RECOMBINANT ADENOVIRUSES AND USE THEREOF

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Background of the Invention

Recombinant adenoviral vectors have been used in the development of vaccines. To date, approximately 55 different adenovirus serotypes have been identified. The subgroup C adenoviruses have been most extensively studied for applications such as vaccination and gene therapy. Adenovirus serotypes 2 and 5 (Ad2 and Ad5), in particular, are widely used in the field. Importantly, Ad5 vector-based vaccines have been shown to elicit potent and protective immune responses in a variety of animal models. Moreover, large-scale clinical trials for HIV vaccination using Ad5-based recombinant vectors are ongoing (see, e.g., WO 01/02607; WO 02/22080; Shiver et al., *Nature*. 415:331-335, 2002; Letvin et al., *Annu. Rev. Immunol.* 20:73-99, 2002; and Shiver and Emini, *Annu. Rev. Med.* 55:355, 2004).

The usefulness of recombinant Ad5 vector-based vaccines for HIV and other pathogens, however, may be limited due to high pre-existing anti-Ad5 immunity in human populations. The presence of anti-Ad5 immunity has been correlated with a reduction in the immunogenicity of Ad5-based vaccines in studies in mice and rhesus monkeys. Early data from phase-1 clinical trials show that this problem may also occur in humans. Although both Ad5-specific neutralizing antibodies (NAbs) and $CD8^+$ T lymphocytes contribute to anti-Ad5 immunity, the Ad5-specific NAbs appear to play the primary role in this process (Sumida et al., *J. Virol.*, 174:7179-7185, 2004).

Accordingly, there is an unmet need in the field for alternative adenoviral vectors that have low seroprevalence and potent immunogenicity.

Summary of the Invention

The entire genomes of three novel simian adenoviruses (sAds), sAd4287, sAd4310A, and sAd4312, have been identified and their entire genomes determined. These adenoviruses exhibit both surprisingly low seroprevalence and potent immunogenicity, which suggests that these viruses may be useful as novel vaccine vector candidates. In a first aspect, this invention features isolated polynucleotides including a nucleotide sequence that is at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of any one of SEQ ID NOs: 1-3, or its complement. SEQ ID NOs: 1, 2, and 3 are the full-length genome sequence of wild-type sAd4287, sAd4310A, and sAd4312, respectively. The isolated polynucleotides of the invention may include at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 15000, 20000, 25000, 30000, or 35000 or more contiguous or non-contiguous nucleotides of a reference polynucleotide molecule (e.g., SEQ ID NOs: 1-3).

In some embodiments, the isolated polynucleotides of the invention include a nucleotide sequence that is at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95%

identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of any one of SEQ ID NOs: 4-12, or its complement. SEQ NOs: 4-12 feature the nucleotide sequences encoding the fiber-1, fiber-2, and hexon proteins of wild-type sAd4287, sAd4310A, and sAd4312. Accordingly, in some embodiments, the nucleotide sequence encoding all or a portion of the fiber-1 protein can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the fiber-1 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 4, 5, and 6, respectively. In some embodiments, the nucleotide sequence encoding all or a portion of the fiber-2 protein can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the fiber-2 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 7, 8, and 9, respectively. In some embodiments, the nucleotide sequence encoding all or a portion of the hexon protein can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the hexon protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 10, 11, and 12, respectively. In some embodiments, the nucleotide sequence can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of one or more hexon protein hypervariable regions (HVRs) (e.g., HVR1 (nt 403 to nt 489), HVR2 (nt 520 to nt 537), HVR3 (nt 592 to nt 618), HVR4 (nt 706 to nt 744), HVR5 (nt 763 to 786), HVR6 (nt 856 to nt 874), and/or HVR7 (nt 1201 to nt 1296) of sAd4287 hexon protein (SEQ ID NO: 10); HVR1 (nt 403 to nt 477), HVR2 (nt 505 to nt 516), HVR3 (nt 571 to nt 591), HVR4 (nt 679 to nt 690), HVR5 (nt 709 to 735), HVR6 (nt 805 to nt 816), and/or HVR7 (nt 1144 to nt 1236) of sAd4310A hexon protein (SEQ ID NO: 11); or HVR1 (nt 403 to nt 474), HVR2 (nt 505 to nt 522), HVR3 (nt 577 to nt 597), HVR4 (nt 685 to nt 726), HVR5 (nt 748 to 777), HVR6 (nt 847 to nt 864), and/or HVR7 (nt 1192 to nt 1284) of sAd4312 hexon protein (SEQ ID NO: 12)).

In some embodiments, the one or more nucleotide sequences encoding one or more hexon protein hypervariable regions (HVRs) of the invention have been substituted with that of another virus (e.g., HVR1 (nt 403 to nt 489), HVR2 (nt 520 to nt 537), HVR3 (nt 592 to nt 618), HVR4 (nt 706 to nt 744), HVR5 (nt 763 to 786), HVR6 (nt 856 to nt 874), and/or HVR7 (nt 1201 to nt 1296) of sAd4287 hexon protein (SEQ ID NO: 10); HVR1 (nt 403 to nt 477), HVR2 (nt 505 to nt 516), HVR3 (nt 571 to nt 591), HVR4 (nt 679 to nt 690), HVR5 (nt 709 to 735), HVR6 (nt 805 to nt 816), and/or HVR7 (nt 1144 to nt 1236) of sAd4310A hexon protein (SEQ ID NO: 11); or HVR1 (nt 403 to nt 474), HVR2 (nt 505 to nt 522), HVR3 (nt 577 to nt 597), HVR4 (nt 685 to nt 726), HVR5 (nt 748 to 777), HVR6 (nt 847 to nt 864), and/or HVR7 (nt 1192 to nt 1284) of sAd4312 hexon protein (SEQ ID NO: 12)) substituted with the corresponding HVR sequences of one or more other viruses, e.g., an adenovirus, e.g., an adenovirus that has a lower seroprevalence compared to that of Ad5, such as subgroup B (Ad11, Ad34, Ad35, and Ad50) and subgroup D (Ad15, Ad24, Ad26, Ad48, and Ad49) adenoviruses as well as simian adenoviruses (e.g., Pan9, also known as AdC68)). In other embodiments, the nucleotide sequence includes an adenoviral vector backbone of Ad5, Ad11, Ad15, Ad24, Ad26, Ad34, Ad48, Ad49, Ad50, or Pan9/AdC68 having a substitution of all or a portion of one or more of the above hexon HVRs of sAd4287, sAd4310A, and/or sAd4312.

In some embodiments, the isolated polynucleotides of the invention include a nucleotide sequence that is at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of any one of SEQ ID NOs: 13-18, or its complement. SEQ ID NOs: 13-18 feature the nucleotide sequences encoding the knob domain of the fiber-1 and fiber-2 proteins of wild-type sAd4287, sAd4310A, and sAd4312. In some embodiments, the nucleotide sequence encoding all or a portion of the knob domain of fiber-1 can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the knob domain of the fiber-1 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 13, 14, and 15, respectively. In some embodiments, the nucleotide sequence encoding all or a portion of the knob domain of fiber-2 can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the knob domain of the fiber-2 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 16, 17, and 18, respectively. In some embodiments, one or more nucleotide sequences encoding a knob domain of a fiber protein (e.g., a fiber-1 or fiber-2 protein) of the invention (SEQ ID NOs: 13-18) have been substituted with that of another virus.

In a second aspect, the invention features recombinant vectors including an isolated polynucleotide of the invention, the recombinant vectors including a nucleotide sequence that is at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of any one of SEQ ID NOs: 34-51. In some embodiments, the vector is an sAd4297 adenoviral vector including all or a portion of any one of SEQ ID NOs: 34-39. In some embodiments, the vector is an sAd4310A adenoviral vector including all or a portion of any one of SEQ ID NOs: 40-45. In some embodiments, the vector is an sAd4312 adenoviral vector including all or a portion of any one of SEQ ID NOs: 46-51. In other embodiments, more than one (e.g., 2, 3, or 4) of the vectors described by SEQ ID NOs: 34-51 may be used to establish a plasmid system for the generation of a recombinant adenovirus of the invention.

In an embodiment of the first or second aspect of the invention, the isolated polynucleotides and/or recombinant vectors are used to generate recombinant adenoviruses wherein all or a portion of the adenoviruses is derived from any one of SEQ ID NOs: 1-3. In some embodiments, the recombinant adenovirus includes an isolated polynucleotide including a deletion in or of the E1 region (e.g., nt 474 to nt 3085 of sAd4287 (SEQ ID NO: 1); nt 474 to nt 3088 of sAd4310A (SEQ ID NO: 2); and nt 487 to nt 3100 of sAd4312 (SEQ ID NO: 3)). A recombinant adenoviral vector that includes this deletion is rendered replication-defective. In some embodiments, the replication-defective virus may also include a deletion in or of the E3 region (e.g., nt 25973 to nt 28596 of sAd4287 (SEQ ID NO: 1); nt 25915 to nt 28496 of sAd4310A (SEQ ID NO: 2); and nt 25947 to nt 28561 of sAd4312 (SEQ ID NO: 3)) and/or E4 region (e.g., nt 31852 to nt 34752 of sAd4287 (SEQ ID NO: 1); nt 31750 to nt 34048 of sAd4310A (SEQ ID NO: 2); and nt 31818 to nt 34116 of sAd4312 (SEQ ID NO: 3)). In other embodiments, the recombinant adenovirus includes one or more of the E1, E3, and/or E4 regions and is replication-competent.

According to a preferred embodiment, the recombinant adenovirus further includes a heterologous nucleotide sequence encoding an antigenic or therapeutic gene product of interest, or

fragment thereof. In some embodiments, the antigenic gene product, or fragment thereof, includes a bacterial, viral, parasitic, or fungal protein, or fragment thereof.

The bacterial protein, or fragment thereof, may be from *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium leprae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Francisella tularensis*, *Brucella*, *Burkholderia mallei*, *Yersinia pestis*, *Corynebacterium diphtheria*, *Neisseria meningitidis*, *Bordetella pertussis*, *Clostridium tetani*, or *Bacillus anthracis*. Examples of preferred gene products, or fragments thereof, from *Mycobacterium* strains include 10.4, 85A, 85B, 85C, CFP-10, Rv3871, and ESAT-6 gene products or fragments thereof.

The viral protein, or fragment thereof, may be from a virus of the *Retroviridae* family, which includes the human immunodeficiency virus (HIV; e.g., types 1 and 2), and human T-lymphotropic virus Types I and II (HTLV-1 and HTLV-2, respectively); *Flaviviridae* family (e.g., a member of the *Flavivirus, Pestivirus,*and *Hepacivirus* genera), which includes the hepatitis C virus (HCV), Yellow fever virus; tick-borne viruses, such as the Gadgets Gully virus, Kadam virus, Kyasanur Forest disease virus, Langat virus, Omsk hemorrhagic fever virus, Powassan virus, Royal Farm virus, Karshi virus, tick-borne encephalitis virus, Neudoerfl virus, Sofjin virus, Louping ill virus and the Negishi virus; seabird tick-borne viruses, such as the Meaban virus, Saumarez Reef virus, and the Tyuleniy virus; mosquito-borne viruses, such as the 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; and viruses with no known arthropod vector, such as the 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, Rio Bravo virus, Tamana bat virus, and the Cell fusing agent virus; *Arenaviridae* family, which includes the Ippy virus, Lassa virus (e.g., the Josiah, LP, or GA391 strain), lymphocytic choriomeningitis virus (LCMV), 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, Whitewater Arroyo virus, Chapare virus, and Lujo virus; *Bunyaviridae* family (e.g., a member of the *Hantavirus, Nairovirus, Orthobunyavirus*, and *Phlebovirus* genera), which includes the Hantaan virus, Sin Nombre virus, Dugbe virus, Bunyamwera virus, Rift Valley fever virus, La Crosse virus, Punta Toro virus (PTV), California encephalitis virus, and Crimean-Congo hemorrhagic fever (CCHF) virus; *Filoviridae* family, which includes the Ebola virus (e.g., the Zaire, Sudan, Ivory Coast, Reston, and Uganda strains) and the Marburg virus (e.g., the Angola, Ci67, Musoke, Popp, Ravn and Lake Victoria strains); *Togaviridae* family (e.g., a member of the *Alphavirus genus*), which includes the Venezuelan equine encephalitis virus (VEE), Eastern equine encephalitis virus (EEE), Western equine encephalitis virus (WEE), Sindbis virus, rubella virus, Semliki Forest virus, Ross River virus, Barmah Forest virus, O'nyong'nyong virus, and the chikungunya virus; *Poxviridae* family (e.g., a member of the *Orthopoxvirus* genus), which includes the smallpox virus, monkeypox virus, and vaccinia virus; *Herpesviridae* family, which includes the herpes simplex virus (HSV; types 1, 2, and 6), human herpes virus (e.g., types 7 and 8), cytomegalovirus (CMV),

Epstein-Barr virus (EBV), Varicella-Zoster virus, and Kaposi's sarcoma associated-herpesvirus (KSHV); *Orthomyxoviridae* family, which includes the influenza virus (A, B, and C), such as the H5N1 avian influenza virus or H1N1 swine flu; *Coronaviridae* family, which includes the severe acute respiratory syndrome (SARS) virus; *Rhabdoviridae* family, which includes the rabies virus and vesicular stomatitis virus (VSV); *Paramyxoviridae* family, which includes the human respiratory syncytial virus (RSV), Newcastle disease virus, hendravirus, nipahvirus, measles virus, rinderpest virus, canine distemper virus, Sendai virus, human parainfluenza virus (e.g., 1, 2, 3, and 4), rhinovirus, and mumps virus; *Picornaviridae* family, which includes the poliovirus, human enterovirus (A, B, C, and D), hepatitis A virus, and the coxsackievirus; *Hepadnaviridae* family, which includes the hepatitis B virus; *Papillomaviridae* family, which includes the human papillomavirus; *Parvoviridae* family, which includes the adeno-associated virus; *Astroviridae* family, which includes the astrovirus; *Polyomaviridae* family, which includes the JC virus, BK virus, and SV40 virus; *Calciviridae* family, which includes the Norwalk virus; or *Reoviridae* family, which includes the rotavirus. In a preferred embodiment, the viral protein, or fragment thereof, is from human immunodeficiency virus (HIV), human papillomavirus (HPV), hepatitis A virus (Hep A), hepatitis B virus (HBV), hepatitis C virus (HCV), *Variola major*, *Variola minor*, monkeypox virus, measles virus, rubella virus, mumps virus, varicella zoster virus (VZV), poliovirus, rabies virus, Japanese encephalitis virus, herpes simplex virus (HSV), cytomegalovirus (CMV), rotavirus, influenza, Ebola virus, yellow fever virus, or Marburg virus. In a most preferred embodiment, the viral protein, or fragment thereof, from HIV is Gag, Pol, Env, Nef, Tat, Rev, Vif, Vpr, or Vpu.

