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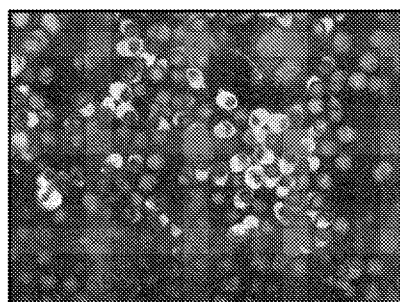


FIG. 1A

(57) Abstract: The present disclosure provides for proliferation-incompetent cells to deliver inactivated Zika viruses and GM-CSF to a subject, and induce or enhance both humoral and cellular immune responses against Zika virus antigens.



WHOLE-CELL BASED VACCINE AGAINST ZIKA VIRUS

Cross Reference to Related Application

5 This application claims priority to U.S. Provisional Application No. 62/352,796 filed on June 21, 2016, which is incorporated herein by reference in its entirety.

Technical Field

10 The present disclosure relates to methods and compositions for the prevention and/or treatment of flavivirus infection. In particular, the present disclosure relates to whole-cell vaccine compositions that trigger protective immunity against Zika virus infection.

Background of the Disclosure

15 Zika virus (ZIKV) is an emerging mosquito-borne virus that affects all major continents with the exception of Europe and has been deemed a global emergency by the WHO. Zika virus belongs to the Flavivirus genus of the *Flaviviridae* family. ZIKV infection carries high risk for pregnant women as it has been causally linked to severe fetal brain anomalies such as microcephaly, intracranial calcifications, fetal brain disruption sequence as well as ocular anomalies (Rasmussen et al. N Engl J Med, 2016 May 19;374(20):1981-7). Furthermore, ZIKV
20 infection likely triggers an increased risk for Guillain-Barré syndrome (Broutet et al. N Engl J Med, 2016 Apr 21;374(16):1506-9).

25 In urban and suburban environments, ZIKV is transmitted in a human-mosquito-human transmission cycle. While two species in the stegomyia subgenus of aedes — *A. aegypti* and *A. albopictus* have been linked with nearly all known Zika virus outbreaks, two other species, *A. hensilli* and *A. polynesiensis*, are believed to be vectors in the Yap and French Polynesia outbreaks, respectively (Paterson et al. N Engl J Med 2016; 374:1552-1563).

 In addition to mosquito transmission, evidence indicates that ZIKV can be transmitted from the mother to the fetus during pregnancy. Additionally, sexual transmission to partners has also been reported. Finally, although the transmission of ZIKV through a blood transfusion has

not been reported, it is likely to occur, given the transmission of other, related flaviviruses, through this route.

5 Currently, there are no vaccines for ZIKV or other specific preventative methods, and thus, prevention and control measures focus on avoiding mosquito bites, reducing sexual transmission, and controlling the mosquito vector. Therefore, there remains a need for immediate and long-term prevention and control strategies.

Summary

The present disclosure provides for an immunogenic composition comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least one cytokine.

5 The present disclosure also provides for an immunogenic composition comprising: a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and a second population of cells expressing at least one cytokine.

10 The present disclosure provides for a method of preventing or treating flavivirus infection (e.g., Zika virus infection) in a subject, the method comprising administering to the subject an immunogenic composition comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least one cytokine.

15 The present disclosure provides for a method of preventing or treating flavivirus infection (e.g., Zika virus infection) in a subject, the method comprising administering to the subject an immunogenic composition comprising: a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and a second population of cells expressing at least one cytokine.

20 The present disclosure provides for a method of preventing or treating flavivirus infection (e.g., Zika virus infection) in a subject, the method comprising: (a) administering to the subject a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (b) administering to the subject a second population of cells expressing at least one cytokine.

In certain embodiments, the first population of cells and the second population of cells are of the same type of cells. In certain embodiments, the first population of cells and the second population of cells are of different types of cells.

25 Non-limiting examples of flaviviruses include Zika virus, the dengue virus (DENV), the yellow fever virus (YFV), the West Nile virus (WNV), the Japanese encephalitis virus (JEV), the St. Louis encephalitis virus, and the tick-borne encephalitis virus.

In certain embodiments, the cytokine is granulocyte macrophage colony-stimulating factor (GM-CSF), interferon alpha (IFN- α), interleukin-2 (IL-2), interleukin-12 (IL-12), tumor necrosis factor alpha (TNF-alpha), or combinations thereof. In certain embodiments, the cytokine is GM-CSF. In one embodiment, the GM-CSF is human GM-CSF.

5 In certain embodiments, the at least one flavivirus antigen is an inactivated or attenuated flavivirus. In certain embodiments, the at least one Zika virus antigen is an inactivated or attenuated Zika virus. In certain embodiments, the Zika virus is inactivated by irradiation, such as gamma irradiation, ultraviolet (UV) irradiation, and/or heat.

10 In certain embodiments, the cells are pancreatic cancer cells, colon cancer cells, lung cancer cells, breast cancer cells, ovarian cancer cells, melanoma cancer cells, or combinations thereof.

In certain embodiments, the cells are epithelial cells. In certain embodiments, the cells are antigen presenting cells (APCs), such as dendritic cells.

15 In certain embodiments, the cells are proliferation-incompetent. In certain embodiments, the cells are rendered proliferation-incompetent by irradiation.

In certain embodiments, the virus is inactivated by irradiation in the range of 20-40 kGy (kilogray).

The subject may be a woman. In certain embodiments, the subject is a woman who is pregnant.

20

Brief Description of the Drawings

Figures 1A-1D. Detection by immunostaining of viral proteins in the cells for use as whole-cell based vaccine against ZIKV. Mouse neuroblastoma cell line N2a was transfected with pcDNA-3.1 vector containing mouse GM-CSF gene. The transfected cells were designated as N2a-GMSCF or N2a-GM. N2a-GM cells were then infected with ZIKV strain MR776 (Uganda). Viral infection was monitored by staining viral E2 protein using anti-E2 antibodies 4G2. Figures 1A and 1B: cells having an infectivity rate of about 40% - 50%. Figures 1C and 1D: cells having an infectivity rate of about 80% - 90%.

Figure 2 shows results from the cellular enzyme-linked immunosorbent assay (cELISA) assay. Sera from mice immunized with N2a-GM (naïve) cells did not react either with N2a-GM (naïve) cells ("Group 1 on N2A-GM-naïve") or with N2a-GM-ZIKV cells ("Group 1 on N2A-GM-ZIKA"). Sera from mice vaccinated with N2a-GM-ZIKV cells was immunologically reactive to N2a-GM-ZIKV cells ("Group 2 on N2A-GM-ZIKA"), but not reactive to N2a-GM cells ("Group 2 on N2A-GM-naïve").

Figure 3 shows antibody response (vertical bars according to the left y-axis, cELISA assay on N2a-GM-ZIKV cells) and antibody neutralization potential (solid line with closed circles, according to the right y-axis, PRNT) for three groups of mice immunized with N2a-GM-ZIKV cells (group A), N2a-ZIKV cells (group B), or purified ZIKV (group C), respectively.

Figure 4 shows antibody response (vertical bars according to the left y-axis, cELISA assay on N2a-GM-ZIKV cells) for, as well as the number of colonies infected with ZIKV in the presence of a fixed dilution (1:3000) of the serum (solid line with closed circles, according to the right y-axis) from, three groups of mice immunized with N2a-GM-ZIKV cells (group A), N2a-ZIKV cells (group B), or purified ZIKV (group C), respectively.

Figures 5A-5K. Testing ZIKV infectivity in different cell types. Different types of cells, including Vero cells (Figures 5A and 5B), N2a (naïve) cells (Figures 5A and 5B), N2a-GMSCF

cells (Figures 5E and 5F), N2a new cell line (Figures 5G and 5H), human fibroblasts WS-1 (Figures 5I and 5J), human fibroblasts PCS201-012 (Figure 5K), were infected with ZIKV-containing cell culture media (P2) and immunostained using anti-E2 antibodies 4G2 at different time points post-infection, e.g., 24 hours, or 48 hours post-infection. Figures 5A, 5C, 5E, 5G, and 5I show cells 24 hours post-infection. Figures 5B, 5D, 5F, 5H, 5J and 5K show cells 48 hours post-infection.

DETAILED DESCRIPTION

The present disclosure provides methods and compositions for the prevention and/or treatment of Zika virus infection. In certain embodiments, the immunotherapy uses cells comprising Zika virus antigens and expressing a cytokine, such as granulocyte macrophage colony-stimulating factor (GM-CSF). In certain embodiments, the present disclosure relates to immunotherapy using cells comprising Zika virus antigens and cells expressing a cytokine, such as GM-CSF. The immunotherapy induces or enhances immune responses against Zika viruses.

In certain embodiments, the present disclosure relates to compositions and to methods for inducing or enhancing an immune response to a flavivirus antigen (e.g., a Zika virus antigen). An immune response to the target antigen may provide a prophylactic or therapeutic effect.

Viral infections can be treated and/or prevented by administering reagents that modulate the immune system. The present compositions and methods inhibit and/or treat the flavivirus infection (e.g., Zika virus infection), and/or ameliorating one or more symptoms associated with the flavivirus infection (e.g., Zika virus infection). The present compositions and methods are useful in the prophylaxis and/or treatment of a disease caused by flaviviruses (e.g., Zika viruses).

An appropriate cell expressing at least one virus antigen (e.g., cell comprising at least one inactivated or attenuated virus) can be modified to express one or more cytokines, and administered to an uninfected subject, to serve as a vaccine and elicit an enhanced immune response to confer the ability to resist subsequent infection by the virus. Alternatively, the antigen and the cytokines may be administered without a host cell to a subject (e.g., as cell lysate). Furthermore, the present composition can be administered to a subject infected by the viruses to treat the viral infection.

In certain embodiments, the present composition and method relate to a whole cell-based vaccine composition. In certain embodiments, the present whole cell vaccine composition provides multiple antigens that can be targeted by both the innate and adaptive immune systems. In certain embodiments, the present whole-cell based vaccine serves as an adjuvant on its own, because of their ability to stimulate the immune system in a non-specific manner. In certain embodiments, the present whole-cell vaccine comprises allogeneic cells providing MHC-allotypes (alternative histocompatibility complexes), which are powerful stimulators of the

immune response. In certain embodiments, the present whole cell-based vaccine offers a natural delivery of viral antigens to antigen presenting cells (APC), as the primary recognition of infectious agents involves recognition of infected cells, rather than individual viral particles.

5 In certain embodiments, the present composition and method deliver both GM-CSF and inactivated Zika virus to a subject, and therefore, establish both humoral as well as cellular immunity against Zika virus antigens in a subject.

10 In certain embodiments, the present disclosure provides for an immunogenic composition comprising: cells comprising at least one flavivirus antigen or a fragment thereof (e.g., at least one Zika virus antigen or a fragment thereof), wherein the cells also express a cytokine (such as GM-CSF).

In certain embodiments, the present disclosure provides for an immunogenic composition comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least one cytokine.

15 In certain embodiments, the present disclosure provides for an immunogenic composition comprising a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen) or a fragment thereof, and a second population of cells expressing at least one cytokine (such as GM-CSF). In one embodiment, cells that express a cytokine (e.g., GM-CSF) and cells that comprise at least one flavivirus antigen (e.g., at least one Zika virus antigen) (such as inactivated flavivirus, e.g., inactivated Zika virus) are of the same type of cells. In another
20 embodiment, cells that express at least one cytokine (such as GM-CSF) and cells that comprise at least one flavivirus antigen (e.g., at least one Zika virus antigen) (such as inactivated flavivirus, e.g., inactivated Zika virus) are of different types of cells.

25 In the composition, the ratio of the number of the first population cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen) to the number of the second population of cells expressing at least one cytokine may range from about 50:1 to about 1:10, 40:1 to about 1:8, 30:1 to about 1:6, 20:1 to about 1:5, 20:1 to about 1:3, from about 20:1 to about 1:2, from about 15:1 to about 1:1.5, from about 10:1 to about 1:1, from about 8:1 to about 1:1, from about 6:1 to about 1:2, from about 5:1 to about 1:1, from about 4:1 to about 1:1, from about 3:1 to about 1:1, from about 2:1 to about 1:1, from about 1.5:1 to about 1:1, from about
30 0.8:1 to about 1:1, from about 0.6:1 to about 1:1, from about 0.5:1 to about 1:1, from about 0.4:1

to about 1:1, or from about 0.3:1 to about 1:1. In certain embodiments, the ratio of the number of the first population cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen) to the number of the second population of cells expressing at least one cytokine may be about 1:1, about 2:1, about 3:1, about 4:1, or about 5:1.

5 The cell may comprise at least one virus antigen, or may comprise nucleic acids encoding at least one virus antigen. In one embodiment, the cell comprises at least one inactivated virus.

In certain embodiments, the present composition and method use the irradiated whole-cell pancreatic vaccine (GVAX), which is a vaccine derived from human pancreatic cell lines (PANC 6.03 and PANC 10.05). Pancreatic GVAX has been genetically modified to secrete
10 human granulocyte macrophage-colony stimulating factor (GM-CSF), an immune-modulatory cytokine that can activate antigen presenting cells (APCs, monocytes, and DCs) locally at the vaccine site (Dranoff et al. Immunol Rev 188:147–154 (2002); Mach et al. Curr Opin Immunol 12:571–575 (2000); Dranoff et al. Proc Natl Acad Sci USA 90:3539–3543(1993). Furthermore, macrophages, granulocytes and NKT cells have also been shown to respond to GM-CSF, thereby
15 improving tumor antigen presentation. Autologous and allogeneic cancer cells that have been genetically modified to express a cytokine, e.g., GM-CSF, followed by administration to a patient for the treatment of cancer may be used in the present methods and compositions. U.S. Patent Nos. 5,637,483; 5,904,920; 6,277,368; 6,350,445 and 9,669,081, each of which is incorporated by reference herein. A form of GM-CSF-expressing genetically modified cancer
20 cells for the treatment of pancreatic cancer is described in U.S. Patent Nos. 6,033,674 and 5,985,290, both of which are incorporated by reference herein. A universal immunomodulatory cytokine-expressing bystander cell line is also described in U.S. Pat. No. 6,464,973, incorporated by reference herein.

The cell may express the cytokine. The cell may express and secrete the cytokine. Non-
25 limiting examples of cytokines that may be used in the present composition and method include GM-CSF, IL-2, IL-4, TNF-alpha, IL-6, CD2, ICAM, and combinations thereof. In one embodiment, the cell expresses GM-CSF.

In certain embodiments, the present disclosure provides for an immunogenic composition comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus
30 antigen), and (ii) expressing at least one immunomodulatory molecule.

In certain embodiments, the present disclosure provides for an immunogenic composition comprising a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and a second population of cells expressing at least one immunomodulatory molecule.

5 For example, the immunomodulatory molecule can be a recombinant protein cytokine, chemokine, or immunostimulatory agent or nucleic acid encoding cytokines, chemokines, or immunostimulatory agents designed to enhance the immunologic response. Non-limiting examples of immunomodulatory cytokines include interferons (e.g., IFN- α , IFN- β and IFN- γ), interleukins (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-15, IL-20, 10 and IL-21), tumor necrosis factors (e.g., TNF- α and TNF- β), erythropoietin (EPO), FLT-3 ligand, glp10, TCA-3, MCP-1, MIF, MIP-1 α , MIP-1 β , Rantes, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF), as well as functional fragments of any of the foregoing. Any immunomodulatory chemokine that binds to a chemokine receptor, i.e., a CXC, CC, C, or 15 CX3C chemokine receptor, also can be used in the present composition and method. Examples of chemokines include, but are not limited to, Mip1 α , Mip-1 β , Mip-3 α (Larc), Mip-3 β , Rantes, Hcc-1, Mpif-1, Mpif-2, Mcp-1, Mcp-2, Mcp-3, Mcp-4, Mcp-5, Eotaxin, Tarc, Elc, I309, IL-8, Gcp-2 Gro- α , Gro- β , Gro- γ , Nap-2, Ena-78, Gcp-2, Ip-10, Mig, I-Tac, Sdf-1, and Bca-1 (Blc), as well as functional fragments of any of the foregoing.

20 As used herein, the terms "immunogenic composition" and "vaccine composition" are interchangeable.

The cells may be proliferation-incompetent. In certain embodiments, methods may be used to inactivate cells to make them incapable of cell division but retaining the ability to express cytokines. The cells may be unable to undergo mitosis, but still retain the capability to express 25 proteins such as cytokines. Cells may be inactivated by rendering them proliferation incompetent by irradiation. In certain embodiments, a dose ranging from about 3500 rads to about 30,000 rads is used to irradiate cells. In certain embodiments, methods such as treatments with mitomycin C, cycloheximide, and analogous agents, and/or incorporation of a suicide gene by the cell etc. are used to render the cells proliferation-incompetent.

As used herein, the term "proliferation-incompetent" or "inactivated" cells refers to cells that are unable to undergo multiple rounds of mitosis, but still retain the capability to express proteins such as cytokines and/or virus antigens. This may be achieved through numerous methods known to those skilled in the art. Non-limiting examples of methods to inactivate cells, or render cells proliferation-incompetent, include, treatments that inhibit at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or substantially 100% of the cells from further proliferation. In one embodiment, the cells are irradiated at a dose of from about 50 to about 200 rads/min or from about 120 to about 140 rads/min. In certain embodiments, the irradiation level (dose) is about 2,500 rads, about 5,000 rads, about 10,000 rads, about 15,000 rads or about 20,000 rads.

In one embodiment of the invention, cells are rendered proliferation incompetent by irradiation prior to administration to the subject.

The present composition may elicit T-cell, and/or B-cell, responses against a flavivirus.

The present vaccine composition can be used for prevention and/or treatment of flavivirus infections (e.g., Zika virus infections). Additionally, vaccine(s) of the present disclosure can be used to prevent flavivirus infection (e.g., Zika virus infection) of a fetus during pregnancy. For example, administration of a vaccine to a woman before and/or during pregnancy can prevent flavivirus infection (e.g., Zika virus infection) of a fetus.

In one embodiment, the present disclosure provides a method of preventing and/or treating flavivirus infection (e.g., Zika virus infection) in a subject, the method comprising the step of administering to the subject a vaccine composition comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least one cytokine.

In one embodiment, the present disclosure provides a method of preventing and/or treating flavivirus infection (e.g., Zika virus infection) in a subject, the method comprising the step of administering to the subject a vaccine composition comprising: a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and a second population of cells expressing at least one cytokine.

In one embodiment, the present disclosure provides a method of eliciting an immune response to a flavivirus (e.g., Zika virus) in a subject, the method comprising the step of administering to the subject a vaccine composition comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least one cytokine.

5 In one embodiment, the present disclosure provides a method of eliciting an immune response to a flavivirus (e.g., Zika virus) in a subject, the method comprising the step of administering to the subject a vaccine composition comprising: a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and a second population of cells expressing at least one cytokine.

10 In one embodiment, the present disclosure provides a method of preventing and/or treating flavivirus infection (e.g., Zika virus infection) in a subject, the method comprising the step of: (a) administering to the subject a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (b) administering to the subject a second population of cells expressing at least one cytokine.

15 In one embodiment, the present disclosure provides a method of eliciting an immune response to a flavivirus (e.g., Zika virus) in a subject, the method comprising the step of: (a) administering to the subject a first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (b) administering to the subject a second population of cells expressing at least one cytokine.

20 The first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and the second population of cells expressing at least one cytokine may be co-administered to a subject. The first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen) may be administered to a subject prior to, concurrently with, or subsequent to, the second population of cells expressing at least one
25 cytokine.

As used herein, the term "co-administering" refers to a process where the first population of cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen) and the second population of cells expressing at least one cytokine are encountered by the subject's immune system at essentially the same time. The two populations of cells may or may not be
30 administered by the same vehicle. If they are administered in two separate vehicles, they can be

administered sufficiently closely, both in time and by route of administration, that they are encountered essentially simultaneously by the subject's immune system to achieve the desired specificity.

