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Adenoviral vectors comprising partial deletions of E3

Abstract

This disclosure provides replication-incompetent adenoviral vectors useful in vaccine development and gene therapy. The disclosed vectors comprise a selective deletion of E3 and are particularly useful for preparation of vaccines development and for gene therapy using toxic transgene products that result in vector instability that occurs when the entire E3 domain is deleted.

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References Cited

U.S. PATENT DOCUMENTS

Patent No.	Issued Date	Patentee Name	U.S. Cl.	CPC
5814486	12/1997	Cohen et al.	N/A	N/A
6936255	12/2004	Wettendorff et al.	N/A	N/A
8962816	12/2014	Ertl et al.	N/A	N/A
9624510	12/2016	Ertl et al.	N/A	N/A
9724406	12/2016	Ertl et al.	N/A	N/A
10328146	12/2018	Ertl et al.	N/A	N/A
11207402	12/2020	Ertl et al.	N/A	N/A
2004/0253210	12/2003	Robert-Guroff et al.	N/A	N/A
2005/0095270	12/2004	Staecker et al.	N/A	N/A
2013/0315871	12/2012	Roy et al.	N/A	N/A
2014/0065105	12/2013	Wilson et al.	N/A	N/A
2014/0248305	12/2013	Ertl et al.	N/A	N/A
2019/0167813	12/2018	Ertl et al.	N/A	N/A

FOREIGN PATENT DOCUMENTS

Patent No.	Application Date	Country	CPC
1336619	12/2002	EP	N/A
2006120034	12/2005	WO	N/A
2007071997	12/2006	WO	N/A

OTHER PUBLICATIONS

European Search Report, EP17837398, date of mailing Apr. 20, 2020. cited by applicant
International Search Report and Written Opinion, PCT/US17/43315, date of malling Nov. 30, 2017. cited by applicant
International Search Report, PCT/US2007/018939, date of mailing May 16, 2008. cited by applicant
Singapore Search Report, SG Application No. 11201900808S, mailing date Apr. 29, 2020. cited by applicant
Altstein, et al., “Immunization with influenza A NP-Expressing Vaccinia Virus Recombinant Protects Mice Against Experimental Infection with Hyuman and Avian Influenza Viruses”, Archives of Virology, vol. 151, No. 5, May 2006, pp. 921-931. cited by applicant

