transformed into DH10B *E. coli* and liquid cultures from colonies were grown at 30°C overnight. DNA preps were confirmed using restriction digest and verified by sequencing reactions. Plasmid midipreps (Qiagen) were used for transfection to recovery infectious virions.

[0139] Plasmid Transfection. All plasmid transfections were done using Lipofectamine 2000 (Invitrogen) following manufacture's recommended protocol.

[0140] Recovery and purification of VSV-ZENV vaccine vectors. VSV ZENV, VSV ZprME, VSVm-ZENV, and VSVmμ-ZprME were recovered using established VSV recovery methods. In brief, 1.5×10^6 293Ts were seeded into 6 well plates and allowed to adhere overnight. The next day they were infected with VTF7-3 at multiplicity of infection (MOI) of 2.5 in serum free media for 45min. After the incubation, cells were washed with SFM and given DMEM with 5% low-IgG FBS (Life technologies). The VTF7-3 infected 293Ts were transfected using Lipofectamine 2000 (Invitrogen) with 0.5μg of pBS-VSV-N, 0.83μg of pBS-VSV-P, 0.17μg of pBS-VSV-L, and 5μg of the respective pVSV ZENV vector. The transfection mix was prepared in 100μL Optimem (Invitrogen) and added to the well. The next day, the

media was filtered through 0.2µm syringe onto fresh 293Ts to remove vTF7-3. After 24-48hrs, if cytopathic effect (CPE) was observed, the milieu was collected and VSV virions were plaque isolated and further amplified using ultracentrifugation using a 10% Optiprep cushion. Virus titers were determined by standard plaque assay using BHK-21-WI cells.

[0141] Virus infections. Cells were seeded in 6- or 12-well plates. Adherent cells were allowed to adhere overnight and were 80-90% confluent unless otherwise indicated. Adherent cells were infected with rVSVs at the indicated MOI in a reduced volume of serum-free DMEM for 1 hour with agitation at 15-min intervals. The cells were then washed with 1x PBS twice, and complete medium was added back to the cells.

[0142] Western blotting. Infected cells were collected and incubated in RIPA lysis buffer with protease inhibitor cocktail (Sigma) for 30 min at 4°C with gentle agitation. Cell debris was removed by centrifugation for 10 min at 15,000 g. Protein concentration was quantitated using Coomassie blue (Thermo Scientific), and the optical density was read at 595 nm. Equal amounts of protein (20µg) were separated using SDS– 10% PAGE and transferred to a polyvinylidene difluoride membrane (PVDF). Membranes were blocked with 5% milk powder in PBS– 0.1% Tween 20 (PBS-T) at room temperature and then probed with primary antibodies against VSV-G (Sigma 1:5,000), Flavivirus (4G2 mouse anti-flavivirus specific antibody provided by Dr. David Watkins at 1:1000), β-actin (Sigma 1:10,000), and HA (Sigma at 1:2000). Membranes were then washed with PBS-T and probed with 2nd antibodies. The image was resolved using an enhanced chemiluminescence system ECL (Thermo Scientific) and detected by autoradiography (Kodak).

[0143] Growth kinetic assays. A total of 5x10⁵ HeLa cells/well were seeded in a six-well plate. Cells were infected with either VSV-GFP, VSVm, VSV-ZENV, VSV-ZprME, VSVm - ZENV, and VSVm -ZprME at an MOI of 0.001 in serum-free DMEM for 1 hour, with agitation every 15 min. Next, the cells were washed twice with 1x PBS, and 3 ml of complete medium was added to each well. The culture supernatants were harvested at the indicated times and kept at -80°C until virus titer was measured using a standard plaque assay with BHK-21-WI cells.

[0144] Immunofluorescence of VSV ZENV infected cells. HeLa cells seeded on coverslips were infected with recombinant VSV-ZENV viruses at M.O.I. 10 for 6 hours. Cell were fixed with 4% paraformaldehyde for 15 minutes in at room temperature, blocked with 5% BSA for 1 hour, and then immunostained with mouse anti-HA antibody (sigma, at 1:500) followed by

fluor488 conjugated goat-anti-mouse IgG (Invitrogen, at 1:1000). Cells were counterstained with Dapi. Images were taken with Leica LSM confocal microscope at the Image Core Facility, University of Miami.

[0145] Mouse studies. Female C57BL/6 and Balb/c mice were purchase from Jackson Laboratory. All mice were between 6 to 8 weeks old. Mice care and study were conducted under approval from the Institutional Animal Care and Use Committee of the University of Miami.

[0146] Toxicity Assessment. 8 week old Female Balb/C mice were injected with a high dose of 5×10^7 or 1×10^8 PFU of rVSV diluted in PBS, intramuscularly. Mice were monitored for signs of distress and survival twice a week.

[0147] Vaccine studies. To determine the efficacy of VSV ZENV vaccine, 8 week old female C57BL/6 mice were vaccinated with 2×10^6 PFUs of VSVm, VSV-ZENV, VSV-ZprME, VSVm-ZENV or VSVm-ZprME (n=5 per group) intravenously and then boosted on day 22. Female Balb/c mice were vaccinated with 2×10^6 PFUs of VSVm, VSV-ZENV, VSV-ZprME, VSVm-ZENV or VSVm-ZprME (n=5 per group) intravenously or intramuscularly and then boosted on day 14. Prior to vaccination, mice were pre-bled to establish baseline IgG levels. Blood was collected periodically using sub mandibular bleed method under anesthesia. Serum was isolated from whole blood and antibody titer was analyzed using ZIKV IgG ELISA analysis and neutralizing antibody assay.

[0148] ZIKV IgG ELISA analysis. 96-well PVC microtiter plates were coated with recombinant ZIKV ENV protein (MyBioSource) at $0.25 \mu\text{g/ml}$ for overnight at 4°C . After washing with PBS, plates were blocked with 5% BSA for 1 hour, incubated with appropriately diluted serum draw from vaccinated or control mice for 2 hours, followed by incubation with HRP conjugated anti-mouse IgG (1:5000) for 1 hour. HRP signal was developed with TMB (3,3',5,5'-tetramethylbenzidine) for 30 minutes at room temperature and stopped reaction with 1M HCl. Optical density was read at 450nm on a plate reader. A serial of 4G2 antibody dilution was used as the standard to quantitate the anti-serum.

[0149] Zika Neutralization Assay. Vero cells were plated 5×10^5 cells/well in 6 well plates and allowed to adhere overnight. Serial serum dilutions were made to 1/80. Serum was heat inactivated at 56°C for 30 minutes. Zika virus was diluted to 30 PFU/well, in 199 medium containing 5% FBS and mixed with the diluted serum sample (1/80) and incubated for 1 hour at

room temperature. After incubation, 200µl of inoculum was added to each well and incubated for 1hr with agitation every 10-15 minutes. After the adsorption period, the inoculum was discarded and replaced with 4ml of 199 medium 5% FBS 0.8% of high viscosity carboxymethyl cellulose. The plates were incubated for a period of 6-7 days. After the incubation period the monolayers were fixed with 10% formalin for 1 hour and stained with 1% crystal violet in ethanol for 30 seconds. Plates were destained with gentle H₂O wash and the plaques were counted. The % neutralization was calculated using serum free sample as a control.

[0150] ZIKV Plaque Reduction Neutralization Titration (PRNT) Assay. Vero cells were plated 2.5×10^5 cells/well in 612 well plates and allowed to adhere overnight. Serum was heat inactivated at 56°C for 30 minutes. Serial serum dilutions were made. ZIKV was diluted to 50 PFUs/well, mixed with the diluted serum samples and incubated for 3 hours at 37 °C. After incubation, 100µl of inoculum was added to Vero cells and incubated for 1 hour with agitation every 10-15 minutes. After the adsorption period, the inoculum was discarded and replaced with 2ml of 199 medium with 10% FBS and 0.8% of high viscosity carboxymethyl cellulose. The plates were incubated for a period of 4 days. After the incubation period the monolayers were fixed with 10% formalin for 1 hour and stained with 1% crystal violet in ethanol for 30 seconds. Plates were destained with gentle H₂O wash and the plaques were counted. The percentage of neutralization was calculated using virus control without serum. PRNT50 was calculated using probit analysis as described in Cutchins et al, 1960.