The present disclosure relates to compositions and methods of regulating the immune response of a subject to a virus antigen by administering a mixture of the virus antigen and one or more cytokines, and/or adhesion or accessory molecules, in such a manner that the immune system of the subject is stimulated. In certain embodiments, the antigen, the cytokine, and/or the adhesion or accessory molecule, are co-administered in a therapeutically effective amount, which results in the systemic immune response.

The present composition and method provide for transfer or release of the cytokine, and/or the adhesion or accessory molecule, in direct proximity or in combination with the virus antigen. The cytokine and the virus antigen may be administered in the same vehicle, or may be administered in two separate vehicles. If they are administered in two separate vehicles, they are administered sufficiently closely, both in time and by route of administration, that they are encountered essentially simultaneously by the individual's immune system. That is, the cytokine, and/or the adhesion or accessory molecules, can be co-administered with the virus antigen in any manner which provides transfer or delivery of the cytokine in the context of the virus antigen in relation to which the immune response is to be regulated. For example, this can be accomplished by using slow or sustained release delivery systems, or direct injection. In certain embodiment, as a result of the co-administration, the nonspecific cytokine has the specific effect of amplifying or altering the specific immune response to the virus antigen. The emphasis is on local interaction of the cytokine and the virus antigen to mimic the physiological occurrence of simultaneous presentation of cytokine and antigen, to maximize efficacy and minimize toxicity.

The present composition (vaccine composition or immunogenic composition) functions by preparing the immune system to mount a response to a virus. In certain embodiments, a vaccine can comprise an antigen, which is a virus or a component of the virus, or a fragment thereof, that is introduced into a subject to be vaccinated in a non-toxic, non-infectious and/or non-pathogenic form. In certain embodiments, virus antigens include whole live attenuated viruses (modified to reduce their virulence) or inactivated viruses, individual viral components (e.g., protein or polysaccharides) and the genetic material of the virus (e.g., RNA or DNA). The

antigen in the vaccine causes the subject's immune system to be "primed" or "sensitized" to the virus from which the antigen is derived. Subsequent exposure of the immune system of the subject to the virus results in a rapid, robust and/or specific immune response, that controls or destroys the virus before it can multiply and infect or damage sufficient number of cells in the host organism to cause manifestation of disease symptoms.

In one embodiment, cells which express the virus antigen also express the cytokine (e.g., genetically engineered to express the cytokine). The resulting modified cells presents to the host immune system not only the antigen against which an immune response is sought, but also the cytokines, and/or adhesion or accessory molecules, whose expression determines the type and extent of the resulting immune response.

In certain embodiments, the subject's systemic immune response is increased or enhanced compared to the immune response in the absence of the present composition. The systemic response is sufficient for the subject to mount an immune response against the virus or virus antigen.

In certain embodiments, a "prophylactic effect" is an inhibition of one or more symptoms associated with the viral infection for which the vaccine(s) are being administered. In certain embodiments, a "therapeutic effect" is a lessening of one or more symptoms associated with the viral infection for which the vaccine(s) are being administered.

As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition (e.g., afflicted by viral infection) in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition (e.g., afflicted by viral infection).

As used herein, "susceptible to" or "prone to" or "predisposed to" a specific disease or condition (e.g., viral infection) and the like refers to a subject who based on genetic, environmental, health, and/or other risk factors is more likely to be infected by the virus than the general population. An increase in likelihood of being infected by the virus may be an increase of about 10%, 20%, 50%, 100%, 150%, 200%, or more.

As used herein, the terms "treat," "treating," "treatment," and the like refer to reducing or ameliorating a disorder (e.g., viral infection) and/or symptoms associated therewith.

The term "effective amount" refers to an amount of the composition or cells that is sufficient to stimulate, enhance, or elicit, the immune response of a subject against a flavivirus (e.g., Zika virus).

An "immunological response" or "immune response" to an antigen, or vaccine or composition comprising the antigen, is the development in a mammalian subject of a humoral and/or a cellular immune response to the antigen. An "immunological response" or "immune response" as used herein encompasses at least one or more of the following effects: the production of antibodies by B-cells; and/or the activation of suppressor T-cells and/or T-cells directed specifically to an antigen or antigens present in the vectors, composition or vaccine of interest. In some embodiments, the "immunological response" or "immune response" encompasses the inactivation of suppressor T-cells. A "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells, including without limitation NK cells and macrophages. Functionally cellular immunity includes antigen specific cytotoxic T cells (CTL). Antigen specific T cells, CTL, or cytotoxic T cells as used herein refers to cells which have specificity for peptide antigens presented in association with proteins encoded by the major histocompatibility complex (MHC) or human leukocyte antigens (HLA) as the proteins are referred to in humans. CTLs of the present invention include activated CTL which have become triggered by specific antigen in the context of MHC; and memory CTL or recall CTL to refer to T cells that have become reactivated as a result of re-exposure to antigen as well as cross-reactive CTL. CTLs of the present disclosure include CD4⁺ and CD8⁺ T cells. Activated antigen specific CTLs of the present disclosure promote the destruction and/or lysis of cells of the subject infected with the pathogen or cancer cell to which the CTL are specific via amongst other things, secretion of chemokines and cytokines including without limitation macrophage inflammatory protein 1a (MIP-1a), MIP-1B, and RANTES; and secretion of soluble factors that suppress the disease state. Cellular immunity of the present disclosure also refers to antigen specific response produced by the T helper subset of T cells. Helper T cells act to help stimulate the function, and focus the activity of nonspecific effector cells against cells displaying peptide in association with MHC molecules on their surface. A cellular immune response also refers to the production of cytokines, chemokines and other such molecules produced by activated T cells and/or other white blood cells including those derived from CD4 and CD8 T cells and NK cells.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a mammalian subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host. The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays known in the art, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., *J. Immunol.* (1993) 151:4189-4199; Doe et al., *Eur. J. Immunol.* (1994) 24:2369-2376. Methods of measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-cells (e.g., by the tetramer technique) (reviewed by McMichael et al., *J. Exp. Med.* 187(9):1367-1371, 1998; Mcheyzer-Williams et al, *Immunol. Rev.* 150:5-21, 1996; Lalvani et al, *J. Exp. Med.* 186:859-865, 1997). An immunological response, or immune response, as used herein encompasses one which stimulates the production of CTLs, and/or the production or activation of helper T-cells and/or an antibody-mediated immune response.

The present method and composition may regulate the immune response to a flavivirus (e.g., Zika virus) in a subject. The term "regulating the immune response" refers to any alteration in any cell type involved in the immune response. The definition is meant to include an increase or decrease in the number of cells, an increase or decrease in the activity of the cells, or any other changes which can occur within the immune system. The cells may be, but are not limited to, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells, or neutrophils. The definition encompasses both a stimulation or enhancement of the immune system to develop a sufficiently potent response to a deleterious target, as well as a suppression of the immune system to avoid a destructive response to a desirable target. In the case of stimulation of the immune system, the definition includes future protection against subsequent viral challenge.

Zika Virus and Flaviviruses

The present compositions and methods can be used for the treatment and/or prevention of infection caused by a flavivirus. The Flavivirus family (Flaviviridae) are single-stranded (+) RNA virus. In certain embodiments, flaviviruses include arthropod-transmitted human pathogens. Non-limiting examples of flaviviruses include Zika virus, the dengue virus (DENV), the yellow fever virus (YFV), the West Nile virus (WNV), the Japanese encephalitis virus (JEV), the St. Louis encephalitis virus, and the tick-borne encephalitis virus.

The present compositions and methods can be used for the treatment and/or prevention of infection caused by Zika virus, including any strain of Zika virus. Within the mosquito-borne clade of flaviviruses, ZIKV is a member of the Spondweni group; both genetically and serologically, ZIKV is related closely to the four serotypes of DENV with approximately 43% amino acid identity and extensive antibody cross-reactivity (Alkan et al. *J. Virol.* 89, 11773–11785 (2015). Current data suggests that ZIKV is likely to have originated in East Africa and subsequently spread to West Africa and then to Asia, resulting in distinct lineages (Nigerian strain, MR766 strain, and the Asian strain). All strains currently associated with the outbreak in the Americas belong to the Asian genotype and are most closely related to strains from Yap, Cambodia, Thailand, and French Polynesia. (Petersen et al., *N Engl J Med* 2016; 374:1552–1563).

In certain embodiments, in order to generate a vaccine composition with broad specificity and cross-reactivity against more than one Zika virus strain, cells used for the generation of the vaccine composition are infected with two or more strains of Zika viruses. In certain embodiments, cells infected with distinct strains of Zika viruses are combined and formulated into one vaccine composition.

A virus antigen (e.g., a Zika virus antigen) may refer to any protein, carbohydrate, nucleic acid (RNA or DNA), or other component capable of eliciting an immune response. A virus antigen (e.g., a Zika virus antigen) includes, but is not limited to, the whole virus with all of its associated antigens as an antigen, as well as any component separated from the virus, proteins purified from the virus, or unique carbohydrate moieties associated with the virus. In certain embodiments, the virus antigen comprises the flavivirus (or Zika virus) E-glycoprotein or flavivirus envelope glycoprotein, capsid protein, nucleocapsid, and/or a viral glycoprotein. In certain embodiments, the virus antigen (e.g., a Zika virus antigen) comprises at least one

nonstructural protein and/or at least one structural protein of the virus (e.g., Zika virus). In certain embodiments, the Zika virus antigen comprises at least one nonstructural protein and/or at least one structural protein of the virus (e.g., Zika virus).

The present cell may contain at least one virus antigen (e.g., at least one flavivirus antigen, or at least one Zika virus antigen). For example, the present cell may contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more virus antigens (e.g., flavivirus antigens, or Zika virus antigens).

Non-limiting examples of the virus antigens include the envelope (E) protein, the membrane (M) protein, viral RNA, a capsid (C) protein, a structural protein, a membrane precursor (PrM) protein, a part or complete envelope, a nonstructural protein (e.g., NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) of the virus (e.g., flavivirus, or Zika virus). Bollati et al., Structure and functionality in flavivirus NS-proteins: Perspectives for drug design, Antiviral Research, 2010, 87(2):125-148. Kostyuchenko et al., Structure of the thermally stable Zika virus, Nature (2016) doi:10.1038/nature17994.

In one embodiment, the virus antigen is a viral peptide, polypeptide or protein. In one embodiment, the antigens of a flavivirus (e.g., Zika virus) are at least one inactivated or attenuated whole virus.

An antigen refers to a molecule containing one or more epitopes (either linear, conformational or both) or immunogenic determinants that will stimulate a host's immune-system, such as a mammal's immune system, to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen." An antigen may be a virus (e.g., an inactivated virus, or an attenuated virus), a whole protein, a truncated protein, a fragment of a protein or a peptide. Antigens may be naturally occurring, genetically engineered variants of the protein, or may be codon optimized for expression in a particular mammalian subject or host. Generally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature). Antibodies such as anti-idiotypic antibodies, or fragments thereof, and synthetic peptide mimotopes, that is synthetic peptides

which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Furthermore, an "antigen" refers to a protein which includes modifications, such as deletions, additions and substitutions, generally conservative in nature, to the naturally occurring sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the antigens. Antigens of the present disclosure may also be codon optimized by methods known in the art to improve their expression or immunogenicity in the host. As used herein, a "cross reaction" immunogenic determinant refers to a determinant, epitope, or antigen which is capable of eliciting an immune response to related but not identical antigenic determinants.

Viruses (e.g., Zika virus) may be inactivated by irradiation, a physical method, a chemical method, or a combination thereof.

Viruses (e.g., Zika virus) can be inactivated by gamma irradiation, ultraviolet (UV) irradiation, heat, or other methods known in the art.

Viruses (e.g., Zika virus) may be inactivated using a physical and/or chemical method. A range of inactivation agents or methods have been described to inactivate viruses for vaccine purposes. Examples of viral inactivation methods include, gamma irradiation (Martin et al. Vaccine 28(18):3143–3151 (2010b)); Alsharifi and Mullbacher Immunol Cell Biol 88(2):103–104 (2010)), UV treatment (Budowsky et al. Arch Virol 68 (3–4):239–247 (1981)), heat (Nims and Plavsic, N Engl J Med 352(14):1411–1412 (2012)), ascorbic acid (Madhusudana et al. Int J Infect Dis 8(1):21–25 (2004)), ethylenimine derivatives (Larghi and Nebel, J Clin Microbiol 11(2):120–122 (1980)), psorlens (Maves et al. Vaccine 29(15):2691–2696 (2011)), hydrogen peroxide (Amanna et al. Nat Med 18(6):974–979 (2012)), and other methods (Stauffer et al. Recent Pat Antiinfect Drug Discov 1(3):291–296 (2006)). Sanders et al. provide a detailed discussion of inactivated viral vaccines in *Vaccine Analysis: Strategies, Principles, and Control*, Chapter 2. Springer, 2014.

In certain embodiments, a virus (e.g., ZIKV) can be inactivated by gamma irradiation. A person skilled in the art would recognize that the suitable irradiation dose to inactivate a virus in

cells may vary upon the virus, specific viral strain, number of cells carrying a virus, etc. In certain embodiments, the gamma irradiation dose for inactivation of the virus (e.g., ZIKV) ranges from about 20 kGy to about 40 kGy, from about 25 kGy to about 40 kGy, from about 25 kGy to about 35 kGy, from about 20 kGy to about 35 kGy, or from about 25 kGy to about 30 kGy.

In certain embodiments, a virus (e.g., ZIKV) can be inactivated using UV irradiation. In certain embodiments, UV with a wavelength ranging from about 230 nm to about 280 nm (e.g., at energies 900-1000 Joule/m²) is used for the inactivation of a virus (e.g., ZIKV). In certain embodiments, UV with a wavelength of about 254 nm is used for the inactivation of a virus (e.g., ZIKV).

In certain embodiments, a virus (e.g., ZIKV) is inactivated by heat treatment at a temperature ranging from about 45°C to about 80°C, from about 45°C to about 75°C, from about 50°C to about 75°C, from about 50°C to about 70°C, or from about 50°C to about 60°C.

In certain embodiments, a virus (e.g., ZIKV) is inactivated by irradiation or heat treatment for about 10 minutes to about 5 hours, about 20 minutes to about 3 hours, about 20 minutes to about 2 hours, about 30 minutes to about 1 hour, or longer.

In one embodiment, the regimen used for inactivation of Zika virus in infected cells also renders the cells proliferation incompetent.

In certain embodiments, the virus (e.g., Zika virus) is inactivated by a chemical agent, such as formalin (formaldehyde), beta-propiolactone (BPL), hydrogen peroxide, glutaraldehyde, N-acetyleneimine, binary ethyleneimine, tertiary ethyleneimine, ascorbic acid, caprylic acid, psolarens, detergents including non-ionic detergents etc. U.S. Patent Publication No. 20170014502.

In order to test the presence of ZIKV in a subject, methods relying on the detection of viral nucleic acid by RT-PCR and/or the detection of IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) may be used. While the detection of viral nucleic acid in serum provides a definitive diagnosis, in most cases viremia is transient, and diagnosis by RT-PCR has been most successful within 1 week after the onset of clinical illness. However, viral RNA can be detected in serum approximately 10 weeks after infection in a pregnant woman

whose fetus has implications of congenital infection. There is data that suggest that ZIKV RNA can be detected longer in urine than in serum. If these findings are further verified, the period during which a definitive diagnosis of Zika virus infection can be established by RT-PCR would be extended (Paterson et al. N Engl J Med 2016; 374:1552-1563). Testing regimens for the
5 diagnosis of prenatal and antenatal Zika virus infection may rely on the RT-PCR of amniotic fluid. In addition to RT-PCR, immunohistochemical testing have been used to detect Zika virus infection in tissues of fetal losses and full-term infants who died shortly after birth.

Cells

10 The present disclosure provides methods and compositions for inducing or enhancing an immune response against a virus antigen in a subject. The present cells may express or comprise at least one virus antigen (e.g., at least one Zika virus antigen). In certain embodiments, when the cell is an antigen-presenting cell (APC), expressing the virus antigen in the cell may further include presenting the expressed antigen on the surface of the cell.

15 In certain embodiments, the present vaccine composition comprises a whole cell vaccine, e.g., an allogeneic or autologous whole cell vaccine.

The present cells may be normal (non-cancer) cells and/or cancer cells. Numerous cancer and non-cancer cells lines are commercially available from American Type Culture Collection (ATCC)(<http://www.atcc.org>). Non-limiting examples of cells include, epithelial cells,
20 fibroblasts, keratinocytes, endothelial cells, monocytes, dendritic cells, B-cells and T-cells.

Cells used for the preparation of the vaccine compositions of the present disclosure may be autologous or allogeneic.

In certain embodiments, the present cells comprise human cancer cells that are genetically modified to secrete GM-CSF. In certain embodiments, the present cells are pancreatic cancer cells, colon cancer cells, lung cancer cells, breast cancer cells, ovarian cancer cells,
25 prostate cancer cells, melanoma cells, or combinations thereof. In one embodiment, the present vaccine composition comprises allogeneic pancreatic ductal adenocarcinoma (PDA) tumor cells engineered to secrete GM-CSF. For example, the vaccine comprises GM-CSF-secreting PDA vaccine (GVAX).

In certain embodiments, the cells are permissive to infection by ZIKV. In one embodiment, vaccine compositions and methods of the present disclosure can employ any cell type which is permissive for ZIKV infection, where the cells are maintained in a surviving mode immediately upon infection with Zika virus (i.e., cells are not killed by the virus immediately following the infection).

In certain embodiments, the ability of the cells to secrete GM-CSF facilitates the recruitment and activation of DCs and macrophages, which increases the effectiveness of antigen presentation to T-cells.

In certain embodiments, the present cells comprise skin immune cells, including dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells (J. Virol. 2015 Sep 1; 89(17): 8880–8896). In certain embodiments, the present cells comprise epithelial cells. In certain embodiments, the present cells comprise neuroprogenitor cells. Mlakar et al., N. Engl. J. Med. 374,951–958. (2016); Sarno et al. PloS Negl. Trop. Dis. 10 (2016); Tang et al., Cell Stem Cell 18(5):587-90 (2016).

In certain embodiments, the present cells are Vero cells, N2a cells, human fibroblast WS-1 cells, human fibroblast PCS201-012 cells, MRC-5 cells, WI-38 cells, BHK-21 cells, CHO cells, C6/C3, or combinations thereof.

At least one virus antigen (e.g., Zika virus antigen) may be introduced into, or loaded onto, the present cells. In certain embodiments, nucleic acids encoding at least one virus antigen (e.g., Zika virus antigen) may be introduced into the cells.

The cells may be antigen-presenting cells (APCs). Non-limiting examples of antigen-presenting cells including dendritic cells, macrophages, B cells, cells of myeloid lineage, Langerhans cells, epithelial cells, or any nucleated cells.

The APC may be autologous or allogeneic. The APC may be isolated from a subject. The APC may also be derived from cells isolated from a subject.

Dendritic Cells (DCs) can be generated *in vivo* or *ex vivo* from immature precursors (e.g., monocytes), CD34+ cells (i.e., cells expressing CD34), etc. U.S. Patent No. 8,728,806.

In certain embodiments, the cell may co-express human leucocyte antigen (HLA). The HLA expression by the cell may be the result of a natural process or due to recombinant

expression of HLA. The HLA expression may be as a result of HLA expression by the cell by endogenous processes. Alternatively, the HLA expression may occur as a result of recombinant engineering and protein production.

5 Cells may be contacted with nucleic acids encoding one or more virus antigens (e.g., Zika virus antigens). For example, cells can be transfected with expression vectors or infected with viral vectors for introducing nucleic acids encoding one or more virus antigens (e.g., Zika virus antigens) into the cells.

10 Other techniques for introducing nucleic acids into cells include, but are not limited to, electroporation, microinjection, hypotonic shock, scrape loading, cationic liposomes, and calcium phosphate coprecipitation.

 The time and amount of antigens, or nucleic acids encoding the antigens, necessary for the antigen presenting cells to process and present the antigens can be determined, for example, by assaying T cell cytotoxic activity in vitro or using antigen-presenting cells as targets of CTLs.

