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54 Titre : MVA-BN and AD26.ZEBOV or AD26.FILO prime-boost regimen.

57 Abrégé :

Compositions and methods are described for generating an improved effective immune response against an immunogen in humans. The enhanced immune response, is obtained by using an MVA vector as a prime, an adenovirus vector as a first boost, and an adenovirus vector as a second boost. The compositions and methods can be used to provide a protective immunity against a disease, such as an infection of one or more subtypes of Ebola and Marburg filoviruses, in humans.

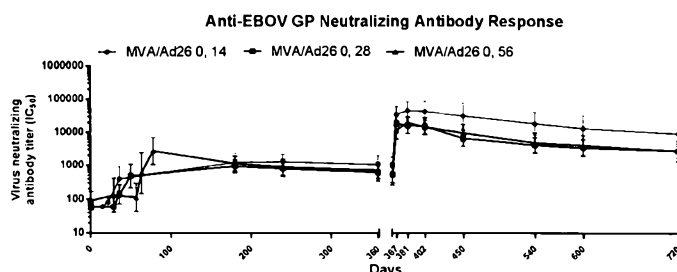


Fig. 3

METHODS AND COMPOSITIONS FOR ENHANCING IMMUNE RESPONSES**STATEMENT OF GOVERNMENT SUPPORT**

This invention was made with government support under contract
5 HHSN272200800056C, awarded by the National Institute of Health. The government has
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REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a sequence listing, which is submitted electronically via EFS-
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reference in its entirety.

FIELD OF THE INVENTION

15 This invention relates to methods and compositions for enhancing an immune response
in a human subject. In particular, the methods and compositions provide a strong induction of B
cell and T cell activity against an immunogen in a human subject, which can be used to provide
an effective treatment and/or protection against a disease, such as a tumor or an infectious
20 disease, more particularly an infection by a filovirus, in the human subject.

BACKGROUND OF THE INVENTION

Vaccines can be used to provide immune protection against pathogens, such as viruses,
bacteria, fungi, or protozoans, as well as cancers, such as HPV vaccine.

25 Infectious diseases are the second leading cause of death worldwide after
cardiovascular disease but are the leading cause of death in infants and children (Lee and
Nguyen, 2015, *Immune Network*, 15(2):51-7). Vaccination is the most efficient tool for
preventing a variety of infectious diseases. The goal of vaccination is to generate a pathogen-
specific immune response providing long-lasting protection against infection. Despite the
30 significant success of vaccines, development of safe and strong vaccines is still required due to
the emergence of new pathogens, re-emergence of old pathogens and suboptimal protection
conferred by existing vaccines. Recent important emerging or re-emerging diseases include:

severe acute respiratory syndrome (SARS) in 2003, the H1N1 influenza pandemic in 2009, and Ebola virus in 2014. As a result, there is a need for the development of new and effective vaccines against emerging diseases.

Cancer is one of the major killers in the Western world, with lung, breast, prostate, and colorectal cancers being the most common (Butterfield, 2015, BMJ, 350:h988). Several clinical approaches to cancer treatment are available, including surgery, chemotherapy, radiotherapy, and treatment with small molecule signaling pathway inhibitors. Each of these standard approaches has been shown to modulate antitumor immunity by increasing the expression of tumor antigens within the tumor or causing the release of antigens from dying tumor cells and by promoting anti-tumor immunity for therapeutic benefit. Immunotherapy is a promising field that offers alternative methods for treatment of cancer. Cancer vaccines are designed to promote tumor-specific immune responses, particularly cytotoxic CD8⁺ T cells that are specific to tumor antigens. Clinical efficacy must be improved in order for cancer vaccines to become a valid alternative or complement to traditional cancer treatments. Considerable efforts have been undertaken so far to better understand the fundamental requirements for clinically-effective cancer vaccines. Recent data emphasize that important requirements, among others, are (1) the use of multi-epitope immunogens, possibly deriving from different tumor antigens; (2) the selection of effective adjuvants; (3) the association of cancer vaccines with agents able to counteract the regulatory milieu present in the tumor microenvironment; and (4) the need to choose the definitive formulation and regimen of a vaccine after accurate preliminary tests comparing different antigen formulations (Fenoglio et al., 2013, Hum Vaccin Immunother, (12):2543-7). A new generation of cancer vaccines, provided with both immunological and clinical efficacy, is needed to address these requirements.

Ebolaviruses, such as Zaire ebolavirus (EBOV) and Sudan ebolavirus (SUDV), and the closely related Marburg virus (MARV), are associated with outbreaks of highly lethal Ebola Hemorrhagic Fever (EHF) and Marburg Hemorrhagic Fever (MHF) in humans and primates in North America, Europe, and Africa. These viruses are filoviruses that are known to infect humans and non-human primates with severe health consequences, including death. Filovirus infections have resulted in case fatality rates of up to 90% in humans. EBOV, SUDV, and MARV infections cause EHF or MHF with death often occurring within 7 to 10 days post-infection. EHF presents as an acute febrile syndrome manifested by an abrupt fever, nausea, vomiting, diarrhea, maculopapular rash, malaise, prostration, generalized signs of increased

vascular permeability, coagulation abnormalities, and dysregulation of the innate immune response. Much of the disease appears to be caused by dysregulation of innate immune responses to the infection and by replication of virus in vascular endothelial cells, which induces death of host cells and destruction of the endothelial barrier. Filoviruses can be spread by small particle aerosol or by direct contact with infected blood, organs, and body fluids of human or NHP origin. Infection with a single virion is reported to be sufficient to cause EHF in humans. Presently, there is no therapeutic or vaccine approved for treatment or prevention of EHF or MHF. Supportive care remains the only approved medical intervention for individuals who become infected with filoviruses.

As the cause of severe human disease, filoviruses continue to be of concern as both a source of natural infections, and also as possible agents of bioterrorism. The reservoir for filoviruses in the wild has not yet been definitively identified. Four subtypes of Ebolaviruses have been described to cause EHF, i.e., those in the Zaire, Sudan, Bundibugyo and Ivory Coast episodes (Sanchez A. et al., 1996, PNAS USA, 93:3602–3607). These subtypes of Ebolaviruses have similar genetic organizations, e.g., negative-stranded RNA viruses containing seven linearly arrayed genes. The structural gene products include, for example, the envelope glycoprotein that exists in two alternative forms, a secreted soluble glycoprotein (ssGP) and a transmembrane glycoprotein (GP) generated by RNA editing that mediates viral entry (Sanchez A. et al., 1996, PNAS USA, 93:3602–3607).

It has been suggested that immunization can be useful in protecting against Ebola infection because there appears to be less nucleotide polymorphism within Ebola subtypes than among other RNA viruses (Sanchez A. et al., 1996, PNAS USA, 93:3602–3607). Until recently, developments of preventive vaccines against filoviruses have had variable results, partly because the requirements for protective immune responses against filovirus infections are poorly understood. Additionally, the large number of filoviruses circulating within natural reservoirs complicates efforts to design a vaccine that protects against all species of filoviruses.

Currently, there are several vaccine antigen delivery platforms that demonstrated various levels of protection in non-human primates (NHPs) exposed with high infectious doses of filoviruses. Vaccine candidates are in development based on a variety of platform technologies including replication competent vectors (e.g. Vesicular Stomatitis Virus; Rabies virus; Parainfluenza Virus); replication incompetent vectors (Adenovirus, Modified Vaccinia Ankara Virus); protein subunits inclusive of Virus Like Particles expressed in bacterial cells,

insect cells, mammalian cells, plant cells; DNA vaccines; and /or live and killed attenuated filovirus (Friedrich et al., 2012, *Viruses*, 4(9):1619-50). The EBOV glycoprotein GP is an essential component of a vaccine that protects against exposures with the same species of EBOV. Furthermore, inclusion of the GP from EBOV and SUDV, the two most virulent species of ebolaviruses, can protect monkeys against EBOV and SUDV intramuscular exposures, as well as exposures with the distantly related Bundibugyo (BDBV), Taï Forest ebolavirus (TAFV; formerly known as Ivory Coast or Cote d'Ivoire) species. Likewise, inclusion of the GP from MARV can protect monkeys against intramuscular and aerosol MARV exposures. The development of medical countermeasures for these viruses is a high priority, in particular the development of a PAN-filovirus vaccine – that is one vaccine that protects against all pathogenic filoviruses.

Replication-defective adenovirus vectors (rAd) are powerful inducers of cellular immune responses and have therefore come to serve as useful vectors for gene-based vaccines particularly for lentiviruses and filoviruses, as well as other nonviral pathogens (Shiver et al., 2002, *Nature*, 415(6869): 331-5; Hill et al., 2010, *Hum Vaccin* 6(1): 78-83; Sullivan et al., 2000, *Nature*, 408(6812): 605-9; Sullivan et al., 2003, *Nature*, 424(6949): 681-4; Sullivan et al., 2006, *PLoS Med*, 3(6): e177; Radosevic et al., 2007, *Infect Immun*, 75(8):4105-15; Santra et al., 2009, *Vaccine*, 27(42): 5837-45).

Adenovirus-based vaccines have several advantages as human vaccines since they can be produced to high titers under GMP conditions and have proven to be safe and immunogenic in humans (Asmuth et al., 2010, *J Infect Dis* 201(1): 132-41; Kibuuka et al., 2010, *J Infect Dis* 201(4): 600-7; Koup et al., 2010, *PLoS One* 5(2): e9015; Catanzaro et al., 2006, *J Infect Dis*, 194(12): 1638-49; Harro et al., 2009, *Clin Vaccine Immunol*, 16(9): 1285-92). While most of the initial vaccine work was conducted using rAd5 due to its significant potency in eliciting broad antibody and CD8⁺ T cell responses, pre-existing immunity to rAd5 in humans may limit efficacy (Catanzaro et al., 2006, *J Infect Dis*, 194(12): 1638-49; Cheng et al., 2007, *PLoS Pathog*, 3(2): e25; McCoy et al., 2007, *J Virol*, 81(12): 6594-604; Buchbinder et al., 2008, *Lancet*, 372(9653): 1881-93). This property might restrict the use of rAd5 in clinical applications for many vaccines that are currently in development including Ebolavirus (EBOV) and Marburg virus (MARV).

Replication-defective adenovirus vectors, rAd26 and rAd35, derived from adenovirus serotype 26 and serotype 35, respectively, have the ability to circumvent Ad5 pre-existing

immunity. rAd26 can be grown to high titers in Ad5 E1-complementing cell lines suitable for manufacturing these vectors at a large scale and at clinical grade (Abbink, et al., 2007, *J Virol*, 81(9):4654-63), and this vector has been shown to induce humoral and cell-mediated immune responses in prime-boost vaccine strategies (Abbink, et al., 2007, *J Virol*, 81(9):4654-63; Liu et al., 2009, *Nature*, 457(7225): 87-91). rAd35 vectors grow to high titers on cell lines suitable for production of clinical-grade vaccines (Havenga et al., 2006, *J Gen Virol*, 87: 2135-43), and have been formulated for injection as well as stable inhalable powder (Jin et al., 2010, *Vaccine* 28(27): 4369-75). These vectors show efficient transduction of human dendritic cells (de Gruijl et al., 2006, *J Immunol*, 177(4): 2208- 15; Lore et al., 2007, *J Immunol*, 179(3): 1721-9), and thus have the capability to mediate high level antigen delivery and presentation.

Modified Vaccinia Ankara (MVA) virus is related to Vaccinia virus, a member of the genera Orthopoxvirus in the family of Poxviridae. Poxviruses are known to be good inducers of CD8 T cell responses because of their intracytoplasmic expression. However, they may be poor at generating CD4 MHC class II restricted T cells (see for example Haslett et al., 2000, *Journal of Infectious Diseases*, 181 : 1264-72, page 1268). MVA has been developed for use as a viral vector for recombinant gene expression or as recombinant vaccine.

Strains of MVA having enhanced safety profiles for the development of safer products, such as vaccines or pharmaceuticals, have been developed by Bavarian Nordic. MVA was further developed by Bavarian Nordic and is designated MVA-BN, a representative sample of which was deposited on August 30, 2000 at the European Collection of Cell Cultures (ECACC) under Accession No. V00083008. MVA-BN is further described in WO 02/42480 (US 2003/0206926) and WO 03/048184 (US 2006/0159699), both of which are incorporated by reference herein in their entirety.

MVA-BN can attach to and enter human cells where virally-encoded genes are expressed very efficiently. MVA-BN is replication incompetent, meaning that the virus does not replicate in human cells. In human cells, viral genes are expressed, and no infectious virus is produced. MVA-BN is classified as Biosafety Level 1 organism according to the Centers for Disease Control and Prevention in the United States. Preparations of MVA-BN and derivatives have been administered to many types of animals, and to more than 2000 human subjects, including immune-deficient individuals. All vaccinations have proven to be generally safe and well tolerated. Despite its high attenuation and reduced virulence, in preclinical studies MVA-BN has been shown to elicit both humoral and cellular immune responses to vaccinia and to

heterologous gene products encoded by genes cloned into the MVA genome [E. Harrer et al. (2005), *Antivir. Ther.* 10(2):285-300; A. Cosma et al. (2003), *Vaccine* 22(1):21-9; M. Di Nicola et al. (2003), *Hum. Gene Ther.* 14(14):1347-1360; M. Di Nicola et al. (2004), *Clin. Cancer Res.*, 10(16):5381-5390].

Protective immunity to infection relies on both the innate and adaptive immune response. The adaptive immune response includes production of antibodies by B cells (humoral immune response) and the cytotoxic activity of CD8⁺ effector T cells (cellular immune response) and CD4⁺ T cells, also known as helper T cells, who play a key role in both the humoral and the cellular immune response.

Several assays can be used to detect immune responses, including, e.g., ELISA (enzyme-linked immunosorbent assay), ELISPOT (enzyme-linked immunospot), and ICS (intracellular cytokine staining). ELISA assays analyze, e.g., levels of secreted antibodies or cytokines. When ELISA assays are used to determine levels of antibodies that bind to a particular antigen, an indicator of the humoral immune response, they may also reflect CD4⁺ T cell activity, as the production of high-affinity antibodies by B cells depends on the activity of CD4⁺ helper T cells. ELISPOT and ICS are single-cell assays that analyze, e.g., T cell responses to a particular antigen. ELISPOT assays measure the secretory activity of individual cells, and ICS assays analyze levels of intracellular cytokine. CD4⁺ specific and CD8⁺ specific T cell responses can be determined using ICS assays.

There are published papers testing methods for using MVA-Ad prime-boost regimens in animals, such as monkeys and mice. For example, Barouch et al. (2012, *Nature*, 482(7383):89-93) found that, in monkeys, a heterologous regimen comprising MVA/Ad26 was “comparatively less efficacious than Ad26/MVA or Ad35/Ad26, which reduced viral load set-points by greater than 100-fold.” In particular, the cellular immune response to SIV Gag, Pol, and Env in rhesus monkeys was less-pronounced for the MVA/Ad26 prime-boost regimen administered on a 0-24 week schedule than for the opposite Ad26/MVA regimen, as measured by IFN-gamma ELISPOT and ICS assays. The antibody response was also less effective for the MVA/Ad26 regimen than for the Ad26/MVA regimen, as evidenced by an ELISA assay, though to a lesser extent. Roshorm et al. (2012, *Eur J Immunol*, 42(12):3243-55) found that an MVA/ChAdV68 prime-boost regimen administered in mice on a 0-4 week schedule was no more effective at inducing an immune response to HIV Gag than the opposite ChAdV68/MVA regimen, as measured by an ICS assay for CD8⁺ T cell activity. Gilbert et al. (2002, *Vaccine*,

20(7-8):1039-45) found that an MVA/Ad5 prime-boost regimen administered in mice on a 0-14 day schedule was slightly less effective in producing an immune response to Plasmodium CS than the opposite Ad5/MVA regimen, as measured by an ELISPOT assay. The MVA/Ad5 regimen was even less effective than the Ad5/MVA regimen when both were administered on a 0-10 day schedule. Additionally, the MVA/Ad5 regimen was less effective in protecting immunized mice against a challenge infection (80% vs. 100% protection). None of these reports indicate that an MVA/Ad regimen can result in a stronger humoral and/or cellular immune response in humans, than an Ad/MVA regimen. WO/2016/036971 describes that, different from the previously reported animal studies, for a given prime boost interval, the use of an MVA vector as a prime and an adenovirus vector as a boost generates a superior immune response against an immunogen, characterized by a stronger induction of T cell activity and/or a higher level of antibody response specific to the immunogen, compared to the use of an Ad/MVA regimen. When administered at similar intervals, higher levels of those induced immune responses persist after MVA/Ad vaccination in comparison to Ad/MVA vaccination.

There is an unmet need for improved vaccines that elicit broad and strong immune responses in humans against antigenic proteins, and particularly vaccines that provide protective immunity against the deadly Ebola and Marburg filoviruses.

BRIEF SUMMARY OF THE INVENTION

In one general aspect, the invention relates to a method of generating an enhanced immune response in a human subject by priming the immune response with an MVA vector, followed by an initial boosting of the immune response with an adenovirus vector, and a further boosting of the immune response with an adenovirus vector.

In certain embodiments of the invention, MVA-prime and adenovirus-boost combinations of replication incompetent vectors generate an enhanced immune response to an antigenic protein or an immunogenic polypeptide thereof in a human subject, and the immune response is further enhanced by a second boost with an adenovirus vector. The antigenic protein or immunogenic polypeptide thereof can be any antigenic protein or immunogenic polypeptide thereof. For example, the antigenic protein or immunogenic polypeptide thereof can be derived from a pathogen, e.g., a virus, a bacterium, a fungus, a protozoan, or a tumor.

Accordingly, one general aspect of the invention relates to a method of enhancing an immune response in a human subject, the method comprising:

- a. administering to the human subject a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding a first antigenic protein or an immunogenic polypeptide thereof for priming the immune response;
- 5 b. administering to the subject a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding a second antigenic protein or an immunogenic polypeptide thereof for boosting the immune response; and
- 10 c. administering to the subject a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding a third antigenic protein or an immunogenic polypeptide thereof for further boosting the immune response,

to thereby obtain an enhanced immune response in the human subject, wherein the first, second and third antigenic proteins share at least one antigenic determinant.

- 15 In a preferred embodiment of the invention, the enhanced immune response comprises an enhanced antibody response against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject.

- In a preferred embodiment of the invention, the enhanced immune response comprises an enhanced CD4+ and/or CD8+ T cell response against the at least one antigenic determinant
- 20 shared by the first, second and third antigenic proteins in the human subject.

In a preferred embodiment, the first, second and third antigenic proteins are identical or substantially identical.

Another aspect of the invention relates to a method of eliciting an immune response in a human subject, the method comprising:

- 25 a. administering to the human subject a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding a first antigenic protein or an immunogenic polypeptide thereof for priming the immune response;
- 30 b. administering to the subject a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding a second antigenic protein or an immunogenic polypeptide thereof for boosting the immune response; and

- c. administering to the subject a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding a third antigenic protein or an immunogenic polypeptide thereof for further boosting the immune response,

5 to thereby obtain an enhanced immune response in the human subject relative to the immune response that would be observed if the third composition is not administered, wherein the first, second and third antigenic proteins share at least one antigenic determinant.

In a preferred embodiment of the invention, the enhanced immune response generated by the method comprises an enhanced antibody response against the at least one antigenic
10 determinant shared by the first, second and third antigenic proteins in the human subject. Such a response can, e.g., be characterized by the presence of a high proportion of responders, such as more than 50%, 60%, 70%, 80%, 90%, or 100% of subjects tested.

In one embodiment of the invention, the enhanced immune response generated by the method comprises an enhanced CD8+ T cell response against the at least one antigenic
15 determinant shared by the first, second and third antigenic proteins in the human subject [e.g. a response characterized by the presence of a high proportion of CD8+ responders, such as more than 50%, 60%, 70%, 80%, 90%, or 100% of subjects tested as determined by an ICS assay, with a median total cytokine response of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5% or more]. In another embodiment of the invention, the enhanced CD8+ T cell response generated by the
20 method comprises an increase or induction of polyfunctional CD8+ T cells specific to the at least one antigenic determinant shared by the first, second and third antigenic proteins. Such polyfunctional CD8+ T cells express more than one cytokine, such as two or more of IFN-gamma, IL-2 and TNF-alpha.

In one embodiment of the invention, the enhanced immune response generated by the
25 method comprises an enhanced CD4+ T cell response against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject [e.g. a response characterized by the presence of a high proportion of CD4+ responders, such as more than 50%, 60%, 70%, 80%, 90%, or 100% of subjects tested as determined by an ICS assay, with a median total cytokine response of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5% or more]. In
30 another embodiment of the invention, the enhanced CD4+ T cell response generated by the method comprises an increase or induction of polyfunctional CD4+ T cells specific to the at least one antigenic determinant shared by the first, second and third antigenic proteins. Such

polyfunctional CD4+ T cells express more than one cytokine, such as two or more of IFN-gamma, IL-2 and TNF-alpha.

In another preferred embodiment of the invention, the enhanced immune response further comprises an enhanced antibody response against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject. Such a response can, e.g. be characterized by the presence of a high proportion of responders, such as more than 50%, 60%, 70%, 80%, 90%, or 100% of subjects tested.

In another embodiment of the invention, the enhanced immune response further comprises an enhanced CD8+ T cell response against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject [e.g. a response characterized by the presence of a high proportion of CD8+ responders, such as more than 50%, 60%, 70%, 80%, 90%, or 100% of subjects tested as determined by an ICS assay, with a median total cytokine response of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5% or more]. In one embodiment of the invention, the enhanced CD8+ T cell response generated by the method comprises an increase or induction of polyfunctional CD8+ T cells specific to the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject.

In a more preferred embodiment of the invention, the enhanced immune response comprises an enhanced CD4+ T cell response, an enhanced antibody response and an enhanced CD8+ T cell response, against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject.

In a preferred embodiment, the first, second and third antigenic proteins are identical or substantially identical.

In an embodiment of the invention, the first and second adenovirus vectors are different vectors, for example, one is rAd26 and the other is rAd35. In a preferred embodiment of the invention, each of the first and second adenovirus vectors is a rAd26 vector.

In another preferred embodiment of the invention, the boosting step (b) is conducted 1-12 weeks after the priming step (a). In a preferred embodiment of the invention, the boosting step (b) is conducted 2-12 weeks after the priming step (a). In another preferred embodiment of the invention, the boosting step (b) is conducted 4-12 weeks after the priming step (a). In another preferred embodiment of the invention, the boosting step (b) is conducted 1 week after the priming step (a). In another preferred embodiment of the invention, the boosting step (b) is conducted 2 weeks after the priming step (a). In another preferred embodiment of the invention,

the boosting step (b) is conducted 4 weeks after the priming step (a). In another preferred embodiment of the invention, the boosting step (b) is conducted 8 weeks after the priming step (a).