Alves, et al., “Antibody Response in Mice Immunized with a Plasmid DNA Encoding the Colonization Factor Antigen I of Enterotoxigenic *Escherichia coli*”, FEMS Immunology Medical Microbiology, vol. 23, No. 4, Apr. 1999, pp. 321-330. cited by applicant
Bayer, et al., “Improved vaccine protection against retrovirus infection after co-administration of adenoviral vectors encoding viral antigens and type I interferon subtypes.”, 2011 Retrovirology 8:75 (15 pages). cited by applicant
Casimiro, et al., “Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with DNA and recombinant adenoviral vaccine vectors expressing Gag.”, 2005, J Virol 79(24):15547-15555. cited by applicant
Casimiro, et al., “Comparative Immunogenicity in Rhesus Monkeys of DNA Plasmid, Recombinant Vaccinia Virus, and Replication-Defective Adenovirus Vectors Expressing a Human Immunodeficiency Virus Type 1 gag Gene”, 2003, J Virol 77(11):6305-5313. cited by applicant
Cervasi, et al., “Immunological and Virological Analyses of Rhesus Macaques Immunized with Chimpanzee Adenoviruses Expressing the Simian Immunodeficiency Virus Gag/Tat Fusion Protein and Challenged Intrarectally with Repeated Low Doses of SIVmac”, Journal of Virology, 87(17), Sep. 2013, 9420-9430. cited by applicant
Chawla, et al., “Adenovirus-vectored vaccines”, Dev Biol Stand, Mar. 16, 2008, vol. 18, No. 3, pp. 293-307, Table 1. cited by applicant
Chen, et al., “Adenovirus-Based Vaccines: Comparison of Vectors from Three Species of Adenoviridae.”, 2010, Journal of Virology 84(20):10522-32. cited by applicant
Chen, et al., “Direct Observation of Xe and Kr Adsorption in a Xe-Selective Microporous Metal-Organic Framework.”, J. Am. Chem. Soc., May 2015, 137, 7007-7010. cited by applicant
Engram, et al., “Vaccine-induced, simian immunodeficiency virus-specific CD8+ T cells reduce virus replication but do not protect from simian immunodeficiency virus disease progression.”, 2009, J Immunol 183:706-717. cited by applicant
Hazama, et al., “Adjuvant-independent enhanced immune responses to recombinant Herpes Simplex Virust Type 1 Glycoprotein D by fusion biologically active interlukin-2”, Vaccine, vol. 11, No. 6, 1993, pp. 629-636. (Abstract Only). cited by applicant
He, et al., “A simplified system for generating recombinant adenoviruses.”. 1998, PNAS 95:2509-14. cited by applicant
Hinuma, et al., “A novel strategy for converting recombinant viral protein into high immunogeic antigen”, FEBS Letters, vol. 288, No. 1/2, Aug. 1991, pp. 138-142 (Abstract Only). cited by applicant
Horwitz, “Function of adenovirus E3 proteins and their interactions with immunoregulatory cell proteins.”, 2004, J Gene Med 6:S172-S183 (Abstract only). cited by applicant
Lasaro, et al., “Antibody-inducing properties of a prototype bivalent Herpes Simplex virus/Enterotoxigenic *Escherichia coli* DNA Vaccine”, FEMS Immunology and Medical Microbiology, vol. 35, No. 1, Jan. 21, 2003, pp. 25-31. cited by applicant
Lasaro, et al., “Anti-Tumor DNA Vaccines based on the Expression of Human Papillomavirus-16 E6/E7 Oncoproteins Genetically Fused With The Glycoprotein D from Herpes Simplex Virus-1”, Microbes and Infection, vol. 7, No. 15, Dec. 2005, pp. 1541-1550. (Abstract Only). cited by applicant
Lasaro, et al., “Human papillomavirus-associated cervical cancer: Prophylactic and therapeutic vaccines.”, Gene Therapy Molecular Biology, 2004, vol. 8, pp. 291-306. cited by applicant
Lasaro, et al., “New insights on adenovirus as vaccine vectors”, Molecular Therapy, vol. 17, No. 8, Aug. 1, 2009, pp. 1333-1339. cited by applicant
Lasaro, et al., “Vaccine-induced T cells Provide Partial Protection Against High-dose Rectal SIVmac239 Challenge of Rhesus Macaques”, Molecular Therapy, 19(2), Feb. 2011, 417-426. cited by applicant
Lewis, et al., “Response of a simian immunodeficiency virus (SIVmac251) to raltegravir: a basis