15 Other methods that can detect the presence of antigen on the surface of antigen-presenting cells are also contemplated by the presented invention. The antigen-presenting cells loaded with the antigen can be used to stimulate CTL proliferation *in vivo* or *ex vivo*. The ability of the loaded dendritic cells to stimulate a CTL response can be measured by assaying the ability of the effector cells to lyse target cells. For example, the non-radioactive LDH cytotoxicity assay or the europium release assay can be used. Volgmann et al., J. Immunol. Methods 119:45-51, 1989.

20 Expression can be optionally effected by targeting the expression construct to specific cells, such as with a viral vector or a receptor ligand, or by using a tissue-specific promoter, or combinations thereof.

 In certain embodiments, the present cells are loaded with one or more virus antigens (e.g., Zika virus antigens). As used herein, a cell "loaded" (or "pulsed") with a peptide shall mean that the cell has been incubated with the peptide under conditions permitting entry into, and/or attachment onto, the cell of the peptide. For example, APCs (e.g., dendritic cells) can be incubated with one or more virus antigens (e.g., Zika virus antigens) under conditions that are needed to load the MHC of the APC (e.g., the dendritic cell). Suitable conditions for antigen loading are provided that permit an APC to contact, process and/or present one or more antigens

on its MHC, whether intracellularly or on the cell surface. The incubation time may range from about 10 minutes to about 3 days or longer, from about 30 minutes to about 36 hours, from about 1 hour to about 28 hours, from about 2 hours to about 24 hours, from about 4 hours to about 24 hours, from about 4 hours to about 16 hours, from about 16 hours to about 24 hours, from about 20 hours to about 28 hours, from about 2 hours to about 4 hours, from about 1 hour to about 12 hours, from about 2 hours to about 8 hours, from about 3 hours to about 5 hours, for less than about a week, illustratively, for about 1 minute to about 48 hours, about 2 minutes to about 36 hours, about 3 minutes to about 24 hours, about 4 minutes to about 12 hours, about 6 minutes to about 8 hours, about 8 minutes to about 6 hours, about 10 minutes to about 5 hours, about 15 minutes to about 4 hours, about 20 minutes to about 3 hours, about 30 minutes to about 2 hours, about 40 minutes to about 1 hour, about 16 hours, about 20 hours, about 24 hours, about 28 hours, about 1 hour, about 2 hours, or about 4 hours. The incubation temperature may range from about 4°C to about 37°C, from about 25°C to about 37°C, about 4°C, about 25°C, or about 37°C.

The concentration of the peptide for loading may range from about 1 µg/ml to about 1 mg/ml, from about 5 µg/ml to about 800 µg/ml, from about 10 µg/ml to about 600 µg/ml, from about 15 µg/ml to about 400 µg/ml, from about 10 µg/ml to about 200 µg/ml, from about 10 µg/ml to about 100 µg/ml, from about 50 µg/ml to about 100 µg/ml, from about 20 µg/ml to about 100 µg/ml, about 10 µg/ml, about 20 µg/ml, about 30 µg/ml, about 50 µg/ml, about 60 µg/ml, about 80 µg/ml, or about 100 µg/ml.

One or more virus antigens (e.g., Zika virus antigens) may be introduced into APCs. Methods of introducing a virus antigen (e.g., Zika virus antigen) into an APC include, but are not limited to, non-covalent complex formation (Chariot), osmotic lysis of pinocytic vesicles (Influx pinocytic cell-loading reagent), electric power (electroporation), lipid-based delivery system (Bioporter), microinjection, small protein transduction domains (PTDs) from viral proteins, bacterial secretion system type III (T3SS), cell-penetrating peptide (CPP) conjugation, and protein transfection.

In certain embodiments of the adoptive immunotherapy methods described above, the cells of interest (i.e., mature DCs) can be purified prior to administration to the subject.

Purification of the cells can be done using a variety of methods known in the art, including

methods in which antibodies to specific cell surface molecules are employed. These methods include both positive and negative selection methods. For example, cells generated in vitro can be isolated by staining the cells with fluorescently labeled antibodies to cell surface markers followed by sorting of the cells that express both of these markers on their cell surface using
5 fluorescence activated cell sorting (FACS). These and other purification/isolation methods are well known to those of skill in the art.

The present cells may be inactivated or rendered proliferation incompetent by any suitable technique known in the art, such as gamma irradiation or UV irradiation. Proliferation
10 incompetent cells are cells that have been treated in such a way that renders them unable to undergo mitosis, but still maintain the capacity to express proteins, such as a cytokine (e.g., GM-CSF). In certain embodiments, for cells infected with ZIKV, cells can be treated to inactivate the virus and rendered proliferation incompetent.

In certain embodiments, the cells are rendered proliferation incompetent using gamma
15 irradiation. A person skilled in the art would recognize that the suitable irradiation dose to inactivate/growth arrest a cell or population of cells may vary upon the cell type and/or number of cells. In one embodiment, the gamma irradiation dose for rendering cells proliferation incompetent ranges from about 100 Gy (Gray) to about 300 Gy. In another embodiment, the gamma irradiation dose for rendering cells proliferation incompetent ranges from about 150 to
20 about 300 Gy.

In certain embodiments, cells are rendered proliferation incompetent using UV irradiation.

In certain embodiments, to prepare cells comprising inactivated flaviviruses (e.g., Zika virus), cells are first infected with flaviviruses (e.g., Zika viruses). Following infection,
25 flaviviruses (e.g., Zika viruses) can be inactivated in such cells to prevent propagation of the viruses upon administration to a subject.

In certain embodiments, the gamma irradiation levels for rendering the cells proliferation incompetent are approximately two orders of magnitude lower than those required for virus inactivation. Thus, irradiating cells at the levels required for the virus inactivation may
30 completely obliterate them, eliminating the capability of these cells to express and secrete GM-

CSF. To circumvent this limitation, cells expressing GM-CSF may be used as bystanders in combination with virus-loaded cells. This immunization scenario can be accomplished by initially dividing cells into two fractions: (i) cells infected with flaviviruses (e.g., Zika viruses); and (ii) cells not infected with flaviviruses (e.g., Zika viruses). Then only fraction (i) of the cells (but not fraction (ii)) will be treated to inactivate the viruses. Under these circumstances, fraction (ii) will not need to be exposed to high radiation levels, as it lacks viruses. Such approach ensures that cells that flaviviruses (e.g., Zika viruses) are irradiated at the levels required for rendering the cells proliferation incompetent, which allows them to maintain the ability to express GM-CSF. The two fractions of cells (cells that lack flaviviruses (e.g., Zika viruses) and are rendered proliferation incompetent, and cells that comprise inactivated flaviviruses (e.g., Zika viruses)) are then combined and administered to a subject.

Cytokines

The present composition may express or comprise at least one cytokine. Cytokines include the general class of hormones of the cells of the immune system, including lymphokines, monokines, and others. The present composition may express or comprise an immunomodulating cytokine, which is any cytokine that is involved in immune system regulation or has an effect upon modulating the immune response.

Cytokines suitable for use in the present vaccine compositions include, but are not limited to, GM-CSF, an interleukin, an interferon, and a tumor necrosis factor. Specific examples of such cytokines include interferon alpha (IFN- α), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-12 (IL-12), TNF-alpha, and granulocyte macrophage-colony stimulating factor (GM-CSF), or combinations thereof. Non-limiting examples of cytokines include, GM-CSF, IL-1 (IL-1alpha or IL-1beta), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, M-CSF, G-CSF, LIF, LT, TGF-beta, gamma-IFN (or alpha-IFN or beta-IFN), TNF-alpha, BCGF, CD2, or ICAM. A. S. Hamblin, 1993, D. Male, ed., Oxford University Press, New York, N.Y. Guidebook to Cytokines and Their Receptors, 1995, N. A. Nicola, ed. (Oxford University Press, New York, N.Y.).

In certain embodiments, the cytokine is substantially similar to the human form of the protein or is derived from the protein of the human sequence (i.e., of human origin). In certain

embodiments, cytokines of other mammals with substantial homology to the human forms of IL-2, GM-CSF, TNF-alpha, and others, may be used in the present composition or method when demonstrated to exhibit similar activity on the immune system. In certain embodiments, the present composition or method uses proteins that are substantially analogous to any particular cytokine, but have relatively minor changes of protein sequence.

In addition to the cytokines, adhesion or accessory molecules or combinations thereof (such as a NKG2D ligand, CD40L, CD80, CD86, and the like), may be employed alone or in combination with the cytokines. Dranoff (2002) *Immunol. Rev.* 188:147-154; Jain, et al. (2003) *Ann. Surg. Oncol.* 10:810-820; Borrello and Pardoll (2002) *Cytokine Growth Factor Rev.* 13:185-193; Chen, et al. (2005) *Cancer Immunol. Immunother.* 27:1-11; Kjaergaard, et al. (2005) *J. Neurosurg.* 103:156-164; Tai, et al. (2004) *J. Biomed. Sci.* 11:228-238; Schwaab, et al. (2004) *J. Urol.* 171:1036-1042; Friese, et al. (2003) *Cancer Res.* 63:8996-9006; Briones, et al. (2002) *Cancer Res.* 62:3195-3199; Vieweg and Dannull (2003) *Urol. Clin. North Am.* 30:633-643; Mincheff, et al. (2001) *Crit. Rev. Oncol. Hematol.* 39:125-132.

In certain embodiments, immunomodulatory cytokines such as GM-CSF can be used as adjuvants to whole-cell based vaccine to enhance the vaccine efficacy. The GM-CSF may be a full-length human GM-CSF, or may have an amino acid sequence 80%, 85%, 90%, or 95% identical to a full length human GM-CSF, which has one or more biological activities of full length GM-CSF. Examples of biological activities of GM-CSF include its capacity to stimulate macrophage differentiation and proliferation, or activation of antigen presenting dendritic cells. Additionally, the GM-CSF sequence may include one or more mutations which can be amino acid substitutions, deletions, or additions. Methods for the evaluation and the measurement of the biological activity of GM-CSF proteins are known in the art. U.S. Patent No. 7,371,370. In certain embodiments, cytokines, antigens, or hormones of other mammals with substantial homology to the human forms of the cytokines, antigens, and hormones, will be useful in the invention when demonstrated to exhibit similar activity on the immune system.

In addition to cytokines, vaccine compositions of the present disclosure can be engineered to secrete cytokines and factors that promote homing of DCs to germinal centers as well as licensing of dendritic cells. Such factors include lipopolysaccharide (LPS) (ligand for

Toll-like receptor 4), Poly(I:C) (recognized by Toll-like receptor 3), and CpG (ligand for Toll-like receptor -9).

Vectors

The virus antigen(s) may be introduced into the cell, or loaded onto the cell, using any vector. The cell may be genetically engineered using any vector to express a cytokine.

As used herein, the term "vector" refers to a polynucleotide capable of transporting another nucleic acid to which it has been linked. The present vectors can be, for example, a plasmid vector, a single- or double-stranded phage vector, or a single- or double-stranded RNA or DNA viral vector. Such vectors include, but are not limited to, chromosomal, episomal and virus-derived vectors, e.g., vectors derived from bacterial plasmids, bacteriophages, yeast episomes, yeast chromosomal elements, and viruses such as baculoviruses, papova viruses, SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, cosmids and phagemids.

Expression vectors can be used to replicate and/or express the nucleotide sequence encoding, e.g., a virus antigen, and/or a cytokine in a cell (e.g., a mammalian cell such as a human cell). A variety of expression vectors useful for introducing into cells the polynucleotides of the inventions are well known in the art.

Recombinant vectors are prepared using standard techniques known in the art, and contain suitable control elements operably linked to the nucleotide sequence encoding the target antigen. See, for example, Plotkin, et al. (eds.) (2003) *Vaccines*, 4 ed., W.B. Saunders, Co., Phila., Pa.; Sikora, et al. (eds.) (1996) *Tumor Immunology* Cambridge University Press, Cambridge, UK; Hackett and Ham (eds.) *Vaccine Adjuvants*, Humana Press, Totowa, N.J.; Isaacson (eds.) (1992) *Recombinant DNA Vaccines*, Marcel Dekker, NY, N.Y.; Morse, et al. (eds.) (2004) *Handbook of Cancer Vaccines*, Humana Press, Totowa, N.J.), Liao, et al. (2005) *Cancer Res.* 65:9089-9098; Dean (2005) *Expert Opin. Drug Deliv.* 2:227-236; Arlen, et al. (2003) *Expert Rev. Vaccines* 2:483-493; Dela Cruz, et al. (2003) *Vaccine* 21:1317-1326; Johansen, et al. (2000) *Eur. J. Pharm. Biopharm.* 50:413-417; Excler (1998) *Vaccine* 16:1439-1443; Disis, et al. (1996) *J. Immunol.* 156:3151-3158). Peptide vaccines are described (see, e.g.,

McCabe, et al. (1995) *Cancer Res.* 55:1741-1747; Minev, et al. (1994) *Cancer Res.* 54:4155-4161; Snyder, et al. (2004) *J. Virology* 78:7052-7060.

Non-limiting viral vectors include retroviral vectors, adenoviral vectors, adeno-associated viral vectors, lentiviral vectors, sindbis viral vectors, herpesviral vectors, SV-40 vectors, and pox
5 viral vectors, such as vaccinia viral vectors, baculoviral vectors, alphaviral vectors, Fowl pox viral vectors, AV-pox viral vectors, modified vaccinia Ankara (MVA) viral vectors, and other recombinant viruses.

In one embodiment, the vector is an adeno-associated virus (AAV, or adenovirus-associated virus) vector. See also, e.g., Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-
10 300; U.S. Pat. No. 5,436,146). Any of the AAV serotypes may be used, including, but not limited to, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, etc.

Adenoviruses are described in, e.g., Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234;
15 Kozarsky and Wilson, 1993, *Curr. Opin. Genetics Develop.* 3:499-503; Bout et al., 1994, *Human Gene Therapy* 5:3-10; PCT Publication No. WO 94/12649; and Wang et al., 1995, *Gene Therapy* 2:775-783). U.S. Patent No. 7,244,617.

Greater detail about retroviral vectors is available in Boesen et al., 1994, *Biotherapy* 6:291-302. In one embodiment, the present expression vector is a lentivirus (including human
20 immunodeficiency virus (HIV)), which is a sub-type of retrovirus.

Plasmids that may be used as the present expression vector include, but are not limited to, pcDNA3.1, pcDNA3.1-hygro, pGL3, pCDM8 (Seed, 1987, "An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2", *Nature*, 840-842) and pMT2PC (Kaufman et al., 1987, "Translational efficiency of polycistronic mRNAs and their
25 utilization to express heterologous genes in mammalian cells", *EMBO J.* 6:187-193). Any suitable plasmid may be used in the present invention.

Bacterial vectors include, for example, *Salmonella*, *Shigella*, *Yersinia*, *Lactobacillus*, *Streptococcus*, *Bacille Calmette-Guerin*, *Bacillus anthracis*, and *Escherichia coli*. The bacterium
30 can be engineered to contain a nucleic acid encoding a recombinant antigen, a heterologous antigen, or an antigen derived from a tumor, cancer cell, or infective agent. Moreover, the

bacterium can be modified to be attenuated. In another aspect, the non-listerial bacterial vaccine can be absent of any nucleic acid encoding a recombinant antigen (see, e.g., Xu, et al. (2003) Vaccine 21:644-648; Pasetti, et al. (2003) J. Virol. 77:5209-5219; Loessner and Weiss (2004) Expert Opin. Biol. Ther. 4:157-168; Grangette, et al. (2002) Vaccine 20:3304-3309; Byrd, et al. (2002) Vaccine 20:2197-2205; Edelman, et al. (1999) Vaccine 17:904-914; Domenech, et al. (2005) Microbes and Infection 7:860-866).

Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

In certain embodiments, a vector comprising a nucleic acid sequence encoding a cytokine (and/or a nucleic acid sequence encoding a virus antigen) may be transferred to a cell *in vitro*, using any of a number of methods known in the art, which include electroporation, membrane fusion with liposomes, Lipofectamine treatment, incubation with calcium phosphate-DNA precipitate, DEAE-dextran mediated transfection, infection with modified viral nucleic acids, direct microinjection into single cells, etc. Procedures for the cloning and expression of modified forms of a native protein using recombinant DNA technology are generally known in the art, as described in Ausubel, et al., 1992 and Sambrook, et al., 1989.

Immunotherapy

In one embodiment, vaccine compositions and methods of the present disclosure can be used to prevent and/or treat symptoms and adverse outcomes associated with flavivirus infection (e.g., ZIKV infection). In another embodiment, vaccine compositions and methods of the present disclosure can be used for the prevention of microcephaly and other brain anomalies associated with Zika infection in newborns. In further embodiment, vaccine compositions and methods of the present disclosure can be used to prevent negative outcomes associated with Zika infection in adults, such as increased risk of Guillain-Barré syndrome.

The present composition may be administered to a subject to prevent or treat viral infection, and/or the symptoms associated therewith. In certain embodiments, the present composition may be administered to a subject prior to infection by the virus (e.g., Zika virus). In

certain embodiments, the present composition may be administered to a subject after the subject is infected by the virus (e.g., Zika virus). In certain embodiments, the present composition may be administered to a subject after the subject is infected by the virus (e.g., Zika virus), and during, or after, the incubation period for Zika virus. Bearcroft WG, Trans R Soc Trop Med Hyg 1956 Sep;50(5):442-8).

Common symptoms attributable to ZIKV include macular or papular rash, fever, conjunctivitis, rash, arthralgia, arthritis or arthralgia, nonpurulent conjunctivitis, myalgia, headache, retro-orbital pain, edema, vomiting, or a combination of these symptoms. The rash is generally maculopapular and pruritic, and fever, when present, is generally short-term and low-grade (Brasil et al. N Engl J Med, 2016 Mar 4). Other symptoms that have been noted in association with acute infection include hematospermia, transient dull and metallic hearing, swelling of the hands and ankles, and subcutaneous bleeding (Paterson et al. N Engl J Med 2016; 374:1552-1563).

In another embodiment, vaccine compositions and methods of the present disclosure can be used for the prevention and/or treatment of adverse fetal outcomes associated with ZIKV infection. In one embodiment, a vaccine composition of the present disclosure is administered to a woman prior to pregnancy. In another embodiment, a vaccine composition of the present disclosure is administered to a woman during pregnancy.

Vaccine compositions of the present disclosure can be used to increase a systemic immune response in a subject beyond that which would occur in the absence of administration of the present composition. The systemic response in a subject induced upon administration of such vaccine composition may be sufficient to either partially or completely reject flavivirus infection (e.g., ZIKV infection).

In certain embodiments, cells expressing GM-CSF and/or cells comprising inactivated flaviviruses (e.g., Zika virus) can be rendered proliferation incompetent and formulated into single vaccine composition. Final vaccine composition can be formulated to contain varying ratios of cells expressing GM-CSF and cells comprising inactivated flaviviruses (e.g., Zika virus).

In certain embodiments, cells expressing GM-CSF and cells comprising inactivated flaviviruses (e.g., Zika virus) are rendered proliferation incompetent and formulated

into two separate vaccine compositions. Under such circumstances, where there are two or more separate vaccine compositions, vaccine compositions can be administered simultaneously, separately or sequentially.

5 In certain embodiments, the present composition comprises GVAX comprising at least one virus antigen (e.g., Zika virus antigen). GVAX may refer to an inactivated tumor cell containing a nucleic acid that encodes granulocyte macrophage-colony stimulation factor (GM-CSF), where the tumor cell line was CT-26 cells, a cell line that expresses the gp70. AH1 is an epitope of gp70, an immunodominant antigen of CT26 cells. Yoshimura, et al. (2006) Cancer Res. 66:1096-1104; Jain, et al. (2003) Annals Surgical Oncol. 10:810-820; Zhou, et al. (2005) 10 Cancer Res. 65:1079-1088; Chang, et al. (2000) Int. J. Cancer 86:725-730; Borrello and Pardoll (2002) Cytokine Growth Factor Rev. 13:185-193; Thomas, et al. (1998) Human Gene Ther. 9:835-843). U.S. Patent No. 9,198,960.