The parasitic protein, or fragment thereof, may be from *Toxoplasma gondii*, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae*, *Trypanosoma* spp., or *Legionella* spp. Examples of particularly preferred parasitic proteins that may be cloned into the vectors of the present invention include those from *Plasmodium falciparum*, such as the circumsporozoite (CS) protein and Liver Specific Antigens 1 or 3 (LSA-1 or LSA-3).

The fungal protein, or fragment thereof, may be from *Aspergillus*, *Blastomyces dermatitidis*, *Candida*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum var. capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Zygomycetes* spp., *Absidia corymbifera*, *Rhizomucor pusillus*, or *Rhizopus arrhizus*. Examples of fungal gene products, or fragments thereof, include any cell wall mannoprotein (e.g., Afmp1 of *Aspergillus fumigatus*) or suface-expressed glycoprotein (e.g., SOWgp of *Coccidioides immitis*).

The therapeutic gene products, or fragments thereof, may be interferon (IFN) proteins, Factor VIII, Factor IX, erythropoietin, alpha-1 antitrypsin, calcitonin, glucocerebrosidase, growth hormone, low density lipoprotein (LDL), receptor IL-2 receptor and its antagonists, insulin, globin, immunoglobulins, catalytic antibodies, the interleukins, insulin-like growth factors, superoxide dismutase, immune responder modifiers, parathyroid hormone and interferon, nerve growth factors, tissue plasminogen activators, and/or colony stimulating factors, or fragments thereof.

A third aspect of the invention features a method of treating a subject (e.g., a human) having a disease (e.g., HIV or cancer) by administering a recombinant sAd adenovirus vector of the second aspect of the invention to the subject. In a preferred embodiment, the recombinant sAd adenovirus of the invention includes an antigenic gene product, or fragment thereof, that promotes an immune response against an infective agent in a subject at risk of exposure to, or exposed to, the infective agent. In some embodiments, the infective agent is a bacterium, a virus, a parasite, or a fungus, such as those described

above. In one non-limiting example, the administration of a sAd adenovirus of the invention expressing an HIV Gag protein, or fragment thereof, to an HIV-positive subject or a subject with acquired immune deficiency syndrome (AIDS) can stimulate an immune response in the subject against HIV, thereby treating the subject. In another embodiment, the recombinant sAd adenovirus of the invention includes a therapeutic gene product, or fragment thereof, such as an interferon (IFN) protein, or fragment thereof, that provides therapy to a subject having a disease caused by a non-infective agent, such as cancer, by stimulating a favorable immune response in the subject against neoplasia and/or by providing gene therapy, thereby treating the subject. Other non-limiting examples of diseases that may be treated include any human health disease, such as tuberculosis, leprosy, typhoid fever, pneumonia, meningitis, staphylococcal scalded skin syndrome (SSSS), Ritter's disease, tularemia (rabbit fever), brucellosis, Glanders disease, bubonic plague, septicemic plague, pneumonic plague, diphtheria, pertussis (whooping cough), tetanus, anthrax, hepatitis, smallpox, monkeypox, measles, mumps, rubella, chicken pox, polio, rabies, Japanese encephalitis, herpes, mononucleosis, influenza, Ebola virus disease, hemorrhagic fever, yellow fever, Marburg virus disease, toxoplasmosis, malaria, trypanosomiasis, legionellosis, aspergillosis, blastomycosis, candidiasis (thrush), coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis, sporotrichosis, or sinus-orbital zygomycosis. Treatment of these diseases may be by administration of a recombinant sAd vector of the invention that encodes or expresses on its surface an immune response-stimulating antigen from the selected infective agent.

In some embodiments, the recombinant adenovirus or adenoviral vector is administered intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in cremes, or in lipid compositions. In one preferred embodiment, the recombinant adenovirus or adenoviral vector is administered as a pharmaceutical composition that includes a pharmaceutically acceptable carrier, diluent, or excipients, and may optionally include an adjuvant. In some embodiments, the subject is administered at least one dose (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more doses) of the composition. In other embodiments, the subject is administered at least two doses (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more doses) of the composition. In yet another embodiment, the pharmaceutical composition is administered to the subject as a prime boost or in a prime boost regimen. The subject can be administered at least about $1x10^3$ viral particles (vp)/dose or between $1x10^1$ and $1x10^{14}$ vp/dose, preferably between $1x10^3$ and $1x10^{12}$ vp/dose, and more preferably between $1x10^5$ and $1x10^{11}$ vp/dose. The pharmaceutical composition may be administered, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 35, 40, 45, 50, 55, or 60 minutes, 2, 4, 6, 10, 15, or 24 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, or even 3, 4, or 6 months pre-exposure or pre-diagnosis, or may be administered to the subject 15-30 minutes or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 20, 24, 48, or 72 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, 3, 4, 6, or 9 months, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 years or longer post-diagnosis or post-exposure or to the infective agent. When treating disease (e.g., AIDS or cancer), the pharmaceutical compositions of the invention may be administered to the subject either before the occurrence of symptoms or a definitive diagnosis or after diagnosis or symptoms become evident. The pharmaceutical composition may be administered, for example, immediately after

diagnosis or the clinical recognition of symptoms or 2, 4, 6, 10, 15, or 24 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, or even 3, 4, or 6 months after diagnosis or detection of symptoms.

In a fourth aspect, the invention features a method of producing a recombinant adenovirus of the invention that includes culturing a cell in a suitable medium; transfecting the cell with an isolated polynucleotide of the first aspect of the invention or a recombinant vector of the second aspect of the invention; allowing replication of the polynucleotide or vector in the cell; and harvesting the produced recombinant adenovirus from the medium and/or cell. In some embodiments, the cell is a bacterial, plant, or mammalian cell. In a preferred embodiment, the mammalian cell is a PER.55K cell or a Chinese hamster ovary (CHO) cell.

Definitions

By "adenovirus" is meant a medium-sized (90-100 nm), nonenveloped icosahedral virus that includes a capsid and a double-stranded linear DNA genome. The adenovirus can be a naturally occurring, but isolated, adenovirus (e.g., sAd4287, sAd4310A, or sAd4312) or a recombinant adenovirus (e.g., replication-defective or replication competent sAd4287, sAd4310A, or sAd4312, or a chimeric variant thereof).

As used herein, by "administering" is meant a method of giving a dosage of a pharmaceutical composition (e.g., a recombinant adenovirus of the invention) to a subject. The compositions utilized in the methods described herein can be administered, for example, intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in cremes, or in lipid compositions. 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).

Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

By "deletion" of an adenoviral genomic region is meant the partial or complete removal, the disruption (e.g., by an insertion mutation), or the functional inactivation (e.g., by a missense mutation) of a specified genomic region (e.g., the E1, E2, E3, and/or E4 region), or any specific open-reading frame within the specified region.

By "gene product" is meant to include mRNAs or other nucleic acids (e.g., microRNAs) transcribed from a gene as well as polypeptides translated from those mRNAs. In some embodiments, the gene product is from a virus (e.g., HIV) and many include, for example, any one or more of the viral proteins, or fragments thereof, described in, for example, pending U.S. Pub. No. 2012/0076812. In some embodiments, the gene product is a therapeutic gene product, including, but not limited to, interferon proteins, Factor VIII, Factor IX, erythropoietin, alpha-1 antitrypsin, calcitonin, glucocerebrosidase, growth hormone, low density lipoprotein (LDL), receptor IL-2 receptor and its antagonists, insulin, globin, immunoglobulins, catalytic antibodies, the interleukins, insulin-like growth factors, superoxide dismutase,

immune responder modifiers, parathyroid hormone and interferon, nerve growth factors, tissue plasminogen activators, and colony stimulating factors.

By "heterologous nucleic acid molecule" is meant any exogenous nucleic acid molecule that can be incorporated into, for example, an adenovirus of the invention, or polynucleotide or vector thereof, for subsequent expression of a gene product of interest, or fragment thereof, encoded by the heterologous nucleic acid molecule. In a preferred embodiment, the heterologous nucleic acid molecule encodes an antigenic or therapeutic gene product, or fragment thereof, that is a bacterial, viral, parasitic, or fungal protein, or fragment thereof (e.g., a nucleic acid molecule encoding one or more HIV or SIV Gag, Pol, Env, Nef, Tat, Rev, Vif, Vpr, or Vpu gene products, or fragments thereof). The heterologous nucleic acid molecule is one that is not normally associated with the other nucleic acid molecules found in the wild-type adenovirus.

By "isolated" is meant separated, recovered, or purified from a component of its natural environment.

By "pharmaceutical composition" is meant any composition that contains a therapeutically or biologically active agent, such as a recombinant adenoviral vector of the invention, preferably including a heterologous nucleotide sequence encoding an antigenic or therapeutic gene product of interest, or fragment thereof, that is suitable for administration to a subject and that treats a disease (e.g., cancer or AIDS) or reduces or ameliorates one or more symptoms of the disease. For the purposes of this invention, pharmaceutical compositions include vaccines, and pharmaceutical compositions suitable for delivering a therapeutic or biologically active agent can include, for example, 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 (see, e.g., U.S. Pub. No. 2012/0076812).

By "portion" or "fragment" is meant a part of a whole. A portion may comprise at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the entire length of an polynucleotide or polypeptide sequence region. For polynucleotides, for example, a portion may include at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 15000, 20000, 25000, 30000, 35000 or more contiguous nucleotides of a reference polynucleotide molecule. For polypeptides, for example, a portion may include at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 350 or more contiguous amino acids of a reference polypeptide molecule.

By "promotes 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, macrophages, neutrophils, and natural

killer cells) directed against, for example, one or more infective agents (e.g., a bacterium, virus, parasite, fungus, or combination thereof) or protein targets in a subject to which the pharmaceutical composition (e.g., a vaccine) has been administered.

By "recombinant," with respect to a vector or virus, is meant a vector or virus that has been manipulated *in vitro*, such as a vector or virus that includes a heterologous nucleotide sequence (e.g., a sequence encoding an antigenic or therapeutic gene product) or a vector or virus bearing an alteration, disruption, or deletion in a viral E1, E3, and/or E4 region relative to a wild-type viral E1, E3, and/or E4 region.

By "sequence identity" or "sequence similarity" is meant that the identity or similarity between two or more amino acid sequences, or two or more nucleotide sequences, is expressed in terms of the identity or similarity between the sequences. Sequence identity can be measured in terms of "percentage (%) identity," wherein the higher the percentage, the more identity shared between the sequences. Sequence similarity can be measured in terms of percentage similarity (which takes into account conservative amino acid substitutions); the higher the percentage, the more similarilty shared between the sequences. Homologs or orthologs of nucleic acid or amino acid sequences possess a relatively high degree of sequence identity/similarity when aligned using standard methods. Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

A "subject" is a vertebrate, such as a mammal (e.g., primates and humans). Mammals also include, but are not limited to, farm animals (such as cows), sport animals (e.g., horses), pets (such as cats, and dogs), mice, and rats. A subject to be treated according to the methods described herein (e.g., a subject having a disease such as cancer and/or a disease caused by an infective agent, e.g., a bacterium, virus, fungus, or parasite) 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 a biological agent, such as a virus).

As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, such as clinical results. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of extent of disease, disorder, or condition; stabilization (i.e., not worsening) of a state of disease, disorder, or condition; prevention of spread of disease, disorder, or condition; delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable. "Palliating" a disease, disorder, or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment.

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

By "vector" is meant a composition that includes one or more genes (non-structural or structural), or fragments thereof, from a viral species, such as an adenoviral species (e.g., sAd4287, sAd4310A, or sAd4312), that may be used to transmit one or more heterologous genes from a viral or non-viral source to a host or subject. 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 vector can be used to infect cells of a subject, which, in turn, promotes the translation of the heterologous gene(s) of the viral vector into a protein product.

The term "virus," as used herein, is defined as an infectious agent that is unable to grow or reproduce outside a host cell and that infects mammals (e.g., humans) or birds.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figure 1 is a schematic map of the genomic organization of sAd4287.

Figure 2 is a schematic map of plasmid sAdApt4287.Empty.

Figure 3 is a schematic map of plasmid pBr/sAd4287.pIX-pV.

Figure 4 is a schematic map of plasmid pBr/sAd4287.PsiI-rITR.

Figure 5 illustrates the cloning strategy used to obtain plasmid pBr/sAd4287.PsiI-rITR.dE3 and a schematic map of pBr/sAd4287.PsiI-rITR.dE3 relative to that of its parental plasmid pBr/sAd4287.PsiI-rITR.

Figure 6 shows a schematic map of plasmid pBr/sAd4287.PsiI-rITR.dE3.dE4 relative to that of its parental plasmid pBr/sAd4287.PsiI-rITR.dE3.