[0151] ZIKV plaque assay. Vero cells were plated at 2.5×10^5 cells per well in 12-well plates and allowed to adhere overnight. A total of 0.2 g of brain tissue from euthanized mice was homogenized in 500 ml of Medium 199. After three cycles of freezing at -80°C and thawing, lysate was cleared by centrifugation. Serial 10-fold dilutions were made in Medium 199. A total of 100 ml of serially diluted tissue lysate was added to Vero cells and incubated for 1 hour, with agitation every 10-15 min. After the adsorption period, the inoculum was discarded and replaced with 2 ml of Medium 199 with 10% FBS and 0.8% high-viscosity carboxymethyl cellulose, and the plates were incubated for 4 days. After the incubation period, the monolayers were fixed with 10% formalin for 1 hour and stained with 1% Crystal violet in ethanol for 30 sec. Plates were rinsed with a gentle H₂O wash and air dried. Plaques were counted, and viral titer was calculated as PFU/g of tissue.

[0152] Interferon γ ELISA and Intracellular Cytokine Staining. ZIKV specific cytotoxic T lymphocyte response was accessed using splenocytes isolated from vaccinated mice. Cells were stimulated with 2 μ g/ml of overlapping 15-amino acid peptides covering the prME region of ZIKV (custom synthesized from GenScript). For ELISA analysis, cell media were collected 72 hours post stimulation, and interferon γ (IFN γ) level was assessed using mouse IFN γ ELISA kit (R&D System) following the manufacturer's instruction. For Flow cytometry, cells were stimulated for either 6 or 72 hours. Brefeldin-A was added to the cells at 3 μ g/ml 6 hours before analysis. Cells were then washed, stained with cell surface marker, permeabilized with Cytofix/Cytoperm (BD Biosciences) and then stained with IFN γ . Data was acquired using an LSRII flow cytometer.

[0153] Statistical Analysis. All statistical analysis was performed by Student's t test unless specified. The data were considered to be significantly different when $P < 0.05$.

[0154] Results

[0155] Generation of rVSV expressing Zika envelope and premembrane/envelope antigens. Recombinant VSV constructs were successfully generated using traditional cloning techniques using the G-L foreign gene expression site engineered into two VSV vectors, VSV and VSV- Δ M (VSVm) (Fig. 1A). The VSV- Δ M (VSVm) construct has a mutation in the matrix protein (amino acid 52-54: DTY to AAA) such that following infection, this mutant cannot block host cell mRNA export and is thus considerably attenuated due to innate immune proteins being translated (Heiber and Barber, J Virol. 85:10440-10450, 2011; Faria et al., Mol Cell 17:93-102, 2005). The Zika envelope (ZENV) and Zika premembrane/envelope (ZprME) genes with a C-terminal HA tag were first synthesized and inserted in a pcDNA3.1 expression vector. Expression was confirmed in 293T cells using antibodies against HA and a flavivirus specific antibody 4G2 that cross reacts with both Dengue virus and ZIKV envelope proteins (Fig. 1B). Two versions of the ZIKV ENV were designed since it was not clear whether inclusion of the TM region would better enhance expression and/or immunogenicity or not (Konishi and Mason, J Virol 67:1672-1675, 1993; Iyer et al., Vaccine 27:893-903, 2009). After expression was confirmed, the two ZIKV ENV constructs were ligated into both VSV and VSVm backbones and infectious rVSV recovered and virus concentrations determined by plaque assay (Heiber et al., *supra*; Fernandez et al., J. Virol. 76:895-904, 2002). Hela cells were infected at MOI 10 and

western blot was performed 6 hours post infection (hpi) to confirm ZIKV envelope and VSV-G expression (Fig. 1C). The CT-HA tag allowed for the detection of both the unprocessed full length ZprME and the shortened ZENV as indicated by the anti-HA immunoblot.

Immunofluorescent microscopy was also performed using rVSV infected HeLa cells to further establish expression of ZENV antigens after infection. This data indicated using 4G2 as well as anti-HA antibody that Zika ENV and prME are efficiently expressed by rVSV in vitro (Fig. 1D).

[0156] *Characterization of growth kinetics of VSV-ZIKV constructs.* The insertion of foreign genes into VSV can produce unintended consequence to the replication of rVSV's and drastically affect the viral lifecycle (Wertz et al., J Virol. 76:7642-7650, 2002). A multi-cycle growth kinetic assay was thus performed in HeLa cells and verified the incorporation of the ZENV genes in either VSV backbone (VSV or VSVm) did not significantly affect the replicative capacity of VSV. HeLa cells were infected at MOI of 0.001 and the viral titer was analyzed at various time points. All of the various rVSVs displayed robust growth kinetics with slight deviation in the kinetics but were nonetheless fully replication competent (Fig. 2). Therefore, the incorporation of the ZENV constructs into rVSV does not significantly affect replication.

[0157] *Toxicity Assessment.* VSV has routinely displayed a well-tolerated safety profile (Balachandran et al., IUBMB Life 50:135-138, 2000). Nevertheless, to confirm that the newly created VSV-ZENV constructs are safe, high concentrations of rVSV were injected into naïve Balb/c mice that were several logs higher than that normally used in vaccination studies (typically 2×10^6 PFU). Mice were thus injected with 5×10^7 and 1×10^8 PFUs of rVSV's expressing ZENV variants and were not found to display any overt malaise or general observable adverse effects. No deaths of the inoculated mice were observed after a month post-inoculation (Fig. 3).

[0158] *Vaccination study.* To assess the efficacy of the different rVSVs as an effective vaccine vector, 6-week female C57BL/6 mice were injected intravenously with 2×10^6 PFUs of rVSVs and boosted again with the same dose, two weeks later. Serum from the immunized mice was analyzed by ELISA as shown in Fig. 4. ELISA was used to measure the antibody titer (IgG) using solid phase recombinant ZIKV ENV protein produced from insect cells. The results from the ELISA indicated that the VSVm-ZprME construct was the most effective at generating anti-ZENV antibody using this immunization regime, followed by the VSV-ZprME construct. Less

robust responses were seen using the VSV-ZENV and VSVm- ZENV constructs (Fig. 4B, 4F, Fig. 9). To complement this approach, a neutralization assay was performed to preliminarily evaluate whether the antibodies generated to ZENV were able to neutralize ZIKA virus infection in vitro. This analysis indicated that rVSV's expressing ZENV were able to generate neutralizing antibody. However, this analysis conflicted with the ELISA assay in that the VSV- Δ M-ZENV and VSV-ZENV candidate vaccines exhibited the ability to generate higher neutralizing antibody (Fig. 4D).

[0159] In a additional assay, a plaque reduction neutralization assay (PRNT) was performed to preliminarily evaluate whether the antibodies generated to ZENV were able to neutralize ZIKV infection in vitro. Results indicated that rVSVs expressing ZprME were able to generate neutralizing antibody and a majority of mice from VSVm-ZprME inoculated group generated high titer of neutralization antibody with PRNT50 factor above 1000 (Fig 4E, Figure 9). The effects of the different rVSVs were also assessed through different inoculation routes (intravenously and intramuscularly) in naïve Balb/c mice. Similar effects were observed by ELISA analysis. As shown in Fig. 4, intravenously injected VSV-ZprME and VSVm-ZprME were able to generate anti-ZENV antibody at much higher titer compared to intramuscular administration of the same type of virus. Similar to intramuscular inoculation, less robust responses were seen using the VSV-ZENV and VSVm ZENV constructs. The ability of the vaccines to generate CTL was evaluated. High amounts of IFN- γ were produced following stimulation of retrieved splenocytes with the synthetic ZprME peptide pool (15 mer), as shown by ELISA analysis (Fig. 4G). Further, flow cytometry analysis showed significant increases in CD8+ /CD44high /IFN- γ + T cell populations (Fig. 4F). Taken together, the data indicate that rVSV expressing ZprME could provide an effective approach to create immune responses that may prevent ZIKV infection.

[0160] Also, the rVSV based ZENV constructs are able to generate protective serum IgG against ZIKV envelope. Surprisingly, VSVm-ZprME generated the highest antibody titer among all the constructs, suggesting the possibility that activated cellular innate immune response facilitates antigen presentation and adaptive immunity establishment (Fig. 5). Wild type MEFs were infected with VSV or VSVm at M.O.I. 5. Culture medium was collect 8 or 24 hours post infection and measured for mouse interferon β using Elisa kit (PBL Assay Science).