15 According to the methods of the present disclosure, vaccine compositions described herein may be used for the prevention and/or treatment of flavivirus infection (e.g., Zika virus infection) in a subject, either alone or in combination with other methods suitable for the prevention and/or treatment of flavivirus infection (e.g., Zika virus infection).

Methods are provided for administration of the present immunogenic composition to a subject in need of immunostimulation.

20 Dosages may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from in vitro studies can provide useful guidance on the proper doses for patient administration. Studies in animal models can also be used for guidance regarding effective dosages for treatment of flavivirus infection (e.g., Zika virus infection) in accordance with the present disclosure.

25 In certain embodiments, one or more than one administration of the present compositions can be delivered to the subject in a course of treatment. Dependent upon the particular course of treatment, multiple administrations may be given at a single time point with the treatment repeated at various time intervals. For example, an initial or priming (or prime) treatment may be followed by one or more booster (or boost) treatments. In certain embodiments, the priming (or 30 prime) and booster (or boost) treatments are delivered by the same route of administration and/or

at about the same site. When multiple doses are administered, the first immunization dose may be higher than subsequent immunization doses.

In certain embodiments, a single administration (dose) of the present composition may comprise about 10^5 to about 10^9 cells, e.g., 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 5×10^7 , 1×10^8 , 1×10^9 cells. The number of the cells may be adjusted according, for example, to the level of cytokine and/or antibodies produced by a given vaccine composition.

In some embodiments, vaccine composition of the present disclosure is administered in a dose that is capable of producing at least 1, 5, 10, 25, 75, 100, 200, 300, 400, 500 ng of GM-CSF per 24 hours per one million cells. In some embodiments, vaccine composition comprising cells comprising Zika inactivated virus is administered in a dose that is capable of producing at least 500 ng of a particular flavivirus antigen (e.g., ZIKV antigen) per 24 hours per one million cells. Determination of optimal cell dosage and ratios is a matter of routine determination and within the skill of a practitioner of ordinary skill.

In one embodiment, vaccine compositions of the present disclosure are administered in a dose that ranges between 100 to 500 million cells per immunization.

In one embodiment, the vaccine is administered intradermally. In one embodiment, the pharmaceutical composition of administered vaccine is no more than 200 μ L in volume. In another embodiment, vaccine compositions of present disclosure may be administered at more than one site, particularly in instances where higher doses of vaccine are needed.

The duration of the therapy depends on the kind of disease being treated, the age and condition of the patient, the stage and type of the patient's disease, and how the patient's body responds to the treatment. Drug administration may be performed at different intervals (e.g., daily, weekly, or monthly). Therapy may be given in on-and-off cycles that include rest periods so that the patient's body has a chance to build healthy new cells and regain its strength.

The present cells may be administered with a pharmaceutically acceptable diluent, carrier, and/or excipient, in unit dosage form.

When treating the viral infection, administration may begin before the subject is symptomatic, or when the subject is symptomatic.

Any appropriate route of administration may be employed, for example, administration may be topical, parenteral, intravenous, intraarterial, subcutaneous, intratumoral, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intrahepatic, intracapsular, intrathecal, intracisternal, intraperitoneal, intranasal, aerosol, suppository, or oral administration. For
5 example, therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

The administration of the present cells or composition (vaccination) may be given once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten
10 times, eleven times, twelve times, thirteen times, fourteen times, fifteen times, or more, within a treatment regime to a subject/patient. The administration of the present cells or composition (vaccination) may be given every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, every 8 days, every 9 days, every 10 days, every 11 days, every 12 days, every 13 days, every 14 days, every 16 days, every 18 days, every 20 days, every 1 month, every 2
15 months, every 3 months, every 6 months, or at different frequencies.

The present cells may be administered to a subject (e.g., a human subject) at a dose ranging from about 1×10^3 cells to about 1×10^{12} cells, from about 1×10^4 cells to about 1×10^{10} cells, from about 1×10^5 cells to about 1×10^9 cells, from about 1×10^6 cells to about 1×10^8 cells, from about 1×10^6 cells to about 1×10^7 cells, from about 1×10^7 cells to about 1×10^8 cells, about 1×10^5 cells, about 1×10^6 cells, about 1×10^7 cells, about 1×10^8 cells, or
20 about 1×10^9 cells.

In certain embodiments, the present cells are administered at a concentration of about 10^2 cells/ml to about 10^{12} cells/ml, about 10^3 cells/ml, about 10^4 cells/ml, about 10^5 cells/ml, about 10^6 cells/ml, about 10^7 cells/ml, about 10^8 cells/ml, about 10^9 cells/ml, about 10^{10} cells/ml, or
25 about 10^{11} cells/ml. In certain embodiments, the total volume of the present composition administered is about 0.001 mL-about 10 mL, e.g., 0.01 mL, 0.1 mL, or 1 mL. In one embodiment, the present composition is administered at a concentration of about 2×10^7 cells/ml in a total volume of about 0.1 mL. In one embodiment, the present composition is administered subcutaneously.

In certain embodiments, the vaccine is administered twice or more, e.g., 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 15 times, 20 times, 25 times, 30 times, 35 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more. For example, the vaccine is administered at least once per week, e.g., at least twice per week, at least three times
5 per week, at least four times per week, at least five times per week, at least six times per week, at least seven times per week. In certain embodiments, the vaccine is administered at least once per day, e.g., at least twice per day, at least every eight hours, at least every four hours, at least every two hours, or at least every hour. In certain embodiments, the present compositions are administered for a duration of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3
10 weeks, 4 weeks, five weeks, six weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 2 years, 3 years, 4 years, 5 years or more. In certain embodiments, the present composition is administered one dose every two weeks for 4 to 6 weeks or until the viral infection is treated.

The present invention provides a method for eliciting in a subject an immune response to
15 at least one virus antigen. The method comprises administering to the subject cells comprising at least one virus antigen (or loaded with at least one virus antigen), or cells comprising nucleic acids encoding at least one virus antigen. The cells may also express at least one cytokine. The present cells or composition, when administered to the subject, may elicit an immune response to the virus (e.g., Zika virus), and/or may elicit an immune response to at least one virus antigen.

20 In certain embodiments, the present invention provides a method of preventing and/or treating viral infection, the method comprising administering to a subject a therapeutically or prophylactically effective amount of a pharmaceutical composition (e.g., an immunogenic composition, or a vaccine composition), wherein the composition comprises: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least
25 one cytokine.

In certain embodiments, the present invention provides a method of preventing and/or treating viral infection, the method comprising administering to a subject a therapeutically or prophylactically effective amount of a pharmaceutical composition (e.g., an immunogenic composition, or a vaccine composition), wherein the composition comprises: a first population of
30 cells comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and a

second population of cells expressing at least one cytokine, such as GM-CSF.

In certain embodiments, the present cells (e.g., antigen-presenting cells) comprising one or more virus antigens (or a fragment thereof) may be used to contact lymphocytes under conditions sufficient to produce virus antigen-specific lymphocyte capable of eliciting an

5 immune response against the virus. Thus, the antigen-presenting cells also can be used to provide lymphocytes, including T lymphocytes and B lymphocytes, for eliciting an immune response against a cell that comprises a virus antigen. In one embodiment, a preparation of T lymphocytes is contacted with the antigen-presenting cells described above for a period of time, for priming the T lymphocytes to the at least one virus antigen presented by the antigen-presenting cells.
10 Accordingly, T lymphocytes and B lymphocytes that are primed to respond to cells that comprise a virus antigen can be prepared.

T lymphocytes can be obtained from any suitable source such as peripheral blood, spleen, and lymph nodes. The T lymphocytes can be used as crude preparations or as partially purified or substantially purified preparations, which can be obtained by standard techniques including,
15 but not limited to, methods involving immunomagnetic or flow cytometry techniques using antibodies.

In another embodiment, T cells can be removed from a subject and treated in vitro with the present cells (e.g., antigen-presenting cells), wherein the resulting CTL are reinfused autologously or allogeneically to the subject.

20 Accordingly, the antigen-primed antigen-presenting cells of the present disclosure and the antigen-specific T lymphocytes generated with these antigen-presenting cells can be used as immunomodulating compositions for prophylactic or therapeutic applications for viral infection. In some embodiments, the virus antigen-primed antigen-presenting cells of the present disclosure can be used for generating CD8⁺ CTL, CD4⁺ CTL, and/or B lymphocytes for adoptive transfer
25 to the subject. Thus, for example, virus antigen-specific CTLs can be adoptively transferred for therapeutic purposes in subjects afflicted with viral infection.

The present compositions or methods may function to provide or enhance an immune response. Generally, the immune response can include humoral immune response, cell-mediated immune response, or both. For example, antigen presentation through an immunological pathway
30 involving MHC class II molecules or direct B-cell stimulation can produce a humoral response;

and, antigens presented through a pathway involving MHC I molecules can elicit cell-mediated immune response. A humoral response can be determined by a standard immunoassay for antibody levels in a serum sample from the subject receiving the pharmaceutical composition. A cellular immune response is a response that involves T cells and can be determined in vitro or in vivo. For example, a general cellular immune response can be determined as the T cell proliferative activity in cells (e.g., peripheral blood leukocytes (PBLs)) sampled from the subject at a suitable time following the administering of a pharmaceutical composition. Following incubation of e.g., PBMCs with a stimulator for an appropriate period, [³H]thymidine incorporation can be determined. The subset of T cells that is proliferating can be determined using flow cytometry. T cell cytotoxicity can also be determined.

The immune response that is elicited or enhanced may be sufficient for prophylactic or therapeutic treatment of viral infection, or a symptom associated therewith. Accordingly, a beneficial effect of the present compositions and/or methods will generally at least in part be immune-mediated, although an immune response need not be positively demonstrated in order for the compositions and methods described herein to fall within the scope of the present disclosure.

While one round of vaccination may be sufficient to generate sustained and protective immune response, periodic booster (or boost) treatments may be administered. In one embodiment, booster (or boost) treatments are administered 6-12 months following the initial vaccine administration, or following previous booster (or boost) administration.

An effective amount of a prime or boost vaccine may be given in one dose, but is not restricted to one dose. Thus, the administration can be two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more, administrations of the vaccine. Where there is more than one administration of a vaccine the administrations can be spaced by time intervals of one minute, two minutes, three, four, five, six, seven, eight, nine, ten, or more minutes, by intervals of about one hour, two hours, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, and so on. The administrations can also be spaced by time intervals of one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, ten days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, and

combinations thereof. The invention is not limited to dosing intervals that are spaced equally in time, but encompass doses at non-equal intervals, such as a priming schedule consisting of administration at 1 day, 4 days, 7 days, and 25 days, just to provide a non-limiting example.

The following may be taken into consideration in determining the relative timing of the prime vaccine and boost vaccine. It has been found that administration of an antigen, or nucleic acid encoding an antigen, can stimulate expansion of antigen-specific immune cells, resulting in a peak, followed by contraction of the number of antigen specific immune cells (see, e.g., Badovinac, et al. (2002) *Nature Immunol.* 3:619-626). Initiation of the boost vaccination can be administered before the peak is reached, coincident with the peak, or after the peak.

Administration of the boost vaccination can be initiated when a population of antigen-specific immune cells has expanded (increased in number) to at least 20% the maximal number of antigen-specific immune cells that is eventually attained; to at least 30%; to at least 40%; to at least 50%; to at least 60%; to at least 70%; to at least 80%; to at least 90%; to at least 95%; to at least 99% the maximal number of antigen-specific immune cells that is eventually attained.

Additional schedules of prime-boost vaccines are available, for example, the boost vaccination can be initiated when the population of antigen-specific cells has contracted to under 90% the maximal number of antigen-specific cells; under 80%; under 70%; under 60%; under 50%; under 40%; under 30%; under 20%; under 10%; under 5%; under 1.0%; under 0.5%; under 0.1%; under 0.05%; or under 0.01% the maximal number of antigen-specific immune cells. The antigen-specific cells can be identified as specific for a vector-specific antigen (specific for empty vector), or specific for a heterologous antigen expressed by a nucleic acid contained in the vector.

In other aspects, administration of the boost vaccination can be initiated at about 5 days after the prime vaccination is initiated; about 10 days after the prime vaccination is initiated; about 15 days; about 20 days; about 25 days; about 30 days; about 35 days; about 40 days; about 45 days; about 50 days; about 55 days; about 60 days; about 65 days; about 70 days; about 75 days; about 80 days, about 6 months, and about 1 year after administration of the prime vaccination is initiated.

The boost vaccination can be administered 5-10 days after the prime vaccination; 10-15 days after the prime vaccination; 15-20 days after the prime vaccination; 20-25 days after the

prime vaccination; 25-30 days after the prime vaccination; 30-40 days after the prime vaccination; 40-50 days after the prime vaccination; 50-60 days after the prime vaccination; 60-70 days after the prime vaccination; and so on.

5 In some embodiments of the invention the boost dose will enhance the prime dose immune response by at least two-fold, at times between about three- and five-fold or five-fold to ten-fold, or from ten-fold to 100-fold or greater. In some embodiments of the invention the prime dose and boost dose will have a synergistic effect on the immune response. In some
10 embodiments of the invention the enhanced immune response will include a T-cell response, and in some embodiments the T-cell response will be a CD8⁺ T-cell response. In some embodiments of the invention the prime dose and boost dose will break the mammal's tolerogenic state towards the target antigen.

Treating a subject using the present compositions and methods may refer to reducing the symptoms of the disease, reducing the occurrence of the disease, reducing the severity of the disease, and/or preventing a disease from occurring. As such, to treat a subject means both
15 preventing disease occurrence (prophylactic treatment) and treating a subject that has a disease (therapeutic treatment). In particular, treating a subject is accomplished by providing or enhancing an immune response in the subject.

The present cell may comprise one or more virus antigens, including 2, 3, 4, 5, 6, 7, 8, 9, 10 or more antigens. In certain embodiments, the present cell comprises one or more inactivated (or attenuated) viruses (e.g., one or more inactivated (or attenuated) Zika viruses). Additionally,
20 multiple, independently generated cells can be administered to a subject.

The present cells may be autologous, allogenic (e.g., from a different donor subject that is MHC matched or mismatched with the recipient subject) or heterologous to the recipient subject. The cells can be introduced into a subject by any mode that elicits the desired immune response
25 to the virus (e.g., Zika virus).

Vaccine compositions of the present disclosure can be administered, for example, intramuscularly, intradermally, subdermally, subcutaneously, orally, intraperitoneally, intrathecally, or intravenously. Modes of administration also include, systemic rectal, topical, intraocular, buccal, intravaginal, intracisternal, intracerebroventricular, intratracheal, nasal,
30 transdermal, within/on implants, or parenteral routes. The term "parenteral" includes

subcutaneous, intrathecal, intravenous, intramuscular, intraperitoneal, or infusion. Compositions comprising a composition of the invention can be added to a physiological fluid, such as blood. Inhaled therapy is also provided.

5 The quantity of the vaccine compositions described in the present disclosure for effective therapy will depend upon a variety of factors, including the type of strain of ZIKV infection, means of administration, physiological state of the patient, other medications administered, and other factors. The vaccination or administration parameters, the dosing schedule, etc., can be determined by routine experimentation. A mammalian subject can initially be given a low dose of the vaccine composition. The dose and/or the relative amounts of the vaccine composition can
10 be varied while monitoring the immune response.

Suitable pharmaceutically acceptable carriers for the vaccine compositions of the present disclosure refers to fluid vehicles that can be injected into a host without significant adverse effects. Suitable pharmaceutically acceptable carriers known in the art include, sterile water,
15 saline, glucose solution, and physiologically acceptable aqueous buffers or solutions, including phosphate-buffered saline. Carriers may include auxiliary agents including, diluents, stabilizers (i.e., sugars and amino acids), preservatives, wetting agents, emulsifying agents, pH buffering agents, viscosity enhancing additives, colors and the like. Appropriate amounts of the cells are mixed with the selected carrier to form the final vaccine composition. In one embodiment, all the
20 components of the vaccine can be provided together in one carrier, but if desired, one or more components can be provided in a separate carrier and administered in combination with the other components.

The surrogate point of vaccination can include the specific anti-ZIKV antibody levels (titers), T-cell activation (by the IFN-gamma levels) and viremia levels upon virus challenge.
25 The inventors will perform these experiments in rhesus monkeys since they are susceptible to ZIKV infection and display almost all manifestations characteristic of human infection. Additionally, a set of experiments using non-human primates will be performed in order to establish whether the vaccination approach of the present disclosure protects fetuses from infection and the development of neurologic conditions.

30

Pharmaceutical Compositions

The present disclosure provides a pharmaceutical composition (e.g., an immunogenic composition or a vaccine composition) comprising: cells comprising at least one virus antigen or a fragment thereof (e.g., at least one Zika virus antigen or a fragment thereof), wherein the cells
5 also express a cytokine, such as GM-CSF.

The present disclosure also provides a pharmaceutical composition (e.g., an immunogenic composition or a vaccine composition) comprising: cells (i) comprising at least one flavivirus antigen (e.g., at least one Zika virus antigen), and (ii) expressing at least one cytokine.

10 In some embodiments, the composition further comprises an adjuvant as described above. When administered to a subject, the pharmaceutical composition elicits or enhance an immune response to a virus (e.g., Zika virus).

In one embodiment, the present pharmaceutical composition comprises antigen-presenting cells contacted *in vitro* or *ex vivo* with at least one virus antigen. In another
15 embodiment, the present invention provides a composition comprising antigen-presenting cells contacted *in vitro* with nucleic acids encoding at least one virus antigen.

The present pharmaceutical composition can be useful as vaccine compositions for prophylactic or therapeutic treatment of a viral infection, such as for preventing or treating Zika virus infection in the subject.

20 In certain embodiments, the present composition is administered to a subject, either alone, or in combination with one or more other modalities of therapy.

The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, diluent, or excipient. Techniques for formulating and administering also can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., latest edition.

25 Pharmaceutically acceptable carriers known in the art include, but are not limited to, sterile water, saline, glucose, dextrose, or buffered solutions. Agents such as diluents, stabilizers (e.g., sugars and amino acids), preservatives, wetting agents, emulsifying agents, pH buffering agents, additives that enhance viscosity, and the like. Preferably, the medium or carrier will produce minimal or no adverse effects.

30 The pharmaceutical composition may further comprise an adjuvant. Preferably, the

adjuvant employed provides for increased immunogenicity. The adjuvant can be one that provides for slow release of antigen (e.g., a liposome), or it can be an adjuvant that is immunogenic. For example, the adjuvant can be a known adjuvant or other substance that promotes nucleic acid uptake, recruits immune system cells to the site of administration, or facilitates the immune activation of responding lymphoid cells. Adjuvants include, but are not limited to, oil and water emulsions, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, synthetic polymers such as poly amino acids and co-polymers of amino acids, saponin, paraffin oil, and muramyl dipeptide. U.S. Patent No. 9,011,835.

In some embodiments, the adjuvant is comprised of incomplete Freund's adjuvant (Montanide ISA 51) or *Corynebacterium granulosum* P40.

The vaccine compositions of the invention may further comprise various excipients, adjuvants, carriers, auxiliary substances, modulating agents, and the like. A carrier, which is optionally present, is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycollic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee J P, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan D T, et al., *Vaccine* 11(2):149-54, 1993. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants").

Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as toxins derived from *E. coli*. Such adjuvants include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (International Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles

using a microfluidizer such as Model 110 Y microfluidizer (Microfluidics, Newton, Mass.), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi.TM. adjuvant system (RAS), (Ribi Immunochem, Hamilton, Mont.) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (Detoxu); (3) saponin adjuvants, such as Stimulon.TM. (Cambridge Bioscience, Worcester, Mass.) may be used or particle generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), beta chemokines (MIP, 1-alpha, 1-beta Rantes, etc.); (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an E. coli heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (see, e.g., International Publication Nos. WO93/13202 and WO92/19265); and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition.