Figure 7 illustrates the cloning strategy used to obtain plasmid sAdApt4287.E1btg.Empty and a schematic map of sAdApt4287.E1btg.Empty relative to that of its parental plasmid sAdApt4287.Empty.

Figure 8 is a schematic map of the genomic organization of sAd4310 #13-1 (sAd4310A).

Figure 9 is a schematic map of plasmid sAdApt4310A.Empty.

Figure 10 is a schematic map of plasmid pBr/sAd4310A.pIX-pV.

Figure 11 is a schematic map of plasmid pBr/sAd4310A.RsrII-rITR.

Figure 12 shows a schematic map of pBr/sAd4310A.RsrII-rITR.dE3 relative to that of its parental plasmid pBr/sAd4310A.RsrII-rITR.

Figure 13 shows a schematic map of plasmid pBr/sAd4310A.RsrII-rITR.dE3.dE4 relative to that of its parental plasmid pBr/sAd4310A.RsrII-rITR.dE3.

Figure 14 illustrates the cloning strategy used to obtain plasmid sAdApt4310A.E1btg.Empty and a schematic map of sAdApt4310A.E1btg.Empty relative to that of its parental plasmid sAdApt4310A.Empty.

Figure 15 is a schematic map of the genomic organization of sAd4312.

Figure 16 is a schematic map of plasmid sAdApt4312.Empty.

Figure 17 is a schematic map of plasmid pBr/sAd4312.pIX-pV.

Figure 18 is a schematic map of plasmid pBr/sAd4312.pV-rITR.

Figure 19 illustrates the cloning strategy used to obtain plasmid pBr/sAd4312.pV-rITR.dE3 and a schematic map of pBr/sAd4312.pV-rITR.dE3 relative to that of its parental plasmid pBr/sAd4312.pV-rITR.

Figure 20 shows a schematic map of plasmid pBr/sAd4312.pV-rITR.dE3.dE4 relative to that of its parental plasmid pBr/sAd4312.pV-rITR.dE3.

Figure 21 is a schematic map of plasmid sAdApt4312.E1btg.Empty.

Figure 22A is a pie chart showing the relative sAd4287-specific neutralizing antibody (NAb) responses in sub-Saharan humans (n=144; top) and rhesus monkeys (n=108; bottom). The relative number of individuals that fall within each of the four NAb titer categories (<18 = negative, 18-200=low, 201-1000=high, and >1000=high), as assessed by luciferase-based virus neutralization assays, is shown.

Figure 22B is a pie chart showing the relative sAd4310A-specific neutralizing antibody (NAb) responses in sub-Saharan humans (n=144; top) and rhesus monkeys (n=108; bottom). The relative number of individuals that fall within each of the four NAb titer categories (<18 = negative, 18-200=low, 201-1000=high, and >1000=high), as assessed by luciferase-based virus neutralization assays, is shown.

Figure 22C is a pie chart showing the relative sAd4312-specific neutralizing antibody (NAb) responses in sub-Saharan humans (n=144; top) and rhesus monkeys (n=108; bottom). The relative number of individuals that fall within each of the four NAb titer categories (<18 = negative, 18-200=low, 201-1000=high, and >1000=high), as assessed by luciferase-based virus neutralization assays, is shown.

Figure 23A is a graph showing the cellular responses induced by sAd4287 vectors bearing SIVmac239 Gag in C57BL/6 mice immunized with 10^7, 10^8, and 10^9 viral particles (vp) of the vector, as assessed by measuring the $CD8^+$ T cell response via D^b/AL11 tetramer binding assays at days 0, 7, 14, 21, and 28 post-immunization.

Figure 23B is a graph showing the cellular responses induced by sAd4310A vectors bearing SIVmac239 Gag in C57BL/6 mice immunized with 10^7, 10^8, and 10^9 viral particles (vp) of the vector, as assessed by measuring the $CD8^+$ T cell response via D^b/AL11 tetramer binding assays at days 0, 7, 14, 21, and 28 post-immunization.

Figure 24A is a graph showing the cellular responses induced by sAd4287, sAd4310A, and replication-competent sAd4287 (rcsAd4287) at 10^9 vp as determined by IFN-γ ELISPOT assays using splenocytes from C57BL/6 mice on day 28 post-immunization. IFN-γ ELISPOT responses were measured to overlapping Gag peptides (Gag), the dominant $CD8^+$ T cell epitope AL11, the sub-dominant $CD8^+$ T epitope KV9, and the $CD4^+$ T cell epitope DD13.

Figure 24B is a graph showing the cellular responses induced by sAd4287, sAd4310A, and rcsAd4287 at 10^8 vp as determined by IFN-γ ELISPOT assays using splenocytes from C57BL/6 mice on day 28 post-immunization. IFN-γ ELISPOT responses were measured to Gag, the dominant $CD8^+$ T cell epitope AL11, the sub-dominant $CD8^+$ T epitope KV9, and the $CD4^+$ T cell epitope DD13.

Figure 24C is a graph showing the cellular responses induced by sAd4287, sAd4310A, and rcsAd4287 at 10^7 vp as determined by IFN-γ ELISPOT assays using splenocytes from C57BL/6 mice on day 28 post-immunization. IFN-γ ELISPOT responses were measured to Gag, the dominant $CD8^+$ T cell epitope AL11, the sub-dominant $CD8^+$ T epitope KV9, and the $CD4^+$ T cell epitope DD13.

Detailed Description

We have previously identified a variety of novel viruses, including several novel adenoviruses, from rhesus monkeys as part of a metagenomics study (Handley et al. *Cell*. 151(2):253-266, 2012). In the present invention, we isolated, amplified, and purified three novel simian adenoviruses (sAds), sAd4287, sAd4310 #13-1 (sAd4310A), and sAd4312. The three sAds were obtained from the rhesus monkey metagenomics study described above. These viruses are entirely novel and their full sequences have never previously been reported. As these viruses have not yet been officially "named," they do not

yet have an official adenovirus number. Accordingly, the nomenclature used throughout represents our internal laboratory designation.

The complete genome sequence of the novel sAds as well as the vector systems we generated for each of the viruses is described in detail below. We generated recombinant sAd4287, sAd4310A, and sAd4312 vectors expressing a variety of transgenes, including luciferase and SIV Gag. In addition, we demonstrated that these vectors (i) have extremely and surprisingly low seroprevalence in human populations and (ii) exhibit potent immunogenicity in mice. This combination of low baseline anti-vector immunity and potent immunogenicity suggests that these novel adenoviral vectors can be useful in the generation of vaccines against diseases, such as cancer and those caused by an infective agent.

Polynucleotides of the Invention

As a first aspect, the invention provides polynucleotide sequences related to the three novel sAds (sAd4287, sAd4310A, and sAd4312). The isolated polynucleotides may include a nucleotide sequence that is at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of any one of the full-length genome sequence of wild-type sAd4287 (SEQ ID NO: 1), sAd4310A (SEQ ID NO: 2), or sAd4312 (SEQ ID NO: 3), or their complement. The isolated polynucleotides of the invention may include at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 15000, 20000, 25000, 30000, 35000 or more contiguous or non-contiguous nucleotides of SEQ ID NOs: 1-3.

In some embodiments, the polynucleotides of the invention may be used as primers that are between 10-100 nucleotides in length, more particularly between 10-30 nucleotides in length (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length), and can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to any one of SEQ ID NOs: 52-123.

In some embodiments, the polynucleotides of the invention include all or a portion of the nucleotide sequence encoding the fiber-1, fiber-2, and/or hexon protein of wild-type sAd4287, sAd4310A, and/or sAd4312. In some embodiments, the nucleotide sequence encoding all or a portion of the fiber-1 protein can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the fiber-1 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 4, 5, and 6, respectively. The polypeptide sequences of the fiber-1 protein of wild-type sAd4287, sAd4310A, and sAd4312 correspond to SEQ ID NOs: 19, 20, and 21, respectively. In some embodiments, the nucleotide sequence encoding all or a portion of the fiber-2 protein can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the fiber-2 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 7, 8, and 9, respectively. The polypeptide sequences of the fiber-2 protein of wild-type sAd4287, sAd4310A, and sAd4312 correspond to SEQ ID NOs: 22, 23, and 24, respectively. In some embodiments, the nucleotide sequence encoding all or a portion of the hexon protein can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the hexon protein of wild-type sAd4287, sAd4310A, or sAd4312, which

corresponds to SEQ ID NO: 10, 11, and 12, respectively. The polypeptide sequences of the hexon protein of wild-type sAd4287, sAd4310A, and sAd4312 correspond to SEQ ID NOs: 25, 26, and 27, respectively.

In other embodiments, the polynucleotides of the invention include all or a portion of the nucleotide sequence encoding the knob domain of fiber-1 of wild-type sAd4287, sAd4310A, and/or sAd4312. In some embodiments, the nucleotide sequence encoding all or a portion of the knob domain of fiber-1 can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the knob domain of the fiber-1 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 13, 14, or 15, respectively. The polypeptide sequences of the knob domain of the fiber-1 protein of wild-type sAd4287, sAd4310A, and sAd4312 correspond to SEQ ID NOs: 28, 29, and 30, respectively. In some embodiments, the nucleotide sequence encoding all or a portion of the knob domain of fiber-2 can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to the nucleotide sequence encoding the knob domain of the fiber-2 protein of wild-type sAd4287, sAd4310A, or sAd4312, which corresponds to SEQ ID NO: 16, 17, and 18, respectively. The polypeptide sequences of the knob domain of the fiber-2 protein of wild-type sAd4287, sAd4310A, and sAd4312 correspond to SEQ ID NOs: 31, 32, and 33, respectively.

In other embodiments, the polynucleotides of the invention include all or a portion of one or more of the nucleotide sequences encoding the fiber-1, fiber-2, hexon, fiber-1 knob, and/or fiber-2 knob proteins of sAd4287, sAd4310A, and/or sAd4312 and nucleotide sequence from one or more adenoviral vectors including Ad11, Ad15, Ad24, Ad26, Ad34, Ad35, Ad48, Ad49, Ad50, and/or Pan9 (also known as AdC68) directed to the generation of chimeric adenoviral vectors, as discussed below. In other embodiments, the polynucleotides of the invention include all or a portion of one or more of the nucleotide sequences encoding the fiber-1, fiber-2, hexon, fiber-1 knob, and/or fiber-2 knob proteins of sAd4287, sAd4310A, and/or sAd4312 and nucleotide sequence that can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to nucleotide sequence from one or more adenoviral vectors including Ad11, Ad15, Ad24, Ad26, Ad34, Ad35, Ad48, Ad49, Ad50, and/or Pan9 (also known as AdC68). In other embodiments, the polynucleotides of the invention include nucleotide sequence from one or more adenoviral vectors including Ad5, Ad11, Ad15, Ad24, Ad26, Ad34, Ad35, Ad48, Ad49, Ad50, and/or Pan9 (also known as AdC68) and all or a portion of one or more of a nucleotide sequence that can be at least 90% identical (e.g., at least 91%, 92%, 93%, or 94% identical), at least 95% identical (e.g., at least 96%, 97%, 98%, or 99% identical), or 100% identical to all or a portion of one or more of the nucleotide sequences encoding the fiber-1, fiber-2, hexon, fiber-1 knob, and/or fiber-2 knob proteins of sAd4287, sAd4310A, and/or sAd4312.

Vectors of the Invention

The present invention also features recombinant vectors including any one or more of the polynucleotides described above. In some embodiments, one vector of the invention can be used in conjunction with one or more other vectors (e.g., 1, 2, 3, or more vectors) of the invention as a vector system, which can be used to generate recombinant replication-defective sAds (rdsAds) or replication-

competent sAds (rcsAds) of the invention. Accordingly, the invention features novel adenovirus vector systems for each of the three novel sAds (sAd4287, sAd4310A, and sAd4312) described herein. Such vector systems to generate replication-defective adenoviruses are known in the art and have been applied to generate replication competent adenovirus-free batches based of, for example, Ad5, Ad11, Ad35 and Ad49 (see, e.g., WO 97/00326, WO 00/70071; WO 02/40665; U.S. Pub. No. 2005/0232900, all incorporated herein by reference). However, the vectors and vector systems of the present invention, applied towards the sAds sAd4287, sAd4310A, and sAd4312 are novel.

In some embodiments, the vectors of the invention can contain the E1 region (e.g., nt 474 to nt 3085 of sAd4287 (SEQ ID NO: 1); nt 474 to nt 3088 of sAd4310A (SEQ ID NO: 2); and nt 487 to nt 3100 of sAd4312 (SEQ ID NO: 3)) of the specific sAd (e.g., sAd4287, sAd4310A, and sAd4312) for the purposes of producing replication-competent sAd (rcsAd). Such vectors are exemplified, for example, in the .E1btg.Empty vectors of the invention (see, e.g., Figures 7, 14, and 21, which depict the .E1btg.Empty vectors of the invention for each of the three novel adenoviruses).

In some embodiments, the vectors of the invention can contain the left-end sAd sequences and an expression/transgene cassette (see, e.g., Figure 3, depicting the pBr/sAd4287.pIX-pV vector that includes the left part of the sAd4287 genome from approximately pIX to pV). In some embodiments, the expression cassette of the vector replaces or disrupts the E1 region of the specific adenovirus. In preferred embodiments, the expression cassette includes a promoter (e.g., a CMV promoter, e.g., a CMVlong promoter) that stimulates expression of a transgene, and optionally a poly-adenylation signal (e.g., a heterologous nucleotide sequence encoding an antigenic gene product of interest, e.g., a bacterial, viral, parasitic, fungal, or therapeutic protein, or fragment thereof) (see, e.g., Figures 2, 9, and 16, depicting .Empty vectors of the invention for each of the three novel adenoviruses). The E1 region can be deleted (either partially or completely), disrupted, or rendered inactive by one or more mutations.

