Poxvirus vectors encoding HIV antigens, and methods of use thereof

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Poxvirus vectors encoding HIV antigens, and methods of use thereof

Abstract

Poxvirus vectors encoding a synthetic HIV envelope antigen and other HIV antigens, as well as compositions containing such poxvirus vectors and uses of such poxvirus vectors as vaccines to provide improved immunity against HIV, are provided. Also provided are vaccine combinations containing the disclosed poxvirus vectors, adenovirus vectors encoding one or more HIV antigens, and one or more isolated HIV antigenic polypeptides, and methods of using the vaccine combinations to provide improved immunity against HIV.

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Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Section 371 of International Application No. PCT/IB2018/054386, filed Jun. 14, 2018, which was published in the English language on Dec. 20, 2018 under International Publication No. WO 2018/229711 A1, which claims the benefit of U.S. Provisional Application No. 62/520,079, filed Jun. 15, 2017, and the disclosures of which are incorporated herein by reference.

Claims

The invention claimed is:

1. A poxvirus vector comprising nucleic acid encoding: (a) a first HIV envelope (Env) antigen comprising the amino acid sequence of SEQ ID NO: 18; (b) a second HIV Env antigen different from the first HIV Env antigen; (c) a third antigen and a fourth antigen, being two different HIV Gag antigens; and (d) a fifth antigen and a sixth antigen, being two different HIV Pol antigens.

2. The poxvirus vector of claim 1, wherein: the second HIV Env antigen comprises the amino acid sequence of SEQ ID NO: 5, the third and fourth antigens comprise the amino acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2, respectively; and the fifth and the sixth antigens comprise the amino acid sequence of SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

3. The poxvirus vector of claim 1, wherein the third and the fifth antigens are fused into a first Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 28, and the fourth and the sixth antigens are fused into a second Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 29.

4. The poxvirus vector of claim 1, wherein the first HIV Env antigen is encoded by SEQ ID NO: 41.

5. The poxvirus vector of claim 3, wherein the first HIV Env antigen is encoded by SEQ ID NO: 41; the second HIV Env antigen is encoded by SEQ ID NO: 39; the first Gag-Pol fusion antigen is encoded by SEQ ID NO: 38; and the second Gag-Pol fusion antigen is encoded by SEQ ID NO: 40.

6. The poxvirus vector of claim 1, wherein the poxvirus vector is a recombinant Modified Vaccinia virus Ankara (MVA) vector.

7. The poxvirus vector of claim 6, wherein the MVA vector comprises MVA-BN or derivatives thereof.

8. The poxvirus vector of claim 6, wherein the poxvirus vector is a recombinant MVA vector, and wherein the first Gag-Pol fusion antigen and the second Env antigen are inserted into intergenic region (IGR) 44/45 of the MVA genome, and the second Gag-Pol fusion antigen and the first Env antigen are inserted into IGR 88/89 of the MVA genome.

9. The poxvirus vector of claim 8, wherein the first Gag-Pol fusion antigen and the second Gag-Pol fusion antigen are each under control of a separate Pr13.5 promoter, and the first Env antigen and the second Env antigen are each under control of a separate PrHyb promoter.

10. A vaccine comprising a poxvirus vector according to claim 1 and a pharmaceutically acceptable carrier.

11. A vaccine combination comprising: (a) a first vaccine composition comprising an immunologically effective amount of a poxvirus vector according to claim 1; and at least one of: (b) (i) a second vaccine composition comprising an immunogenically effective amount of one or more adenovirus vectors encoding one or more of the first, second, third, fourth, fifth and sixth antigens; and (b) (ii) a third vaccine composition comprising one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide, wherein the first composition, and second and/or third composition are present in the same composition or in one or more different compositions.

12. The vaccine combination of claim 11, wherein the second vaccine composition comprises recombinant Ad26 vectors together encoding SEQ ID NOs: 18, 5, 1, 2, 3 and 4.

13. The vaccine combination of claim 11, wherein the one or more isolated HIV antigenic polypeptides in the third vaccine composition comprise: (i) a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or (ii) a polypeptide comprising residues 30-724 of SEQ ID NO: 36; or (iii) both polypeptides (i) and (ii).

14. A method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising administering to the subject the vaccine of claim 10.

15. A method of inducing an immune response against a human immunodeficiency virus in a subject in need thereof, the method comprising administering to the subject: (a) a first vaccine comprising one or more recombinant Ad26 adenovirus vectors, encoding one or more of SEQ ID NOs: 1, 2, 3, 4, 5 and 18; and (b) a second vaccine comprising a poxvirus vector according to claim 1, wherein the first vaccine is a priming vaccine and the second vaccine is a boosting vaccine, or wherein the second vaccine is a priming vaccine and the first vaccine is a boosting vaccine.

16. The method of claim 15, further comprising administering to the subject one or more isolated HIV antigenic polypeptides, at about the same time as the boosting vaccine, wherein the one or more HIV antigenic polypeptides comprise (i) a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or (ii) a polypeptide comprising residues 30-724 of SEQ ID NO: 36; or (iii) both polypeptides (i) and (ii), and wherein the one or more isolated HIV antigenic polypeptides is in the same composition as the boosting vaccine or in a composition separate from the boosting vaccine.

17. The method according to claim 14, wherein the subject is a subject that has been infected with HIV.

18. A method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising administering to the subject the vaccine combination of claim 11.

19. A method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising administering to the subject the vaccine combination of claim 12.

20. A method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising administering to the subject the vaccine combination of claim 13.

Description

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "Sequence Listing 688097_331U1", creation date of Dec. 3, 2019, and having a size of about 170 KB. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus (HIV) affects millions of people worldwide, and the prevention of HIV through an efficacious vaccine remains a very high priority, even in an era of widespread antiretroviral treatment. HIV-1 is the most common and pathogenic strain of the virus, with more than 90% of HIV/AIDS cases deriving from infection with HIV-1 group M. The M group is subdivided further into clades or subtypes. An efficacious vaccine ideally would be capable of eliciting both potent cellular responses and broadly neutralizing antibodies capable of neutralizing HIV-1 strains from different clades.

The high genetic variability of HIV-1 makes the development of a HIV-1 vaccine an unprecedented challenge. In order to improve coverage of potential T-cell epitopes, and improve cellular responses, "mosaic" HIV-1 Gag, Pol and Env antigens, derived from HIV Group Antigen (Gag), Polymerase (Pol), and Envelope (Env) proteins, were described by others and developed in an attempt to provide maximal coverage of potential T-cell epitopes (e.g., Barouch et al, Nat Med 2010, 16: 319-323). The mosaic antigens are similar in length and domain structure to wild-type, naturally occurring HIV-1 antigens.

For example, mosaic HIV antigens described and used in vaccines include those described in Barouch et al, supra, and WO 2010/059732 such as:

(a) Gag mosaic antigens including: (a)(i) a first mosaic Gag sequence ("mos1Gag") having the amino acid sequence as set forth herein in SEQ ID NO: 1, and (a)(ii) a second mosaic Gag sequence ("mos2Gag") having the amino acid sequence as set forth herein in SEQ ID NO: 2;

(b) Pol mosaic antigens including: (b)(i) a first mosaic Pol sequence ("mos1Pol") having the amino acid sequence as set forth herein in SEQ ID NO: 3, and (b)(ii) a second mosaic Pol sequence ("mos2Pol") having the amino acid sequence as set forth herein in SEQ ID NO: 4; and

(c) Env mosaic antigens including: (c)(i) a first mosaic Env sequence ("mos1Env") having the amino acid sequence as set forth herein in SEQ ID NO: 5, and (c)(ii) a second mosaic Env sequence ("mos2Env") having the amino acid sequence as set forth herein in SEQ ID NO: 6.

Sequences encoding these antigens have been cloned in vectors, for example, such as recombinant adenoviral vectors, e.g., recombinant adenovirus serotype 26

(rAd26), and these recombinant vectors were previously used as vaccines to generate immune responses to the antigens (see e.g. Barouch et al, supra; and WO 2010/059732). For example, the mos1Gag and mos1Pol mosaic antigen sequences are typically combined into a fusion protein of Gag and Pol ("mos1GagPol"), and the coding sequence of which is cloned into a first Ad26 vector ("rAd26.mos1GagPol"); and the mos2Gag and mos2Pol antigen sequences are combined into another fusion protein of Gag and Pol ("mos2GagPol"), and the coding sequence of which is cloned into a second Ad26 vector ("rAd26.mos2GagPol"). Constructs encoding mos1Env and mos2Env are typically cloned into separate Ad26 vectors ("rAd26.mos1Env" and "rAd26.mos2Env", respectively).

A set of such mosaic antigens as described above gives good global coverage of Group M HIV-1 isolates, where rAd26 vectors encoding mosaic 1 antigen sequences (e.g., rAd26.mos1GagPol and rAd26.mos1Env) favor clade B and CRF01 HIV-1 subtypes, and rAd26 vectors encoding mosaic 2 antigen sequences (e.g., rAd26.mos2GagPol and rAd26.mos2Env) favor clade C strains. Mosaic HIV-1 Gag, Pol, and Env antigens expressed in rAd26 vectors can be used to improve both the breadth and depth of antigen-specific T-lymphocyte responses in rhesus monkeys, without compromising the magnitude of both cellular and humoral responses when compared with consensus or natural sequence HIV-1 antigens (Barouch et al, supra; and WO 2010/059732).

However, upon further development efforts on the vaccine components described above, it was found that rAd26.mos2Env showed non-optimal cell surface expression and immune response in non-human primates, but moreover displayed a hitherto unreported, unexpected and unpredictable non-optimal genetic stability during the manufacturing process as compared to the other rAd26 vectors, such as rAd26.mos1Env. Thus, vaccines containing rAd26.mos2Env may result in non-optimal immune responses against Clade C HIV-1 subtypes, since the mos2Env mosaic antigen favors clade C HIV-1 strains. Accordingly, there is a need for an alternative to the mos2Env antigen in vaccines against HIV that can be used to induce improved immune responses against HIV-1 clade C.

Poxvirus vectors, such as Modified Vaccinia virus Ankara (MVA), can be used to encode antigens of interest for vaccination purposes. There is a need in the art for poxvirus vectors encoding novel combinations of HIV antigens.

BRIEF SUMMARY OF THE INVENTION

The invention relates to synthetic human immunodeficiency virus (HIV) envelope proteins that have improved cell surface expression and genetic stability as compared to the previously described mos2Env antigen and a novel poxvirus vector comprising nucleic acid sequence encoding the synthetic HIV envelope proteins. The invention also relates to compositions and methods of using such novel poxvirus vectors comprising nucleic acid sequence encoding the synthetic HIV envelope proteins to induce increased immune responses against HIV-1, particularly HIV-1 clade C and B, preferably when used in combination with other HIV antigens.

In particular aspects, the invention relates to poxvirus vectors, preferably Modified Vaccinia virus Ankara (MVA) vectors, comprising nucleic acid encoding the synthetic HIV envelope protein and preferably comprising a nucleic acid sequence encoding further HIV antigens.

In one general aspect, the invention relates to a nucleic acid encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 8. The synthetic HIV envelope protein can further comprise a signal sequence, for instance a signal sequence having the amino acid sequence selected from the group consisting of SEQ ID NOs: 9-12. In one embodiment, the signal sequence has the amino acid sequence of SEQ ID NO: 9.

In certain embodiments, the synthetic HIV envelope protein further comprises a transmembrane domain, preferably a transmembrane domain having the amino acid sequence of SEQ ID NO: 13. In certain embodiments, the synthetic HIV envelope protein further comprises a fragment of a cytoplasmic domain, preferably a fragment of a cytoplasmic domain comprising the amino acid sequence of SEQ ID NO: 14, or the N-terminal amino acids 1-4 thereof (i.e., NRVR). In embodiments wherein the synthetic HIV envelope protein further comprises a transmembrane domain and a fragment of a cytoplasmic domain, it is preferred that the protein also comprises the amino acid sequence of SEQ ID NO: 37, which is fused to the carboxyl-terminus (C-terminus) of SEQ ID NO: 8 and the amino-terminus (N-terminus) of the transmembrane region.

In a most preferred embodiment, the invention relates to a poxvirus vector comprising a nucleic acid sequence encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 17, SEQ ID NO: 18, or aa 1-686 of SEQ ID NO: 19. Most preferably the synthetic HIV envelope protein encoded by the nucleic acid comprises or consists of the amino acid sequence of SEQ ID NO: 18.

In preferred embodiments, the poxvirus vector comprises a nucleic acid encoding a synthetic HIV envelope protein as described above, and at least one additional HIV antigen. In a preferred embodiment, the poxvirus vector is a Modified Vaccinia virus Ankara (MVA) vector. Most preferably the MVA vector comprises MVA-BN or derivatives thereof.

In one preferred embodiment, the poxvirus vector comprises (a) nucleic acid encoding a first HIV envelope (Env) antigen comprising the amino acid sequence of SEQ ID NO: 18, and preferably further comprises nucleic acid encoding: (b) a second HIV Env antigen different from the first HIV Env antigen; (c) a third antigen and fourth antigen, being two different HIV Gag antigens; and (d) a fifth antigen and sixth antigens, being two different HIV Pol antigens. In certain preferred embodiments, (b) the second HIV Env antigen comprises the amino acid sequence of SEQ ID NO: 5; (c) the third and fourth antigens comprise the amino acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2, respectively; and (d) the fifth and the sixth antigens comprise the amino acid sequence of SEQ ID NO: 3 and SEQ ID NO: 4, respectively. In certain embodiments, the third and fifth antigens are fused into a first Gag-Pol fusion antigen, preferably comprising SEQ ID NO: 28; and the fourth and sixth antigens are fused into a second Gag-Pol fusion antigen, preferably comprising SEQ ID NO: 29. In certain particular embodiments, the first HIV Env antigen is encoded by SEQ ID NO: 41. In one or more particular embodiments, the second HIV Env antigen is encoded by SEQ ID NO: 39; the first Gag-Pol fusion antigen is encoded by SEQ ID NO: 38; and the second Gag-Pol fusion antigen is encoded by SEQ ID NO: 40.

In embodiments wherein the poxvirus vector is an MVA vector, such as an MVA vector comprising MVA-BN or derivatives thereof, the first Gag-Pol fusion antigen and the second Env antigen are preferably inserted into intergenic region (IGR) 44/45 of the MVA genome, and the second Gag-Pol fusion antigen and the first Env antigen are preferably inserted into IGR 88/89 of the MVA genome. More preferably, the first Gag-Pol fusion antigen and the second Gag-Pol fusion antigens are each under control of a separate promoter, preferably a Pr13.5 promoter, and the first Env antigen and the second Env antigen are each under control of a separate promoter, preferably a PrHyb promoter.

Another general aspect of the invention relates to a composition, preferably a vaccine composition, comprising an immunogenically effective amount of a poxvirus vector comprising a nucleic acid sequence encoding a synthetic HIV envelope protein according to an embodiment of the invention and preferably further comprising a nucleic acid sequence encoding one or more additional HIV antigens, and a carrier, wherein the nucleic acid encoding the synthetic HIV envelope protein is operably linked to a promoter sequence. In one embodiment, the composition further comprises an adenovirus vector, preferably an adenovirus 26 vector, encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18. In certain embodiments, the composition comprises a poxvirus vector, preferably an MVA vector, encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18, and preferably encoding further HIV antigens. In certain embodiments, the compositions of the invention further comprise additional expression vectors encoding additional HIV antigens and/or isolated HIV antigenic polypeptide.

In another general aspect, the invention relates to a vaccine combination for inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof. In one embodiment, the vaccine combination comprises: (a) a first vaccine composition comprising an immunogenically effective amount of a vector, preferably a poxvirus vector, more preferably an MVA vector, encoding (i) a first HIV envelope (Env) protein being a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and preferably further comprising nucleic acid encoding: (ii) a second HIV Env antigen different from the first HIV Env antigen; (iii) a third antigen and a fourth antigen, being two different HIV Gag antigens; and (iv) a fifth antigen and a sixth antigen, being two different HIV Pol antigens; and at least one of: (b) (i) a second vaccine composition comprising an immunogenically effective amount of one or more vectors, preferably one or more adenovirus vectors, more preferably one or more adenovirus 26 vectors, encoding one or more of the first, second, third, fourth, fifth, and sixth HIV antigens, preferably encoding one or more HIV antigens comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29; and/or (b) (ii) a third vaccine composition comprising one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide, for instance, a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, and/or a polypeptide comprising residues 30-724 of SEQ ID NO: 36, wherein the first composition and the second and/or third compositions are present in the same composition or in one or more different compositions.

In one embodiment wherein the vaccine combination comprises a second vaccine composition, the second vaccine composition comprises one or more recombinant adenovirus 26 vectors encoding one or more antigens comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29, more preferably comprising two, three, or four recombinant adenovirus 26 vectors together encoding SEQ ID NOs: 1, 2, 3, 4, 5, and 18.

Yet another general aspect of the invention relates to methods of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need

thereof, comprising administering to the subject a composition, such as a vaccine composition, or vaccine combination according to an embodiment of the invention. The invention also relates to methods of inducing an immune response against an HIV comprising priming and boosting the immune response using a composition or a vaccine combination according to an embodiment of the invention.

In a particular embodiment, a method of inducing an immune response against a HIV in a subject in need thereof comprises administering to the subject: (a) a first vaccine comprising one or more recombinant adenovirus vectors, preferably Ad26 vectors, encoding one or more of SEQ ID NOs: 1, 2, 3, 4, 5, and 18; and (b) a second vaccine comprising a poxvirus vector, preferably an MVA vector, encoding a first HIV envelope (Env) protein being a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and preferably encoding further HIV antigens, preferably a second HIV Env antigen different from the first HIV Env antigen, a third antigen and a fourth antigen being two different HIV Gag antigens, and a fifth antigen and sixth antigen being two different HIV Pol antigens, more preferably one or more HIV antigens encoding the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 28, and 29, wherein the first vaccine is a priming vaccine and the second vaccine is a boosting vaccine, or wherein the second vaccine is a priming vaccine and the first vaccine is a boosting vaccine. In certain embodiments, one or more isolated HIV antigenic polypeptides preferably comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide, for example, a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, and/or a polypeptide comprising residues 30-724 of SEQ ID NO: 36 are administered to the subject at about the same time as the boosting vaccine in the same composition as the boosting vaccine or in a composition separate from the boosting vaccine.

Another aspect of the invention relates to a cell, preferably an isolated cell, comprising a vector according to an embodiment of the invention.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS 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.

In the drawings:

FIGS. 1A-1C are schematic representations of the structure of HIV envelope proteins; FIG. 1A shows a full length HIV envelope protein; FIG. 1B shows the structure of a soluble single chain HIV envelope protein according to an embodiment of the invention in which the transmembrane domain (TM) is replaced with a GCN4 trimerization domain, and the furin cleavage site is mutated (sC4); FIG. 1C shows the structure of a membrane bound HIV envelope protein according to an embodiment of the invention comprising a transmembrane domain and a fragment of a cytoplasmic domain (C4D7);

FIG. 2 shows expression levels of the soluble sC1 HIV envelope protein, which is based on the mos2Env mosaic antigen sequence with an additional C-terminal trimerization domain, and a soluble synthetic HIV envelope protein (sC4) according to an embodiment of the invention; expression was measured by quantitative Western blot using a polyclonal antibody against gp120; plasmids encoding sC1 or sC4 were transiently expressed twice, and each transfection was quantified twice by densitometry; the sC1 protein showed very low expression levels compared to the sC4 synthetic HIV envelope protein, which showed relatively high expression levels;

FIGS. 3A and 3B show the binding of synthetic HIV envelope proteins with monoclonal antibody 17b (mAb17b) in the presence (light gray) and absence (dark gray) of soluble CD4 as determined by ELISA assay; FIG. 3A shows binding of sC1; FIG. 3B shows binding of sC4;

FIG. 4 is an image of a Western blot from a native polyacrylamide gel electrophoresis of the sC1 protein, and the sC4 synthetic HIV envelope protein;

FIG. 5 shows the relative cell surface expression levels of the membrane-bound C1, C1D7, C4 and C4D7 synthetic HIV envelope proteins by FACS analysis of cells expressing these proteins using an anti-gp120 polyclonal antibody (GP120), and by binding to broadly neutralizing antibodies PG9 (PG9) and PG16 (PG16) that are quaternary-structure dependent and preferentially bind to correctly folded Env trimer;

FIG. 6 is a graphical representation of the stability of adenovirus vectors containing sequences encoding synthetic HIV envelope proteins of the invention including full-length C4 (FLC4), C4D7, and sC4 after multiple viral passages; recombinant adenovirus 26 vectors were generated in PER.C6 cells; after the initial 3 passages for

transfection and plaque purification, 5 plaques were selected and upscaled for 10 passages in T25 format, resulting in a total viral passage number (vpn) of 13; the stability after vpn 3, 5, 10, and 13 as determined by E1 transgene cassette polymerase chain reaction (PCR) is shown; for example, 3/5 means 3 plaques were stable out of 5 plaques tested, and 5/5 means 5 plaques were stable out of 5 plaques tested;

FIGS. 7A and 7B show virus neutralization titers against HIV-1 envelope pseudotyped virus particles (EVPs) in a TZM-bl cell-based neutralization assay in rabbits; log 10-transformed IC.sub.50 values of the high-adenoviral vector dosed groups were measured against EVPs VSV-G (negative control) and MW965.26 (Tier 1A clade C) at weeks 1, 8, 14, and 20; each dot represents the log 10-transformed IC.sub.50 value of an individual rabbit, with the group mean indicated by a horizontal line; HD: Highest Dilution tested (upper solid line); LD: Lowest Dilution tested (lower solid line); LOB: limit of background, 95 percentile value of compiled negative samples (dotted line); Log 10 IC.sub.50 values exceeding the LD or HD threshold were set at the corresponding line; a one-way non-parametric comparison with control using the Dunn method for joint ranking was done for each time point; statistically significant differences are indicated in the graphs: *=P<0.05, **=P<0.01, and ***=P<0.001; FIG. 7A shows the results with VSV-G (negative control); and FIG. 7B shows the results with MW965.26 (Tier 1A clade C).