The dosage and regimen will be determined, at least in part, be determined by the potency of the modality, the vaccine delivery employed, the need of the subject.

The pharmaceutical composition can be administered in a therapeutically or a prophylactically effective amount. Administering the pharmaceutically acceptable composition of the present invention to the subject can be carried out using known procedures, and at dosages and for periods of time sufficient to achieve a desired effect. For example, a therapeutically or prophylactically effective amount of the pharmaceutical composition can vary according to factors such as the age, sex, and weight of the subject. Dosage regime can be adjusted by one of ordinary skill in the art to elicit the desired immune response including immune responses that provide therapeutic or prophylactic effects.

The pharmaceutically acceptable composition can be administered to the subject at any suitable site. The route of administering can be parenteral, intramuscular, subcutaneous, intradermal, intraperitoneal, intranasal, intravenous (including via an indwelling catheter), via an afferent lymph vessel, or by any other route and the subject's condition. Preferably, the dose will
5 be administered in an amount and for a period of time effective in bringing about a desired response, be it eliciting the immune response or the prophylactic or therapeutic treatment of the viral infection and/or symptoms associated therewith.

Administering can be properly timed, and can depend on the clinical condition of the subject, the objectives of administering, and/or other therapies also being contemplated or
10 administered. In some embodiments, an initial dose can be administered, and the subject monitored for an immunological and/or clinical response. Suitable means of immunological monitoring include using patient's peripheral blood lymphocyte (PBL) as responders. An immunological reaction also can be determined by a delayed inflammatory response at the site of administering.

15 One or more doses subsequent to the initial dose can be given as appropriate, typically on a monthly, semimonthly, or a weekly basis, until the desired effect is achieved. Thereafter, additional booster or maintenance doses can be given as required, particularly when the immunological or clinical benefit appears to subside.

Single or multiple administrations of the present composition can be carried out with cell
20 numbers and treatment being selected by a care provider (e.g., a physician). In certain embodiments, the present cells are administered in a pharmaceutically acceptable carrier. Suitable carriers can be the growth medium in which the cells were grown, or any suitable buffering medium such as phosphate buffered saline. The cells can be administered alone or as an adjunct therapy in conjunction with other therapeutics.

25 In one embodiment, the present composition is administered systemically, e.g., by injection. Alternately, one can administer locally rather than systemically, for example, via injection directly into tissue. The pharmaceutical composition may be in a depot or sustained release formulation. Furthermore, one can administer in a targeted drug delivery system, for example, in a liposome that is coated with tissue-specific antibody. The liposomes can be
30 targeted to and taken up selectively by the tissue.

Pharmaceutical compositions may be administered directly, endoscopically, intratracheally, intratumorally, intravenously, intralesionally, intramuscularly, intraperitoneally, regionally, percutaneously, topically, intrarterially, intravesically, or subcutaneously.

Compositions may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more times, and they may be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, or 1, 2, 3, 4, 5, 6, 7 days, or 1, 2, 3, 4, 5 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months.

These compositions can be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. In one embodiment, a therapeutic composition containing an agent of this invention is administered in a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The present pharmaceutical composition can be given subsequent to, preceding, or contemporaneously with, other therapies including therapies that also elicit an immune response in the subject, and/or other antiviral therapies. For example, the subject may previously or concurrently be treated by anti-viral agents, surgery, other forms of immunotherapy, anti-angiogenic agents, and hormonal agents. Such other therapies preferably are provided in such a way so as not to interfere with the immunogenicity of the compositions of the present invention.

Methods of Measuring Immune Response

A variety of in vitro and in vivo assays are known in the art for measuring an immune response, including measuring humoral and cellular immune responses, which include but are not limited to, standard immunoassays, such as RIA, ELISA assays; intracellular staining; T cell assays including for example, lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., J. Immunol. (1993)

151:4189-4199; Doe et al., Eur. J. Immunol. (1994) 24:2369-2376. Recent methods of measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-cells (e.g., by the tetramer technique) (reviewed by McMichael, A. J., and O'Callaghan, C. A., J. Exp. Med. 187(9):1367-1371, 1998; Mcheyzer-Williams, M. G., et al., Immunol. Rev. 150:5-21, 1996; Lalvani, A., et al., J. Exp. Med. 186:859-865, 1997). Assays for measuring an immune response also include the cellular enzyme-linked immunosorbent assay (cELISA) assay, plaque reduction neutralization test (PRNT), etc.

The release of cytokines (e.g., IFN- γ , TNF- α , and/or IL-17) may be assayed by, e.g., ELISpot assay, to determine immune responses.

The cytokine ELISPOT (Enzyme-Linked ImmunoSPOT) assay is designed to enumerate cytokine-secreting cells. The assay has the advantage of detecting only activated/memory T cells and has the ability to detect cytokine release in response to antigen by a single cell thereby permitting direct calculation of responder T cell frequencies. The high sensitivity and easy performance, allowing the determination of peptide-reactive T cells without prior *in vitro* expansion, makes the ELISPOT assay well suited to monitor T cell responses. Tanguay et al., 1994. Lymphokine Cytokine Res. 13: 259. Carter et al., 1997. Curr. Opin. Immunol. 9: 177. In illustrative embodiments disclosed herein, the enzyme-linked immunospot (ELISPOT) assay (BD Biosciences) is used to detect and analyze individual cells that secrete interferon-gamma (IFN-gamma). The ELISPOT assay is capable of detecting cytokine producing cells from both activated naive and memory T-cell populations and derives its specificity and sensitivity by employing high affinity capture and detection antibodies and enzyme-amplification. Additional information regarding the use of ELISPOT assay is provided in J. Immunol. Methods. 2001, 254(1-2):59. Briefly, cells are incubated in the wells of the ELISPOT plate pre-coated with a high-affinity monoclonal antibody to which the cytokine, produced during incubation, will bind. Subsequently, cells are washed away. Areas in which the cytokines have been bound are detected with a combination of biotinylated anti-cytokine detection antibodies and ϕ -labeled goat anti-biotin antibodies. The last step in the assay is the addition of a reagent allowing the precipitation of silver on ϕ revealing the site of cytokine secretion (i.e., spot formation).

Animal models, e.g. non-human primates, are known in the art. For example, the mouse is an accepted model for human immune response. Mouse NK cell response to tumors is an accepted model for human NK cell response to tumors. Additionally, mouse T cells are a model for human T cells, mouse dendritic cells (DCs) are a model for human DCs, mouse NKT cells are a model for human NKT cells, mouse innate response is an accepted model for human innate response, and so on. Model studies are disclosed, for example, for CD8⁺ T cells, central memory T cells, and effector memory T cells (see, e.g., Walzer, et al. (2002) *J. Immunol.* 168:2704-2711); the two subsets of NK cells (see, e.g., Chakir, et al. (2000) *J. Immunol.* 165:4985-4993; Smith, et al. (2000) *J. Exp. Med.* 191:1341-1354; Ehrlich, et al. (2005) *J. Immunol.* 174:1922-1931; Peritt, et al. (1998) *J. Immunol.* 161:5821-5824); NKT cells (see, e.g., Couedel, et al. (1998) *Eur. J. Immunol.* 28:4391-4397; Sakamoto, et al. (1999) *J. Allergy Clin. Immunol.* 103:S445-S451; Saikh, et al. (2003) *J. Infect. Dis.* 188:1562-1570; Emoto, et al. (1997) *Infection Immunity* 65:5003-5009; Taniguchi, et al. (2003) *Annu. Rev. Immunol.* 21:483-513; Sidobre, et al. (2004) *Proc. Natl. Acad. Sci.* 101:12254-12259); monocytes/macrophages (Sunderkotter, et al. (2004) *J. Immunol.* 172:4410-4417); the two lineages of DCs (Boonstra, et al. (2003) *J. Exp. Med.* 197:101-109; Donnenberg, et al. (2001) *Transplantation* 72:1946-1951; Becker (2003) *Virus Genes* 26:119-130; Carine, et al. (2003) *J. Immunol.* 171:6466-6477; Penna, et al. (2002) *J. Immunol.* 69:6673-6676; Alferink, et al. (2003) *J. Exp. Med.* 197:585-599). Mouse innate response, including the Toll-Like Receptors (TLRs), is a model for human innate immune response, as disclosed (see, e.g., Janssens and Beyaert (2003) *Clinical Microb. Revs.* 16:637-646). Mouse neutrophils are an accepted model for human neutrophils (see, e.g., Kobayashi, et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:10948-10953; Torres, et al. (2004) 72:2131-2139; Sibieli, et al. (1999) *Infection Immunity* 67:1125-1130; Tvinnereim, et al. (2004) *J. Immunol.* 173:1994-2002). Murine immune response to *Listeria* is an accepted model for human response to *Listeria* (see, e.g., Kolb-Maurer, et al. (2000) *Infection Immunity* 68:3680-3688; Brzoza, et al. (2004) *J. Immunol.* 173:2641-2651).

The immunological efficacy of the present methods and compositions may be determined based on the Distribution Free Resampling (DFR) method.

Immune responses to vaccines were assessed by harvesting splenocytes, a source that provides cells of the immune system, including T cells and dendritic cells (DCs). Antigen-

specific immune responses were measured by incubating splenocytes with one or more peptides and measuring immune cell activity, where activity was determined by intracellular staining (ICS) and Enzyme-Linked ImmunoSpot (ELISPOT) assays. In some assays, only a single peptide was added, where the peptide contained only one epitope of a viral antigen. In other
5 assays, an entire library of peptides was added, encompassing the entire length of the antigen.

ICS assays involve permeabilizing the splenocytes, and treating with an antibody that binds cytokines that have accumulated inside the immune cell, where the antibody allows fluorescent tagging. Brefeldin blocks protein transport, and provokes the accumulation of cytokines within the immune cell.

Kits

The present disclosure further pertains to a kit containing the present pharmaceutical composition. The kit or container holds an effective amount of a pharmaceutical composition for carrying out the methods or producing the compositions described herein and/or instructions for
15 producing or using the compositions for prophylactic use in, or therapy of, a patient or subject having or suspected of having or at risk of viral infection.

The kit may comprise instructions for administering the present composition to a subject having or at risk of viral infection (e.g., Zika virus infection). The instructions will generally include information about the use of the composition for the treatment or prevention of viral
20 infection (e.g., Zika virus infection). In other embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of viral infection or symptoms thereof; precautions; warnings; indications; counter-indications; overdose information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the
25 container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container. U.S. Patent Publication No. 2017/0106067. A kit may also contain instructions for combining the components so as to formulate an immunogenic composition suitable for administration to a mammal.

The compositions of the present invention can be supplied in unit dosage or kit form. Kits can comprise various components of the pharmaceutical composition or vaccines thereof provided in separate containers as well as various other active ingredients or agents.

Administering to both human and non-human vertebrates is contemplated within the scope of the present invention. Veterinary applications also are contemplated. Generally, the subject is any living organism in which an immune response can be elicited. Examples of subjects include, without limitation, humans, livestock, dogs, cats, mice, rats, and transgenic species thereof.

In some embodiments, the kit comprises a sterile container that contains a therapeutic or prophylactic composition; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

Definitions

As used herein, "attenuation" and "attenuated" encompasses a virus, or gene in the virus, and the like, that is modified to reduce toxicity to a host. The host can be a human or animal host, or an organ, tissue, or cell. The virus can be attenuated to reduce binding to a host cell, to reduce spread from one host cell to another host cell, or to reduce intracellular growth in a host cell. Attenuation can be assessed by measuring, e.g., an indicator of toxicity, the LD₅₀, the rate of clearance from an organ, or the competitive index (see, e.g., Auerbuch, et al. (2001) Infect. Immunity 69:5953-5957). Generally, an attenuation results an increase in the LD₅₀ by at least 25%; more generally by at least 50%; most generally by at least 100% (2-fold); normally by at least 5-fold; more normally by at least 10-fold; most normally by at least 50-fold; often by at least 100-fold; more often by at least 500-fold; and most often by at least 1000-fold; usually by at least 5000-fold; more usually by at least 10,000-fold; and most usually by at least 50,000-fold; and most often by at least 100,000-fold. "Attenuated gene" encompasses a gene that mediates toxicity, pathology, or virulence, to a host, growth within the host, or survival within the host, where the gene is mutated in a way that mitigates, reduces, or eliminates the toxicity, pathology, or virulence. "Mutated gene" encompasses deletions, point mutations, insertion mutations, and

frameshift mutations in regulatory regions of the gene, coding regions of the gene, non-coding regions of the gene, or any combination thereof.

"Effective amount" as used in treatment encompasses, without limitation, an amount that can ameliorate, reverse, mitigate, or prevent a symptom or sign of a medical condition or disorder. Unless dictated otherwise, explicitly or otherwise, an "effective amount" is not limited to a minimal amount sufficient to ameliorate a condition, or to an amount that results in an optimal or a maximal amelioration of the condition. "Effective amount" within the context of administration of a vaccine composition is that which causes an immune response in the mammal. In certain embodiments, an "effective amount" of a vaccine composition or an immunogenic composition refers to the amount of target antigen which elicits a measurable immune response in a mammalian subject as compared to the immune response in the mammalian subject in the absence of administration of the antigen.

In one embodiment, a subject is a mammal such as a primate, and, more preferably, a human. Examples of non-human primates include marmosets, monkeys, chimpanzees, gorillas, orangutans, and gibbons. The term "subject" also includes domesticated animals, such as cats, dogs, etc., livestock (for example, cattle (cows), horses, pigs, sheep, goats, etc.), laboratory animals (for example, ferret, chinchilla, mouse, rabbit, rat, gerbil, guinea pig, etc.) and avian species (for example, chickens, turkeys, ducks, pheasants, pigeons, doves, parrots, cockatoos, geese, etc.). Subjects can also include fish (for example, zebrafish, goldfish, tilapia, salmon, and trout), amphibians and reptiles. As used herein, a "subject" is the same as a "patient," and the terms can be used interchangeably.

By "control" or "reference" is meant a standard of comparison. As used herein, "changed as compared to a control" sample or subject is understood as having a level that is statistically different than a sample from a normal, untreated, or control sample. Control samples include, for example, cells in culture, one or more laboratory test animals, or one or more human subjects. Methods to select and test control samples are within the ability of those in the art.

As used herein, "operably linked" is understood as joined, preferably by a covalent linkage, e.g., joining an amino-terminus of one peptide, e.g., expressing an enzyme, to a carboxy terminus of another peptide, e.g., expressing a signal sequence to target the protein to a specific cellular compartment; joining a promoter sequence with a protein coding sequence, in a manner

that the two or more components that are operably linked either retain their original activity, or gain an activity upon joining such that the activity of the operably linked portions can be assayed and have detectable activity, e.g., enzymatic activity, protein expression activity.

5 The following are examples of the present invention and are not to be construed as limiting.

Example 1 Overview of ZIKV vaccine Studies

10 Whole-cell vaccination (WCV) against ZIKV is produced using irradiated human cancer cells, which can be infected with the virus and which can also be genetically modified to express and secrete cytokines that promote antigen presenting cells to engulf virus-infected cells, process the viral antigens, and present them to T-cells.

15 Previously described GVAX pancreas vaccine is comprised of human pancreatic cancer cell lines transfected with GM-CSF. For pancreatic cancer treatment, these cells are grown *in vitro*, irradiated by gamma-rays and administered intradermally as a therapeutic vaccine. Previous studies have shown GVAX expressing GM-CSF is capable of inducing a robust humoral and T-cell response against pancreatic tumors *in vivo*.

20 In order to generate ZIKV using GVAX expressing GM-CSF, the inventors will first test permissivity (permissiveness) of GVAX pancreas cells (PANC 6.03-PANC-10.05-GM-CSF) to support ZIKV infection. In parallel, the inventors will evaluate permissivity of GVAX pancreas cells to ZIKV infection to the cell lines known to be permissive to ZIKV. As described in the present disclosure, various cell types have been shown to be susceptible to ZIKV infection, where nonlimiting examples include Vero, LLC-MK2, HEK293T, etc. Following the transfection of cells with ZIKV, permissivity of GVAX cells can be evaluated using a number of
25 techniques, including RT-PCR, ZIKV plaque assay, MOI, and/or flow cytometry using anti-ZIKV antibody 4G2.

 A mouse epithelial cell line (that will be used for the generation of the vaccine and then tested it in the preclinical animal model) will also be selected based on its capability to support the virus. Once identified, such mouse epithelial cell line will be transfected with murine GM-

CSF and used for immunology studies in mice (anti-ZIKA antibody titers, ELISAPOT for T-cell competence etc.)

WCV is usually rendered proliferation incompetent by gamma irradiation at levels 150-300 Gy. This regimen makes cells unable to proliferate and disintegrate with time, while preserving their ability to express and secrete factors introduced into the cells by transfection (e.g. GM-CSF). Gamma-irradiation can also be used for virus inactivation. However, the irradiation levels for virus inactivation are almost 100 times higher than those required for rendering the cells proliferation incompetent, i.e. 15,000-30,000 Gy. Thus, at 15,000-30,000 Gy, the cells will likely be killed and, therefore, would lose the ability to secrete immunostimulating cytokines. In order to accurately determine whether radiation levels required for the inactivation of virus would render Zika infected GVAX pancreas cells unable to secrete functional GM-CSF, the inventors will irradiate both Zika virus-infected and non-infected cells and monitor their ability to secrete GM-CSF and maintain the viable virus.

In case the gamma-irradiation regimen required for full Zika virus inactivation results in failure of cells to secrete GM-CSF, the inventors will next explore the by-stander approach, wherein the Zika virus-permissive GM-CSF-transfected cell line will be split in two fractions: one fraction will be infected with Zika virus and irradiated under the regimen required for a complete viral inactivation. Another fraction (comprising non-infected cells) will be irradiated under the regimen required for rendering the cells proliferation incompetent. Both fractions will be combined and used in a vaccine composition. In conclusion, non-infected cells will serve as a source of GM-CSF, while Zika- infected cells will serve as a source of viral antigens.

In addition to using cells infected with Zika virus for the purposes of vaccine preparation, the inventors will also test cells transfected with Zika replicon. Under such circumstances, there would be no need for the inactivation of Zika virus, and cells would be rendered proliferation incompetent using standard methods in the art.

Following the generation of vaccine composition comprising proliferation incompetent cells that (i) express GM-CSF, and (ii) comprise inactivated Zika virus, the inventors will evaluate the vaccine composition using *in vivo* animal models, including mouse models as well as non-human primates Macaque rhesus. Since rodents (mice) are susceptible to ZIKV infection, fully immunocompetent mice will be vaccinated with vaccine compositions described here. In

order to assess the efficacy and immunogenic potential of Zika vaccine, the inventors will measure various parameters, including, but not limited to, anti-ZIKV antibody titers, T-cell activation (ELISPOT) and inhibition of infection *in vitro* using immune serum.

5 Following animal vaccination, the levels of anti-ZIKV neutralizing antibodies as well as the levels of T-cell response will be assessed *in vitro* using ELISA, plaque neutralization assay, ELISPOT and/or other immunological techniques. In one set of experiments, the inventors will use a mouse model wherein animals will be vaccinated with a vaccine derived from ZIKA virus permissive mouse cell line and transfected with mGM-CSF. In experiments using a non-human primate model, the inventors will use GM-CSF Vero transfected cells, which will be either
10 infected with Zika virus or transfected with Zika replicon.

 Following the vaccination, animals will be challenged with Zika virus. The viremia will be monitored in vaccinated animals and compared to non-vaccinated group. In order to model the repeating mosquito bites, the effect of repeating infection will also be evaluated. In addition to testing the efficacy of the ZIKV vaccine in adult animals, the inventors will also
15 asses the effectiveness of vaccine composition described here in a developing embryo and/or fetus. For example, adult animals will be vaccinated and allowed to breed. During the gestation period, animals will be challenged with Zika virus and newborns will be examined for the persistence of the virus in their body and for the neurologic manifestation of the viral infection. The purpose of this experimental design is to test whether the initial viremia, which is created by
20 a mosquito bite, can be quenched at the onset of infection by the existing anti-viral immunity.