In some embodiments, the vectors of the invention can contain the left part of the sAd sequences (see, e.g., Figure 3, depicting the pBr/sAd4287.pIX-pV vector that includes the left part of the sAd4287 genome from approximately pIX to pV), which includes the penton base and 52K coding regions of the sAd (see, e.g., Figures 3, 10, and 17, depicting the .pIX-pV vectors of the invention for each of the three novel adenoviruses).

In other embodiments, the vectors of the invention can contain the right part of the sAd sequences (see, e.g., Figure 4, depicting the pBr/sAd4287.PsiI.rITR vector that includes the right part of the sAd4287 genome from approximately pVII to the right ITR (rITR)) (see, e.g., Figures 4, 11, and 18, depicting the .pV-rITR vectors of the invention for each of the three novel adenoviruses). In some embodiments, these vectors may further have a deleted, disrupted, or mutated E3 (e.g., nt 25973 to nt 28596 of sAd4287 (SEQ ID NO: 1); nt 25915 to nt 28496 of sAd4310A (SEQ ID NO: 2); and nt 25947 to nt 28561 of sAd4312 (SEQ ID NO: 3); see Figures 5, 12, and 19, depicting the .dE3 vectors of the invention for each of the three novel adenoviruses) and/or E4 region (e.g., nt 31852 to nt 34752 of sAd4287 (SEQ ID NO: 1); nt 31750 to nt 34048 of sAd4310A (SEQ ID NO: 2); and nt 31818 to nt 34116 of sAd4312 (SEQ ID NO: 3); see Figures 6, 13, and 20, depicting the .dE3.dE4 vectors of the invention for each of the three novel adenoviruses), which are not required for replication and packaging of the adenoviral particle. Deletion of the E3 region is generally preferred if large transgene sequences are to be incorporated into the vector since the genome size which can be packaged into a functional particle is limited to approximately 105% of the wild type size. Although not applied herein, it is to be understood

that other modifications may be introduced in the adenoviral genome, such as deletion of the E2A region, or most if not all of the entire E4 region. In some embodiments, a cell transfected with a vector of the invention can complement these deficiencies by delivering the functionality of the missing regions. The E2A region can be provided by, for instance, a temperature sensitive E2A mutant, or by delivering the E4 functions. Cells that can be used to complement a deficiency of an adenoviral gene (e.g., a E1, E3, and/or E4 deletion) of a vector of the invention include, for example, PER.55K, PER.C6®, and 293 cells. All such systems are known in the art and such modifications of the adenoviral genomes are within the scope of the present invention, which in principal relates to the three novel sAd4287, sAd4310A, and sAd4312 genomic sequences, and the use thereof. As described above, any one vector of the invention can be used in conjunction with one or more other vectors of the invention. In some embodiments, vectors are used which encode both left and right sides of the sAd genome in order to generate a given sAd of the invention.

The present invention also features vectors for the generation of chimeric adenoviruses which include a portion of the sAd4287, sAd4310A, or sAd4312 genome as well as a portion of the genome of one or more other viruses. In some embodiments, the chimeric adenoviral vectors of the invention may include a substitution of all or a portion of the hexon and/or fiber protein. In some embodiments, the portion of the hexon protein substituted with that of another virus is one or more of the hexon protein hypervariable regions (HVRs), for example, HVR1 (nt 403 to nt 489), HVR2 (nt 520 to nt 537), HVR3 (nt 592 to nt 618), HVR4 (nt 706 to nt 744), HVR5 (nt 763 to 786), HVR6 (nt 856 to nt 874), and/or HVR7 (nt 1201 to nt 1296) of sAd4287 hexon protein (SEQ ID NO: 10); HVR1 (nt 403 to nt 477), HVR2 (nt 505 to nt 516), HVR3 (nt 571 to nt 591), HVR4 (nt 679 to nt 690), HVR5 (nt 709 to 735), HVR6 (nt 805 to nt 816), and/or HVR7 (nt 1144 to nt 1236) of sAd4310A hexon protein (SEQ ID NO: 11); or HVR1 (nt 403 to nt 474), HVR2 (nt 505 to nt 522), HVR3 (nt 577 to nt 597), HVR4 (nt 685 to nt 726), HVR5 (nt 748 to 777), HVR6 (nt 847 to nt 864), and/or HVR7 (nt 1192 to nt 1284) of sAd4312 hexon protein (SEQ ID NO: 12). In some embodiments, the portion of the fiber protein substituted with that of another virus is the fiber knob domain. In some embodiments, the substituted regions are replaced with a region derived from an adenovirus that has a lower seroprevalence compared to that of Ad5, such as subgroup B (Ad11, Ad34, Ad35, and Ad50) and subgroup D (Ad15, Ad24, Ad26, Ad48, and Ad49) adenoviruses as well as simian adenoviruses (e.g., Pan9, also known as AdC68). In some embodiments, an adenoviral vector backbone of Ad5, Ad11, Ad15, Ad24, Ad26, Ad34, Ad48, Ad49, Ad50, or Pan9/AdC68 includes a substitution of all or a portion of one or more of the above hexon HVRs of sAd4287, sAd4310A, and/or sAd4312.

Adenoviruses of the Invention

As discussed above, a recombinant adenovirus of the invention derived, at least in part, from sAd4287, sAd4310A, and/or sAd4312 can be generated using the above-described vectors of the invention. These adenoviruses may be rcsAds or rdsAds. rdsAds will include a deleted, disrupted, or mutational inactivation of the E1 region, and may further include a deletion, disruption, or mutational inactivation of the E2, E3, and/or E4 regions. In some embodiments, the adenovirus of the invention may include an antigenic or therapeutic gene product, or fragment thereof, including a bacterial, viral, parasitic, or fungal protein, or fragment thereof. In a preferred embodiment, the antigenic gene product, or fragment thereof, when expressed in a host, or host cells, is capable of eliciting a strong immune response. In some embodiments, the bacterial protein, or fragment thereof, may be derived from

Mycobacterium tuberculosis, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium leprae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Francisella tularensis*, *Brucella*, *Burkholderia mallei*, *Yersinia pestis*, *Corynebacterium diphtheria*, *Neisseria meningitidis*, *Bordetella pertussis*, *Clostridium tetani*, or *Bacillus anthracis*. In some embodiments, the viral protein, or fragment thereof, may be derived from a virus of a viral family selected from the group consisting of *Retroviridae*, *Flaviviridae*, *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, *Togaviridae*, *Poxviridae*, *Herpesviridae*, *Orthomyxoviridae*, *Coronaviridae*, *Rhabdoviridae*, *Paramyxoviridae*, *Picornaviridae*, *Hepadnaviridae*, *Papillomaviridae*, *Parvoviridae*, *Astroviridae*, *Polyomaviridae*, *Calciviridae*, and *Reoviridae*. In some embodiments, the virus is human immunodeficiency virus (HIV), human papillomavirus (HPV), hepatitis A virus (Hep A), hepatitis B virus (HBV), hepatitis C virus (HCV), *Variola major*, *Variola minor*, monkeypox virus, measles virus, rubella virus, mumps virus, varicella zoster virus (VZV), poliovirus, rabies virus, Japanese encephalitis virus, herpes simplex virus (HSV), cytomegalovirus (CMV), rotavirus, influenza, Ebola virus, yellow fever virus, or Marburg virus. In some embodiments, the parasitic protein, or fragment thereof, is from *Toxoplasma gondii*, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae*, *Trypanosoma* spp., or *Legionella* spp. In some embodiments, the fungal protein, or fragment thereof, is from *Aspergillus*, *Blastomyces dermatitidis*, *Candida*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum var. capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Zygomycetes* spp., *Absidia corymbifera*, *Rhizomucor pusillus*, or *Rhizopus arrhizus*. In some embodiments, the therapeutic gene products may be interferon (IFN) proteins, Factor VIII, Factor IX, erythropoietin, alpha-1 antitrypsin, calcitonin, glucocerebrosidase, growth hormone, low density lipoprotein (LDL), receptor IL-2 receptor and its antagonists, insulin, globin, immunoglobulins, catalytic antibodies, the interleukins, insulin-like growth factors, superoxide dismutase, immune responder modifiers, parathyroid hormone and interferon, nerve growth factors, tissue plasminogen activators, and/or colony stimulating factors (see, e.g., U.S. Pat. No. 6,054,288, incorporated by reference herein). In some embodiments, the IFN protein has an amino acid sequence substantially identical (e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identical) to the sequence of a human IFN-α (e.g., IFN-α -1a, IFN-α -1b, IFN-α-2a, IFN-α-2b, and consensus IFN-α (conIFN-α); Figure 1), a human IFN-β (e.g., IFN-β-1a and IFN-β-1b), a human IFN-γ), or an IFN-τ or a polypeptide that demonstrates the same or similar biological activity to an interferon (e.g., at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the activity of a human IFN-α, a human IFN-β, a human IFN-γ, an IFN-τ, or a conIFN-α (see, e.g., U.S. Pat. No. 4,695,623 and U.S. Pub. No. 2011/0000480, incorporated by reference herein, for examples of specific IFN sequences).

Non-limiting examples of bacterial gene products, or fragments thereof, include 10.4, 85A, 85B, 86C, CFP-10, Rv3871, and ESAT-6 gene products, or fragments thereof, of *Mycobacterium*; O, H, and K antigens, or fragments thereof, of *E. coli*; and protective antigen (PA), or fragments thereof, of *Bacillus anthracis*. Non-limiting examples of viral gene products, or fragments thereof, include Gag, Pol, Nef, Tat, Rev, Vif, Vpr, or Vpu, or fragments thereof, of HIV and other retroviruses (see, e.g., U.S. Pub. No. 2012/0076812, incorporated by reference herein); 9D antigen, or fragments thereof, of HSV; Env, or fragments thereof, of all envelope protein-containing viruses. Non-limiting examples of parasitic gene products, or fragments thereof, include circumsporozoite (CS) protein, gamete surface proteins Pfs230 and Pfs48/45, and Liver Specific Antigens 1 or 3 (LSA-1 or LSA-3), or fragments thereof, of *Plasmodium*

falciparum. Non-limiting examples of fungal gene products, or fragments thereof, include any cell wall mannoprotein (e.g., Afmp1 of *Aspergillus fumigatus*) or suface-expressed glycoprotein (e.g., SOWgp of *Coccidioides immitis*).

Methods of Prophylaxis or Treatment Using Compositions of the Invention

The pharmaceutical compositions of the invention can be used as vaccines for treating a subject (e.g., a human) with a disease (e.g., cancer or a disease caused by an infective agent, e.g., AIDS). In particular, the compositions of the invention can be used to treat (pre- or post-exposure) infection by bacteria, including *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium leprae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Francisella tularensis*, *Brucella*, *Burkholderia mallei*, *Yersinia pestis*, *Corynebacterium diphtheria*, *Neisseria meningitidis*, *Bordetella pertussis*, *Clostridium tetani*, or *Bacillus anthracis*; viruses of a viral family selected from the group consisting of *Retroviridae*, *Flaviviridae*, *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, *Togaviridae*, *Poxviridae*, *Herpesviridae*, *Orthomyxoviridae*, *Coronaviridae*, *Rhabdoviridae*, *Paramyxoviridae*, *Picornaviridae*, *Hepadnaviridae*, *Papillomaviridae*, *Parvoviridae*, *Astroviridae*, *Polyomaviridae*, *Calciviridae*, and *Reoviridae*; parasites, including *Toxoplasma gondii*, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae*, *Trypanosoma* spp., or *Legionella* spp.; and fungi, including *Aspergillus*, *Blastomyces dermatitidis*, *Candida*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum var. capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Zygomycetes* spp., *Absidia corymbifera*, *Rhizomucor pusillus*, or *Rhizopus arrhizus*.

Accordingly, in other non-limiting embodiments, the pharmaceutical compositions of the invention can be used to treat a subject (e.g., a human) with acquired immune deficiency syndrome (AIDS), cancer, tuberculosis, leprosy, typhoid fever, pneumonia, meningitis, staphylococcal scalded skin syndrome (SSSS), Ritter's disease, tularemia (rabbit fever), brucellosis, Glanders disease, bubonic plague, septicemic plague, pneumonic plague, diphtheria, pertussis (whooping cough), tetanus, anthrax, hepatitis, smallpox, monkeypox, measles, mumps, rubella, chicken pox, polio, rabies, Japanese encephalitis, herpes, mononucleosis, influenza, Ebola virus disease, hemorrhagic fever, yellow fever, Marburg virus disease, toxoplasmosis, malaria, trypanosomiasis, legionellosis, aspergillosis, blastomycosis, candidiasis (thrush), coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis, sporotrichosis, or sinus-orbital zygomycosis.

Pharmaceutical Formulation and Administration of the Compositions of the Invention

Administration

The pharmaceutical compositions of the invention can be administered to a subject (e.g., a human), pre- or post-exposure to an infective agent (e.g., bacteria, viruses, parasites, fungi) or pre- or post-diagnosis of a disease of a disease without an etiology traceable to an infective agent (e.g., cancer), to treat, prevent, ameliorate, inhibit the progression of, or reduce the severity of one or more symptoms of the disease in the subject. For example, the compositions of the invention can be administered to a subject to treat having AIDS. Examples of symptoms of diseases caused by a viral infection, such as AIDS, that can be treated using the compositions of the invention include, for example, fever, muscle

aches, coughing, sneezing, runny nose, sore throat, headache, chills, diarrhea, vomiting, rash, weakness, dizziness, bleeding under the skin, in internal organs, or from body orifices like the mouth, eyes, or ears, shock, nervous system malfunction, delirium, seizures, renal (kidney) failure, personality changes, neck stiffness, dehydration, seizures, lethargy, paralysis of the limbs, confusion, back pain, loss of sensation, impaired bladder and bowel function, and sleepiness that can progress into coma or death. These symptoms, and their resolution during treatment, may be measured by, for example, a physician during a physical examination or by other tests and methods known in the art.