FIG. 8 is a graphical representation of inserts into specified locations of the MVA genome for vector MVA-mBN414; Pr13.5 and PrHyb are promoter sequences; IGRs are intergenic regions;

FIGS. 9A, 9B and 9C show immune responses raised in rabbits on day 85 following immunization with Ad26.Mos.HIV (abbreviated as Ad26), either alone or combined with MVA-mBN414 (abbreviated in FIGS. 9A-9C as "MVA"), clade C gp140 (abbreviated as GP140) or a combination thereof; Males (M) and Females (F) are shown separately; FIGS. 9A and 9B show clade C gp140 and Mosaic gp140-specific ELISA titers, respectively; each dot represents the log 10-transformed relative potency value (log 10 EU/ml) of an individual rabbit, with group-mean indicated as a horizontal line; ULOQ, upper limit of quantitation (upper solid line), LLOQ lower limit of quantitation (lower solid line), LOB, limit of background (dotted line); all values below the LOB were set at the LOB level; statistical analysis consisted of an across sex Tobit model; for the comparison of group 1, 2, and 3, a Tukey correction was applied; statistically significant differences are indicated in the graphs: *P<0.05, **P<0.01, ***P<0.001; FIG. 9C shows virus neutralization titers against HIV-1 BaL envelope pseudotyped virus particles in a TZM-bl cell-based neutralization assay using rabbit serum; each dot represents the log 10-transformed IC.sub.50 value of an individual rabbit, with the group mean indicated by a horizontal line; HD: Highest Dilution tested (upper solid line); LD: Lowest Dilution tested (lower solid line); LOB: limit of background, 95 percentile value of compiled negative samples (dotted line); log 10 IC.sub.50 values exceeding the LD or HD threshold were set at the corresponding line; statistical analysis consisted of an across sex Tobit model; for the comparison of group 1, 2, and 3, a Tukey correction was applied; statistically significant differences are indicated in the graphs: *P<0.05, **P<0.01, ***P<0.001; and

FIGS. 10A-10E show immune responses raised in mice following prime-only (week 5) or prime-boost (week 7) immunization with Ad26.Mos4.HIV (abbreviated as Ad26) followed by MVA-mBN414 prime-boost or homologous MVA-mBN414 (abbreviated in FIGS. 10A-10E as "MVA") prime-boost; Groups 1 and 2 were primed in week 0 with 2.5.times.10.sup.9 or 2.5.times.10.sup.8 vp of Ad26.Mos4.HIV, respectively, and boosted in week 5 with 2.8.times.10.sup.6 or 2.8.times.10.sup.5 TCID.sub.50 of MVA-mBN414, respectively; Groups 3 and 4 were immunized in week 0 and week 5 with 2.8.times.10.sup.6 or 2.8.times.10.sup.5 TCID.sub.50 of MVA-mBN414, respectively; Group 5 (control) was primed with 2.5.times.10.sup.9 Ad26.Empty and boosted with 2.8.times.10.sup.6 TCID.sub.50 of MVA-BN-empty; FIGS. 10A and 10B show Mosaic gp140-specific ELISA titers; each dot represents the log 10-transformed endpoint titer of an individual mouse, with group-mean indicated as a horizontal line; UD, upper limit of dilution; LD, lowest dilution; all values below the LD were set at the LD level; statistical analysis consisted of an across dose Tobit model; statistically significant differences are indicated in the graphs: *P<0.05, **P<0.01, ***P<0.001; FIGS. 10C-10E show Interferon-gamma (IFN-.gamma.) ELISPOT data at week 7 of the study; each dot represents the spot-forming cell (SFC) count per 10.sup.6 splenocytes of an individual mouse, with group-mean indicated as a horizontal line; LOD: limit of detection, 95 percentile value of compiled unstimulated controls (dotted line); Env-, Gag- and Pol-specific responses were determined by stimulation with the immuno-dominant Env-, Gag- and Pol-peptides IHIGPGRAFYTAGDI (SEQ ID NO: 44), AMQMLKETI (SEQ ID NO: 45), and YYDPSKDLI (SEQ ID NO: 46), respectively; statistically significant differences are indicated in the graphs: *P<0.05, **P<0.01, ***P<0.001.

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.

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. Any of the aforementioned terms of "comprising", "containing", "including", and "having", whenever used herein in the context of an aspect or embodiment of the invention can be replaced with the term "consisting of" or "consisting essentially of" to vary scopes of the disclosure.

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 who will be or has been administered a vector, composition or vaccine combination according to embodiments 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.

The invention generally relates to synthetic HIV envelope proteins, nucleic acid and vectors encoding the synthetic HIV envelope proteins, and methods of inducing an immune response against HIV with vectors encoding the synthetic HIV envelope proteins and optionally encoding further HIV antigens, alone or in combination with one or more additional vectors encoding one or more additional HIV antigens and/or in combination with one or more additional isolated HIV antigenic polypeptides.

Human immunodeficiency virus (HIV) is a member of the genus Lentivirinae, which is part of the family of Retroviridae. Two species of HIV infect humans: HIV-1 and HIV-2. HIV-1 is the most common strain of HIV virus, and is known to be more pathogenic than HIV-2. As used herein, the terms "human immunodeficiency virus" and "HIV" refer, but are not limited to, HIV-1 and HIV-2.

HIV is categorized into multiple clades with a high degree of genetic divergence. As used herein, the term "HIV clade" or "HIV subtype" refers to related human immunodeficiency viruses classified according to their degree of genetic similarity. There are currently three groups of HIV-1 isolates: M, N and O. Group M (major strains) consists of at least ten clades, A through J. Group O (outer strains) can consist of a similar number of clades. Group N is a new HIV-1 isolate that has not been categorized in either group M or 0.

As used herein, the terms "HIV antigenic polypeptide," "HIV antigenic protein," "HIV antigen," and "HIV immunogen" refer to a polypeptide capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against HIV in a subject. The antigenic polypeptide or antigen can be a protein of the HIV, a fragment or epitope thereof, or a combination of multiple HIV proteins or portions thereof that can induce an immune response or produce an immunity, e.g., protective immunity, against the HIV in a subject.

Preferably, an antigenic polypeptide or antigen is capable of raising in a host a protective immune response, e.g., inducing an immune response against a viral disease or infection, and/or producing an immunity in (i.e., vaccinates) a subject against a viral disease or infection, that protects the subject against the viral disease or infection. For example, the antigenic polypeptide or antigen can comprise a protein or fragments thereof from Simian Immunodeficiency Virus (SIV) or an HIV, such as the HIV or SIV envelope gp160 protein, the HIV or SIV matrix/capsid proteins, and the HIV or SIV gag, pol and env gene products.

An HIV antigenic polypeptide or antigen can be any HIV-1 or HIV-2 antigen or fragment thereof. Examples of HIV antigens include, but are not limited to gag, pol, and env gene products, which encode structural proteins and essential enzymes. Gag, pol, and env gene products are synthesized as polyproteins, which are further processed into multiple other protein products. The primary protein product of the gag gene is the viral structural protein gag polyprotein, which is further processed into MA, CA, SP1, NC, SP2, and P6 protein products. The pol gene encodes viral enzymes (Pol, polymerase), and the primary protein product is further processed into RT, RNase H, IN, and PR protein products. The env gene encodes structural proteins, specifically glycoproteins of the virion envelope. The primary protein product of the env gene is gp160, which is further processed into gp120 and gp41. Other examples of HIV antigens include gene regulatory proteins Tat and Rev; accessory proteins Nef, Vpr, Vif and Vpu; capsid proteins, nucleocapsid proteins, and p24 viral protein.

In certain embodiments, the HIV antigenic polypeptide or antigen comprises an HIV Gag, Env, or Pol antigen, or any antigenic portion or epitope or combination thereof, preferably an HIV-1 Gag, Env, or Pol antigen or any antigenic portion or epitope or combination thereof.

HIV antigenic polypeptides can also be mosaic HIV antigens. As used herein, "mosaic antigen" refers to a recombinant protein assembled from fragments of natural sequences. Mosaic antigens resemble natural antigens, but are optimized to maximize the coverage of potential T-cell epitopes found in the natural sequences, which improves the breadth and coverage of the immune response. Mosaic HIV antigens for use with the invention are preferably mosaic Gag, Pol, and/or Env antigens, and more preferably a mosaic HIV-1 Gag, Pol, and/or Env antigens. As used herein, "a mosaic HIV Gag, Pol, and/or Env antigen" specifically refers to a mosaic antigen comprising multiple epitopes derived from one or more of the Gag, Pol and/or Env polyprotein sequences of HIV.

In one embodiment, a mosaic HIV antigen for use with the invention is a mosaic HIV Gag antigen with epitopes derived from the sequences of gag gene products (examples are provided in SEQ ID NOs: 1, 2); a mosaic HIV Pol antigen with epitopes derived from the sequences of pol gene products (examples are provided in SEQ ID NOs: 3, 4); or a mosaic HIV Env antigen with epitopes derived from the sequences of env gene products (examples are provided in SEQ ID NOs: 5, 6; also the synthetic antigens of the invention, e.g. in SEQ ID NOs: 8, 17, 18, 19, can be considered mosaic HIV Env antigens). In certain embodiments, a mosaic HIV antigen for use with the invention comprises a combination of epitopes derived from sequences of gag, pol, and/or env gene products. Illustrative and non-limiting examples include mosaic Env-Pol antigens with epitopes derived from the sequences of env and pol gene products; mosaic Gag-Pol antigens with epitopes derived from the sequences of gag and pol gene products (examples are provided in SEQ ID NOs: 28, 29); and mosaic Gag-Env antigens with epitopes derived from the sequences of gag and env gene products. The sequences of gag, pol, and env gene products can be derived from one or more clades.

Examples of mosaic HIV Gag, Pol and/or Env antigens that can be used in the invention include those described in, e.g., US20120076812; Barouch et al., Nat Med 2010, 16:319-323; and Barouch et al., Cell 155:1-9, 2013, all of which are incorporated herein by reference in their entirety. Preferably, mosaic HIV Gag, Pol, and/or Env antigens for use with the present invention include, but are not limited to, mos1Gag (SEQ ID NO: 1), mos2Gag (SEQ ID NO: 2), mos1Pol (SEQ ID NO: 3), mos2Pol (SEQ ID NO: 4), mos1Env (SEQ ID NO: 5), mos2Env (SEQ ID NO: 6), mos1GagPol (SEQ ID NO: 28), mos2GagPol (SEQ ID NO: 29), and combinations thereof.

As used herein, each of the terms "HIV envelope protein," "env protein," and "Env" refers to a protein that is expressed on the envelope of an HIV virion and enables an HIV to target and attach to the plasma membrane of HIV infected cells, or a fragment or derivative thereof that can induce an immune response or produce an immunity against the HIV in a subject in need thereof. The HIV env gene encodes the precursor protein gp160, which is proteolytically cleaved into the two mature envelope glycoproteins, gp120 and gp41. The cleavage reaction is mediated by a host cell protease, furin, at a sequence highly conserved in retroviral envelope glycoprotein precursors. More specifically, gp160 trimerizes to (gp160).sub.3 and then undergoes cleavage into the two noncovalently associated gp120 and gp41. Viral entry is subsequently mediated by a trimer of gp120/gp41 heterodimers. Gp120 is the receptor binding fragment, and binds to the CD4 receptor on a target cell that has such a receptor, such as, e.g., a T-helper cell. Gp41, which is noncovalently bound to gp120, is the fusion fragment and provides the second step by which HIV enters the cell. Gp41 is originally buried within the viral envelope, but when gp120 binds to a CD4 receptor, gp120 changes its conformation causing gp41 to become exposed, where it can assist in fusion with the host cell. Gp140 is the uncleaved ectodomain of trimeric gp160, i.e., (gp160).sub.3, that has been used as a surrogate for the native state of

the cleaved, viral spike.

According to embodiments of the invention, an "HIV envelope protein" can be a gp160, gp140, gp120, gp41 protein, combinations, fusions, truncations or derivatives thereof. For example, an "HIV envelope protein" can include a gp120 protein noncovalently associated with a gp41 protein. It can also include a stabilized trimeric gp140 protein that can have or can be modified to include a trimerization domain that stabilizes trimers of gp140. Examples of trimerization domains include, but are not limited to, the T4-fibritin "foldon" trimerization domain; the coiled-coil trimerization domain derived from GCN4; and the catalytic subunit of E. coli aspartate transcarbamoylase as a trimer tag. An "HIV envelope protein" can also be a truncated HIV envelope protein including, but not limited to, envelope proteins comprising a C-terminal truncation in the ectodomain (i.e. the domain that extends into the extracellular space), a truncation in the gp41, such as a truncation in the transmembrane domain of gp41, or a truncation in the cytoplasmic domain of gp41. An "HIV envelope protein" can further be a derivative of a naturally occurring HIV envelope protein having sequence mutations, e.g., in the furin cleavage sites, and/or so-called SOSIP mutations.

Preferably, an "HIV envelope protein" is a "synthetic HIV envelope protein." As used herein, the term "synthetic HIV envelope protein" refers to a non-naturally occurring HIV envelope protein that is optimized to induce an immune response or produce an immunity against one or more naturally occurring HIV strains in a subject in need thereof. Mosaic HIV Env proteins are examples of synthetic HIV Env proteins, and the invention provides synthetic HIV Env antigens, e.g. the ones comprising SEQ ID NOs: 8, 17, 18, or 19.

As used herein, "TCID.sub.50" refers to Tissue Culture Infectious Dose 50 given as TCID.sub.50. The TCID.sub.50 can be determined using various methods known to the skilled person such as for example a Tissue Culture Infectious Dose 50 (TCID.sub.50) assay. The TCID.sub.50 assay is a method for titrating the infectivity of Modified Vaccinia virus Ankara (MVA) vectors, using 10-fold dilutions in a 96-well format as described in Example 2 of WO 03/053463. The infectivity of a poxvirus such as MVA can be determined using various methods known to the skilled person such as for example by a Flow Cytometry based assay or a Tissue Culture Infectious Dose .sub.50 (TCID.sub.50) assay. In one exemplary aspect, a titration of MVA is performed in a TCID.sub.50-based assay using 10-fold dilutions in a 96-well format. At the endpoint of the assay, infected cells are visualized using an anti-vaccinia virus antibody and an appropriate staining solution. Primary CEF cells are prepared and cultivated in RPMI including 10% serum and 1% Gentamycin using T-flasks for 2-3 days at a given density following trypsinization and seeding into 96-well plates at a density of 1.times.10.sup.5cells/mL using RPMI with 7% serum. The expected titer of the sample dictates the number of 10-fold serial dilutions, which are performed across a deep-well plate from column 1 to e.g. 10 using 100 .mu.L for transfer into the next well. Following dilution, 100 .mu.L are seeded per well of 96-well plates. Cells are incubated for 5 days at 34-38.degree. C. and 4-6% CO.sub.2 to allow infection and viral replication.

Five days post infection, cells are stained with an MVA specific antibody. For the detection of the specific antibody, a horseradish peroxidase (HRP) coupled secondary antibody is used. The MVA specific antibody can be an anti-vaccinia virus antibody, rabbit polyclonal, or an IgG fraction (Quartett, Berlin, Germany #9503-2057), for example. The secondary antibody can be anti-rabbit IgG antibody, or HRP coupled goat polyclonal (Promega, Mannheim, Germany, #W4011), for example. The secondary antibody is visualized using a precipitating TMB substrate. Every well with cells that are positive in the color reaction are marked as positive for the calculation of the TCID.sub.50. The titer is calculated by using the Spearman-Kaerber method of calculation. The data can also be represented as a log of virus titer which is the relative difference for any given time-point from T=0 time-point.

An alternative method for quantification of virus concentration is by viral plaque assay, which is a standard method well known to the skilled person to determine virus concentration in terms of infectious dose. Briefly, a confluent monolayer of host cells is infected with virus at various dilutions and covered with a semi-solid medium. A viral plaque is formed when a virus infects a cell in the cell monolayer and the number of plaques can be counted in combination with the dilution factor to calculate the number of plaque forming units per sample volume (pfu/mL). The pfu/mL represents the number of infective particles within the sample. Due to distinct differences in assay methods and principles, TCID.sub.50 and pfu/mL or other infectivity assay results are not necessarily equivalent. For MVA, both methods (TCID.sub.50 and viral plaque assay) can be used, and generally the dosage of an MVA vector for clinical administration to humans is provided in pfu, or in TCID.sub.50. The dosage of an adenovirus vector can also be given in pfu or TCID.sub.50. For administration to humans, generally the dosage of an adenovirus vector is given in viral particles (vp), and concentrations are expressed in vp/mL.

Synthetic HIV Envelope Proteins and Coding Sequences Thereof

Embodiments of the invention relate to novel poxvirus vectors, preferably MVA vectors, comprising nucleic acid sequence encoding synthetic HIV envelope proteins and preferably comprising nucleic acid sequence encoding further HIV antigens.

In one embodiment, a synthetic HIV envelope protein comprises the amino acid sequence of SEQ ID NO: 8, or SEQ ID NO:8 having one or more mutations selected from the group consisting of (i) I529P, (ii) K480E, and (iii) a combination of EK479-480RRRR, I529P, A471C and T575C. SEQ ID NO: 8 comprises a synthetic mature gp120 and a synthetic truncated gp41 without the transmembrane region, nor the cytoplasmic domain. SEQ ID NO: 8 is a non-naturally occurring sequence comprised of a chimera of sequences from the mos2Env mosaic antigen (SEQ ID NO: 6), and other HIV envelope protein sequences. The sequence of the synthetic Env antigen comprising SEQ ID NO: 8 is optimized to provide broad coverage and an enhanced T-cell response against HIV clade C (as compared to the mos2Env antigen (SEQ ID NO: 6)). In certain embodiments, further amino acids can be added to SEQ ID NO: 8 or one of its variants defined herein.

In certain embodiments, the synthetic HIV envelope protein further comprises a signal sequence. The synthetic HIV envelope protein is synthesized with a signal sequence that is cleaved from the nascent polypeptide chain during its transport into the lumen of the endoplasmic reticulum (ER). In principle, any known signal sequence could be used. Preferably an HIV Env signal sequence or a variant thereof is used. Different signal sequences have been used in the art for HIV Env proteins (see e.g. WO 2014/107744). In certain embodiments, the signal sequence comprises SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12. In one preferred embodiment, the signal sequence comprises SEQ ID NO: 9.

In certain embodiments, the synthetic HIV envelope protein further comprises a transmembrane domain. The transmembrane domain anchors the synthetic HIV envelope protein to the ER membrane, and contributes to membrane assembly and function of the HIV envelope. Preferably, the transmembrane domain comprises SEQ ID NO: 13.

In another embodiment, the synthetic HIV envelope protein comprises a gp41 having a truncated cytoplasmic domain. The gp41 has an unusually long cytoplasmic domain at its carboxyl end, typically about 150 amino acids (Edwards et al., J. Virology, 2002, 76:2683-2691). Truncation of the cytoplasmic domain was reported to induce exposure of conserved regions in the ectodomain of HIV-1 Env protein (Id.). The truncated cytoplasmic domain in a synthetic HIV envelope of the invention can range from one to about 140 amino acids, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, or 140 amino acids of a full-length cytoplasmic domain. In certain embodiments the truncated cytoplasmic domain is derived from amino acids 704-862 of SEQ ID NO: 17 (i.e. from the cytoplasmic domain of the C4 molecule of the invention), by truncation after a given amino acid up to the C-terminus. In a preferred embodiment, the synthetic HIV envelope protein comprises a truncated cytoplasmic domain having 1 to 10 amino acids residues, more preferably 4 to 8 amino acid residues, and most preferably 7 amino acid residues of an HIV gp41 cytoplasmic domain. The cytoplasmic domain or fragment thereof of a synthetic HIV envelope protein is located C-terminal to the extracellular domain (ectodomain), and when the synthetic HIV envelope protein also comprises a transmembrane domain, the cytoplasmic domain or fragment thereof is located C-terminal to the transmembrane domain. See, e.g., FIGS. 1A and 1C. In a particular embodiment, the synthetic HIV envelope protein comprises a gp41 with a truncated cytoplasmic domain having the amino acid sequence of SEQ ID NO: 14 or a fragment thereof, such as residues 1-4 thereof (i.e. NRVR). Other truncated cytoplasmic domains have been described and could be used (e.g. Schiernle et al., PNAS 1997; Abrahamyan et al., J Virol 2005).

In embodiments wherein the synthetic HIV envelope protein further comprises a transmembrane domain and a fragment of a cytoplasmic domain, it is preferred that the protein also comprises the amino acid sequence of SEQ ID NO: 37, which contains residues 655-682 of SEQ ID NO: 18, wherein the amino acid sequence of SEQ ID NO: 37 is fused to the C-terminus of SEQ ID NO: 8 and the N-terminus of the transmembrane domain.

In a particularly preferred embodiment of the invention, the synthetic HIV envelope protein further comprises a transmembrane domain, such as that having the amino acid sequence of SEQ ID NO: 13, and a truncated cytoplasmic domain or a fragment of a cytoplasmic domain, such as that having the amino acid sequence of SEQ ID NO: 14 or residues 1-4 of SEQ ID NO: 14 (i.e., NRVR). Most preferably, the synthetic HIV envelope protein comprises or consists of the amino acid sequence of SEQ ID NO: 18, with or without the signal sequence (i.e., amino acid resides 1-29 of SEQ ID NO: 18).

In another embodiment, the synthetic HIV envelope protein comprises a trimerization domain that replaces an Env transmembrane region. The trimerization domain increases the stability of an Env trimeric structure. Preferably, the synthetic HIV envelope protein comprises a gp140 polypeptide that is modified to include a trimerization domain that stabilizes trimers of gp140. Examples of trimerization domains include, but are not limited to, the T4-fibritin "foldon" trimerization domain, such as that comprising the amino acid sequence of SEQ ID NO: 16; the coiled-coil trimerization domain derived from GCN4, such as that comprising the amino acid sequence of SEQ

ID NO: 15; the catalytic subunit of E. coli aspartate transcarbamoylase as a trimer tag; or matrillin-based trimerization motifs. If present, the trimerization domain typically is located C-terminal to the extracellular domain (see FIG. 1B). In certain preferred embodiments where the synthetic HIV envelope protein comprises a trimerization domain, the synthetic HIV envelope protein comprises the amino acid sequence of SEQ ID NO: 19, with or without the signal sequence (i.e., amino acid residues 1-29 of SEQ ID NO: 19). These embodiments with trimerization domains are mainly useful for soluble ectodomain variants of the synthetic HIV envelope protein. In certain embodiments of such soluble variants of the invention, it is possible to mutate the furin cleavage site (e.g. mutation of Lys to Glu at position 480 in SEQ ID NO: 8) to inactivate this cleavage site, so that the protein will be a single chain; this combines well with a trimerization domain, especially with the GCN4 trimerization domain of SEQ ID NO: 19.

Alternative versions of such soluble ectodomain variants of the synthetic HIV envelope protein without use of trimerization domains are also embodiments of the invention, and can be prepared from SEQ ID NO: 8 by combining mutations that optimize the furin cleavage site (e.g., replacing the Gly-Lys dipeptide at positions 479-480 by four Arg residues) as well as so-called SOSIP mutations (e.g., I to P mutation at position 529, and introduction of a disulfide bridge between positions 471 and 575 by replacement of the respective Ala and Thr at those positions in SEQ ID NO: 8 each with a Cys residue). This yields a protein having the amino acid sequence of SEQ ID NO: 8 with the following combination of mutations: EK479-480RRRR, I529P, A471C and T575C.