[0161] ZIKV is also known to infect individuals for long periods, indicating that the generation of effective cytotoxic T cell (CTL) activity may be important in helping to eliminate infection in the presence of antibody. Thus, the ability of the ZIKV vaccines to generate CTLs was further evaluated. High amounts of interferon γ were produced following stimulation of retrieved splenocytes using synthetic ZprME peptide pool (15 mers) as shown by ELISA analysis (Fig. 6A). Further, flow cytometry analysis showed significant increases in CD8⁺/CD44^{high}/IFN γ ⁺ T cell populations (Fig. 6B). Taken together, this data indicates that rVSV expressing ZprME could provide an effective approach to create immune responses that may prevent ZIKV infection and be useful as an effective vaccine.

[0162] *Assessment of Maternal Protection by VSV-ZIKV Constructs* Although ZIKV does not usually cause disease in weaned wild type (WT) mice (>3 weeks old), suckling WT mice (~1 week old) are susceptible to ZIKV infection. Therefore, the protection efficacy of the present rVSV based ZIKV vaccine through maternal antibody transmission was assessed. Neonatal mice have been shown to be susceptible to ZIKV infection (strain MR766, 90% similar to Brazil Isolates [Paraiba, 2015]) (Figure 7). Neonatal mice born to VSVm-prME vaccinated or naïve female C56BL/6 mice were infected with high dose ZIKV (MR766) (7×10^5 PFUs; average mosquito bite is approximately 1×10^4), intraperitoneally, and monitored for signs of disease as well as lethality daily (Fig. 8A). All mice born to naïve females developed neurological disease signs, including hindlimb paralysis, before succumbing to infection by day 10 (Fig. 8B, D). In comparison, the majority of mice born to VSVm-prME vaccinated females exhibited no morbidity or mortality (Fig. 8C, D). High copy numbers of ZIKV were detected in brain tissue of mice born to naïve females; however, ZIKV was not able to replicate in progeny of VSVm-ZprME-vaccinated mothers (Fig. 8E, 8F). These results suggested that the VSVm-prME vaccine has the potential in protecting prenatal and neonatal development from ZIKV infection likely through the transmission of maternal IgG.

[0163] *Discussion*

[0164] Mosquito-borne ZIKV has been implicated in the cause of microcephaly in gestating fetuses and is now a major public health concern worldwide (Focosi et al., *supra*, Rasmussen et al., *supra*). There is an urgent need for effective vaccines that prevent ZIKV infection. Here it has been demonstrated that rVSV's can be successfully generated to carry and express ZIKV

envelope proteins that may have the capacity to produce protective neutralizing antibody against ZIKV infection. Given the little biological data presently available on Zika virus, it is difficult to predict which ZENV construct may be the most effective immunogen and generate neutralizing antibody to the Zika virus (Konishi et al., *supra*; Iyer et al., *supra*; Schafer et al., PLoS One 6:e24505, 2011). It has been reported that the TM domain of flavivirus envelope proteins can either enhance or suppress efficient expression of the ENV product. Conformational variations may also effect the exposure of key immunogenicity regions. For this reason two VSV constructs were used herein (VSV and VSVm), that expressed either ZENV with (prME) or without (ENV) the TM domain. Surprisingly, the prME constructs generated the most anti-ZENV IgG, as well as the most neutralizing antibody. Greater vaccine efficacy were seen in VSVm-prME group following the inoculation regime. This analysis indicates that it is not readily predictable which ZENV product will be the most efficacious at generating protective antibody to the ZENV protein. This data also indicates that the rVSV with a mutation in the matrix gene gave the higher antibody responses, suggesting that, since this virus cannot block host cell mRNA export including type I interferons, host defense countermeasures may rapidly eliminate this virus (Faria et al., *supra*; Obuchi et al., J Virol 77:8843-8856, 2003) but also facilitate establishing of the adaptive immunity, which provides an additional safety measure for using this attenuated version as vaccine. The results indicate that rVSV may be a suitable platform for the development of effective vaccines against ZIKV.

[0165] Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

WHAT IS CLAIMED:

1. A recombinant vesicular stomatitis virus (VSV) vector comprising a nucleic acid expressing an envelope protein (ENV) of a Zika virus.
2. The VSV vector of claim 1, wherein the envelope protein lacks the transmembrane region (prmENV).
3. The VSV vector of claim 1 or 2 wherein the vector comprises a mutation in the VSV matrix protein (M).
4. The VSV vector of claim 3 wherein the mutation is VSVM51.
5. The VSV vector of any one of the preceding claims wherein the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L, optionally wherein the VSV lacks G-protein function.
6. The VSV vector of any one of the preceding claims wherein the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).
7. An immunogenic composition comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.
8. The immunogenic composition of claim 7 wherein the envelope protein lacks the transmembrane region (prmENV).
9. The immunogenic composition of claim 7 or 8 wherein the vector comprises a mutation in the VSV matrix protein (M).
10. The immunogenic composition of claim 9 wherein the mutation is VSVM51.
11. The immunogenic composition of any one claims 7 to 11 wherein the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L, optionally wherein the VSV lacks G-protein function.
12. The immunogenic composition of any one claims 7 to 12 wherein the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).
13. A vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant.

14. The vaccine of claim 13 wherein the envelope protein lacks the transmembrane region (prmENV).

15. The vaccine of claim 13 or 14 wherein the vector comprises a mutation in the VSV matrix protein (M).

16. The vaccine of claim 15 wherein the mutation is VSVM51.

17. The vaccine of any one claims 13 to 16 wherein the Zika virus envelope nucleic acid sequence is inserted between the vesicular stomatitis virus vector genes, G and L, optionally wherein the VSV lacks G-protein function.

18. The vaccine of any one claims 13 to 17 wherein the Zika virus envelope nucleic acid sequence is complementary DNA (cDNA).

19. A nucleic acid vaccine comprising a pharmaceutically acceptable carrier and a vesicular stomatitis virus (VSV) vector comprising at least one nucleic acid molecule encoding a Zika virus envelope protein, wherein the at least one nucleic acid molecule is expressed in a vaccine recipient, and wherein the expression product induces an immune response against Zika virus in the recipient.

20. A method of immunizing a subject against Zika virus infection by administering to the subject a substance that is: a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

21. A method of diminishing a Zika virus infection in a subject, comprising administering to the subject a) an immunogenic composition comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

22. A method of reducing or alleviating one or more symptoms of a Zika virus infection in a subject, comprising administering to the subject a) an immunogenic composition

comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a vesicular stomatitis virus (VSV) vector comprising an envelope protein (ENV) of a Zika virus.

23. The method of claim 22 wherein the one or more symptoms is selected from the group consisting of fever, rash, joint pain, conjunctivitis, muscle pain, headache and symptoms of Guillain-Barre syndrome.

24. The method of any one of claims 20 to 23 wherein the administration induces antibodies in the subject that neutralize Zika virus infection.

25. A recombinant viral vector comprising a nucleic acid expressing an envelope protein (ENV) of a Zika virus.

26. The vector of claim 25, wherein the envelope protein lacks the transmembrane region (prmENV).

27. An immunogenic composition comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus.

28. The immunogenic composition of claim 27 wherein the envelope protein lacks the transmembrane region (prmENV).

29. A vaccine comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant.

30. A nucleic acid vaccine comprising a pharmaceutically acceptable carrier and a viral vector comprising at least one nucleic acid molecule encoding a Zika virus envelope protein, wherein the at least one nucleic acid molecule is expressed in a vaccine recipient, and wherein the expression product induces an immune response against Zika virus in the recipient.

31. A method of immunizing a subject against Zika virus infection by administering to the subject a substance that is: a) an immunogenic composition comprising a viral vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising recombinant viral vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus.

32. A method of diminishing a Zika virus infection in a subject, comprising administering to the subject a) an immunogenic composition comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus.

33. A method of reducing or alleviating one or more symptoms of a Zika virus infection in a subject, comprising administering to the subject a) an immunogenic composition comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus; b) a vaccine comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus and an adjuvant; or c) a nucleic acid vaccine comprising a recombinant viral vector comprising an envelope protein (ENV) of a Zika virus.