The compositions utilized in the methods described herein can be formulated, for example, for administration intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, by gavage, in cremes, or in lipid compositions.

The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated). Formulations suitable for oral or nasal 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, tablets, or gels, each containing a predetermined amount of the chimeric Ad5 vector composition of the invention. The pharmaceutical composition may also be an aerosol formulation for inhalation, for example, to the bronchial passageways. Aerosol formulations may be mixed with pressurized, pharmaceutically acceptable propellants (e.g., dichlorodifluoromethane, propane, or nitrogen). In particular, administration by inhalation can be accomplished by using, for example, an aerosol containing sorbitan trioleate or oleic acid, for example, together with trichlorofluoromethane, dichlorofluoromethane, dichlorotetrafluoroethane, or any other biologically compatible propellant gas.

Immunogenicity of the composition of the invention may be significantly improved if it is co-administered with an immunostimulatory agent or adjuvant. Suitable adjuvants well-known to those skilled in the art include, for example, aluminum phosphate, aluminum hydroxide, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, glycolipid analogs, octodecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, DDA, cytokines, and other adjuvants and derivatives thereof.

Pharmaceutical compositions according to the invention described herein may be formulated to release the composition immediately upon administration (e.g., targeted delivery) or at any predetermined time period after administration using controlled or extended release formulations. Administration of the pharmaceutical composition in controlled or extended release formulations is useful where the composition, either alone or in combination, has (i) a narrow therapeutic index (e.g., the difference between the plasma concentration leading to harmful side effects or toxic reactions and the plasma concentration leading to a therapeutic effect is small; generally, the therapeutic index, TI, is defined as the ratio of median lethal dose (LD_{50}) to median effective dose (ED_{50})); (ii) a narrow absorption window at the site of release (e.g., the gastro-intestinal tract); or (iii) a short biological half-life, so that frequent dosing during a day is required in order to sustain a therapeutic level.

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

The compositions of the invention may be administered to provide pre-exposure prophylaxis or after a subject has been diagnosed with a disease having a disease without an etiology traceable to an infective agent (e.g., cancer) or a subject exposed to an infective agent, such as a bacterium, virus, parasite, or fungus. The composition may be administered, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 35, 40, 45, 50, 55, or 60 minutes, 2, 4, 6, 10, 15, or 24 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, or even 3, 4, or 6 months pre-exposure or pre-diagnosis, or may be administered to the subject 15-30 minutes or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 20, 24, 48, or 72 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, 3, 4, 6, or 9 months, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 years or longer post-diagnosis or post-exposure to the infective agent.

When treating disease (e.g., AIDS or cancer), the compositions of the invention may be administered to the subject either before the occurrence of symptoms or a definitive diagnosis or after diagnosis or symptoms become evident. For example, the composition may be administered, for example, immediately after diagnosis or the clinical recognition of symptoms or 2, 4, 6, 10, 15, or 24 hours, 2, 3, 5, or 7 days, 2, 4, 6 or 8 weeks, or even 3, 4, or 6 months after diagnosis or detection of symptoms.

The compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation may be administered in powder form or combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the recombinant replication-defective sAd vector containing a heterologous nucleic acid encoding an antigenic gene product, or fragment thereof, (e.g., an sAd4287, sAd4310A, or sAd4312 HIV Gag delivery vector) and, if desired, one or more immunomodulatory agents, such as in a sealed package of tablets or capsules, or in a suitable dry powder inhaler (DPI) capable of administering one or more doses.

Dosages

The dose of the compositions of the invention (e.g., the number of antigenic gene product-encoding recombinant sAd vectors) or the number of treatments using the compositions of the invention may be increased or decreased based on the severity of, occurrence of, or progression of, the disease in the subject (e.g., based on the severity of one or more symptoms of, e.g., viral infection or cancer).

The pharmaceutical compositions of the invention can be administered in a therapeutically effective amount that provides an immunogenic and/or protective effect against an infective agent or target protein for a disease caused by a non-infective agent. For example, the subject can be

administered at least about $1x10^3$ viral particles (vp)/dose or between $1x10^1$ and $1x10^{14}$ vp/dose, preferably between $1x10^3$ and $1x10^{12}$ vp/dose, and more preferably between $1x10^5$ and $1x10^{11}$ vp/dose.

Viral particles include nucleic acid molecules encoding an antigenic gene product or fragment thereof (e.g., viral structural and non-structural proteins) and are surrounded by a protective coat (a protein-based capsid with hexon and fiber proteins, which may be derived from a single sAd of the invention or a chimeric variant thereof). Viral particle number can be measured based on, for example, lysis of vector particles, followed by measurement of the absorbance at 260 nm (see, e.g,. Steel, Curr. Opin. Biotech., 1999).

The dosage administered depends on the subject to be treated (e.g., the age, body weight, capacity of the immune system, and general health of the subject being treated), the form of administration (e.g., as a solid or liquid), the manner of administration (e.g., by injection, inhalation, dry powder propellant), and the cells targeted (e.g., epithelial cells, such as blood vessel epithelial cells, nasal epithelial cells, or pulmonary epithelial cells). The composition is preferably administered in an amount that provides a sufficient level of the antigenic or therapeutic gene product, or fragment thereof (e.g., a level of an antigenic gene product that elicits an immune response without undue adverse physiological effects in the host caused by the antigenic gene product).

In addition, single or multiple administrations of the compositions of the present invention may be given (pre- or post-exposure and/or pre- or post-diagnosis) to a subject (e.g., one administration or administration two or more times). For example, subjects who are particularly susceptible to, for example, 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, for example, measuring amounts of neutralizing secretory and serum antibodies. The dosages may then be adjusted or repeated as necessary to trigger the desired level of immune response. For example, the immune response triggered by a single administration (prime) of a composition of the invention may not sufficiently potent and/or persistent to provide effective protection. Accordingly, in some embodiments, repeated administration (boost), such that a prime boost regimen is established, can significantly enhance humoral and cellular responses to the antigen of the composition.

Alternatively, the efficacy of treatment can be determined by monitoring the level of the antigenic or therapeutic gene product, or fragment thereof, expressed in a subject (e.g., a human) following administration of the compositions of the invention. For example, the blood or lymph of a subject can be tested for antigenic or therapeutic gene product, or fragment thereof, using, for example, standard assays known in the art (see, e.g., Human Interferon-Alpha Multi-Species ELISA kit (Product No. 41105) and the Human Interferon-Alpha Serum Sample kit (Product No. 41110) from Pestka Biomedical Laboratories (PBL), Piscataway, New Jersey).

A single dose of the compositions of the invention may achieve protection, pre-exposure or pre-diagnosis. In addition, a single dose administered post-exposure or post-diagnosis can function as a treatment according to the present invention.

A single dose of the compositions of the invention can also be used to achieve therapy in subjects being treated for a disease. Multiple doses (e.g., 2, 3, 4, 5, or more doses) can also be administered, in necessary, to these subjects.

Carriers, Excipients, Diluents

The compositions of the invention include sAd5 vectors containing a heterologous nucleic acid molecule encoding an antigenic or therapeutic gene product, or fragment thereof. Therapeutic formulations of the compositions of the invention are prepared using standard methods known in the art by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (20th edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA). Acceptable carriers, include saline, or buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagines, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, PLURONICS™, or PEG.

Optionally, but preferably, the formulation contains a pharmaceutically acceptable salt, preferably sodium chloride, and preferably at about physiological concentrations. Optionally, the formulations of the invention can contain a pharmaceutically acceptable preservative. In some embodiments the preservative concentration ranges from 0.1 to 2.0%, typically v/v. Suitable preservatives include those known in the pharmaceutical arts. Benzyl alcohol, phenol, m-cresol, methylparaben, and propylparaben are preferred preservatives. Optionally, the formulations of the invention can include a pharmaceutically acceptable surfactant at a concentration of 0.005 to 0.02%.

Examples

The following examples are to illustrate the invention. They are not meant to limit the invention in any way.

The practice of this invention may employ, unless otherwise indicated, conventional techniques of molecular biology, cell biology, and recombinant DNA, which are within the skill of the person skilled in the art (see, e.g., Green and Sambrook. *Molecular Cloning: A Laboratory Manuel, 4th edition, 2012*; Ausubel, et al. *Current Protocols in Molecular Biology,* 1987; *Methods in Enzymology.* Academic Press, Inc.; and MacPherson et al. *PCR2: A Practical Approach,* 1995).

Example 1. Sequence of simian adenovirus sAd4287

The total genome sequence of simian adenovirus sAd4287 was determined following the isolation, amplification, and purification of the novel virus obtained from the rhesus monkey metagenomics study of Handley et al. (*Cell.* 151(2):253-266, 2012). The obtained sequence of the sAd4287 genome (35079 nucleotides (nt)) is given as SEQ ID NO: 1. A schematic genome structure of sAd4287 is depicted in Figure 1. Using the full genomic sequence in an NCBI web-based BLAST search, the most closely related virus to sAd4287 was identified as simian adenovirus 1 (sAd1) ATCC VR-195 (query coverage: 93%; maximum identity: 98%). NCBI web-based BLAST searches were also performed to assess homology of three major capsid proteins of sAd4287 (fiber-1, fiber-2, and hexon proteins). The most closely related protein to sAd4287 fiber-1 was identified as sAd1 fiber-1 (query coverage: 100%; maximum identity: 74%). The most closely related protein to sAd4287 fiber-2 was identified as sAd7 long

fiber (query coverage: 100%; maximum identity: 97%). The most closely related protein to sAd4287 hexon was identified as sAd1 hexon (query coverage: 100%; maximum identity: 93%).

Example 2. Generation of recombinant sAd4287 viruses

Here, the construction of an sAd4287 plasmid-based system to generate recombinant sAd4287 vectors in a safe and efficient manner is described. The plasmid system consists of a first plasmid, referred to as an adapter plasmid, which contains sAd4287 nucleotides 1 to 460 including the left inverted terminal repeat (ITR) and packaging signal, an expression cassette and an sAd4287 fragment corresponding to nucleotides 2966 to 5466. The expression cassette comprises the human CMV promoter, a multiple cloning site (MCS), and the SV40 polyadenylation signal (polyA) as previously described (see, e.g., WO 00/70071). The adapter plasmid is based on pAdApt26.Empty (Abbink, et al. *J. Virol.* 81(9): 4654–4663, 2007), albeit now generated to comprise the sAd4287-derived sequences instead of the Ad26-derived sequences. Furthermore, the system consists of other plasmids together constituting sAd4287 sequences between nucleotide 2966 and 35079 that may be deleted for E1 region (nt 474 to nt 3085 of SEQ ID NO: 1), E3 region (nt 25973 to nt 28596 of SEQ ID NO: 1), and/or E4 region (nt 31852 to nt 34752 of SEQ ID NO: 1) sequences.

Generation of adapter plasmid sAdApt4287.Empty

Plasmids that were used for harboring the sAd4287 sequences were prepared. Primers (sAd4287.1A.fwd and sAd4287.1A.rev, SEQ ID NOs: 52 and 53, respectively) were designed to obtain the first 460 nucleotides of sAd4287 by PCR, with PacI and SalI at the 5'- and 3'-end of the resulting PCR product, respectively. A second set of primers (sAd4287.1B.fwd and sAd4287.1B.rev, SEQ ID NOs: 54 and 55, respectively) was designed to obtain pIX (nt 2966) through 2.5 kb upstream (nt 5466), with AflII and PacI designed on the 5'- and 3'- end, respectively. A third set of PCR primers (sAd4287.TGC.fwd and sAd4287.TGC.rev, SEQ ID NOs: 56 and 57, respectively) were designed to obtain the transgene cassette from AdApter plasmid pAdApt26.Empty (Abbink, et al. *J. Virol.* 81(9): 4654–4663, 2007) from start of the CMV to end of the polyA with a SalI and AflII site designed on the 5'- and 3'-end, respectively. These three PCR fragments were ligated together with the pAdApt bacterial backbone obtained by PacI digestion from pAdApt26 in a 4-point ligation, resulting in sAdApt4287.Empty (SEQ ID NO: 34). A schematic map of sAdApt4287.Empty is depicted in Figure 2. This adapter plasmid contains left-end sAd4287 sequences (1-460 and 2966-5466) with the E1 region replaced by an expression/transgene cassette including the CMV promoter.

Generation of pBr/sAd4287.pIX-pV

To enable cloning of an sAd4287 HpaI-HindIII restriction fragment, which encompasses the 52K protein of sAd4287, a new plasmid was generated by inserting two PCR fragments in a pBr backbone. For this, primers (SEQ ID NOs: 58 and 59) were designed to obtain a PCR fragment from start of pIX over the HpaI site in wild-type sAd4287 (nt 2966 to nt 8311) with a PacI and a SbfI designed on the 5'- and 3'-end, respectively. A second PCR fragment was generated from HindIII (nt 12761) to the end of pV (nt 16679), with a SbfI and PacI site designed on the 5'- and 3'-end, respectively. The second PCR fragment was generated using a second primer set (SEQ ID NOs: 60 and 61). These PCR fragments were ligated (PacI-SbfI-PacI) into a pBr backbone, obtained from pBr/Ad26.SfiI (see, e.g., WO

2007/104792) by PacI digestion, resulting in the pBr/sAd4287.pIX-pV shuttle vector. Finally, the sAd4287 HpaI-HindIII restriction fragment obtained from the sAd4287 wild-type genome was ligated into the pBr/sAd4287.pIX-pV shuttle vector digested with HpaI-HindIII, resulting in the complete pBr/sAd4287.pIX-pV plasmid (SEQ ID NO: 35). A schematic map of pBr/sAd4287.pIX-pV is depicted in Figure 3.

