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(71) Applicants (for all designated States except US):

TRUSTEES OF BOSTON UNIVERSITY [US/US];
One Silber Way, Boston, MA 02215 (US). BOSTON
MEDICAL CENTER CORPORATION [US/US]; One
Boston Medical Center Place, Boston, MA 02118 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): GEISBERT,
Thomas, William [US/US]; 68 Bay State Road #1,
Boston, MA 02215 (US). CONNOR, John, Hazard [US/
US]; 160 Pine Street Unit 14, Auburndale, MA 02466
(US). EBIHARA, Hideki [JP/JP]; 4-6-1 Shirokanedai,
Minato-ku Tokyo, 108-8639 (JP).

(74) Agent: CLARK, Paul, T.; Clark & Elbing LLP, 101 Fed-
eral Street, Boston, MA 02110 (US).

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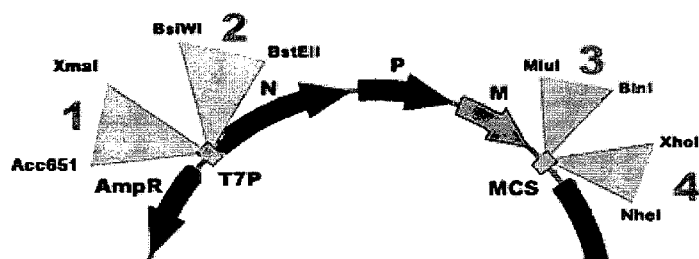
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Figure 1



(57) Abstract: The present invention features compositions and methods for the treatment or inhibition of one or more viruses using a recombinant viral vector that encodes and expresses at least one viral gene from two or more viruses. The viral genes encoded within the recombinant viral vector may be gene sequences from different viruses or may be sequences from different strains of the same virus.

MULTIVALENT VACCINE VECTOR FOR THE TREATMENT AND INHIBITION OF VIRAL INFECTION

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BACKGROUND OF THE INVENTION

Viral infections, in particular infections associated with hemorrhagic fever viruses, may cause severe symptoms in humans and nonhuman primates that can be fatal. With the exception of a vaccine for Junin virus, there are currently no vaccines or treatments targeting hemorrhagic fever viruses licensed for human use. Because of the multitude of strains and species of pathogenic hemorrhagic fever viruses, protection against hemorrhagic fever viruses may require multiple different vaccination regimens. However, interference between the vaccines may occur upon administration, potentially disrupting the efficacy of the vaccinations. Therefore, there is a need in the art for a single vaccine that could provide broad protection against a number of different viruses or different strains of the same virus.

SUMMARY OF THE INVENTION

The present invention features compositions and methods for the treatment or inhibition of one or more viruses using a recombinant viral vector that encodes and expresses all or part of multiple viral proteins (e.g., two, three, or four viral proteins) from one or more viruses. The viral genes contained within the recombinant viral vector may be gene sequences from different viruses, gene sequences from different strains of the same virus, or combinations thereof.

In one embodiment, the invention features a recombinant viral vector that includes all or a portion of at least one gene that is not endogenous to the vector from three or more (e.g., four) different viruses or different viral strains. Alternatively, the vector may contain all or a portion of at least two genes from one of three or more different viruses or different viral strains. In one aspect, the gene(s) of the viral vector encode viral glycoproteins, capsid proteins, tegument proteins, or non-structural proteins, or fragments thereof. Preferably, the vector is a vesicular stomatitis virus (VSV) vector that does not encode the VSV glycoprotein. In one aspect, the vector may contain a VSV matrix protein having one or more mutations that decrease cytopathogenicity of the vector.

In another embodiment, the vector is included in a pharmaceutical composition (e.g., a vaccine). In one aspect, the vaccine treats, reduces, or inhibits infection by at least one of two or more different viruses. In another aspect, the vaccine alleviates one or more symptoms associated with viral infection (e.g., hemorrhagic fever viral infection). Preferably, the composition includes a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant. The pharmaceutical composition is suitable for administration to a human that has been infected with or exposed to at least one of two or more different viruses (e.g., hemorrhagic fever viruses). Preferably, the pharmaceutical composition contains between 1×10^1 and 1×10^8 pfu of the viral vector, more preferably at least 1×10^3 pfu of the viral vector.

In yet another embodiment, the invention features a method of inhibiting or treating infection by three or more different viruses (e.g., hemorrhagic fever viruses) or different strains of the same virus in a subject by administering to the subject the vector described herein in an amount sufficient to inhibit or treat the infection. As described herein, the vector is included in a pharmaceutical composition that inhibits or treats infection by at least one, preferably two, three, or four viruses. In one aspect, the pharmaceutical composition is administered to the subject prior to exposure to the viruses. In another aspect, the composition is administered following exposure of the subject to the viruses. Preferably, the composition is administered to a human by, e.g., injection.

In another embodiment, the invention features a method of inducing an immune response against infection by three or more different viruses in a subject by administering to the subject the vector described herein in an amount sufficient to inhibit or treat the infection.

Preferably, the viruses of the invention are hemorrhagic fever viruses, such as arenaviruses (e.g., Lassa virus (e.g., the Josiah, LP, or GA391 strain), Junin virus, or Machupo virus), filoviruses (e.g., Ebola virus (e.g., the Zaire species, Sudan species, Ivory Coast species, Uganda species, or a new strain or species of Ebola virus) or Marburg virus (e.g., the Angola, Ci67, Musoke, Popp, or Ravn strain)), bunyaviruses, or flaviviruses. Alternatively, the virus of the invention may be, for example, hepatitis C virus, respiratory syncytial virus, Sindbis virus, poliovirus, hepatitis B

virus, human papilloma virus, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, rubella, coxsackevirus, enterovirus, hepatitis A virus, astrovirus, rabies virus, influenza virus A, influenza virus B, measles, mumps, La Crosse virus, California encephalitis virus, Eastern equine encephalitis virus, JC virus, BK virus, herpes simplex virus (type 1), herpes simplex virus (type two), human herpes simplex virus (type six), human herpes virus (type seven), human herpes virus (type eight), human adenovirus, human cytomegalovirus, smallpox virus, Norwalk virus, coronavirus, parainfluenza, rotavirus, Varicella-Zoster virus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus, or any strains of these viruses.

As used herein, by “administering” is meant a method of giving a dosage of a pharmaceutical composition to a subject. The compositions utilized in the methods described herein can be administered by a route selected from, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perilingual, nasal, rectal, 5 topical, and oral. Parenteral administration includes intra-arterial, intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated).