Figure 1A

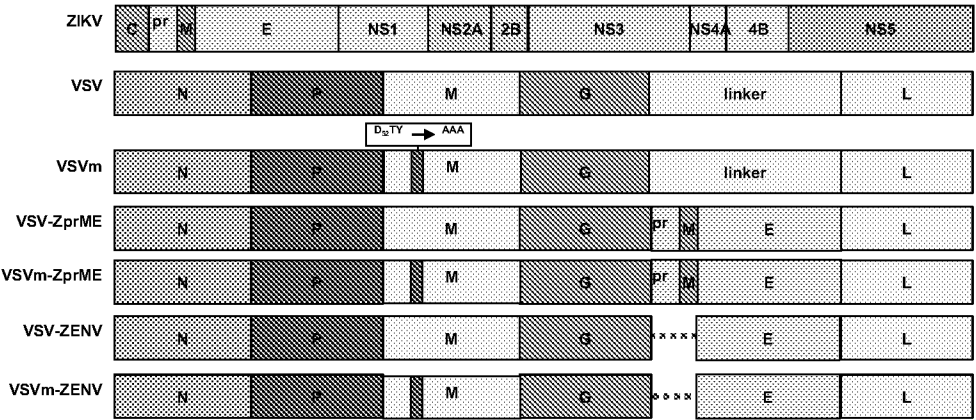


Figure 1B

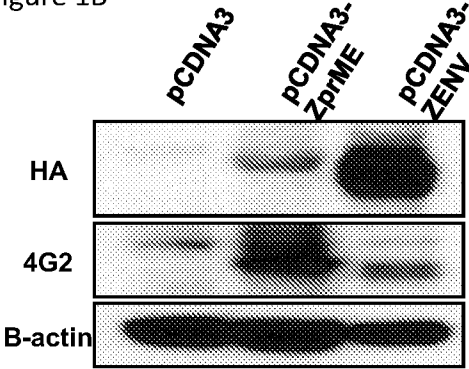


Figure 1C

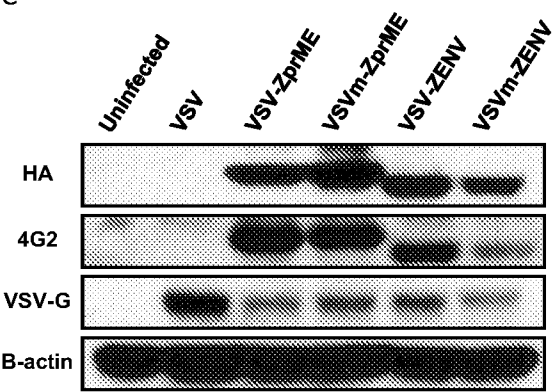


Figure 1D

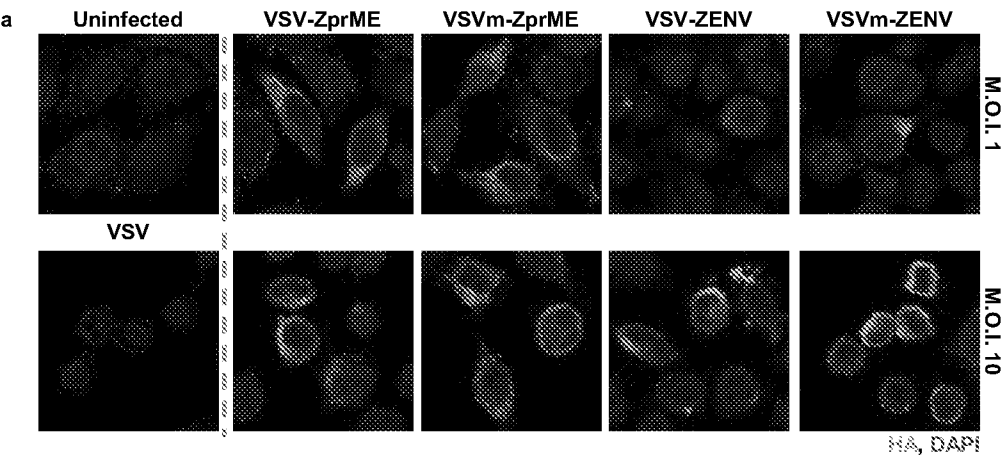


Figure 2

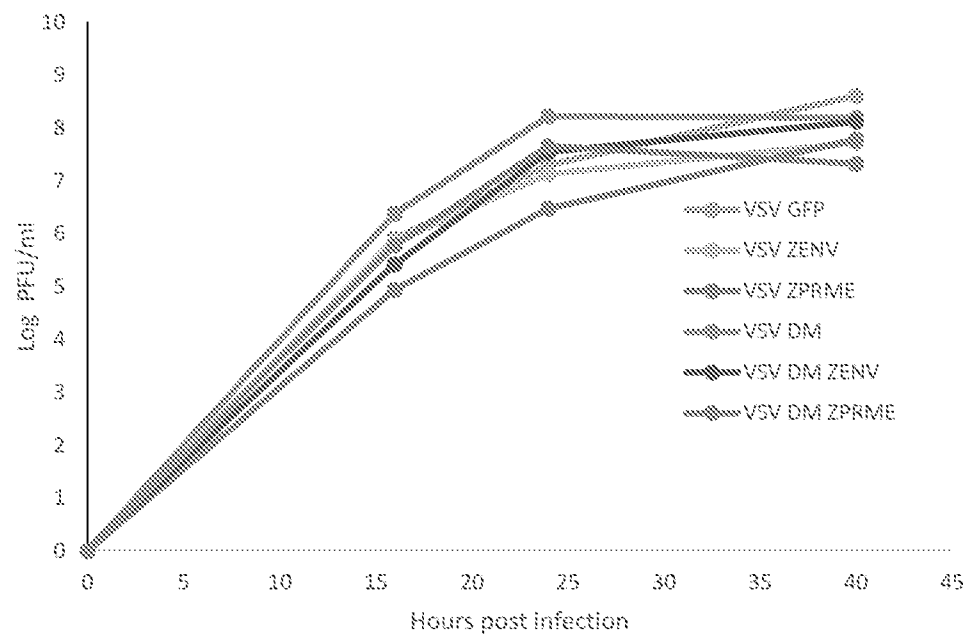


Figure 3

Virus	% survival
VSVΔM 5x10 ⁷	100
VSV ZENV 5x10 ⁷	100
VSV ZPRME 5x10 ⁷	100
VSV ΔM ZENV 5x10 ⁷	100
VSV ΔM ZPRME 5x10 ⁷	100
VSVΔM 1x10 ⁸	100
VSV ZENV 1x10 ⁸	100
VSV ZPRME1x10 ⁸	100
VSV ΔM ZENV 1x10 ⁸	100
VSV ΔM ZPRME 1x10 ⁸	100