Generation of pBr/sAd4287.PsiI-rITR

pBr/sAd4287.PsiI-rITR contains sAd4287 sequences from the PsiI site at nucleotide 14053 to the end of the right inverted terminal repeat (rITR). To enable cloning of this sequence first a new plasmid was generated by inserting two PCR fragments in a pBr backbone. The two PCR fragments were generated such that they could be ligated together and cloned into a pBr-based backbone using the PacI restriction site. Primers were designed to obtain a PCR fragment from before PsiI site at nt 14053 to ~4kb upstream over the NdeI site (nt 18186) at nt 18234, with a PacI and a SbfI site designed on the 5'- and 3'-end, respectively. A second set of primers was designed to obtain a PCR fragment from before PmeI site at nt30022 until the end of rITR at nt35079, with an SbfI and PacI site designed at the 5'- and 3'-end, respectively. The sequences of the primers used to generate these two PCR fragments is set forth in SEQ ID NOs: 62-65. These PCR fragments were ligated into a pBr backbone obtained from pBr/Ad26.SfiI by PacI-SbfI digestion, resulting in the pBr/sAd4287.PsiI-rITR shuttle vector. Finally, the NotI-AsiSI fragment (nt 16639 – nt 34032) was obtained from the wild-type sAd4287 genome and ligated into the pBr/sAd4287.PsiI.rITR shuttle vector, resulting in the complete pBr/sAd4287.PsiI-rITR plasmid (SEQ ID NO: 36). A schematic map of pBr/sAd4287.PsiI-rITR is depicted in Figure 4.

Generation of pBr/sAd4287.PsiI-rITR.dE3

pBr/sAd4287.PsiI-rITR was modified to delete part of the E3 region, which spans approximately nt 25973 to nt 28596 of sAd4287, and which is not required for replication and packaging of the adenoviral particle. To create the pBr/sAd4287.PsiI-rITR.dE3, two PCR fragments were generated. The first PCR fragment contained the pVIII from AscI to 140 bp after the polyA of pVIII (nt 8291-11192). The forward primer (SEQ ID NO: 66) was directed against the ApaLI in 100K and the reverse primer (SEQ ID NO: 67) has a SpeI site designed in it. The second PCR contains the Fiber region starting 100 bp before the polyA of the E3 region until the unique XbaI restriction site in the Fiber-2 region (nt 13177-14824). The forward primer, directed 100bp in front of the polyA of E3, will have a SpeI site designed in it (SEQ ID NO: 68). The reverse primer was directed to the XbaI site (SEQ ID NO: 69). These two PCR fragments were ligated into pBr/sAd4287.PsiI-rITR with a 3-point ligation, with AscI-SpeI-XbaI, to generate pBr/sAd4287.PsiI-rITR.dE3 (SEQ ID NO: 37). Figure 5 depicts a schematic map of pBr/sAd4287.PsiI-rITR.dE3 as well as an overview of the cloning strategy set forth above to generate the E3-deleted plasmid.

Generation of pBr/sAd4287.PsiI-rITR.dE3.dE4

pBr/sAd4287.PsiI-rITR.dE3 was modified to delete part of the E4 region, which spans approximately nt 31852 to nt 34752 of sAd4287, and specifically E4orf1-E4orf4. The modified plasmid, pBr/sAd4287.PsiI-rITR.dE3.dE4 (SEQ ID NO: 38), resulted in an enlarged cloning capacity with a 1409 bp gain of space. To create the pBr/sAd4287.PsiI-rITR.dE3.dE4, two PCR products were generated. The first PCR fragment starts at the XbaI site until the start of E4orf6. The sequences of the forward and

reverse primers used to generate this first PCR fragment are set forth in SEQ ID NOs: 72 and 73, respectively. The second PCR fragment starts directly in front of the E4orf1 until the NotI site. The sequences of the forward and reverse primers used to generate this second PCR fragment are set forth in SEQ ID NOs: 74 and 75, respectively. These PCR fragments have 30-bp overlaps with flanking regions at the XbaI and Not I site and a 15-bp overlap with each other (30 bp total). The PCR fragments were assembled into pBr/sAd4287.PsiI-rITR.dE3 digested with XbaI and NotI by Gibson Assembly (New England BioLabs), resulting in pBr/sAd4287.PsiI-rITR.dE3.dE4. Figure 6 depicts a schematic map of pBr/sAd4287.PsiI-rITR.dE3.dE4 relative to pBr/sAd4287.PsiI-rITR.dE3.

Generation of sAdApt4287.E1btg.Empty

To clone the E1 region of sAd4287 (approximately nt 474 to nt 3085 of SEQ ID NO: 1) into sAdApt4287.Empty for the purposes of producing replication-competent sAd4287 (rcsAd4287), a PCR fragment was generated from the wild-type sAd4287 with the forward primer (SEQ ID NO: 70) starting ~30bp before the NgoMIV site in the IITR region until ~10bp after the polyA of the E1 region (nt 218 to nt 3137). The reverse primer (SEQ ID NO: 71) has a ~30bp overlap with the start of the CMV promoter in the sAdApt4287.Empty and includes the SalI restriction site. This PCR fragment was cloned into sAdApt4287.Empty, digested with NgoMIV and SalI, with Gibson Assembly (New England BioLabs), resulting in sAdApt4287.E1btg.Empty (SEQ ID NO: 39). A schematic map of sAdApt4287.E1btg.Empty and the cloning strategy described above is depicted in Figure 7.

Example 3. Sequence of simian adenovirus sAd4310 #13-1 (sAd4310A)

The total genome sequence of simian adenovirus sAd4310 #13-1 (sAd4310A) was determined as described above for sAd4287. The obtained sequence of the sAd4310A genome (34391 nucleotides) is given as SEQ ID NO: 2. A schematic map of the genome structure of sAd4310A is depicted in Figure 8. Using the full genomic sequence in an NCBI web-based BLAST search, the most closely related virus to sAd4310A was identified as simian adenovirus 1 (sAd1) ATCC VR-195 (query coverage: 97%; maximum identity: 98%). NCBI web-based BLAST searches were also performed to assess homology of three major capsid proteins of sAd4310A (fiber-1, fiber-2, and hexon proteins). The most closely related protein to sAd4310A fiber-1 was identified as sAd1 fiber-1 (query coverage: 100%; maximum identity: 99%). The most closely related protein to sAd4310A fiber-2 was identified as sAd1 fiber-2 (query coverage: 100%; maximum identity: 99%). The most closely related protein to sAd4310A hexon was identified as human Ad31 hexon (query coverage: 100%; maximum identity: 87%).

Example 4. Generation of recombinant sAd4310A viruses

Here, the construction of an sAd4310A plasmid-based system to generate recombinant sAd4310A vectors in a safe and efficient manner is described. The plasmid system consists of a first plasmid, referred to as an adapter plasmid, which contains sAd4310A nucleotides 1 to 461 including the left inverted terminal repeat (IITR) and packaging signal, an expression cassette and an sAd4310A fragment corresponding to nucleotides 2903 to 5410. The expression cassette comprises the human CMV promoter, a multiple cloning site (MCS), and the SV40 polyadenylation signal (polyA) as previously described (see, e.g., WO 00/70071). The adapter plasmid is based on pAdApt26.Empty (Abbink, et al. *J. Virol.* 81(9): 4654–4663, 2007), albeit now generated to comprise the sAd4310A-derived sequences

instead of the Ad26-derived sequences. Furthermore, the system consists of other plasmids together constituting sAd4310A sequences between nucleotide 2903 and 34391 that may be deleted for E1 region (nt 474 to nt 3088 of SEQ ID NO: 2), E3 region (nt 25915 to nt 28496 of SEQ ID NO: 2), and/or E4 region (nt 31750 to nt 34048 of SEQ ID NO: 2) sequences.

Generation of adapter plasmid sAdApt4310A.Empty

Plasmids that were used for harboring the sAd4310A sequences were prepared. Primers (sAd4310A.1A.fwd and sAd4310A.1A.rev, SEQ ID NOs: 76 and 77, respectively) were designed to obtain the first 461 nucleotides of sAd4310A by PCR, with PacI and SalI at the 5'- and 3'-end of the resulting PCR product, respectively. A second set of primers (sAd4310A.1B.fwd and sAd4310A.1B.rev, SEQ ID NOs: 78 and 79, respectively) was designed to obtain pIX (nt 2903) through approximately 2.5 kb upstream (nt 5410), with AflII and PacI designed on the 5'- and 3'- end, respectively. A third set of PCR primers (sAd4310A.TGC.fwd and sAd4310A.TGC.rev, SEQ ID NOs: 80 and 81, respectively) were designed to obtain the transgene cassette from AdApter plasmid pAdApt26.Empty (Abbink, et al. *J. Virol.* 81(9): 4654–4663, 2007) from start of the CMV to end of the polyA with a SalI and AflII site designed on the 5'- and 3'-end, respectively. These three PCR fragments were ligated together with the pAdApt bacterial backbone obtained by PacI digestion from pAdApt26 in a 4-point ligation, resulting in sAdApt4310A.Empty (SEQ ID NO: 40). A schematic map of sAdApt4310A.Empty is depicted in Figure 9. This adapter plasmid contains left-end sAd4310A sequences (1-461 and 2903-5410) with the E1 region replaced by an expression/transgene cassette including the CMV promoter.

Generation of pBr/sAd4310A.pIX-pV

To enable cloning of an sAd4310A SrfI-SnaBI restriction fragment, which encompasses the 52K protein of sAd4310A, a new plasmid was generated by inserting two PCR fragments in a pBr backbone. For this, primers (SEQ ID NOs: 82 and 83) were designed to obtain a PCR fragment from start of pIX over the SrfI site in wild-type sAd4310A (nt 2903 to nt 7224) with a PacI and a SbfI designed on the 5'- and 3'-end, respectively. A second PCR fragment was generated from SnaBI (nt 12098) in pIIIa to pVI (nt 17365), with a SbfI and PacI site designed on the 5'- and 3'-end, respectively. The second PCR fragment was generated using a second primer set (SEQ ID NOs: 84 and 85). These PCR fragments were ligated (PacI-SbfI-PacI) into a pBr backbone, obtained from pBr/Ad26.SfiI (see, e.g., WO 2007/104792) by PacI digestion, resulting in the pBr/sAd4310A.pIX-pV shuttle vector. Finally, the sAd4310A SrfI-SnaBI restriction fragment obtained from the sAd4310A wild-type genome was ligated into the pBr/sAd4310A.pIX-pV shuttle vector digested with SrfI-SnaBI, resulting in the complete pBr/sAd4310A.pIX-pV plasmid (SEQ ID NO: 41). A schematic map of pBr/sAd4310A.pIX-pV is depicted in Figure 10.

Generation of pBr/sAd4310A.RsrII-rITR

pBr/sAd4310A.RsrII-rITR contains sAd4310A sequences from the RsrII site at nucleotide 14882 to the end of the right inverted terminal repeat (rITR) at nucleotide 34391. To enable cloning of this sequence first a new plasmid was generated by inserting two PCR fragments in a pBr backbone. The two PCR fragments were generated such that they could be ligated together and cloned into a pBr-based backbone using the PacI restriction site. Primers (sAd4310A.3A.fwd and sAd4310A.3A.rev, SEQ ID NOs:

86 and 87, respectively) were designed to obtain a PCR fragment from the RsrII site at nt 14882 to ~4.5kb upstream over the SalI site (nt 19189) to nt 19224, with a PacI and a SbfI site designed on the 5'- and 3'-end, respectively. A second set of primers (sAd4310A.3B.fwd and sAd4310A.3B.rev, SEQ ID NOs: 88 and 89, respectively) was designed to obtain a PCR fragment from before the PmeI site at nt 29829 until the end of the rITR at nt 34391, with an SbfI and PacI site designed at the 5'- and 3'-end, respectively. These PCR fragments were ligated into a TOPO vector using the commercially available Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen). The two PCR fragments were digested as PCR fragments or from the TOPO® clone with PacI and SbfI and subsequently ligated into a pBr backbone obtained from pBr/Ad26.SfiI digested with PacI. Finally, SalI-XbaI fragment (nt 19190 – nt 30014) was obtained from the wild-type sAd4310A genome and ligated into the pBr/sAd4310A.RsrII.rITR shuttle vector, resulting in the complete pBr/sAd4310A.RsrII-rITR plasmid (SEQ ID NO: 42). A schematic map of pBr/sAd4310A.RsrII-rITR is depicted in Figure 11.

Generation of pBr/sAd4310A.RsrII-rITR.dE3

pBr/sAd4310A.RsrII-rITR was modified to delete part of the E3 region, which spans approximately nt 25915 to nt 28496 of sAd4310A, and which is not required for replication and packaging of the adenoviral particle. To create the pBr/sAd4310A.RsrII-rITR.dE3 with Gibson Assembly, two PCR fragments were generated. The first PCR fragment (dE3AG) contained from approximately 50 bp before the SfiI site at nt 7644to 140 bp after the polyA of pVIII. The forward primer and reverse primer have sequences set forth in SEQ ID NOs: 90 and 91, respectively, wherein the reverse primer was designed to have an approximately 25-bp overlap with the second PCR fragment. The second PCR fragment (dE3BG) starts at nt 14641 (approximately 100 bp before the polyA of the E3 region) until approximately 50 bp after the XbaI site at nt 16252. The forward primer and reverse primer for the second PCR have sequences set forth in SEQ ID NOs: 92 and 93, respectively, wherein the forward primer was designed to have an approximately 25-bp overlap with the first PCR fragment. The two PCR fragments were assembled with Gibson Assembly, with the pBr/sAd4310A.RsrII.rITR digested with SfiI and XbaI. The resulting plasmid, pBr/sAd4310A.RsrII.rITR.dE3 (SEQ ID NO: 43), is depicted in Figure 12, along with the parental plasmid, pBr/sAd4310A.RsrII.rITR.