5 In another preferred embodiment of the invention, the further boosting step (c) is conducted at least 4 weeks after the boosting step (b). In a preferred embodiment of the invention, the further boosting step (c) is conducted at least 5 weeks after the boosting step (b). In still another preferred embodiment, the further boosting step (c) is conducted at least 6 weeks after the boosting step (b). For example, the further boosting step (c) can be conducted 6 weeks to 5 years after the boosting step (b), such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
10 21, 22, 23, 24 or 25 weeks, or 7, 8, 9, 10, 11 or 12 months, or 2, 3, 4 or 5 years after the boosting step (b). Optionally, the further boosting step (c) can be repeated one or more times as needed.

In an embodiment of the invention, the antigenic protein is derived from a pathogen, such as a virus, a bacterium, a fungus, or a protozoan. In another embodiment of the invention,
15 the antigenic protein is derived from a tumor, preferably a cancer.

In an embodiment of the invention, the first polynucleotide, the second polynucleotide and the third polynucleotide encode for the same antigenic protein or immunogenic polypeptide thereof. In another embodiment of the invention, the first polynucleotide, the second polynucleotide and the third polynucleotide encode for different immunogenic polypeptides or
20 epitopes of the same antigenic protein. In yet another embodiment of the invention, the first polynucleotide, the second polynucleotide and the third polynucleotide encode for different, but related, antigenic proteins or immunogenic polypeptide thereof. For example, the related antigenic proteins can be substantially similar proteins derived from the same antigenic protein, or different antigenic proteins derived from the same pathogen or tumor.

25 According to embodiment of the invention, a method of the invention provides a protective immunity to the human subject against a disease associated with the antigenic protein, such as a tumor or an infectious disease.

In one preferred embodiment, the prime-boost-boost combination of replication incompetent MVA and adenovirus vectors enhances a protective immune response against a
30 tumor in a human subject.

In another preferred embodiment, the prime-boost-boost combination of replication incompetent MVA and adenovirus vectors enhances an immune response against a pathogen,

more preferably one or more filovirus subtypes, such as the Ebola and/or Marburg filoviruses, in a human subject.

The filovirus subtypes according to the invention can be any filovirus subtype. In a preferred embodiment, the filovirus subtypes are selected from the group of Zaire, Sudan, Reston, Bundibugyo, Taï Forest and Marburg viruses. The antigenic proteins can be any protein from any filovirus comprising an antigenic determinant. In a preferred embodiment the antigenic proteins are glycoproteins or nucleoproteins. The antigenic proteins encoded by the MVA vectors or adenovirus vectors comprised in the first, second and third compositions according to the invention can be any antigenic protein or an immunogenic polypeptide or an antigenic determinant thereof from any filovirus.

In certain embodiments, a first composition comprising a MVA vector is used for the priming step (a) of the invention, a second composition comprising at least one adenovirus vector is used for the boosting step (b) of the invention, and a third composition comprising at least one adenovirus vector is used for the boosting step (c) of the invention.

In a preferred embodiment, the MVA vector in the first composition comprises a nucleic acid encoding antigenic proteins of at least four filovirus subtypes. Preferably, the MVA vector comprises a nucleic acid encoding one or more antigenic proteins having the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5, or an immunogenic polypeptide or antigenic determinant thereof. Most preferably, the MVA vector comprises a nucleic acid encoding four antigenic proteins having the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5.

In certain embodiments, the at least one adenovirus vector in the second composition or the third composition comprises a nucleic acid encoding an antigenic protein of at least one filovirus subtype. The at least one filovirus subtype encoded by the adenovirus can be selected from any of the four filovirus subtypes encoded by the MVA vector, or a new subtype not encoded by the MVA vector. In a preferred embodiment, the first and second adenovirus vectors each independently encode at least one of the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, or an immunogenic polypeptide or antigenic determinant thereof.

In another embodiment, each of the second and third compositions comprises more than one adenovirus vectors. Each of the adenovirus vectors comprises a nucleic acid encoding an antigenic protein of at least one filovirus subtype. The antigenic proteins encoded by the more

than one adenovirus vectors can be the same or different antigenic proteins. For example, the second or third composition can comprise an adenovirus vector comprising a nucleic acid encoding a first antigenic protein having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

5 The second or third composition can further comprise another adenovirus vector comprising a nucleic acid encoding a second antigenic protein having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5. The second or third composition can additionally comprise a third adenovirus vector comprising a nucleic acid encoding a third antigenic protein having an amino acid sequence
10 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

In one embodiment, the second composition and the third composition are the same composition.

In another embodiment, the second composition and the third composition are different
15 compositions. For example, they can comprise different adenovirus vectors encoding the same antigenic proteins. They can also comprise the same adenovirus vectors encoding different antigenic proteins. They can comprise different adenovirus vectors encoding different antigenic proteins. They can even comprise the same adenovirus vectors encoding the same antigenic proteins, but formulated with different excipients or carriers.

20 In a preferred embodiment, each of the second and third compositions, which preferably have the same composition, comprises a first adenovirus vector comprising a nucleic acid encoding an antigenic protein having the amino acid sequence of SEQ ID NO:1, or immunogenic polypeptide or antigenic determinant thereof. In another embodiment, each of the second and third compositions, which preferably have the same composition, further comprises
25 a second adenovirus vector comprising a nucleic acid encoding an antigenic protein having the amino acid sequence of SEQ ID NO:2, or immunogenic polypeptide or antigenic determinant thereof. In yet another embodiment, each of the second and third compositions, which preferably have the same composition, additionally comprises a third adenovirus vector comprising a nucleic acid encoding an antigenic protein having the amino acid sequence of SEQ
30 ID NO:3, or immunogenic polypeptide or antigenic determinant thereof.

It is contemplated that the methods, vaccines, and compositions described herein can be embodied in a kit. For example, in one embodiment, the invention can include a kit comprising:

- (a) a first composition comprising an immunologically effective amount of a MVA vector comprising a nucleic acid encoding a first antigenic protein or an immunogenic polypeptide thereof of at least one filovirus subtype or a substantially similar antigenic protein or an immunogenic polypeptide thereof, together with a pharmaceutically acceptable carrier;
- (b) a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a nucleic acid encoding a second antigenic protein or an immunogenic polypeptide thereof of at least one filovirus subtype or a substantially similar antigenic protein or an immunogenic polypeptide thereof, together with a pharmaceutically acceptable carrier; and
- (c) a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a nucleic acid encoding a third antigenic protein or an immunogenic polypeptide thereof of at least one filovirus subtype or a substantially similar antigenic protein or an immunogenic polypeptide thereof, together with a pharmaceutically acceptable carrier;

wherein the first composition is a priming composition, each of the second and third compositions is a boosting composition, and wherein the first, second and third antigenic proteins share at least one antigenic determinant. In a preferred embodiment, the second and third compositions are identical.

In a preferred embodiment, the adenovirus vectors comprised in the combination vaccine or kit of the invention or the adenovirus vectors used for generating a protective immune response against at least one of the filovirus subtypes, are rAd26 or rAd35 vectors, more preferably rAd26. In another preferred embodiment, the MVA vector used in the combination vaccine or kit or method of the invention is a MVA-BN vector.

In a preferred embodiment, the invention relates to a combination vaccine, a kit or a use wherein the MVA vector, preferably a MVA-BN vector, in the first composition comprises a nucleic acid encoding one or more antigenic proteins from four different filovirus subtypes having the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5, or an immunogenic polypeptide thereof, preferably all four of the antigenic proteins; the adenovirus vector, preferably a rAd26 vector, in the second composition comprises a nucleic

acid encoding an antigenic protein having the amino acid sequence of SEQ ID NO: 1, or an immunogenic polypeptide thereof; and the adenovirus vector, preferably a rAd26 vector, in the third composition comprises a nucleic acid encoding the antigenic protein having the amino acid sequence of SEQ ID NO: 1, or an immunogenic polypeptide thereof.

5 In another embodiment, the MVA vector, preferably a MVA-BN vector, in the first composition, and the first and second adenovirus vectors, preferably a rAd26 vector, in the second and third compositions, each independently comprise a nucleic acid encoding at least one antigenic protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5, or an antigenic
10 determinant or immunogenic polypeptide thereof.

In yet another preferred embodiment, the invention relates to a combination vaccine, a kit or a use wherein the MVA vector in composition (a) comprises a nucleic acid encoding one or more antigenic proteins from four different filovirus subtypes having SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5, or an immunogenic polypeptide thereof; and
15 wherein each of the second composition and the third composition comprises at least one adenovirus vector comprising a nucleic acid encoding an antigenic protein having the amino acid sequence of SEQ ID NO: 1 or immunogenic polypeptide thereof, at least one adenovirus vector comprising a nucleic acid encoding an antigenic protein having the amino acid sequence of SEQ ID NO: 2 or immunogenic polypeptide thereof, or at least one adenovirus vector
20 comprising a nucleic acid encoding an antigenic protein with SEQ ID NO: 3 or immunogenic polypeptide thereof.

In yet another preferred embodiment, the invention relates to a combination vaccine, a kit or a use wherein the MVA vector in composition (a), preferably a MVA-BN vector, comprises a nucleic acid encoding four antigenic proteins from four different filovirus subtypes having SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5; and wherein each of
25 the second composition and the third composition comprises at least one adenovirus vector, preferably a rAd26 vector, comprising a nucleic acid encoding an antigenic protein having SEQ ID NO: 1, at least one adenovirus vector, preferably a rAd26 vector, comprising a nucleic acid encoding an antigenic protein having SEQ ID NO: 2, and at least one adenovirus vector,
30 preferably a rAd26 vector, comprising a nucleic acid encoding an antigenic protein having SEQ ID NO: 3.

According to various embodiments of the invention, the priming vaccination is

conducted at week 0, followed by an initial boosting vaccination at week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or later, and a further boosting vaccination 4-96 weeks after the initial boosting vaccination. Preferably, the initial boosting vaccination is administered at week 1-10, more preferably at week 1, 2 or 3, and the further boosting vaccination is administered at least 6 weeks after the initial boosting vaccination, such as 6 to 24 weeks, more preferably 8-16 weeks, after the administration of the initial boosting vaccination. The further boosting can also be conducted later, for example 40-60 weeks after the administration of the initial boosting vaccination.

In a preferred embodiment of the invention, the method comprises a priming vaccination with an immunologically effective amount of one or more MVA vectors, preferably MVA-BN vectors, expressing one or more antigenic proteins or immunogenic polypeptides thereof, preferably filovirus glycoproteins, followed by a boosting vaccination with an immunologically effective amount of one or more adenovirus vectors, preferably Ad26 vectors, expressing one or more antigenic proteins or immunogenic polypeptides thereof, preferably filovirus glycoproteins or substantially similar glycoproteins, and a further boosting vaccination with an immunologically effective amount of one or more adenovirus vectors, preferably Ad26 vectors, expressing one or more antigenic proteins or immunogenic polypeptides thereof, preferably filovirus glycoproteins or substantially similar glycoproteins.

In preferred embodiments of the invention, the one or more filoviruses are Ebolaviruses or Marburg viruses. The Ebolavirus can be of any species, for example, Zaire ebolavirus (EBOV) and Sudan ebolavirus (SUDV), Reston, Bundibugyo, Taï Forest. The Marburg virus (MARV) can be of any species. Exemplary amino acid sequences of suitable filovirus antigenic proteins are shown in SEQ ID NO: 1 to SEQ ID NO: 5.

The invention also relates to use of the first, second and third compositions according to embodiments of the invention for enhancing an immune response in a human subject, wherein the first composition is administered to the human subject for priming the immune response, the second composition is administered to the human subject for boosting the immune response, and the third composition is administered to the human subject for further boosting the immune response, to thereby obtain an enhanced immune response against the antigenic protein in the human subject.

The invention further relates to:

- a. a first composition comprising an immunologically effective amount of a

MVA vector comprising a first polynucleotide encoding an antigenic protein or an immunogenic polypeptide thereof;

- b. a second composition comprising an immunologically effective amount of an adenovirus vector comprising a second polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for boosting the immune response; and
- c. a third composition comprising an immunologically effective amount of an adenovirus vector comprising a third polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for boosting the immune response;

the first, second and third compositions for use in inducing an enhanced immune response against the antigenic protein in a human subject, wherein the first composition is administered to the human subject for priming the immune response, the second composition is administered to the human subject for boosting the immune response, and the third composition is administered to the human subject for further boosting the immune response.

In one preferred embodiment, the antigenic protein or an immunogenic polypeptide thereof encoded by the first polynucleotide is derived from a pathogen or a tumor. In another preferred embodiment, the antigenic protein or an immunogenic polypeptide thereof encoded by the first polynucleotide is derived from a filovirus. In yet another embodiment, the antigenic proteins comprise the amino acid sequences selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5. Most preferably, the MVA vector in the first composition comprises a polynucleotide encoding the antigenic proteins having the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5.

More preferably, the adenovirus vector in the second or third composition comprises a polynucleotide encoding at least one antigenic protein having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In a more preferred embodiment, the adenovirus vector comprises a polynucleotide encoding the antigenic protein having the amino acid sequence of SEQ ID NO: 1. Preferably said adenovirus vector is an rAd26 vector. More preferably, the second and third composition each comprise an rAd26 vector comprising a polynucleotide encoding at least one antigenic protein having the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

In yet another preferred embodiment, the MVA vector in composition (a) comprises a nucleic acid encoding four antigenic proteins from four different filovirus subtypes having SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5; and both the second and third composition comprise at least one adenovirus comprising a nucleic acid encoding an antigenic protein having SEQ ID NO: 1, at least one adenovirus comprising a nucleic acid encoding an antigenic protein having SEQ ID NO: 2, and at least one adenovirus comprising a nucleic acid encoding an antigenic protein having SEQ ID NO: 3.

The invention further relates to:

- a. a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding an antigenic protein or an immunogenic polypeptide thereof;
- b. a second composition comprising an immunologically effective amount of an adenovirus vector comprising a second polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for boosting the immune response; and
- c. a third composition comprising an immunologically effective amount of an adenovirus vector comprising a third polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for boosting the immune response;

wherein the first composition is administered to a human subject for priming the immune response, the second composition is administered to the human subject for boosting the immune response, and the third composition is administered to the human subject for further boosting the immune response to thereby induce an enhanced humoral and/or cellular immune response in the human subject relative to the humoral and/or cellular immune response that would be observed if the third composition would not be administered.

In one embodiment of the invention, the enhanced immune response generated by said first, second and third compositions comprises an increase of the antibody response against the antigenic protein in the human subject combined with a CD4⁺ and CD8⁺ response [e.g., a response characterized by the presence of a high proportion of CD4⁺ and CD8⁺ responders, such as more than 50%, 60%, 70%, 80%, 90% or 100% of subjects tested as determined by an ICS assay, with a median total cytokine response of about 0.2%, 0.3%, 0.4%, 0.5% or more]. In

another embodiment of the invention, the enhanced CD4⁺ and CD8⁺ T cell responses generated by said compositions a. and b. comprises an increase or induction of polyfunctional CD4⁺ and CD8⁺ T cells specific to the antigenic protein. Such polyfunctional CD4⁺ T cells express more than one cytokine, such as two or more of IFN-gamma, IL-2 and TNF-alpha.

5

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood that the invention is not limited to the precise embodiments shown in the drawings.

10 The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

In the drawings:

15 Figures 1a and 1b show Ebola virus (EBOV), Marburg virus (MARV) and Sudan Virus (SUDV) glycoprotein (GP)-specific binding antibody responses (Fig. 1a) and neutralizing antibody responses (Fig. 1b). The immunoglobulin G (IgG) responses, assessed by enzyme-linked immunosorbent assay (ELISA, Battelle Biomedical Research Center; in ELISA units/mL), are displayed with geometric mean and 95% confidence interval. The neutralizing antibody responses, assessed by pseudovirus neutralization assay (psVNA, Monogram; in 20 50% inhibitory concentration (IC₅₀) titer), are displayed with geometric mean and 95% confidence interval. The values at the bottom of each graph represent the median reportable value (first row) and the responder rate (second row). The lower limit of quantification (LLOQ) is indicated by the dashed red line. Vaccination time points are indicated with a dotted vertical line. Positive samples are displayed with an orange dot.

25 Figure 2 shows Ebola virus (EBOV) glycoprotein (GP)-specific binding antibody responses. The anti-EBOV GP immunoglobulin G (IgG) responses, assessed by enzyme-linked immunosorbent assay (ELISA, Battelle Biomedical Research Center; in ELISA units/mL), are displayed with geometric mean and 95% confidence interval. Green: 14-day interval; Blue: 28-day interval; Black: 56-day interval

30 Figure 3 shows Ebola virus (EBOV) glycoprotein (GP)-specific neutralizing antibody responses. The anti-EBOV GP neutralizing antibody responses, assessed by pseudovirus neutralization assay (psVNA, Monogram; in 50% inhibitory concentration (IC₅₀) titer), are

displayed with geometric mean and 95% confidence interval. Green: 14-day interval; Blue: 28-day interval; Black: 56-day interval

Figures 4a-4c show individual Ebola virus (EBOV) glycoprotein (GP)-specific responses (interferon [IFN]- γ enzyme-linked immunospot [ELISpot] on frozen peripheral blood mononuclear cells [PBMC]). The anti-EBOV GP IFN- γ responses, as assessed by HIV Vaccine Trials Network (HVTN), are displayed in spot-forming units per million PBMC (SFU/10⁶ PBMC). The error bars indicate the median values with the interquartile ranges. The values at the top of each graph represent the median reportable value and the responder rate. The lower limit of quantification (LLOQ) is indicated by the dashed grey line.

Figure 5a-5c show individual Ebola virus (EBOV) glycoprotein (GP)-specific CD4⁺ T cell responses (intracellular cytokine staining [ICS] on frozen peripheral blood mononuclear cells [PBMC]). The anti-EBOV GP CD4⁺ T cell responses, as assessed by HIV Vaccine Trials Network (HVTN), are displayed as the percentage of CD4⁺ T cells secreting interferon (IFN)- γ , interleukin (IL)-2, and/or tumor necrosis factor (TNF)- α . The error bars indicate the median values with the interquartile ranges. The values at the top of each graph represent the median reportable value and the responder rate. The lower limit of quantification (LLOQ) is indicated by the dashed grey line.

Figures 6a-6c show individual Ebola virus (EBOV) glycoprotein (GP)-specific CD8⁺ T cell responses (intracellular cytokine staining [ICS] on frozen peripheral blood mononuclear cells [PBMC]). The anti-EBOV GP CD8⁺ T cell responses, as assessed by HIV Vaccine Trials Network (HVTN), are displayed as the percentage of CD4⁺ T cells secreting interferon (IFN)- γ , interleukin (IL)-2, and/or tumor necrosis factor (TNF)- α . The error bars indicate the median values with the interquartile ranges. The values at the top of each graph represent the median reportable value and the responder rate. The lower limit of quantification (LLOQ) is indicated by the dashed grey line.

DETAILED DESCRIPTION OF THE INVENTION

Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art

with respect to any inventions disclosed or claimed.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein. It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term “comprising” can be substituted with the term “containing” or “including” or sometimes when used herein with the term “having”. When used herein “consisting of” excludes any element, step, or ingredient not specified in the claim element. When used herein, “consisting essentially of” does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. In each instance herein any of the terms “comprising”, “consisting essentially of” and “consisting of” can be replaced with either of the other two terms.

As used herein, the conjunctive term “and/or” between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by “and/or”, a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or” as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement

of the term “and/or.”

As used herein, “subject” means any animal, preferably a mammal, most preferably a human, to whom will be or has been treated by a method according to an embodiment of the invention. The term “mammal” as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

As used herein, the term “protective immunity” or “protective immune response” means that the vaccinated subject is able to control an infection or a disease related to an antigenic protein or immunogenic polypeptide thereof against which the vaccination was done. Usually, the subject having developed a “protective immune response” develops only mild to moderate clinical symptoms or no symptoms at all. Usually, a subject having a “protective immune response” or “protective immunity” against a certain antigenic protein will not die as a result of an infection or disease related to the antigenic protein.

The antigenic protein can be a native protein from a pathogen or a tumor, or a modified protein based on a native protein from a pathogen or a tumor.

As used herein, the term “pathogen” refers to an infectious agent such as a virus, a bacterium, a fungus, a parasite, or a prion that causes disease in its host.

As used herein, the term “enhanced” when used with respect to an immune response, such as a CD4⁺ T cell response, an antibody response, or a CD8⁺ T cell response, refers to an increase in the immune response in a human subject administered with a prime-boost combination of replication incompetent MVA and adenovirus vectors according to the invention, relative to the corresponding immune response observed from the human subject administered with a reverse prime-boost combination, wherein the adenovirus vector is provided as a prime and the MVA vector is provided to boost the immune response, using the same prime-boost interval.

As used herein, the term “dominant CD4⁺ or CD8⁺ T cell response” refers to a T cell immune response that is characterized by observing high proportion of immunogen-specific CD4⁺ T cells within the population of total responding T cells following vaccination. The total immunogen-specific T-cell response can be determined by an IFN-gamma ELISPOT assay. The immunogen-specific CD4⁺ or CD8⁺ T cell immune response can be determined by an ICS assay. For example, a dominant CD4⁺ T cell response can comprise an antigen specific CD4⁺ T cell response that is more than 50%, such as 51%, 60%, 70%, 80%, 90% or 100% of the total

antigen specific T-cell responses in the human subject. Preferably, the dominant CD4+ T cell response also represents 0.1% or more, such as 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, or more of the total cytokine responses in the human subject.

As used herein, the term “enhanced antibody response” refers to an antibody response in a human subject administered with a prime-boost combination of replication incompetent MVA and adenovirus vectors according to the invention, that is increased by a factor of at least 1.5, 2, 2.5, or more relative to the corresponding immune response observed from the human subject administered with a reverse prime-boost combination, wherein the adenovirus vector is provided as a prime and the MVA vector is provided to boost the immune response, using the same prime-boost interval.

As used herein, the term “polyfunctional” when used with respect to CD4+ or CD8+ T cells means T cells that express more than one cytokine, such as at least two of: IL-2, IFN-gamma, and TNF-alpha.

An “adenovirus capsid protein” refers to a protein on the capsid of an adenovirus (e.g., Ad 26 or Ad 35) that is involved in determining the serotype and/or tropism of a particular adenovirus. Adenoviral capsid proteins typically include the fiber, penton and/or hexon proteins. As used herein a “Ad26 capsid protein” or a “Ad35 capsid protein” can be, for example, a chimeric capsid protein that includes at least a part of an Ad26 or Ad35 capsid protein. In certain embodiments, the capsid protein is an entire capsid protein of Ad26 or of Ad35. In certain embodiments, the hexon, penton and fiber are of Ad26 or of Ad35.