Figure 4A

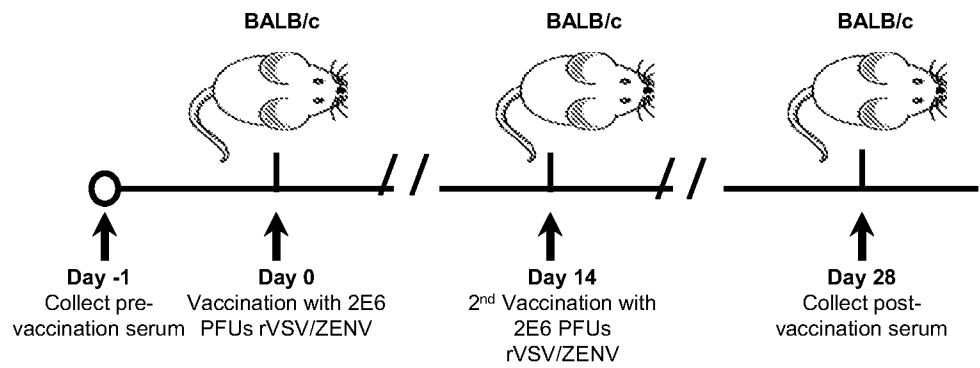


Figure 4B

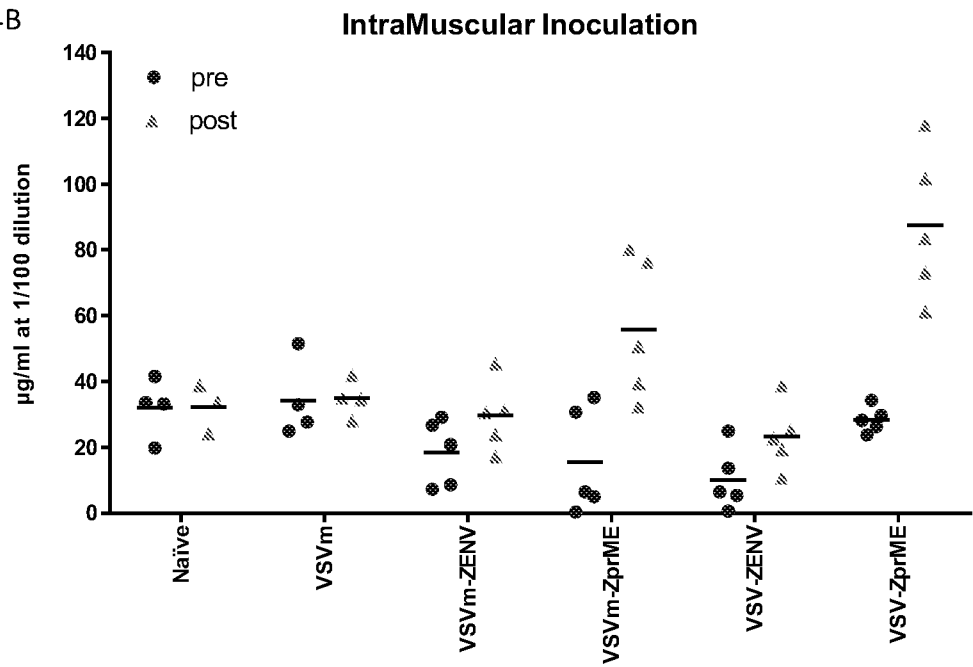


Figure 4C

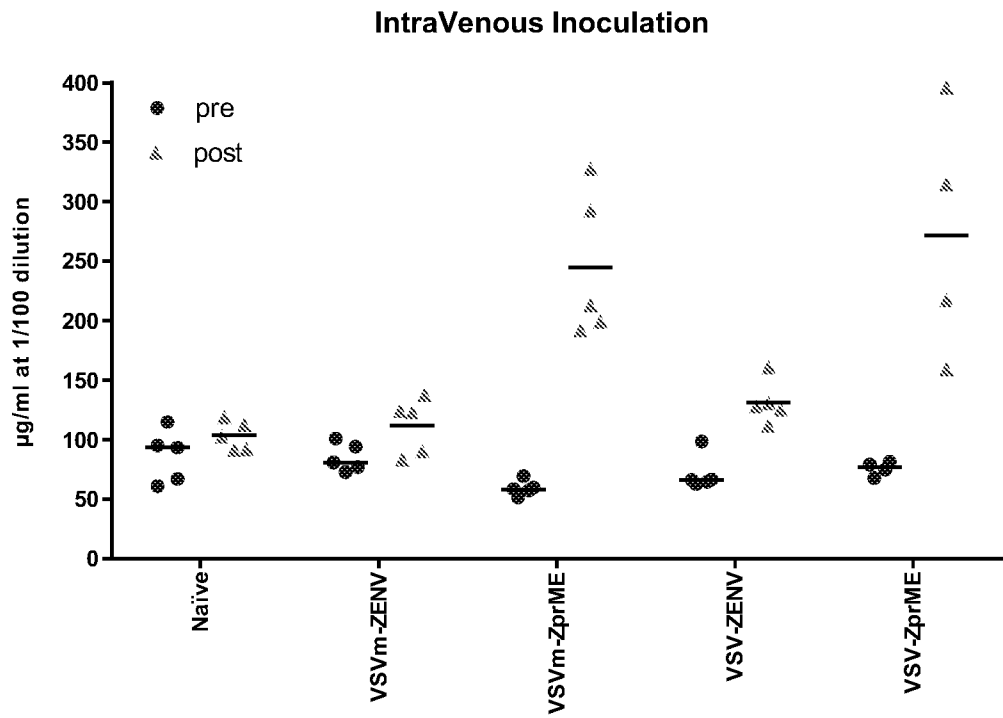


Figure 4D

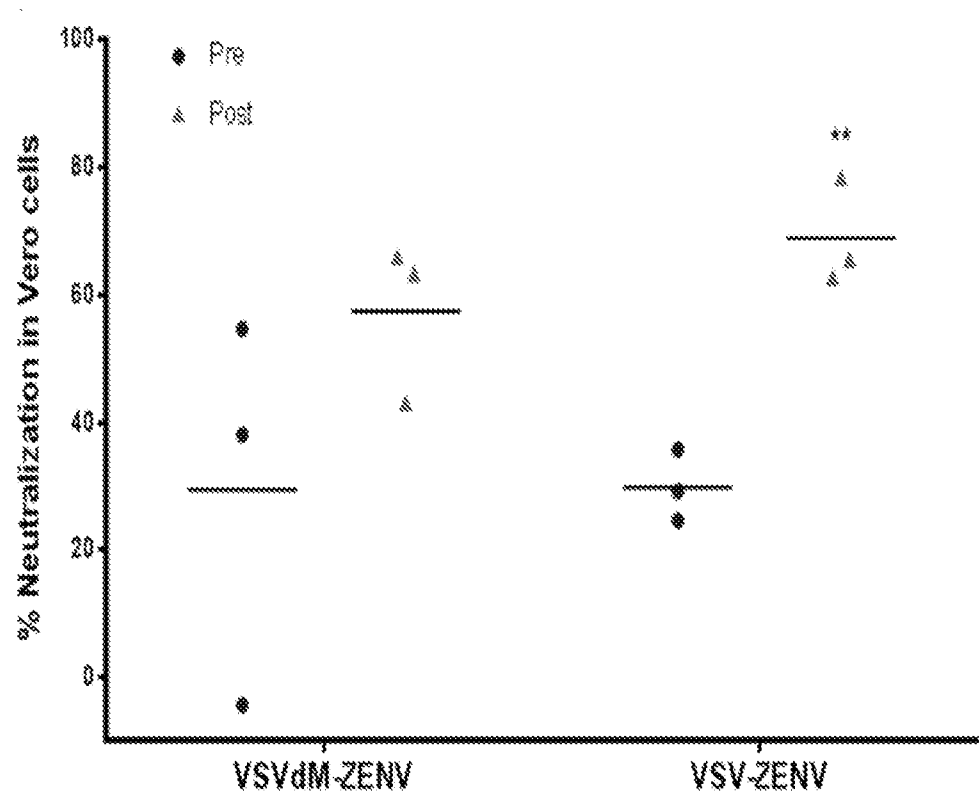


Figure 4E

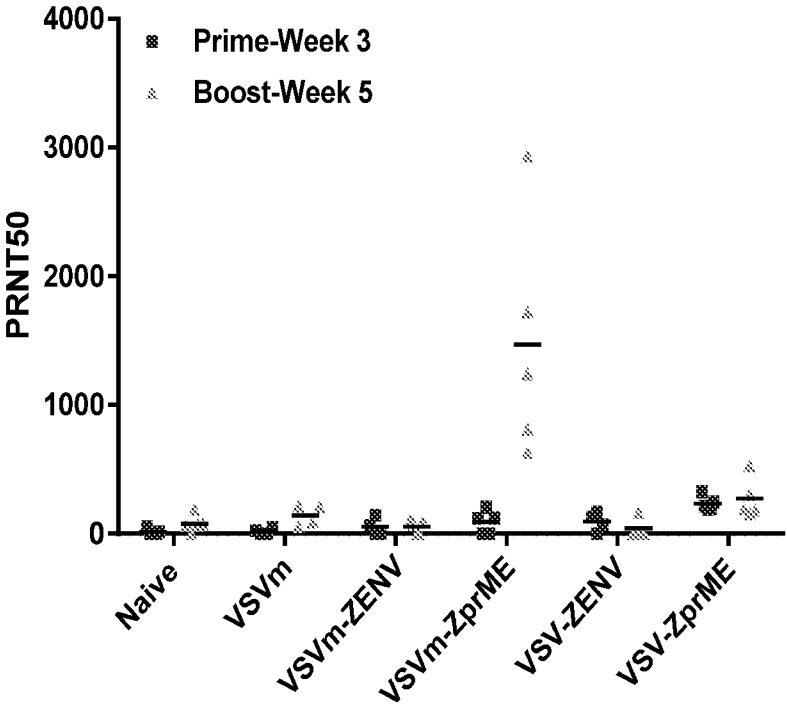


Figure 4F

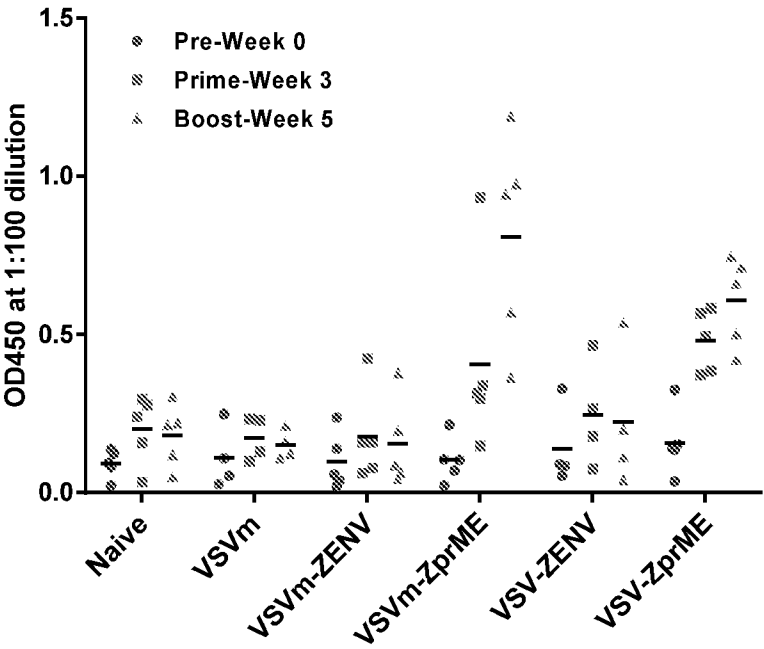


Figure 4G

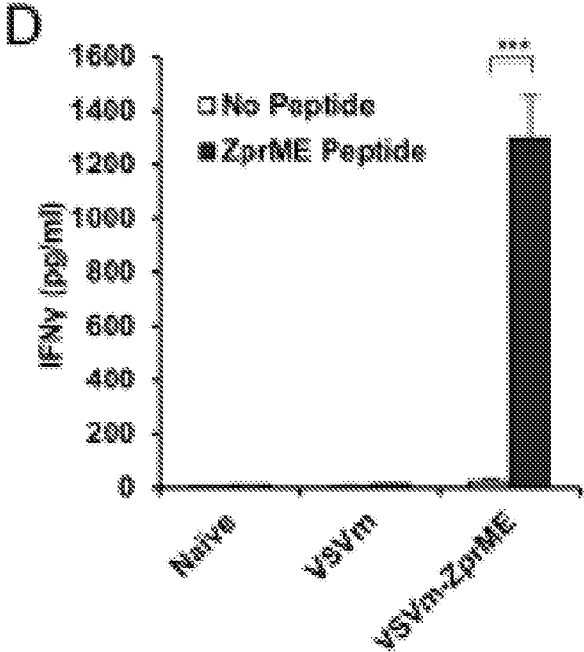


Figure 4H

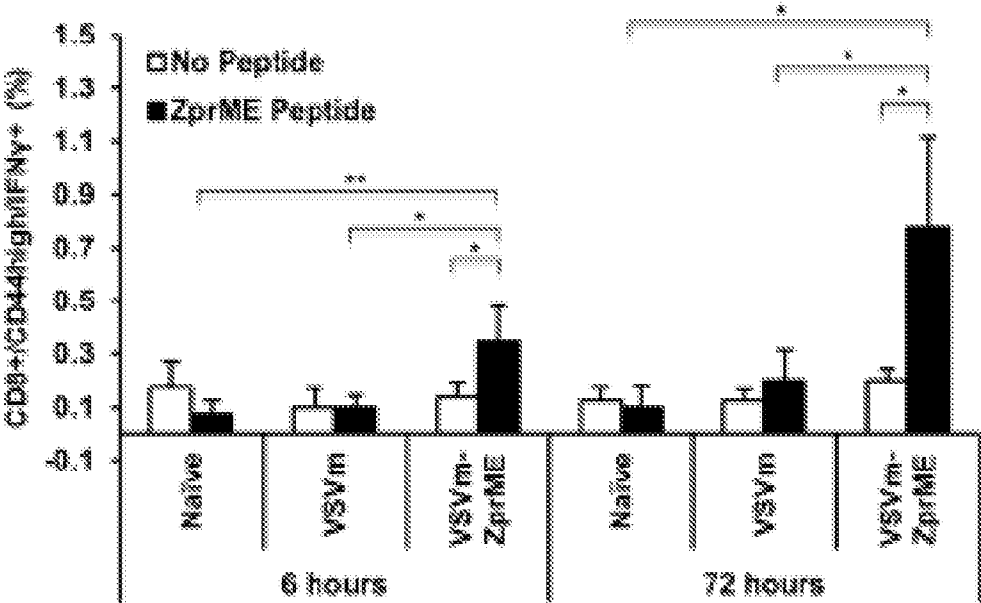


Figure 5

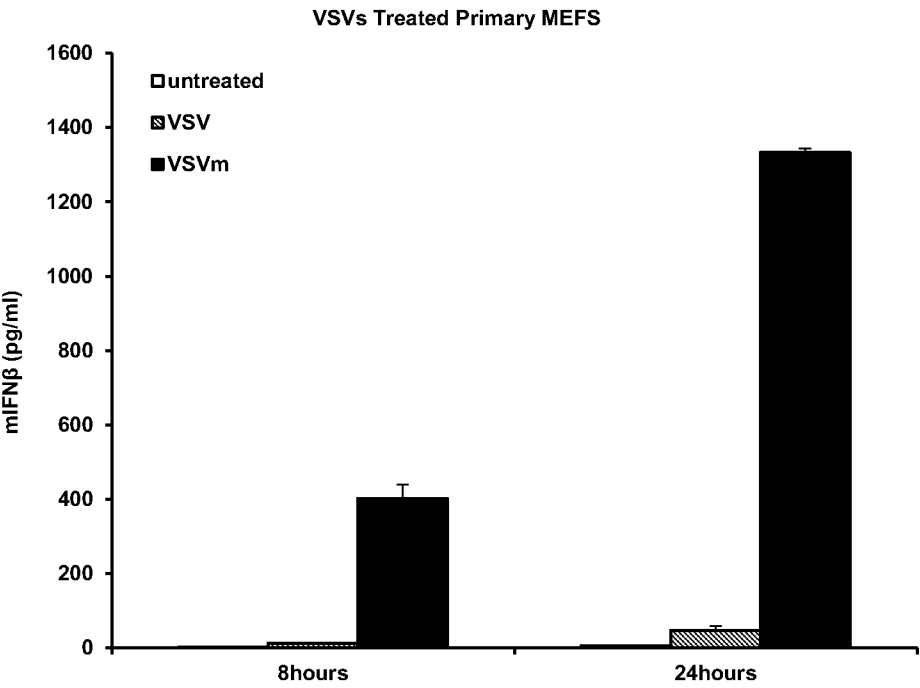


Figure 6A

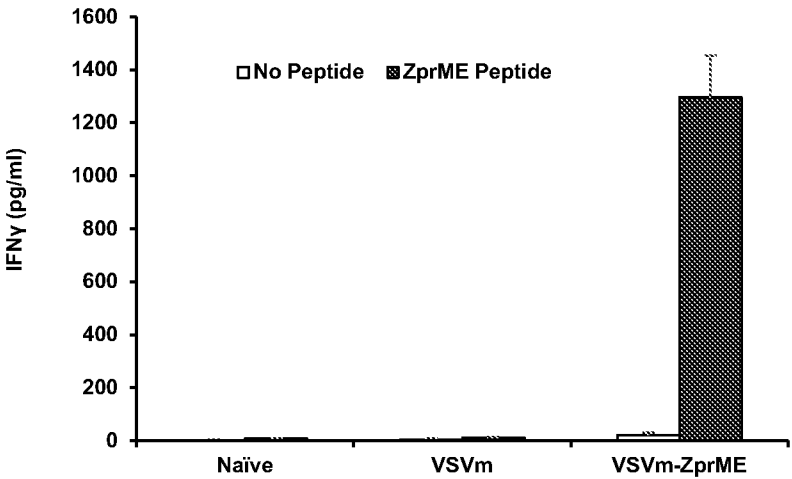


Figure 6B

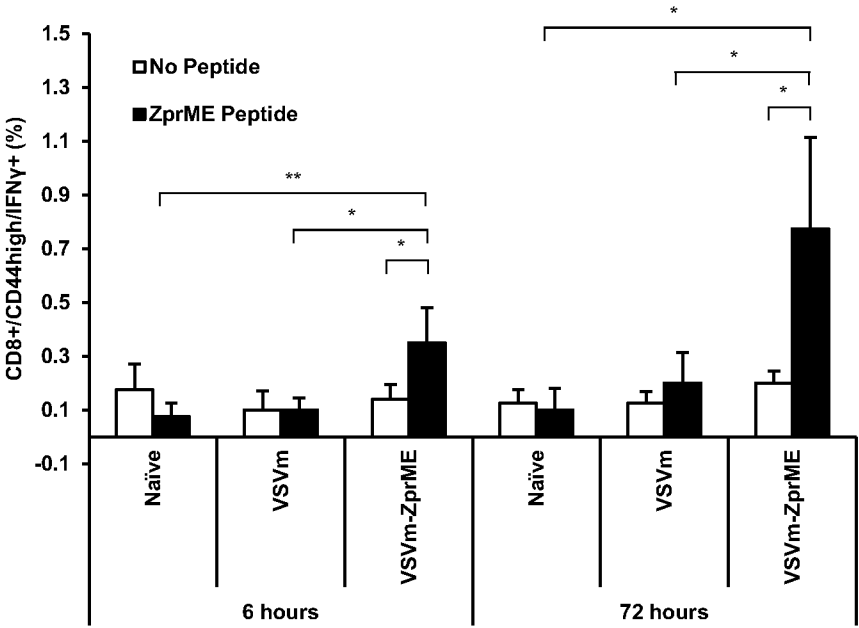


Figure 7

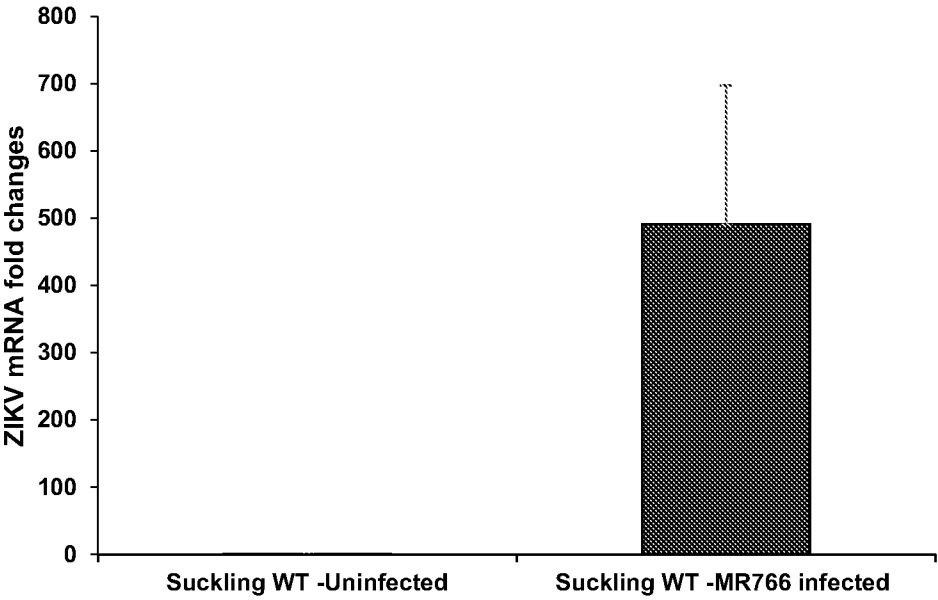


Figure 8A

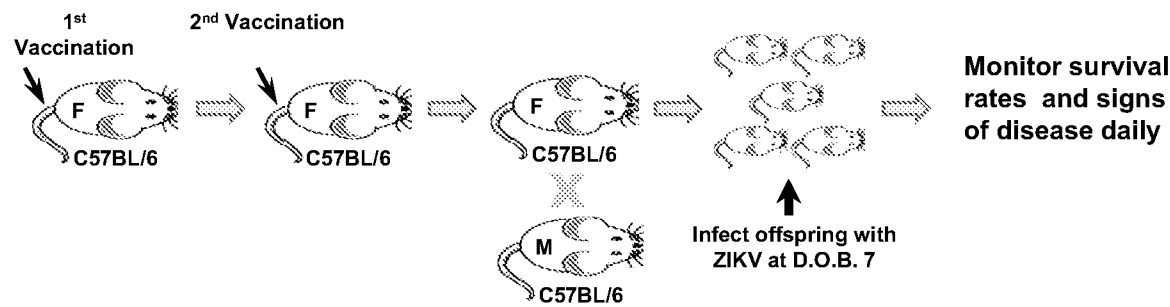


Figure 8B

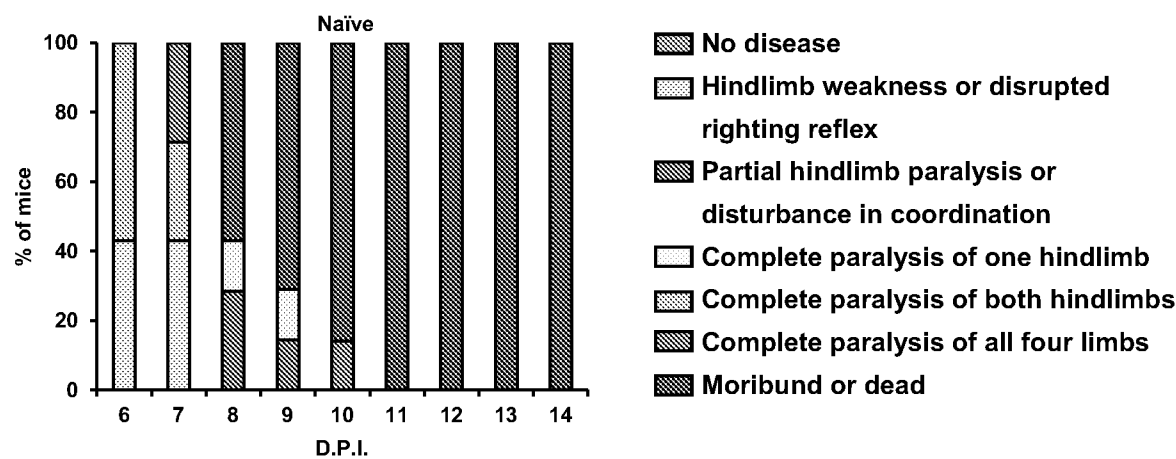


Figure 8C

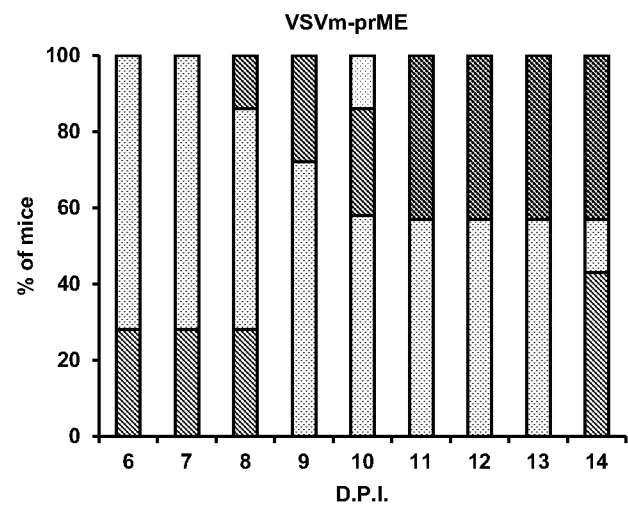


Figure 8D

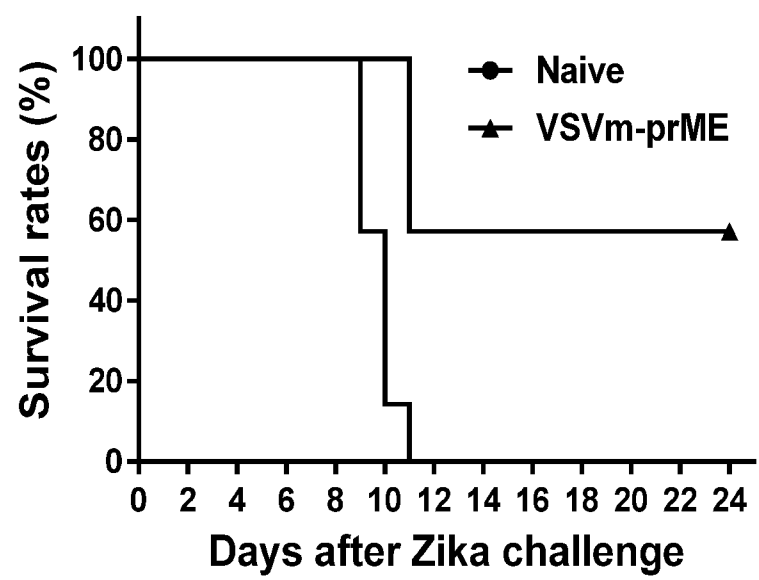


Figure 8E

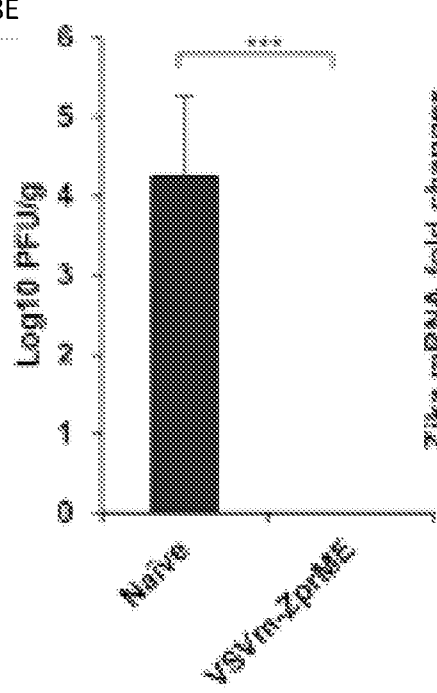


Figure 8F

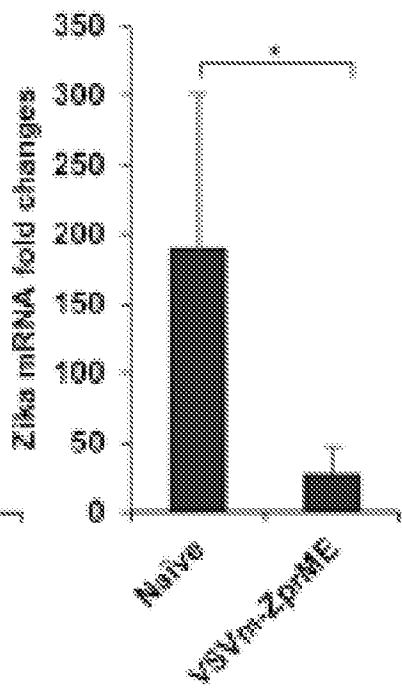


Figure 9

Group	Mouse Number	Duplicate	OD450			PRNT50	
			Week 0	Week 3	Week 5	Week 3	Week 5
Naïve	Naïve 1	1	0.093	0.287	0.324	N/D	N/D
		2	0.069	0.267	0.283	N/D	134.48
	Naïve 2	1	0.013	0.031	0.026	N/D	N/D
		2	0.032	0.037	0.073	N/D	N/D
	Naïve 3	1	0.086	0.249	0.193	N/D	159.88
		2	0.159	0.231	0.234	N/D	219.52
	Naïve 4	1	0.07	0.29	0.211	112.8	N/D
		2	0.12	0.301	0.231	N/D	139.69