One possible modification to further increase the trimer content of a synthetic HIV envelope protein of the invention (comprising SEQ ID NO: 8), is modification of Ile to Pro at position 529. This can be effective for both soluble and membrane-bound variants.

Vectors

In one general aspect, the invention relates to vectors comprising nucleic acid sequence encoding a synthetic HIV envelope protein, and preferably comprising nucleic acid sequence encoding at least one additional HIV antigen. According to embodiments of the invention, the vectors can comprise any of the synthetic HIV envelope proteins described herein. In a particular embodiment of the invention, the vector is a poxvirus vector, preferably an MVA vector, comprising nucleic acid sequence encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 17, SEQ ID NO: 18, or SEQ ID NO: 19, and more preferably SEQ ID NO: 18 or amino acid residues 30-711 of SEQ ID NO: 18.

According to embodiments of the invention, the nucleic acid sequence encoding the synthetic HIV envelope protein is operably linked to a promoter, meaning that the nucleic acid is under the control of a promoter. The promoter can be a homologous promoter (i.e., derived from the same genetic source as the vector) or a heterologous promoter (i.e., derived from a different vector or genetic source). Non-limiting examples of suitable promoters for the adenoviral vectors include the cytomegalovirus (CMV) promoter and the Rous Sarcoma virus (RSV) promoter. Preferably, the promoter is located upstream of the nucleic acid within an expression cassette. An exemplary CMV promoter sequence that can be operably linked to nucleic acid sequence encoding the synthetic HIV envelope protein is shown in SEQ ID NO: 24.

Non-limiting examples of suitable promoters for the poxvirus vectors include the 30K promoter, the 13 promoter, the PrS promoter, the PrS5E promoter, the Pr7.5K, the Pr13.5 long promoter, the PrHyb promoter, the 40K promoter, the MVA-40K promoter, the FPV 40K promoter, 30k promoter, the PrSynIIm promoter, and the PrLE1 promoter. Additional promoters are further described in WO 2010/060632, WO 2010/102822, WO 2013/189611 and WO 2014/063832, which are incorporated fully by reference herein. In more preferred embodiments, the HIV antigens, when incorporated as part of a poxvirus vector according to the invention, are operably linked to the Pr13.5long promoter (SEQ ID NO: 42) and/or the PrHyb promoter (SEQ ID NO: 43).

According to embodiments of the invention, a vector can be an expression vector. Expression vectors include, but are not limited to, vectors for recombinant protein expression and vectors for delivery of nucleic acid into a subject for expression in a tissue of the subject, such as a viral vector. Examples of viral vectors suitable for use with the invention include, but are not limited to adenoviral vectors, adeno-associated virus vectors, poxvirus vectors, MVA vectors, enteric virus vectors, Venezuelan Equine Encephalitis virus vectors, Semliki Forest Virus vectors, Tobacco Mosaic Virus vectors, lentiviral vectors, etc. The vector can also be a non-viral vector. Examples of non-viral vectors include, but are not limited to plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages, etc.

In certain embodiments of the invention, the vector is an adenovirus vector. 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 adenovirus, such as a chimpanzee adenovirus or a gorilla adenovirus). Preferably, the adenovirus is a human adenovirus (HAdV, or AdHu), or a simian adenovirus such as chimpanzee or gorilla adenovirus (ChAd, AdCh, or SAdV), or a rhesus monkey adenovirus (RhAd). In the invention, a human adenovirus is meant if referred to as Ad without indication of species, e.g. the brief notation "Ad26" means the same as HAdV26, which is human adenovirus serotype 26. Also as used herein, the notation "rAd" means recombinant adenovirus, e.g., "rAd26" refers to recombinant human adenovirus 26.

Most advanced studies have been performed using human adenoviruses, and human adenoviruses are preferred according to certain aspects of the invention. In certain preferred embodiments, a 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, 50, 52, etc. According to a particularly preferred embodiment of the invention, an adenovirus is a human adenovirus of serotype 26. Advantages of these serotypes include a low seroprevalence and/or low pre-existing neutralizing antibody titers in the human population, and experience with use in human subjects in clinical trials.

Simian adenoviruses generally also have a low seroprevalence and/or low pre-existing neutralizing antibody titers in the human population, and a significant amount of work has been reported using chimpanzee adenovirus vectors (e.g. U.S. Pat. No. 6,083,716; 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: 849-62; and review by Lasaro and Ertl, 2009, Mol Ther 17: 1333-39). Hence, in other 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.

Preferably, the adenovirus vector is a replication deficient recombinant viral vector, such as rAd26, rAd35, rAd48, rAd5HVR48, etc.

In a preferred embodiment of the invention, the adenoviral vectors comprise capsid proteins from rare serotypes including Ad26. In the typical embodiment, the vector is an rAd26 virus. An "adenovirus capsid protein" refers to a protein on the capsid of an adenovirus (e.g., Ad26, Ad35, rAd48, rAd5HVR48 vectors) 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 "capsid protein" for a particular adenovirus, such as an "Ad26 capsid protein" can be, for example, a chimeric capsid protein that includes at least a part of an Ad26 capsid protein. In certain embodiments, the capsid protein is an entire capsid protein of Ad26. In certain embodiments, the hexon, penton and fiber are of Ad26.

One of ordinary skill in the art 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 a first serotype 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 Ad26 (i.e., the vector is rAd26). In some embodiments, the adenovirus is replication deficient, e.g., because it contains a deletion in the E1 region of the genome. For adenoviruses being derived from non-group C adenovirus, such as 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 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). However, such adenoviruses will not be capable of replicating in non-complementing cells that do not express the E1 genes of Ad5.

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

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-stranded or single-stranded.

The adenovirus vectors useful in the invention are typically replication deficient. In these embodiments, the virus is rendered replication deficient 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 a gene of interest, such as a gene encoding a synthetic HIV envelope protein (usually linked to a promoter), or a gene encoding an HIV antigenic polypeptide (usually linked to a promoter) within the region. In some embodiments, the vectors of the invention can contain deletions in other regions, such as the E2, E3 or E4 regions, or insertions of heterologous genes linked to a promoter within one or more of these regions. 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.

A packaging cell line is typically used to produce sufficient amounts of adenovirus vectors for use in the invention. A packaging cell is a cell that comprises those genes that have been deleted or inactivated in a replication deficient vector, thus allowing the virus to replicate in the cell. Suitable packaging cell lines for adenoviruses with a deletion in the E1 region include, for example, PER.C6, 911, 293, and E1 A549.

In a preferred embodiment of the invention, the vector is an adenovirus vector, and more preferably a rAd26 vector, most preferably a rAd26 vector with at least a deletion in the E1 region of the adenoviral genome, e.g. such as that described in Abbink, J Virol, 2007. 81(9): p. 4654-63, which is incorporated herein by reference. Typically, the nucleic acid sequence encoding the synthetic HIV envelope protein and/or other HIV antigens is cloned into the E1 and/or the E3 region of the adenoviral genome.

In a preferred aspect of the invention, the vector encoding the synthetic HIV antigen described herein is a poxvirus vector. In a particularly preferred aspect, the vector is a Modified Vaccinia virus Ankara (MVA) vector. In additional preferred embodiments, the MVA virus vector is MVA-BN or derivatives thereof.

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. 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 (CEF).

However, due to the often severe post-vaccination complications associated with vaccinia viruses, there were several attempts to generate a more attenuated, safer vaccine. 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. Strains of MVA having enhanced safety profiles for the development of safer products, such as vaccines or pharmaceuticals, have been developed, for example by Bavarian Nordic. MVA was further passaged by Bavarian Nordic and is designated MVA-BN. A representative sample of MVA-BN was deposited on Aug. 30, 2000 at the European Collection of Cell Cultures (ECACC) under Accession No. V00083008. MVA-BN is further described in WO 02/42480 (see also e.g., U.S. Pat. Nos. 6,761,893 and 6,913,752 and US 2003/0206926) and WO 03/048184 (US 2006/0159699), which are incorporated by reference herein in their entireties. 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.

In various embodiments, the MVA or MVA used for generating the recombinants suitable for the present invention are MVA-572, MVA-575, MVA-1721, MVA as deposited as ATCC.RTM. VR-1508.TM., MVA as deposited as ATCC.RTM. VR-1566.TM., ACAM3000 MVA, MVA-BN or any similarly attenuated MVA strain. In preferred embodiments, the MVA used for generating the recombinants are MVA-575, MVA as deposited as ATCC.RTM. VR-1508.TM., MVA as deposited as ATCC.RTM. VR-1566.TM., ACAM3000 MVA and MVA-BN. More preferably the MVA used for generating the recombinants is MVA-BN.

MVA-572 was deposited at the European Collection of Animal Cell Cultures (ECACC, Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom) with the deposition number ECACC V94012707 on Jan. 27, 1994. MVA-575 was deposited under ECACC V00120707 on Dec. 7, 2000. Acam3000 MVA was deposited at the American Type Culture Collection (ATCC) under Accession No.: PTA-5095 on Mar. 27, 2003 (American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110-2209, USA). MVA-1721 was deposited as CNCM 1721 at the Collection Nationale de Cultures de Microorganisms, Institute Pasteur. MVA-BN was deposited on Aug. 30, 2000 at the ECACC under number V00083008. MVA-BN has been described in WO 02/042480.

Also encompassed by the invention are derivatives or variants of any of the MVA viruses or MVA-BN described herein. "Derivatives" or "variants" of MVA or MVA-BN refer to MVA or MVA-BN viruses exhibiting essentially the same replication characteristics as the MVA or MVA-BN to which it refers, but exhibiting differences in one or more parts of their genomes. Viruses having the same "replication characteristics" as the deposited virus are viruses that replicate with similar amplification ratios as the deposited strain in CEF cells and the cell lines HaCat (Boukamp et al. (1988), J Cell Biol 106: 761-771), the human bone osteosarcoma cell line 143B (ECACC No. 91112502), the human embryo kidney cell line 293 (ECACC No. 85120602), and the human cervix adenocarcinoma cell line HeLa (ATCC No. CCL-2). Tests and assay to determine these properties of MVA, its derivatives and variants are well known to the skilled person, such as the cell line permissivity assay as described in WO 02/42480. In an exemplary cell line permissivity assay, mammalian cell lines are infected with the parental and derivative or variant MVA virus at a low multiplicity of infection per cell, i.e., 0.05 infectious units per cell (5.times.10.sup.4 TCID.sub.50). Following absorption of 1 hour the virus inoculum is removed and the cells washed three times to remove any remaining unabsorbed viruses. Fresh medium supplemented with 3% FCS is added and infections are left for a total of 4 days (at 37.degree. C., 5% CO.sub.2) where viral extracts can be prepared. The infections are stopped by freezing the plates at -80.degree. C. for three times. Virus multiplication and cytopathic effects (CPE) are subsequently determined on CEF cells using methods well known to the skilled person such as those described in Carroll and Moss (1997), Virology 238, 198-211.

More specifically, MVA-BN or a derivative or variant of MVA-BN preferably has 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 assays for these properties of MVA variants are described in WO 02/42480 or in the exemplary cell line permissivity assay as described above.

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. Pat. No. 6,761,893.

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. Pat. 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 recombinant poxvirus vectors 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 poxviruses disclosed herein, different methods are 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 poxvirus 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 poxviral 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 poxvirus. Recombination between homologous poxviral DNA in the plasmid and the viral genome, respectively, can generate a poxvirus 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, such as one or more of the HIV antigen encoding nucleic acids provided in the present disclosure; 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, .beta.-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 this case, this gene shall be introduced into a different insertion site of the poxviral genome, and 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 homologous recombination in E. coli or another bacterial species between a poxvirus genome 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.

One or more nucleic acid sequences encoding at least one HIV antigen according to embodiments of the invention can be inserted into any suitable part of the poxvirus or poxviral vector. In a preferred aspect, the poxvirus used for the present invention includes an MVA virus. Suitable parts of the MVA virus into which one or more nucleic acids of the present disclosure can be inserted include non-essential parts of the MVA virus.

For MVA virus, non-essential parts of the MVA genome can be intergenic regions or the known deletion sites 1-6 of the MVA genome. Alternatively or additionally, non-essential parts of the recombinant MVA can be a coding region of the MVA genome which is non-essential for viral growth. However, the insertion sites are not restricted to these preferred insertion sites in the MVA genome, since it is within the scope of the present invention that the antigens and nucleic acids and any accompanying promoters as described herein can be inserted anywhere in the viral genome as long as it is possible to obtain recombinants that can be amplified and propagated in at least one cell culture system, such as Chicken Embryo Fibroblasts (CEF cells).

Preferably, the nucleic acids of the present invention are inserted into one or more intergenic regions (IGR) of the MVA. The terms "intergenic region" and "IGR" refer preferably to those parts of the viral genome located between two adjacent open reading frames (ORF) of the MVA genome, preferably between two essential ORFs of the MVA virus genome. For MVA, in certain embodiments, the IGR is selected from IGR 07/08, IGR 44/45, IGR 64/65, IGR 88/89, IGR 136/137, and IGR 148/149. In more preferred embodiments, the nucleic acids of the present invention are inserted in the IGR 44/45 and IGR 88/89 regions.

According to embodiments of the invention, and as noted above, any of the synthetic HIV envelope proteins and/or HIV antigens described herein can be expressed in the vectors of the invention. In view of the degeneracy of the genetic code, the skilled person is well aware that several nucleic acid sequences can be designed that encode the same protein, according to methods entirely routine in the art. The nucleic acid encoding the synthetic HIV envelope protein and/or HIV antigens can optionally be codon-optimized to ensure proper expression in the treated host (e.g., human). Codon-optimization is a technology widely applied in the art. Some non-limiting examples of sequences encoding a synthetic HIV envelope protein of the invention are provided in SEQ ID NOs: 26 (used in adenovirus vectors in the examples), and 41 (used in MVA vectors in the examples); and some non-limiting examples of sequences encoding further HIV antigens for use in the invention are provided in SEQ ID NOs: 20-22 (used in adenovirus vectors in the examples), and 38-40 (used in MVA vectors in the examples).

The invention also provides cells, preferably isolated cells, comprising any of the vectors described herein. The cells can be used for recombinant protein production, or for the production of viral particles.

Also disclosed is a method of making a synthetic HIV antigenic polypeptide. The method comprises transfecting a host cell with an expression vector comprising nucleic acid encoding the synthetic HIV antigenic polypeptide operably linked to a promoter, growing the transfected cell under conditions suitable for expression of the synthetic HIV antigenic polypeptide, and isolating the synthetic HIV antigenic polypeptide from the cell. The synthetic HIV antigenic polypeptide can be isolated or collected from the cell by any method known in the art including affinity chromatography, etc. Techniques used for recombinant protein expression are well known to one of ordinary skill in the art in view of the present disclosure.

The invention also relates to a method for manufacturing a vector encoding a synthetic HIV antigenic polypeptide of the invention, the method comprising culturing a cell that comprises the vector, to propagate and multiply the vector during said culturing, and isolating the vector that encodes the synthetic HIV antigenic polypeptide of the invention from the cell culture, e.g. from the cells, from the culture medium, or both. The vector can be further purified according to methods known in the art.

In certain embodiments, the invention provides a poxvirus vector, preferably an MVA vector, such as an MVA-BN vector, comprising a nucleic acid sequence encoding a synthetic HIV antigen. The poxvirus vector comprises nucleic acid sequence encoding a synthetic HIV envelope (Env) antigen according to the invention, such as that comprising the amino acid sequence of SEQ ID NO: 18, and optionally further comprises nucleic acid sequence encoding at least one additional HIV antigen. In preferred embodiments, the poxvirus vector and more preferably a MVA vector, comprises nucleic acid sequence encoding a first HIV Env antigen which is a synthetic HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 18, and at least one additional HIV antigen, such as Gag, Pol, and/or Env antigens, preferably one or more additional HIV antigens comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 28, and 29.

For example, in a particular embodiment, a poxvirus vector can comprise nucleic acid encoding a first HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 18; a second HIV Env antigen different than the first HIV Env antigen; a third antigen and a fourth antigen, being two different HIV Gag antigens; and a fifth antigen and a sixth antigen, being two different HIV Pol antigens. The Gag and Pol antigens can be fused into a first Gag-Pol fusion antigen and a second Gag-Pol fusion antigen, such as those Gag-Pol fusion antigens comprising the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

In certain exemplary embodiments, the poxvirus vector comprises nucleic acid encoding one or more amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29. In one or more specific embodiments, the poxvirus vector comprises nucleic acid encoding the amino acid sequences of SEQ ID NOs: 1-5, and 18, more preferably SEQ ID NOs: 5, 18, 28, and 29.

In other certain exemplary embodiments a vector is an adenovirus vector comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 25, 26 and 27, preferably SEQ ID NO: 26. Further exemplary embodiments include adenovirus vectors encoding other HIV antigens, such adenovirus vectors comprising one or more nucleic acid sequences selected from the group consisting of SEQ ID NOs: 20, 21, and 22. In other certain embodiments, the vector is a poxvirus vector, preferably an MVA vector, more preferably MVA-BN, the vector comprising one or more nucleic acid sequences selected from the group consisting of SEQ ID NOs: 38, 39, 40, and 41. In one or more specific embodiments the poxvirus vector comprises SEQ ID NOs: 38, 39, 40, and 41.

The nucleic acid sequence encoding the one more HIV antigens can be inserted into any appropriate insertion site of the poxvirus vector genome as described herein. In particular embodiments wherein the poxvirus vector is an MVA vector, such as MVA-BN or derivatives thereof, encoding one or more Gag-Pol fusion antigens, nucleic acid sequence encoding a first Gag-Pol fusion antigen can be inserted into intergenic region (IGR) 44/45 of the MVA genome and nucleic acid sequence encoding a second Gag-Pol fusion antigen can be inserted into IGR 88/89 of the MVA genome. Additionally, nucleic acid sequence encoding HIV Env antigens can be inserted into the IGR 44/45 and/or IGR 88/89 of the MVA genome. In one or more specific embodiments, the poxvirus vector comprises nucleic acid sequence encoding a Gag-Pol fusion antigen comprising SEQ ID NO: 28 and nucleic acid sequence encoding an HIV Env antigen comprising SEQ ID NO: 5 into IGR 44/45 of the MVA genome and/or nucleic acid sequence encoding a Gag-Pol fusion antigen comprising SEQ ID NO: 29 and nucleic acid sequence encoding an HIV Env antigen comprising SEQ ID NO: 18 into IGR 88/89 of the MVA genome. Preferably, the Gag-Pol fusion antigens are each under control of a separate promoter, preferably a Pr13.5 promoter, such as that shown in SEQ ID NO: 42 and/or the Env antigens are each under control of a separate promoter, preferably a PrHyb promoter, such as that shown in SEQ ID NO: 43.

Compositions

In another general aspect, the invention relates to a composition comprising a vector comprising a nucleic acid encoding a synthetic HIV envelope protein and a carrier. Preferably, the composition is a vaccine composition, which is described in greater detail below. According to embodiments of the invention, any of vectors described herein can be included in the composition. Preferably, the vector is a viral vector, more preferably an adenovirus vector or a poxvirus vector, and even more preferably an adenovirus 26 vector or an MVA vector.

In one embodiment, a composition of the invention comprises a poxvirus vector, preferably an MVA vector, comprising nucleic acid sequence encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 18, or SEQ ID NO: 19, and more preferably the amino acid sequence of SEQ ID NO: 18. In certain preferred embodiments, the vector is an MVA-BN vector, or derivative thereof. In one or more specific embodiments, a composition of the invention comprises a poxvirus vector, MVA vector, or MVA-BN vector comprising nucleic acid encoding at least a first HIV envelope antigen comprising the amino acid sequence of SEQ ID NO: 18. Most preferably, such vector further comprises nucleic acid sequence encoding: (b) a second HIV Env antigen different from the first HIV Env antigen; (c) a third antigen and a fourth antigen, being two different HIV Gag antigens; and (d) a fifth antigen and a sixth antigen, being two different HIV Pol antigens. In preferred embodiments, the second HIV Env antigen comprises the amino acid sequence of SEQ ID NO: 5, the third and fourth antigens comprise the amino acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2, respectively; and the fifth and the sixth antigens comprise the amino acid sequence of SEQ ID NO: 3 and SEQ ID NO: 4, respectively. In certain embodiments, the third and the fifth antigens are fused into a first Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 28, and the fourth and the sixth antigens are fused into a second Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 29.

According to embodiments of the invention, a composition comprising a poxvirus vector according to the invention can be used together with one or more additional vectors encoding one or more additional HIV antigens, and/or one or more isolated HIV antigenic polypeptides. The additional vectors and/or HIV antigenic polypeptides can be present in the same composition or in one or more different compositions. Preferably, the one or more additional vectors are viral vectors, such as adenovirus vectors, more preferably adenovirus 26 vectors, or poxvirus vectors, more preferably MVA vectors. The one or more additional vectors can encode any HIV antigen known to those skilled in the art in view of the present disclosure. Most preferably, the one or more additional vectors are adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29.

In one aspect, the invention provides a combination vaccine comprising one or more vectors together comprising nucleic acid sequences encoding (a) a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (b) a second HIV envelope protein, preferably comprising the amino acid sequence of SEQ ID NO: 5; (c) a third antigen and a fourth antigen, being two different HIV Gag antigens, preferably comprising the amino acid sequences of SEQ ID NOs: 1 and 2, respectively; and (d) a fifth antigen and a sixth antigen, being two different HIV Pol antigens, preferably comprising the amino acid sequences of SEQ ID NOs: 3 and 4, respectively. In one or more specific embodiments, the third and fifth antigens are fused into a first Gag-Pol fusion antigen, preferably comprising the amino acid sequence of SEQ ID NO: 28 and the fourth and sixth antigens are fused into a second Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 29. The vectors can each be in separate compositions, or they can be combined in a single composition. The multiple nucleic acids in the vector(s) are intended to be administered to one subject, which will result in an immune response to HIV that is broader than the immune response that would be obtained upon administration of either vector alone. The multiple nucleic acid sequences could also be present on one single vector.

According to embodiments of the invention, the one or more vectors can be adenovirus vectors, preferably adenovirus 26 vectors, and/or poxvirus vectors, preferably MVA vectors. The compositions comprising adenovirus and/or poxvirus vectors can optionally further comprise one or more isolated HIV antigenic polypeptides. Any isolated HIV antigenic polypeptide can be used in the compositions of the invention in view of the present disclosure. In certain preferred embodiments, the one or more isolated HIV antigenic polypeptides comprises a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, a polypeptide comprising residues 30-724 of SEQ ID NO: 36, or a combination thereof.

In one or more specific embodiments, a combination comprises an adenovirus vector, preferably an adenovirus 26 vector, comprising nucleic acid encoding one or more HIV antigens preferably selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29 and a poxvirus vector, preferably a MVA vector, comprising nucleic acid encoding one or more HIV antigens preferably selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29. Preferably, one or more adenovirus vectors,

preferably adenovirus 26 vectors together encode SEQ ID NOs: 1-5 and 18; and the poxvirus vector, preferably MVA vector, encodes SEQ ID NOs: 1-5 and 18. The vectors can be present in one composition, or in one or more different compositions.