for a new treament for simian AIDS and an animal model for studying lentiviral persistence during antiretroviral therapy", Retrovirology, 7(21), 2010, 1-19. cited by applicant
Lichtenstein, et al., "Functions and mechanisms of action of the adenovirus E3 proteins.", 2004, International Reviews of Immunology 23: 75-111. cited by applicant
McCoy, et al., "Effect of Preexisting Immunity to Adenovirus Human Serotype 5 Antigens on the Immune Responses of Nonhuman Primates to Vaccine Regimens Based on Human- or Chimpanzee-Derived Adenovirus Vectors", Journal of Virology, 81(12), Jun. 2007, 6594-6604. cited by applicant
Michel, et al., "Enhanced Immunogenicity of HPV 16 E7 Fusion Proteins in DNA Vaccination", Virology, 2002, vol. 294, pp. 47-59. cited by applicant
Patel, et al., "DNA and virus particle vaccination protects against acquisition and confers control of viremia upon heterologous simian immunodeficiency virus challenge", PNAS, 110(8), Feb. 2013, 2975-2980. cited by applicant
Plonka, et al., "Light Hydrocarbon Adsorption Mechanisms in Two Calcium-Based Microporous Metal Organic Frameworks.", Chem. Mater. 2016, 28, 1636-1646 (Abstract Only). cited by applicant
Saha, et al., "A Fused Gene of Nucleoprotein (NP) and Herpes Simplex Virus Genes (VP22) Induces Highly Protective Immunity Against Different Subtypes of Influenza Virus", Virology, vol. 354, No. 1, Oct. 10, 2006, pp. 48-57. cited by applicant
Santra, et al., "Heterologous Prime/Boost Immunizations of Rhesus Monkeys Using Chimpanzee Adenovirus Vectors", Vaccine, 27(42), Sep. 2009, 5837-5845. cited by applicant
Shiver, et al., "Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity.", 2002, Nature 415:331-335. cited by applicant
Small, et al., "Viruses—From Pathogens to Vaccine Carriers", Curr. Opin. Virol., 1(4), Oct. 2011, 241-245. cited by applicant
Tatsis, et al., "Adenovirus Vector-Induced Immune Responses in Nonhuman Primates: Responses to Prime Boost Regimens.", 2009, Journal of Immunology, 182, pp. 6587-6599. cited by applicant
Tatsis, et al., "Chimpanzee-origin adenovirus vectors as vaccine", 2006, Gene Therapy 13:421-429. cited by applicant
Tatsis, et al., "Multiple Immunizations with Adenovirus and MVA vectors improve CD8+ T cell functionality and mucosal homing", Virology, 367(1), Oct. 2007, 156-167. cited by applicant
Watson, et al., "Herpes Simplex Virus Type=1 Glycoprotein D Gene: Nucleotide Sequence and Expression in *Escherichia coli*", Science, vol. 218, Oct. 22, 1982, pp. 381-384 (Abstract Only). cited by applicant
Xiang, "Chimpanzee Adenovirus Antibodies in Humans, Sub-Saharan Africa", 2006, Emerging Infectious Diseases 12(10):1596-1599. cited by applicant
Zago, et al., "Use of herpes simplex virus and pseudorabies virus chimeric glycoprotein D molecules to identify regions critical for membrane fusion.", PNAS, 2004, vol. 101, No. 50, pp. 17498-17503. cited by applicant
Zhou, et al., "A Universal Influenza A Vaccine Based on Adenovirus Expressing Matrix-2 Ectodomain and Nucleoprotein Protects Mice from Lethal Challenge", Molecular Therapy, 18(12), Dec. 2010, 2182-2189. cited by applicant
Zhou, et al., "An efficient method of directly cloning chimpanzee adenovirus as a vaccine vector", Natl. Protoc., 5(11), Nov. 2010, 1775-1785. cited by applicant
Zolla-Pazner, et al., "Analysis of V2 Antibody Responses Induced in Vaccinees in the ALVAC/AIDSVAX HIV-1 Vaccine Efficacy Trial", PLOS One, 8(1), Jan. 2013, 1-11. cited by applicant

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This application is a continuation of, and claims priority to, U.S. patent application Ser. No. 17/037,966, filed Sep. 30, 2020, now allowed, which is a continuation of U.S. patent application Ser. No. 16/530,359, filed Aug. 2, 2019, issued as U.S. Pat. No. 10,822,619, which is a continuation of U.S. patent application Ser. No. 15/453,579, filed Mar. 8, 2017, issued as U.S. Pat. No. 10,407,696, which is a continuation of U.S. patent application Ser. No. 14/190,787, filed Feb. 26, 2014, issued as U.S. Pat. No. 9,624,510, which claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 61/771,370, filed on Mar. 1, 2013, each of which is hereby incorporated herein by reference in its entirety.

TECHNICAL FIELD
(1) This disclosure relates generally to adenoviral vectors.
DETAILED DESCRIPTION
(2) This disclosure provides replication-incompetent adenoviral vectors useful in vaccine development and gene therapy. These vectors comprise a selective deletion of the viral genomic region E3 but retain anti-apoptotic function otherwise provided by E3-encoded proteins. This is achieved either by retaining portions of E3 or by including in the vector open reading frames (ORFs) that encode anti-apoptotic proteins. The disclosed vectors are particularly useful for vaccine development and for gene therapy in which the encoded protein products are toxic and result in vector instability.
(3) In some embodiments, a replication-incompetent adenovirus vector comprises a deletion of E3 ORF3, ORF4, ORF5, ORF6, and ORF7; and comprises at least one open reading frame encoding an anti-apoptotic protein. The anti-apoptotic protein can be a native protein of the adenovirus, such as the proteins encoded by ORF8 or ORF9, or can be an apoptotic protein from a different source, e.g., a p53 inhibitor, Bcl-XL, BCL2, BCL2L1, BCL2A1, BAG1, TRAF1, BIRC3, BIRC5, BAK1, cIAP1, c-IAP2, XIAP, or API5. These proteins and nucleotide sequences encoding them are well known in the art. A replication-incompetent adenovirus vector can comprise one open reading frame encoding an anti-apoptotic protein or can comprise several such open reading frames (e.g., 2, 3, 4).
(4) An adenovirus vector can be rendered replication-incompetent by various means, including, but not limited to, a complete deletion of E1 or a functional deletion of E1 (i.e., a deletion of less than the entire E1a and E1b loci, but sufficient to disable the function of the E1genes, and mutations at functional sites).
(5) The disclosed vectors can be generated using basic cloning techniques and can be used thereafter to express a variety of different protein products.
(6) In some embodiments, the serotype of the replication-incompetent adenovirus vector is a human serotype (e.g., a serotype of group A, group B, group C, group D, group E, group F). Human serotypes include, but are not limited to, Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, and Ad50.
(7) In other embodiments, the serotype of the replication-incompetent adenovirus vector is a chimpanzee serotype (e.g., Ad C1, Ad C3, Ad C6, Ad C7, Ad C68).
(8) Replication-incompetent adenovirus vectors disclosed herein are particularly useful when an antigenic protein is toxic to cell machinery upon expression by the vector. For example, the disclosed replication-incompetent adenovirus vectors carrying a coding sequence for HIVgp140 can be readily rescued, express the gp140 protein, and remain stable after 12 serial passages.