By “an amount sufficient to treat” is meant the amount of a composition 10 administered to improve, inhibit, or ameliorate a condition of a subject, or a symptom of a disorder, in a clinically relevant manner (e.g., improve, inhibit, or ameliorate infection by one or more viruses or viral strains or one or more symptoms that occur following infection). Any improvement in the subject is considered sufficient to achieve treatment. Preferably, an amount sufficient to treat is an amount that reduces, 15 inhibits, or prevents the occurrence or one or more symptoms of a viral infection (e.g., symptoms that result from infection by at least one and preferably two or more viruses or viral strains) or is an amount that reduces the severity of, or the length of time during which a subject suffers from, one or more symptoms of the infection (e.g., by at least 10%, 20%, or 30%, more preferably by at least 50%, 60%, or 70%, and most 20 preferably by at least 80%, 90%, 95%, 99%, or more, relative to a control subject that is not treated with a composition of the invention). A sufficient amount of the pharmaceutical composition used to practice the methods described herein (e.g., the treatment of viral infection(s)) varies depending upon the manner of administration

and the age, body weight, and general health of the subject being treated. A physician or researcher can decide the appropriate amount and dosage regimen.

As used herein, the term “gene” refers to a nucleic acid molecule that either directly or indirectly encodes all or a portion of a nucleic acid or protein product that has a defined biological activity. Exemplary genes contained in the recombinant viral vector of the present invention include, e.g., genes that encode viral glycoproteins, capsid proteins, tegument proteins, and non-structural proteins, or fragments thereof.

By “glycoprotein” is meant a glycoprotein polypeptide, in secreted or transmembrane-bound form, that is encoded by a virus, or any fragment or mutation of the glycoprotein polypeptide, so long as it has the ability to induce or enhance an immune response that confers a protective or therapeutic benefit to the subject, e.g., against a virus (e.g., a hemorrhagic fever virus). The glycoprotein may also include any polypeptide or fragment thereof that is substantially identical (e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or even 100% identical) to the viral glycoprotein over at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 or more contiguous residues.

By “inducing an immune response” is meant eliciting a humoral response (e.g., the production of antibodies) or a cellular response (e.g., the activation of T cells) directed against two or more viruses or viral strains (e.g., two, three, four, or more viruses or viral strains) in a subject to which the pharmaceutical composition (e.g., a vaccine) has been administered.

By “pharmaceutical composition” is meant any composition that contains a therapeutically or biologically active agent (e.g., at least one nucleic acid molecule that encodes all or part of two or more viral proteins (e.g., glycoproteins, capsid proteins, tegument proteins, or non-structural proteins) or all or a portion of at least two viral proteins (e.g., glycoproteins, capsid proteins, tegument proteins, or non-structural proteins), either incorporated into a viral vector or independent of a viral vector) that is suitable for administration to a subject and that is capable of inducing an immune response against at least two different viruses (e.g., two, three, four, or more viruses). For the purposes of this invention, pharmaceutical compositions suitable for delivering a therapeutic or biologically active agent can include, e.g., tablets, gelcaps, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels, hydrogels, oral gels, pastes, eye drops, ointments, creams, plasters,

drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. Any of these formulations can be prepared by well-known and accepted methods of art. See, for example, *Remington: The Science and Practice of Pharmacy* (21st ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2005, and *Encyclopedia of Pharmaceutical Technology*, ed. J. Swarbrick, Informa Healthcare, 2006, each of which is hereby incorporated by reference.

By “pharmaceutically acceptable diluent, excipient, carrier, or adjuvant” is meant a diluent, excipient, carrier, or adjuvant which is physiologically acceptable to the subject while retaining the therapeutic properties of the pharmaceutical composition with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable diluents, excipients, carriers, or adjuvants and their formulations are known to one skilled in the art.

By “recombinant,” with respect to a viral vector, is meant a vector that includes all or a portion of a viral genome that has been incorporated into one or more delivery vehicles and that has been manipulated *in vitro*, e.g., using recombinant nucleic acid techniques to introduce changes to the viral genome (e.g., to include two or more heterologous viral nucleic acid sequences). An example of a recombinant viral vector of the invention is a vector that includes all or part of the VSV genome and that includes a nucleic acid sequence that encodes all or part of, e.g., two or more heterologous viral gene products, such as glycoproteins of at least two different viruses or viral strains (e.g., the glycoproteins of at least two different hemorrhagic fever viruses) that are not endogenous to the viral genome of the viral vector.

By “subject” is meant any animal, e.g., a mammal (e.g., a human).

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

one or more risk factors (e.g., exposure to at least two different viruses or viral strains).

By "treating" is meant administering a pharmaceutical composition for prophylactic and/or therapeutic purposes. Prophylactic treatment may be administered, for example, to a subject who is not yet ill, but who is susceptible to, or otherwise at risk of, a particular disorder, e.g., infection with at least two (and preferably three, four, or more) different viruses or viral strains (e.g., at least two different hemorrhagic fever viruses or viral strains). Therapeutic treatment may be administered, for example, to a subject already suffering from a disorder in order to improve or stabilize the subject's condition (e.g., a subject already infected with at least one virus). Thus, in the claims and embodiments described herein, treating is the administration to a subject either for therapeutic or prophylactic purposes. In some instances, as compared with an equivalent untreated control, treatment may ameliorate a disorder or a symptom thereof by, e.g., 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% as measured by any standard technique. In some instances, treating can result in the inhibition of viral infection by at least two (and preferably three, four, or more) different viruses or viral strains, the treatment of the infection(s), and/or the amelioration of symptoms (e.g., hemorrhagic fever) of the infection(s). Confirmation of treatment can be assessed by detecting an improvement in the treated subject (e.g., the absence of symptoms in the subject), or by the inability to detect the presence of one or more viruses (e.g., one or more hemorrhagic fever viruses) in the treated subject.

The term "vaccine," as used herein, is defined as material used to provoke an immune response and confer immunity after administration of the vaccine to a subject.

As used herein, a "viral vector" is a composition that includes all or a portion of two or more (e.g., three, four, or more) viral genes from two or more different viruses or viral strains that is able to transmit the genetic information to a host or subject so that the host or subject can mount an immune response against the protein product of viral genes or against the nucleic acid encoding the viral genes. The nucleic acid material of the viral vector may be encapsulated, e.g., in a lipid membrane or by structural proteins (e.g., capsid proteins) that may include one or more viral polypeptides (e.g., a glycoprotein). The viral genes of the viral vector may

include, e.g., a nucleic acid that encodes one or more polypeptides (e.g., glycoproteins, capsid proteins, tegument proteins, or non-structural proteins, or fragments thereof) of at least two (and preferably three, four, or more) different viruses or viral strains. The viral vector can be used to infect cells of a subject, which, in turn, promotes the translation into a protein product of the viral genes of the viral vector (e.g., a glycoprotein). The viral vector may also be, e.g., a pseudotyped virus that includes one or more of the polypeptides encoded by the genome of the virus. The viral vector itself can be used to stimulate an immune response that is protective against infection by the virus(es) (e.g., two or more hemorrhagic fever viruses) or that treats infection by the virus(es). Alternatively, the viral vector can be administered to a subject so that it infects one or more cells of the subject, which then promotes expression of the one or more viral genes of the viral vector and stimulates an immune response that is protective against infection by the virus(es) or that treats infection by the virus(es).