According to certain embodiments of the invention, a composition, such as a vaccine composition, comprises an immunogenically effective amount of a vector, such as a viral vector. As used herein, "an immunogenically effective amount" or "immunologically effective amount" means an amount of a composition sufficient to induce a desired immune effect or immune response in a subject in need thereof. In one embodiment, an immunogenically effective amount means an amount sufficient to induce an immune response in a subject in need thereof. In another embodiment, an immunogenically effective amount means an amount sufficient to produce immunity in a subject in need thereof, e.g., provide a protective effect against a disease such as a viral infection. An immunogenically effective amount can vary depending upon a variety of factors, such as the physical condition of the subject, age, weight, health, etc.; the particular application, whether inducing immune response or providing protective immunity; the specific recombinant vector administered; the immunogen or antigenic polypeptide encoded by the recombinant vector administered; the specific antigenic polypeptide administered; and the particular disease, e.g., viral infection, for which immunity is desired. An immunogenically effective amount can readily be determined by one of ordinary skill in the art in view of the present disclosure.

As general guidance, an immunogenically effective amount when used with reference to a recombinant viral vector such as an adenoviral vector can be for instance about 10.sup.8 viral particles to about 10.sup.12 viral particles, for example 10.sup.8, 10.sup.9, 10.sup.10, 10.sup.11, or 10.sup.12 viral particles. A single dose of adenoviral vectors for administration to humans in certain embodiments is between 10' and 10.sup.11 viral particles. An immunogenically effective amount when used with reference to a recombinant viral vector such as a poxviral vector can be for instance about 10.sup.4 to 10.sup.11 TCID.sub.50, 10.sup.5 to 10.sup.10 TCID.sub.50, 10.sup.6 to 10.sup.9 TCID.sub.50, or 10.sup.7 to 10.sup.8 TCID.sub.50, such as 10.sup.4, 10.sup.5, 10.sup.6, 10.sup.7, 10.sup.8, 10.sup.9, 10.sup.10, or 10.sup.11 TCID.sub.50. A preferred dose for the subjects (preferably a human) comprises 10.sup.5 to 10.sup.10 TCID.sub.50, including a dose of 10.sup.5 TCID.sub.50, 10.sup.6 TCID.sub.50, 10.sup.7 TCID.sub.50, 10.sup.8 TCID.sub.50, 10.sup.9 TCID.sub.50, or 10.sup.10 TCID.sub.50. The immunogenically effective amount of a poxviral vector such as an MVA vector can alternatively and conveniently be expressed in plaque forming units (pfu), and can for instance be about 10.sup.5 to about 10.sup.11 pfu, e.g. about 10.sup.5, 10.sup.6, 10.sup.7, 10.sup.8, 10.sup.9, 10.sup.10 or 10.sup.11 pfu, preferably about 10.sup.7 to 10.sup.9 pfu, and more preferably about 10.sup.8 pfu, such as for instance about 0.5.times.10.sup.8, 1.times.10.sup.8, 2.times.10.sup.8, 3.times.10.sup.8, 4.times.10.sup.8, or 5.times.10.sup.8 pfu. In certain embodiments, the immunogenically effective amount of an MVA vector according to the invention administered to a human subject is about 1.times.10.sup.7 to 1.times.10.sup.9 pfu, preferably about 1.times.10.sup.8 pfu, preferably in a volume of 0.1 mL to 1 mL, e.g. 0.5 mL.

An immunogenically effective amount of a vector, such as an MVA vector and/or adenovirus vector, can be administered in a single composition, or in multiple compositions, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 compositions (e.g., tablets, capsules or injectables), wherein the administration of the multiple capsules or injections collectively provides a subject with the immunogenically effective amount. It is also possible to administer an immunogenically effective amount to a subject, and subsequently administer another dose of an immunogenically effective amount to the same subject, in a so-called prime-boost regimen. Further booster administrations can optionally be added to the regimen, as needed. This general concept of a prime-boost regimen is well known to the skill person in the vaccine field and is described in greater detail below.

Compositions of the invention may further comprise a carrier. A carrier can include one or more pharmaceutically acceptable excipients such as binders, disintegrants, swelling agents, suspending agents, emulsifying agents, wetting agents, lubricants, flavorants, sweeteners, preservatives, dyes, solubilizers and coatings. The precise nature of the carrier or other material can depend on the route of administration, e.g., intramuscular, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal or intraperitoneal routes. For liquid injectable preparations, for example, suspensions and solutions, suitable carriers and additives include water, glycols, oils, alcohols, preservatives, coloring agents and the like. For solid oral preparations, for example, powders, capsules, caplets, gelcaps and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. For nasal sprays/inhalant mixtures, the aqueous solution/suspension can comprise water, glycols, oils, emollients, stabilizers, wetting agents, preservatives, aromatics, flavors, and the like as suitable carriers and additives.

Compositions of the invention can be formulated in any matter suitable for administration to a subject to facilitate administration and improve efficacy, including, but not limited to, oral (enteral) administration and parenteral injections. The parenteral injections include intravenous injection or infusion, intra-arterial injection, subcutaneous injection, intramuscular injection, and intra-articular injection. Compositions of the invention can also be formulated for other routes of administration including

transmucosal, ocular, rectal, long acting implantation, sublingual administration, under the tongue, from oral mucosa bypassing the portal circulation, inhalation, or intranasal.

According to certain embodiments of the invention, a composition comprises an immunogenically effective amount of purified or partially purified vector, for instance adenovirus vector, such as an adenovirus 26 vector, or poxvirus vector, such as MVA or MVA-BN, the vector comprising a nucleic acid encoding a synthetic HIV envelope protein of the invention and optionally one or more additional HIV antigens. Said compositions can be formulated as a vaccine (also referred to as an "immunogenic composition") according to methods well known in the art.

In certain embodiments of the invention, a composition can further comprise one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide. In general, when used with reference to a polypeptide, such as an isolated antigenic polypeptide, an immunogenically effective amount can range from, e.g. about 0.3 to about 3000 microgram (.mu.g), e.g. 1-1000 .mu.g, e.g. 10-500 .mu.g, e.g. about 50 or 250 .mu.g. As a non-limiting example, it is possible to combine administration of the one or more vectors encoding the synthetic HIV Env antigen of the invention (e.g., having SEQ ID NO: 18) and optionally one or more additional HIV antigens (e.g., having SEQ ID NOs: 1-5, 28, and/or 29) with administration of an isolated HIV Env polypeptide, e.g. 250 .mu.g of HIV clade C Env trimer protein having amino acids 30-708 of SEQ ID NO: 7 or 250 .mu.g of HIV mosaic Env trimer protein having amino acids 30-708 of SEQ ID NO: 36.

In some embodiments, compositions of the invention can further optionally comprise an adjuvant to enhance immune responses. 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 vectors encoding synthetic HIV envelope proteins of the invention and optionally one or more additional HIV antigens and/or HIV antigenic polypeptides used in combination with vectors encoding synthetic HIV envelope proteins of the invention and optionally one or more additional HIV antigens.

Adjuvants suitable for use with the invention should be ones that are potentially safe, well tolerated and effective in people, such as for instance QS-21, Detox-PC, MPL-SE, MoGM-CSF, TiterMax-G, CRL-1005, GERBU, TERamide, PSC97B, Adjumer, PG-026, GSK-I, GcMAF, B-alethine, MPC-026, Adjuvax, CpG ODN, Betafectin, aluminum salts (e.g. AdjuPhos), Adjuplex, and MF59. 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 the present disclosure.

In a preferred embodiment, the adjuvant is an aluminum salt, such as aluminum phosphate, e.g. AdjuPhos. In certain embodiments, the aluminum phosphate is preferably present in or administered with a composition with isolated HIV antigenic polypeptide, such as gp140.

The preparation and use of immunogenic compositions are well known to those of ordinary 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 polyethylene glycol can also be included.

For instance recombinant adenovirus vector may be stored in the buffer that is also used for the Adenovirus World Standard (Hoganson et al., 2002, Bioprocessing J 1: 43-8): 20 mM Tris pH 8, 25 mM NaCl, 2.5% glycerol. Another useful adenovirus formulation buffer suitable for administration to humans is 20 mM Tris, 2 mM MgCl.sub.2, 25 mM NaCl, sucrose 10% w/v, polysorbate-80 0.02% w/v. Another formulation buffer that is suitable for recombinant adenovirus comprises 10-25 mM citrate buffer pH 5.9-6.2, 4-6% (w/w) hydroxypropyl-beta-cyclodextrin (HBCD), 70-100 mM NaCl, 0.018-0.035% (w/w) polysorbate-80, and optionally 0.3-0.45% (w/w) ethanol. Obviously, many other buffers can be used, and several examples of suitable formulations for the storage and for pharmaceutical administration of purified vectors are known.

An exemplary preparation and storage of poxviral vectors, including MVA and MVA-BN can be based on the experience in the preparation of poxvirus vaccines used for vaccination against smallpox, as described, for example, in Stickl, H. et al., Dtsch. med. Wschr. 99, 2386-2392 (1974).

In an exemplary embodiment, purified poxvirus is stored at -80.degree. C. with a titer of 5.times.10.sup.8 TCID.sub.50/ml formulated in 10 mM Tris, 140 mM NaCl, pH 7.7. For the preparation of vaccine shots, e.g., 10.sup.2-10.sup.8 particles of the virus can be lyophilized in phosphate-buffered saline (PBS) in the presence of 2% peptone and 1% human albumin in an ampoule, preferably a glass ampoule. Alternatively, the vaccine shots can be prepared by stepwise, freeze-drying of the virus in a formulation. In certain embodiments, the formulation contains additional additives such as mannitol, dextran, sugar, glycine, lactose, polyvinylpyrrolidone, or other

additives, such as, including, but not limited to, antioxidants or inert gas, stabilizers or recombinant proteins (e.g. human serum albumin) suitable for in vivo administration. The ampoule is then sealed and can be stored at a suitable temperature, for example, between 4.degree. C. and room temperature for several months. However, as long as no need exists, the ampoule is stored preferably at temperatures below -20.degree. C.

In various embodiments involving vaccination or therapy, the lyophilisate is dissolved in 0.1 to 0.5 ml of an aqueous solution, preferably physiological saline or Tris buffer, and administered either systemically or locally, i.e., by parenteral, subcutaneous, intravenous, intramuscular, intranasal, intradermal, or any other path of administration known to a skilled practitioner. Optimization of the mode of administration, dose, and number of administrations is within the skill and knowledge of one skilled in the art.

An advantage of embodiments wherein the vector or vector combination encodes both HIV antigens comprising SEQ ID NOs: 18 and 5, is increased breadth of the immune response (covering strains from clades B and C).

In certain embodiments, a composition or a vaccine combination of the invention further comprises on the same or other vectors, nucleic acid encoding an HIV antigenic polypeptide comprising the amino acid sequence of SEQ ID NO: 28 (mos1GagPol) and/or SEQ ID NO: 29 (mos2GagPol).

In a particular embodiment, a composition or a vaccine combination of the invention comprises a first adenovirus vector, preferably an adenovirus 26 vector, encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 18, and further comprises a second adenovirus vector, preferably an adenovirus 26 vector, encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 5, and one or more additional adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 28 or SEQ ID NO: 29. For example, a composition or a vaccine combination according to an embodiment of the invention can comprise four adenovirus vectors, preferably adenovirus 26 vectors, with a first vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; a second vector encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 5; a third vector encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 28; and a fourth vector encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 29. Preferably, the poxvirus vector of the invention can be part of a vaccine combination with those adenovirus vectors. Such poxvirus vector, preferably an MVA vector, e.g. an MVA-BN vector, in a preferred embodiment encodes each of SEQ ID NOs: 18, 5, 28 and 29.

In a particularly preferred embodiment of the invention, a composition or a vaccine combination comprises a poxvirus vector, preferably an MVA vector, preferably MVA-BN, comprising nucleic acid sequence encoding six different HIV antigens, namely the antigens encoded by SEQ ID NO: 18 (mos2S Env), SEQ ID NO: 5 (mos1 Env), SEQ ID NO: 1 (mos1 Gag), SEQ ID NO: 2 (mos2 Gag), SEQ ID NO: 3 (mos1 Pol), and SEQ ID NO: 4 (mos2 Pol), wherein SEQ ID NOs: 1 and 3 can optionally be fused (SEQ ID NO: 28; mos1GagPol) and SEQ ID NOs: 2 and 4 can optionally be fused (SEQ ID NO: 29: mos2GagPol). An advantage is that only a single vector needs to be manufactured, purified, formulated, tested, stored, shipped, and administered for administering these six HIV antigens. Also, it is known that poxviral vectors, such as MVA, including MVA-BN, provide good immune responses against the antigens encoded therein. Moreover, they can typically be advantageously used together with other vector platforms, such as with adenoviral, e.g. Ad26, vectors in prime-boost regimens to generate further improved immune responses. For example, the poxvirus vector comprising nucleic acid sequence encoding the six different HIV antigens encoded by SEQ ID NOs: 1-5 and 18 can be used together with one or more adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens encoded by SEQ ID NOs: 1-5 and 18, preferably wherein the one or more adenovirus vectors together encode SEQ ID NOs: 1-5 and 18.

As mentioned above, in some embodiments, the composition or a vaccine combination further comprises one or more isolated HIV antigenic polypeptides. Any HIV antigenic polypeptide known to those skilled in the art in view of the present disclosure can be further included in a composition or a vaccine combination of the invention, including, but not limited to an HIV envelope protein (e.g., gp160, gp140, gp120, or gp41), preferably a stabilized trimeric gp140 protein, such as a stabilized clade C or clade A gp140 protein. In a preferred embodiment, the isolated HIV antigenic polypeptide is a stabilized HIV clade C trimeric gp140 protein, such as that comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7 (residues 1-29 of SEQ ID NO: 7 are in the signal sequence). An alternative or additional HIV Env polypeptide that could be used in addition to the clade C gp140 protein or alone, is a mosaic Env trimer protein, for instance having an amino acid sequence as disclosed in amino acids 30-724 of SEQ ID NO: 36 (corresponding to SEQ ID NO: 2 of WO 2014/107744, in which residues 1-29 of SEQ ID NO: 36 are in the signal sequence). In certain embodiments, the HIV antigenic polypeptides comprise both (i) a clade C gp140 protein comprising amino acid residues 30-708 of SEQ ID NO: 7, and (ii) a mosaic gp140 protein comprising amino acid residues 30-724 of SEQ ID NO: 36.

The invention also relates to a method of producing a composition or a vaccine combination of the invention. According to embodiments of the invention, a method of producing a composition or a combination comprises combining a vector comprising nucleic acid encoding the synthetic HIV envelope protein of the invention with a carrier, and optionally one or more additional vectors encoding one or more additional HIV antigenic polypeptides and/or one or more isolated HIV antigenic polypeptides. One of ordinary skill in the art will be familiar with conventional techniques used to prepare such compositions.

Vaccine and Vaccine Combinations

Other general aspects of the invention relate to vaccines and vaccine combinations. In certain embodiments, the compositions of the invention described herein are vaccines. As used herein, the term "vaccine" refers to a composition comprising an immunologically effective amount of an expression vector, preferably a viral vector, encoding a synthetic HIV envelope protein of the invention and optionally further encoding one or more additional HIV antigens that can provide protective immunity or a protective immune response to a subject, or to vaccinate a subject. According to embodiments of the invention, upon administration of the composition to a subject, the expression vector expresses the encoded synthetic HIV envelope protein and optionally further encoded HIV antigens, and the expressed synthetic HIV envelope protein and optionally further encoded HIV antigens are presented to the immune system of the subject, thereby inducing the required response to produce immunity, or induce an immune response.

Thus, in another general aspect, the invention provides a vaccine for inducing an immune response against a human immunodeficiency virus (HIV) in a subject. According to embodiments of the invention, the vaccine comprises a composition comprising an immunogenically effective amount of an expression vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18. Preferably, the expression vector is a viral vector, more preferably an adenovirus vector, e.g., adenovirus 26 vector, and most preferably a poxvirus vector, e.g., MVA or MVA-BN vector.

According to embodiments of the invention, vaccine compositions can further comprise one or more additional vectors, e.g., viral vectors, such as adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more additional HIV antigenic polypeptides and/or one or more isolated HIV antigenic polypeptides. The synthetic HIV envelope protein, additional vectors and/or one or more isolated HIV antigenic polypeptides can be formulated in the same composition or one or more different compositions in the vaccine.

The invention also relates to vaccine combinations for priming and boosting an immune response to one or more HIV clades in a subject in need thereof using one or more vectors, optionally in combination with an isolated antigenic polypeptide. Thus, in another general aspect, the invention provides a vaccine combination for inducing an immune response against an HIV in a subject comprising: (a) a first vaccine composition comprising an immunologically effective amount of a poxvirus vector comprising nucleic acid sequence encoding a first HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 18, and optionally further comprising nucleic acid sequence encoding further HIV antigens, preferably one or more HIV antigens comprising the amino acid sequences selected from SEQ ID NOs: 1-5, 28, and 29; and at least one of: (b) (i) a second vaccine composition comprising an immunogenically effective amount of one or more adenovirus vectors encoding one or more HIV antigens comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29; and (b) (ii) a third vaccine composition comprising one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide, wherein the first composition, and second and/or third composition are present in the same composition or in one or more different compositions.

In certain embodiments thereof, the first vaccine composition comprises an MVA vector encoding a first HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 18, a second HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 5, third and fourth HIV Gag antigens comprising the amino acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2, respectively, and fifth and sixth HIV Pol antigens comprising the amino acid sequence of SEQ ID NO: 3 and SEQ ID NO: 4, respectively. In certain embodiments, the Gag and Pol antigens of SEQ ID NOs: 1 and 3 are combined and present as a Gag-Pol fusion antigen comprising SEQ ID NO: 28, and/or the Gag and Pol antigens of SEQ ID NOs: 2 and 4 are combined and present as a Gag-Pol fusion antigen comprising SEQ ID NO: 29.

In certain embodiments thereof, the second vaccine composition comprises an Ad26 vector encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 18, an Ad26 vector encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 5, an Ad26 vector encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 28, and an Ad26 vector encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 29.

In certain embodiments of the invention, a vaccine combination comprises one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide. Preferably an isolated HIV antigenic polypeptide comprises residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or residues 30-724 of SEQ ID NO: 36. In certain embodiments, two isolated HIV antigenic polypeptides are administered together in one composition, for instance a first isolated HIV antigenic polypeptide that comprises residues 30-708 of the amino acid sequence of SEQ ID NO: 7, and a second isolated HIV antigenic polypeptide that comprises residues 30-724 of SEQ ID NO: 36. The isolated HIV antigenic polypeptide can be present in a third composition or in the first and/or second compositions. The first or second composition can be administered together with the one or more isolated HIV antigenic polypeptides, preferably gp140, for the priming and/or boosting administrations.

As used herein, the terms "co-delivery", "co-administration" or "administered together with" refers to simultaneous administration of two or more components, such as a viral expression vector and an isolated antigenic polypeptide, or multiple viral expression vectors. "Simultaneous administration" can be administration of the two or more components at least within the same day. When two components are "administered together with," they can be administered in separate compositions sequentially within a short time period, such as 24, 20, 16, 12, 8 or 4 hours, or within 1 hour or less, such as essentially simultaneously, or they can be administered in a single composition at the same time.

Another general aspect of the invention relates to a kit comprising a vaccine combination according to an embodiment of the invention.

Other embodiments of the synthetic HIV envelope protein, expression vectors, additional expression vectors, HIV antigens encoded by the expression vectors, and isolated HIV antigenic polypeptide etc. that can be used in the vaccine combinations of the invention are discussed in detail above and in the illustrative examples below.

Method for Inducing Protective Immunity Against HIV Infection

The invention also relates to a method of inducing an immune response against one or more HIV clades in a subject in need thereof. The methods described herein include methods of priming and boosting an immune response using one or more expression vectors optionally in combination with one or more isolated antigenic polypeptides.

According to embodiments of the invention, "inducing an immune response" when used with reference to the methods and compositions described herein encompasses providing protective immunity and/or vaccinating a subject against an infection, such as a HIV infection, for prophylactic purposes, as well as causing a desired immune response or effect in a subject in need thereof against an infection, such as a HIV infection, for therapeutic purposes, i.e., therapeutic vaccination. "Inducing an immune response" also encompasses providing a therapeutic immunity for treating against a pathogenic agent, i.e., HIV. Typically, for prophylactic vaccination, compositions and vaccines are administered to subjects who have not been previously infected with HIV, whereas for therapeutic vaccination, compositions and vaccines are administered to a subject already infected with HIV. The immune response can be a cellular immune response and/or a humoral immune response.

As used herein, the term "protective immunity" or "protective immune response" means that the vaccinated subject is able to control an infection with the pathogenic agent 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 agent will not die as a result of the infection with said agent.

As used herein, the term "therapeutic immunity" or "therapeutic immune response" means that the HIV infected vaccinated subject is able to control an infection with the pathogenic agent, i.e., HIV, against which the vaccination was done. Typically, the administration of the primer and booster vaccine compositions according to embodiments of the invention will have a therapeutic aim to generate an immune response against HIV after HIV infection or development of symptoms characteristic of HIV infection. Preferably, the methods of the invention are for therapeutic purposes, such as for therapeutic vaccination, in which the compositions and vaccines described herein are administered to a subject already infected with HIV. Thus, the patient population for treatment according to the methods of the invention described herein is preferably HIV-infected subjects, and more preferably HIV-infected human subjects. The terms "HIV infection" and "HIV-infected" as used herein refer to invasion of a human host by HIV. As used herein, "an HIV-infected subject" refers to a subject in whom HIV has invaded and subsequently replicated and propagated within the host, thus causing the host to be infected with HIV or have an HIV infection or symptoms thereof.

In one general aspect, a method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject comprises administering to the subject a composition comprising an immunogenically effective amount of an expression vector, preferably a poxvirus vector (e.g., MVA or MVA-BN) comprising a nucleic acid sequence encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18, and preferably encoding further HIV antigens as described herein. Any of the compositions described herein can be used in a method of inducing an immune response against HIV in a subject. The composition can further comprise one or more additional vectors, for instance adenovirus, encoding the same or one or more additional HIV antigens and/or one or more additional isolated HIV antigenic polypeptides. It is also possible to encode the one or more additional HIV antigens in the same vector as the vector encoding the HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18. This is particularly suitable for poxvirus vectors such as MVA, including MVA-BN, as shown herein.

In another general aspect, a method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject comprises administering to the subject: (a) a first vaccine comprising one or more recombinant adenovirus vectors, preferably Ad26 vectors, encoding one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 18, 28, and 29; and (b) a second vaccine comprising a poxvirus vector, preferably an MVA vector encoding SEQ ID NO: 18 and preferably further encoding one or more HIV antigens comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-5, 28, and 29, wherein steps (a) and (b) are conducted in either order, with one of the steps for priming immunization and the other step for boosting immunization.