	Naïve 5	1	0.077	0.15	0.118	29.5	N/D
		2	0.198	0.166	0.119	N/D	135.25
VSVm	VSVm1	1	0.231	0.229	0.217	N/D	N/D
		2	0.267	0.236	0.207	N/D	110.58
	VSVm2	1	0.01	0.136	0.118	N/D	177.23
		2	0.043	0.123	0.129	47.9	254.3
	VSVm3	1	0.093	0.231	0.132	103.3	198.8
		2	0.124	0.227	0.191	N/D	219.4
	VSVm4	Deceased					
	VSVm5	1	0.038	0.097	0.102	N/D	178.8
		2	0.069	0.103	0.118	N/D	N/D
VSVm-ZENV	VSVm-ZENV1	1	0.015	0.062	0.063	N/D	119.73
		2	0.027	0.061	0.064	N/D	67.67
	VSVm-ZENV2	1	0.066	0.179	0.081	132.3	N/D
		2	0.047	0.143	0.094	N/D	N/D
	VSVm-ZENV3	1	0.138	0.166	0.186	139.4	N/D
		2	0.14	0.153	0.207	155.0	N/D
	VSVm-ZENV4	1	0.046	0.083	0.024	N/A	143.44
		2	0.028	0.073	0.063	N/A	N/D
	VSVm-ZENV5	1	0.239	0.466	0.399	N/D	4.2
		2	0.235	0.381	0.357	N/D	215.194
VSVm-ZprME	VSVm-ZprME1	1	0.119	0.986	0.974	113.7	480.5
		2	0.09	0.879	1.404	130.3	783.4
	VSVm-ZprME2	1	0.027	0.137	0.505	N/D	1750.2
		2	0.019	0.159	0.635	N/D	1695.4
	VSVm-ZprME3	1	0.097	0.232	0.754	188.7	3078.0
		2	0.043	0.361	1.197	228.9	2799.1
	VSVm-ZprME4	1	0.256	0.345	0.914	N/D	771.1
		2	0.171	0.284	0.971	N/D	853.2
	VSVm-ZprME5	1	0.109	0.365	0.351	120.6	1498.3
		2	0.096	0.313	0.374	121.2	987.1
VSV-ZENV	VSV-ZENV1	Deceased					
	VSV-ZENV2	1	0.31	0.492	0.511	N/D	N/D
		2	0.348	0.438	0.562	N/D	N/D
	VSV-ZENV3	1	0.075	0.077	0.032	N/D	121.35
		2	0.032	0.073	0.049	144.2	213.8