(9) Accordingly, in some embodiments, the antigenic protein is all or an antigenic portion of an HIV-1 envelope protein. In some embodiments, the antigenic protein is all or an antigenic portion of an HCV envelope protein. In some embodiments, the antigenic protein is all or an antigenic portion of a protein of *M. tuberculosis*. In some embodiments, the antigenic protein is all or an antigenic portion of a protein of *Plasmodium* (e.g., *P. falciparum*).
(10) In some embodiments, the antigenic protein is all or an antigenic portion of a protein of an infectious eukaryotic organism, such as a *Plasmodium* (e.g., *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae Plasmodium* diarrhea), and fungi such as *Candida* (e.g., *Candida albicans*), *Aspergillus* (e.g., *Aspergillus fumigatus*), *Cryptococcus* (e.g., *Cryptococcus neoformans*), *Histoplasma* (e.g., *Histoplasma capsulatum*), *Pneumocystis* (e.g., *Pneumocystis jirovecii*), and *Coccidioides* (e.g., *Coccidioides immitis*).
(11) In some embodiments, the antigenic protein is all or an antigenic portion of a protein of an infectious virus, such as an influenza virus, retrovirus (e.g., HIV, Rous Sarcoma Virus (RSV), human endogenous retrovirus K (HERV-K)), human endogenous retrovirus K (HERV-K), papillomavirus (e.g., human papilloma virus), picornavirus (e.g., Hepatitis A, Poliovirus), hepadnavirus (e.g., Hepatitis B), flavivirus (e.g., Hepatitis C, Yellow Fever virus, Dengue Fever virus, Japanese encephalitis virus, West Nile virus), togavirus (e.g., chikungunya virus, Eastern equine encephalitis (EEE) virus, Western equine encephalitis (WEE) virus, Venezuelan equine encephalitis (VEE) virus), herpesvirus (e.g., Cytomegalovirus), paramyxovirus (Parainfluenza virus, Pneumonia virus, Bronchiolitis virus, common cold virus, Measles virus, Mumps virus), rhabdovirus (e.g., Rabies virus), Filovirus (e.g., Ebola virus), bunyavirus (e.g., Hantavirus, Rift Valley Fever virus), calicivirus (e.g., Norovirus), or reovirus (e.g., Rotavirus, Epstein-Barr virus, Herpes simplex virus types 1 & 2).
(12) In some embodiments, the antigenic protein is all or an antigenic portion of a protein of an infectious gram-negative bacterium or gram-positive bacterium, *Bacillus* (e.g., *Bacillus anthracis*), *Mycobacterium* (e.g., *Mycobacterium tuberculosis, Mycobacterium Leprae*), *Shigella* (e.g., *Shigella sonnei, Shigella dysenteriae, Shigella flexneri*), *Helicobacter* (e.g., *Helicobacter pylori*), *Salmonella* (e.g., *Salmonella enterica, Salmonella typhi, Salmonella typhimurium*), *Neisseria* (e.g., *Neisseria gonorrhoeae, Neisseria meningitidis*), *Moraxella* (e.g., *Moraxella catarrhalis*), *Haemophilus* (e.g., *Haemophilus influenzae*), *Klebsiella* (e.g., *Klebsiella pneumoniae*), *Legionella* (e.g., *Legionella pneumophila*), *Pseudomonas* (e.g., *Pseudomonas aeruginosa*), *Acinetobacter* (e.g., *Acinetobacter baumannii*), *Listeria* (e.g., *Listeria monocytogenes*), *Staphylococcus* (e.g., methicillin-resistant, multidrug-resistant, or oxacillin-resistant *Staphylococcus aureus*), *Streptococcus* (e.g., *Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae*), *Corynebacterium* (e.g., *Corynebacterium diphtheria*), *Clostridium* (e.g., *Clostridium botulinum, Clostridium tetani, Clostridium difficile*), *Chlamydia* (e.g., *Chlamydia pneumonia, Chlamydia trachomatis*), *Camphylobacter* (e.g., *Camphylobacter jejuni*), *Bordetella* (e.g., *Bordetella pertussis*), *Enterococcus* (e.g., *Enterococcus faecalis, Enterococcus* faecum, Vancomycin-resistant *enterococcus* (VRE)), *Vibrio* (e.g., *Vibrio cholerae*), *Yersinia* (e.g., *Yersinia pestis*), *Burkholderia* (e.g., *Burkholderia cepacia* complex), *Coxiella* (e.g., *Coxiella burnetti*), *Francisella* (e.g., *Francisella tularensis*), and *Escherichia* (e.g., enterotoxigenic, enterohemorrhagic or Shiga toxin-producing *E. coli*, such as ETEC, EHEC, EPEC, EIEC, EAEC)).
(13) Production, purification and quality control procedures for Ad vectors are well established..sup.17 Once a vector backbone is created, molecular cloning can be used to create an adenoviral plasmid comprising a coding sequence for an antigenic protein (“transgene”). The plasmid can be transfected into packaging cells that provide E1 of a suitable adenovirus serotype in trans. Packaging cells are well known in the art, and cells lines such as HEK293 can be used for this purpose. Viral particles are then harvested once plaques become visible. Fresh cells can then be infected to ensure continued replication of the adenovirus. Quality can be assessed using Southern blotting or other methods, such as restriction enzyme mapping, sequences, and PCR, to confirm the