In some embodiments, a method of inducing an immune response further comprises administering to the subject one or more isolated HIV antigenic polypeptides, preferably one or more HIV antigenic polypeptides comprising (i) a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or (ii) a polypeptide comprising residues 30-724 of SEQ ID NO: 36, or (iii) both polypeptides (i) and (ii). The one or more isolated HIV antigenic polypeptides can be present in the same composition as the first and/or second composition or in one or more additional compositions. In a preferred embodiment, the one or more isolated HIV antigenic polypeptides is administered at about the same time as the composition used for the boosting immunization. In certain embodiments, the one or more isolated HIV antigenic polypeptides are present in the same composition as the boosting vaccine. In other embodiments, the one or more isolated HIV antigenic polypeptides are present in a composition separate from the boosting vaccine. In certain embodiments, the isolated HIV antigenic polypeptide is in a composition comprising an adjuvant, for instance aluminum phosphate.

In a particular embodiment of a method of inducing an immune response according to the invention, the first composition comprises an adenovirus vector, preferably an adenovirus 26 vector, encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and a second adenovirus vector, preferably an adenovirus 26 vector, encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 5; the second composition comprises a poxvirus vector, preferably an MVA vector, such as an MVA-BN vector, according to the invention comprising nucleic acid encoding the synthetic HIV antigen comprising the amino acid of SEQ ID NO: 18, and preferably further comprises nucleic acid sequence encoding one or more HIV antigens comprising the amino acid sequences of SEQ ID NOs: 1-5, 28, and 29, more preferably HIV antigens comprising the amino acid sequences of SEQ ID NOs: 5, 28, and 29; wherein the first composition is administered to the subject one or more times for priming immunization, and the second composition is administered to the subject one or more times for boosting immunization, or wherein the first composition is administered to the subject one or more times for boosting immunization and the second composition is administered to the subject one or more times for priming immunization. In preferred embodiments, the first composition further comprises a third adenovirus vector, preferably an Ad26 vector, encoding an HIV antigenic polypeptide comprising the amino acid sequence of SEQ ID NO: 28, and a fourth adenovirus vector, preferably an Ad26 vector, encoding an HIV antigenic polypeptide comprising the amino acid sequence of SEQ ID NO: 29.

In another particular embodiment of a method of inducing an immune response, the first composition comprises a first adenovirus vector, preferably an adenovirus 26 vector, encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and a second adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigenic polypeptide comprising the amino acid sequence of SEQ ID NO: 5; the second composition comprises a poxvirus vector, preferably a MVA vector, more preferably MVA-BN, comprising nucleic acid sequence encoding HIV antigens comprising the amino acid sequences of SEQ ID NOs: 1-5 and 18, more preferably SEQ ID NOs: 5, 18, 28, and 29, and combinations thereof; wherein the first composition is administered to the subject, one or more times for priming immunization, and the second composition is administered to the subject one or more times for boosting immunization, optionally together with one or more isolated HIV antigenic polypeptides; or wherein the second composition is administered to the subject, one or more times for priming immunization, and the first composition is administered to the subject one or more times for boosting immunization, optionally together with one or more isolated HIV antigenic polypeptides. In preferred embodiments, the first composition further comprises a third adenovirus vector, preferably an Ad26 vector, encoding a HIV antigenic polypeptide comprising the amino acid sequence of SEQ ID NO: 28, and a fourth adenovirus vector, preferably an Ad26 vector, encoding a HIV antigenic polypeptide comprising the amino acid sequence of

SEQ ID NO: 29.

Administration of the immunogenic compositions comprising the expression vectors and/or antigenic polypeptides is typically intramuscular, intradermal or subcutaneous. However, other modes of administration such as intravenous, rectal, cutaneous, oral, nasal, etc. can be envisaged as well. Intramuscular administration of the immunogenic compositions can be achieved by using a needle to inject a suspension of the expression vectors, e.g. adenovirus vectors, and/or antigenic polypeptides. 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 intramuscular, 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. Likewise, the isolated antigenic polypeptide will be in the form of a parenterally acceptable solution having a suitable pH, isotonicity, and stability. Those of ordinary 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 can also be employed.

Typically, administration of the vaccine compositions according to embodiments of the invention will have a therapeutic aim to generate an immune response against an HIV antigen after infection or development of symptoms. In other embodiments, the expression vectors, e.g., adenovirus vectors and/or poxvirus vectors, and/or HIV antigenic polypeptides can be administered for prophylactic purposes before infection or development of symptoms.

The immunogenic compositions containing the expression vectors, e.g., adenovirus vectors and/or poxvirus vectors, and/or antigenic polypeptides are administered to a subject, giving rise to an anti-HIV immune response in the subject. An amount of a composition sufficient to induce a detectable immune response is defined to be an "immunogenically effective dose" or "immunogenically effective amount." In a typical embodiment of the invention, the immune response is a therapeutic 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, or in a veterinary context a veterinarian, 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 adenovirus vectors and/or poxvirus vectors such as MVA vectors and optional formulation of such particles into compositions, the vectors can be administered to an individual, particularly a human or other primate. Delivery to a non-human mammal need not be for a therapeutic purpose, but can be for use in an experimental context, for instance in investigation of mechanisms of immune responses to the synthetic HIV envelope protein and other HIV antigens expressed by the adenovirus vectors and/or poxvirus vectors of the invention.

In one embodiment of the disclosed methods, one or more adenovirus vectors encoding one or more HIV antigens disclosed herein are used to prime the immune response. One or more isolated HIV antigenic polypeptides can be used together with the one or more adenovirus vectors for the priming immunization. In another embodiment, one or more poxviral vectors, preferably MVA or MVA-BN, the poxviral vectors encoding one or more HIV antigens of the present invention are used to prime the immune response. One or more isolated HIV antigenic polypeptides can be used together with the one or more poxviral vectors for the priming immunization. The priming immunization can be administered only once, but can optionally also be administered multiple times, for example, initial priming administration at time 0, followed by another priming administration about 1-24 weeks after the initial priming administration. One or more isolated HIV antigenic polypeptides optionally together with one or more additional adenovirus or poxvirus vectors encoding one or more additional HIV antigens can be used to boost the immune response.

Following the priming administration, one or more of the adenoviral vectors of the present invention or the poxviral vectors of the present invention can be used in one or more boosting immunizations. A boosting immunization can also be administered once or multiple times, for example, first at about 4-52 weeks after the initial priming administration, optionally followed by another boosting administration at for instance about 8-100 weeks after the initial priming administration. In certain other embodiments, one or more adenovirus vectors of the present invention are administered together with one or more poxviral vectors of the present invention for the priming and/or boosting immunization. The immune response induced by the immunization is monitored.

Prime-boost regimens are generally preferred for generation of strong immune responses. It is possible to administer the same vector multiple times, referred to as homologous prime-boost. It is typically preferred according to the invention to apply a heterologous prime-boost regimen, which in this context indicates that the priming and boosting vectors are different. In certain such heterologous prime-boost regimen embodiments for instance, the priming is with adenoviral vector, e.g. Ad26, and boosting is with poxviral vector, e.g. MVA, for instance MVA-BN. In other such heterologous prime-boost regimen embodiments for instance, the priming is with poxviral vector, e.g. MVA, such as MVA-BN, and boosting is with adenoviral vector, e.g. Ad26. Optionally in prime-boost regimens, isolated HIV antigenic polypeptide such as gp140 can be administered at about the same time as the priming or boosting administration of such adenoviral or poxviral vector.

In one exemplary and non-limiting embodiment, a subject is administered four different adenovirus 26 vectors, together encoding HIV antigens comprising SEQ ID NOs: 5, 18, 28 and 29, wherein the vectors are present in a 1:1:1:1 ratio and are administered at a total dose of 5.times.10.sup.10 viral particles in 0.5 mL by intramuscular injection at weeks 0 and 12, followed by administration of an MVA vector encoding HIV antigens comprising SEQ ID NOs: 5, 18, 28 and 29, at a dose of about 10.sup.8 plaque forming units per 0.5 mL injection administered intramuscularly at weeks 24 and 48.

It is readily appreciated by those skilled in the art that the regimen for the priming and boosting administrations can be adjusted based on the measured immune responses after the administrations. For example, the boosting compositions are generally administered weeks or months or even years after administration of the priming composition.

According to embodiments of the invention, an adjuvant can be administered together with the isolated HIV antigenic polypeptide as part of the priming and/or boosting immunization. Any adjuvant can be used together with the isolated HIV antigenic polypeptide in view of the present disclosure, and in certain embodiments the adjuvant is an aluminum salt, such as aluminum phosphate.

In a preferred embodiment of the invention, the adenovirus vectors used in the methods disclosed herein include a rAd26 vector and the poxvirus vectors used in the methods disclosed herein include an MVA vector.

In one exemplary embodiment, an rAd26 vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 is used to prime the immune response in combination with an rAd26 vector encoding an HIV antigen having the amino acid sequence of SEQ ID NO: 5. One or more additional rAd26 vectors encoding one or more additional HIV antigens having the amino acid sequences selected from the group consisting SEQ ID NOs: 1-4, 28 and 29 can also be administered together with the other rAd26 vectors to prime the immune response. In certain embodiments, the priming administration can be re-administered before any boosting immunization is administered. An MVA vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and further encoding SEQ ID NOs: 5, 28, and 29 is used to boost the immune response in these embodiments. Optionally, an isolated HIV antigenic polypeptide, such as that comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or that comprising residues 30-724 of the amino acid sequence of SEQ ID NO: 36, or a combination of at least two of such isolated HIV antigenic polypeptides, is administered together with the MVA vector to boost the immune response. Preferably, an adjuvant is further administered with the isolated HIV antigenic polypeptide in the boosting immunization.

In another exemplary embodiment, an MVA or MVA-BN vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and further encoding SEQ ID NOs: 5, 28, and 29 is used to prime the immune response. In certain embodiments, the priming administration is re-administered before any boosting immunization is administered. Subsequent to the priming administration one or more boosting immunization(s) is/are administered, the boosting immunization comprises an rAd26 vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 in combination with an rAd26 vector encoding an HIV antigen having the amino acid sequence of SEQ ID NO: 5. One or more additional rAd26 vectors encoding one or more additional HIV antigenic polypeptides having the amino acid sequences selected from the group consisting SEQ ID NOs: 1-4, 28 and 29 preferably are also administered together with the other rAd26 vectors to boost the immune response. Optionally, an isolated HIV antigenic polypeptide, such as that comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or that comprising residues 30-724 of the amino acid sequence of SEQ ID NO: 36, or a combination of at least two of such isolated HIV antigenic polypeptides, is administered together with the rAd26 vectors to boost the immune response.

In a particularly exemplary embodiment, an immune response is primed by administration of four HIV antigens encoded on adenoviral vectors, preferably rAd26 vectors,

the four antigens that are encoded being: (i) a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18, (ii) HIV Env antigen having the amino acid sequence of SEQ ID NO: 5, (iii) HIV Gag-Pol fusion antigen having the amino acid sequence of SEQ ID NO: 28, and (iv) HIV Gag-Pol fusion antigen having the amino acid sequence of SEQ ID NO: 29. Each of these four antigens can be encoded on a separate adenoviral vector, preferably a rAd26 vector, administered at a total dose of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.times.10.sup.10 viral particles (vp), e.g. about 5.times.10.sup.10 vp (for all vectors together). The vectors can be pre-mixed, e.g. in a 1:1:1:1 ratio. The administration of adenovirus vectors is preferably via intramuscular injection. The priming administration can be re-administered after the initial priming administration. In this embodiment, an immune response is boosted by administration of an MVA or MVA-BN vector encoding four HIV antigens comprising SEQ ID NO: 18, SEQ ID NO: 5, SEQ ID NO: 28, and SEQ ID NO: 29 at a dosage of 10.sup.5 to 10.sup.11 pfu, e.g. a dose of 10.sup.7 pfu, 10.sup.8 pfu, or 10.sup.9 pfu, or at a dosage of 10.sup.5 to 10.sup.10 TCID.sub.50, e.g 10.sup.7, 10.sup.8 or 10.sup.9 TCID.sub.50. Preferably, the dosage is 2.times.10.sup.5 to 5.times.10.sup.8 pfu. Preferably, the dose for humans comprises at least 5.times.10.sup.7 pfu, e.g., at least 1.times.10.sup.8 pfu, or alternatively at least 2.times.10.sup.7 TCID.sub.50, at least 3.times.10.sup.7 TCID.sub.50, at least 5.times.10.sup.7 TCID.sub.50, e.g. at least 1.times.10.sup.8 TCID.sub.50, or at least 2.times.10.sup.8 TCID.sub.50. The MVA or MVA-BN administration to boost the immune response can be performed any time after the initial priming administration. The boosting administration can be repeated after the initial boosting administration. All administrations of MVA according to this embodiment can be performed, for instance, via the intramuscular or subcutaneous route.

Alternatively, the MVA vector can be used for priming administration and the Ad26 vectors for boosting administration, all essentially as indicated above except in reversed order of administering the adenoviral and poxviral vector types. Optionally, isolated gp140 protein can be administered together with boosting administration. For example, isolated Env gp140 protein, e.g. clade C gp140 protein (comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7), or mosaic gp140 protein (comprising residues 30-724 of the amino acid sequence of SEQ ID NO: 36), or clade C gp140 protein and mosaic gp140 protein, at a total dose of about 50-300 .mu.g protein, e.g. 50 or 250 microgram of clade C gp140 protein, or e.g. 50 or 250 microgram of mosaic gp140 protein, or e.g. 50 or 250 microgram of a combination of clade C gp140 protein and mosaic gp140 protein (e.g. in a 1:1 ratio, either mixed together or separately administered) can be administered together with the poxvirus vector for the boosting immunization. Preferably, the gp140 protein is administered together with an adjuvant, e.g. aluminum phosphate.

In certain embodiments, a method of inducing an immune response according to the invention further comprises administering a latent viral reservoir purging agent. Cells latently infected with HIV carry integrated virus that is transcriptionally silent, making it difficult to effectively eradicate HIV infection in treated subjects. As used herein, "reservoir purging agent" and "latent viral reservoir purging agent" refer to a substance that reduces the latent pool of HIV by reactivating HIV reservoirs, such as by inducing expression of quiescent HIV. Examples of latent viral reservoir purging agents suitable for use with the invention include, but are not limited to, histone deacetylase (HDAC) inhibitors and modulators of toll-like receptors (e.g., TLR7), such as those described in WO2016/007765 and WO2016/177833, which are herein incorporated by reference in their entireties. The latent viral reservoir purging agent can be administered before, after, or co-administered with one or more of the priming and boosting immunizations described herein. The vaccination of a combination of adenovirus 26 vectors encoding Gag, Pol and Env antigens as a prime, followed by MVA vectors encoding such antigens as a boost, in combination with TLR7 stimulation has shown to result in improved virologic control and delayed viral rebound following discontinuation of antiretroviral therapy in rhesus monkey model studies, demonstrating the potential of therapeutic vaccination combined with innate immune stimulation to aim at functional cure for HIV infection (Borducchi E. N., et al, 2016, Nature 540: 284-287 (doi: 10/1038/nature20583)).

In certain embodiments of the invention, the priming and boosting immunizations described herein for inducing an immune response can be combined with standard treatment, e.g., antiretroviral therapy (ART). Subjects treated according to the priming/boosting immunizations of the invention can also undergo ART with any antiretroviral drugs known in the art in view of the present disclosure. ART are medications that treat HIV, although the drugs do not kill or cure the virus. However, when taken in combination they can prevent the growth of the virus. When the virus is slowed down, so is HIV disease. Antiretroviral drugs are referred to as ARV. Combination ARV therapy (cART) is referred to as highly active ART (HAART). One of ordinary skill in the art will be able to determine the appropriate antiretroviral treatment, frequency of administration, dosage of the ART, etc. so as to be compatible with administration of the priming/boosting immunizations of the invention.

Examples of antiretroviral drugs used for ART include, but are not limited to nucleoside reverse transcriptase inhibitors (NRTIs, non-limiting examples of which include zidovudine, didanosine, stavudine, lamivudine, abacavir, tenofovir, combivir [combination of zidovudine and lamivudine], trizivir [combination of zidovudine, lamivudine and abacavir], emtricitabine, truvada [combination of emtricitabine and tenofovir], and epzicom [combination of abacavir and lamivudine]), non-nucleoside reverse transcriptase inhibitors (NNRTIs, non-limiting examples of which include nevirapine, delavirdine, efavirenz, etravirine, and rilpivirine), protease inhibitors (PIs, non-limiting examples of which include saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir/ritonavir, atazanavir, fosamprenavir, tipranavir, darunavir), integrase inhibitors (INSTIs, non-limiting examples including raltegravir, elvitegravir, and dolutegravir), and fusion inhibitors, entry inhibitors and/or chemokine receptor antagonists (FIs, CCR5 antagonists;

non-limiting examples including enfuvirtide, aplaviroc, maraviroc, vicriviroc, and cnicriviroc).

In other embodiments, subjects undergo interruption (also referred to as discontinuation, used interchangeably herein) of ART after completion of a priming/boosting immunization according to embodiments of the invention. In some embodiments, subjects can undergo antiretroviral analytical treatment interruption (ARV ATI) after completion of a priming boosting immunization according to embodiments of the invention. "Antiretroviral analytical treatment interruption" and "ARV ATI" as used in the invention refer to discontinuation of treatment with antiretroviral drugs in order to assess viral suppression and viremic control in the absence of continued ART. Typically, subjects can undergo ARV ATI, i.e., ART can be discontinued, when the subject has plasma HIV RNA levels at less than 50 copies/mL for at least about 52 weeks, but a subject can still undergo ARV ATI even if the subject has one or more blips (i.e., instances) of plasma HIV RNA greater than 50 copies/ml to less than 200 copies/ml within this period, provided that the screening immediately prior to ARV ATI shows less than 50 copies/ml of plasma HIV RNA.

According to embodiments of the invention, the ART can be stopped at about 4-20 weeks after the last booster vaccine is administered. In certain embodiments, for subjects who are on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART, a boosted protease inhibitor can be administered in place of the NNRTI for about 1-2 weeks prior to stopping ART to reduce the risk of developing NNRTI resistance. It is also possible to administer an activator (e.g. a histone deacetylase inhibitor or TLR7 modulator) during the ATI stage to activate any (e.g. latent) HIV reservoir and thereby improve the immune response.

Subjects undergoing ARV ATI can be monitored, e.g., by measuring plasma HIV RNA levels. For example, monitoring after the initiation of ARV ATI can occur up to two times per week during the first six weeks when rebound viremia is most likely to occur. "Rebound viremia" is defined as plasma HIV RNA levels of greater than 1,000 copies/ml after ARV ATI. ART can be re-initiated in subjects with rebound viremia. Preferably, a subject treated according to the methods of the invention will maintain viremic control after ART interruption. As used herein, "maintain viremic control" is defined as at least 24 weeks with plasma HIV RNA of less than 50 copies/mL after ARV ATI. The "maintained viremic control" criterion is still deemed to be met if there are one or more instances of plasma HIV RNA greater than 50 copies/ml to less than 1000 copies/ml, as long as the subject does not have plasma HIV RNA levels above 1000 copies/ml on two consecutive determinations at least one week apart.

Typically (not using the methods of the instant invention) human HIV-infected subjects have a return of viremia after 2-3 weeks following ART interruption. Without wishing to be bound by any theories, it is believed that the priming/boosting immunization according to embodiments of the invention among individuals with fully suppressed HIV will result in a measurable immune response and maintain viremic control after ARV ATI in at least certain individuals. In some embodiments, subjects can discontinue ART after being treated according to a method of the invention. Discontinuation of ART can be for long periods of time (e.g., at least 24 weeks, preferably longer, e.g. at least about 28, 32, 36, 40, 44, 48, 52 weeks, 16 months, 18, 20, 22, 24 months, or even longer). Such periods of time in which ART is stopped or discontinued are referred to as a "holiday" or "ART holiday" or "treatment holiday". In other embodiments, vaccine therapy according to the methods of the invention can provide HIV remission, meaning that viral suppression is maintained in the absence of ART.

EMBODIMENTS

Embodiment 1 is a poxvirus vector comprising nucleic acid encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18.

Embodiment 2 is a poxvirus vector comprising nucleic acid encoding: (a) a first HIV envelope (Env) antigen comprising the amino acid sequence of SEQ ID NO: 18; (b) a second HIV Env antigen different from the first HIV Env antigen; (c) a third antigen and a fourth antigen, being two different HIV Gag antigens; and (d) a fifth antigen and a sixth antigen, being two different HIV Pol antigens.

Embodiment 3 is the poxvirus vector of embodiment 2, wherein the second HIV Env antigen comprises the amino acid sequence of SEQ ID NO: 5, the third and fourth antigens comprise the amino acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2, respectively; and the fifth and the sixth antigens comprise the amino acid sequence of SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

Embodiment 4 is the poxvirus vector of embodiments 2 or 3, wherein the third and the fifth antigens are fused into a first Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 28, and the fourth and the sixth antigens are fused into a second Gag-Pol fusion antigen that comprises the amino acid sequence of SEQ ID NO: 29.

Embodiment 5 is the poxvirus vector of any one of embodiments 1-4, wherein the first HIV Env antigen is encoded by SEQ ID NO: 41.

Embodiment 6 is the poxvirus vector of any one of embodiments 4-5, wherein the first HIV Env antigen is encoded by SEQ ID NO: 41; the second HIV Env antigen is encoded by SEQ ID NO: 39; the first Gag-Pol fusion antigen is encoded by SEQ ID NO: 38; and the second Gag-Pol fusion antigen is encoded by SEQ ID NO: 40.

Embodiment 7 is the poxvirus vector of any one of embodiments 1-6, wherein the nucleic acid encoding the antigen(s) is operably linked to a promoter sequence.

Embodiment 8 is the poxvirus vector of any one of embodiments 1-7, wherein the poxvirus vector is a recombinant Modified Vaccinia virus Ankara (MVA) vector.

Embodiment 9 is the poxvirus vector of embodiment 8, wherein the MVA vector comprises MVA-BN or derivatives thereof.

Embodiment 10 is the poxvirus vector of embodiment 8 or 9, wherein the first Gag-Pol fusion antigen and the second Env antigen are inserted into intergenic region (IGR) 44/45 of the MVA genome, and the second Gag-Pol fusion antigen and the first Env antigen are inserted into IGR 88/89 of the MVA genome.

Embodiment 11 is the poxvirus vector of any one of embodiments 7-10, wherein the first Gag-Pol fusion antigen and the second Gag-Pol fusion antigens are each under control of a separate Pr13.5 promoter, and the first Env and the second Env antigens are each under control of a separate PrHyb promoter.