The terms “adjuvant” and “immune stimulant” are used interchangeably herein, and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to the adenovirus and/or MVA vectors of the invention.

The term “corresponding to”, when applied to positions of amino acid residues in sequences, means corresponding positions in a plurality of sequences when the sequences are optimally aligned.

The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, (e.g., glycoproteins of filovirus and polynucleotides that encode them) refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison

algorithms or by visual inspection.

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

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, *Current Protocols in Molecular Biology*, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) *J. Mol. Biol.* 215: 403-410 and Altschuel et al. (1977) *Nucleic Acids Res.* 25: 3389- 3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative

alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W , T , and X determine the sensitivity and speed of the alignment. The BLASTN program (for
 5 nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, $M=5$, $N=-4$, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

In addition to calculating percent sequence identity, the BLAST algorithm also performs
 10 a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest
 15 sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a
 20 polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below.

The term "substantially similar" in the context of the filovirus antigenic proteins of the
 25 invention indicates that a polypeptide comprises a sequence with at least 90%, preferably at least 95% sequence identity to the reference sequence over a comparison window of 10-20 amino acids. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the
 30 reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the

number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

It is discovered in the invention that heterologous prime-boost combinations, in particular, MVA priming followed by Ad26 boosting, are surprisingly effective in generating protective immune responses in human subjects.

Antigenic proteins

Any DNA of interest can be inserted into the viral vectors described herein to be expressed heterologously from the vectors. Foreign genes for insertion into the genome of a virus in expressible form can be obtained using conventional techniques for isolating a desired gene. For organisms which contain a DNA genome, the genes encoding an antigen of interest can be isolated from the genomic DNA; for organisms with RNA genomes, the desired gene can be isolated from cDNA copies of the genome. The antigenic protein can also be encoded by a recombinant DNA that is modified based on a naturally occurring sequence, e.g., to optimize the antigenic response, gene expression, etc.

In certain embodiments of the invention, MVA-prime and adenovirus-boost combinations of replication incompetent vectors generate an enhanced immune response to an antigenic protein or an immunogenic polypeptide thereof in a human subject. The antigenic protein can be any antigenic protein related to an infection or disease.

According to embodiments of the invention, the antigenic protein or immunogenic polypeptide thereof can be isolated from, or derived from, a pathogen, such as a virus (e.g., filovirus, adenovirus, arbovirus, astrovirus, coronavirus, coxsackie virus, cytomegalovirus, Dengue virus, Epstein-Barr virus, hepatitis virus, herpesvirus, human immunodeficiency virus, human papilloma virus, human T-lymphotropic virus, influenza virus, JC virus, lymphocytic choriomeningitis virus, measles virus, molluscum contagiosum virus, mumps virus, norovirus, parovirus, poliovirus, rabies virus, respiratory syncytial virus, rhinovirus, rotavirus, rubella virus, smallpox virus, varicella zoster virus, West Nile virus, etc.), a bacteria (e.g., *Campylobacter jejuni*, *Escherichia coli*, *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Streptococcus*, etc.), a fungus (e.g., *Coccidioides immitis*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Candida species*, *Aspergillus species*, etc.), a protozoan (e.g., *Plasmodium*, *Leishmania*, *Trypanosome*, *cryptosporidium*, *isospora*, *Naegleria fowleri*,

Acanthamoeba, Balamuthia mandrillaris, Toxoplasma gondii, Pneumocystis carinii, etc.), or a cancer (e.g., bladder cancer, breast cancer, colon and rectal cancer, endometrial cancer, kidney cancer, leukemia, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer, thyroid cancer, etc.).

In some embodiments, nucleic acids express antigenic domains rather than the entire antigenic protein. These fragments can be of any length sufficient to be immunogenic or antigenic. Fragments can be at least four amino acids long, preferably 8-20 amino acids, but can be longer, such as, e.g., 100, 200, 660, 800, 1000, 1200, 1600, 2000 amino acids long or more, or any length in between.

In some embodiments, at least one nucleic acid fragment encoding an antigenic protein or immunogenic polypeptide thereof is inserted into a viral vector. In another embodiment, about 2-8 different nucleic acids encoding different antigenic proteins are inserted into one or more of the viral vectors. In some embodiments, multiple immunogenic fragments or subunits of various proteins can be used. For example, several different epitopes from different sites of a single protein or from different proteins of the same species, or from a protein ortholog from different species can be expressed from the vectors.

Filovirus antigenic proteins

The Ebola viruses, and the genetically-related Marburg virus, are filoviruses associated with outbreaks of highly lethal hemorrhagic fever in humans and primates in North America, Europe, and Africa (Peters, C.J. et al. in: Fields Virology, eds. Fields, B.N. et al. 1161-1176, Philadelphia, Lippincott-Raven, 1996; Peters, C.J. et al. 1994 Semin Virol 5:147-154).

Although several subtypes have been defined, the genetic organization of these viruses is similar, each containing seven linearly arrayed genes. Among the viral proteins, the envelope glycoprotein exists in two alternative forms, a 50-70 kilodalton (kDa) secreted protein (sGP) and a 130 kDa transmembrane glycoprotein (GP) generated by RNA editing that mediates viral entry (Peters, C.J. et al. in: Fields Virology, eds. Fields, B.N. et al. 1161-1176, Philadelphia, Lippincott-Raven, 1996; Sanchez, A. et al. 1996 PNAS USA 93:3602-3607). Other structural gene products include the nucleoprotein (NP), matrix proteins VP24 and VP40, presumed nonstructural proteins VP30 and VP35, and the viral polymerase (reviewed in Peters, C.J. et al. in: Fields Virology, eds. Fields, B.N. et al. 1161-1176, Philadelphia, Lippincott-Raven, 1996).

The nucleic acid molecules comprised in the adenovirus and MVA vectors may encode structural gene products of any filovirus species, such as subtypes of Zaire (type species, also

referred to herein as ZEBOV), Sudan (also referred to herein as SEBOV), Reston, Bundibugyo, and Ivory Coast. There is a single species of Marburg virus (also referred to herein as MARV).

The adenoviral vectors and MVA vectors of the invention can be used to express antigenic proteins which are proteins comprising an antigenic determinant of a wide variety of
 5 filovirus antigens. In a typical and preferred embodiment, the vectors of the invention include nucleic acid encoding the transmembrane form of the viral glycoprotein (GP). In other embodiments, the vectors of the invention may encode the secreted form of the viral glycoprotein (ssGP), or the viral nucleoprotein (NP).

One of skill will recognize that the nucleic acid molecules encoding the filovirus
 10 antigenic protein can be modified, e.g., the nucleic acid molecules set forth herein can be mutated, as long as the modified expressed protein elicits an immune response against a pathogen or disease. Thus, as used herein, the term “antigenic protein” or “filovirus protein” refers to a protein that comprises at least one antigenic determinant of a filovirus protein described above. The term encompasses filovirus glycoproteins (i.e., gene products of a
 15 filovirus) or filovirus nucleoprotein as well as recombinant proteins that comprise one or more filovirus glycoprotein determinants. The term antigenic proteins also encompasses antigenic proteins that are substantially similar.

In some embodiments, the protein can be mutated so that it is less toxic to cells (see e.g., WO/2006/037038) or can be expressed with increased or decreased level in the cells. The
 20 invention also includes vaccines comprising a combination of nucleic acid molecules. For example, and without limitation, nucleic acid molecules encoding GP, ssGP and NP of the Zaire, Sudan, Marburg and Ivory Coast/Tai Forest Ebola strains can be combined in any combination, in one vaccine composition.

Adenoviruses

An adenovirus according to the invention belongs to the family of the Adenoviridae and preferably is one that belongs to the genus Mastadenovirus. It can be a human adenovirus, but also an adenovirus that infects other species, including but not limited to a bovine adenovirus (e.g. bovine adenovirus 3, BAdV3), a canine adenovirus (e.g. CAdV2), a porcine adenovirus (e.g. PAdV3 or 5), or a simian adenovirus (which includes a monkey adenovirus and an ape
 30 adenovirus, such as a chimpanzee adenovirus or a gorilla adenovirus). Preferably, the adenovirus is a human adenovirus (HAdV, or AdHu; in the invention a human adenovirus is meant if referred to Ad without indication of species, e.g. the brief notation “Ad5” means the

same as HAdV5, which is human adenovirus serotype 5), or a simian adenovirus such as chimpanzee or gorilla adenovirus (ChAd, AdCh, or SAdV).

Most advanced studies have been performed using human adenoviruses, and human adenoviruses are preferred according to certain aspects of the invention. In certain preferred
 5 embodiments, the recombinant adenovirus according to the invention is based upon a human adenovirus. In preferred embodiments, the recombinant adenovirus is based upon a human adenovirus serotype 5, 11, 26, 34, 35, 48, 49 or 50. According to a particularly preferred embodiment of the invention, an adenovirus is a human adenovirus of one of the serotypes 26 or 35.

10 An advantage of these serotypes is a low seroprevalence and/or low pre-existing neutralizing antibody titers in the human population. Preparation of rAd26 vectors is described, for example, in WO 2007/104792 and in Abbink et al., (2007) Virol 81(9): 4654-63, both of which are incorporated by reference herein in their entirety. Exemplary genome sequences of Ad26 are found in GenBank Accession EF 153474 and in SEQ ID NO:1 of WO 2007/104792.
 15 Preparation of rAd35 vectors is described, for example, in US Patent No. 7,270,811, in WO 00/70071, and in Vogels et al., (2003) J Virol 77(15): 8263-71, all of which are incorporated by reference herein in their entirety. Exemplary genome sequences of Ad35 are found in GenBank Accession AC_000019 and in Fig. 6 of WO 00/70071.

Simian adenoviruses generally also have a low seroprevalence and/or low pre-existing
 20 neutralizing antibody titers in the human population, and a significant amount of work has been reported using chimpanzee adenovirus vectors (e.g. US6083716; WO 2005/071093; WO 2010/086189; WO 2010085984; Farina et al, 2001, J Virol 75: 11603-13; Cohen et al, 2002, J Gen Virol 83: 151-55; Kobinger et al, 2006, Virology 346: 394-401; Tatsis et al., 2007, Molecular Therapy 15: 608-17; see also review by Bangari and Mittal, 2006, Vaccine 24: 849-
 25 62; and review by Lasaro and Ertl, 2009, Mol Ther 17: 1333-39). Hence, in other preferred embodiments, the recombinant adenovirus according to the invention is based upon a simian adenovirus, e.g. a chimpanzee adenovirus. In certain embodiments, the recombinant adenovirus is based upon simian adenovirus type 1, 7, 8, 21, 22, 23, 24, 25, 26, 27.1, 28.1, 29, 30, 31.1, 32, 33, 34, 35.1, 36, 37.2, 39, 40.1, 41.1, 42.1, 43, 44, 45, 46, 48, 49, 50 or SA7P.

30 Adenoviral Vectors rAd26 and rAd35

In a preferred embodiment according to the invention the adenoviral vectors comprise capsid proteins from two rare serotypes: Ad26 and Ad35. In the typical embodiment, the vector

is an rAd26 or rAd35 virus.

Thus, the vectors that can be used in the invention comprise an Ad26 or Ad35 capsid protein (e.g., a fiber, penton or hexon protein). One of skill will recognize that it is not necessary that an entire Ad26 or Ad35 capsid protein be used in the vectors of the invention. Thus, 5 chimeric capsid proteins that include at least a part of an Ad26 or Ad35 capsid protein can be used in the vectors of the invention. The vectors of the invention may also comprise capsid proteins in which the fiber, penton, and hexon proteins are each derived from a different serotype, so long as at least one capsid protein is derived from Ad26 or Ad35. In preferred embodiments, the fiber, penton and hexon proteins are each derived from Ad26 or each from 10 Ad35.

One of skill will recognize that elements derived from multiple serotypes can be combined in a single recombinant adenovirus vector. Thus, a chimeric adenovirus that combines desirable properties from different serotypes can be produced. Thus, in some embodiments, a chimeric adenovirus of the invention could combine the absence of pre-existing immunity of the 15 Ad26 and Ad35 serotypes with characteristics such as temperature stability, assembly, anchoring, production yield, redirected or improved infection, stability of the DNA in the target cell, and the like.

In certain embodiments the recombinant adenovirus vector useful in the invention is derived mainly or entirely from Ad35 or from Ad26 (i.e., the vector is rAd35 or rAd26). In 20 some embodiments, the adenovirus is replication deficient, e.g. because it contains a deletion in the E1 region of the genome. For the adenoviruses of the invention, being derived from Ad26 or Ad35, it is typical to exchange the E4-orf6 coding sequence of the adenovirus with the E4-orf6 of an adenovirus of human subgroup C such as Ad5. This allows propagation of such adenoviruses in well-known complementing cell lines that express the E1 genes of Ad5, such as 25 for example 293 cells, PER.C6 cells, and the like (see, e.g. Havenga et al, 2006, J Gen Virol 87: 2135-43; WO 03/104467). In certain embodiments, the adenovirus is a human adenovirus of serotype 35, with a deletion in the E1 region into which the nucleic acid encoding the antigen has been cloned, and with an E4 orf6 region of Ad5. In certain embodiments, the adenovirus is a human adenovirus of serotype 26, with a deletion in the E1 region into which the nucleic acid 30 encoding the antigen has been cloned, and with an E4 orf6 region of Ad5. For the Ad35 adenovirus, it is typical to retain the 3' end of the E1B 55K open reading frame in the adenovirus, for instance the 166 bp directly upstream of the pIX open reading frame or a

fragment comprising this such as a 243 bp fragment directly upstream of the pIX start codon, marked at the 5' end by a Bsu36I restriction site, since this increases the stability of the adenovirus because the promoter of the pIX gene is partly residing in this area (see, e.g. Havenga et al, 2006, supra; WO 2004/001032).

5 The preparation of recombinant adenoviral vectors is well known in the art.

Preparation of rAd26 vectors is described, for example, in WO 2007/104792 and in Abbink et al., (2007) Virol 81(9): 4654-63. Exemplary genome sequences of Ad26 are found in GenBank Accession EF 153474 and in SEQ ID NO:1 of WO 2007/104792. Preparation of rAd35 vectors is described, for example, in US Patent No. 7,270,811 and in Vogels et al.,
10 (2003) J Virol 77(15): 8263-71. An exemplary genome sequence of Ad35 is found in GenBank Accession AC_000019.

In an embodiment of the invention, the vectors useful for the invention include those described in WO2012/082918, the disclosure of which is incorporated herein by reference in its entirety.

15 Typically, a vector useful in the invention is produced using a nucleic acid comprising the entire recombinant adenoviral genome (e.g., a plasmid, cosmid, or baculovirus vector). Thus, the invention also provides isolated nucleic acid molecules that encode the adenoviral vectors of the invention. The nucleic acid molecules of the invention can be in the form of RNA or in the form of DNA obtained by cloning or produced synthetically. The DNA can be double-
20 stranded or single-stranded.

The adenovirus vectors useful the invention are typically replication defective. In these embodiments, the virus is rendered replication-defective by deletion or inactivation of regions critical to replication of the virus, such as the E1 region. The regions can be substantially deleted or inactivated by, for example, inserting the gene of interest (usually linked to a
25 promoter). In some embodiments, the vectors of the invention may contain deletions in other regions, such as the E2, E3 or E4 regions or insertions of heterologous genes linked to a promoter. For E2- and/or E4-mutated adenoviruses, generally E2- and/or E4-complementing cell lines are used to generate recombinant adenoviruses. Mutations in the E3 region of the adenovirus need not be complemented by the cell line, since E3 is not required for replication.

30 A packaging cell line is typically used to produce sufficient amount of adenovirus vectors of the invention. A packaging cell is a cell that comprises those genes that have been deleted or inactivated in a replication-defective vector, thus allowing the virus to replicate in the

cell. Suitable cell lines include, for example, PER.C6, 911, 293, and E1 A549.

In some embodiments, the Adenovirus virus may express genes or portions of genes that encode antigenic peptides. These foreign, heterologous or exogenous peptides or polypeptides can include sequences that are immunogenic such as, for example, tumor-specific antigens

5 (TSAs), bacterial, viral, fungal, and protozoal antigens.

As noted above, a wide variety of filovirus glycoproteins can be expressed in the vectors. If required, the heterologous gene encoding the filovirus glycoproteins can be codon-optimized to ensure proper expression in the treated host (e.g., human). Codon-optimization is a technology widely applied in the art. Typically, the heterologous gene is cloned into the E1
10 and/or the E3 region of the adenoviral genome.

The heterologous filovirus gene can be under the control of (i.e., operably linked to) an adenovirus-derived promoter (e.g., the Major Late Promoter) or can be under the control of a heterologous promoter. Examples of suitable heterologous promoters include the CMV promoter and the RSV promoter. Preferably, the promoter is located upstream of the
15 heterologous gene of interest within an expression cassette.

In a preferred embodiment of the invention, the adenovirus vectors useful for the invention can comprise a wide variety of filovirus glycoproteins known to those of skill in the art. In a further preferred embodiment of the invention, the rAd vector(s) comprises one or more GPs selected from the group consisting of GPs of Zaire ebolavirus (EBOV), GPs of Sudan
20 ebolavirus (SUDV), GPs of Marburg virus (MARV), and GPs substantially similar thereto.

MVA vectors

MVA vectors useful for the invention utilize attenuated virus derived from Modified Vaccinia Ankara virus which is characterized by the loss of their capabilities to reproductively replicate in human cell lines.

25 In some embodiments, the MVA virus may express genes or portions of genes that encode antigenic peptides. These foreign, heterologous or exogenous peptides or polypeptides can include sequences that are immunogenic such as, for example, tumor-specific antigens (TSAs), bacterial, viral, fungal, and protozoal antigens.

In other embodiments, the MVA vectors express a wide variety of filovirus
30 glycoproteins as well as other structural filovirus proteins, such as VP40 and nucleoprotein (NP). In one aspect, the invention provides a recombinant modified vaccinia virus Ankara (MVA) comprising a nucleotide sequence encoding an antigenic determinant of a filovirus

glycoprotein (GP), in particular an envelope glycoprotein. In another aspect, the invention provides a recombinant MVA vector comprising a heterologous nucleotide sequence encoding an antigenic determinant of a filovirus glycoprotein, in particular an envelope glycoprotein, and a heterologous nucleotide sequence encoding an antigenic determinant of a further filovirus protein.

MVA has been generated by more than 570 serial passages on chicken embryo fibroblasts of the dermal vaccinia strain Ankara [Chorioallantois vaccinia virus Ankara virus, CVA; for review see Mayr et al. (1975), *Infection* 3, 6-14] that was maintained in the Vaccination Institute, Ankara, Turkey for many years and used as the basis for vaccination of humans. However, due to the often severe post-vaccination complications associated with vaccinia viruses, there were several attempts to generate a more attenuated, safer smallpox vaccine.

During the period of 1960 to 1974, Prof. Anton Mayr succeeded in attenuating CVA by over 570 continuous passages in CEF cells [Mayr et al. (1975)]. It was shown in a variety of animal models that the resulting MVA was avirulent [Mayr, A. & Danner, K. (1978), *Dev. Biol. Stand.* 41: 225-234]. As part of the early development of MVA as a low dose pre-smallpox vaccine, there were clinical trials using MVA-517 in combination with Lister Elstree [Stickl (1974), *Prev. Med.* 3: 97-101; Stickl and Hochstein-Mintzel (1971), *Munch. Med. Wochenschr.* 113: 1149-1153] in subjects at risk for adverse reactions from vaccinia. In 1976, MVA derived from MVA-571 seed stock (corresponding to the 571st passage) was registered in Germany as the primer vaccine in a two-stage parenteral smallpox vaccination program. Subsequently, MVA-572 was used in approximately 120,000 Caucasian individuals, the majority children between 1 and 3 years of age, with no reported severe side effects, even though many of the subjects were among the population with high risk of complications associated with vaccinia (Mayr et al. (1978), *Zentralbl. Bacteriol. (B)* 167:375-390). MVA-572 was deposited at the European Collection of Animal Cell Cultures as ECACC V94012707.

As a result of the passaging used to attenuate MVA, there are a number of different strains or isolates, depending on the number of passages conducted in CEF cells. For example, MVA-572 was used in a small dose as a low dose pre-vaccine in Germany during the smallpox eradication program, and MVA-575 was extensively used as a veterinary vaccine. MVA as well as MVA- BN lacks approximately 15% (31 kb from six regions) of the genome compared with ancestral CVA virus. The deletions affect a number of virulence and host range genes, as well

as the gene for Type A inclusion bodies. MVA-575 was deposited on December 7, 2000, at the European Collection of Animal Cell Cultures (ECACC) under Accession No. V00120707. The attenuated CVA-virus MVA (Modified Vaccinia Virus Ankara) was obtained by serial propagation (more than 570 passages) of the CVA on primary chicken embryo fibroblasts.

Even though Mayr et al. demonstrated during the 1970s that MVA is highly attenuated and avirulent in humans and mammals, certain investigators have reported that MVA is not fully attenuated in mammalian and human cell lines since residual replication might occur in these cells [Blanchard et al. (1998), *J. Gen. Virol.* 79:1159-1167; Carroll & Moss (1997), *Virology* 238:198-211; U.S. Patent No. 5,185,146; Ambrosini et al. (1999), *J. Neurosci. Res.* 55: 569]. It is assumed that the results reported in these publications have been obtained with various known strains of MVA, since the viruses used essentially differ in their properties, particularly in their growth behavior in various cell lines. Such residual replication is undesirable for various reasons, including safety concerns in connection with use in humans.

Strains of MVA having enhanced safety profiles for the development of safer products, such as vaccines or pharmaceuticals, have been developed by Bavarian Nordic. MVA was further developed by Bavarian Nordic and is designated MVA-BN, a representative sample of which was deposited on August 30, 2000 at the European Collection of Cell Cultures (ECACC) under Accession No. V00083008. MVA-BN is further described in WO 02/42480 (US 2003/0206926) and WO 03/048184 (US 2006/0159699), both of which are incorporated by reference herein in their entirety.

MVA-BN can attach to and enter human cells where virally-encoded genes are expressed very efficiently. MVA-BN is strongly adapted to primary chicken embryo fibroblast (CEF) cells and does not replicate in human cells. In human cells, viral genes are expressed, and no infectious virus is produced. MVA-BN is classified as Biosafety Level 1 organism according to the Centers for Disease Control and Prevention in the United States. Preparations of MVA-BN and derivatives have been administered to many types of animals, and to more than 2000 human subjects, including immune-deficient individuals. All vaccinations have proven to be generally safe and well tolerated. Despite its high attenuation and reduced virulence, in preclinical studies MVA-BN has been shown to elicit both humoral and cellular immune responses to vaccinia and to heterologous gene products encoded by genes cloned into the MVA genome [E. Harrer et al. (2005), *Antivir. Ther.* 10(2):285-300; A. Cosma et al. (2003), *Vaccine* 22(1):21-9; M. Di Nicola et al. (2003), *Hum. Gene Ther.* 14(14):1347-1360; M. Di Nicola et al.