presence of the transgene and the lack of gene rearrangements or undesired deletions.
(14) Vaccine compositions comprising adenovirus particles made using replication-incompetent adenovirus vectors disclosed herein can be used to induce immunity against the encoded antigenic protein.
(15) Vaccines can be formulated using standard techniques and can comprise, in addition to a replication-incompetent adenovirus vector encoding a desired protein, a pharmaceutically acceptable vehicle, such as phosphate-buffered saline (PBS) or other buffers, as well as other components such as antibacterial and antifungal agents, isotonic and absorption delaying agents, adjuvants, and the like. In some embodiments vaccine compositions are administered in combination with one or more other vaccines.
(16) Dosage units of vaccine compositions can be provided. Such dosage units typically comprise 10.sup.8 to 10.sup.11 adenoviral particles (e.g., 10.sup.8, 5×10.sup.8, 10.sup.9, 5×10.sup.9, 10.sup.10, 5×10.sup.10, 10.sup.11, 10.sup.8 to 10.sup.9, 10.sup.8 to 10.sup.10, 10.sup.9 to 10.sup.10, 10.sup.9 to 10.sup.11, 5×10.sup.8 to 10.sup.9, 5×10.sup.8 to 10.sup.10, 5×10.sup.9 to 10.sup.10, 5×10.sup.9 to 10.sup.11, 10.sup.8 to 5×10.sup.9, 10.sup.8 to 5×10.sup.10, 10.sup.9 to 5×10.sup.10, 10.sup.9 to 10.sup.11, 5×10.sup.8 to 5×10.sup.9, 5×10.sup.8 to 5×10.sup.10, 5×10.sup.9 to 5×10.sup.10, 5×10.sup.9 to 10.sup.11).
(17) Immune responses against one or more encoded proteins or antigenic portions thereof can be induced using the disclosed vaccine compositions or dosage units. Methods of administration include, but are not limited to, mucosal (e.g., intranasal), intraperitoneal, intramuscular, intravenous, and oral administration. Immune responses can be assessed using suitable methods known in the art, as disclosed, for example, in WO2012/02483.
(18) Those skilled in the art will appreciate that there are numerous variations and permutations of the above described embodiments that fall within the scope of the appended claims.