Embodiment 12 is an isolated cell comprising the poxvirus vector of any one of embodiments 1-11.

Embodiment 13 is a composition comprising a vector of any one of embodiments 1-11, and a carrier.

Embodiment 14 is a vaccine comprising an immunogenically effective amount of a poxvirus vector according to any one of embodiments 1-11, and a pharmaceutically acceptable carrier.

Embodiment 15 is a vaccine combination, comprising: (a) a first vaccine composition comprising an immunogenically effective amount of a poxvirus vector, preferably a MVA vector, according to any one of embodiments 1-11; and at least one of (b) (i) a second vaccine composition comprising an immunogenically effective amount of one or more adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens, the one or more HIV antigens preferably comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-5, 18, 28, and 29; and (b) (ii) a third vaccine composition comprising one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptide optionally further comprising an adjuvant, preferably aluminum phosphate, wherein the first composition, and second and/or third composition are present in the same composition or in one or more different compositions.

Embodiment 16 is the vaccine combination according to embodiment 15, wherein the one or more isolated HIV antigenic polypeptides in the third vaccine composition comprises (i) a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or (ii) a polypeptide comprising residues 30-724 of the amino acid sequence of SEQ ID NO: 36, or (iii) both polypeptides (i) and (ii).

Embodiment 17 is the vaccine combination of any one of embodiments 15-16, wherein the second vaccine composition comprises recombinant adenovirus 26 vectors together encoding SEQ ID NOs: 1-5 and 18, preferably wherein SEQ ID NOs: 1 and 3 are fused together as SEQ ID NO: 28 and/or SEQ ID NOs: 2 and 4 are fused together as SEQ ID NO: 29.

Embodiment 18 is the vaccine combination of embodiment 17, wherein the second vaccine composition comprise four recombinant Ad26 vectors, the first recombinant Ad26 vector encoding SEQ ID NO: 5; the second recombinant Ad26 vector encoding SEQ ID NO: 18; the third recombinant Ad26 vector encoding SEQ ID NOs: 1 and 3, preferably SEQ ID NO: 28; and the fourth recombinant Ad26 vector encoding SEQ ID NOs: 2 and 4, preferably SEQ ID NO: 29.

Embodiment 19 is a method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising

administering to the subject the composition of embodiment 13, the vaccine of embodiment 14, or the vaccine combination of any one of embodiments 15-18.

Embodiment 20 is a composition of embodiment 13, a vaccine of embodiment 14, or a vaccine combination of any one of embodiments 15-18 for use in inducing an immune response against a human immunodeficiency virus (HIV).

Embodiment 21 is a composition of embodiment 13 or a vaccine of embodiment 14, further comprising one or more additional expression vectors encoding one or more additional HIV antigens, and/or one or more isolated HIV antigenic polypeptides.

Embodiment 22 is a composition of embodiment 13 or a vaccine of embodiment 14, further comprising an adenovirus vector, preferably an adenovirus 26 vector, encoding a synthetic HIV envelope protein comprising or consisting of the amino acid sequence of SEQ ID NO: 18.

Embodiment 23 is a composition or vaccine according to embodiment 22, further comprising a second adenovirus vector, preferably an adenovirus 26 vector, encoding an HIV antigen comprising the amino acid sequence of SEQ ID NO: 5, and optionally one or more additional adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more additional HIV antigens comprising the amino acid sequences of SEQ ID NOs: 1-4, 28 and 29, preferably SEQ ID NOs: 28 and 29, more preferably wherein SEQ ID NOs: 28 and 29 are encoded separately on a third and fourth adenovirus vector, preferably adenovirus 26 vectors.

Embodiment 24 is a method of producing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising administering to the subject a composition or vaccine or vaccine combination according to any one of embodiments 13-23.

Embodiment 25 is a method of producing a composition or a vaccine combination, comprising combining the poxvirus vector of any one of embodiments 1-11 with a carrier, and optionally one or more additional vectors encoding one or more additional HIV antigens and/or one or more isolated HIV antigenic polypeptides in one or more compositions, together with a carrier. 10208f Embodiment 26 is a method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, the method comprising administering to the subject: (i) a first vaccine comprising an immunogenically effective amount of one or more recombinant adenovirus vectors, preferably Ad26 vectors, encoding one or more HIV antigens comprising an amino acid sequence of any one or more of SEQ ID NOs: 1-5, 18, 28, and 29, and a carrier; (ii) a second vaccine comprising a poxvirus vector according to any one of embodiments 1-11, and a carrier; and (iii) optionally, a third vaccine comprising one or more polypeptides comprising an immunogenically effective amount of an isolated HIV antigenic polypeptides, and a carrier and optionally further comprising an adjuvant, preferably aluminum phosphate, wherein steps (i) and (ii) are conducted in either order, with one of the steps for priming immunization and the other step for boosting immunization, and wherein the optional third vaccine is administered together with the first composition or the second composition for the priming and/or boosting immunization.

Embodiment 27 is a method according to embodiment 26, wherein the third composition is administered at about the same time as the composition used for the boosting vaccine.

Embodiment 28 is a method according to embodiment 26 or 27, wherein the one or more isolated HIV antigenic polypeptides comprise (i) a polypeptide comprising residues 30-708 of the amino acid sequence of SEQ ID NO: 7; or (ii) a polypeptide comprising residues 30-724 of SEQ ID NO: 36; or (iii) both polypeptides (i) and (ii), and wherein the one or more isolated HIV antigenic polypeptides are in the same composition as the boosting vaccine and/or priming vaccine or in a composition separate from the boosting vaccine and/or priming vaccine.

Embodiment 29 is a method according to any one of embodiments 26-28, wherein (i) the first vaccine comprises an adenovirus vector, preferably an adenovirus 26 vector, encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and a second adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 5, and optionally one or more additional expression vectors, preferably adenovirus vectors, more preferably adenovirus 26 vectors, encoding one or more additional HIV antigens; (ii) the second vaccine comprises a poxvirus vector, preferably an MVA vector, encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18 and preferably encoding further HIV antigens comprising one or more of the amino acid sequences of SEQ ID NOs: 1-5, 28 and 29; and (iii) the third vaccine comprises an isolated HIV antigenic polypeptide having residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or residues 30-724 of SEQ ID NO: 36; wherein the first vaccine is administered to the subject one or more times for priming

immunization, the second vaccine is administered to the subject one or more times for boosting immunization, and the third vaccine is administered to the subject together with the second vaccine one or more times for the boosting immunization.

Embodiment 30 is a method according to embodiment 29, wherein the first vaccine comprises one or more additional adenovirus 26 vectors encoding one or more HIV antigens comprising the amino acid sequences of SEQ ID NOs: 1-4, 28, and 29, preferably SEQ ID NOs: 28 and 29.

Embodiment 31 is a method according to embodiment 30, wherein the first vaccine comprises a third adenovirus vector encoding SEQ ID NO: 28 and a fourth adenovirus vector encoding SEQ ID NO: 29.

Embodiment 32 is a vaccine combination comprising the following components:

(i) an Ad26 vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18;

(ii) an Ad26 vector encoding an HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 5;

(iii) an Ad26 vector encoding an HIV Gag-Pol fusion protein comprising the amino acid sequence of SEQ ID NO: 28;

(iv) an Ad26 vector encoding an HIV Gag-Pol fusion protein comprising the amino acid sequence of SEQ ID NO: 29, and

(v) an MVA vector encoding a first HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18, a second HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 5, a first Gag-Pol fusion protein comprising the amino acid sequence of SEQ ID NO: 28 and a second Gag-Pol fusion protein comprising the amino acid sequence of SEQ ID NO: 29.

Embodiment 33 is a vaccine combination according to embodiment 32, further comprising the following component:

(vi) (vi, a): isolated HIV antigenic polypeptide having residues 30-708 of the amino acid sequence of SEQ ID NO: 7, or (vi, b): residues 30-724 of the amino acid sequence of SEQ ID NO: 36, or (vi, c): both (vi, a) and (vi, b), wherein (vi, a), (vi, b), or (vi, c) optionally further comprise an adjuvant.

Embodiment 34 is a method of inducing an immune response against a human immunodeficiency virus (HIV) in a human subject in need thereof, the method comprising: (a) administering to the subject: (i) a rAd26 vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (ii) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 5; (iii) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 28; and (iv) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 29; preferably wherein the rAd26 vectors are administered in a ratio of about 1:1:1:1 at a total dose of about 1-10.times.10.sup.10 viral particles (vp), e.g. 5.times.10.sup.10 vp;

(b) optionally repeating step (a);

(c) administering to the subject: (i) an MVA or MVA-BN vector comprising nucleic acid encoding (i,a) a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (i,b) a HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 5; (i,c) a HIV Gag-Pol fusion antigen comprising the amino acid sequence of SEQ ID NO: 28; (i,d) a HIV Gag-Pol fusion antigen comprising the amino acid sequence of SEQ ID NO: 29; preferably wherein the MVA is administered at a dose of 10.sup.5 to 10.sup.11 pfu, e.g., at a dose of 10' pfu, 10.sup.8 pfu, 10.sup.9 pfu, or 10.sup.10 pfu, or at a dose of 10.sup.6 to 10.sup.10 TCID.sub.50, e.g. between 10.sup.7 and 10.sup.9 TCID.sub.50; and (d) optionally repeating step (c).

Embodiment 35 is a method of inducing an immune response against a human immunodeficiency virus (HIV) in a human subject in need thereof, the method comprising: (a) administering to the subject: (i) an MVA or MVA-BN vector comprising nucleic acid encoding (i,a) a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (i,b) a HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 5; (i,c) a HIV Gag-Pol fusion antigen comprising the amino acid sequence

of SEQ ID NO: 28; (i,d) a HIV Gag-Pol fusion antigen comprising the amino acid sequence of SEQ ID NO: 29; preferably wherein the MVA is administered at a dose of 10.sup.5 to 10.sup.11 pfu, e.g., at a dose of 10.sup.7 pfu, 10.sup.8 pfu, 10.sup.9 pfu, or 10.sup.10 pfu, or at a dose of 10.sup.6 to 10.sup.10 TCID.sub.50, e.g. between 10.sup.7 and 10.sup.9 TCID.sub.50;

(b) optionally repeating step (a);

(c) administering to the subject: (i) an MVA or MVA-BN vector comprising nucleic acid encoding (i,a) a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (i,b) a HIV Env antigen comprising the amino acid sequence of SEQ ID NO: 5; (i,c) a HIV Gag-Pol fusion antigen comprising the amino acid sequence of SEQ ID NO: 28; (i,d) a HIV Gag-Pol fusion antigen comprising the amino acid sequence of SEQ ID NO: 29; preferably wherein the MVA is administered at a dose of 10.sup.5 to 10.sup.11 pfu, e.g., at a dose of 10.sup.7 pfu, 10.sup.8 pfu, 10.sup.9 pfu, or 10.sup.10 pfu, or at a dose of 10.sup.6 to 10.sup.10 TCID.sub.50, e.g. between 10.sup.7 and 10.sup.9 TCID.sub.50; and optionally one or more of (ii, a) isolated HIV gp140 protein having the sequence of amino acids 30-708 of SEQ ID NO: 7; (ii, b) isolated HIV gp140 protein having the sequence of amino acids 30-724 of SEQ ID NO: 36; and (iii) aluminum phosphate adjuvant; wherein optionally the isolated HIV gp140 proteins are administered in a ratio of about 1:1 at a total dose of about 50-300 microgram, e.g. 250 microgram; and (d) optionally repeating step (c).

Embodiment 36 is a method of inducing an immune response against HIV in a human subject in need thereof, the method comprising:

(a) administering to the subject a MVA or MVA-BN vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18, SEQ ID NO: 5, SEQ ID NO: 28, and SEQ ID NO: 29, wherein the MVA or MVA-BN vector is administered at a dose of 10.sup.5 to 10.sup.11 pfu, e.g. at a dose of 10.sup.7 pfu, 10.sup.8 pfu, 10.sup.9 pfu, or 10.sup.10 pfu, e.g. at a dose of 2.times.10.sup.7 to 5.times.10.sup.8pfu, e.g. at a dose of about 1.times.10.sup.8 pfu, or at a dose of 10.sup.6 to 10.sup.10 TCID.sub.50, e.g. between 10.sup.7 and 10.sup.9 TCID.sub.50, (b) optionally repeating step (a); (c) administering to the subject: (i) a rAd26 vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (ii) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 5; (iii) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 28; (iv) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 29; and optionally one or more of: (v, a) isolated HIV gp140 protein having the sequence of amino acids 30-708 of SEQ ID NO: 7; (v, b) isolated HIV gp140 protein having the sequence of amino acids 30-724 of SEQ ID NO: 36; (v, c) both (v, a) and (v, b); and (v, d) aluminum phosphate adjuvant; preferably wherein the rAd26 vectors are administered in a ratio of about 1:1:1:1 at a total dose of about 1-10.times.10.sup.10 viral particles (vp), e.g. 5.times.10.sup.10 vp and wherein optionally the isolated HIV gp140 proteins are administered in a ratio of about 1:1 at a total dose of about 50-300 microgram, e.g. 250 microgram; and (d) optionally repeating step (c).

Embodiment 37 is a method of inducing an immune response against HIV in a human subject in need thereof, the method comprising:

(a) administering to the subject: (i) a rAd26 vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18; (ii) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 5; (iii) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 28; and (iv) a rAd26 vector encoding an antigen comprising the amino acid sequence of SEQ ID NO: 29; preferably wherein the rAd26 vectors are administered in a ratio of about 1:1:1:1 at a total dose of about 1-10.times.10.sup.10 viral particles (vp), e.g. 5.times.10.sup.10 vp; (b) optionally repeating step (a); (c) administering to the subject (i) an MVA or MVA-BN vector encoding a synthetic HIV envelope protein comprising the amino acid sequence of SEQ ID NO: 18, SEQ ID NO: 5, SEQ ID NO: 28, and SEQ ID NO: 29, wherein preferably the MVA or MVA-BN vector is administered at a dose of 10.sup.5 to 10.sup.11 pfu, e.g. at a dose of 10.sup.7 pfu, 10.sup.8 pfu, 10.sup.9 pfu, or 10.sup.10 pfu, e.g. at a dose of 2.times.10.sup.7 to 5.times.10.sup.8 pfu, e.g. at a dose of about 1.times.10.sup.8 pfu, or at a dose of 10.sup.6 to 10.sup.10 TCID.sub.50, e.g. between 10.sup.7 and 10.sup.9 TCID.sub.50; and (ii, a) isolated HIV gp140 protein having the sequence of amino acids 30-708 of SEQ ID NO: 7; or (ii, b) isolated HIV gp140 protein having the sequence of amino acids 30-724 of SEQ ID NO: 36; or (ii, c) two isolated HIV gp140 proteins wherein a first isolated HIV gp140 protein has the sequence of amino acids 30-708 of SEQ ID NO: 7 and a second isolated HIV gp140 protein has the sequence of amino acids 30-724 of SEQ ID NO: 36, preferably wherein these proteins are administered in a ratio of about 1:1; and (iii) aluminum phosphate adjuvant; preferably wherein the isolated HIV gp140 protein is or isolated HIV gp140 proteins are administered at a total dose of about 50-300 microgram, e.g. 250 microgram; and (d) optionally repeating step (c).

Embodiment 38 is the method of any of embodiments 26-31 or 34-37, wherein the subject has been infected with HIV prior to the first step of administering a vector or vaccine component.

Embodiment 39 is the method of any of embodiments 26-31 or 34-38, further comprising administering a latent viral reservoir purging agent to the subject.

Embodiment 40 is the method of embodiment 39, wherein the latent viral reservoir purging agent is a TLR7 modulator.

Embodiment 41 is the method of any of embodiments 26-31 or 34-40, wherein the subject is further undergoing antiretroviral therapy (ART).

Embodiment 42 is the method of embodiment 41, wherein the subject undergoes interruption of ART after completion of a priming/boosting immunization.

Embodiment 43 is the method of embodiment 42, wherein the interruption of ART is initiated after completion of an Ad26 priming immunization and MVA boosting immunization, optionally wherein the MVA boosting immunization is administered together with one or more isolated HIV Env gp140 proteins.

Embodiment 44 is a composition of any one of embodiments 13 or 21-23, a vaccine of any one of embodiments 14 or 21-23, or a vaccine combination of any one of embodiments 15-18 or 32-33, for use in treating and/or preventing a human immunodeficiency virus (HIV) infection and/or disease.

Embodiment 45 is a composition of any one of embodiments 13 or 21-23, a vaccine of any one of embodiments 14 or 21-23, or a vaccine combination of any one of embodiments 15-18 or 32-33, for use in manufacturing a medicament for treating and/or preventing a human immunodeficiency virus (HIV) infection and/or disease.

Embodiment 46 is use of a composition of any one of embodiments 13 or 21-23, a vaccine of any one of embodiments 14 or 21-23, or a vaccine combination of any one of embodiments 15-18 or 32-33, for manufacturing a medicament for treating and/or preventing a human immunodeficiency virus (HIV) infection and/or disease.

EXAMPLES

Example 1: Design of HIV Envelope Antigen Sequences

Several HIV envelope antigen sequences were designed having sequence similarity to the mosaic HIV antigen mos2Env (SEQ ID NO: 6; previously also described in WO 2010/059732). The newly designed, membrane bound, sequences were based on (a combination of) fully natural wild-type sequences from HIV envelope proteins, or a chimera of mos2Env sequence and wild-type HIV envelope protein sequences. In addition to full length envelope protein sequences (see FIG. 1A), sequences having a C-terminal truncations of the cytoplasmic domain were also designed (see, e.g., FIG. 1C). See also e.g., Schiernle et al., PNAS 1997; Abrahamyan et al., J Virol 2005; Edwards et al., J Virology, 2002, 76:2683-2691. Soluble variants were also prepared by C-terminal truncation before the transmembrane (TM) region, which was replaced by a trimerization domain, such as a GCN4 trimerization domain (see, e.g., FIG. 1B). These soluble variants were further converted into a single chain variant by mutation of the furin-cleavage site, thus inhibiting the processing of the extracellular domain of the envelope protein into gp120 and gp41 subunits.

Of all constructs generated and tested, constructs based on C4 had the most optimal properties, e.g., good manufacturability, folding, immunogenicity, etc. and these were selected for further studies. A soluble variant of the C4 construct having a GCN4 trimerization domain in place of the transmembrane domain (sC4, FIG. 1B), and a variant comprising a 7-amino acid fragment of the cytoplasmic domain (C4D7, FIG. 1C) were also generated and tested in further studies. The amino acid sequences of C4, sC4, and C4D7 are shown in SEQ ID NOs: 17, 19, and 18, respectively. Sequences encoding these are shown in SEQ ID NOs: 25, 27, and 26, respectively. Construct C1 has an extracellular domain sequence based on the mos2Env sequence (SEQ ID NO: 6). A soluble variant of construct C1 having a GCN4 trimerization domain in place of the transmembrane domain (sC1), and a variant comprising a 7-amino acid fragment of the cytoplasmic domain (C1D7), similar to sC4 and C4D7 as shown in FIGS. 1B and 1C, respectively, were also generated. Construct C1 and its variants were used in further studies for comparison purposes, since these are essentially based on the mos2Env sequence of the prior art. The amino acid sequences of C1, sC1 and C1D7 are shown in SEQ ID NOs: 31, 30, and 32, respectively. Nucleic acid sequences encoding these are shown in SEQ ID NOs: 34, 33, and 35, respectively. Other constructs that were tested were less optimal than the ones based on construct C4, and were not taken into further development.

Example 2: Expression and Folding of Synthetic HIV Envelope Proteins

The expression level, folding, and cell-surface expression of synthetic HIV envelope proteins were measured.

Expression Levels

HEK293F cells were transiently transfected with a plasmid encoding the soluble synthetic HIV envelope proteins sC1 and sC4 as described in Example 1. Expression levels of the soluble protein were measured in the supernatant using quantitative Western blot (QWB). The results are shown in FIG. 2. The low expression levels for sC1 (which essentially corresponds to mos2Env with an added transmembrane domain) are in line with our recent insights for mos2Env. As demonstrated by the results, the sC4 variant of the invention showed significantly higher expression levels than the sC1 variant (control).

Protein Folding

Protein folding was tested by measuring the binding of soluble synthetic HIV envelope proteins to an antibody (MAb 17b) known to bind the co-receptor binding site of the HIV envelope protein, which is exposed only after binding of CD4, by enzyme-linked immunosorbent assay (ELISA). In particular, binding of purified sC4 was tested for binding to MAb 17b with prior binding of sC4 to CD4, and without prior binding of sC4 to CD4. Purified sC1 was used as a control. Binding of MAb 17b to sC4 without prior CD4 binding to the envelope protein is an indication of partially unfolded or pre-triggered envelope protein (i.e., an unstable Env that adopts the "open" conformation in the absence of CD4 binding). The results of the ELISA assay are shown in FIGS. 3A and 3B.

As shown in FIG. 3B, sC4 shows strong binding to MAb 17b with prior binding to CD4, but no detectable binding to MAb 17b without prior binding to CD4. In contrast, as shown in FIG. 3A, sC1 showed much lower binding to MAb 17 both with and without prior binding to CD4. The results suggest that sC4 has a correct folding pattern, with no exposure of the co-receptor binding site prior to CD4 binding.

Protein folding was also analyzed by native polyacrylamide gel electrophoresis (PAGE) of sC1 and sC4 to evaluate the quaternary structure of the soluble protein variants, and possible incorrect disulfide bridge formation between protomers. After electrophoresis on a native gel, protein in the gel was detected by Western blot analysis. As shown by the results in FIG. 4, the majority of sC4 is present in a trimeric state, which is the correct quaternary structure.

Taken together, the results of the protein folding experiments demonstrate that the sC4 soluble synthetic HIV envelope protein has the desired folding profile, which is improved as compared to the folding profile of the existing mos2Env antigen (represented by sC1).

Cell Surface Expression

Cell surface expression of the membrane-bound variants of HIV envelope proteins C1 (full length), C4 (full length, see FIG. 1A), C1D7, and C4D7 was also studied. HEK293T cells were transiently transfected with only eGFP-encoding plasmid (negative control, NC), or with eGFP-encoding plasmid together with an expression construct encoding an HIV envelope protein variant. Two days post-transfection, cells were subjected to fluorescence activated cell sorting (FACS)-analysis upon exposure to several poly- and monoclonal antibodies directed against gp120, and secondary antibodies, and then examined for envelope protein cell-surface expression levels. Quality of the envelope variants was assessed by determining the overall expression levels using an anti-gp120 polyclonal antibody, and by assessing relative binding of the broadly neutralizing antibodies PG9 and PG16, which are quaternary-structure dependent, and preferentially bind to correctly folded envelope trimer.