Generation of pBr/sAd4310A.RsrII-rITR.dE3.dE4

pBr/sAd4310A.RsrII-rITR.dE3 was modified to delete part of the E4 region, which spans approximately nt 31750 to nt 34048 of sAd4310A, and specifically E4orf1-E4orf4. The modified plasmid, pBr/sAd4310A.RsrII-rITR.dE3.dE4 (SEQ ID NO: 44), resulted in an enlarged cloning capacity with a 1394 bp gain of space. To create the pBr/sAd4310A.RsrII-rITR.dE3.dE4 plasmid, two PCR products were generated. The first PCR fragment starts at the XbaI site until the start of E4orf6. The sequences of the forward and reverse primers used to generate this first PCR fragment are set forth in SEQ ID NOs: 96 and 97, respectively. The second PCR fragment starts directly in front of the E4orf1 until the NotI site. The sequences of the forward and reverse primers used to generate this second PCR fragment are set forth in SEQ ID NOs: 98 and 99, respectively. These PCR fragments have 30-bp overlaps with flanking regions at the XbaI and Not I site and a 15-bp overlap with each other (30 bp total). The PCR fragments were assembled by Gibson Assembly (New England BioLabs) into pBr/sAd4310A.RsrII-rITR.dE3 digested with XbaI and NotI, resulting in pBr/sAd4310A.RsrII-rITR.dE3.dE4 (SEQ ID NO: 44). Figure 13

depicts a schematic map of pBr/sAd4310A.RsrII-rITR.dE3.dE4 relative to the parental plasmid pBr/sAd4310A.RsrII-rITR.dE3.

Generation of sAdApt4310A.E1btg.Empty

To clone the E1 region of sAd4310A (nt 474 to nt 3088 of SEQ ID NO: 2) into sAdApt4310A.Empty for the purposes of producing replication-competent sAd4310A (rcsAd4310A), a PCR fragment was generated from the wild-type sAd4310A with the forward primer (SEQ ID NO: 94) starting ~40bp before the BstZ17I site in the IITR region until ~10bp after the polyA of the E1 region (nt 150 to nt 3131). The reverse primer (SEQ ID NO: 95) has a ~30bp overlap with the start of the CMV promoter in the sAdApt4310A.Empty and includes the SalI restriction site. This PCR fragment was cloned into sAdApt4310A.Empty, digested with BstZ17I and SalI, with Gibson Assembly (New England BioLabs), resulting in sAdApt4310A.E1btg.Empty (SEQ ID NO: 45). A schematic map of sAdApt4310A.E1btg.Empty and the cloning strategy described above is depicted in Figure 14.

Example 5. Sequence of simian adenovirus sAd4312

The total genome sequence of simian adenovirus sAd4312 was determined as described above for sAd4287 and sAd4310A. The obtained sequence of the sAd4312 genome (34475 nucleotides) is given as SEQ ID NO: 3. A schematic map of the genome structure of sAd4312 is depicted in Figure 15. Using the full genomic sequence in an NCBI web-based BLAST search, the most closely related virus to sAd4312 was identified as simian adenovirus 1 (sAd1) ATCC VR-195 (query coverage: 90%; maximum identity: 98%). NCBI web-based BLAST searches were also performed to assess homology of three major capsid proteins of sAd4312 (fiber-1, fiber-2, and hexon proteins). The most closely related protein to sAd4312 fiber-1 was identified as human Ad52 fiber-1 (query coverage: 100%; maximum identity: 99%). The most closely related protein to sAd4312 fiber-2 was identified as sAd7 long fiber (query coverage: 99%; maximum identity: 73%). The most closely related protein to sAd4312 hexon was identified as human Ad40 hexon (query coverage: 100%; maximum identity: 89%).

Example 6. Generation of recombinant sAd4312 viruses

Here, the construction of an sAd4312 plasmid-based system to generate recombinant sAd4312 vectors in a safe and efficient manner is described. The plasmid system consists of a first plasmid, referred to as an adapter plasmid, which contains sAd4312 nucleotides 1 to 472 including the left inverted terminal repeat (IITR) and packaging signal, an expression cassette and an sAd4312 fragment corresponding to nucleotides 2939 to 5510. The expression cassette comprises the human CMV promoter, a multiple cloning site (MCS), and the SV40 polyadenylation signal (polyA) as previously described (see, e.g., WO 00/70071). The adapter plasmid is based on pAdApt26.Empty (Abbink, et al. *J. Virol.* 81(9): 4654–4663, 2007), albeit now generated to comprise the sAd4312-derived sequences instead of the Ad26-derived sequences. Furthermore, the system consists of other plasmids together constituting sAd4312 sequences between nucleotide 2939 and 344475 that may be deleted for E1 region (nt 487 to nt 3100 of SEQ ID NO: 3), E3 region (nt 25947 to nt 28561 SEQ ID NO: 3), and/or E4 region (nt 31818 to nt 34116 SEQ ID NO: 3) sequences.

Generation of adapter plasmid sAdApt4312.Empty

Plasmids that were used for harboring the sAd4312 sequences were prepared. Primers (sAd4312.1A.fwd and sAd4312.1A.rev, SEQ ID NOs: 100 and 101, respectively) were designed to obtain the first 472 nucleotides of sAd4312 by PCR, with PacI and SalI at the 5'- and 3'-end of the resulting PCR product, respectively. A second set of primers (sAd4312.1B.fwd and sAd4312.1B.rev, SEQ ID NOs: 102 and 103, respectively) was designed to obtain pIX (nt 2939) through approximately 2.5 kb upstream (nt 5510), with AflII and PacI designed on the 5'- and 3'- end, respectively. A third set of PCR primers (sAd4312.TGC.fwd and sAd4312.TGC.rev, SEQ ID NOs: 104 and 105, respectively) were designed to obtain the transgene cassette from AdApter plasmid pAdApt26.Empty (Abbink, et al. *J. Virol.* 81(9): 4654–4663, 2007) from start of the CMV to end of the polyA with a SalI and AflII site designed on the 5'- and 3'-end, respectively. These three PCR fragments were ligated together with the pAdApt bacterial backbone obtained by PacI digestion from pAdApt26 in a 4-point ligation, resulting in sAdApt4312.Empty (SEQ ID NO: 46). A schematic map of sAdApt4312.Empty is depicted in Figure 16. This adapter plasmid contains left-end sAd4312 sequences (1-472 and 2939-5510) with the E1 region replaced by an expression/transgene cassette including the CMV promoter.

Generation of pBr/sAd4312.pIX-pV

To enable cloning of an sAd4312 BsiWI-BsiWI restriction fragment, a new plasmid was generated by inserting two PCR fragments in a pBr backbone. For this, primers (SEQ ID NOs: 106 and 107) were designed to obtain a PCR fragment from start of pIX over the BsiWI site in wild-type sAd4312 (nt 2939 to nt 6791) with a PacI and a NdeI designed on the 5'- and 3'-end, respectively. A second PCR fragment was generated from pV (nt 15564) to the RsrII site at the end of pVI (nt 17698), with a NdeI and PacI site designed on the 5'- and 3'-end, respectively. The second PCR fragment was generated using a second primer set (SEQ ID NOs: 108 and 109). These PCR fragments were cloned into a TOPO vector using the commercially available Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen), resulting in the pBr/sAd4312.pIX-pV shuttle vector. Finally, the sAd4312 BsiWI-BsiWI restriction fragment obtained from the sAd4312 wild-type genome was ligated into the pBr/sAd4312.pIX-pV shuttle vector digested with BsiWI and screened for orientation, resulting in the complete pBr/sAd4312.pIX-pV plasmid (SEQ ID NO: 47). A schematic map of pBr/sAd4312.pIX-pV is depicted in Figure 17.

Generation of pBr/sAd4312.pV-rITR

pBr/sAd4312.pV-rITR contains sAd4312 sequences from the start of pV at nucleotide 15215 to the end of the right inverted terminal repeat (rITR) at nucleotide 34475. To enable cloning of this sequence first a new plasmid was generated by inserting two PCR fragments in a pBr backbone. The two PCR fragments were generated such that they could be ligated together and cloned into a pBr-based backbone using the PacI restriction site. Primers (sAd4312.3A.fwd and sAd4312.3A.rev, SEQ ID NOs: 110 and 111, respectively) were designed to obtain a PCR fragment from the start of pV at nt 15215 to ~2.5kb upstream over the RsrII site to nt 17698, with a PacI and a SbfI site designed on the 5'- and 3'-end, respectively. A second set of primers (sAd4312.3B.fwd and sAd4312.3B.rev, SEQ ID NOs: 112 and 113, respectively) was designed to obtain a PCR fragment from before the XbaI site at nt 31015 until the end of the rITR at nt 34475, with an SbfI and PacI site designed at the 5'- and 3'-end, respectively. These PCR fragments were ligated into a TOPO vector using the commercially available Zero Blunt® TOPO®

PCR Cloning Kit (Invitrogen). The two PCR fragments were digested from the TOPO® clones with SbfI and PacI and subsequently ligated into a pBr backbone obtained from pBr/Ad26.SfiI digested with PacI, resulting in the pBr/sAd4312.pV-rITR shuttle vector. Finally, the NotI-XbaI fragment (nt 16412 – nt 31083) was obtained from the wild-type sAd4312 genome and ligated into the pBr/sAd4312.pV-rITR shuttle vector, resulting in the complete pBr/sAd4312.pV-rITR plasmid (SEQ ID NO: 48). A schematic map of pBr/sAd4312.pV-rITR is depicted in Figure 18.

Generation of pBr/sAd4312.pV-rITR.dE3

pBr/sAd4312.pV-rITR was modified to delete part of the E3 region, which spans approximately nt 487 to nt 3100 of sAd4312, and which is not required for replication and packaging of the adenoviral particle. To create the pBr/sAd4312.pV-rITR.dE3, two PCR fragments were generated. The first PCR fragment contains the pVIII from AscI to 140bp after the polyA of pVIII (nt 9859 to nt 12302). The forward primer (sAd4312.dE3A.fwd, SEQ ID NO: 114) is directed against the AscI in 100K, and the reverse primer (sAd4312.dE3A.rev, SEQ ID NO: 115) has a SpeI site designed in it.

The second PCR contains the fiber region starting 100 bp before the polyA of the E3 region until the unique restriction site, XbaI, in the fiber-2 region (nt 14378 to nt 17020). The forward primer (sAd4312.dE3B.fwd, SEQ ID NO: 116), directed 100 bp in front of the polyA of E3, has a SpeI site designed in it. The reverse primer (sAd4312.dE3B.fwd, SEQ ID NO: 117) is directed to the XbaI site. These two PCR fragments were ligated into pBr/sAd4312.pV-rITR with a 3-point ligation, with AscI-SpeI-XbaI. The resulting plasmid, pBr/sAd4312.pV-rITR.dE3 (SEQ ID NO: 49), is depicted in Figure 19, along with the parental plasmid, pBr/sAd4312.pV-rITR.

Generation of pBr/sAd4312.pV-rITR.dE3.dE4

pBr/sAd4312.pV-rITR.dE3 was modified to delete part of the E4 region, which spans approximately nt 25947 to nt 28561 of sAd4312, and specifically E4orf1-E4orf4. The modified plasmid, pBr/sAd4312.pV-rITR.dE3.dE4 (SEQ ID NO: 50), resulted in an enlarged cloning capacity with a 1393 bp gain of space. To create the pBr/sAd4312.pV-rITR.dE3.dE4 plasmid, two PCR products were generated. The first PCR fragment starts at the NdeI site until the start of E4orf6. The sequences of the forward and reverse primers used to generate this first PCR fragment are set forth in SEQ ID NOs: 120 and 121, respectively. The second PCR fragment starts directly in front of the E4orf1 until the NotI site. The sequences of the forward and reverse primers used to generate this second PCR fragment are set forth in SEQ ID NOs: 122 and 123, respectively. These PCR fragments have 30-bp overlaps with flanking regions at the NdeI and NotI site and a 15-bp overlap with each other (30 bp total). The PCR fragments were assembled into pBr/sAd4312.pV-rITR.dE3 digested with XbaI and NotI, resulting in pBr/sAd4312.pV-rITR.dE3.dE4 (SEQ ID NO: 50). Figure 20 depicts a schematic map of pBr/sAd4312.pV-rITR.dE3.dE4 and that of the parental plasmid, pBr/sAd4312.pV-rITR.dE3.

Generation of sAdApt4312.E1btg.Empty

To clone the E1 region of sAd4312 (nt 487 to 3100 SEQ ID NO: 3) into sAdApt4312.Empty for the purposes of producing replication-competent sAd4312 (rcsAd4312), a PCR fragment was generated from the wild-type sAd4312 which included the complete E1 region of sAd4312. The forward primer (SEQ ID NO: 118) is directed to ~40bp in front of the first BstZ17I site in the IITR region. The reverse primer (SEQ

ID NO: 119) has a ~30bp overlap with the start of the CMV promoter in the sAdApt4312.Empty. The generated PCR fragment was cloned into sAdApt4312.Empty, digested with BstZ17I and SalI, with Gibson Assembly (New England BioLabs), resulting in sAdApt4312.E1btg.Empty (SEQ ID NO: 51). In this cloning step, only the AdApt plasmid was digested; the PCR product was not digested with restriction enzymes. A schematic map of sAdApt4312.E1btg.Empty and the cloning strategy described above is depicted in Figure 21.