(2004), Clin. Cancer Res., 10(16):5381-5390].

“Derivatives” or “variants” of MVA refer to viruses exhibiting essentially the same replication characteristics as MVA as described herein, but exhibiting differences in one or more parts of their genomes. MVA-BN as well as a derivative or variant of MVA-BN fails to reproductively replicate in vivo in humans and mice, even in severely immune suppressed mice. More specifically, MVA-BN or a derivative or variant of MVA-BN has preferably also the capability of reproductive replication in chicken embryo fibroblasts (CEF), but no capability of reproductive replication in the human keratinocyte cell line HaCat [Boukamp et al (1988), J. Cell Biol. 106: 761-771], the human bone osteosarcoma cell line 143B (ECACC Deposit No. 91112502), the human embryo kidney cell line 293 (ECACC Deposit No. 85120602), and the human cervix adenocarcinoma cell line HeLa (ATCC Deposit No. CCL-2). Additionally, a derivative or variant of MVA-BN has a virus amplification ratio at least two fold less, more preferably three-fold less than MVA-575 in Hela cells and HaCaT cell lines. Tests and assay for these properties of MVA variants are described in WO 02/42480 (US 2003/0206926) and WO 03/048184 (US 2006/0159699).

The term “not capable of reproductive replication” or “no capability of reproductive replication” is, for example, described in WO 02/42480, which also teaches how to obtain MVA having the desired properties as mentioned above. The term applies to a virus that has a virus amplification ratio at 4 days after infection of less than 1 using the assays described in WO 02/42480 or in U.S. Patent No. 6,761,893, both of which are incorporated by reference herein in their entirety.

The term “fails to reproductively replicate” refers to a virus that has a virus amplification ratio at 4 days after infection of less than 1. Assays described in WO 02/42480 or in U.S. Patent No. 6,761,893 are applicable for the determination of the virus amplification ratio.

The amplification or replication of a virus is normally expressed as the ratio of virus produced from an infected cell (output) to the amount originally used to infect the cell in the first place (input) referred to as the “amplification ratio”. An amplification ratio of “1” defines an amplification status where the amount of virus produced from the infected cells is the same as the amount initially used to infect the cells, meaning that the infected cells are permissive for virus infection and reproduction. In contrast, an amplification ratio of less than 1, i.e., a decrease in output compared to the input level, indicates a lack of reproductive replication and therefore

attenuation of the virus.

The advantages of MVA-based vaccine include their safety profile as well as availability for large scale vaccine production. Preclinical tests have revealed that MVA-BN demonstrates superior attenuation and efficacy compared to other MVA strains (WO 02/42480). An additional property of MVA-BN strains is the ability to induce substantially the same level of immunity in vaccinia virus prime/vaccinia virus boost regimes when compared to DNA-prime/vaccinia virus boost regimes.

The recombinant MVA-BN viruses, the most preferred embodiment herein, are considered to be safe because of their distinct replication deficiency in mammalian cells and their well-established avirulence. Furthermore, in addition to its efficacy, the feasibility of industrial scale manufacturing can be beneficial. Additionally, MVA-based vaccines can deliver multiple heterologous antigens and allow for simultaneous induction of humoral and cellular immunity.

MVA vectors useful for the invention can be prepared using methods known in the art, such as those described in WO/2002/042480 and WO/2002/24224, the relevant disclosures of which are incorporated herein by references.

In another aspect, replication deficient MVA viral strains may also be suitable such as strain MVA-572, MVA-575 or any similarly attenuated MVA strain. Also suitable can be a mutant MVA, such as the deleted chorioallantois vaccinia virus Ankara (dCVA). A dCVA comprises del I, del II, del III, del IV, del V, and del VI deletion sites of the MVA genome. The sites are particularly useful for the insertion of multiple heterologous sequences. The dCVA can reproductively replicate (with an amplification ratio of greater than 10) in a human cell line (such as human 293, 143B, and MRC-5 cell lines), which then enable the optimization by further mutation useful for a virus-based vaccination strategy (see WO 2011/092029).

In a preferred embodiment of the invention, the MVA vector(s) comprise a nucleic acid that encode one or more antigenic proteins selected from the group consisting of GPs of Zaire ebolavirus (EBOV), GPs of Sudan ebolavirus (SUDV), GPs of Marburg virus (MARV), the NP of Tai Forest virus and GPs or NPs substantially similar thereto.

The filovirus protein can be inserted into one or more intergenic regions (IGR) of the MVA. In certain embodiments, the IGR is selected from IGR07/08, IGR 44/45, IGR 64/65, IGR 88/89, IGR 136/137, and IGR 148/149. In certain embodiments, less than 5, 4, 3, or 2 IGRs of the recombinant MVA comprise heterologous nucleotide sequences encoding antigenic

determinants of a filovirus envelope glycoprotein and/or a further filovirus protein. The heterologous nucleotide sequences may, additionally or alternatively, be inserted into one or more of the naturally occurring deletion sites, in particular into the main deletion sites I, II, III, IV, V, or VI of the MVA genome. In certain embodiments, less than 5, 4, 3, or 2 of the naturally occurring deletion sites of the recombinant MVA comprise heterologous nucleotide sequences encoding antigenic determinants of a filovirus envelope glycoprotein and/or a further filovirus protein.

The number of insertion sites of MVA comprising heterologous nucleotide sequences encoding antigenic determinants of a filovirus protein can be 1, 2, 3, 4, 5, 6, 7, or more. In certain embodiments, the heterologous nucleotide sequences are inserted into 4, 3, 2, or fewer insertion sites. Preferably, two insertion sites are used. In certain embodiments, three insertion sites are used. Preferably, the recombinant MVA comprises at least 2, 3, 4, 5, 6, or 7 genes inserted into 2 or 3 insertion sites.

The recombinant MVA viruses provided herein can be generated by routine methods known in the art. Methods to obtain recombinant poxviruses or to insert exogenous coding sequences into a poxviral genome are well known to the person skilled in the art. For example, methods for standard molecular biology techniques such as cloning of DNA, DNA and RNA isolation, Western blot analysis, RT-PCR and PCR amplification techniques are described in *Molecular Cloning, A laboratory Manual* (2nd Ed.) [J. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)], and techniques for the handling and manipulation of viruses are described in *Virology Methods Manual* [B.W.J. Mahy et al. (eds.), Academic Press (1996)]. Similarly, techniques and know-how for the handling, manipulation and genetic engineering of MVA are described in *Molecular Virology: A Practical Approach* [A.J. Davison & R.M. Elliott (Eds.), The Practical Approach Series, IRL Press at Oxford University Press, Oxford, UK (1993)(see, e.g., Chapter 9: Expression of genes by Vaccinia virus vectors)] and *Current Protocols in Molecular Biology* [John Wiley & Son, Inc. (1998)(see, e.g., Chapter 16, Section IV: Expression of proteins in mammalian cells using vaccinia viral vector)].

For the generation of the various recombinant MVAs disclosed herein, different methods can be applicable. The DNA sequence to be inserted into the virus can be placed into an E. coli plasmid construct into which DNA homologous to a section of DNA of the MVA has been inserted. Separately, the DNA sequence to be inserted can be ligated to a promoter. The promoter-gene linkage can be positioned in the plasmid construct so that the promoter-gene

linkage is flanked on both ends by DNA homologous to a DNA sequence flanking a region of MVA DNA containing a non-essential locus. The resulting plasmid construct can be amplified by propagation within *E. coli* bacteria and isolated. The isolated plasmid containing the DNA gene sequence to be inserted can be transfected into a cell culture, e.g., of chicken embryo fibroblasts (CEFs), at the same time the culture is infected with MVA. Recombination between homologous MVA DNA in the plasmid and the viral genome, respectively, can generate an MVA modified by the presence of foreign DNA sequences.

According to a preferred embodiment, a cell of a suitable cell culture as, e.g., CEF cells, can be infected with a poxvirus. The infected cell can be, subsequently, transfected with a first plasmid vector comprising a foreign or heterologous gene or genes, preferably under the transcriptional control of a poxvirus expression control element. As explained above, the plasmid vector also comprises sequences capable of directing the insertion of the exogenous sequence into a selected part of the poxviral genome. Optionally, the plasmid vector also contains a cassette comprising a marker and/or selection gene operably linked to a poxviral promoter.

Suitable marker or selection genes are, e.g., the genes encoding the green fluorescent protein, β -galactosidase, neomycin-phosphoribosyltransferase or other markers. The use of selection or marker cassettes simplifies the identification and isolation of the generated recombinant poxvirus. However, a recombinant poxvirus can also be identified by PCR technology. Subsequently, a further cell can be infected with the recombinant poxvirus obtained as described above and transfected with a second vector comprising a second foreign or heterologous gene or genes. In case, this gene shall be introduced into a different insertion site of the poxviral genome, the second vector also differs in the poxvirus-homologous sequences directing the integration of the second foreign gene or genes into the genome of the poxvirus. After homologous recombination has occurred, the recombinant virus comprising two or more foreign or heterologous genes can be isolated. For introducing additional foreign genes into the recombinant virus, the steps of infection and transfection can be repeated by using the recombinant virus isolated in previous steps for infection and by using a further vector comprising a further foreign gene or genes for transfection.

Alternatively, the steps of infection and transfection as described above are interchangeable, i.e., a suitable cell can at first be transfected by the plasmid vector comprising the foreign gene and, then, infected with the poxvirus. As a further alternative, it is also possible

to introduce each foreign gene into different viruses, co-infect a cell with all the obtained recombinant viruses and screen for a recombinant including all foreign genes. A third alternative is ligation of DNA genome and foreign sequences in vitro and reconstitution of the recombined vaccinia virus DNA genome using a helper virus. A fourth alternative is

5 homologous recombination in E.coli or another bacterial species between a vaccinia virus genome, such as MVA, cloned as a bacterial artificial chromosome (BAC) and a linear foreign sequence flanked with DNA sequences homologous to sequences flanking the desired site of integration in the vaccinia virus genome.

The heterologous filovirus gene can be under the control of (i.e., operably linked to) one

10 or more poxvirus promoters. In certain embodiments, the poxvirus promoter is a Pr7.5 promoter, a hybrid early/late promoter, or a PrS promoter, a PrS5E promoter, a synthetic or natural early or late promoter, or a cowpox virus ATI promoter.

Immunogenic Compositions

Immunogenic compositions are compositions comprising an immunologically effective

15 amount of purified or partially purified adenovirus or MVA vectors for use in the invention. Said compositions can be formulated as a vaccine (also referred to as an “immunogenic composition”) according to methods well known in the art. Such compositions may include adjuvants to enhance immune responses. The optimal ratios of each component in the formulation can be determined by techniques well known to those skilled in the art in view of

20 the present disclosure.

The preparation and use of immunogenic compositions are well known to those of skill in the art. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or

25 polyethylene glycol can be included.

The compositions of the invention can comprise MVA or adenovirus vectors expressing one or more antigenic proteins or immunogenic polypeptides thereof. These antigenic peptides or polypeptides can include any sequences that are immunogenic, including but not limited to, tumor-specific antigens (TSAs), bacterial, viral, fungal, and protozoal antigens. For example,

30 the antigenic protein or immunogenic polypeptide thereof can be derived from a pathogen, e.g., a virus, a bacterium, a fungus, a protozoan, or it can also be derived from a tumor. In one or more preferred aspects, the compositions of the invention comprise MVA or adenovirus vectors

expressing one or more antigenic proteins from a filovirus, such as the Ebola and/or Marburg filoviruses.

The filovirus subtypes according to the invention can be any filovirus subtype. In a preferred embodiment, the filovirus subtypes are selected from the group of Zaire, Sudan, Reston, Bundibugyo, Taï Forest and Marburg. The antigenic proteins can be any protein from any filovirus comprising an antigenic determinant. In a preferred embodiment the antigenic proteins are glycoproteins or nucleoproteins. The antigenic proteins encoded by the MVA vectors or adenovirus vectors comprised in the first and second composition according to the invention can be any antigenic protein from any filovirus.

In a preferred embodiment, the antigenic proteins encoded by the MVA vectors or adenovirus vectors have the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and/or SEQ ID NO: 5.

The immunogenic compositions useful in the invention can comprise adjuvants.

Adjuvants suitable for co-administration in accordance with the invention should be ones that are potentially safe, well tolerated and effective in people including QS-21, Detox-PC, MPL- SE, MoGM-CSF, TiterMax-G, CRL- 1005, GERBU, TERamide, PSC97B, Adjumer, PG-026, GSK-I, GcMAF, B-aethine, MPC-026, Adjuvax, CpG ODN, Betafectin, Alum, and MF59.

Other adjuvants that can be administered include lectins, growth factors, cytokines and lymphokines such as alpha-interferon, gamma interferon, platelet derived growth factor (PDGF), granulocyte-colony stimulating factor (gCSF), granulocyte macrophage colony stimulating factor (gMCSF), tumor necrosis factor (TNF), epidermal growth factor (EGF), IL-I, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12 or encoding nucleic acids therefore.

The compositions of the invention can comprise a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g., intramuscular, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal or intraperitoneal routes.

Method for Enhancing an Immune Response

The invention provides an improved method of priming and boosting an immune response to any antigenic protein or immunogenic polypeptide thereof in a human subject using

an MVA vector in combination with an adenoviral vector.

According to one general aspect of the invention, a method of enhancing an immune response in a human subject comprises:

- a. administering to the human subject a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding an antigenic protein or an immunogenic polypeptide thereof for priming the immune response;
- b. administering to the subject a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for boosting the immune response; and
- c. administering to the subject a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for further boosting the immune response,

to thereby obtain an enhanced immune response against the antigenic protein in the human subject.

According to embodiments of the invention, the enhanced immune response comprises an enhanced antibody response against the antigenic protein in the human subject.

Preferably, the enhanced immune response further comprises an enhanced CD4+ response or an enhanced CD8+ T cell response against the antigenic protein in the human subject. The enhanced CD4+ T cell response generated by a method according to an embodiment of the invention can be, for example, an increase or induction of a dominant CD4+ T cell response against the antigenic protein, and/or an increase or induction of polyfunctional CD4+ T cells specific to the antigenic protein in the human subject. The polyfunctional CD4+ T cells express more than one cytokine, such as two or more of IFN-gamma, IL-2 and TNF-alpha. The enhanced CD8+ T cell response generated by a method according to an embodiment of the invention can be, for example, an increase or induction of polyfunctional CD8+ T cells specific to the antigenic protein in the human subject.

More preferably, the enhanced immune response resulting from a method according to an embodiment of the invention comprises an enhanced CD4+ T cell response, an enhanced antibody response and an enhanced CD8+ T cell response, against the antigenic protein in the

human subject.

In one or more embodiments of the invention, one or more MVA vectors are used to prime the immune response, and one or more rAd26 or rAd35 vectors are used to boost the immune response in step b and c of a method according to an embodiment of the invention. The same adenovirus vectors, such as one or more rAd26 or rAd35 vectors, can be used in both the second and third compositions. The second and third compositions can also have different adenovirus vectors.

The antigens in the respective priming and first and second boosting compositions need not be identical, but should share antigenic determinants or be substantially similar to each other.

Administration of the immunogenic compositions comprising the vectors is typically intramuscular or subcutaneous. However other modes of administration such as intravenous, cutaneous, intradermal or nasal can be envisaged as well. Intramuscular administration of the immunogenic compositions can be achieved by using a needle to inject a suspension of the adenovirus vector. An alternative is the use of a needleless injection device to administer the composition (using, e.g., Biojector(TM)) or a freeze-dried powder containing the vaccine.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the vector will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required. A slow-release formulation may also be employed.

Typically, administration will have a prophylactic aim to generate an immune response against an antigen before infection or development of symptoms. Diseases and disorders that can be treated or prevented in accordance with the invention include those in which an immune response can play a protective or therapeutic role. In other embodiments, the MVA and adenovirus vectors can be administered for post-exposure prophylactics.

The immunogenic compositions containing the MVA vectors are administered to a subject, giving rise to an immune response in the subject. An amount of a composition sufficient to induce a detectable immune response is defined to be an "immunologically effective dose." As shown below, the immunogenic compositions of the invention induce a humoral as well as a

cell-mediated immune response. In a typical embodiment the immune response is a protective immune response.

The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g., decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. ed., 1980.

Following production of MVA and adenovirus vectors and optional formulation of such particles into compositions, the vectors can be administered to an individual, particularly a human.

In one exemplary regimen, the adenovirus vector is administered (e.g., intramuscularly) in a volume ranging between about 100 μ l to about 10 ml containing concentrations of about 10^4 to 10^{12} virus particles/ml. Preferably, the adenovirus vector is administered in a volume ranging between 0.25 and 1.0 ml. More preferably the adenovirus vector is administered in a volume of 0.5 ml.

Typically, the adenovirus is administered in an amount of about 10^9 to about 10^{12} viral particles (vp) to a human subject during one administration, more typically in an amount of about 10^{10} to about 10^{12} vp. In a preferred embodiment, the adenovirus vector is administered in an amount of about 5×10^{10} vp. In another preferred embodiment, the adenovirus vector is administered in an amount of about 0.8×10^{10} vp. In another preferred embodiment, the adenovirus vector is administered in an amount of about 2×10^{10} vp. In another preferred embodiment, the adenovirus vector is administered in an amount of about 4×10^{10} vp. In certain embodiments, adenoviruses are formulated as a trivalent composition, wherein three adenoviruses with each a different insert, are mixed together. In a trivalent composition, each distinct adenovirus is preferably present in an amount of about 4×10^{10} vp. In said trivalent composition, the total number of adenovirus particles per dose amounts to about 1.2×10^{11} vp. In another preferred embodiment, each distinct adenovirus in the trivalent composition is present in an amount of about 1×10^{11} vp. In said trivalent composition the total number of adenovirus particles per dose then amounts to about 3×10^{11} vp. The initial vaccination is followed by a boost as described above.

In one exemplary regimen, the MVA vector is administered (e.g., intramuscularly) in a volume ranging between about 100 μ l to about 10 ml of saline solution containing a dose of about 1×10^7 TCID₅₀ to 1×10^9 TCID₅₀ (50% Tissue Culture Infective Dose) or Inf.U. (Infectious Unit). Preferably, the MVA vector is administered in a volume ranging between 0.25 and 1.0 ml. More preferably the MVA vector is administered in a volume of 0.5 ml.

Typically, the MVA vector is administered in a dose of about 1×10^7 TCID₅₀ to 1×10^9 TCID₅₀ (or Inf.U.) to a human subject during one administration. In a preferred embodiment, the MVA vector is administered in an amount of about 5×10^7 TCID₅₀ to 5×10^8 TCID₅₀ (or Inf.U.). In a more preferred embodiment, the MVA vector is administered in an amount of about 5×10^7 TCID₅₀ (or Inf.U.). In a more preferred embodiment, the MVA vector is administered in an amount of about 1×10^8 TCID₅₀ (or Inf.U.). In another preferred embodiment, the MVA vector is administered in an amount of about 1.9×10^8 TCID₅₀ (or Inf.U.). In yet another preferred embodiment, the MVA vector is administered in an amount of about 4.4×10^8 TCID₅₀ (or Inf.U.). In a more preferred embodiment, the MVA vector is administered in an amount of about 5×10^8 TCID₅₀ (or Inf.U.)

The compositions can, if desired, be presented in a kit, pack or dispenser, which can contain one or more unit dosage forms containing the active ingredient. The kit, for example, may comprise metal or plastic foil, such as a blister pack. The kit, pack, or dispenser can be accompanied by instructions for administration.

The compositions of the invention can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Boosting compositions are administered two or more times, weeks or months after administration of the priming composition, for example, about 1 or 2 weeks or 3 weeks, or 4 weeks, or 6 weeks, or 8 weeks, or 12 weeks, or 16 weeks, or 20 weeks, or 24 weeks, or 28 weeks, or 32 weeks or one to two years after administration of the priming composition.

Preferably, the initial boosting inoculation is administered 1-12 weeks or 2-12 weeks after priming, more preferably 1, 2, 4 or 8 weeks after priming. In a preferred embodiment, the initial boosting inoculation is administered 4 or 8 weeks after priming. In additional preferred embodiments, the initial boosting is conducted at least 1 week, or at least 2 weeks, or at least 4 weeks after priming. In still another preferred embodiment, the initial boosting is conducted 4-12 weeks or 4-8 weeks after priming.

In a preferred embodiment of the invention, at least one further boosting inoculation is administered at least 4 weeks after the initial boosting inoculation. In still a preferred embodiment of the invention, the further boosting inoculation is administered at least 5 weeks after the initial boosting inoculation. In yet another preferred embodiment, the further boosting inoculation is administered at least 6 weeks after the initial boosting inoculation. For example, the further boosting inoculation can be administered 6 weeks to 5 years after the boosting step (b), such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 weeks, or 7, 8, 9, 10, 11 or 12 months, or 2, 3, 4 or 5 years, after the initial boosting inoculation. Optionally, the further boosting step (c) can be repeated one or more times as needed.

In a more preferred embodiment according to this method, an MVA vector, such as a MVA-BN vector, is used for the priming followed by an initial boosting with an adenovirus vector, such as a rAd26 vector, and a further boosting with an adenovirus vector, such as an rAd26 vector. Preferably, the initial boosting composition is administered 1-12 weeks after priming, more preferably 1, 2, 4 or 8 weeks after priming, and the further boosting composition is administered 4-96 weeks after initial boosting, more preferably 8-60 weeks, or preferably 10-60 weeks after initial boosting, such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 weeks, or 7, 8, 9, 10, 11 or 12 months, or 2, 3, 4 or 5 years, after the initial boosting inoculation. In a preferred embodiment, the initial boosting composition is administered 8 weeks after priming, and the further boosting composition is administered 360 days after priming. In another preferred embodiment, the initial boosting composition is administered 2 week after priming, and the further boosting composition is administered 360 days after priming. In another preferred embodiment, the boosting composition is administered 4 weeks after priming, and the further boosting composition is administered 360 days after priming. In yet another preferred embodiment, the boosting composition is administered 2 weeks after priming, and the further boosting composition is administered 13 weeks after priming. In yet another preferred embodiment, the initial boosting composition is administered 1 week after priming, and the further boosting composition is administered 13 weeks after priming.

In a preferred embodiment, each of the first and second boosting compositions comprises an Ad26 vector, more preferably encoding the same antigenic protein(s) or immunogenic polypeptide(s) thereof.