The results of the cell surface expression experiments are shown in FIG. 5. The surface expression levels of truncated variants C1D7 and C4D7 as measured using an anti-gp120 antibody, are much higher than the surface expression levels of their full length counterparts, C1 and C4, respectively. This confirms that deletion of 144 residues from the carboxy-terminus of Env increases envelope surface expression levels. The full length C4 construct of the invention also showed improved PG9 and PG16 binding as compared to full length C1, suggesting that the C4 envelope sequence is properly folded (i.e., a trimer) on the cell surface.

The results also demonstrate that the C1D7 variant, which is essentially Mos2Env with an added transmembrane domain and 7 amino acids of the cytoplasmic domain, can be surface-expressed on HEK293T cells. This is in contrast to the soluble construct in Ad26.mos2Env, which cannot be expressed at detectable levels on the surface

when transfected to A549 cells. However, relative binding to PG9 and PG16 is barely detectable above background, suggesting that the C1D7 envelope sequence is poorly folded and is probably not present as an intact trimer on the cell surface.

Overall, the C4D7 envelope variant has the most optimal antibody binding profile, with higher gp120 expression than its full-length counterpart C4, and with greater than 15-fold increased PG9 and PG16 binding compared to C1 and C1D7 (FIG. 5).

Example 3: Stability of Vectors Encoding HIV Envelope Sequences

Previous work in our laboratories (unpublished) indicated that adenovirus 26 (Ad26) vectors encoding the mos2Env antigen sequence showed relatively high VP/IU ratios (indicating lower quality of adenovirus product batches) and moreover that such vectors displayed stability issues. Accordingly, it was important to test the stability of the synthetic HIV envelope protein constructs of the invention in an adenovirus background.

Recombinant Ad26 (rAd26) vectors encoding HIV antigen sequences of the invention C4, C4D7, and sC4 as described above in Example 1 were generated in PER.C6 cells (referred to as "rAd26.C4", "rAd26.C4D7", and "rAd26.sC4", respectively). Vector clones (plaques) were picked and scaled-up for the generation of research batches. A maximum of 5 viral clones (plaques) were scaled-up to T25 format and serially passaged for 10 passages in T25 format (passages 1-3 being the transfection and plaque purification steps, followed by 10 passages in T25 format, resulting in a total of 13 passages). Genetic stability was assessed at viral passage number (vpn) 3, 5, 10 and 13 by an E1 transgene cassette PCR assay, followed by sequencing at vpn 13. The results are shown in FIG. 6.

The rAd26 vectors encoding full length C4 (rAd26.C4) showed poor growth characteristics, as determined by no full cytopathogenic effect (CPE) in 2-3 days; genetic instability, as determined by deletions of the E1 transgene cassette region; or a combination thereof (FIG. 6). Due to the poor growth characteristics and observed genetic instability, this vector encoding full length C4 was not pursued further.

In contrast, for the rAd26 vectors encoding C4D7 (rAd26.C4D7) and sC4 (rAd26.sC4), all propagated plaques remained genetically stable during the course of the experiment (FIG. 6). Thus, the novel sC4 and C4D7 constructs outperform the original mos2Env construct with respect to stability in an adenoviral vector background. The genetic stability testing up to vpn 13 represents propagation several passages beyond that used in the industrial scale preparation of the vectors.

Example 4: Expression and in Vivo Antigenicity of HIV Envelope Sequences in Adenovirus Vectors

Expression and antigenicity of rAd26.C4D7 and rAd26.sC4 were assessed separately or in combination with a recombinant Ad26 vector encoding mos1Env (SEQ ID NO: 5) (hereinafter "rAd26.mos1Env") in vector-transduced A549 cells (human cell line) in vitro (data not shown). Flow cytometry analysis demonstrated that all antigens were expressed in cell cultures transduced with either 2.times.10.sup.4 viral particles (vp) of the single envelope antigens as controls, or with 1.times.10.sup.4 vp of the 2 combined Env antigens by adenovirus transduction. All transductions additionally contained single doses (1.times.10.sup.4 vp) of adenovirus vectors encoding mos1GagPol ("rAd26.mos1GagPol") and mos2GagPol ("rAd26.mos2GagPol") (Barouch et al, Nat Med 2010, 16:319-323), so that the assessed vector combinations exhibited the same relative ratios of the different adenoviral vectors as intended for pre-clinical and clinical use. Preferably, the vectors encoding synthetic HIV envelope proteins of the invention are combined with vectors encoding the mos1GagPol and the mos2GagPol antigens for clinical use.

The combination of rAd26.mos1Env and rAd26.C4D7 yielded a maximal coverage of the assessed epitopes as determined by monoclonal antibody binding. Particularly, the exposure of the PG16 epitope, which was contributed by transformation with Ad26.C4D7 is promising for vaccine use since PG16 represents a broadly neutralizing monoclonal antibody recognizing the V1/V2 loop region of HIV-1 Env (Walker et al, Science 326:258-9, 2009). Hence, the synthetic HIV envelope protein of the invention derived from the C4 sequence increases the breadth of the immune response against the HIV envelope protein compared to the immune response generated by mos1Env only. Vaccine-induced antibody responses directed towards the envelope protein region have been shown to correlate with protection from HIV-1 infection in the RV144 study (Haynes et al, N Engl J Med. 336:1275-86, 2012), and thus the synthetic HIV envelope protein of the invention is a promising candidate to include in HIV vaccine regimens.

Example 5: Immunogenicity of Vectors Encoding Synthetic HIV Envelope Proteins

The synthetic HIV envelope protein sequences of the invention in an Ad26 vector background were tested in rabbits to determine if these constructs were an immunogenic alternative to the rAd26.mos2Env construct.

The immunogenicity of adenovirus vector encoding mos1Env (rAd26.mos1Env; SEQ ID NO: 5) was tested alone, and in combination with adenovirus vectors encoding synthetic HIV envelope proteins of the invention (rAd26.C4D7 and rAd26.sC4; comprising SEQ ID NO: 8, in particular SEQ ID NOs: 18 and 19, respectively). In all cases, adenovirus 26 vectors encoding mos1GagPol and mos2GagPol antigens (rAd26.mos1GagPol [SEQ ID NO: 28] and rAd26.mos2GagPol [SEQ ID NO: 29], respectively) were also administered. More specifically, the immunogenicity of rAd26.mos1Env alone (trivalent vaccine: rAd26.mos1GagPol, rAd26.mos2GagPol and rAd26.mos1Env) was compared to the immunogenicity of rAd26.mos1Env in combination with one of rAd26.C4D7 or rAd26.sC4 (tetravalent vaccine: administration of either rAd26.mos1GagPol, rAd26.mos2GagPol, rAd26.mos1Env and rAd26.C4D7; or administration of rAd26.mos1GagPol, rAd26.mos2GagPol, rAd26.mos1Env and rAd26.sC4). This comparison of the trivalent vaccine, which lacks any vectors encoding the synthetic HIV envelope proteins of the invention, with the tetravalent vaccine, which contains vectors encoding the synthetic HIV envelope proteins of the invention, allows for a determination of whether the HIV envelope proteins of the invention contribute to the breadth of protection.

Administration was done in vaccine regimens, wherein these Ad26 vectors were administered at weeks 0 and 6 as a double prime, and a clade C gp140 protein (a trivalent Env gp140 protein having SEQ ID NO: 7 without the signal peptide sequence of residues 1-29, see also WO 2010/042942) at weeks 12 and 18 as a double boost (see e.g. Barouch et al, 2015, Science 349: 320-324). Table 1 describes the vaccine regimens used for the current study. rAd26.Empty refers to a control vector lacking any gene encoding a sequence for an HIV antigenic protein. Each group contained six rabbits.

TABLE-US-00001 TABLE 1 Vaccine regimens tested in immunogenicity study in rabbits First and second Immunizations Third and fourth immunizations Total Dose Group adeno vectors Dose (vp) dose (vp) protein boost (ug) Adjuvant N= 1 rAd26.Mos1Env 2.5 .times. 10.sup.10 5 .times. 10.sup.10 GP140 (clade C) 10 AdjuPhos 250 .mu.g 6 rAd26.Mos1GagPol 1.25 .times. 10.sup.10 rAd26.Mos2GagPol 1.25 .times. 10.sup.10 2 rAd26.Mos1Env 1.25 .times. 10.sup.10 5 .times. 10.sup.10 GP140 (clade C) 10 AdjuPhos 250 .mu.g 6 rAd26.C4D7 1.25 .times. 10.sup.10 rAd26.Mos1GagPol 1.25 .times. 10.sup.10 rAd26.Mos2GagPol 1.25 .times. 10.sup.10 3 rAd26.Mos1Env 1.25 .times. 10.sup.10 5 .times. 10.sup.10 GP140 (clade C) 10 AdjuPhos 250 .mu.g 6 rAd26.sC4 1.25 .times. 10.sup.10 rAd26.Mos1GagPol 1.25 .times. 10.sup.10 rAd26.Mos2GagPol 1.25 .times. 10.sup.10 control rAd26 Empty 5 .times. 10.sup.10 5 .times. 10.sup.10 NA 0 AdjuPhos 250 .mu.g 6

The comparison of the trivalent Ad26 vaccine (lacking the novel Env antigens of the invention) with the tetravalent Ad26 vaccine (which comprises the novel sC4 or C4D7 Env antigens) allows for testing whether the novel antigens of the invention contribute to breadth of protection. An established TZM-bl cell-based neutralization assay [Montefiori D C. Methods Mol Biol 2009, 485:395-405; Sarzotti-Kelsoe M et al., J Immunol Methods 2014, 409:131-146] was used to measure neutralizing activity of the vaccine candidates.

The results are shown in FIG. 7, and were statistically analyzed by using the trivalent vaccine (group 1 in Table 1) as control group and comparing to each of the novel tetravalent vaccines (groups 2 and 3 in Table 1).

Overall, the novel C4-derived (i.e. encoding Env proteins comprising SEQ ID NO: 8, being an alternative for mos2Env) adenovirus constructs were immunogenic after two homologous intramuscular immunizations in rabbits.

Neutralization capacity of rabbit immune sera against Tier 1B pseudoviruses was absent (data not shown), which is not unexpected as it was known that such viruses are more difficult to neutralize.

Pseudovirus neutralization capacity of rabbit immune sera against a clade B Tier 1A virus was unaffected by the addition of new components (data not shown). This demonstrates that the novel antigen did not negatively interfere with immunogenicity of the existing clade B antigen present in the vaccine (although the new components were directed to clade C, such undesirable interference could not be excluded a priori before it had been tested).

Pseudovirus neutralization capacity of rabbit immune sera against a clade C Tier 1A virus was significantly enhanced in the quadrivalent novel C4D7 containing adeno

(quadrivalent, group 2), compared to trivalent (having only mos1Env) immunization alone (group 1) (FIG. 7 panel B). In addition, pseudovirus neutralization capacity of rabbit immune sera against a clade C Tier 1A virus at week 8 was significantly enhanced in the tetravalent novel sC4 containing adenovirus (quadrivalent, group 3), compared to trivalent (having only mos1Env) immunization alone (group 1) (FIG. 7 panel B).

In conclusion, the C4D7 and sC4 constructs encoded in Ad26 were immunogenic and addition thereof expanded the binding- and neutralization capacity of a vaccine that has mos1Env (mainly clade B) as sole Ad26-encoded Env component, towards clade C strains (FIG. 7B).

Example 6: Immunogenicity of Vaccine Regimens Including Vectors Encoding Synthetic HIV Envelope Proteins of the Invention

One further rabbit study assessed the tetravalent vector combination Ad26.Mos4.HIV (consisting of four adenoviral vectors: Ad26.Mos1GagPol [encoding SEQ ID NO: 28], Ad26.Mos2GagPol [encoding SEQ ID NO: 29], Ad26.Mos1Env [encoding SEQ ID NO: 5] and Ad26.Mos2SEnv [the name "C4D7" as used above is also referred to as "Mos2S"; this vector encodes the novel SEQ ID NO: 18 according to the invention], in a 1:1:1:1 mixture at a total dose of 5.times.10.sup.9 vp,) applied intramuscularly as double prime immunizations in weeks 0 and 6, in combination with recombinant HIV-1 Env protein boosts using clade C gp140 [having the sequence of amino acid residues 30-708 of SEQ ID NO: 7], Mosaic gp140 [having the sequence of amino acid residues 30-724 of SEQ ID NO: 36], or a combination of clade C gp140 and Mosaic gp140, in weeks 13 and 19. These protein boosts were applied intramuscularly at a total dose of 10 or 50 micrograms of protein combined with 250 micrograms aluminum phosphate adjuvant formulated on the day of immunization.

Results indicate that all tested regimens were immunogenic in all animals, inducing high antibody titers and moderate neutralization activity against Tier 1 Env pseudotyped viruses. If Mosaic gp140 was used as vaccine antigen, either alone or in combination with clade C gp140, Mosaic gp140-specific ELISA titers and clade B pseudovirus recognition were significantly increased at week 15 in comparison to the reference group boosted with clade C gp140 alone. The overall effect size of the improvement was moderate, and bigger for the group boosted with the bivalent clade C gp140-Mosaic gp140 combination compared to Mosaic gp140 alone. At week 21 of the study, these differences were lost and immune responses measured for the cohorts receiving bivalent clade C gp140-Mosaic gp140 boosts or monovalent clade C gp140 boosts were statistically indistinguishable.

The bivalent protein regimen showed comparable induction of clade C ELISA titers and pseudovirus recognition as the clade C gp140 alone boosted regimen, indicating that the inclusion of the clade B-related immunogen Mosaic gp140 had no negative effect on clade C antigen coverage, whilst significantly enhancing clade B coverage at week 15 of the study.

The data confirm that the Ad26.Mos2SEnv vector encoding a synthetic Env antigen according to the invention can be successfully used in vaccine regimens.

Example 7: Construction of MVA Vectors Encoding Synthetic HIV Envelope Proteins of the Invention in Combination with Other HIV Antigens

In the instant example, an MVA-BN vector was generated (termed "MVA-mBN414"), comprising a nucleic acid encoding the novel HIV mos2SEnv antigen described herein as SEQ ID NO: 18 (also referred to as C4D7). The MVA-mBN414 vector additionally comprised nucleic acids encoding the following HIV antigens: mos1Env (SEQ ID NO: 5); mos1Gag (SEQ ID NO: 1); mos2Gag (SEQ ID NO: 2); mos1Pol (SEQ ID NO: 3); and mos2Pol (SEQ ID NO: 4). In MVA-mBN414 the mos1Gag (SEQ ID NO:1) and mos1Pol (SEQ ID NO: 3) were encoded as a fusion protein (SEQ ID NO:28, "mos1GagPol") and the mos2Gag and mos2Pol were encoded as a fusion protein (SEQ ID NO: 29, "mos2GagPol"). See FIG. 8 for a schematic representation of the inserts into regions of the MVA genome.

We designed a novel nucleic acid (SEQ ID NO: 41) coding for the HIV antigen mos2SEnv (SEQ ID NO: 18); a novel nucleic acid (SEQ ID NO: 39) coding for the HIV antigen mos1Env (SEQ ID NO: 5); a novel nucleic acid (SEQ ID NO: 38) coding for the HIV antigen mos1GagPol (SEQ ID NO: 28); and a novel nucleic acid (SEQ ID NO: 40) coding for the HIV antigen mos2GagPol (SEQ ID NO: 29). The novel nucleic acids were designed for human expression, minimal homology among each other, and reduced poly-nt stretches as well as repetitive elements.

The PrMVA13.5 long promoter (SEQ ID NO: 42) was included in front of the ATG start codon of both the mos1GagPol and mos2GagPol antigen sequences. The PrHyb promoter (SEQ ID NO: 43) was included in front of the ATG start codon of both the mos2SEnv and the mos1Env antigen sequences.

The mos1GagPol and mos1Env coding sequences were inserted via SacII and Pad into pBNX208, a transfer vector encoding IGR 44/45 MVA-BN homologous regions, thus allowing insertion into the targeted region (IGR 44/45) of MVA-BN via homologous recombination. Moreover, pBNX208 encodes heGFP and nptII for positive selection as well as repetitive sequences of the IGR 44/45 MVA-BN homologous region Flank 2 or later excision of the selection cassette via homologous recombination in the absence of selective pressure. The mos2GagPol and mos2Env coding sequences were inserted via NotI into pBNX227, a transfer vector encoding IGR88/89 MVA-BN homologous regions, thus allowing insertion into of the targeted region (IGR 88/89) of MVA-BN via homologous recombination. Moreover, pBNX227 encodes mRFP1 and ecogpt for positive selection, which are flanked by two loxP sites for later excision of the selection cassette in the absence of selective pressure following transfection with a plasmid encoding the CRE-recombinase, which catalyzes the precise excision of nucleic acid sequences flanked by their target sequence loxP.

The MVA based vectors were generated in primary chicken fibroblasts (CEF) and produced as described herein. The CEF cells were isolated weekly from chicken embryos and maintained in VP-SFM medium without FBS. Briefly, CEF cells were transfected with MVA vector plasmid, using Fugene according to the instructions provided by the manufacturer (Promega) and a coinfection with MVA-BN was performed. Cells were harvested after two or three days, sonified and further passaged. The virus was plaque purified in CEF cells cultured in a multi-well 96-tissue culture plate following amplification within a single well of a multi-well 12-tissue culture plate. Further amplification was carried out in CEF cells cultured in a single well of a multiwell 6-tissue plate and subsequently in a T175 tissue culture flask.

The MVA-mBN414 is thus an MVA-BN comprising in its IGR 44/45 region a nucleic acid encoding mos1GagPol (SEQ ID NO: 28) under control of a PrMVA13.5long promoter (SEQ ID NO: 42) and a nucleic acid encoding mos1Env (SEQ ID NO: 5) under control of a PrHyb promoter (SEQ ID NO: 43). In the IGR 88/89 region there is a nucleic acid encoding the mos2GagPol (SEQ ID NO: 29) under control of a PrMVA13.5long promoter (SEQ ID NO: 42) and a nucleic acid encoding mos2SEnv (SEQ ID NO: 18) under control of a PrHyb promoter (SEQ ID NO: 43). The MVA-mBN414 vector was used in subsequent experiments in prime-boost regimens with adenovirus vectors encoding antigens described herein.

Example 8: Immunogenicity of MVA-mBN414 in Rabbits

The immunogenicity of MVA-mBN414 (see Example 7) in New Zealand White (NZW) rabbits was assessed in the context of Ad26.Mos.HIV (a trivalent vaccine having 3 Ad26 vectors together encoding HIV antigens having SEQ ID NOs: 1, 2, 3, 4 and 5; in the form of antigens mos1GagPol (SEQ ID NO: 28), mos2GagPol (SEQ ID NO: 29), and mos1Env (SEQ ID NO: 5)) prime, and in comparison to homologous prime-boost with Ad26.Mos.HIV. In addition, the added benefit of co-application of clade C gp140 protein (adjuvanted with aluminum phosphate) at day 42 and day 62 (injection into contra-lateral muscles (i.e., in separate limbs)) was assessed.

The immunization schedule that was used is provided in the following Table 2:

TABLE-US-00002 TABLE 2 Immunization schedule for immunogenicity study in rabbits Immunization day Immunization day N N Group 0 + 22 43 + 64 (female) (male) 1 Ad26.Mos.HIV Ad26.Mos.HIV 7 7 5 .times. 10.sup.10vp 5 .times. 10.sup.10vp 2 MVA-mBN414 7 7 1.8 .times. 10.sup.8 TCID.sub.50 3 MVA-mBN414 7 7 1.8 .times. 10.sup.8 TCID.sub.50 + clade C gp140 250 .mu.g in AdjuPhos .RTM. 425 .mu.g 4 Ad26.Mos.HIV MVA-mBN414 7 7 5 .times. 10.sup.9vp 1.8 .times. 10.sup.7 TCID.sub.50 + clade C gp140 25 .mu.g in AdjuPhos .RTM. 42.5 .mu.g 5 Ad26.Empty BN-MVA.Empty 3 3 5 .times. 10.sup.9vp 1.8 .times. 10.sup.8 TCID.sub.50

The readout assays were a clade C (aa residues 30-708 of SEQ ID NO: 7) and mosaic gp140 (aa residues 30-724 of SEQ ID NO: 36) ELISA, and an HIV-1 pseudovirus neutralization assay. Neutralization capacity of sera from immunized rabbits was tested against HIV-1 ENV pseudotyped virus particles (EPVs) by inhibition of entry to TZM-bl cells. TZM-bl cells express high levels of CD4 and the co-receptors CCR5 and CXCR4 and contain an integrated tat-responsive Luciferase reporter gene under control of an HIV long-terminal repeat sequence. A neutralizing effect of serum-containing HIV-1 Env antibodies against the EPV's results in reduced luciferase expression and thereby a reduced luminescence signal in combination with the luciferin containing substrate. Serum or antibodies were tested in a three-fold serial dilution over 6 steps, starting at 1/20. The maximal luciferase expression was measured by adding only cells+EPVs within one well without serum or antibodies. The background luciferase expression was measured by adding only cells to a well, without serum/antibodies and EPVs. A non-linear 4 parameter curve was fitted between the maximal and background luciferase signal and log 10-transformed IC.sub.50 values were determined as reportable value.

The results are shown in FIG. 9.

The results show that MVA-mBN414 was immunogenic in all rabbits. There was no obvious difference in immunogenicity between male and female animals. The MVA boost in the context of Ad26 prime induced an increase in the HIV-specific humoral immune response (measurable in clade C ELISA, clade B-like Mosaic ELISA and in clade B VNA) compared to homologous Ad26 prime-boost.

Co-administration of clade C gp140 protein with MVA as a boost induced an increase in (homologous) clade C gp140 ELISA and (heterologous) Mosaic gp140 ELISA titers compared to a boost with MVA only.

Example 9: Immunogenicity of MVA-mBN414 in Mice

The immunogenicity of MVA-mBN414 was also evaluated in CBF1 mice, with the aim to assess the immunogenicity of a heterologous Ad26.Mos4.HIV (see Example 6) prime and MVA-mBN414 (see Example 7) boost, in comparison to a homologous MVA prime-boost (i.e. both priming and boosting with MVA-mBN414).

The immunization schedule that was used is provided in the following Table 3:

TABLE-US-00003 TABLE 3 Immunization schedule for immunogenicity study in rabbits Week 0 Week 5 Group Test article: Dose: Test article: Dose: N= 1 Ad26.Mos4.HIV 2.5 .times. 10.sup.9 MVA-mBN414 2.8 .times. 10.sup.6 7 total vp TCID.sub.50 2 2.5 .times. 10.sup.8 2.8 .times. 10.sup.5 7 total vp TCID.sub.50 3 MVA-mBN414 2.8 .times. 10.sup.6 MVA-mBN414 2.8 .times. 10.sup.6 7 TCID.sub.50 TCID.sub.50 4 2.8 .times. 10.sup.5 2.8 .times. 10.sup.5 7 TCID.sub.50 TCID.sub.50 5 Ad26.Empty 2 .times. 10.sup.9 MVA BN- 2.8 .times. 10.sup.6 5 total vp empty TCID.sub.50

The readout assays for humoral immune responses (antigen-specific IgG measurement at weeks 5 and 7) was a mosaic gp140 (aa residues 30-724 of SEQ ID NO: 36) ELISA (see example 8). The readout assay for cellular immune responses (at week 7) was an IFN-.gamma. ELISPOT (assay as described in Khan et al, Int J Cancer, 2017, Mar. 6, doi: 10.1002/ijc.30679. [Epub ahead of print]; 2017, Jul. 14, 141(2), 393-404), using Env, Gag and Pol immunodominant peptides as stimuli.