The term "virus," as used herein, is defined as any virus that infects humans and any viral strains thereof.

Other features and advantages of the invention will be apparent from the detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic diagram of the cloning sites in the pATX VSV Δ G4 vector.

Fig. 2 is a photograph of Vero E6 cells transfected with the pATX VSV Δ G4 vector. The figure shows Vero E6 cell cultures after blind passage of supernatant from primary transfected Vero E6 cells to fresh Vero E6 cells. Figure 2A is a negative control. Figure 2B is the positive rescue of the pATX VSV Δ G4 vector.

Fig. 3 is graph showing the results of a luciferase assay. Vero E6 cells were infected with different recombinant VSV vectors (MOI = 1). Luciferase expression decreased when a gene was cloned into position 2 of the vector. The highest level of luciferase expression was seen when the gene was cloned into position 3 (L, luciferase gene; G, VSV glycoprotein G gene; 1 to 4, exogenous gene position in pATX VSV Δ G4 vector (see, e.g., Figure 1)).

DETAILED DESCRIPTION

The invention described herein features compositions and methods for the treatment or inhibition of at least one virus (and preferably two or more (e.g., three, four, or more) different viruses or viral strains) using a recombinant viral vector that encodes and expresses all or a portion of one or more viral genes from two or more different viruses or viral strains. The viral genes contained within the recombinant vector may be gene sequences (or fragments thereof) from different viruses or may be gene sequences (or fragments thereof) from different strains of the same virus. Formulation of the vector as a vaccine would result in the production of multiple antigens, which would allow for broader protection against different viruses or different viral strains using a single vaccination approach.

Viruses

The compositions and methods of the invention may be used to treat or inhibit infection by two or more different viruses or viral strains. Exemplary human viruses include, e.g., hepatitis C virus, respiratory syncytial virus, Sindbis virus, poliovirus, hepatitis B virus, human papilloma virus, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, Eastern equine encephalitis virus, smallpox virus, Norwalk virus, coronavirus, parainfluenza, rotavirus, rubella, coxsackevirus, enterovirus, hepatitis A virus, astrovirus, rabies virus, influenza virus A, influenza virus B, measles, mumps, La Crosse virus, California encephalitis virus, JC virus, BK virus, herpes simplex virus (type 1), herpes simplex virus (type two), human herpes simplex virus (type six), human herpes virus (type seven), human herpes virus (type eight), human adenovirus, human cytomegalovirus, Varicella-Zoster virus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

In a preferred embodiment of the invention, the compositions and methods are used to treat and inhibit infection by two or more different hemorrhagic fever viruses or viral strains. Hemorrhagic fever viruses include, e.g., arenaviruses, filoviruses, bunyaviruses, and flaviviruses. Exemplary arenaviruses include Lassa virus (e.g., Josiah, LP, and GA391 strains), Ippy virus, lymphocytic choriomeningitis virus, Mobala virus, Mopeia virus, Amapari virus, Flexal virus, Guanarito virus, Junin virus, Latino virus, Machupo virus, Oliveros virus, Paraná virus, Pichinde virus, Pirital virus, Sabiá virus, Tacaribe virus, Tamiami virus, and Whitewater Arroyo virus.

Exemplary filoviruses include Marburg virus (e.g., Angola, Ci67, Musoke, Popp, and Ravn strains) and Ebola virus (e.g., Ivory Coast, Reston, Sudan, Uganda, and Zaire strains). Exemplary bunyaviruses include Hanta virus, Crimean-Congo hemorrhagic fever virus, Nairovirus (e.g., Dugbe virus), Orthobunyavirus (e.g., Bunyamwera virus), and Phlebovirus (e.g., Rift Valley fever virus). Exemplary flaviviruses include, e.g., Gadgets Gully virus, Kadam virus, Kyasanur Forest disease virus, Langat virus, Omsk hemorrhagic fever virus, Powassan virus, Royal Farm virus, tick-borne encephalitis virus, Louping ill virus, Meaban virus, Saumarez Reef virus, Tyuleniy virus, Aroa virus, Dengue virus, Kedougou virus, Cacipacore virus, Koutango virus, Japanese encephalitis virus, Murray Valley encephalitis virus, St. Louis encephalitis virus, Usutu virus, West Nile virus, Yaounde virus, Kokobera virus, Bagaza virus, Ilheus virus, Israel turkey meningoencephalo-myelitis virus, Ntaya virus, Tembusu virus, Zika virus, Banzi virus, Bouboui virus, Edge Hill virus, Jugra virus, Saboya virus, Sepik virus, Uganda S virus, Wesselsbron virus, yellow fever virus, Entebbe bat virus, Yokose virus, Apoi virus, Cowbone Ridge virus, Jutiapa virus, Modoc virus, Sal Vieja virus, San Perlita virus, Bukalasa bat virus, Carey Island virus, Dakar bat virus, Montana myotis leukoencephalitis virus, Phnom Penh bat virus, and Rio Bravo virus.

Infection with, e.g., hemorrhagic fever viruses in humans and nonhuman primates is often fatal. The compositions and methods described herein utilize viral genes or proteins to confer protection against pathogenic species of viruses. The gene(s) present in the viral vector of the invention may encode or contain, e.g., one or more of a glycoprotein gene, capsid protein gene, tegument protein gene, or non-structural protein gene, or a fragment thereof. The viral proteins have the ability to induce or enhance an immune response that confers a protective or therapeutic benefit to the subject. The gene or protein product may have a mutation or deletion (e.g., an internal deletion, truncation of the amino- or carboxy-terminal, or a point mutation), so long as the mutation or deletion does not interfere with the immune response elicited by the viral protein(s) upon administration of the viral vector. The polypeptide or fragment encoded by the vector that is capable of eliciting an immune response may have 5, 6, 7, 8, 9, 10, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 300, 400, 500, 600 or more amino acid residues.

Multiple genes (e.g., genes not endogenous to the viral vector) may be encoded by the viral vector of the present invention. The vector may encode, e.g., two, three, four, or more genes (e.g., glycoprotein genes) from one or more different viruses (e.g., hemorrhagic fever viruses) or viral strains. The vector may include two
 5 or more genes encoding different proteins from the same virus, from different viruses, or from different strains of the same virus.

The viral gene sequences expressed by the vector may be obtained by any suitable means, including, e.g., application of genetic engineering techniques to a viral source, chemical synthesis techniques, recombinant production, or any combination
 10 thereof. The sequences of many viral genomes are published and nucleic acid sequences encoding viral proteins are available from a variety of sources, including, e.g., GenBank and PubMed (e.g., GenBank No. AF272001 for the ZEBOV Mayinga strain or GenBank No. Z12132 for the MARV Musoke strain). GenBank accession numbers for exemplary viral sequences (e.g., nucleic acid sequences and protein
 15 sequences) are listed in Table 1.