Example 7. Seroprevalence of sAd4287, sAd4310A, and sAd4312 in sub-Saharan humans and rhesus monkeys

We next evaluated sAd4287, sAd4310A, and sAd4312 titers in 144 sub-Saharan humans and 108 rhesus monkeys (Figures 22A-22C). Adenovirus-specific neutralizing antibody (NAb) titers were determined by luciferase-based virus neutralization assays as previously described (Sprangers et al. *J. Clin. Microbiol.* 41: 5046-5052, 2003; Barouch et al. *Vaccine*. 29: 5203-5209, 2011). Titers of <18 are regarded as negative by this assay, 18-200 is low, 201-1000 is high, and >1000 is considered very high. It is suspected that titers >200 will likely be suppressive, according to data known in the art. Representative pie charts summarizing the relative number of individuals (humans or monkeys) that fall within each of the four titer categories are depicted for each of the three adenoviruses tested (see Figures 22A-22C).

The results of the seroprevalence studies clearly indicate that the majority of both sub-Saharan humans and rhesus monkeys tested exhibited negative (<18) or low (18-200) NAb titers for each of the three adenoviruses tested (sAd4287, sAd4310A, and sAd4312). These seroprevalence studies indicate that the sAd4287, sAd4310A, and sAd4312 vectors have extremely and surprisingly low seroprevalence in human populations (e.g., sub-Saharan human populations) and monkey populations (e.g., rhesus monkey populations). The extremely low seroprevalence of the sAd vectors of the invention are in marked contrast to the relatively high seroprevalence of Ad5 in human populations. Accordingly, these studies indicate a distinct advantage of using a vaccine comprising all or a portion of a recombinant sAd4287, sAd4310A, and sAd4312, as the neutralizing activities in the majority of both humans and monkeys alike are unlikely to hamper the efficacy of the vaccine.

Example 8. Determination of cellular responses to recombinant adenoviruses of the invention in mice

We next studied whether recombinant replication-defective adenoviruses based on simian adenoviruses of the invention (e.g., sAd4287 or sAd4310A) were able to elicit a significant immune response *in vivo*. For this, vectors were generated that all contained the SIVmac239 Gag insert from Simian Immunodeficiency Virus (SIV). Recombinant DNA, such as the required adapter plasmids, and the recombinant viruses were generated generally as described (Lemckert et al. *J. Virol.* 79:9694-9701, 2005).

C57BL/6 mice were injected intramuscularly with different amounts of viral vectors: 10^7, 10^8, and 10^9 viral particles (vp). All vaccination procedures and cellular immune responses were performed and measured by assessing the $CD8^+$ T cell response via D^b/AL11 tetramer binding assays as previously described (Barouch et al. *J. Immunol.* 172:6290-6297, 2004). Tetrameric H-$2D^b$ complexes folded around the immunodominant SIV Gag AL11 epitope (AAVKNWMTQTL) (Liu et al., J. Virol. 80: 11991-11997,

2006) were prepared and SIV Gag-specific $CD8^+$ T lymphocyte responses were measured on days 0, 7, 14, 21, and 28 post-immunization. For immunogenicity experiments with sAd4287 and sAd4310A, the results are shown in Figures 23A and 23B. From these results, it can be concluded that the adenoviral vectors of the invention exhibit potent immunogenicity in mice, especially with 10^8 or 10^9 vp doses.

To evaluate functional responses, splenocytes from day 28 were utilized in IFN-γ ELISPOT assays. IFN-γ ELISPOT responses were measured to overlapping Gag peptides (Gag), the dominant $CD8^+$ T cell epitope AL11 (AAVKNWMTQTL), the sub-dominant $CD8^+$ T epitope KV9 (KSLYNTVCV), and the $CD4^+$ T cell epitope DD13 (DRFYKSLRAEQTD) (Liu et al., J. Virol. 80: 11991-11997, 2006) at 10^7, 10^8, and 10^9 vp of viral vectors (sAd4287, sAd4310A, and rcsAd4287). As depicted in Figures 24A-24C, the IFN-γ ELISPOT responses increased with increasing amounts of vp, and both Gag and AL11 responses were elevated relative to the responses to KV9 or DD13 epitopes. In addition, these functional responses were elicited only when replication-defective adenoviruses of the invention were used (e.g., sAd4287 and sAd4310A), but not when replication-competent adenoviruses of the invention were used (e.g., rcsAd4287). Collectively, the studies of cellular responses to the recombinant adenoviral vectors of the invention clearly indicate potent immunogenicity in mice.

The combination of low baseline anti-vector immunity (low seroprevalence), potent immunogenicity, and novel biology suggests that the novel adenoviral vectors of the invention can be useful as novel vaccine candidates against human or veterinary pathogens, including, but not limited to, HIV, SIV, cancer, malaria, and tuberculosis, in addition to utility in gene therapy and/or diagnostics.

Other Embodiments

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 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.

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

What is claimed is:

Claims

1. A recombinant adenovirus comprising a nucleotide sequence having:

(i) the sequence of SEQ ID NO: 10, or a complementary sequence thereto;

(ii) the sequence of SEQ ID NO: 11, or a complementary sequence thereto; or

(iii) the sequence of SEQ ID NO: 12, or a complementary sequence thereto; and

wherein the recombinant adenovirus comprises a deletion in or of an E1 region, the deletion rendering the recombinant virus a replication-defective virus.

2. The recombinant adenovirus of claim 1, wherein:

(i) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 10, or a complementary sequence thereto, further comprises a sequence having 90% or greater sequence identity to any one of SEQ ID NOs: 4, 7, 13, and 16, or a complementary sequence thereto;

(ii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 11, or a complementary sequence thereto, further comprises a sequence having 90% or greater sequence identity to any one of SEQ ID NOs: 5, 8, 14, and 17, or a complementary sequence thereto; or

(iii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 12, or a complementary sequence thereto, further comprises a sequence having 90% or greater sequence identity to any one of SEQ ID NOs: 6, 9, 15, and 18, or a complementary sequence thereto.

3. The recombinant adenovirus of claim 1, wherein:

(i) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 10, or a complementary sequence thereto, further comprises the sequence of any one of SEQ ID NOs: 4, 7, 13, and 16, or a complementary sequence thereto;

(ii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 11, or a complementary sequence thereto, further comprises the sequence of any one of SEQ ID NOs: 5, 8, 14, and 17, or a complementary sequence thereto; or

(iii) the nucleotide sequence having the sequence of SEQ ID NO: 12, or a complementary sequence thereto, further comprises the sequence of any one of SEQ ID NOs: 6, 9, 15, and 18, or a complementary sequence thereto.

4. The recombinant adenovirus of claim 1, wherein:

(i) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 10, or a complementary sequence thereto, comprises a sequence having 90% or greater sequence identity to the sequence of SEQ ID NO: 1, or a complementary sequence thereto;

(ii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 11, or a complementary sequence thereto, comprises a sequence having 90% or greater sequence identity to the sequence of SEQ ID NO: 2, or a complementary sequence thereto; or

(iii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 12, or a complementary sequence thereto, comprises a sequence having 90% or greater sequence identity to the sequence of SEQ ID NO: 3, or a complementary sequence thereto.

5. The recombinant adenovirus of claim 1, wherein:

(i) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 10, or a complementary sequence thereto, comprises the sequence of SEQ ID NO: 1, or a complementary sequence thereto;

(ii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 11, or a complementary sequence thereto, comprises the sequence of SEQ ID NO: 2, or a complementary sequence thereto; or

(iii) the recombinant adenovirus comprising the nucleotide sequence of SEQ ID NO: 12, or a complementary sequence thereto, comprises the sequence of SEQ ID NO: 3, or a complementary sequence thereto.

6. The recombinant adenovirus of any one of claims 1-5, further comprising a deletion in or of the E3 region, the E4 region, or both the E3 and E4 regions.

7. A recombinant vector comprising the nucleotide sequence of any one of claims 1-6.

8. The recombinant vector of claim 7, further comprising:

(i) the nucleotide sequence of any one of SEQ ID NOs: 34-39,

(ii) the nucleotide sequence of any one of SEQ ID NOs: 40-45, or

(iii) the nucleotide sequence of any one of SEQ ID NOs: 46-51.

9. The recombinant adenovirus of any one of claims 1-6, further comprising a heterologous nucleotide sequence encoding an antigenic or therapeutic protein of interest, or fragment thereof.

10. The recombinant adenovirus of claim 9, wherein said recombinant adenovirus promotes an antibody immune response against the antigenic or therapeutic protein of interest, or fragment thereof, encoded by the heterologous nucleotide sequence.

11. The recombinant adenovirus of claim 10, wherein said antibody immune response is determined using a D^b/AL11 tetramer binding assay.

12. The recombinant adenovirus of claim 11, wherein said antibody immune response is determined using an IFN-γ ELISPOT assay.

13. The recombinant adenovirus of any one of claims 9-12, wherein said antigenic protein, or fragment thereof, comprises a bacterial, viral, parasitic, or fungal protein, or fragment thereof.

14. The recombinant adenovirus of claim 13, wherein:

(i) said viral protein, or fragment thereof, is from a viral family selected from the group consisting of *Retroviridae*, such as human immunodeficiency virus (HIV), such as an Env, Gag, Pol, Nef, Tat, Rev, Vif, Vpr, or Vpu protein, or a fragment thereof, *Flaviviridae*, such as hepatitis C virus (HCV), yellow fever virus, Zika virus, and Japanese encephalitis virus, *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, such as Ebola virus and Marburg virus, *Togaviridae*, such as rubella virus, *Poxviridae*, such as *Variola major, Variola minor,* and monkeypox virus, *Herpesviridae*, such as varicella zoster virus (VZV), cytomegalovirus (CMV), and herpes simplex virus (HSV), *Orthomyxoviridae*, such as influenza, *Coronaviridae*, *Rhabdoviridae*, such as rabies virus, *Paramyxoviridae*, such as measles virus and mumps virus, *Picornaviridae*, such as hepatitis A virus (Hep A) and poliovirus, *Hepadnaviridae*, such as hepatitis B virus (HBV), *Papillomaviridae*, such as human papillomavirus (HPV), *Parvoviridae*, *Astroviridae*, *Polyomaviridae*, *Calciviridae*, and *Reoviridae*, such as rotavirus; or

(ii) said bacterial protein, or fragment thereof, is from *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium leprae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Francisella tularensis*, *Brucella*, *Burkholderia mallei*, *Yersinia pestis*, *Corynebacterium diphtheria*, *Neisseria meningitidis*, *Bordetella pertussis*, *Clostridium tetani*, or *Bacillus anthracis*; or

(iii) said parasitic protein, or fragment thereof, is from *Toxoplasma gondii*, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae*, *Trypanosoma* spp., or *Legionella* spp; or

(iv) said fungal protein, or fragment thereof, is from *Aspergillus*, *Blastomyces dermatitidis*, *Candida*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum var. capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Zygomycetes* spp., *Absidia corymbifera*, *Rhizomucor pusillus*, or *Rhizopus arrhizus*.

15. The recombinant adenovirus of claim 14, wherein said viral protein is a protein or fragment thereof from a Zika virus.

16. The recombinant adenovirus of claim 14, wherein said viral protein is an envelope glycoprotein or fragment thereof.

17. The recombinant adenovirus of claim 16, wherein said envelope glycoprotein or fragment thereof is from a Zika virus.

18. The recombinant adenovirus of any one of claims 9-17, for use in treating a subject having a disease.

19. The recombinant adenovirus for use according to claim 18, wherein said disease is acquired immune deficiency syndrome (AIDS), cancer, tuberculosis, leprosy, typhoid fever, pneumonia, meningitis, staphylococcal scalded skin syndrome (SSSS), Ritter's disease, tularemia (rabbit fever), brucellosis, Glanders disease, bubonic plague, septicemic plague, pneumonic plague, diphtheria, pertussis (whooping cough), tetanus, anthrax, hepatitis, smallpox, monkeypox, measles, mumps, rubella, chicken pox, polio, rabies, Japanese encephalitis, herpes, mononucleosis, influenza, Ebola virus disease, hemorrhagic fever, yellow fever, Marburg virus disease, toxoplasmosis, malaria, trypanosomiasis, legionellosis, aspergillosis, blastomycosis, candidiasis (thrush), coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis, sporotrichosis, Zika virus infection, or sinus-orbital zygomycosis.

20. The recombinant adenovirus for use according to claim 18 or 19, wherein said subject is human.

21. The recombinant adenovirus for use according to any one of claims 18-20, wherein:

(a) said recombinant adenovirus is formulated for intramuscular, intravenous, intradermal, percutaneous, intraarterial, intraperitoneal, intralesional, intracranial, intraarticular, intraprostatical, intrapleural, intratracheal, intranasal, intravitreal, intravaginal, intrarectal, topical, intratumoral, peritoneal, subcutaneous, subconjunctival, intravesicular, mucosal, intrapericardial, intraumbilical, intraocular, oral, topical, or local administration, for administration by inhalation, injection, infusion, continuous infusion, localized perfusion bathing target cells directly, catheter, lavage, or gavage, or in a creme or lipid composition; and/or

(b) said adenovirus is formulated as a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

22. The recombinant adenovirus for use according to claim 21, wherein said pharmaceutical composition is formulated for administration in at least one or two doses.

23. The recombinant adenovirus for use according to claim 22, wherein said pharmaceutical composition is formulated for administration as a prime boost.

24. A method of producing a recombinant adenovirus of any one of claims 1-6 and 9-17, wherein said method comprises culturing a cell in a suitable medium; transfecting said cell with the nucleotide

sequence of any one of claims 1-6 or the recombinant vector of claim 7 or 8; allowing replication of said nucleotide sequence or vector in said cell; and harvesting produced recombinant adenovirus from said medium and/or said cell.

25. The method of claim 24, wherein said cell is a bacterial, plant, or mammalian cell.

26. The method of claim 25, wherein said mammalian cell is a Chinese hamster ovary (CHO) cell.