In other embodiments, each of the first and second boosting compositions comprises

different adenovirus vectors, such as an Ad26 vector and an Ad35 vector, respectively, and the different adenovirus vectors can encode the same or different antigenic proteins or immunogenic polypeptides thereof.

In one embodiment, the invention relates to a method of enhancing an immune response against a tumor in a human subject. The method comprises:

- a. administering to the human subject a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding an antigenic protein produced by a cell of the tumor, a substantially similar antigenic protein, or an immunogenic polypeptide thereof for priming the immune response;
 - b. administering to the subject a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding the antigenic protein, the substantially similar antigenic protein, or an immunogenic polypeptide thereof for boosting the immune response; and
 - c. administering to the subject a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding the antigenic protein or an immunogenic polypeptide thereof for further boosting the immune response,
- to thereby obtain an enhanced immune response against the tumor in the human subject.

Preferably, the enhanced immune response provides the human subject with a protective immunity against the tumor.

In a preferred embodiment the boosting step b is conducted 1-12 weeks or 2-12 weeks after the priming step a. The boosting step b can also be conducted later than 12 weeks after the priming step a. In additional preferred embodiments, the boosting step b is conducted at least 2 weeks or at least 4 weeks after the priming step a. In still other preferred embodiments, the boosting step b is conducted 4-12 weeks or 4-8 weeks after the priming step a.

In another preferred embodiment of the invention, the further boosting step (c) is conducted at least 4 weeks after the boosting step (b). In a preferred embodiment of the invention, the further boosting step (c) is conducted at least 5 weeks after the boosting step (b). In still another preferred embodiment, the further boosting step (c) is conducted at least 6 weeks after the boosting step (b). For example, the further boosting step (c) can be conducted 6 weeks

to 5 years after the boosting step (b), such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 weeks, or 7, 8, 9, 10, 11 or 12 months, or 2, 3, 4 or 5 years after the boosting step (b).

In another preferred embodiment, the adenovirus vector in each of the second and third compositions is an Ad26 vector.

The antigenic protein produced by a cell of the tumor can be any tumor antigen. In a preferred embodiment, the tumor antigen is a tumor-specific antigen that is present only on tumor cells. The tumor antigen can also be a tumor-associated antigen that is present on some tumor cells and also some normal cells.

According to another embodiment, the invention relates to a method of enhancing an immune response against at least one subtype of filovirus in a human subject. The method comprises:

- a. administering to the human subject a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding an antigenic protein of the at least one filovirus subtype, a substantially similar antigenic protein, or an immunogenic polypeptide thereof, for priming the immune response;
- b. administering to the subject a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding an antigenic protein of the at least one filovirus subtype, a substantially similar antigenic protein, or an immunogenic polypeptide thereof, for boosting the immune response; and
- c. administering to the subject a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding an antigenic protein of the at least one filovirus subtype, a substantially similar antigenic protein, or an immunogenic polypeptide thereof, for further boosting the immune response;

to thereby obtain an enhanced immune response against the at least one subtype of filovirus in the human subject.

Preferably, the enhanced immune response provides the human subject a protective immunity against the at least one subtype of filovirus.

In a preferred embodiment the boosting step b is conducted 1-12 weeks or 2-12 weeks after the first step, more preferably 1, 2, 4, or 8 weeks after priming. In additional preferred embodiments, the boosting step b is conducted at least 1 week or at least 2 weeks after the priming. In still other preferred embodiments, the boosting step b is conducted 4-12 weeks or 4-8 weeks after the priming.

The boosting step can also be conducted later than 12 weeks after priming.

In another preferred embodiment of the invention, the further boosting step (c) is conducted at least 4 weeks after the boosting step (b). In a preferred embodiment of the invention, the further boosting step (c) is conducted at least 5 weeks after the boosting step (b). In still another preferred embodiment, the further boosting step (c) is conducted at least 6 weeks after the boosting step (b). For example, the further boosting step (c) can be conducted 6 weeks to 5 years after the boosting step (b), such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 weeks, or 7, 8, 9, 10, 11 or 12 months, or 2, 3, 4 or 5 years after the boosting step (b).

In another preferred embodiment, the adenovirus vector in each of the second and third compositions is an Ad26 vector.

In yet another preferred embodiment, the antigenic protein is a glycoprotein or a nucleoprotein of a filovirus subtype.

In one embodiment of the invention, the MVA vector in the first composition comprises a polynucleotide encoding antigenic proteins derived from more than one filovirus subtypes. More preferably, the MVA vector in the first composition comprises a polynucleotide encoding four antigenic proteins from four filovirus subtypes having the amino acid sequences of SEQ ID NOs: 1, 2, 4 and 5, or immunogenic polypeptides thereof.

In another embodiment of the invention, each of the second and third compositions comprises at least one adenovirus vector comprising a polynucleotide encoding an antigenic protein derived from a filovirus subtype that is same or different from the filovirus subtype encoded by the MVA vector. For example, the adenovirus vector can comprise a polynucleotide encoding an antigenic protein having the amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5. Preferably, each of the second and third compositions can comprise more than one adenovirus vectors encoding more than one antigenic proteins or immunogenic polypeptides thereof from more than one filovirus subtypes. For example, each of the second and third compositions can comprise one to three adenovirus vectors encoding

one to three of the antigenic proteins have the amino acid sequences of SEQ ID NOs: 1, 2 and 3.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

5 The following examples are offered to illustrate, but not to limit the claimed invention.

Example 2

A randomized, placebo-controlled, observer-blind study is performed to evaluate the safety, tolerability and immunogenicity of a heterologous regimen, which contains: (1) a single dose of MVA-BN-Filo (1×10^8 TCID₅₀) or placebo (0.9% saline) as prime on Day 1; (2) a
10 single dose of Ad26.Filo (9×10^{10} vp) or placebo as the initial boost on Day 15 (i.e., 2 weeks after Day 1); and (3) a single dose of Ad26.Filo (9×10^{10} vp) or placebo as the second boost on Day 92 (13 weeks after Day 1). The Ad26.Filo is a composition that comprises three monovalent replication-incompetent adenoviral vector serotype 26 (Ad26) vectors each encoding either an EBOV Mayinga GP (SEQ ID NO: 1); a SUDV Gulu GP (SEQ ID NO:2); or
15 a MARV Angola GP (SEQ ID NO:3).

This regimen is tested in 7 healthy subjects, aged between 18 and 50 years (inclusive) who have never received an experimental Ebola candidate vaccine before and have no known exposure to EBOV or diagnosis of Ebola disease. Out of the 7 subjects in this group 5 received active vaccination and 2 the placebo.

20 The study consists of a vaccination period in which subjects are vaccinated at their baseline visit (Day 1) followed by a first boost on Day 15, a second boost on Day 92, and a post-boost follow-up period until 1 year post prime. The study vaccination schedules of the relevant group is summarized in Table 4.

Table 4: Study Vaccination Schedule

25

N	n	Day 1	Day 15	Day 92
7	5	- MVA-BN-Filo, 5×10^8 Inf U	Ad26.Filo, 9×10^{10} vp	Ad26.Filo, 9×10^{10} vp
	2	- Placebo	Placebo	Placebo

Immunogenicity is assessed using the immunologic assays summarized in Tables 5 and 6. The exploratory assay package may include, but is not limited to, the listed assays.

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Table 5: Summary of Immunologic Assays (Serology)

Assay	Purpose
Secondary endpoints	
Pseudovirus neutralization assay (psVNA)	Analysis of neutralizing antibodies to EBOV GP, MARV GP and SUDV GP
ELISA	Analysis of antibodies binding to EBOV GP, MARV GP and SUDV GP
Exploratory endpoints	
Adenovirus/MVA neutralization assay	Neutralizing antibodies to adenovirus/MVA

EBOV: Ebola virus; ELISA: enzyme-linked immunosorbent assay; GP: glycoprotein; IgG: immunoglobulin G; MARV: Marburg virus; MVA: Modified Vaccinia Ankara; NP: nucleoprotein; SUDV: Sudan virus; TAFV: Tai Forest virus

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Table 6: Summary of Immunologic Assays (Cellular)

Assay	Purpose
Exploratory endpoints	
ELISpot	T cell IFN- γ responses to EBOV GP, MARV GP and SUDV GP
ICS of frozen PBMC	Analysis of T cell responses to EBOV GP, SUDV GP, MARV GP and/or TAFV NP (including CD4/8, IL-2, IFN- γ , TNF- α and/or activation markers)

EBOV: Ebola virus; ELISpot: enzyme-linked immunospot; GP: glycoprotein; ICS: intracellular cytokine staining; IFN: interferon; IL: interleukin; MARV: Marburg virus; NP: nucleoprotein; PBMC: peripheral blood mononuclear cells; SUDV: Sudan virus; TAFV: Tai Forest virus; TNF: tumor necrosis factor

The clinical study is ongoing. Some initial results are described below.

10 Assessment of immune responses

Immunogenicity has been assessed at baseline (Day 1) and Days 15, 36, 92, 99 and 113 post-prime immunization (i.e. 21 days post-second boost vaccination) using an ELISA assay to analyze antibodies binding to either EBOV GP, MARV GP or SUDV GP, a psVNA to analyze the neutralizing antibody response against either EBOV GP, MARV GP or SUDV GP and a VNA to analyze the neutralizing antibody response against the Ad26 backbone.

Humoral immune responses against EBOV GP

A binding antibody response against EBOV GP was observed in 20% of the subjects 14 days post-prime (Day 15), with the geometric-mean concentration (GMC) <LLOQ (Figure 1).

Twenty-one days post-boost vaccination with Ad26.Filo (post-Dose 2, Day 36), a binding antibody response against EBOV GP was observed in 100% of the subjects with a GMC of 3843 ELISA units/mL (95% CI: 1761; 8388) (Figure 1). GMC stayed in the same range until administration of the third vaccination on Day 92 (2425 ELISA units/mL [95% CI: 1503; 3911]) (Figure 1). Seven days post-Dose 3 (Day 99), a binding antibody response against EBOV GP was observed in 100% of the subjects with a GMC of 13445 ELISA units/mL (95% CI: 4125; 43829) (Figure 1). GMC tended to be slightly increased at Day 113 (15715 ELISA units/mL [95%CI: 7146; 34558]) (Figure 1).

No neutralizing antibody responses against EBOV GP were observed in the subjects 14 days post-prime (Day 15) (neutralizing GMTs <LLOQ) (Figure 1). On Day 92, a neutralizing antibody response against EBOV GP was observed in 100% of the subjects, with a neutralizing GMT of 520 IC₅₀ (95% CI: 168; 1612) (Figure 1). Seven days post-Dose 3 (Day 99), a neutralizing antibody response against EBOV GP was observed in 100% of the subjects, with a neutralizing GMT of 5589 IC₅₀ (95% CI: 1155; 27051) that was further increased by Day 113 (7779 IC₅₀ [95%CI: 2604; 23239]) (Figure 1). On Day 180, both the binding and neutralizing antibody responses had declined compared to the peak responses post-third vaccination (Figure 1).

Humoral immune responses against MARV GP

A binding antibody response against MARV GP was observed in 20% of the subjects 14 days post-prime (Day 15), with GMC<LLOQ (Figure 1). Twenty-one days post-boost vaccination with Ad26.Filo (post-Dose 2, Day 36), a binding antibody response against MARV GP was observed in 100% of the subjects with a GMC of 215 ELISA units/mL (95% CI: 52; 887) (Figure 1). GMC stayed in the same range until administration of the third vaccination on Day 92 (155 ELISA units/mL [95% CI: 36; 665]) (Figure 1). Seven days post-Dose 3 (Day 99), a binding antibody response against MARV GP was observed in 100% of the subjects with a GMC of 510 ELISA units/mL (95% CI: 92; 2827) (Figure 1). GMC tended to stay in the same range at Day 113 (825 ELISA units/mL [95% CI: 230; 2959]) (Figure 1).

No neutralizing antibody responses against MARV GP were observed in the subjects 14 days

post-prime (Day 15) (neutralizing GMTs <LLOQ) (Figure 1). On Day 92, a neutralizing antibody response against MARV GP was observed in 20% of the subjects, with a neutralizing GMT below LLOQ (Figure 1). Seven days post-Dose 3 (Day 99), a neutralizing antibody response against MARV GP was observed in 40% of the subjects, with a neutralizing GMT of 51 IC₅₀ (95% CI: <LLOQ; 275) (Figure 1). Neutralizing GMTs remained similar at Day 113 (55 IC₅₀ [95% CI: <LLOQ; 313]) (Figure 1). On Day 180, both the binding and neutralizing antibody responses had declined compared to the peak responses post-third vaccination (Figure 1).

Humoral immune responses against SUDV GP

10 A binding antibody response against SUDV GP was observed in 80% of the subjects 14 days post-prime (Day 15), with a GMC of 77 ELISA units/mL (95% CI: <LLOQ; 423) (Figure 1). Twenty-one days post-boost vaccination with Ad26.Filo (post-Dose 2, Day 36), a binding antibody response against SUDV GP was observed in 100% of the subjects with a GMC of 1879 ELISA units/mL (95% CI: 803; 4396) (Figure 1). GMC decreased until administration of
15 the third vaccination on Day 92 (726 ELISA units/mL [95% CI: 401; 1313]) (Figure 1). Seven days post-Dose 3 (Day 99), a binding antibody response against SUDV GP was observed in 100% of the subjects with a GMC of 3186 ELISA units/mL (95% CI: 921; 11020) (Figure 1). GMC was similar at Day 113 (3036 ELISA units/mL [95%CI: 1672; 5513]) (Figure 1).

No neutralizing antibody responses against SUDV GP were observed in the subjects 14 days
20 post-prime (Day 15) (neutralizing GMTs <LLOQ) (Figure 1). On Day 92, a neutralizing antibody response against SUDV GP was observed in 60% of the subjects, with a neutralizing GMT of 63 IC₅₀ (95% CI: <LLOQ; 236) (Figure 1). Seven days post-Dose 3 (Day 99), a neutralizing antibody response against SUDV GP was observed in 80% of the subjects, with a neutralizing GMT of 601 IC₅₀ (95% CI: 52; 6995) (Figure 1). Neutralizing GMTs stayed in the
25 same range at Day 113 (100% responders, neutralizing GMT: 884 IC₅₀ [95%CI: 280; 2788]) (Figure 1).

Neutralizing antibody responses against Ad26 backbone

Neutralizing antibodies directed against Ad26 were measured by VNA at baseline and at Day 92, prior to the third vaccination. At baseline, only 1 (20%) subject had a neutralizing antibody
30 response directed against Ad26 (neutralizing GMT: 23 IC₅₀; 95%CI: <LLOQ; 377). Seventy-

seven days after boost vaccination with Ad26.Filo (Day 92), all 5 subjects had a neutralizing antibody response directed against Ad26 with a neutralizing GMT of 203 IC₅₀ (95% CI: <LLOQ; 15674).

5 Example 3

A randomized, placebo-controlled, observer-blind study is performed in Groups 1-3 to evaluate the safety, tolerability and immunogenicity of a heterologous regimen, which contains: (1) a single dose of MVA-BN-Filo (1×10^8 TCID₅₀) or placebo (0.9% saline) as prime on Day 1; (2) a single dose of Ad26.ZEBOV (5×10^{10} vp) or placebo as the first boost at different time point of Day 15, 29 or 57 (i.e., 2, 4, or 8 weeks after prime); and (3) a single dose of Ad26.ZEBOV (5×10^{10} vp) as the second boost on Day 360 (i.e., 1 year post-prime). The Ad26.ZEBOV encodes the EBOV Mayinga GP (SEQ ID NO: 1).

Subjects are enrolled in 3 different groups, comprising 18 (Groups 1 to 3) healthy subjects each. Subjects are randomized in a 5:1 ratio to receive active vaccine or placebo throughout the study. The study vaccination schedules in the different groups are summarized in Table 7.

Table 7: Study Vaccination Schedules

Group	N	n	Day 1	Day 15	Day 29	Day 57	Day 360
1	18	15 3	MVA-BN-Filo Placebo	Ad26.ZEBOV Placebo			Ad26.ZEBOV Placebo
2	18	15 3	MVA-BN-Filo Placebo		Ad26.ZEBOV Placebo		Ad26.ZEBOV Placebo
3	18	15 3	MVA-BN-Filo Placebo			Ad26.ZEBOV Placebo	Ad26.ZEBOV Placebo

N: number of subjects to receive study vaccine

MVA-BN-Filo dose level is 1×10^8 TCID₅₀ (50% Tissue Culture Infective Dose) in all groups;

Ad26.ZEBOV dose level is 5×10^{10} vp (viral particles) in all groups;

Placebo is 0.9% saline in all groups

Safety is assessed by collection of solicited local and systemic adverse events, unsolicited adverse events and serious adverse events, and by physical examination. In addition, standard chemistry, hematologic (including coagulation parameters) and urinalysis parameters are assessed at multiple time points.

Immunogenicity is assessed using the immunologic assays summarized in Tables 8 and 9. The exploratory assay package may include, but is not limited to, the listed assays.

Table 8: Summary of Immunologic Assays (Serology)

Assay	Purpose
Secondary endpoints	
Pseudovirus neutralization assay (psVNA)	Analysis of neutralizing antibodies to EBOV GP
ELISA	Analysis of antibodies binding to EBOV GP
Exploratory endpoints	
Adenovirus/MVA neutralization assay	Neutralizing antibodies to adenovirus/MVA
Molecular antibody characterization	Analysis of anti-EBOV GP, SUDV GP, MARV GP and/or TAFV NP antibody characteristics, including IgG subtyping
EBOV: Ebola virus; ELISA: enzyme-linked immunosorbent assay; GP: glycoprotein; IgG: immunoglobulin G; MARV: Marburg virus; MVA: Modified Vaccinia Ankara; NP: nucleoprotein; SUDV: Sudan virus; TAFV: Tai Forest virus	

Table 9: Summary of Immunologic Assays (Cellular)

Assay	Purpose
Secondary endpoints	
ELISpot	T cell IFN- γ responses to EBOV GP
Exploratory endpoints	
ICS of frozen PBMC	Analysis of T cell responses to EBOV GP (including CD4/8, IL-2, IFN- γ , TNF- α and/or activation markers)
EBOV: Ebola virus; ELISpot: enzyme-linked immunospot; GP: glycoprotein; ICS: intracellular cytokine staining; IFN: interferon; IL: interleukin; PBMC: peripheral blood mononuclear cells; TNF: tumor necrosis factor	

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Assessment of immune responses

Immunogenicity has been assessed at multiple time points, the most important being baseline (Day 1), pre-boost 1 (Day 15, 29 or 57), 21 days post-boost 1 (Day 36, 50 or 78), pre-boost 2 (Day 360), 7 days post-boost 2 (Day 367), 21 days post-boost 2 (Day 381) and approximately one year post-boost 2 (Day 720). An ELISA was used to analyze antibodies binding to EBOV GP, a psVNA to analyze the neutralizing antibody response against EBOV GP, an ELISpot and ICS to analyze the cellular immune responses, a VNA to analyze the neutralizing antibody response against the Ad26 backbone and a plaque reduction neutralization test (PRNT) to analyze the neutralizing antibody response against the MVA backbone.

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Assessment of humoral immune responses

Figure 2 shows the Ebola Zaire glycoprotein specific humoral immune response observed in individuals that were administered an MVA-BN-Filo vector as a prime on day 1, followed by a boost with an Ad26.ZEBOV vector either on day 15, day 29 or 57 and a second boost with Ad26.ZEBOV on day 360. A 20 to 23 fold increase in antibody levels was assessed by ELISA after the second boost at day 360 with Ad26.ZEBOV. The onset of this anamnestic response was very fast and was observed already 7 days after administration of the second boost. At this Day 367, binding antibody responses were much higher compared to the peak responses observed post-dose 2, and continued to increase until 21 days post-boost 2 (Day 381). After Day 381, the response levels gradually decreased towards the end of the study (Day 720) but remained 1.6- to 5.8-fold higher compared to 1 year post-prime. Across all groups the responder rates on Day 720 were between 91% and 100%.

In line with the binding antibody responses, re-exposure to the EBOV GP antigen mimicked by the second boost vaccination on Day 360 induced a marked increase of neutralizing antibody levels 7 days later (Figure 3). The neutralizing antibody responses continued to increase until 21 days post-boost 2 (Day 381), except in the MVA/Ad26 28-day interval group, by when they reached geometric mean neutralizing antibody concentrations that were 27- to 77-fold higher compared to Day 360 (Figure 3). After Day 381, the response levels gradually decreased but the responder rates remained high (between 92% and 100%) until the end of the study (Day 720) (Figure 3).

Assessment of cellular immune responses

Re-exposure to the EBOV GP antigen mimicked by the third vaccination on Day 360 induced an increase in IFN- γ + responses as measured by ELISpot, however, the magnitude of the responses varied across regimens (Figure 4). The highest median response post-boost 2 was observed on Day 367 in subjects who received the MVA/Ad26 14-day interval regimen and a third dose with Ad26 on Day 360 (Figure 4). In this group, the median IFN- γ + response on Day 367 was almost 5 times higher compared to Day 360 (Figure 6). After Day 367, the response level in this group gradually decreased and the responder rate at the end of the study (Day 720) was 80% (Figure 4).

Administration of the second boost vaccination on Day 360 induced a modest increase in the CD4+ T cell responses in all groups by Day 367, while a modest increase in CD8+ T cell responses was measured in only some but not all groups by ICS (Figures 5 and 5).

The CD4+ T cell responses mostly remained below or around the peak value of the acute phase. After Day 367, the response levels as well as the responder rates gradually decreased (Figure 5). The CD8+ T cell responses continued to increase until Day 381 but mostly remained either below or around the peak value of the acute phase. After Day 381, the response levels and responder rates gradually decreased (Figure 6).

Neutralizing antibody responses against the Ad26 backbone

Neutralizing antibody responses against the Ad26 vector as measured by Ad26 neutralization assay were detected in 13.2% of subjects prior to the first dose of Ad26. After the first Ad26 dose but pre-boost 2, neutralizing antibody responses against the Ad26 vector were observed in 98.8% of the subjects. At 21 days after a second dose of Ad26, 100% of subjects had neutralizing antibodies directed against the Ad26 vector. The geometric mean titers measured by Ad26 neutralization assay increased after both Ad26 doses (titer range after the first dose: 869 to 2001; titer range after the second dose: 1460 to 8004) (data not shown).