Table 1

Viral Sequence	GenBank Accession Number
ZEBOV (Mayinga strain) GP	AF272001 AAN37507
SEBOV (Gulu strain) GP	AY316199
SEBOV (Boniface strain) GP	U28134
SEBOV (Maleo strain) GP	U23069
ICEBOV GP	U28006
Marburg virus (Musoke strain) GP	Z12132 YP_001531156 NC_001608
Marburg virus (Popp strain) GP	Z29337
Marburg virus (Ravn strain) GP	AF005734
Lassa virus (Josiah strain) GP	NP_694870
Junin virus GP	AF264234
Machupo virus GP	AY571931
Hanta virus GP	AF288658
Rift Valley fever virus GP	M11157

Dengue fever virus GP	AB194885
West Nile virus GP	AY590229
Tick-borne encephalitis virus GP	EU443284
Hepatitis C GP	AM707978 AM710838 AM709167
Respiratory syncytial virus GP	Z33454 AF086868
Hepatitis B GP	AY847270
Human papilloma virus capsid protein	AM922325
Coxsackievirus capsid protein (VP1)	EU372145
Influenza virus (hemagglutinin)	AF131990
Varicella-Zoster virus GP	E01405 E36499
HIV-1 envelope GP	EU010360

Viral Vectors

The invention described herein relates to a multivalent viral vector that expresses multiple viral genes (e.g., genes from at least two (and preferably three, four, or more) different hemorrhagic fever viruses or viral strains) for the inhibition or treatment of an infection caused by one or more viruses (e.g., different viruses, different strains of the same virus, or combinations thereof).

Any suitable viral vector system can be used to construct the vector of the invention including, e.g., an adenovirus vector, a lentivirus vector, a rhabdovirus vector (e.g., vesicular stomatitis virus), or a poxvirus vector. The viral vector contains at least two genes (and preferably three, four, or more genes) from at least two (and preferably three, four, or more) different viruses or viral strains that are not endogenous to the viral vector chosen as the delivery vehicle. These genes may encode, for example, glycoproteins, capsid proteins, tegument proteins, or non-structural proteins or combinations or fragments thereof. Expression of the viral genes is under the control of regulatory sequences that direct the expression of the genes in a cell (e.g., a host cell). Methods of constructing the viral vector and delivering the vector to a cell are known to one of skill in the art.

The sequence of the viral vector may be modified to decrease the cytopathogenicity (e.g., neurovirulence) of the vector upon its administration to a subject (e.g., in the form of a pharmaceutical composition). For example, the viral vector may contain a deletion of one or more gene sequences that are associated with cytopathogenicity (e.g., a glycoprotein or matrix protein) upon its expression. Alternatively, the gene sequences encoding cytopathogenic viral proteins may contain mutations (e.g., substitutions, insertions, or deletions) that decrease the cytopathogenicity of one or more proteins encoded by the vector gene sequence.

Non-Viral Vectors

Non-viral approaches can also be employed for the introduction of therapeutic nucleic acid molecules or proteins into cells to treat or prevent viral infection. For example, a glycoprotein, or nucleic acid molecule encoding the same, from a virus can be introduced into a cell by lipofection (see, e.g., Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413, 1987; Ono et al., *Neuroscience Letters* 17:259, 1990; Brigham et al., *Am. J. Med. Sci.* 298:278, 1989; Staubinger et al., *Methods in Enzymology* 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu et al., *Journal of Biological Chemistry* 263:14621, 1988; Wu et al., *Journal of Biological Chemistry* 264:16985, 1989), or, less preferably, micro-injection under surgical conditions (Wolff et al., *Science* 247:1465, 1990). Gene transfer can also be achieved by the use of calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes, microparticles, or nanoparticles can also be potentially beneficial for delivery of a nucleic acid molecule or a protein (e.g., a gene that encodes a viral glycoprotein or the glycoprotein encoded thereby) into a cell or into a subject in order to stimulate an immune response against the nucleic acid molecule or polypeptide.

Therapy

Therapy according to the methods described herein may be performed alone or in conjunction with another therapy, and may be provided, e.g., at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the therapy depends on the

age and condition of the subject, the severity of the subject's infection, and how the subject responds to the treatment.

Formulation and Administration of the Pharmaceutical Composition

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

- Pharmaceutical compositions according to the invention described herein may be formulated to release the composition immediately upon administration (e.g., targeted delivery of the viral vector) or at any predetermined time period after
- 20 administration using controlled or extended release formulations that will release the viral vector over time.

- Administration of the pharmaceutical compositions (e.g., vaccines) of the present invention can be by any of the routes known to one of skill in the art. Administration may be by, e.g., intramuscular injection. The compositions utilized in
- 25 the methods described herein can also be administered by a route selected from, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perilingual, nasal, rectal, topical administration, and oral administration. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The preferred method of administration can vary depending on
- 30 various factors, e.g., the components of the composition being administered and the severity of the condition being treated. The composition may be administered as a vaccine (e.g., to inhibit, reduce, or prevent infection by one or more (e.g., two, three, four, or more) different viruses or viral strains) or after a subject has been exposed to

a virus. The composition may be administered, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 35, 40, 45, 50, 55, or 60 minutes post-exposure, or may be administered to the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 20, 24, 48, 72 hours, or longer after being exposed to a virus.

5

Dosage

The pharmaceutical compositions of the invention are administered in such an amount as will be therapeutically effective, immunogenic, and/or protective against a pathogenic strain of a virus (e.g., at least 1×10^3 pfus/dose or between 1×10^1 and 1×10^8 pfus/dose). The dosage administered depends on the subject to be treated (e.g., the manner of administration and the age, body weight, capacity of the immune system, and general health of the subject being treated). The composition is administered in an amount to provide a sufficient level of expression that elicits an immune response without undue adverse physiological effects. A physician or researcher can decide the appropriate amount and dosage regimen.

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

Additional Therapeutic Regimens

If desired, the subject may also receive additional therapeutic regimens. For example, an additional therapeutic agent may be administered with the pharmaceutical compositions described herein at concentrations known to be effective for such therapeutic agents. Particularly useful therapeutic agents include, e.g., antiviral agents, immunostimulatory agents, and other immunization vaccines.

Additional therapeutic agents may be delivered separately or may be admixed into a single formulation together with the pharmaceutical composition. When agents are present in different pharmaceutical compositions, different routes of

administration may be employed.