	VSV-ZENV4	1	0.091	0.233	0.165	124.3	N/D
		2	0.087	0.299	0.236	216.2	N/D
	VSV-ZENV5	1	0.046	0.185	0.102	101.3	N/D
		2	0.121	0.171	0.121	163.1	N/D
VSV-ZprME	VSV-ZprME1	1	0.142	0.506	0.439	235.4	752.0
		2	0.129	0.479	0.4	143.8	310.3
	VSV-ZprME2	1	0.163	0.559	0.547	250.6	165.4
		2	0.123	0.604	0.774	176.5	224.9
	VSV-ZprME3	1	0.279	0.565	0.617	215.9	136.3
		2	0.368	0.569	0.876	170.4	177.5
	VSV-ZprME4	1	0.027	0.368	0.654	253.9	292.9
		2	0.044	0.378	0.769	398.2	316.0
	VSV-ZprME5	1	0.135	0.363	0.472	175.5	150.2
		2	0.169	0.406	0.532	321.4	218.2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/031067

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00; A61K 39/12; C12N 7/00; C12N 7/01 (2017.01)

CPC - A61K 39/12; A61K 2039/5258; C07K 16/1081; C12N 7/00; C12N 2760/14011; C12N 2760/14022; C12N 2760/14034; C12N 2760/20211; C12N 2760/20234; C12N 2760/20241 (2017.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/130.1; 424/159.1; 424/161.1; 424/218.1; 435/320.1; 536/23.72 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	— BETANCOURT et al. "Innate Immune Augmenting Vesicular Stomatitis Virus Expressing Zika Virus Proteins Confers Protective Immunity," The Journal of Immunology, 03 April 2017 (03.04.2017), Vol. 198, No. 8, Pgs. 3023-3028. entire document	1-4, 7-10, 13-16, 19-33