In summary, these examples show that immunization series consisting of priming the immune response with an MVA vector, followed by an initial boosting of the immune response with an adenovirus vector, and a further boosting of the immune response with an adenovirus vector resulted in a robust increase in immune response. A robust increase in binding and neutralizing antibody titers was observed after the last immunization with an adenovirus vector, ranging from 20 to 23 and 19 to 43 fold increase, respectively. Importantly, this was associated with an increase in the percentage of responders and was observed across the large range of vaccination intervals studied. A similar effect was observed for the cellular immune response albeit to a more modest magnitude.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

SEQUENCE LISTING

SEQ ID NO:1

Glycoprotein Ebola virus Zaire, strain Mayinga (Amino Acid sequence):

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15 SEQ ID NO:2

Glycoprotein Ebola virus Sudan, strain Gulu (Amino Acid sequence):

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30 SEQ ID NO:3

Glycoprotein Marburg virus Angola (Amino Acid sequence):

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SEQ ID NO:4

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 NPHAQGIALHLWGAFFLYDRIASTTMYRGKVFTEGNIAAMIVNKTVHKMIFSRQGGYRHMNLT
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 15 TTEERTFSLINRHAIDFLLTRWGGTCKVLGPDCCIGIEDLSKNISEQIDQIKKDEQKEGTGWGL
 GGKWWTSDWGVLTNLGILLLLSIAVLIALSCICRIFTKYIG

SEQ ID NO:5

Nucleoprotein Ebola virus Tai Forest / Ivory coast (Amino Acid sequence):

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 ALNEDNRFITMDDQQFYWPVMNHRNKFMAILQHKK

CLAIMS

We claim:

- 5 1. Use of a combination for the preparation of a medicament for inducing an enhanced immune response in a human subject, comprising:
 - a. a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding a first antigenic protein or an immunogenic polypeptide thereof;
 - 10 b. a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding a second antigenic protein or an immunogenic polypeptide thereof; and
 - c. a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding a third

15 antigenic protein or an immunogenic polypeptide thereof,

wherein the first composition is for priming the immune response, the second composition is for boosting the immune response, and the third composition is for further boosting the immune response, and wherein the first, second and third antigenic proteins share at least one antigenic determinant.

20
2. The use according to claim 1, wherein the enhanced immune response comprises an enhanced antibody response, an enhanced CD8⁺ T cell response, and/or an enhanced CD4⁺ T cell response against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human subject.

25
3. The use according to claim 2, wherein the enhanced CD8⁺ or CD4⁺ T cell response comprises an increase or induction of a dominant CD8⁺ or CD4⁺ T cell response and/or an increase or induction of polyfunctional CD8⁺ or CD4⁺ T cells against the at least one antigenic determinant shared by the first, second and third antigenic proteins in the human

30 subject.

4. The use according to claim 1, wherein the enhanced immune response provides a protective immunity to the human subject against a disease related to at least one of the first, second and third antigenic proteins.
- 5 5. The use according to any one of claims 1, 2, 3 and 4, wherein the second composition is for administration 1-12 weeks, 2-12 weeks, at least 1 week, or at least 2 weeks- after the first composition, and the third composition is for administration 4-96 weeks or at least 4 weeks after the second composition.
- 10 6. The use according to any one of claims 1, 2, 3, 4, and 5, wherein the first, second or third antigenic protein is derived from a pathogen or a tumor.
7. The use according to claim 6, wherein the first, second or third antigenic protein is derived from a filovirus, preferably, the first, second and third antigenic proteins are identical or
15 substantially identical.
8. The use according to claim 7, wherein each of the first, second and third antigenic protein independently comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5, immunogenic
20 polypeptides thereof, and combinations thereof.
9. The use according to claim 8, wherein the MVA vector comprises a polynucleotide encoding at least one antigenic protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 5, or an
25 immunogenic polypeptide thereof; preferably, the MVA vector comprises a polynucleotide encoding four antigenic proteins having the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 5, respectively.
10. The use according to claim 8 or 9, wherein each of the first and the second adenovirus
30 vectors comprises a polynucleotide encoding the antigenic protein having the amino acid sequence of SEQ ID NO: 1.

11. The use according to claim 10, wherein the second and/or third composition further comprises an immunologically effective amount of an adenovirus vector comprising a polynucleotide encoding an antigenic protein having the amino acid sequence of SEQ ID NO:2; and further comprises an immunologically effective amount of an adenovirus vector comprising a polynucleotide encoding an antigenic protein having the amino acid sequence of SEQ ID NO:3.

12. The use according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, wherein each of the first and second adenovirus vectors is an rAd26 vector, and the MVA vector is MVA-BN vector.

13. Use of a combination for the preparation of a medicament for inducing an enhanced immune response against at least one filovirus subtype in a human subject, comprising:

a. a first composition comprising an immunologically effective amount of a MVA vector comprising a first polynucleotide encoding a first antigenic protein of the at least one filovirus subtype, a substantially similar antigenic protein, or an immunogenic polypeptide thereof;

b. a second composition comprising an immunologically effective amount of a first adenovirus vector comprising a second polynucleotide encoding a second antigenic protein of the at least one filovirus subtype, a substantially similar antigenic protein, or an immunogenic polypeptide thereof; and

c. a third composition comprising an immunologically effective amount of a second adenovirus vector comprising a third polynucleotide encoding a third antigenic protein of the at least one filovirus subtype, a substantially similar antigenic protein, or an immunogenic polypeptide thereof;

wherein the first composition is for priming the immune response, the second composition is for boosting the immune response, and the third composition is for further boosting the immune response, and wherein the first, second and third antigenic proteins share at least one antigenic determinant.

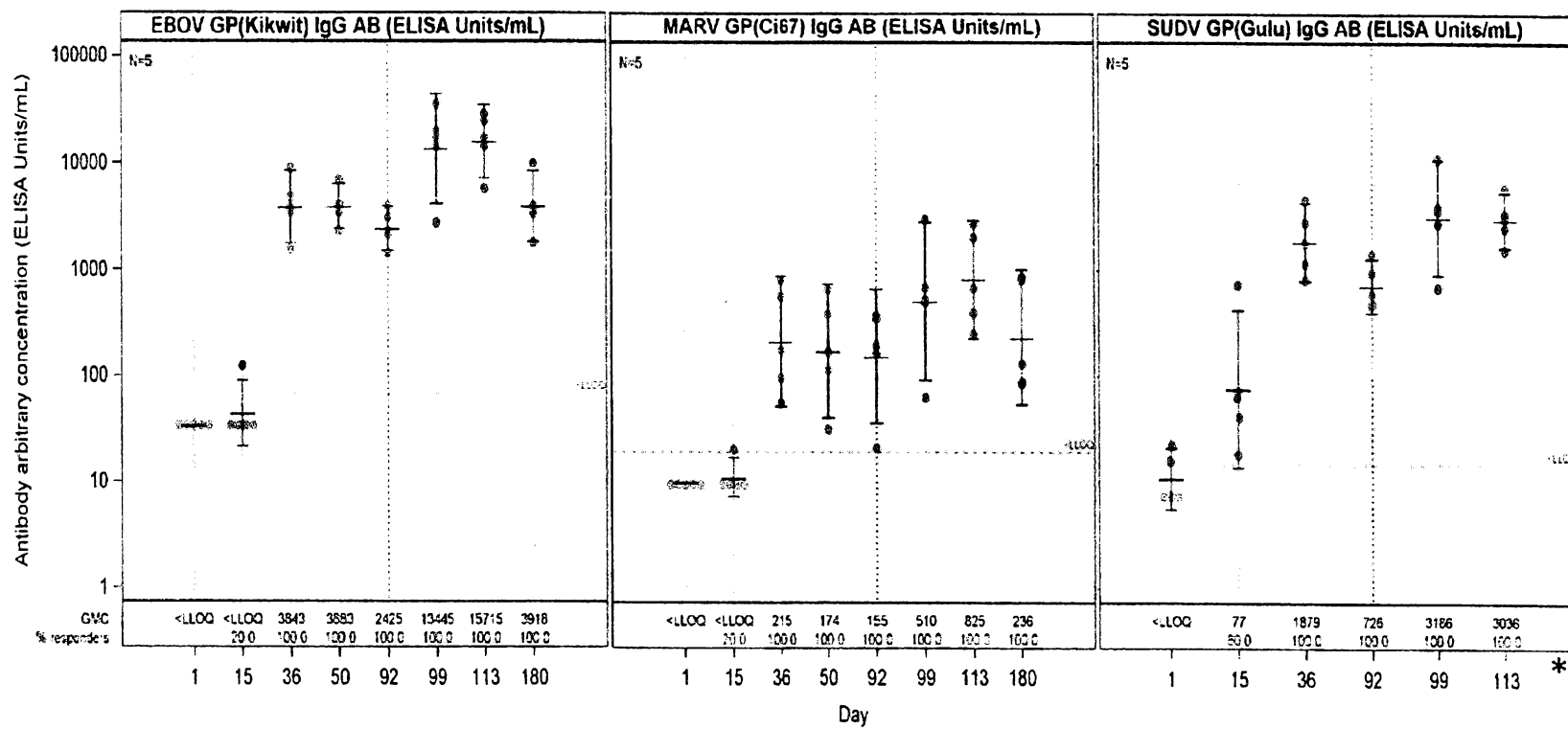


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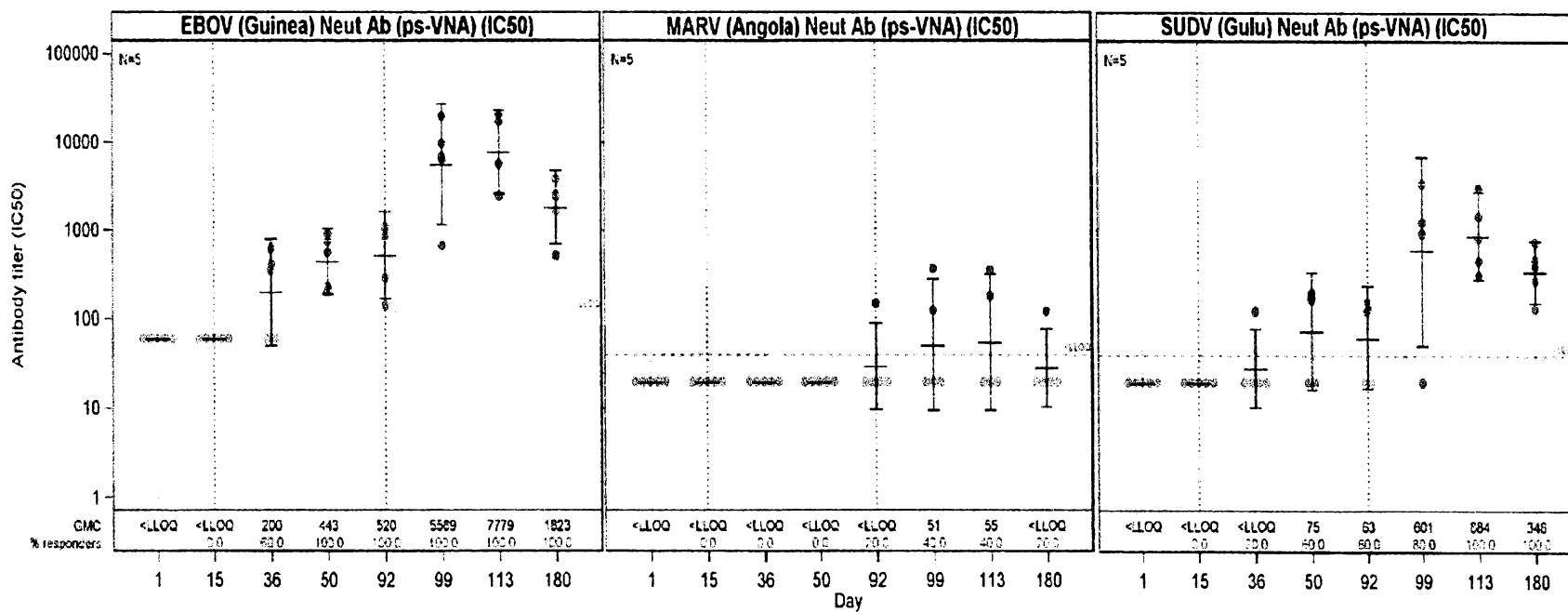


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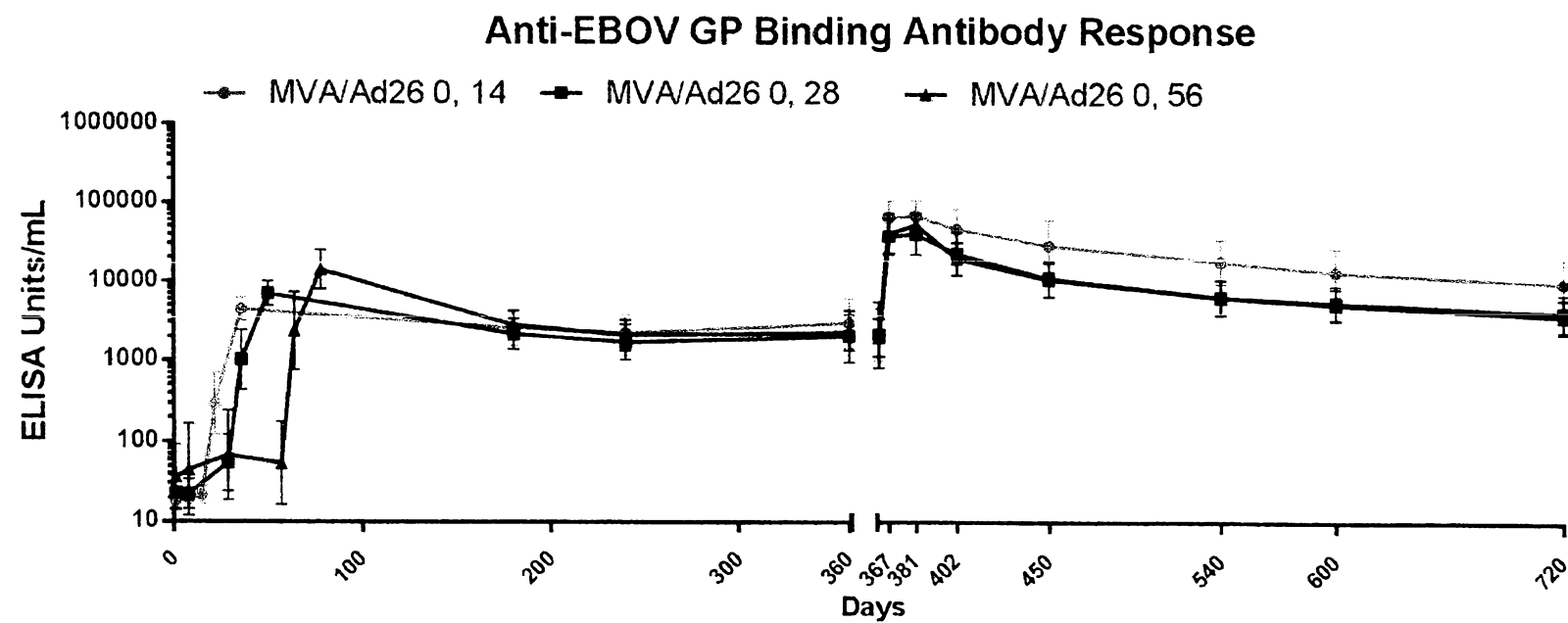


Figure 2

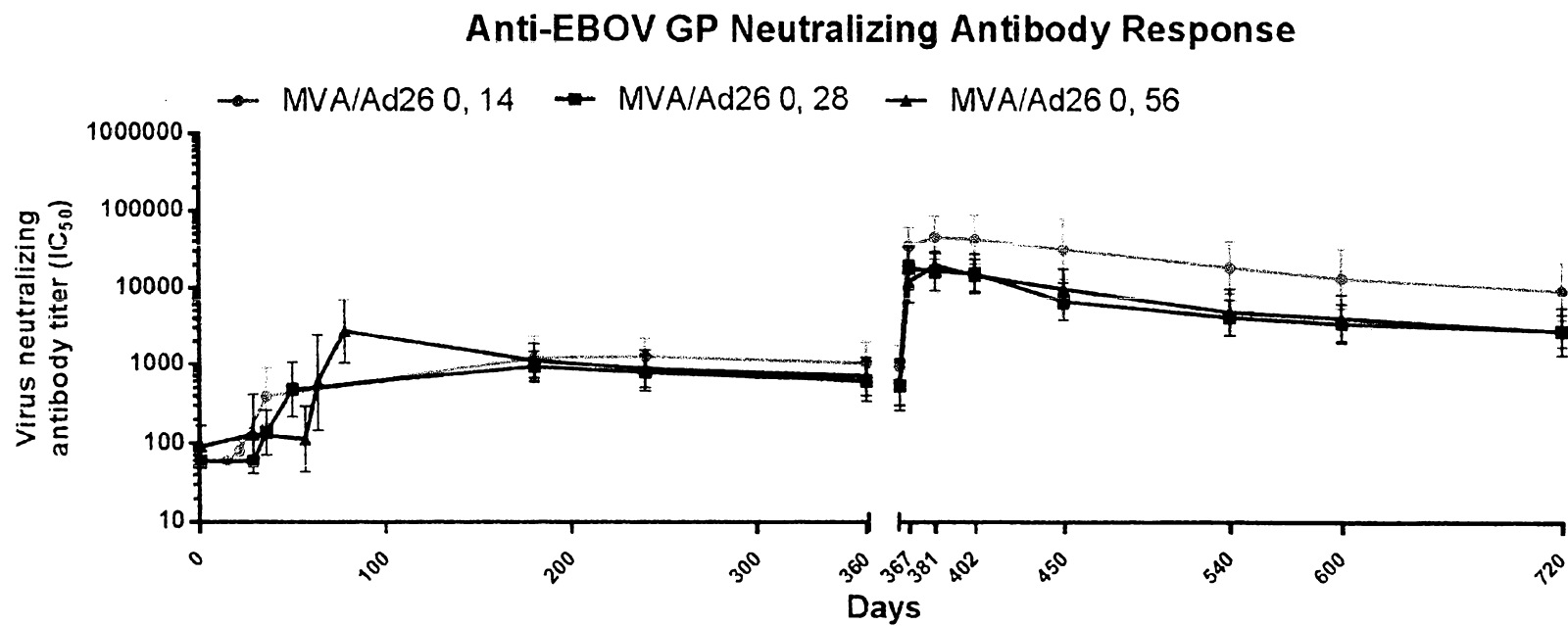


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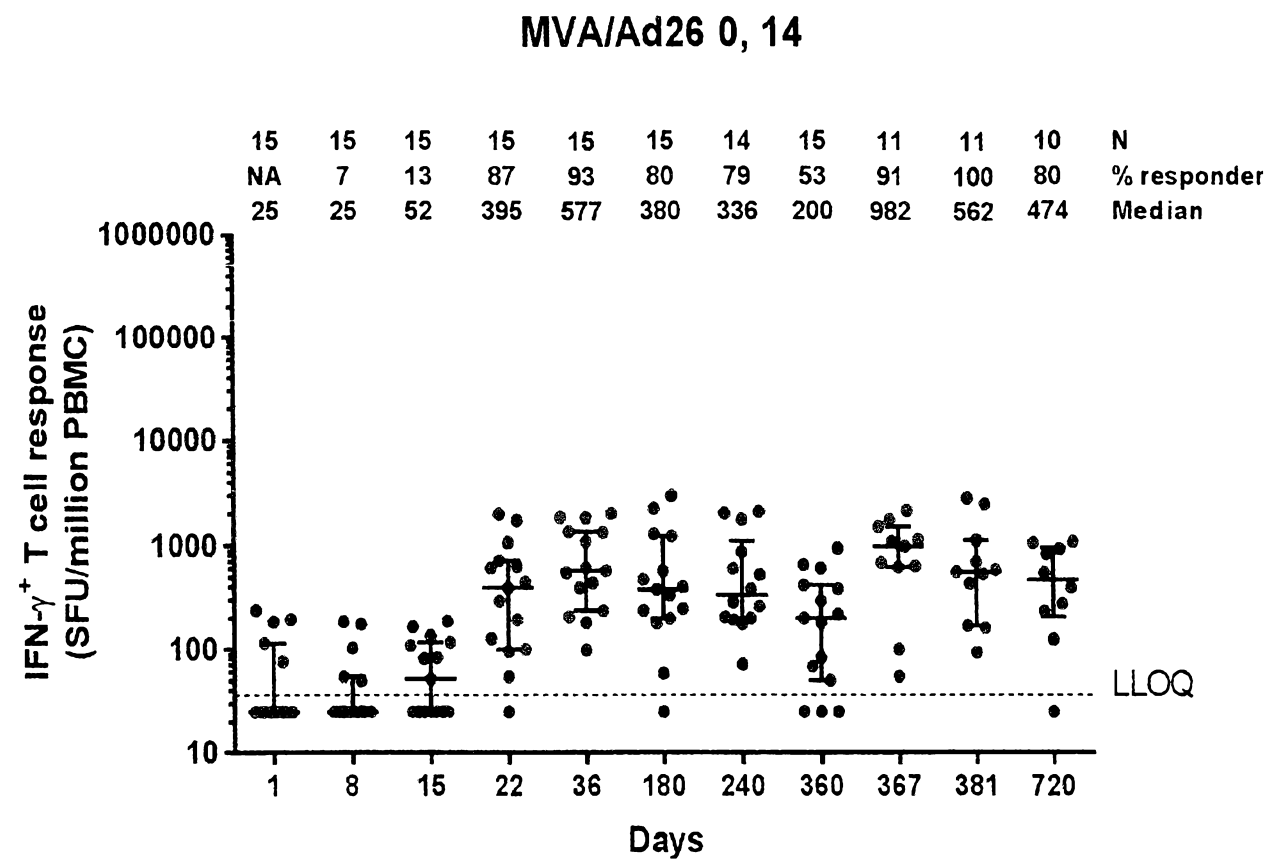