In some instances, the pharmaceutical composition and additional therapeutic agents are administered at least one hour, two hours, four hours, six hours, 10 hours, 12 hours, 18 hours, 24 hours, three days, seven days, fourteen days, or one month apart. The dosage and frequency of administration of each component can be controlled independently. The additional therapeutic agents described herein may be admixed with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for the administration of the compositions of the present invention to a subject. Pharmaceutically acceptable carriers include, for example, water, saline, buffers and other compounds, described, for example, in the Merck Index, Merck & Co., Rahway, New Jersey. A slow release formulation or a slow release apparatus may be also be used for continuous administration. The additional therapeutic regimen may involve other therapies, including modification to the lifestyle of the subject being treated.

Antiviral Agents

Antiviral agents may be used as an additional therapeutic agent, either in combination with the vaccine or in a separate administration. Exemplary antiviral agents are abacavir, aciclovir, acyclovir, adefovir, amantadine, amprenavir, arbidol, atazanavir, atripla, brivudine, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, entry inhibitors, famciclovir, fixed dose combinations, fomivirsen, fosamprenavir, foscarnet, fosfonet, fusion inhibitors, ganciclovir, gardasil, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, integrase inhibitors, interferon type III, interferon type II, interferon type I, interferon, lamivudine, lopinavir, loviride, MK-0518, maraviroc, moroxydine, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitors, reverse transcriptase inhibitors, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, synergistic enhancers, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, and zidovudine. Exemplary antiviral

agents are listed in, e.g., U.S. Patent Nos. 6,093,550 and 6,894,033, hereby incorporated by reference.

Immunostimulatory Agents

5 Immunogenicity of the pharmaceutical composition may be significantly improved if the composition of the present invention is co-administered with an immunostimulatory agent or adjuvant. Exemplary immunostimulatory agents include aluminum phosphate, aluminum hydroxide, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide,
10 glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, DDA, cytokines, and other adjuvants and derivatives thereof.

Immunization Vaccines

15 In some instances, it may be desirable to combine the compositions of the present invention with compositions that induce protective responses against other viruses. For example, the compositions of the present invention can be administered simultaneously, separately, or sequentially with other immunization vaccines, such as those for, e.g., influenza, malaria, tuberculosis, smallpox, measles, rubella, mumps, or
20 any other vaccines known in the art.

EXAMPLES

The present invention is illustrated by the following examples, which are in no way intended to be limiting of the invention.

25

Example 1. Construction of recombinant vectors

A recombinant vesicular stomatitis virus (rVSV) vector lacking the glycoprotein (G) gene was constructed with two cloning sites (restriction sites for MluI/BlnI and XhoI/NheI) for foreign genes positioned between the matrix protein (M) gene and the polymerase (L) gene (see, e.g., Garbutt et al., *J Virol.* 78: 5458-65,
30 2004, and Jones et al., *Nat Med.* 11: 786-90, 2005). An additional multiple cloning site (MCS) positioned upstream of the nucleoprotein (N) gene was added to the vector. The MCS encoded restriction sites for two different restriction enzyme

combinations (Acc651/XmaI and BsiWI/BstEII). This vector (pATX VSV ΔG4) accommodated the insertion of four additional genes into the rVSV vector (Figure 1).

Example 2. Evaluating gene expression of the pATX VSV ΔG4 vector

5 In order to evaluate gene expression in the pATX VSV ΔG4 vector, two genes at different positions within the vector were expressed, including the luciferase gene (as a simple and quantifiable assay of gene expression) and the VSV glycoprotein (VSVG) gene. Expression of VSVG allowed for the production of infectious viral particles. The rescue of the recombinant VSV particles was performed as previously
10 described (see, e.g., Garbutt et al., *J Virol.* 78: 5458-5465, 2004). Following transfection of Vero E6 cells with the pATX VSV ΔG4 vector, cell cultures were screened for the appearance of a VSV-specific cytopathic effect (Figure 2).

 The rescued recombinant VSV vectors expressing the luciferase gene at various positions within the vector were quantified and analyzed for luciferase
15 activity. Vero E6 cells were infected with the different recombinant VSV vectors at a multiplicity of infection (MOI) of 1. Analysis of the different recombinant viruses demonstrated a dramatic decrease in luciferase expression if a gene was cloned into position 2 of the vector, a position upstream of the nucleocapsid gene. The highest level of luciferase expression was obtained by cloning the luciferase gene into
20 position 3, a position downstream of the matrix protein gene (Figure 3).

Example 3. Construction of multivalent rVSV vectors

 Multivalent VSV vaccines expressing hemorrhagic fever viral genes are constructed using any combination of up to four hemorrhagic fever viral genes (or
25 fragments thereof). For example, the glycoprotein gene (GP) of three different species of Ebola virus (e.g., the Zaire strain (ZEBOV), the Sudan strain (SEBOV), and the Ivory Coast strain (ICEBOV)) and a single strain of Marburg virus (e.g., the Musoke strain) are generated by PCR or subcloned from existing plasmids and cloned into different sites of the pATX VSV ΔG4 vector. Two glycoprotein genes can be
30 cloned into positions 3 and 4 (Figure 1) of the pATX VSV ΔG4 vector, as outlined in Table 2. Subsequently, either one or two additional glycoprotein genes can be cloned into positions 1 and 2 (Table 2). Cloning and rescue is performed as previously

described for other recombinant VSV vectors (see, e.g., Garbutt et al., *J Virol.* 78: 5458-5465, 2004).

Table 2

Position 1	Position 2	Position 3	Position 4	Designation
---	---	ZEBOV GP	SEBOV GP	VSV ZEBOV GP / SEBOV GP
---	---	ZEBOV GP	ICEBOV GP	VSV ZEBOV GP / ICEBOV GP
---	---	ICEBOV GP	SEBOV GP	VSV ICEBOV GP / SEBOV GP
---	---	ZEBOV GP	MARV GP	VSV ZEBOV GP / MARV GP
---	---	SEBOV GP	MARV GP	VSV SEBOV GP / MARV GP
---	---	ICEBOV GP	MARV GP	VSV ICEBOV GP / MARV GP
---	SEBOV GP	ZEBOV GP	ICEBOV GP	VSV SEBOV GP / ZEBOV GP / ICEBOV GP
SEBOV GP	ICEBOV GP	ZEBOV GP	MARV GP	VSV SEBOV GP / ICEBOV GP / ZEBOV GP / MARV GP

5

Example 4. Characterization of multivalent rVSV vectors for optimal expression and particle production

Cloning and rescue of the multivalent rVSV vectors are performed as previously described for other recombinant VSV vectors (see, e.g., Garbutt et al., *J Virol.* 78: 5458-5465, 2004). Following successful rescue, the different multivalent rVSV vectors are transfected into Vero E6 cells and other cell lines (e.g., primary macrophages or endothelial cells) and analyzed for their ability to induce viral growth. Depending on the number of exogenous genes in the vector and the position of the genes within the vector, it is expected that the resulting viruses will be attenuated *in vitro*. In particular, insertion of exogenous genes into positions 1 and 2 of the vector is expected to reduce virus growth (see, e.g., Flanagan et al., *J Virol.* 75: 6107-6114, 2001).