 The following general steps will be carried out in mouse studies: (1) permissive mouse epithelial cancer cell line will be permanently transfected with mouse GM-CSF; (2) a fraction of transfected cells will be infected with Zika virus for 24-48 hours, followed by irradiation where the infected cells will be irradiated at, e.g., 25-40 kGy (or other doses as discussed herein) and
25 non-infected cells at 150-250 Gy. Subsequent to irradiation, cells will be combined and injected intradermally in mouse flanks; (3) after three weeks of incubation period, the procedure described in step (2) will be repeated and 8-10 days later blood will be drawn and an antibody titers against ZIKV measured using ELISA and plaque inhibition assay (PIA); (4) upon reaching a sufficient anti-virus antibody levels, both vaccinated and non-vaccinated mice will be

challenged in parallel, and the levels of viremia in blood will be monitored for a period of time spanning from several days to several weeks.

Low levels or the complete absence of viremia in vaccinated mice will serve as a proof of vaccine effectiveness.

5

Example 2 Testing of ZIKV vaccine in Mouse Models of Zika Virus Pathogenesis

Studies in animal models will be carried out to evaluate the efficacy of the vaccines of the present disclosure. Until recently, a limited number of studies have been performed in mice (Bell et al. Arch. Gesamte Virusforsch. 35, 183–193 (1971); Dick et al., Trans. R. Soc. Trop. Med. Hyg. 46, 509–520 (1952); Way et al., J. Gen. Virol. 30, 123–130 (1976)). Although these early studies suggested that ZIKV can replicate and induce injury in cells of the central nervous system (CNS), it remained unclear whether this pathogenesis is related to ZIKV-induced neurodevelopmental defects or Guillain-Barre syndrome (GBS) in humans. While these studies indicated that mice could be infected with ZIKV via intracerebral inoculation, determining mechanisms of pathogenesis and evaluating candidate vaccines requires more clinically relevant inoculation routes.

Recently, Lazear et al. evaluated ZIKV infection and disease in wildtype (WT) C57BL/6 mice, as well as a large panel of immune-deficient transgenic mice, using several strains of ZIKV including a contemporary clinical isolate (Cell Host Microbe (2016) S1931-3128(16)30102-0). Specifically, the authors tested 5- to 6-week-old WT C57BL/6 mice as well as congenic transgenic mice lacking key components of innate antiviral immunity (*Ifnar1*^{-/-}, *Mavs*^{-/-}, *Irf3*^{-/-}, *Irf3*^{-/-} *Irf5*^{-/-} *Irf7*^{-/-} TKO) for susceptibility to disease induced by a contemporary human isolate of ZIKV (H/PF/2013) from French Polynesia, as well as the original ZIKV strain, MR 766. Four to six-week-old *Ifnar1*^{-/-} mice (which cannot respond to IFN-α/β) and *Irf3*^{-/-} *Irf5*^{-/-} *Irf7*^{-/-} TKO mice (which produce almost no IFN-α/β) developed neurological disease and were susceptible to ZIKV infection, while single *Irf3*^{-/-}, *Irf5*^{-/-}, and *Mavs*^{-/-} knockout mice exhibited no overt illness. *Ifnar1*^{-/-} mice sustained high viral loads in the brain and spinal cord, which is consistent with evidence that ZIKV causes neurodevelopmental defects

in human fetuses. Moreover, the testes of *Ifnar1*^{-/-} mice had the highest viral loads, which can be relevant to sexual transmission of ZIKV.

Briefly, *Ifnar1*^{-/-} mice will be injected with inactivated GVAX cells expressing GM-CSF, as well as inactivated GVAX cells infected with various strains of ZIKV. Vaccination will be carried out at least 2 weeks before challenging the animals with the virus. Next, *Ifnar1*^{-/-} animals that have received the vaccine(s) will be challenged with ZIKV of a specific strain by subcutaneous (footpad), intravenous (retro-orbital), or i.p. routes with 10², 10³, or 10⁴ FFU of ZIKV. Survival, weight loss, and disease symptoms will be monitored for 14–30 days, depending on the experiment. Mice will be euthanized at various days following the viral challenge, and liver, spleen, kidney, testes, brain, and spinal cord will be harvested, weighed, and homogenized. Viral burden will be evaluated by qRT-PCR or plaque assay (Lazear et al. Cell Host Microbe (2016) S1931-3128(16)30102-0).

Furthermore, Miner et al. have recently described a mouse model of placental and fetal disease associated with in utero transmission of ZIKV (Cell 2016 May 19;165(5):1081-91).

Female mice lacking type I interferon signaling (*Ifnar1*^{-/-}) crossed to wild-type (WT) males produced heterozygous fetuses exhibiting similar immune status as that observed in human fetuses. Moreover, maternal inoculation at embryonic day 6.5 (E6.5) or E7.5 resulted in fetal demise that was associated with ZIKV infection of the placenta and fetal brain. Thus, this model can be used to test ability of vaccine(s) of the present disclosure to prevent congenital malformations during pregnancy.

Example 3 Generation of cells transfected with DNA encoding GM-CSF and infected with Zika viruses

Mouse neuroblastoma cell line N2a was used to produce whole cell-based vaccine against the Zika virus. N2a cells originate from Balb/C mice and have similar genetic background. Although considered allogeneic (due to a number of somatic mutations occurred in vitro), N2a cells cause no or very little immune response in Balb/C mice.

The N2a cells were transfected with DNA encoding mouse GM-CSF using pcDNA-3.1 vector containing the neo-resistance gene. The transfected cells were designated as N2a-GMSCF or N2a-GM.

N2a-GM cells were infected with ZIKV strain MR776 (Uganda). Viral infection was monitored by staining viral E2 protein using anti-E2 antibodies 4G2 according to the following protocol:

1. Grow N2A-GM cells on coverslips to 70-80% confluency.
2. Transfer the coverslips into 24 well-plates with PBS.
3. Fix the cells with 10% formalin for 15 min; wash twice (PBS).
4. Permeabilize with 0.1% Triton X-100 for 15 min; wash twice (PBS).
5. Block using 2% BSA/PBS for 1 hr; wash once.
6. Add 4G2 antibodies diluted 1:5-1:6 with PBS to cells; incubate 2 hrs at room temperature or overnight at 4°C.
7. Wash 2 times.
8. Add secondary antibody donkey-anti-mouse conjugated with AlexaFluor488 (1:500) in 0.1 % Triton, and incubate for 1 hr.
9. Wash twice, add DAPI. Embed and observe under the microscope.

In different experiments, cells had an infectivity rate of about 40-50% (Figures 1A and 1B), or an infectivity rate of about 80-90% (Figures 1C and 1D).

Example 4 Vaccination of mice using whole cell-based vaccine (cells expressing GM-CSF and infected with Zika viruses)

Two groups of mice were immunized with either N2a-GM cells or N2a-GM-naïve cells (Group 1, n = 4) or N2a-GM-ZIKV cells (Group 2, n = 4). For each group, mice were vaccinated twice within a time frame of two weeks: a first vaccination or prime vaccination, and a second vaccination or boost vaccination. For the first (prime) vaccination, about 10^6 cells at the infectivity rate of about 40-50% were injected subcutaneously into the right flanks of the mice. For the second (boost) vaccination, about 20×10^6 cells at the infectivity rate of about 80-90% were injected subcutaneously in the right flanks of the mice.

Blood from immunized animals was collected 9 days after the second vaccination, and sera was prepared. The antibody titers of the sera were measured using serial dilutions of the sera in standard cellular enzyme-linked immunosorbent assay (cELISA) assay. Two types of cells

were used in cELISA: N2a-GM cells (naïve) and N2a-GM-ZIKV cells at the infectivity rate of about 80-90%. Specifically, cells were grown at regular conditions, collected at 80-90% confluency with 0.25% Trypsin/EDTA. Cell suspension was placed immediately into culture media (10% FBS) to inactivate Trypsin/EDTA. Cell suspension was then spun at 250-300g, and the media was aspirated. Cells were resuspended in ice-cold MetOH (methanol), and placed at -20°C for 20min. PBS was added into the tubes, spun at 450g for 10 min and replaced with fresh PBS, and stored at 4°C for 2-4 days.

The cELISA test was performed as follows.

1. For cELISA, fixed cells are washed with PBS twice, resuspended in blocking buffer (0.3% dry milk in PBS). The multiscreen plate (Millipore, MAHVN4510) was filled with 300 µl blocking buffer. Incubate cells and plate for 1 hr at RT. Empty plate using the suction device, spread cells over the plate, 100 µl/well, 30-50x10³ cells per well.
2. Remove blocking solution, add diluted sera 50 µl/well (e.g., dilution for unknown media is 1:2 with blocking solution), negative controls (blocking solution and fresh culture media) and positive control (if any). Incubate for 2 hrs at RT.
3. Wash plate 5-6 times with blocking solution.
4. Prepare horseradish peroxidase (HRP)-conjugated antibodies (e.g., goat anti-mouse-IgG(H+L)-HRP, or other appropriate conjugates) diluted 1:3000 in the blocking solution. Add conjugate solution to the wells of the multiscreen plate, 50 µl/well, incubate for 1 hr at RT.
5. Wash the plate 6-8 times with the blocking solution.
6. Add Ultra-TMB substrate solution (ThermoFisher, catalog number 34028), 50 µl/well, incubate for 1 hr at RT.
7. Watch for the blue color development. In certain embodiment, it takes 5-10 minutes. In certain embodiment, it takes no more than 30 min. Watch closely for the negative control well which should remain uncolored. If color appears in the negative control (this is usually due to problems with washing procedure), stop reaction immediately with 10% HCl (25 µl/well). If there is no color in negative wells, stop the reaction no later than 30 min after the start of the reaction. When reaction is stopped, blue color turns into yellow.
8. Read absorbance at 450nm.

As shown in Figure 2, sera from mice immunized with N2a-GM (naïve) cells did not react either with N2a-GM (naïve) cells ("Group 1 on N2A-GM-naïve" in Figure 2) or with N2a-GM-ZIKV cells ("Group 1 on N2A-GM-ZIKA" in Figure 2). This indicates that neither N2a cells' antigens nor recombinant mouse GM-CSF secreted by transfected cells had immunogenicity sufficient for an antibody response. Sera from mice vaccinated with N2a-GM-ZIKV cells was immunologically reactive to N2a-GM-ZIKV cells ("Group 2 on N2A-GM-ZIKA" in Figure 2), but not to N2a-GM cells ("Group 2 on N2A-GM-naïve" in Figure 2). This indicates that only immune response directed against ZIKV was detected. This suggests that syngeneic cells N2a did not trigger immune responses, and that only immune response against virus antigens was detected (Figure 2).

Figure 3 shows antibody response (vertical bars according to the left y-axis, cELISA assay on N2a-GM-ZIKV cells) and antibody neutralization potential (solid line according to the right y-axis, PRNT) for three groups of mice immunized with N2a-GM-ZIKV cells (group A), N2a-ZIKV cells (group B), or purified ZIKV (group C), respectively. In the plaque reduction neutralization test (PRNT), neutralization of ZIKV virus-forming plaques was studied in the presence of sera from mice vaccinated with N2a-GM-ZIKV cells (A), N2a-ZIKV cells (B), or purified ZIKV (C). As shown in Figure 3, anti-ZIKV antibody titers are higher in mice immunized with N2a-GM-ZIKV than anti-ZIKV antibody titers in mice immunized with N2a-ZIKV or purified ZIKV. This indicates that the local secretion of GM-CSF by N2a-GM cells facilitates the activation of immune cells and results in the increase of antibody production. The dilution of sera required for 50% inhibition of virus induced plaques is the highest in group A which is in good correlation with the antibody titers.

Figure 4 shows antibody response (vertical bars according to the left y-axis, cELISA assay on N2a-GM-ZIKV cells) for, as well as the number of colonies infected with ZIKV in the presence of a fixed dilution (1:3000) of the serum from, three groups of mice immunized with N2a-GM-ZIKV cells (group A), N2a-ZIKV cells (group B), or purified ZIKV (group C), respectively. Figure 4 is similar to Figure 3 except that in this experiment the number of virus

induced plaques was counted at the fixed sera dilution using sera from mice from all three groups.

The data presented in Figures 3 and 4 were the averages of 7 animals in each group.

PRNT was used to quantify the titer of neutralizing antibody for the Zika virus. Serum from the vaccinated mice was preincubated with the ZIKV to allow the antibodies to react with the viruses. The serum that had been preincubated with the ZIKV was then added to a monolayer of host cells (e.g., N2A cells, or N2A-GM cells). The concentration of serum to reduce the number of plaques by 50% compared to the serum-free virus gives the measure of how much antibody is present or how effective it is. This measurement is denoted as the PRNT50 value. Alternatively or additionally, the dilution of serum to reduce the number of plaques by 50% compared to the serum-free virus gives the measure of how much antibody is present or how effective it is. This measurement is denoted as the 50% neutralization value (Figure 3).

PRNT was done as follows.

To test sera for neutralization, N2A-GM cells suspension in 100 μ l media are plated onto a 96-well plate, at 2×10^4 cells/well and used after about 24 hrs (60-70% confluence), or at 1×10^4 cells/well which can be used after about 48 hrs.

This step was done in separate transfer plates. The serum was diluted in series at step 4 starting with 1/25 growth media), and titrate down with step 4 (last dil. 1:1600). 60 μ l of all dilutions was prepared in triplicates. Several wells with 60 μ l of diluent (media) for ZIKV only which is added later as a control. Single dilution of 1/40 of pre-titrated ZIKV containing media is also prepared. 60 μ l of ZIKV (1:40) are added into all wells with sera, normal mouse serum (NMS) and culture media. The final dilution of sera in preincubation plate increases 2 times and becomes the range from 1/50 to 1/3200. The plate is incubated on a plate shaker at 2500 rpm for 2 hrs at room temperature.

After 2 hrs, 100 μ l of mixtures are added carefully to the N2A cells plate (plated 1 day or 2 days before in 100 μ l of media). Also, fresh pre-titrated ZIKV-containing media is added to several plates to reach the same dilution as in preincubation plate. This is the control for infection which is done without 2 hrs of preincubation. Final dilutions of sera in cell plate are 1:100-1:6400, and ZIKV - 1:160. The plates incubated for 48-72 hrs. Afterwards, the plates are washed with fresh PBS and Methylene blue is added to stain the remaining cell. The virus

induced plaques are seen at the bottom of the plates as white spots which can be counted using plaque counting.

Example 5 Testing ZIKV infectivity in different cell lines

Different types of cells, including Vero cells (Figures 5A and 5B), N2a (naïve) cells (Figures 5A and 5B), N2a-GMCSF cells (Figures 5E and 5F), N2a new cell line (Figures 5G and 5H), human fibroblasts WS-1 (Figures 5I and 5J), human fibroblasts PCS201-012 (Figure 5K), were infected with ZIKV-containing cell culture media (P2) and immunostained using anti-E2 antibodies 4G2 at different time points post-infection, e.g., 24 hours, or 48 hours post-infection.

The cells were immunostained for the virus E2 antigen using anti-E2 antibodies 4G2 (see Example 3 for the staining protocol). Figure 5 show different levels of permissivity of cells for ZIKV infection. At multiplicity of infection (MOI) 1, the highly remissive cell line is Vero (90-100%), while human fibroblasts and N2 cells have low permissivity (3-5%). In order to reach high level of infection in N2a cells which were used in the experiments of Examples 3 and 4, MOI 20 was used.

The practice of the present invention may employ, unless otherwise indicated, conventional techniques including those of molecular biology (including recombinant techniques), immunology, cell biology, biochemistry, and pharmaceutical practice. Such techniques are explained fully in the literature, for example, Molecular Cloning: A Laboratory Manual, second edition, (Sambrook et al.); Methods in Enzymology (Academic Press, Inc.); Current Protocols in Molecular Biology (F. M. Ausubel et al., eds); Current Protocols in Immunology (John Wiley & Sons, Inc., N.Y.), Handbook of Pharmaceutical Excipients (Rowe et al., Eds); Vaccines (Plotkin and Orenstein, 2003); and Vaccine Protocols (Methods in Molecular Medicine) (Robinsin, Cranage and Hudson, 2003).

The scope of the present invention is not limited by what has been specifically shown and described hereinabove. Those skilled in the art will recognize that there are suitable alternatives to the depicted examples of materials, configurations, constructions and dimensions. Numerous references, including patents and various publications, are cited and discussed in the description of this invention. The citation and discussion of such references is provided merely to clarify the

description of the present invention and is not an admission that any reference is prior art to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entirety. Variations, modifications and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention. While certain embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that changes and modifications may be made without departing from the spirit and scope of the invention. The matter set forth in the foregoing description is offered by way of illustration only and not as a limitation.

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What is claimed is:

1. An immunogenic composition comprising: cells (i) comprising at least one Zika virus antigen, and (ii) expressing at least one cytokine.
2. An immunogenic composition comprising: a first population of cells comprising at least one Zika virus antigen, and a second population of cells expressing at least one cytokine.
3. The composition of claims 1 or 2, wherein the cytokine is granulocyte macrophage colony-stimulating factor (GM-CSF), interferon alpha (IFN- α), interleukin-2 (IL-2), interleukin-12 (IL-12), tumor necrosis factor alpha (TNF-alpha), or combinations thereof.
4. The composition of claims 1 or 2, wherein the cytokine is GM-CSF.
5. The composition of claim 4, wherein the GM-CSF is human GM-CSF.
6. The composition of claims 1 or 2, wherein the at least one Zika virus antigen is an inactivated Zika virus.
7. The composition of claims 1 or 2, wherein the cells are pancreatic cancer cells, colon cancer cells, lung cancer cells, breast cancer cells, ovarian cancer cells, melanoma cancer cells, or combinations thereof.
8. The composition of claims 1 or 2, wherein the cells are epithelial cells.
9. The composition of claims 1 or 2, wherein the cells are antigen presenting cells (APCs).
10. The composition of claims 1 or 2, wherein the cells are dendritic cells.
11. The composition of claims 1 or 2, wherein the cells are proliferation-incompetent.

12. The composition of claim 11, wherein the cells are rendered proliferation-incompetent by irradiation.
13. The composition of claims 1 or 2, wherein the Zika virus is inactivated by irradiation.
14. The composition of claim 13, wherein the irradiation is in the range of 20-40 kGy.
15. The composition of claim 2, wherein the first population of cells and the second population of cells are of same type of cells.
16. The composition of claim 2, wherein the first population of cells and the second population of cells are of different types of cells.
17. A method of preventing or treating Zika virus infection in a subject, the method comprising the step of administering to the subject an immunogenic composition comprising: cells (i) comprising at least one Zika virus antigen, and (ii) expressing at least one cytokine.
18. A method of preventing or treating Zika virus infection in a subject, the method comprising the step of administering to the subject an immunogenic composition comprising: a first population of cells comprising at least one Zika virus antigen, and a second population of cells expressing at least one cytokine.
19. A method of preventing or treating Zika virus infection in a subject, the method comprising the steps of:
 - (a) administering to the subject a first population of cells comprising at least one Zika virus antigen, and
 - (b) administering to the subject a second population of cells expressing at least one cytokine.

20. The method of any of claims 17 – 19, wherein the cytokine is granulocyte macrophage colony-stimulating factor (GM-CSF), interferon alpha (IFN- α), interleukin-2 (IL-2), interleukin-12 (IL-12), tumor necrosis factor alpha (TNF-alpha), or combinations thereof.
21. The method of any of claims 17 – 19, wherein the cytokine is GM-CSF.
22. The method of any of claims 17 – 19, wherein the GM-CSF is human GM-CSF.
23. The method of any of claims 17 – 19, wherein the at least one Zika virus antigen is an inactivated Zika virus.
24. The method of any of claims 17 – 19, wherein the cells are pancreatic cancer cells, colon cancer cells, lung cancer cells, breast cancer cells, ovarian cancer cells, melanoma cancer cells, or combinations thereof.
25. The method of any of claims 17 – 19, wherein the cells are epithelial cells.
26. The method of any of claims 17 – 19, wherein the cells are antigen presenting cells (APCs).
27. The method of any of claims 17 – 19, wherein the cells are dendritic cells.
28. The method of any of claims 17 – 19, wherein the cells are proliferation-incompetent.
29. The method of claim 28, wherein the cells are rendered proliferation-incompetent by irradiation.
30. The method of any of claims 17 – 19, wherein the Zika virus is inactivated by irradiation.
31. The method of claim 30, wherein the irradiation is in the range of 20-40 kGy.