Figure 4a

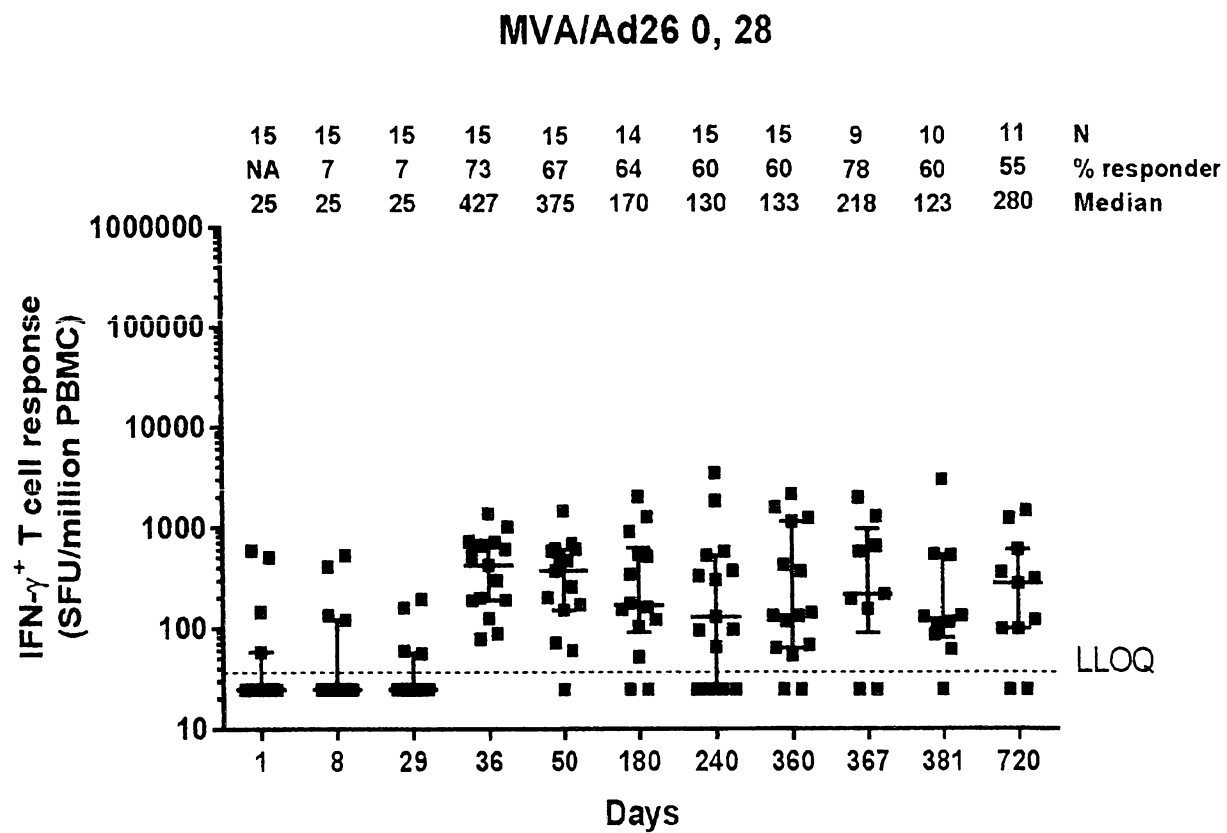


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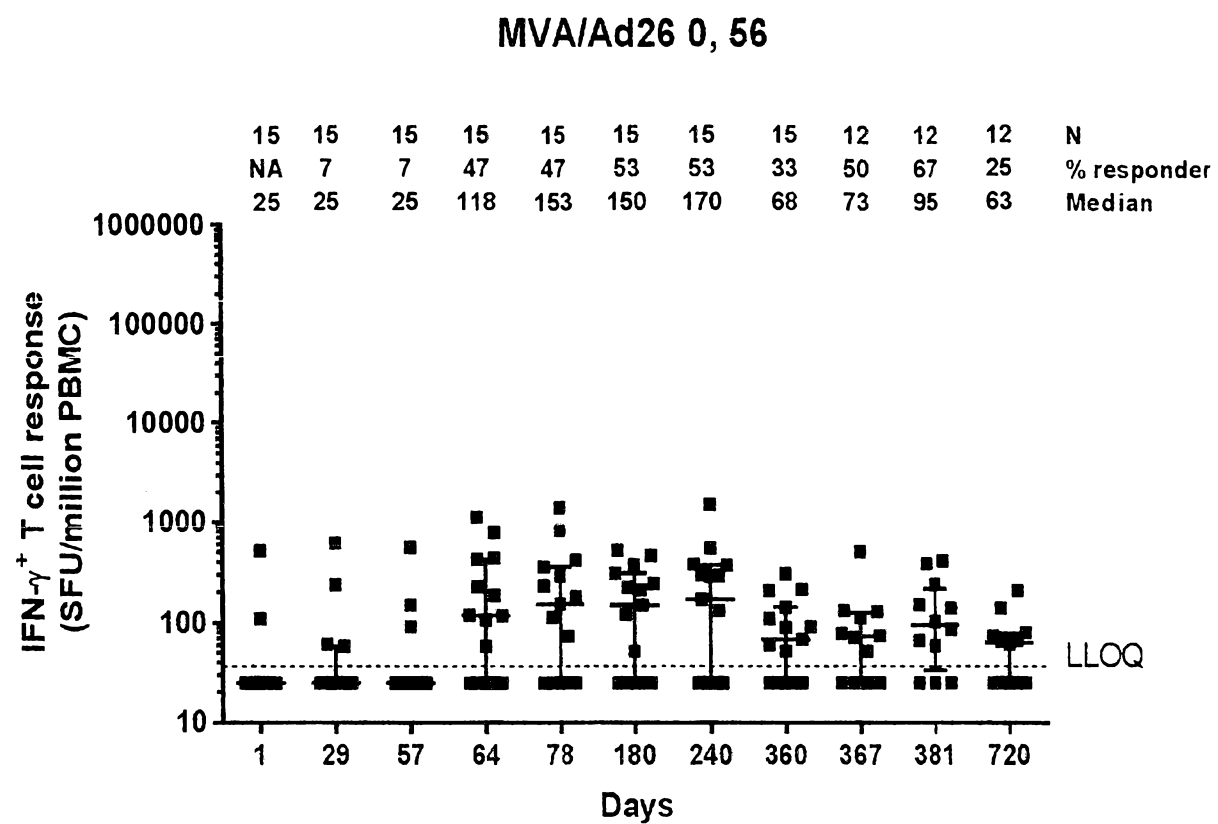


Figure 4c

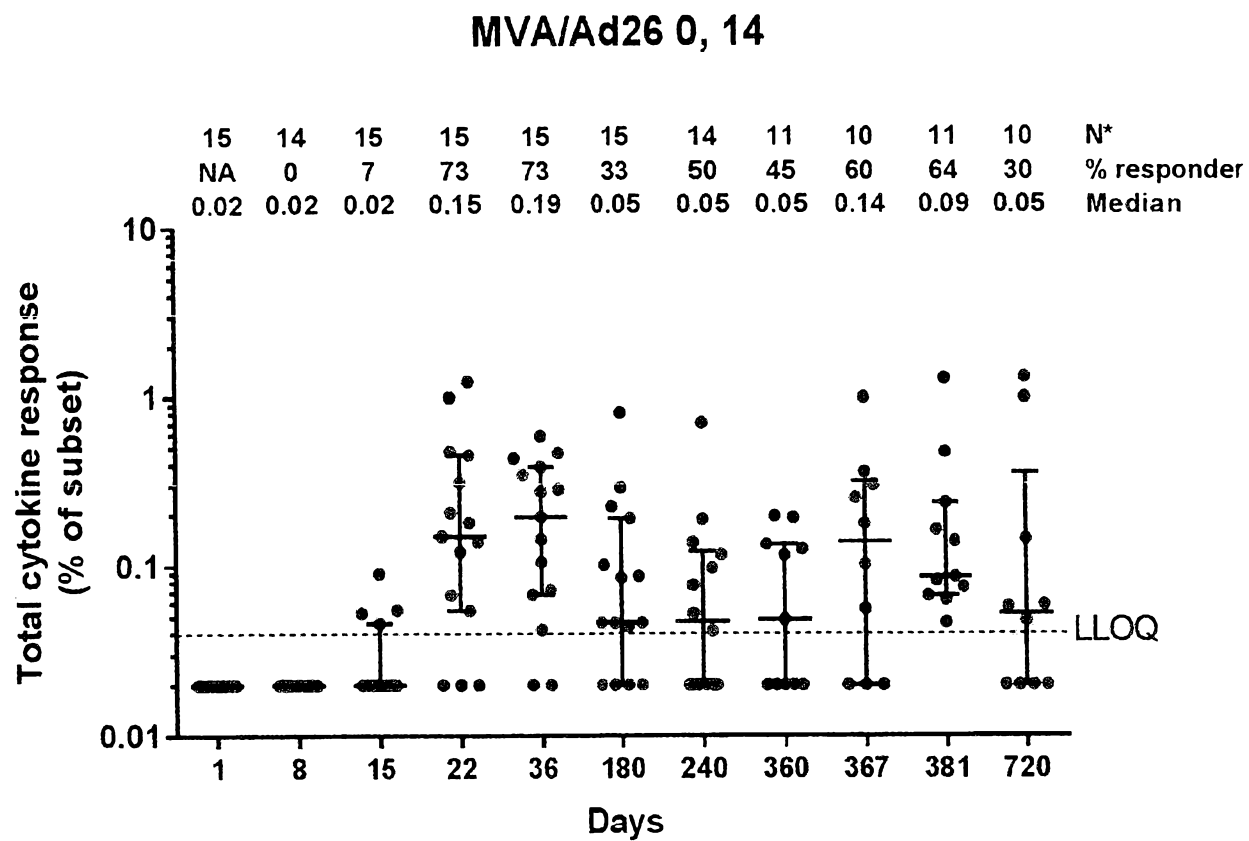


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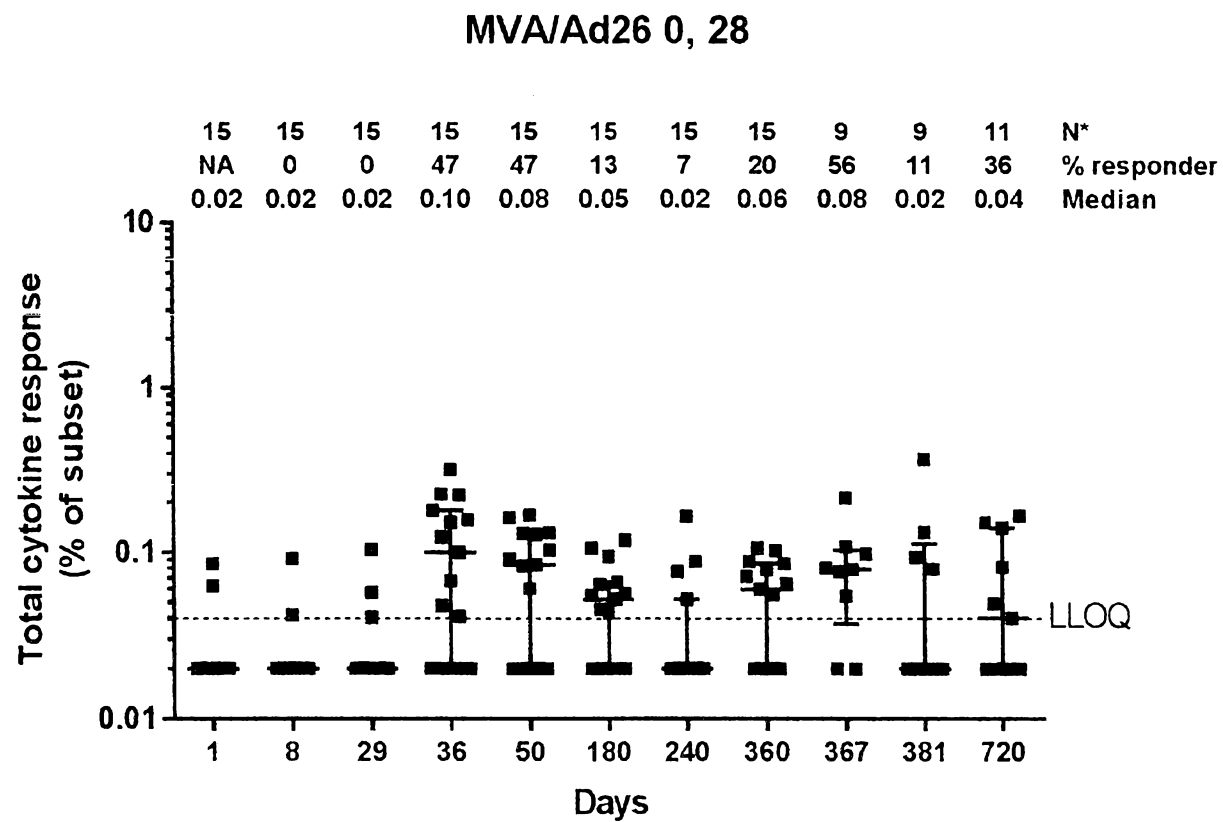


Figure 5b

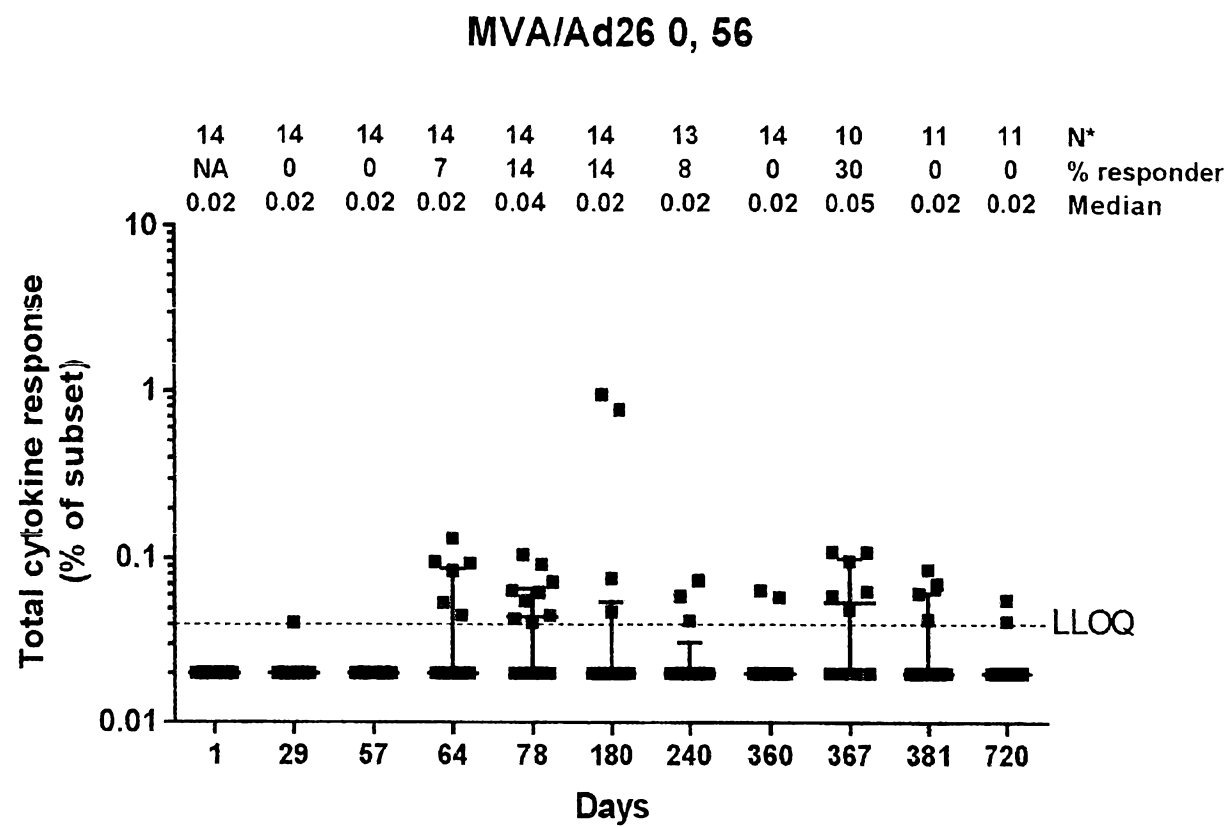


Figure 5c

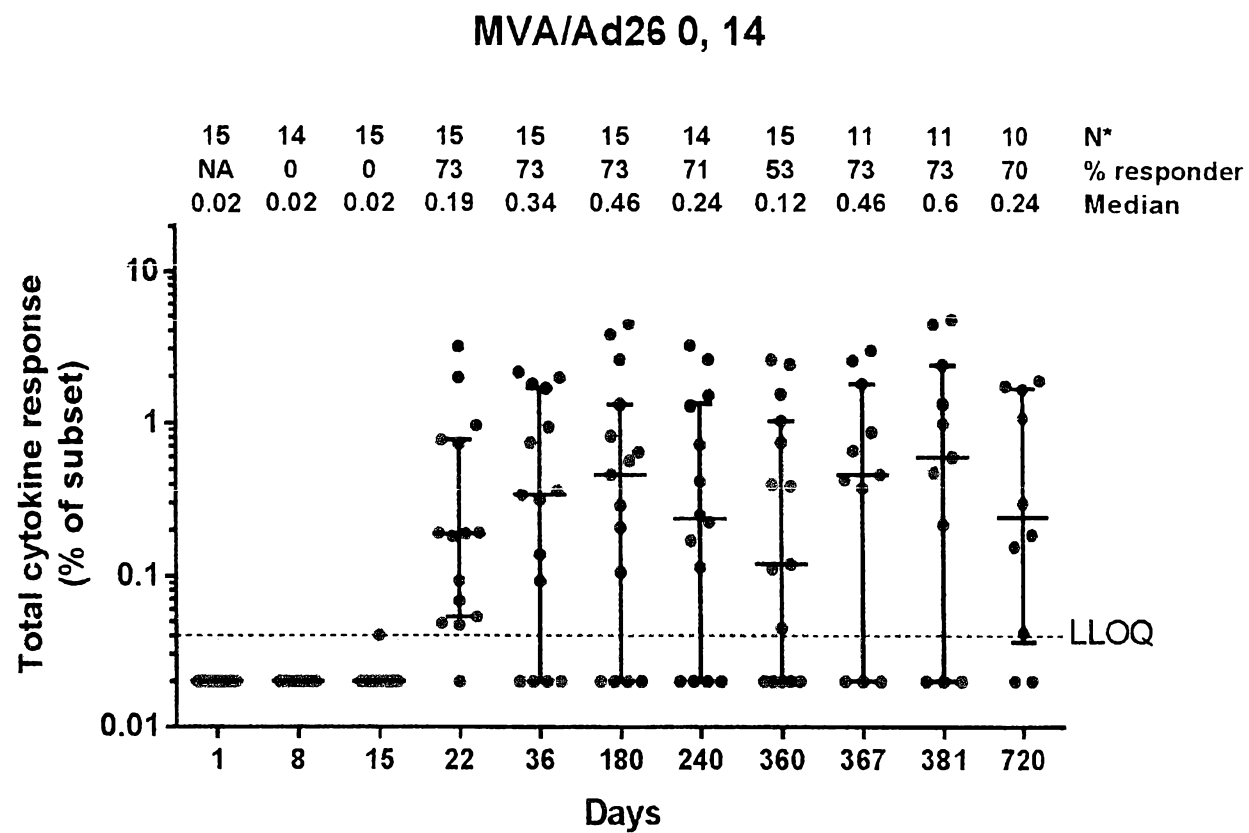


Figure 6a

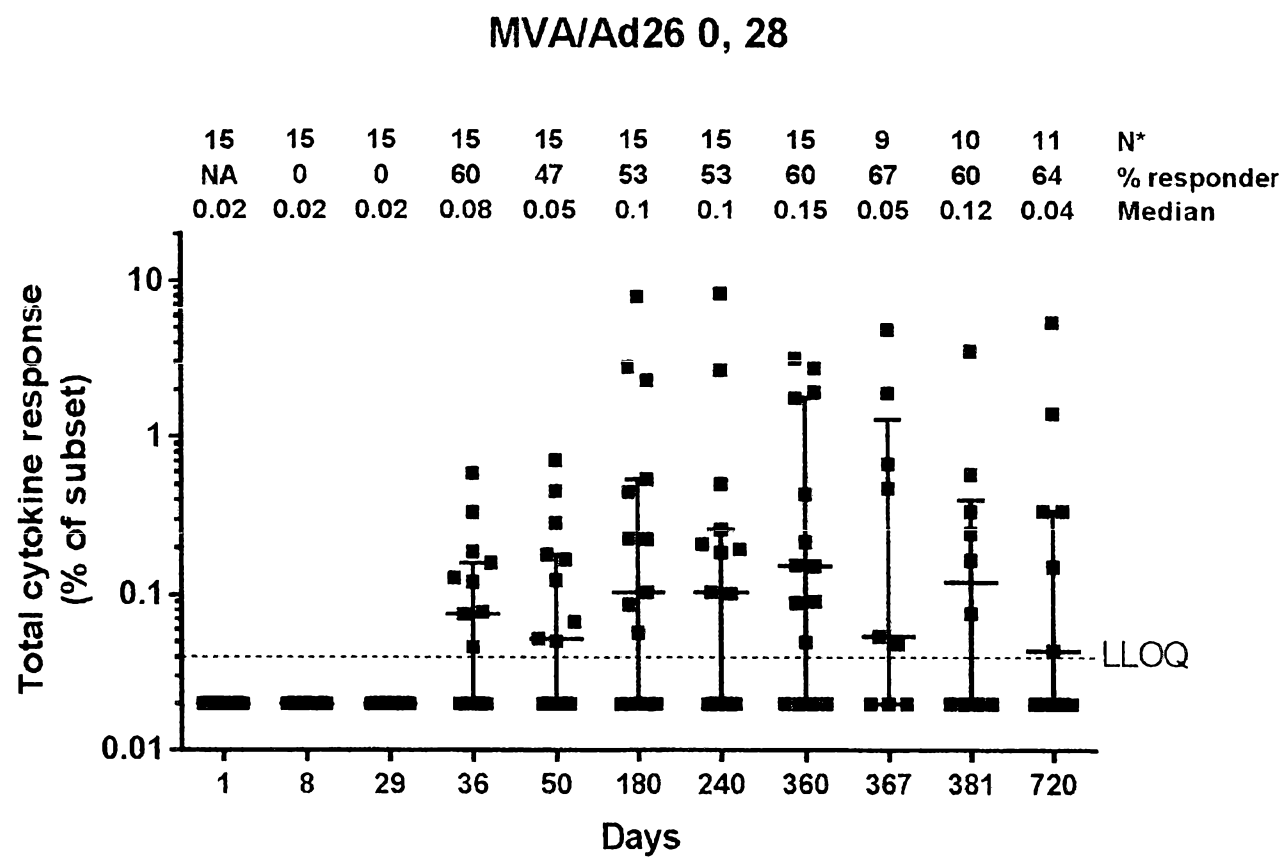


Figure 6b

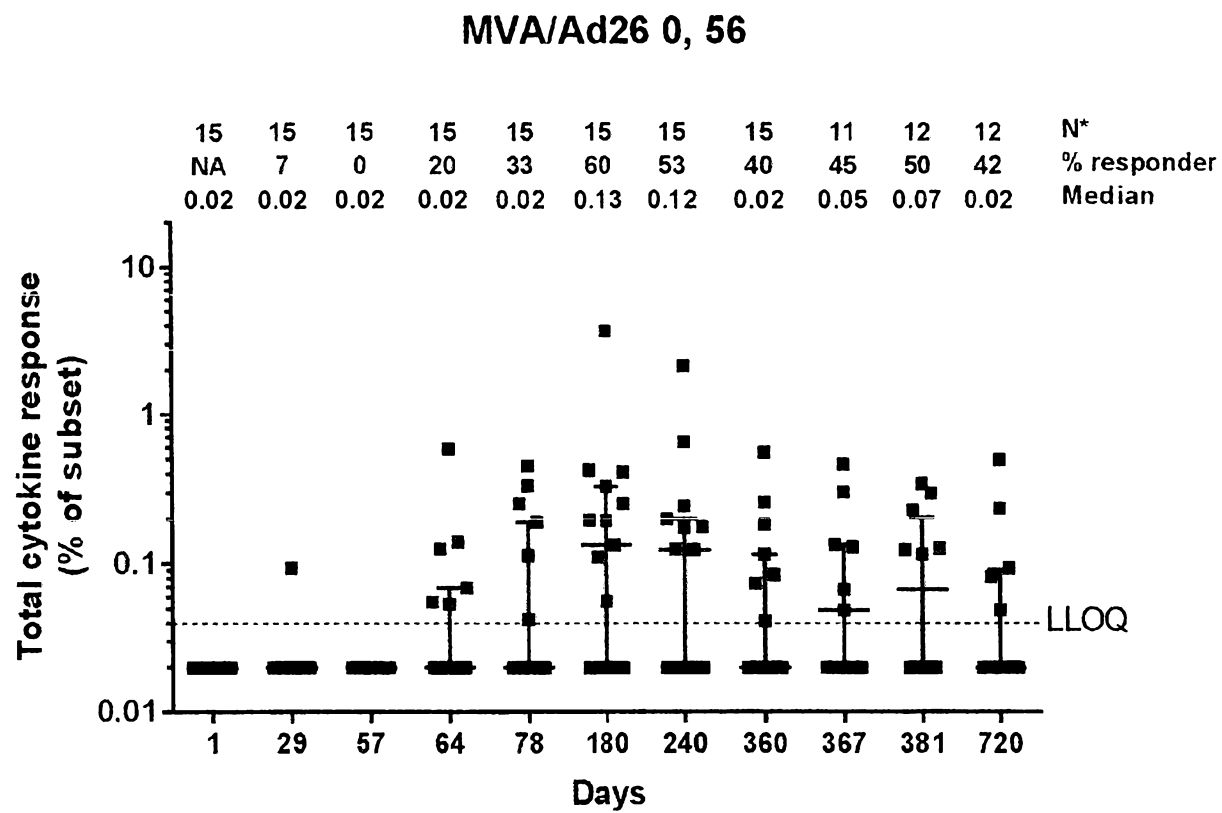


Figure 6c

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Gln Ile Ile His Asp Phe Ile Asp Asn Pro Leu Pro Asn Gln Asp Asn
625 630 635 640