Example 5. Rearrangement and mutation of multivalent rVSV vectors to maximize vaccine efficacy and safety

In the event that vaccines formulated with multivalent VSV vectors that
5 express hemorrhagic fever viral genes show unacceptable neurovirulence in animals, the vector backbone is modified. A significant amount of neurovirulence is associated with the presence of VSVG (see, e.g., Clarke et al., *J Virol.* 81: 2056-2064, 2007) and the glycoprotein gene is completely removed in these vectors. An additional neurovirulence determinant is the viral matrix (M) protein. The M protein
10 is the dominant viral protein that causes cytopathic effects during virus infection. Mutation of the M protein dramatically alters the cytopathic effects of the virus *in vitro* and decreases neurovirulence in animal models (see, e.g., Stojdl et al., *Cancer Cell* 4: 263-275, 2003; Clarke et al., *J Virol.* 81: 2056-2064, 2007; Black et al., *J Virol.* 67: 4814-4821, 1993; Kopecky et al., *J Virol.* 75: 12169-12181, 2001; and
15 Jayakar et al., *J Virol.* 76: 8011-8018, 2002).

To increase the safety of the vaccine strains of the multivalent vector, a set of mutations is sequentially introduced into the M protein gene. Initially, the methionine residue at position 51 is deleted. The alteration of this residue has been shown to remove the inhibition of host gene expression that is normally seen during VSV
20 infection, leading to decreased neurovirulence (Stojdl et al., *Cancer Cell* 4: 263-275, 2003; Cooper et al., *J Virol.* 82: 207-19, 2008). The incorporation of this mutation is labeled as delM51. The effect of altering four additional amino acids in the M protein (amino acid residues 121-124) is also monitored. Altering these residues has been shown to attenuate virus replication through mechanisms related to the alteration of
25 protein synthesis (Connor et al., *J Virol.* 80: 3701-3711, 2006). These mutations are incorporated individually and together in separate recombinant VSV vaccine constructs to assess the effect the mutations have on neurovirulence.

Other Embodiments

All publications and patent applications mentioned in this specification are
30 herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such
5 departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

What is claimed is:

CLAIMS

1. A recombinant viral vector that comprises all or a portion of at least one gene from three or more different viruses, wherein said gene is not endogenous to the vector.
2. The vector of claim 1, wherein said vector comprises all or a portion of at least one gene from three different viruses.
3. The vector of claim 1, wherein said vector comprises all or a portion of at least one gene from four different viruses.
4. The vector of claim 1, wherein said vector comprises all or a portion of at least two genes from one of said three or more different viruses.
5. The vector of claim 1, wherein said viruses comprise three different viruses.
6. The vector of claim 1, wherein said viruses comprise three different strains of the same virus.
7. The vector of claim 1, wherein said viruses are hemorrhagic fever viruses.
8. The vector of claim 7, wherein said hemorrhagic fever viruses are selected from the group consisting of an arenavirus, a filovirus, a bunyavirus, or a flavivirus.
9. The vector of claim 8, wherein said arenavirus is Lassa virus, Junin virus, or Machupo virus.
10. The vector of claim 9, wherein said Lassa virus is the Josiah strain, LP strain, or GA391 strain.

11. The vector of claim 8, wherein said filovirus is Ebola virus or Marburg virus.

12. The vector of claim 11, wherein said Ebola virus is the Zaire species, Sudan species, Ivory Coast species, Uganda species, or a new strain or species of Ebola virus.

13. The vector of claim 11, wherein said Marburg virus is the Angola, Ci67, Musoke, Popp, or Ravn strain.

14. The vector of claim 1, wherein each of said viruses is selected from the group consisting of hepatitis C virus, respiratory syncytial virus, Sindbis virus, poliovirus, hepatitis B virus, human papilloma virus, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, rubella, coxsackevirus, enterovirus, hepatitis A virus, astrovirus, rabies virus, influenza virus A, influenza virus B, measles, mumps, La Crosse virus, California encephalitis virus, Eastern equine encephalitis virus, JC virus, BK virus, herpes simplex virus (type 1), herpes simplex virus (type two), human herpes simplex virus (type six), human herpes virus (type seven), human herpes virus (type eight), human adenovirus, human cytomegalovirus, smallpox virus, Norwalk virus, coronavirus, parainfluenza, rotavirus, Varicella-Zoster virus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

15. The vector of claim 1, wherein said gene is selected from a viral glycoprotein, a capsid protein, a tegument protein, or a non-structural protein, or a fragment thereof.

16. The vector of claim 15, wherein said gene is a viral glycoprotein.

17. The vector of claim 1, wherein said vector is a vesicular stomatitis virus (VSV) vector.

18. The vector of claim 1, wherein said vector does not encode the VSV glycoprotein.

19. The vector of claim 1, wherein said vector comprises a VSV matrix protein having one or more mutations that decrease cytopathogenicity of said vector.

20. A pharmaceutical composition comprising the vector of claim 1.

21. The composition of claim 20, further comprising a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

22. The composition of claim 20, wherein said pharmaceutical composition is suitable for administration to a human.

23. The composition of claim 20, wherein said pharmaceutical composition is a vaccine.

24. The composition of claim 23, wherein said vaccine treats, reduces, or inhibits infection by at least one of said two or more different viruses.

25. The composition of claim 24, wherein said viruses are hemorrhagic fever viruses.

26. The composition of claim 20, wherein said pharmaceutical composition alleviates one or more symptoms associated with viral infection.

27. The composition of claim 26, wherein said viral infection is a hemorrhagic fever viral infection.

28. The composition of claim 20, wherein said pharmaceutical composition is administered to a subject infected with or exposed to at least one of said two or more different viruses.

29. The composition of claim 28, wherein said viruses are hemorrhagic fever viruses.

30. The composition of claim 20, wherein said pharmaceutical composition comprises between 1×10^1 and 1×10^8 pfu of said viral vector.

31. The composition of claim 30, wherein said pharmaceutical composition comprises at least 1×10^3 pfu of said viral vector.

32. A method of inhibiting or treating infection by three or more different viruses in a subject, said method comprising administering to said subject the vector of claim 1 in an amount sufficient to inhibit or treat said infection.

33. The method of claim 32, wherein said viruses comprise three different viruses.

34. The method of claim 32, wherein said viruses comprise three different strains of the same virus.

35. The method of claim 32, wherein said viruses are hemorrhagic fever viruses.

36. The method of claim 35, wherein said hemorrhagic fever viruses are selected from the group consisting of an arenavirus, a filovirus, a bunyavirus, or a flavivirus.