P, X	WO 2016/187118 A1 (BATAVIA BIOSCIENCES INC.) 24 November 2016 (24.11.2016) entire document	1-4, 7-10, 13-16, 19-33
A	— EP 2980099 A1 (BERNHARD-NOCHT-INSTITUT FÜR TROPENMEDIZIN) 03 February 2016 (03.02.2016) entire document	1-4, 7-10, 13-16, 19-33
A	WO 2010/093335 A1 (NANYANG TECHNOLOGICAL UNIVERSITY et al) 19 August 2010 (19.08.2010) entire document	1-4, 7-10, 13-16, 19-33
A	— DAI et al. "Structures of the Zika Virus Envelope Protein and Its Complex with a Flavivirus Broadly Protective Antibody," Cell Host & Microbe, 02 May 2016 (02.05.2016), Vol. 19, Iss. 5, Pgs. 696-704. entire document	1-4, 7-10, 13-16, 19-33
A	— ROSE et al. "Glycoprotein Exchange Vectors Based on Vesicular Stomatitis Virus Allow Effective Boosting and Generation of Neutralizing Antibodies to a Primary Isolate of Human Immunodeficiency Virus Type 1," Journal of Virology, 01 December 2000 (01.12.2000), Vol. 74, No. 23, Pgs. 10903-10910. entire document	1-4, 7-10, 13-16, 19-33

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

* Special categories of cited documents:

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

01 July 2017

Date of mailing of the international search report

28 JUL 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/031067

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5, 6, 11, 12, 17, 18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.