Description

EXAMPLE 1
(1) Construction of pAdC6 020
(2) The E3 region of AdC6 contains 5052 base pairs (bp), with a total of nine open reading frames: ORF1 is 325 bp; ORF2 is 624 bp; ORF3 is 531 bp; ORF4 is 684 bp; ORF5 is 612 bp; ORF6 is 867 bp; ORF7 is 276 bp; ORF8 is 435 bp; and ORF9 is 408 bp. ORF3, ORF4, ORF5, ORF6 and ORF7 were deleted to create the vector “AdC6 020” (the total length of the deleted sequence was 3218 bp).
EXAMPLE 2
(3) Construction of Stable Adenoviral Vectors Comprising HIV Envelope Sequences
(4) pAdC6 020 was used to create two vectors encoding HIV envelope sequences, “AdC6 020-HIVgp140-DU172” and “AdC6 020-HIVgp140-DU422.” The HIV strains that served as donors for the envelope sequences were isolated from clinical materials. The coding sequences were codon-optimized to permit enhanced expression in human cells. To create the pAdC6 020 plasmid, PCR-based cloning strategies were used to obtain the fragment without ORF3, ORF4, ORF5, ORF6 and ORF7 of E3 region. By using that PCR product, the pXY-E3 deleted plasmid was generated. Both pXY-E3 deleted (donor for insert) and pC6 E3 deleted (donor for backbone) plasmids were digested with PspX I and Nco I restriction enzymes to result in the pC6 E3 modified plasmid. Finally the modified E3 fragment was excised out from pC6 E3 modified plasmid by Sbf I digestion and cloned into pAdC6 000-eGFP plasmid (E1 deleted only) with E3 removed by Sbf I digestion. This resulted in pAdC6 020-eGFP. AdC6 vectors comprising the HIV coding sequences and a deletion of E1 or a deletion of E1 and of all the ORFs of E3 could not be rescued. Similar problems were encountered using adenovirus vectors of human serotype 5 (Ad5) and 26 (Ad26).

Upon modifying the E3 deletion within AdC6, Ad5 and Ad26, however, all of those vectors could readily be rescued. These vectors passed quality control assays including restriction enzyme mapping, and Western blots showed that vectors expressed HIV-1 gp140 upon transfection of cells. These vectors were stable after 12 sequential passages shown by restriction enzyme mapping. Vectors rescued successfully by modified E3 region are summarized in the following table.
(5) TABLE-US-00001 Viral Western Blot Viral Vector Name Rescued Stability for transgene Genome AdC6 020-HIVgp140-DU172 yes stable yes correct AdC6 020-HIVgp140-DU422 yes stable yes correct AdC7 010-HIVgp140-DU172 yes stable yes correct AdC7 010-HIVgp140-DU422 yes stable yes correct AdC6 020-SIVgp160 yes N.D. N.D. correct AdC6 020-HIVgag yes N.D. N.D. N.D. Ad5 060-HIVgp140-DU422 yes stable yes correct Ad5 060-SIVgp160 yes N.D. N.D. N.D. Ad26 011-HIVgp140-DU422 yes N.D. N.D. correct N.D.—Not Determined

Claims

1. A method of inducing an immune response to an antigenic protein in a subject, comprising administering to the subject an immunogenic composition comprising a replication-incompetent adenovirus vector comprising: a) open reading frame (ORF) ORF1 and ORF2 of E3 genomic region; b) selective deletion of E3 genomic region consisting of ORF3, ORF4, ORF5, ORF6, and ORF7; c) at least one ORF selected from the group consisting of ORF8 and ORF9; and d) at least one ORF encoding the antigenic protein.
2. The method of claim 1, wherein the serotype of the replication-incompetent adenovirus vector is a human serotype.
3. The method of claim 1, wherein the serotype of the replication-incompetent adenovirus vector is a serotype of a group selected from the group consisting of group A, group B, group C, group D, group E, and group F.
4. The method of claim 1, wherein the replication-incompetent adenovirus vector is of a serotype selected from the group consisting of Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad11, Ad20, Ad21, Ad22, Ad23, Ad24, Ad25, Ad26, Ad28, Ad34, Ad35, Ad40, Ad41, Ad48, Ad49, and Ad50.
5. The method of claim 1, wherein the replication-incompetent adenovirus vector is of a serotype selected from the group consisting of Ad C1, Ad C3, Ad C6, Ad C7, and Ad C68.
6. The method of claim 1, wherein the replication-incompetent vector comprises a deletion in E1.