32. The method of any of claims 17 – 19, wherein the subject is a woman.
33. The method of claim 32, wherein the woman is pregnant.

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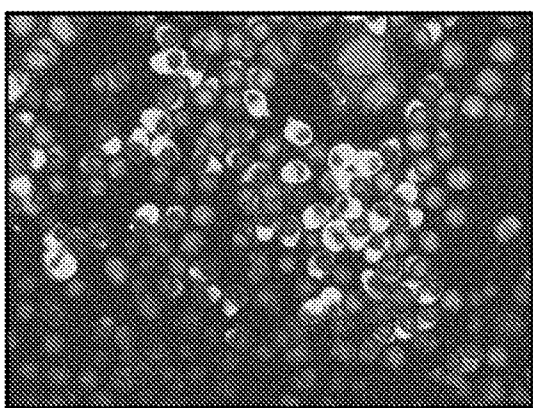


FIG. 1A

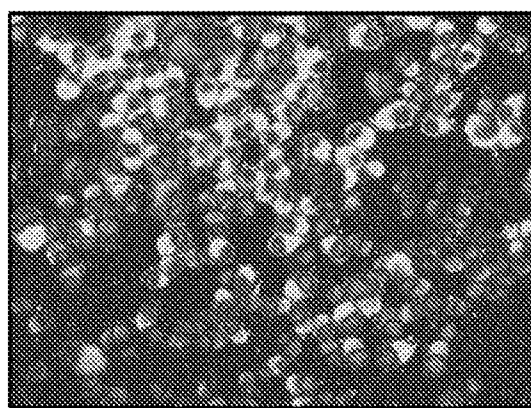


FIG. 1B

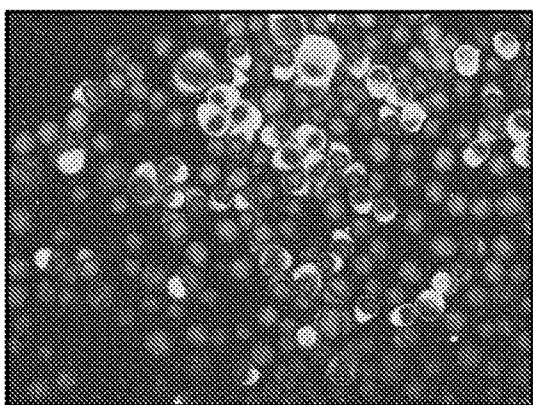


FIG. 1C

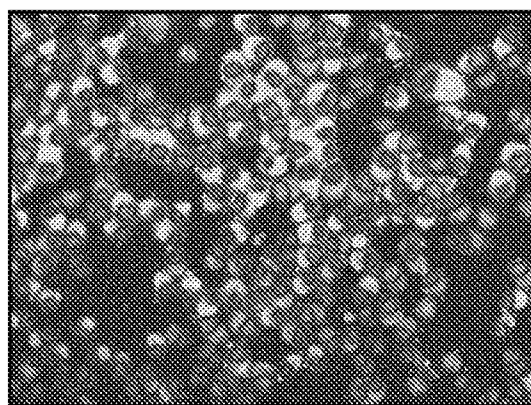


FIG. 1D

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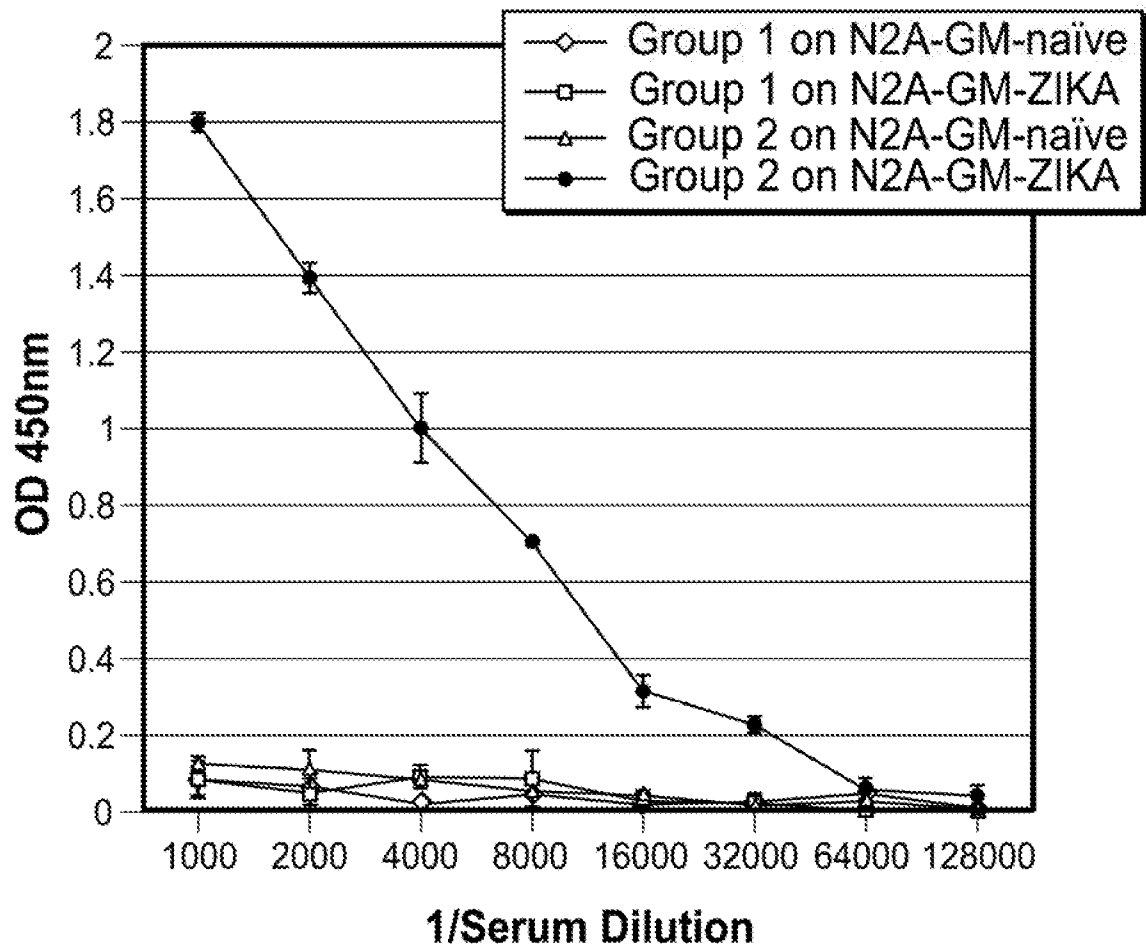


FIG. 2

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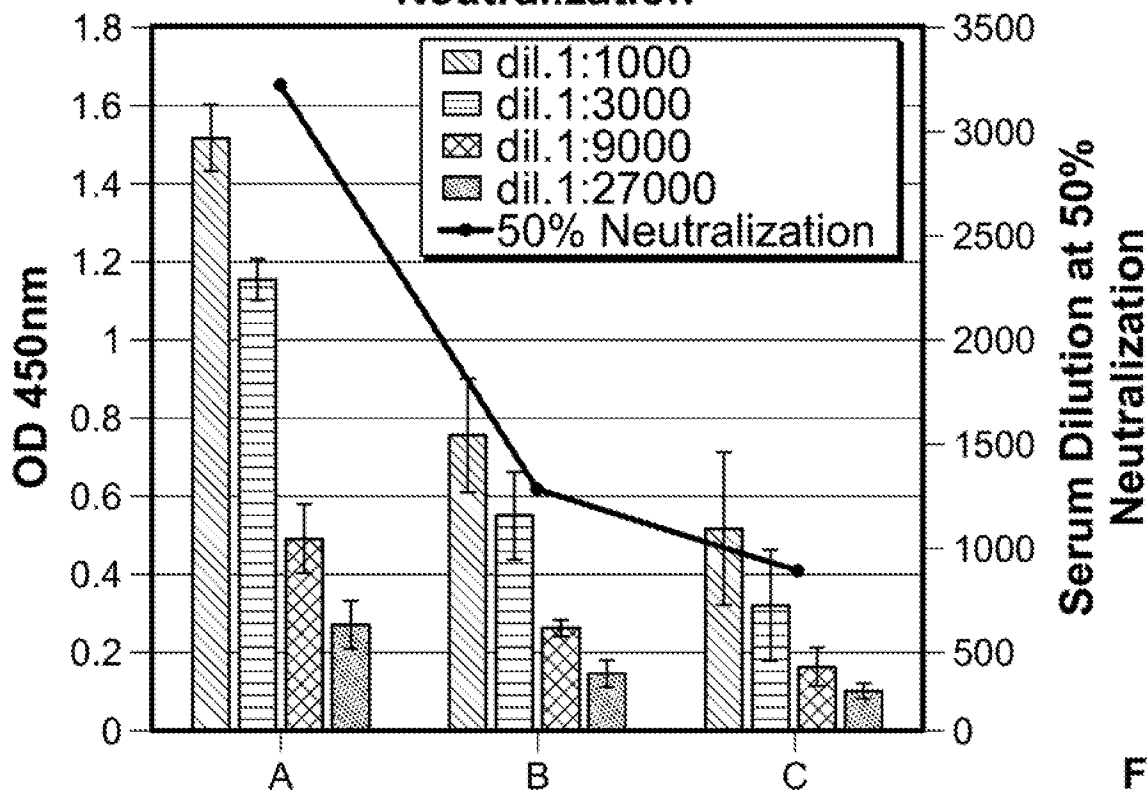
cELISA on N2A-GM-ZIKV Cells and ZIKV Plaque Neutralization

FIG. 3

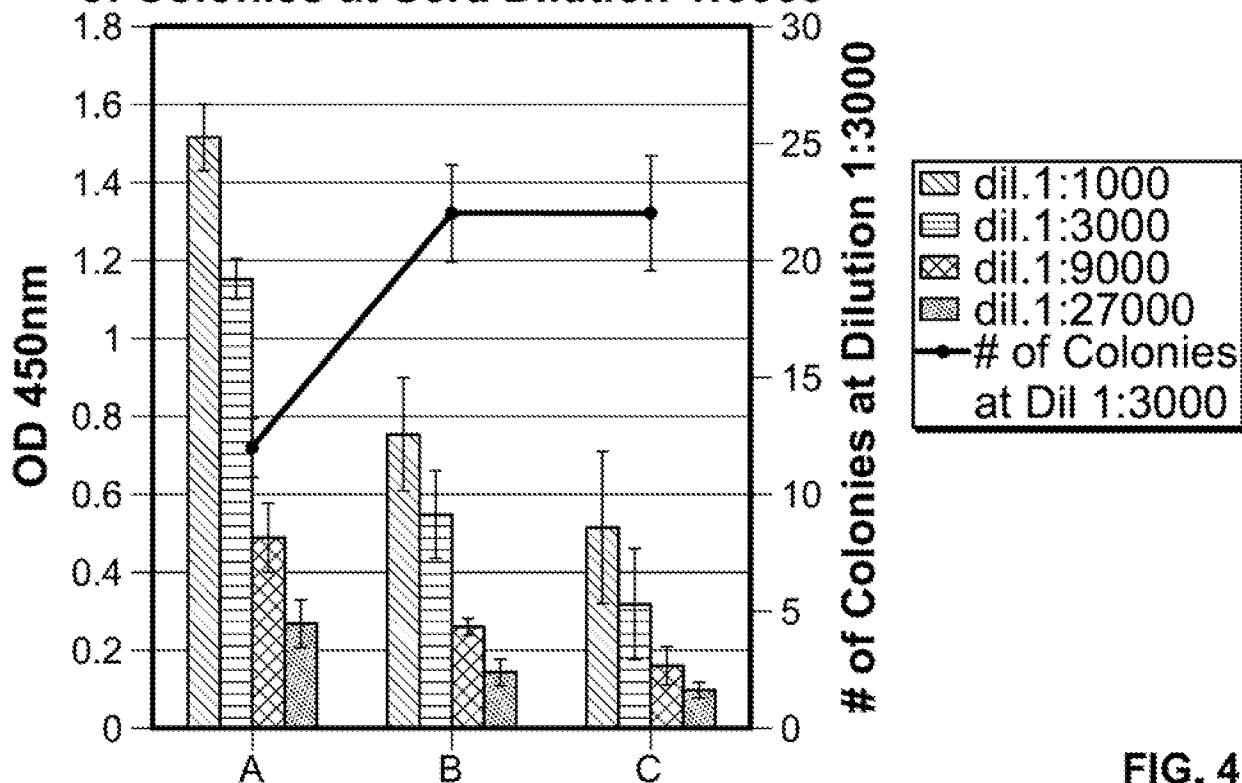
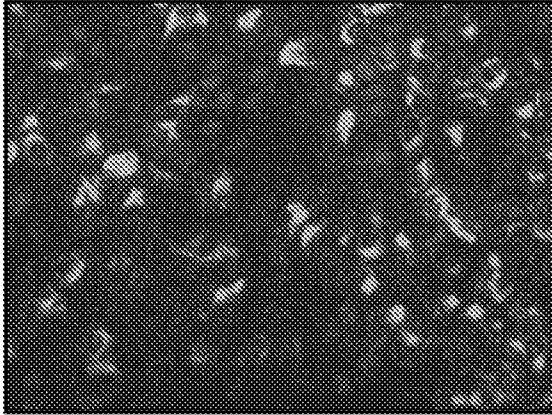
cELISA on N2A-GM-ZIKV Cells and Number of Colonies at Sera Dilution 1:3000

FIG. 4

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(VERO, 24hr)



(VERO, 48hr)

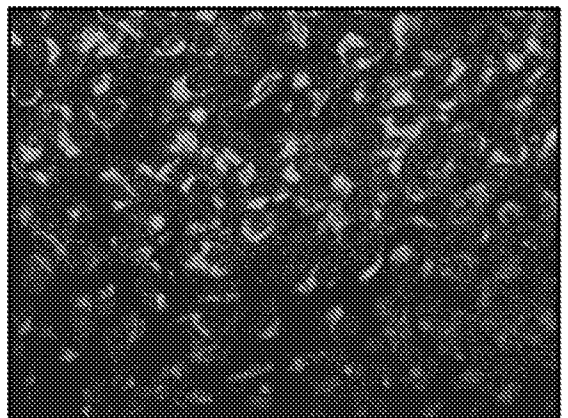
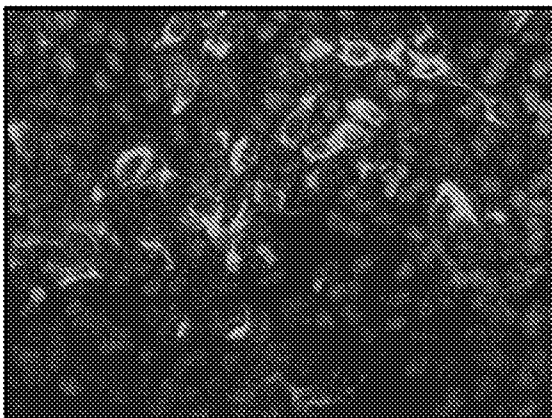
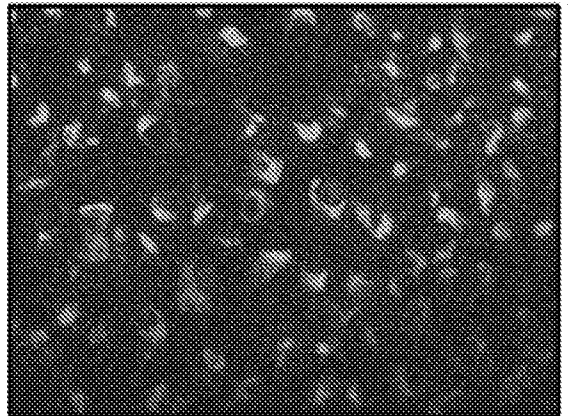
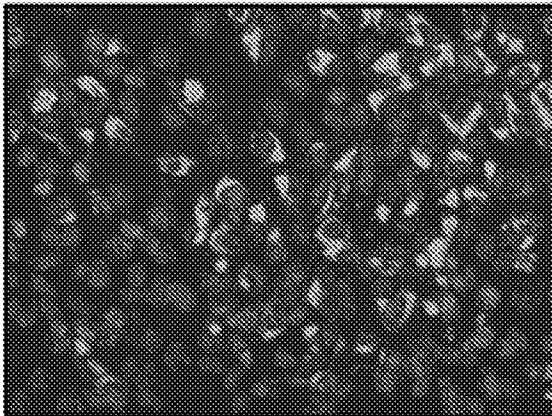
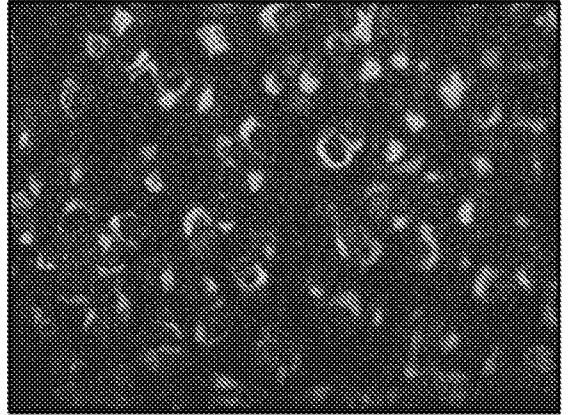
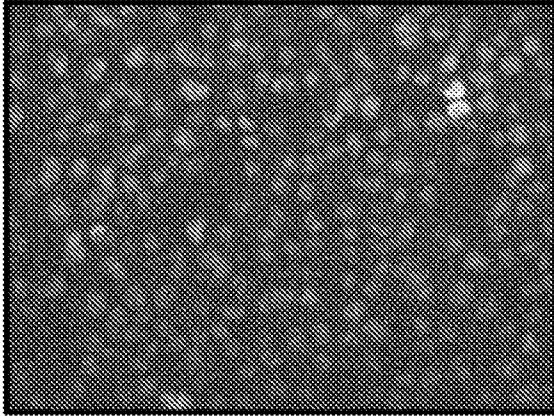


FIG. 5A

FIG. 5B

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(N2A NAÏVE, 24hr)



(N2A NAÏVE, 24hr)

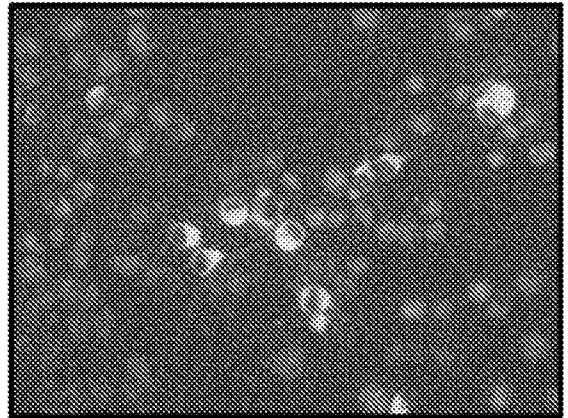
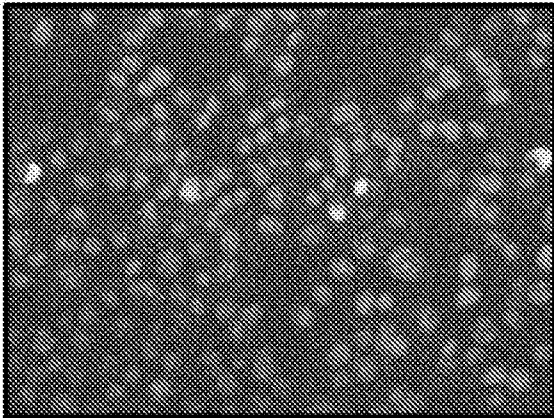
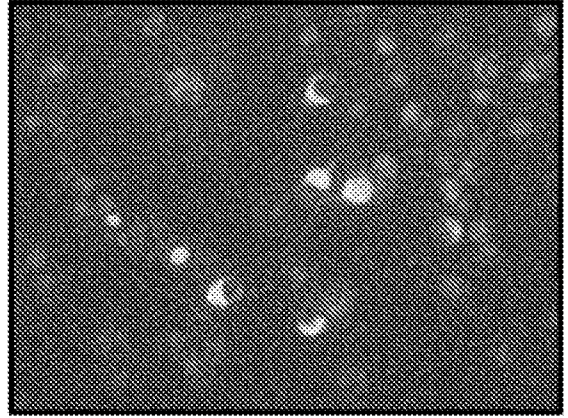