37. The method of claim 36, wherein said arenavirus is Lassa virus, Junin virus, or Machupo virus.

38. The method of claim 37, wherein said Lassa virus is the Josiah strain, LP strain, or GA391 strain.

39. The method of claim 36, wherein said filovirus is Ebola virus or Marburg virus.

40. The method of claim 39, wherein said Ebola virus is the Zaire species, Sudan species, Ivory Coast species, Uganda species, or a new strain or species of Ebola virus.

41. The method of claim 39, wherein said Marburg virus is the Angola, Ci67, Musoke, Popp, or Ravn strain.

42. The method of claim 32, wherein each of said viruses is selected from the group consisting of hepatitis C virus, respiratory syncytial virus, Sindbis virus, poliovirus, hepatitis B virus, human papilloma virus, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, rubella, coxsackevirus, enterovirus, hepatitis A virus, astrovirus, rabies virus, influenza virus A, influenza virus B, measles, mumps, La Crosse virus, California encephalitis virus, Eastern equine encephalitis virus, JC virus, BK virus, herpes simplex virus (type 1), herpes simplex virus (type two), human herpes simplex virus (type six), human herpes virus (type seven), human herpes virus (type eight), human adenovirus, human cytomegalovirus, smallpox virus, Norwalk virus, coronavirus, parainfluenza, rotavirus, Varicella-Zoster virus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

43. The method of claim 32, wherein said vector is in a pharmaceutical composition.

44. The method of claim 43, wherein said pharmaceutical composition inhibits or treats infection by at least one of said viruses.

45. The method of claim 43, wherein said pharmaceutical composition inhibits or treats infection by at least two of said viruses.

46. The method of claim 43, wherein said pharmaceutical composition inhibits or treats infection by at least three of said viruses.

47. The method of claim 43, wherein said pharmaceutical composition inhibits or treats infection by at least four of said viruses.

48. The method of claim 43, wherein said pharmaceutical composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

49. The method of claim 43, wherein said pharmaceutical composition is suitable for administration to a human.

50. The method of claim 43, wherein said subject is administered said pharmaceutical composition prior to exposure to said viruses.

51. The method of claim 43, wherein said subject is administered said pharmaceutical composition following exposure to said viruses.

52. The method of claim 43, wherein said pharmaceutical composition comprises between 1×10^1 and 1×10^8 pfu of said viral vector.

53. The method of claim 43, wherein said pharmaceutical composition comprises at least 1×10^3 pfu of said viral vector.

54. The method of claim 43, wherein said pharmaceutical composition is administered two or more times to said subject.

55. The method of claim 32, wherein said subject is human.

56. The method of claim 43, wherein said pharmaceutical composition is administered by injection.

57. A method of inducing an immune response against infection by three or more different viruses in a subject, said method comprising administering to said subject the vector of claim 1 in an amount sufficient to inhibit or treat said infection.

58. The method of claim 57, wherein said viruses are three different viruses.

59. The method of claim 57, wherein said viruses are three different strains of the same virus.

60. The method of claim 57, wherein said viruses are hemorrhagic fever viruses.

61. The method of claim 60, wherein said hemorrhagic fever viruses are selected from the group consisting of an arenavirus, a filovirus, a bunyavirus, or a flavivirus.

62. The method of claim 61, wherein said arenavirus is Lassa virus, Junin virus, or Machupo virus.

63. The method of claim 62, wherein said Lassa virus is the Josiah strain, LP strain, or GA391 strain.

64. The method of claim 61, wherein said filovirus is Ebola virus or Marburg virus.

65. The method of claim 64, wherein said Ebola virus is the Zaire species, Sudan species, Ivory Coast species, Uganda species, or a new strain or species of Ebola virus.

66. The method of claim 64, wherein said Marburg virus is the Angola, Ci67, Musoke, Popp, or Ravn strain.

67. The method of claim 57, wherein each of said viruses is selected from the group consisting of hepatitis C virus, respiratory syncytial virus, Sindbis virus, poliovirus, hepatitis B virus, human papilloma virus, Epstein-Barr virus, adeno-associated virus, Venezuela encephalitis virus, rubella, coxsackevirus, enterovirus, hepatitis A virus, astrovirus, rabies virus, influenza virus A, influenza virus B, measles, mumps, La Crosse virus, California encephalitis virus, Eastern equine encephalitis virus, JC virus, BK virus, herpes simplex virus (type 1), herpes simplex

virus (type two), human herpes simplex virus (type six), human herpes virus (type seven), human herpes virus (type eight), human adenovirus, human cytomegalovirus, smallpox virus, Norwalk virus, coronavirus, parainfluenza, rotavirus, Varicella-Zoster virus, HIV-1, HIV-2, HTLV-1, HTLV-2, and human parvovirus.

68. The method of claim 57, wherein said vector is in a pharmaceutical composition.

69. The method of claim 68, wherein said pharmaceutical composition inhibits or treats infection by at least one of said viruses.

70. The method of claim 68, wherein said pharmaceutical composition inhibits or treats infection by at least two of said viruses.

71. The method of claim 68, wherein said pharmaceutical composition inhibits or treats infection by at least three of said viruses.

72. The method of claim 68, wherein said pharmaceutical composition inhibits or treats infection by at least four of said viruses.

73. The method of claim 68, wherein said pharmaceutical composition comprises a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant.

74. The method of claim 68, wherein said pharmaceutical composition is suitable for administration to a human.

75. The method of claim 68, wherein said subject is administered said pharmaceutical composition prior to exposure to said viruses.

76. The method of claim 68, wherein said subject is administered said pharmaceutical composition following exposure to said viruses.

77. The method of claim 67, wherein said pharmaceutical composition comprises between 1×10^1 and 1×10^8 pfu of said viral vector.