Asp Asp Asn Trp Trp Thr Gly Trp Arg Gln Trp Ile Pro Ala Gly Ile
645 650 655

Gly Ile Thr Gly Ile Ile Ile Ala Ile Ile Ala Leu Leu Cys Val Cys
660 665 670

Lys Leu Leu Cys
675

<210> 3

<211> 681

<212> PRT

<213> Glycoprotein Marburg virus Angola

<400> 3

Met Lys Thr Thr Cys Leu Leu Ile Ser Leu Ile Leu Ile Gln Gly Val
1 5 10 15

Lys Thr Leu Pro Ile Leu Glu Ile Ala Ser Asn Ile Gln Pro Gln Asn
20 25 30

Val Asp Ser Val Cys Ser Gly Thr Leu Gln Lys Thr Glu Asp Val His
35 40 45

Leu Met Gly Phe Thr Leu Ser Gly Gln Lys Val Ala Asp Ser Pro Leu
50 55 60

Glu Ala Ser Lys Arg Trp Ala Phe Arg Ala Gly Val Pro Pro Lys Asn
65 70 75 80

Val Glu Tyr Thr Glu Gly Glu Glu Ala Lys Thr Cys Tyr Asn Ile Ser
85 90 95

Val Thr Asp Pro Ser Gly Lys Ser Leu Leu Leu Asp Pro Pro Thr Asn
100 105 110

P16944 - Sequence listings

Ile Arg Asp Tyr Pro Lys Cys Lys Thr Ile His His Ile Gln Gly Gln
 115 120 125
 Asn Pro His Ala Gln Gly Ile Ala Leu His Leu Trp Gly Ala Phe Phe
 130 135 140
 Leu Tyr Asp Arg Ile Ala Ser Thr Thr Met Tyr Arg Gly Lys Val Phe
 145 150 155 160
 Thr Glu Gly Asn Ile Ala Ala Met Ile Val Asn Lys Thr Val His Lys
 165 170 175
 Met Ile Phe Ser Arg Gln Gly Gln Gly Tyr Arg His Met Asn Leu Thr
 180 185 190
 Ser Thr Asn Lys Tyr Trp Thr Ser Ser Asn Gly Thr Gln Thr Asn Asp
 195 200 205
 Thr Gly Cys Phe Gly Thr Leu Gln Glu Tyr Asn Ser Thr Lys Asn Gln
 210 215 220
 Thr Cys Ala Pro Ser Lys Lys Pro Leu Pro Leu Pro Thr Ala His Pro
 225 230 235 240
 Glu Val Lys Leu Thr Ser Thr Ser Thr Asp Ala Thr Lys Leu Asn Thr
 245 250 255
 Thr Asp Pro Asn Ser Asp Asp Glu Asp Leu Thr Thr Ser Gly Ser Gly
 260 265 270
 Ser Gly Glu Gln Glu Pro Tyr Thr Thr Ser Asp Ala Ala Thr Lys Gln
 275 280 285
 Gly Leu Ser Ser Thr Met Pro Pro Thr Pro Ser Pro Gln Pro Ser Thr
 290 295 300
 Pro Gln Gln Gly Gly Asn Asn Thr Asn His Ser Gln Gly Val Val Thr
 305 310 315 320
 Glu Pro Gly Lys Thr Asn Thr Thr Ala Gln Pro Ser Met Pro Pro His
 325 330 335
 Asn Thr Thr Thr Ile Ser Thr Asn Asn Thr Ser Lys His Asn Leu Ser
 340 345 350
 Thr Pro Ser Val Pro Ile Gln Asn Ala Thr Asn Tyr Asn Thr Gln Ser
 355 360 365
 Thr Ala Pro Glu Asn Glu Gln Thr Ser Ala Pro Ser Lys Thr Thr Leu
 370 375 380

P16944 - Sequence listings

Leu Pro Thr Glu Asn Pro Thr Thr Ala Lys Ser Thr Asn Ser Thr Lys
 385 390 395 400
 Ser Pro Thr Thr Thr Val Pro Asn Thr Thr Asn Lys Tyr Ser Thr Ser
 405 410 415
 Pro Ser Pro Thr Pro Asn Ser Thr Ala Gln His Leu Val Tyr Phe Arg
 420 425 430
 Arg Lys Arg Asn Ile Leu Trp Arg Glu Gly Asp Met Phe Pro Phe Leu
 435 440 445
 Asp Gly Leu Ile Asn Ala Pro Ile Asp Phe Asp Pro Val Pro Asn Thr
 450 455 460
 Lys Thr Ile Phe Asp Glu Ser Ser Ser Ser Gly Ala Ser Ala Glu Glu
 465 470 475 480
 Asp Gln His Ala Ser Pro Asn Ile Ser Leu Thr Leu Ser Tyr Phe Pro
 485 490 495
 Lys Val Asn Glu Asn Thr Ala His Ser Gly Glu Asn Glu Asn Asp Cys
 500 505 510
 Asp Ala Glu Leu Arg Ile Trp Ser Val Gln Glu Asp Asp Leu Ala Ala
 515 520 525
 Gly Leu Ser Trp Ile Pro Phe Phe Gly Pro Gly Ile Glu Gly Leu Tyr
 530 535 540
 Thr Ala Gly Leu Ile Lys Asn Gln Asn Asn Leu Val Cys Arg Leu Arg
 545 550 555 560
 Arg Leu Ala Asn Gln Thr Ala Lys Ser Leu Glu Leu Leu Leu Arg Val
 565 570 575
 Thr Thr Glu Glu Arg Thr Phe Ser Leu Ile Asn Arg His Ala Ile Asp
 580 585 590
 Phe Leu Leu Ala Arg Trp Gly Gly Thr Cys Lys Val Leu Gly Pro Asp
 595 600 605
 Cys Cys Ile Gly Ile Glu Asp Leu Ser Arg Asn Ile Ser Glu Gln Ile
 610 615 620
 Asp Gln Ile Lys Lys Asp Glu Gln Lys Glu Gly Thr Gly Trp Gly Leu
 625 630 635 640
 Gly Gly Lys Trp Trp Thr Ser Asp Trp Gly Val Leu Thr Asn Leu Gly
 645 650 655

P16944 - Sequence listings

Ile Leu Leu Leu Leu Ser Ile Ala Val Leu Ile Ala Leu Ser Cys Ile
 660 665 670

Cys Arg Ile Phe Thr Lys Tyr Ile Gly
 675 680

<210> 4
 <211> 681
 <212> PRT
 <213> Glycoprotein Marburg virus Musoke

<400> 4

Met Lys Thr Thr Cys Phe Leu Ile Ser Leu Ile Leu Ile Gln Gly Thr
 1 5 10 15

Lys Asn Leu Pro Ile Leu Glu Ile Ala Ser Asn Asn Gln Pro Gln Asn
 20 25 30

Val Asp Ser Val Cys Ser Gly Thr Leu Gln Lys Thr Glu Asp Val His
 35 40 45

Leu Met Gly Phe Thr Leu Ser Gly Gln Lys Val Ala Asp Ser Pro Leu
 50 55 60

Glu Ala Ser Lys Arg Trp Ala Phe Arg Thr Gly Val Pro Pro Lys Asn
 65 70 75 80

Val Glu Tyr Thr Glu Gly Glu Glu Ala Lys Thr Cys Tyr Asn Ile Ser
 85 90 95

Val Thr Asp Pro Ser Gly Lys Ser Leu Leu Leu Asp Pro Pro Thr Asn
 100 105 110

Ile Arg Asp Tyr Pro Lys Cys Lys Thr Ile His His Ile Gln Gly Gln
 115 120 125

Asn Pro His Ala Gln Gly Ile Ala Leu His Leu Trp Gly Ala Phe Phe
 130 135 140

Leu Tyr Asp Arg Ile Ala Ser Thr Thr Met Tyr Arg Gly Lys Val Phe
 145 150 155 160

Thr Glu Gly Asn Ile Ala Ala Met Ile Val Asn Lys Thr Val His Lys
 165 170 175

Met Ile Phe Ser Arg Gln Gly Gln Gly Tyr Arg His Met Asn Leu Thr
 180 185 190

Ser Thr Asn Lys Tyr Trp Thr Ser Ser Asn Gly Thr Gln Thr Asn Asp
 195 200 205

Thr Gly Cys Phe Gly Ala Leu Gln Glu Tyr Asn Ser Thr Lys Asn Gln

P16944 - Sequence listings

210

215

220

Thr Cys Ala Pro Ser Lys Ile Pro Pro Pro Leu Pro Thr Ala Arg Pro
 225 230 235 240

Glu Ile Lys Leu Thr Ser Thr Pro Thr Asp Ala Thr Lys Leu Asn Thr
 245 250 255

Thr Asp Pro Ser Ser Asp Asp Glu Asp Leu Ala Thr Ser Gly Ser Gly
 260 265 270

Ser Gly Glu Arg Glu Pro His Thr Thr Ser Asp Ala Val Thr Lys Gln
 275 280 285

Gly Leu Ser Ser Thr Met Pro Pro Thr Pro Ser Pro Gln Pro Ser Thr
 290 295 300

Pro Gln Gln Gly Gly Asn Asn Thr Asn His Ser Gln Asp Ala Val Thr
 305 310 315 320

Glu Leu Asp Lys Asn Asn Thr Thr Ala Gln Pro Ser Met Pro Pro His
 325 330 335

Asn Thr Thr Thr Ile Ser Thr Asn Asn Thr Ser Lys His Asn Phe Ser
 340 345 350

Thr Leu Ser Ala Pro Leu Gln Asn Thr Thr Asn Asp Asn Thr Gln Ser
 355 360 365

Thr Ile Thr Glu Asn Glu Gln Thr Ser Ala Pro Ser Ile Thr Thr Leu
 370 375 380

Pro Pro Thr Gly Asn Pro Thr Thr Ala Lys Ser Thr Ser Ser Lys Lys
 385 390 395 400

Gly Pro Ala Thr Thr Ala Pro Asn Thr Thr Asn Glu His Phe Thr Ser
 405 410 415

Pro Pro Pro Thr Pro Ser Ser Thr Ala Gln His Leu Val Tyr Phe Arg
 420 425 430

Arg Lys Arg Ser Ile Leu Trp Arg Glu Gly Asp Met Phe Pro Phe Leu
 435 440 445

Asp Gly Leu Ile Asn Ala Pro Ile Asp Phe Asp Pro Val Pro Asn Thr
 450 455 460

Lys Thr Ile Phe Asp Glu Ser Ser Ser Ser Gly Ala Ser Ala Glu Glu
 465 470 475 480

Asp Gln His Ala Ser Pro Asn Ile Ser Leu Thr Leu Ser Tyr Phe Pro

P16944 - Sequence listings

485

490

495

Asn Ile Asn Glu Asn Thr Ala Tyr Ser Gly Glu Asn Glu Asn Asp Cys
 500 505 510

Asp Ala Glu Leu Arg Ile Trp Ser Val Gln Glu Asp Asp Leu Ala Ala
 515 520 525

Gly Leu Ser Trp Ile Pro Phe Phe Gly Pro Gly Ile Glu Gly Leu Tyr
 530 535 540

Thr Ala Val Leu Ile Lys Asn Gln Asn Asn Leu Val Cys Arg Leu Arg
 545 550 555 560

Arg Leu Ala Asn Gln Thr Ala Lys Ser Leu Glu Leu Leu Leu Arg Val
 565 570 575

Thr Thr Glu Glu Arg Thr Phe Ser Leu Ile Asn Arg His Ala Ile Asp
 580 585 590

Phe Leu Leu Thr Arg Trp Gly Gly Thr Cys Lys Val Leu Gly Pro Asp
 595 600 605

Cys Cys Ile Gly Ile Glu Asp Leu Ser Lys Asn Ile Ser Glu Gln Ile
 610 615 620

Asp Gln Ile Lys Lys Asp Glu Gln Lys Glu Gly Thr Gly Trp Gly Leu
 625 630 635 640

Gly Gly Lys Trp Trp Thr Ser Asp Trp Gly Val Leu Thr Asn Leu Gly
 645 650 655

Ile Leu Leu Leu Leu Ser Ile Ala Val Leu Ile Ala Leu Ser Cys Ile
 660 665 670

Cys Arg Ile Phe Thr Lys Tyr Ile Gly
 675 680

<210> 5

<211> 739

<212> PRT

<213> Nucleoprotein Ebola virus Tai Forest / Ivory coast

<400> 5

Met Glu Ser Arg Ala His Lys Ala Trp Met Thr His Thr Ala Ser Gly
 1 5 10 15

Phe Glu Thr Asp Tyr His Lys Ile Leu Thr Ala Gly Leu Ser Val Gln
 20 25 30

Gln Gly Ile Val Arg Gln Arg Val Ile Gln Val His Gln Val Thr Asn
 35 40 45

P16944 - Sequence listings

Leu Glu Glu Ile Cys Gln Leu Ile Ile Gln Ala Phe Glu Ala Gly Val
 50 55 60
 Asp Phe Gln Glu Ser Ala Asp Ser Phe Leu Leu Met Leu Cys Leu His
 65 70 75 80
 His Ala Tyr Gln Gly Asp Tyr Lys Gln Phe Leu Glu Ser Asn Ala Val
 85 90 95
 Lys Tyr Leu Glu Gly His Gly Phe Arg Phe Glu Val Arg Lys Lys Glu
 100 105 110
 Gly Val Lys Arg Leu Glu Glu Leu Leu Pro Ala Ala Ser Ser Gly Lys
 115 120 125
 Ser Ile Arg Arg Thr Leu Ala Ala Met Pro Glu Glu Glu Thr Thr Glu
 130 135 140
 Ala Asn Ala Gly Gln Phe Leu Ser Phe Ala Ser Leu Phe Leu Pro Lys
 145 150 155 160
 Leu Val Val Gly Glu Lys Ala Cys Leu Glu Lys Val Gln Arg Gln Ile
 165 170 175
 Gln Val His Ser Glu Gln Gly Leu Ile Gln Tyr Pro Thr Ala Trp Gln
 180 185 190
 Ser Val Gly His Met Met Val Ile Phe Arg Leu Met Arg Thr Asn Phe
 195 200 205
 Leu Ile Lys Phe Leu Leu Ile His Gln Gly Met His Met Val Ala Gly
 210 215 220
 His Asp Ala Asn Asp Ala Val Ile Ala Asn Ser Val Ala Gln Ala Arg
 225 230 235 240
 Phe Ser Gly Leu Leu Ile Val Lys Thr Val Leu Asp His Ile Leu Gln
 245 250 255
 Lys Thr Glu His Gly Val Arg Leu His Pro Leu Ala Arg Thr Ala Lys
 260 265 270
 Val Lys Asn Glu Val Asn Ser Phe Lys Ala Ala Leu Ser Ser Leu Ala
 275 280 285
 Gln His Gly Glu Tyr Ala Pro Phe Ala Arg Leu Leu Asn Leu Ser Gly
 290 295 300
 Val Asn Asn Leu Glu His Gly Leu Phe Pro Gln Leu Ser Ala Ile Ala
 305 310 315 320

P16944 - Sequence listings

Leu Gly Val Ala Thr Ala His Gly Ser Thr Leu Ala Gly Val Asn Val
 325 330 335
 Gly Glu Gln Tyr Gln Gln Leu Arg Glu Ala Ala Thr Glu Ala Glu Lys
 340 345 350
 Gln Leu Gln Lys Tyr Ala Glu Ser Arg Glu Leu Asp His Leu Gly Leu
 355 360 365
 Asp Asp Gln Glu Lys Lys Ile Leu Lys Asp Phe His Gln Lys Lys Asn
 370 375 380
 Glu Ile Ser Phe Gln Gln Thr Thr Ala Met Val Thr Leu Arg Lys Glu
 385 390 395 400
 Arg Leu Ala Lys Leu Thr Glu Ala Ile Thr Ser Thr Ser Leu Leu Lys
 405 410 415
 Thr Gly Lys Gln Tyr Asp Asp Asp Asn Asp Ile Pro Phe Pro Gly Pro
 420 425 430
 Ile Asn Asp Asn Glu Asn Ser Glu Gln Gln Asp Asp Asp Pro Thr Asp
 435 440 445
 Ser Gln Asp Thr Thr Ile Pro Asp Ile Ile Val Asp Pro Asp Asp Gly
 450 455 460
 Arg Tyr Asn Asn Tyr Gly Asp Tyr Pro Ser Glu Thr Ala Asn Ala Pro
 465 470 475 480
 Glu Asp Leu Val Leu Phe Asp Leu Glu Asp Gly Asp Glu Asp Asp His
 485 490 495
 Arg Pro Ser Ser Ser Ser Glu Asn Asn Asn Lys His Ser Leu Thr Gly
 500 505 510
 Thr Asp Ser Asn Lys Thr Ser Asn Trp Asn Arg Asn Pro Thr Asn Met
 515 520 525
 Pro Lys Lys Asp Ser Thr Gln Asn Asn Asp Asn Pro Ala Gln Arg Ala
 530 535 540
 Gln Glu Tyr Ala Arg Asp Asn Ile Gln Asp Thr Pro Thr Pro His Arg
 545 550 555 560
 Ala Leu Thr Pro Ile Ser Glu Glu Thr Gly Ser Asn Gly His Asn Glu
 565 570 575
 Asp Asp Ile Asp Ser Ile Pro Pro Leu Glu Ser Asp Glu Glu Asn Asn
 580 585 590

p16944 - Sequence listings

Thr Glu Thr Thr Ile Thr Thr Thr Lys Asn Thr Thr Ala Pro Pro Ala
 595 600 605
 Pro Val Tyr Arg Ser Asn Ser Glu Lys Glu Pro Leu Pro Gln Glu Lys
 610 615 620
 Ser Gln Lys Gln Pro Asn Gln Val Ser Gly Ser Glu Asn Thr Asp Asn
 625 630 635 640
 Lys Pro His Ser Glu Gln Ser Val Glu Glu Met Tyr Arg His Ile Leu
 645 650 655
 Gln Thr Gln Gly Pro Phe Asp Ala Ile Leu Tyr Tyr Tyr Met Met Thr
 660 665 670
 Glu Glu Pro Ile Val Phe Ser Thr Ser Asp Gly Lys Glu Tyr Val Tyr
 675 680 685
 Pro Asp Ser Leu Glu Gly Glu His Pro Pro Trp Leu Ser Glu Lys Glu
 690 695 700
 Ala Leu Asn Glu Asp Asn Arg Phe Ile Thr Met Asp Asp Gln Gln Phe
 705 710 715 720
 Tyr Trp Pro Val Met Asn His Arg Asn Lys Phe Met Ala Ile Leu Gln
 725 730 735
 His His Lys

ABSTRACT OF THE DISCLOSURE

Compositions and methods are described for generating an improved effective immune response against an immunogen in humans. The enhanced immune response, is obtained by
5 using an MVA vector as a prime, an adenovirus vector as a first boost, and an adenovirus vector as a second boost. The compositions and methods can be used to provide a protective immunity against a disease, such as an infection of one or more subtypes of Ebola and Marburg filoviruses, in humans.

Planche de l'abrégé

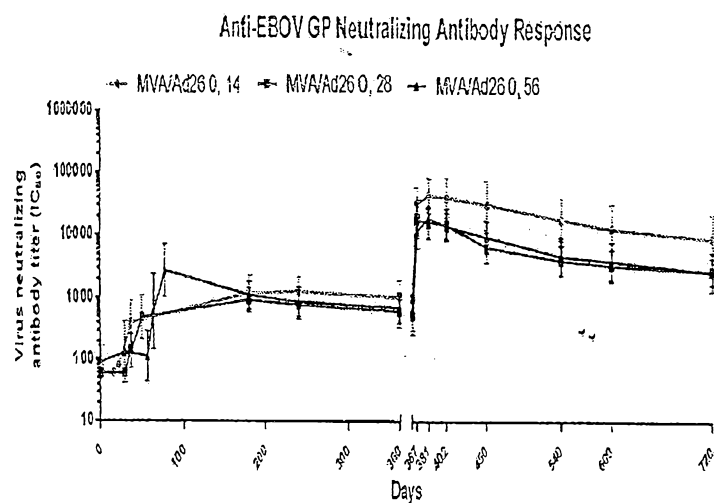


Figure 3