FIG. 5C

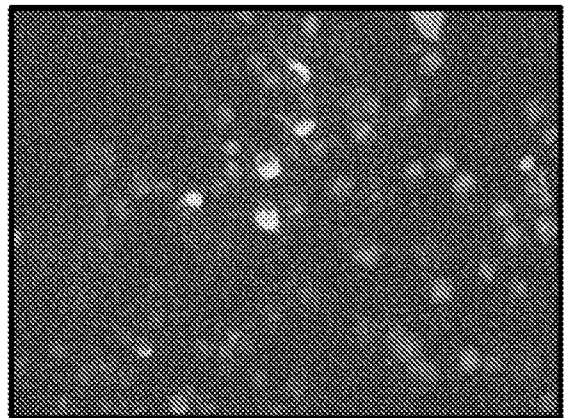
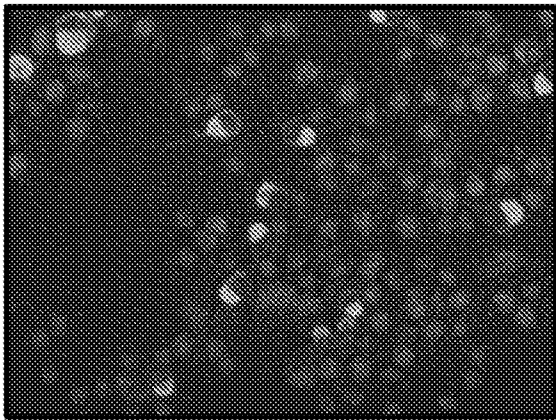


FIG. 5D

(N2A-GMCSF, 24hr)



(N2A-GMCSF, 48hr)

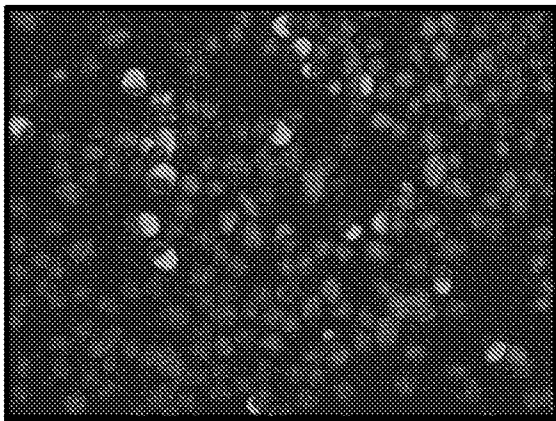
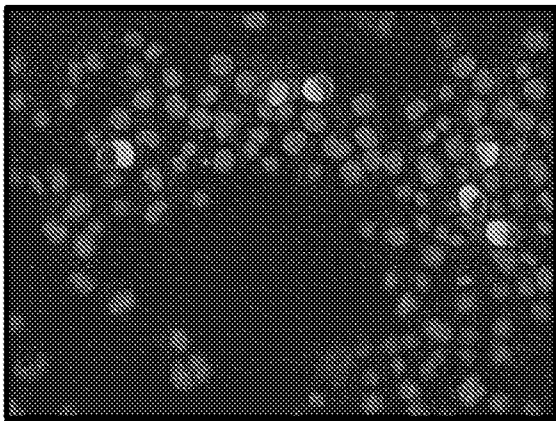
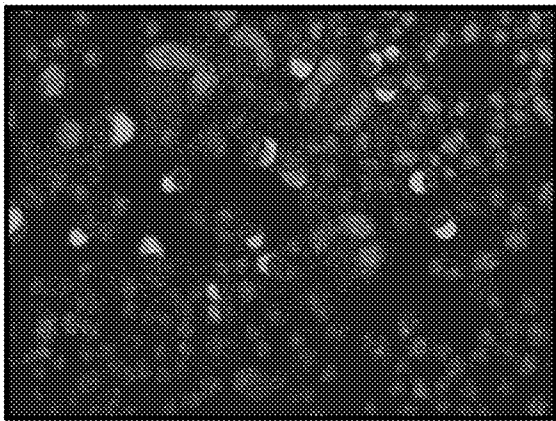
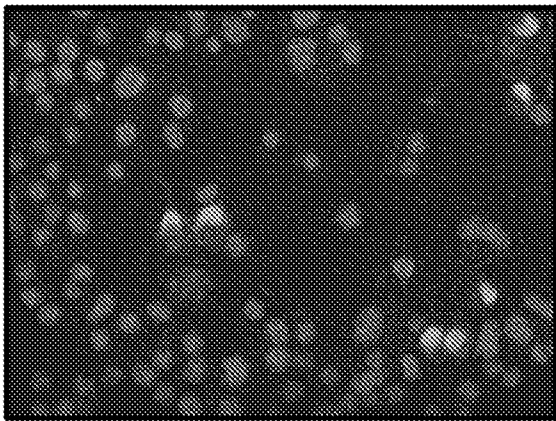
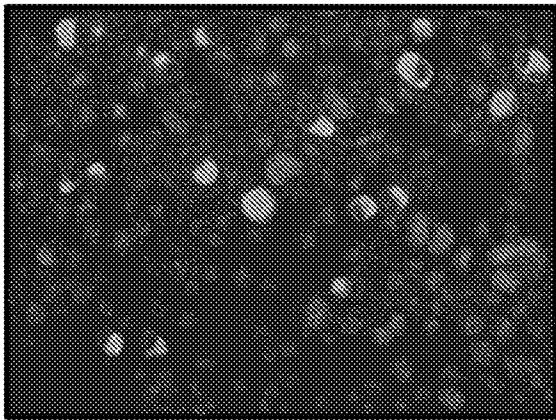
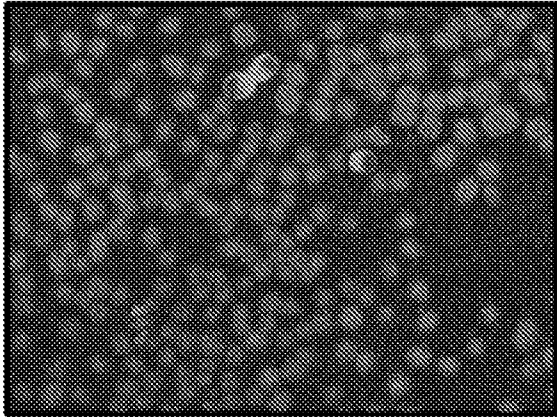


FIG. 5E

FIG. 5F

(N2A New Cell Line, 24hr)



(N2A New Cell Line, 48hr)

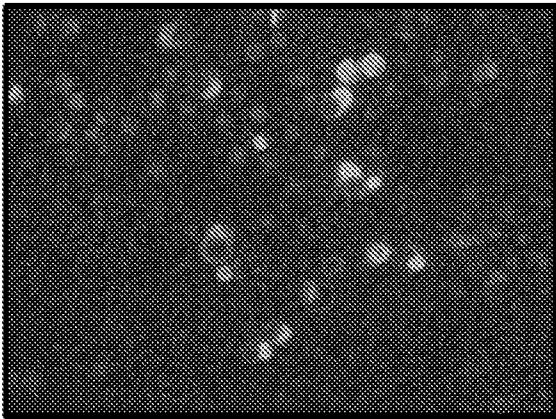
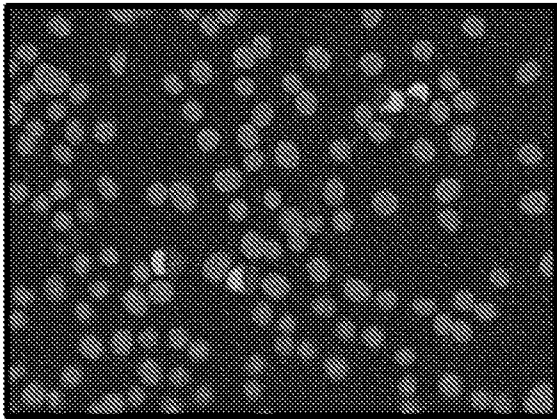
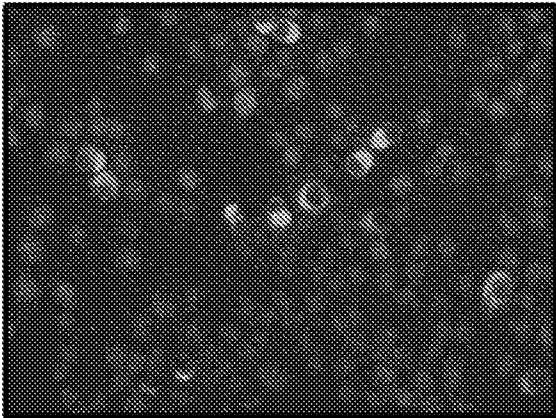
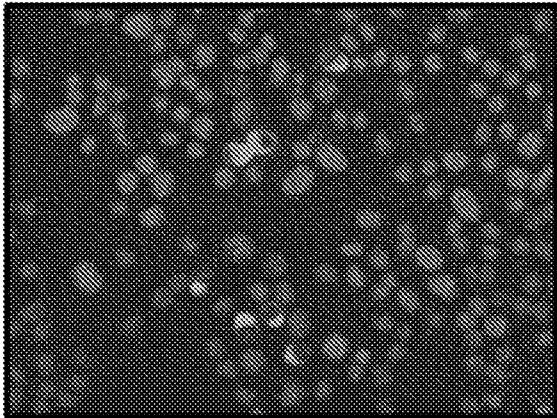
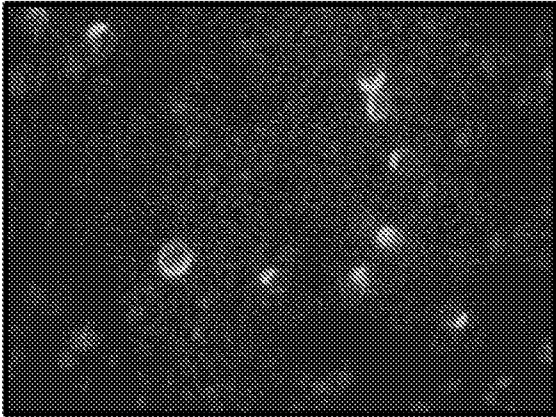
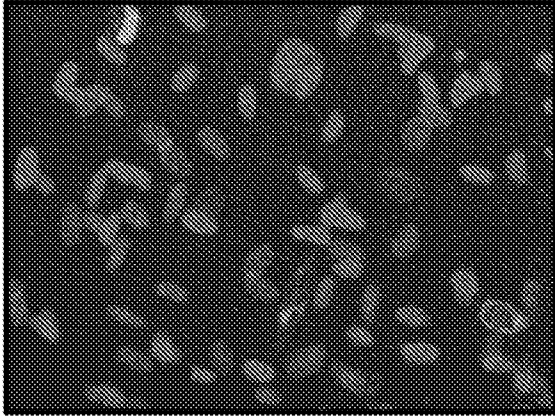


FIG. 5G

FIG. 5H

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(Human Fibroblasts WS-1, 24hr)



(Human Fibroblasts WS-1, 48hr)

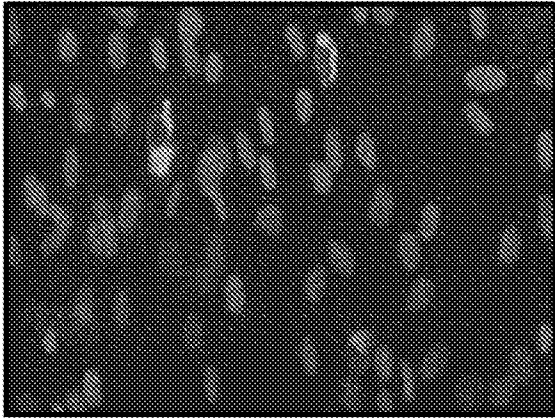
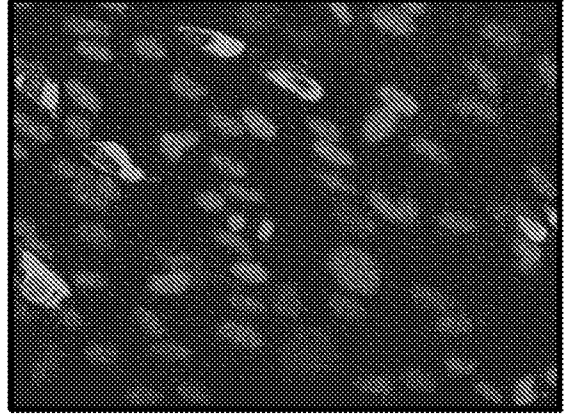


FIG. 5I

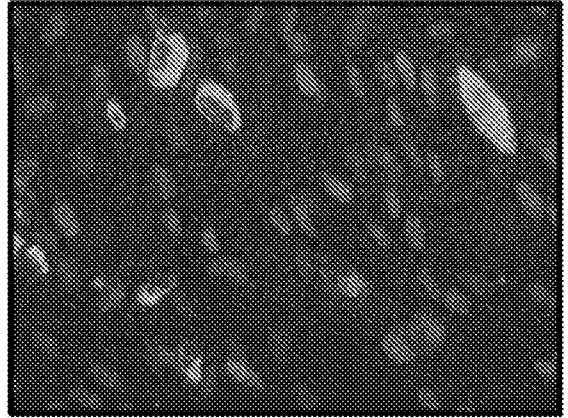


FIG. 5J

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(Human Fibroblasts PCS201-012, 48hr)

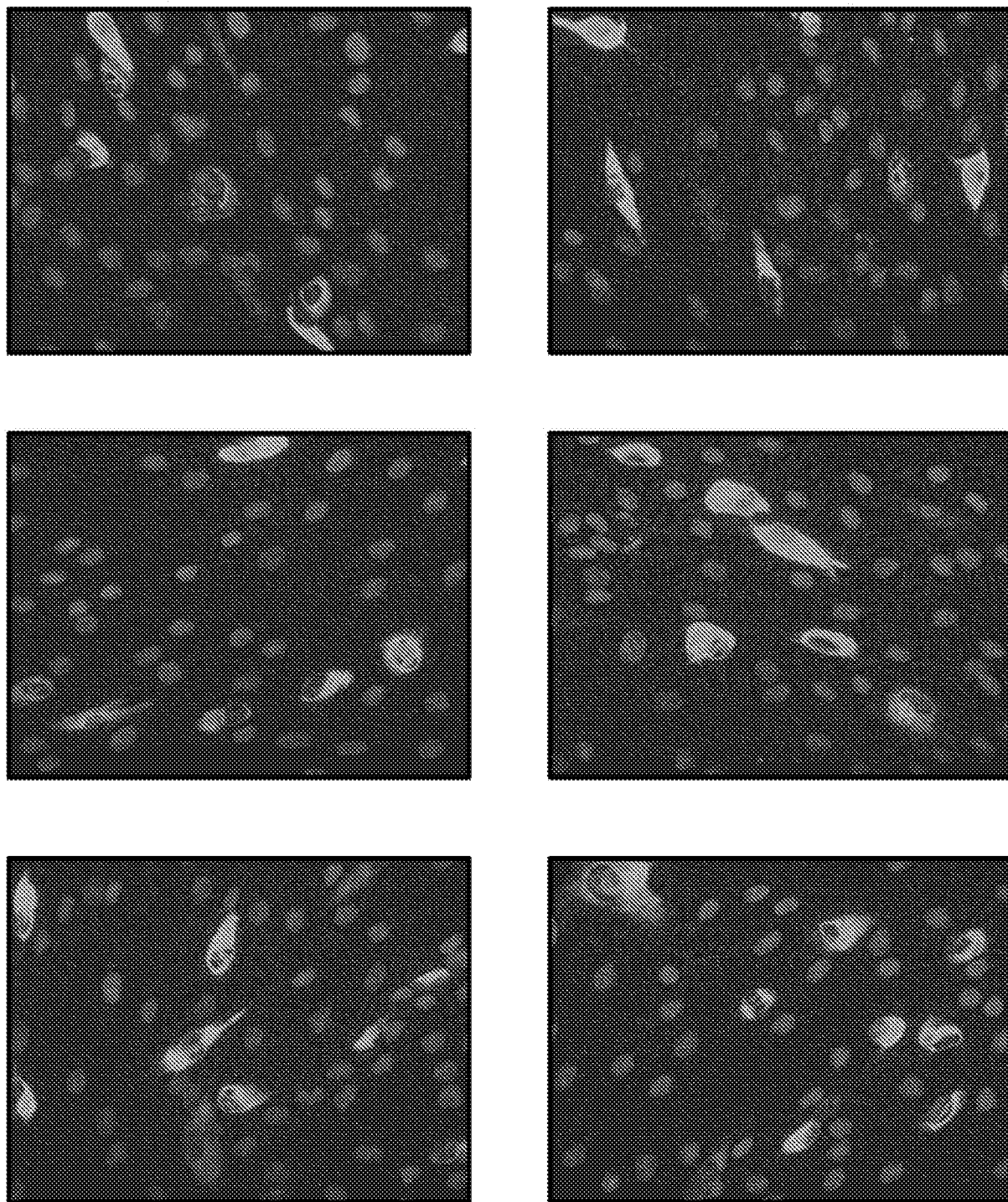


FIG. 5K

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/038340

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/12; A61K 39/39; A61K 47/02; A61K 47/18; A61K 47/26; A61K 47/42 (2017.01)

CPC - A61K 2039/55511; A61K 2039/5555; A61K 2039/55561; A61K 2039/55566; A61K 2039/55572; A61K 2039/58; G01N 33/56983 (2017.08)

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 - 435/29; 435/7.1; 435/235.1 (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.
X	US 2013/0315952 A1 (DEFYRUS, INC.) 28 November 2013 (28.10.2013) entire document	1, 3, 4, 8
Y		5-7, 9-14, 25
Y	US 2005/0002916 A1 (JOOSS et al) 06 January 2005 (06.01.2005) entire document	2, 15-33
Y	HAMEL et al. "Biology of Zika Virus Infection in Human Skin Cells," Journal of Virology, 17 June 2015 (17.06.2015) Vol. 89, No. 17, Pgs. 8880-8896. entire document	2, 15-33
Y	US 5,904,920 A (DRANOFF et al) 18 May 1999 (18.05.1999) entire document	5, 7, 10, 26, 27
Y	KRISHNAN, V. "Bharat Biotech Seeks Govt. Approval of Two Zika Vaccine Candidates," The Hindu, 03 February 2016 (03.02.2016), Pgs. 1-2. entire document	6, 13, 14, 30, 31
Y	US 2012/0003255 A1 (BROWN et al) 05 January 2012 (05.01.2012) entire document	9, 11, 12
Y	US 2011/0020393 A1 (KOMIYA et al) 27 January 2011 (27.01.2011) entire document	14, 31
Y	WO 2013/138670 A1 (ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI) 19 September 2013 (19.09.2013) entire document	32, 33
P, X	US 2017/0014502 A1 (BHARAT BIOTECH INTERNATIONAL LIMITED) 19 January 2017 (19.01.2017) entire document	1-33



Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

11 August 2017

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/038340

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	DAWES et al. "Research and Development of Zika Virus Vaccines," NPJ Vaccines, 28 June 2016 (28.06.2016), Vol. 1, No. 16007, Pgs. 1-7. entire document	1-33