78. The method of claim 68, wherein said pharmaceutical composition comprises at least 1×10^3 pfu of said viral vector.

79. The method of claim 68, wherein said pharmaceutical composition is administered two or more times.

80. The method of claim 57, wherein said subject is human.

81. The method of claim 68, wherein said pharmaceutical composition is administered by injection.

Figure 1

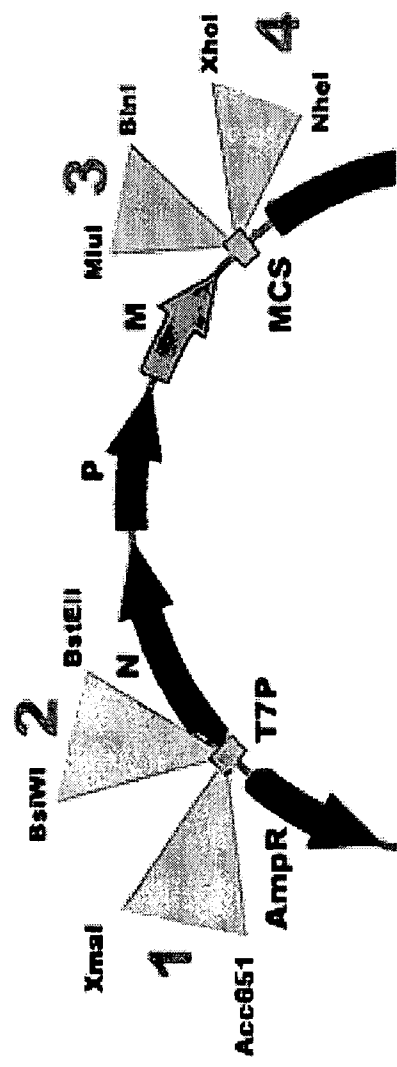
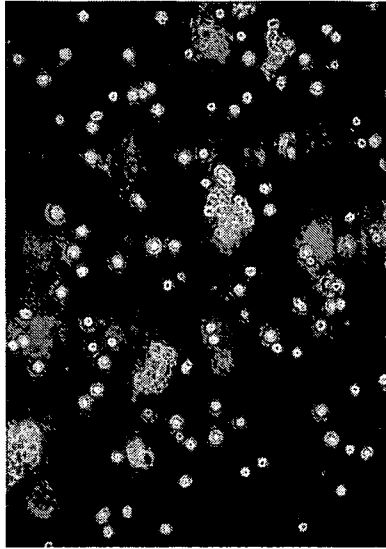
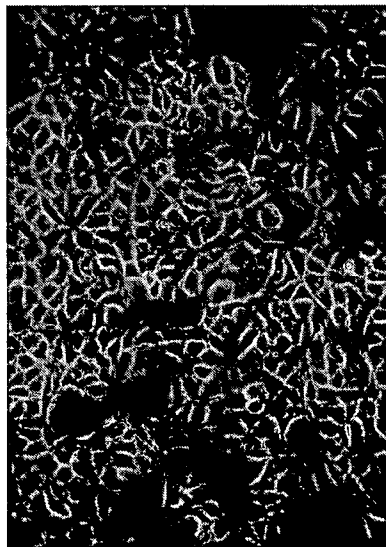


Figure 2

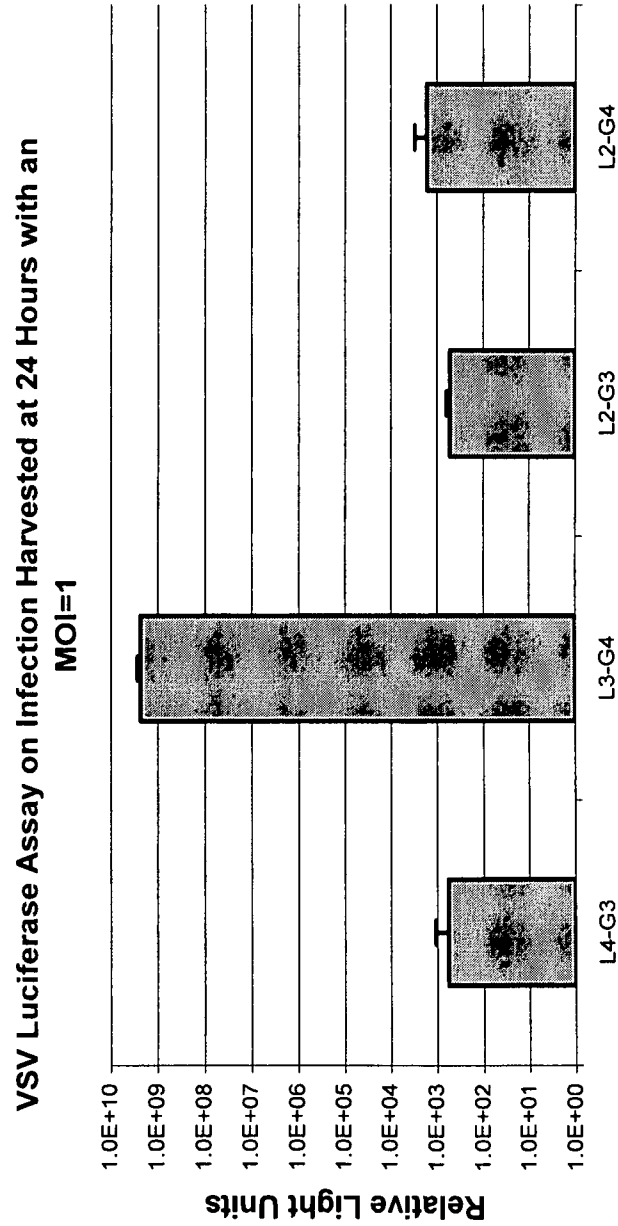


B



A

Figure 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/001821

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/295 C12N15/86 C07K14/145 A61P31/14

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C12N C07K A61P

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/155127 A1 (WANG DANHER [US]) 24 October 2002 (2002-10-24) abstract paragraphs [0011] - [0066], [0163], [0299] - [0317] claims 1-93 figures 1-57 examples 1-3	1-81
X	WANG ET AL: "Complex adenovirus-vectorized vaccine protects guinea pigs from three strains of Marburg virus challenges" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 353, no. 2, 30 September 2006 (2006-09-30), pages 324-332, XP005654108 ISSN: 0042-6822 the whole document	1-81

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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

8 May 2009

Date of mailing of the international search report

08/06/2009

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Ferreira, Roger

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/001821

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GARBUTT MICHAEL ET AL: "Properties of replication-competent vesicular stomatitis virus vectors expressing glycoproteins of filoviruses and arenaviruses." JOURNAL OF VIROLOGY MAY 2004, vol. 78, no. 10, May 2004 (2004-05), pages 5458-5465, XP002527147 ISSN: 0022-538X cited in the application the whole document	1-81
A	SWENSON DANA L ET AL: "Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections." CLINICAL AND VACCINE IMMUNOLOGY : CVI MAR 2008, vol. 15, no. 3, March 2008 (2008-03), pages 460-467, XP002527148 ISSN: 1556-679X Published online 23.01.2008 the whole document	1-81
A	HOLMAN D H ET AL: "Multi-antigen vaccines based on complex adenovirus vectors induce protective immune responses against H5N1 avian influenza viruses" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 26, no. 21, May 2008 (2008-05), pages 2627-2639, XP022639507 ISSN: 0264-410X [retrieved on 2008-03-14] Published online 14.03.2008 the whole document	1-81
A	SCHEPP-BERGLIND JENNIFER ET AL: "Complex adenovirus-mediated expression of West Nile virus C, PreM, E, and NS1 proteins induces both humoral and cellular immune responses." CLINICAL AND VACCINE IMMUNOLOGY : CVI SEP 2007, vol. 14, no. 9, September 2007 (2007-09), pages 1117-1126, XP002527149 ISSN: 1556-6811 Published online 18.07.2007 the whole document	1-81
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/001821

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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