The results of the ELISPOT are shown in FIG. 10. An intracellular cytokine staining (ICS) assay was also performed, which gave similar results (not shown).

The results indicate that priming with Ad26 or MVA induced detectable immune responses at week 5 (FIG. 10A), that are further increased by boost immunization with MVA at week 7 (FIG. 10B) for both, the heterologous Ad-MVA regimen and the homologous MVA-MVA regimen. Heterologous Ad-MVA prime-boost induced significantly higher ELISA titers than the homologous MVA-MVA prime-boost regimen. This was also observed for cellular immune responses measured by ELISPOT against the antigens Env, Gag and Pol at week 7 (FIG. 10C-E). Homologous MVA prime-boost immunization induced a low but detectable cellular immune response against the antigens Gag and Pol that is clearly differentiable from control data.

All in all, the results show that an MVA with HIV antigens according to the invention is immunogenic. In addition, it is shown that such a vector can advantageously be used in prime-boost regimens with adenoviral vectors encoding HIV antigens, and/or with isolated HIV gp140 proteins.

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SEQUENCE LISTINGS

1

461500PRTArtificial Sequencemos1Gag mosaic antigen sequence 1Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp1 5 10 15Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Arg Leu Lys 20 25 30His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro 35 40 45Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu 50 55 60Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn65 70 75 80Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp 85 90 95Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys 100 105 110Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly Asn Ser Ser Gln Val 115 120 125Ser Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His 130 135 140Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu145 150 155 160Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser 165 170 175Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly 180 185 190Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu 195 200 205Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala 210 215 220Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr225 230 235 240Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile 245 250 255Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys 260 265 270Ile Val Arg Met Tyr Ser Pro Val Ser Ile Leu Asp Ile Arg Gln Gly 275 280 285Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu 290 295 300Arg Ala Glu Gln Ala Ser Gln Asp Val Lys Asn Trp Met Thr Glu Thr305 310 315 320Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala 325 330 335Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly 340 345 350Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser 355 360 365Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg 370 375 380Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His385 390 395 400Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys 405 410 415Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn 420 425 430Phe Leu Gly Lys Ile Trp Pro Ser Asn Lys Gly Arg Pro Gly Asn Phe 435 440 445Leu Gln Asn Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg 450 455 460Phe Gly Glu Glu Thr Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp465 470 475 480Lys Glu Met Tyr Pro Leu Ala Ser Leu Lys Ser Leu Phe Gly Asn Asp 485 490 495Pro Ser Ser Gln 5002491PRTArtificial Sequencemos2Gag mosaic antigen sequence 2Met Gly Ala Arg Ala Ser Ile Leu Arg Gly Gly Lys Leu Asp Lys Trp1 5 10 15Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys His Tyr Met Leu Lys 20 25 30His Leu Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Leu Asn Pro 35 40 45Gly Leu Leu Glu Thr Ser Glu Gly Cys Lys Gln Ile Ile Lys Gln Leu 50 55 60Gln Pro Ala Leu Gln Thr Gly Thr Glu Glu Leu Arg Ser Leu Phe Asn65 70 75 80Thr Val Ala Thr Leu Tyr Cys Val His Ala Glu Ile Glu Val Arg Asp 85 90 95Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Gln 100 105 110Gln Lys Thr Gln Gln Ala Lys Glu Ala Asp Gly Lys Val Ser Gln Asn 115 120 125Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Pro Ile 130 135 140Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala145 150 155 160Phe Ser Pro Glu Val Ile Pro Met Phe Thr Ala Leu Ser Glu Gly Ala 165 170 175Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln 180 185 190Ala Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu 195 200 205Trp Asp Arg Leu His Pro Val His Ala Gly Pro Val Ala Pro Gly Gln 210 215 220Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Asn Leu225 230 235 240Gln Glu Gln Ile Ala Trp Met Thr Ser Asn Pro Pro Ile Pro Val Gly 245 250 255Asp Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg 260 265 270Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu 275 280 285Pro Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala Glu 290 295 300Gln Ala Thr Gln Asp Val Lys Asn Trp Met Thr Asp Thr Leu Leu Val305 310 315 320Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Arg Ala Leu Gly Pro 325 330 335Gly Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly 340 345 350Pro Ser His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Thr Asn 355 360 365Ser Thr Ile Leu Met Gln Arg Ser Asn Phe Lys Gly Ser Lys Arg Ile 370 375 380Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn Cys385 390 395 400Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His 405 410 415Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile 420 425 430Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro 435 440 445Glu Pro Thr Ala Pro Pro Ala Glu Ser Phe Arg Phe Glu Glu Thr Thr 450 455 460Pro Ala Pro Lys Gln Glu Pro Lys Asp Arg Glu Pro Leu Thr Ser Leu465 470 475 480Arg Ser Leu Phe Gly Ser Asp Pro Leu Ser Gln 485 4903850PRTArtificial Sequencemos1Pol mosaic antigen sequence 3Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro1 5 10 15Gly Met Asp Gly Pro Arg Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 20 25 30Ile Lys Ala Leu Thr Ala Ile Cys Glu Glu Met Glu Lys Glu Gly Lys 35 40 45Ile Thr Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala 50 55 60Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg65 70 75 80Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile 85 90 95Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala 100 105 110Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Gly Phe Arg Lys 115 120 125Tyr Thr Ala Phe Thr Ile Pro Ser Thr Asn Asn Glu Thr Pro Gly Ile 130 135 140Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala145 150 155 160Ile Phe Gln Cys Ser Met Thr Arg Ile Leu Glu Pro Phe Arg Ala Lys 165 170 175Asn Pro Glu Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly 180 185 190Ser Asp Leu Glu Ile Gly Gln His Arg Ala Lys Ile Glu Glu Leu Arg 195 200 205Glu His Leu Leu Lys Trp Gly Phe Thr Thr Pro Asp Lys Lys His Gln 210 215 220Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys225 230 235 240Trp Thr Val Gln Pro Ile Gln Leu Pro Glu Lys Asp Ser Trp Thr Val 245 250 255Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 260 265 270Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Ala 275 280 285Lys Ala Leu Thr Asp Ile Val Pro Leu Thr Glu Glu Ala Glu Leu Glu 290 295 300Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr305 310 315 320Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly His 325 330 335Asp Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 340 345 350Thr Gly Lys Tyr Ala Lys

Met Arg Thr Ala His Thr Asn Asp Val Lys 355 360 365Gln Leu Thr Glu Ala Val Gln Lys Ile Ala Met Glu Ser Ile Val Ile 370 375 380Trp Gly Lys Thr Pro Lys Phe Arg Leu Pro Ile Gln Lys Glu Thr Trp385 390 395 400Glu Thr Trp Trp Thr Asp Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp 405 410 415Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 420 425 430Lys Asp Pro Ile Ala Gly Val Glu Thr Phe Tyr Val Ala Gly Ala Ala 435 440 445Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asp Arg Gly 450 455 460Arg Gln Lys Ile Val Ser Leu Thr Glu Thr Thr Asn Gln Lys Thr Ala465 470 475 480Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Ser Glu Val Asn 485 490 495Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro 500 505 510Asp Lys Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile 515 520 525Lys Lys Glu Arg Val Tyr Leu Ser Trp Val Pro Ala His Lys Gly Ile 530 535 540Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ser Gly Ile Arg Lys545 550 555 560Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Glu Glu His Glu Lys 565 570 575Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro 580 585 590Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Gln Cys Gln Leu Lys 595 600 605Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln 610 615 620Leu Ala Cys Thr His Leu Glu Gly Lys Ile Ile Leu Val Ala Val His625 630 635 640Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly 645 650 655Gln Glu Thr Ala Tyr Phe Ile Leu Lys Leu Ala Gly Arg Trp Pro Val 660 665 670Lys Val Ile His Thr Ala Asn Gly Ser Asn Phe Thr Ser Ala Ala Val 675 680 685Lys Ala Ala Cys Trp Trp Ala Gly Ile Gln Gln Glu Phe Gly Ile Pro 690 695 700Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu705 710 715 720Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr 725 730 735Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 740 745 750Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Ile Asp Ile Ile Ala Thr 755 760 765Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Ile Lys Ile Gln Asn 770 775 780Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asp Pro Ile Trp Lys Gly Pro785 790 795 800Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn 805 810 815Ser Asp Ile Lys Val Val Pro Arg Arg Lys Val Lys Ile Ile Lys Asp 820 825 830Tyr Gly Lys Gln Met Ala Gly Ala Asp Cys Val Ala Gly Arg Gln Asp 835 840 845Glu Asp 8504850PRTArtificial Sequencemos2Pol mosaic antigen sequence 4Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro1 5 10 15Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 20 25 30Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys 35 40 45Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Ile Phe Ala 50 55 60Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg65 70 75 80Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile 85 90 95Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala 100 105 110Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys 115 120 125Tyr Thr Ala Phe Thr Ile Pro 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Phe Lys Asn Leu Lys 340 345 350Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys 355 360 365Gln Leu Thr Glu Ala Val Gln Lys Ile Ala Thr Glu Ser Ile Val Ile 370 375 380Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp385 390 395 400Glu Ala Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp 405 410 415Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 420 425 430Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala 435 440 445Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asp Arg Gly 450 455 460Arg Gln Lys Val Val Ser Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala465 470 475 480Leu Gln Ala Ile His Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn 485 490 495Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro 500 505 510Asp Lys Ser Glu Ser Glu Leu Val Ser Gln Ile Ile Glu Gln Leu Ile 515 520 525Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile 530 535 540Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Arg Gly Ile Arg Lys545 550 555 560Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Glu Glu His Glu Lys 565 570 575Tyr His Ser Asn Trp Arg Ala Met Ala Ser Glu Phe Asn Leu Pro Pro 580 585 590Ile Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 595

600 605Gly Glu Ala Ile His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln 610 615 620Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His625 630 635 640Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly 645 650 655Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val 660 665 670Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Ser Ala Thr Val 675 680 685Lys Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro 690 695 700Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Ile Asn Lys Glu Leu705 710 715 720Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr 725 730 735Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 740 745 750Ile Gly Glu Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Ser 755 760 765Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn 770 775 780Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asp Pro Leu Trp Lys Gly Pro785 790 795 800Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn 805 810 815Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp 820 825 830Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp 835 840 845Glu Asp 8505685PRTArtificial Sequencemos1Env mosaic antigen sequence 5Met Arg Val Thr Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg Trp1 5 10 15Gly Thr Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala Ala Gly Lys 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Val Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp 100 105 110Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Asn Cys Thr Asp Asp Val Arg Asn Val Thr Asn Asn Ala Thr Asn Thr 130 135 140Asn Ser Ser Trp Gly Glu Pro Met Glu Lys Gly Glu Ile Lys Asn Cys145 150 155 160Ser Phe

Asn Ile Thr Thr Ser Ile Arg Asn Lys Val Gln Lys Gln Tyr 165 170 175Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asp Ser Asn 180 185 190Asn Thr Asn Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln 195 200 205Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala 210 215 220Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Lys Phe Asn Gly225 230 235 240Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile 245 250 255Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu 260 265 270Glu Glu Val Val Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr 275 280 285Ile Met Val Gln Leu Asn Val Ser Val Glu Ile Asn Cys Thr Arg Pro 290 295 300Asn Asn Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe305 310 315 320Tyr Thr Ala Gly Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn 325 330 335Ile Ser Arg Ala Asn Trp Asn Asn Thr Leu Arg Gln Ile Val Glu Lys 340 345 350Leu Gly Lys Gln Phe Gly Asn Asn Lys Thr Ile Val Phe Asn His Ser 355 360 365Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly 370 375 380Glu Phe Phe Tyr Cys Asn Ser Thr Lys Leu Phe Asn Ser Thr Trp Thr385 390 395 400Trp Asn Asn Ser Thr Trp Asn Asn Thr Lys Arg Ser Asn Asp Thr Glu 405 410 415Glu His Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp 420 425 430Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile 435 440 445Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly 450 455 460Asn Asp Thr Ser Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met465 470 475 480Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile 485 490 495Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln 500 505 510Ser Glu Lys Ser Ala Val Gly Ile Gly Ala Val Phe Leu Gly Phe Leu 515 520 525Gly Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Met Thr Leu Thr Val 530 535 540Gln Ala Arg Leu Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu545 550 555 560Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp 565 570 575Gly Ile Lys Gln Leu Gln Ala Arg Val Leu Ala Val Glu Arg Tyr Leu 580 585 590Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile 595 600 605Cys Thr Thr Thr Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu 610 615 620Asp Lys Ile Trp Asn Asn Met Thr Trp Met Glu Trp Glu Arg Glu Ile625 630 635 640Asn Asn Tyr Thr Ser Leu Ile Tyr Thr Leu Ile Glu Glu Ser Gln Asn 645 650 655Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala 660 665 670Ser Leu Trp Asn Trp Phe Asp Ile Ser Asn Trp Leu Trp 675 680 6856684PRTArtificial Sequencemos2Env mosaic antigen sequence 6Met Arg Val Arg Gly Ile Gln Arg Asn Trp Pro Gln Trp Trp Ile Trp1 5 10 15Gly Ile Leu Gly Phe Trp Met Ile Ile Ile Cys Arg Val Met Gly Asn 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Lys 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Met Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Arg Leu Trp Asp 100 105 110Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Glu Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr Tyr Asn Ile 130 135 140Ile His Asn Glu Thr Tyr Lys Glu Met Lys Asn Cys Ser Phe Asn Ala145 150 155 160Thr Thr Val Val Glu Asp Arg Lys Gln Lys Val His Ala Leu Phe Tyr 165 170 175Arg Leu Asp Ile Val Pro Leu Asp Glu Asn Asn Ser Ser Glu Lys Ser 180 185 190Ser Glu Asn Ser Ser Glu Tyr Tyr Arg Leu Ile Asn Cys Asn Thr Ser 195 200 205Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp Pro Ile Pro Ile 210 215 220His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys Cys Asn Asn Lys225 230 235 240Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys 245 250 255Thr His Gly Ile Lys Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly 260 265 270Ser Leu Ala Glu Glu Glu Ile Ile Ile Arg Ser Glu Asn Leu Thr Asn 275 280 285Asn Ala Lys Thr Ile Ile Val His Leu Asn Glu Thr Val Asn Ile Thr 290 295 300Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro305 310 315 320Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln 325 330 335Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr Leu Gln Gly 340 345 350Val Lys Lys Lys Leu Ala Glu His Phe Pro Asn Lys Thr Ile Asn Phe 355 360 365Thr Ser Ser Ser Gly Gly Asp Leu Glu Ile Thr Thr His Ser Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Gly Leu Phe Asn Gly385 390 395 400Thr Tyr Met Pro Asn Gly Thr Asn Ser Asn Ser Ser Ser Asn Ile Thr 405 410 415Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly 420 425 430Arg Ala Met Tyr Ala Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser 435 440 445Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly 450 455 460Val Pro Asn Asp Thr Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg465 470 475 480Asn Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys 485 490 495Pro Leu Gly Val Ala Pro Thr Glu Ala Lys Arg Arg Val Val Glu Ser 500 505 510Glu Lys Ser Ala Val Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly 515 520 525Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln 530 535 540Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu545 550 555 560Arg Ala Ile Glu Ala Gln Gln His Met Leu Gln Leu Thr Val Trp Gly 565 570 575Ile Lys Gln Leu Gln Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln 580 585 590Asp Gln Gln Leu Leu Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys 595 600 605Thr Thr Ala Val Pro Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr 610 615 620Asp Ile Trp Asp Asn Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly625 630 635 640Asn Tyr Thr Gly Glu Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln 645 650 655Gln Glu Lys Asn Glu Lys Asp Leu Leu Ala Leu Asp Ser Trp Lys Asn 660 665 670Leu Trp Asn Trp Phe Asp Ile Thr Asn Trp Leu Trp 675 6807708PRTArtificial Sequencestabilized clade C gp140 trimer C97ZA012- gp140-foldon with cleavage mutations 7Met Arg Val Arg Gly Ile Gln Arg Asn Cys Gln His Leu Trp Arg Trp1 5 10 15Gly Thr Leu Ile Leu Gly Met Leu Met Ile Cys Ser Ala Ala Glu Asn 20 25 30Leu Trp Val Gly Asn Met Trp Val Thr Val Tyr Tyr Gly Val Pro Val 35 40 45Trp Thr Asp Ala Lys Thr Thr Leu Phe Cys Ala Ser Asp Thr Lys Ala 50 55 60Tyr Asp Arg Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro65 70 75 80Thr Asp Pro Asn Pro Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn 85 90 95Phe Asn Met Trp Lys Asn Asp Met Val Asp Gln Met His Glu Asp Ile 100 105 110Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro 115 120 125Leu Cys Val Thr Leu His Cys Thr Asn Ala Thr Phe Lys Asn Asn Val 130 135 140Thr Asn Asp Met Asn Lys Glu Ile Arg Asn Cys Ser Phe Asn Thr Thr145 150 155 160Thr Glu Ile Arg Asp Lys Lys Gln Gln Gly Tyr Ala Leu Phe Tyr Arg 165 170 175Pro Asp Ile Val Leu Leu Lys Glu Asn Arg Asn Asn Ser Asn Asn Ser 180 185 190Glu Tyr Ile Leu Ile Asn Cys Asn Ala Ser Thr Ile Thr Gln Ala Cys 195 200 205Pro Lys Val Asn Phe Asp Pro Ile Pro Ile His Tyr Cys Ala Pro Ala 210 215 220Gly Tyr Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Ser Gly Lys Gly225 230 235 240Pro Cys Asn Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Lys Pro 245 250 255Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Lys Glu 260 265 270Ile Ile Ile Arg Ser Glu Asn Leu Thr Asp Asn Val Lys Thr Ile Ile 275 280 285Val His Leu Asn Lys Ser Val Glu Ile Val Cys Thr Arg Pro Asn Asn 290 295 300Asn Thr Arg Lys Ser Met Arg Ile Gly Pro Gly Gln Thr Phe

Tyr Ala305 310 315 320Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln Ala Tyr Cys Asn Ile Ser 325 330 335Gly Ser Lys Trp Asn Glu Thr Leu Lys Arg Val Lys Glu Lys Leu Gln 340 345 350Glu Asn Tyr Asn Asn Asn Lys Thr Ile Lys Phe Ala Pro Ser Ser Gly 355 360 365Gly Asp Leu Glu Ile Thr Thr His Ser Phe Asn Cys Arg Gly Glu Phe 370 375 380Phe Tyr Cys Asn Thr Thr Arg Leu Phe Asn Asn Asn Ala Thr Glu Asp385 390 395 400Glu Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp 405 410 415Gln Gly Val Gly Arg Ala Met Tyr Ala Pro Pro Ile Ala Gly Asn Ile 420 425 430Thr Cys Lys Ser Asn Ile Thr Gly Leu Leu Leu Val Arg Asp Gly Gly 435 440 445Glu Asp Asn Lys Thr Glu Glu Ile Phe Arg Pro Gly Gly Gly Asn Met 450 455 460Lys Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Ile Glu Leu465 470 475 480Lys Pro Leu Gly Ile Ala Pro Thr Gly Ala Lys Glu Arg Val Val Glu 485 490 495Arg Glu Glu Arg Ala Val Gly Ile Gly Ala Val Phe Leu Gly Phe Leu 500 505 510Gly Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val 515 520 525Gln Ala Arg Gln Leu Leu Ser Ser Ile Val Gln Gln Gln Ser Asn Leu 530 535 540Leu Arg Ala Ile Glu Ala Gln Gln His Met Leu Gln Leu Thr Val Trp545 550 555 560Gly Ile Lys Gln Leu Gln Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu 565 570 575Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile 580 585 590Cys Thr Thr Asn Val Pro Trp Asn Ser Ser Trp Ser Asn Lys Ser Gln 595 600 605Thr Asp Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile 610 615 620Ser Asn Tyr Thr Asp Thr Ile Tyr Arg Leu Leu Glu Asp Ser Gln Thr625 630 635 640Gln Gln Glu Lys Asn Glu Lys Asp Leu Leu Ala Leu Asp Ser Trp Lys 645 650 655Asn Leu Trp Ser Trp Phe Asp Ile Ser Asn Trp Leu Trp Tyr Ile Lys 660 665 670Ser Arg Ile Glu Gly Arg Gly Ser Gly Gly Tyr Ile Pro Glu Ala Pro 675 680 685Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp Gly Glu Trp Val Leu Leu 690 695 700Ser Thr Phe Leu7058625PRTArtificial SequenceC4 fragment gp120-truncated gp41 without signal peptide and transmembrane domain 8Met Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys1 5 10 15Asp Ala Lys Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu 20 25 30Lys Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp 35 40 45Pro Asn Pro Gln Glu Ile Val Leu Gly Asn Val Thr Glu Asn Phe Asn 50 55 60Met Trp Lys Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Ser65 70 75 80Leu Trp Asp Ala Ser Leu Glu Pro Cys Val Lys Leu Thr Pro Leu Cys 85 90 95Val Thr Leu Asn Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr

100 105 110Tyr Asn Ile Ile His Asn Glu Thr Tyr Lys Glu Met Lys Asn Cys Ser 115 120 125Phe Asn Ala Thr Thr Val Val Glu Asp Arg Lys Gln Lys Val His Ala 130 135 140Leu Phe Tyr Arg Leu Asp Ile Val Pro Leu Asp Glu Asn Asn Ser Ser145 150 155 160Glu Lys Ser Ser Glu Asn Ser Ser Glu Tyr Tyr Arg Leu Ile Asn Cys 165 170 175Asn Thr Ser Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp Pro 180 185 190Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys Cys 195 200 205Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser Thr 210 215 220Val Gln Cys Thr His Gly Ile Lys Pro Val Val Ser Thr Gln Leu Leu225 230 235 240Leu Asn Gly Ser Leu Ala Glu Glu Glu Ile Ile Ile Arg Ser Glu Asn 245 250 255Leu Thr Asn Asn Ala Lys Thr Ile Ile Val His Leu Asn Glu Thr Val 260 265 270Asn Ile Thr Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg 275 280 285Ile Gly Pro Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp 290 295 300Ile Arg Gln Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr305 310 315 320Leu Gln Gly Val Lys Lys Lys Leu Ala Glu His Phe Pro Asn Lys Thr 325 330 335Ile Lys Phe Ala Pro His Ser Gly Gly Asp Leu Glu Ile Thr Thr His 340 345 350Thr Phe Asn Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Asn Leu 355 360 365Phe Asn Glu Ser Asn Ile Glu Arg Asn Asp Ser Ile Ile Thr Leu Pro 370 375 380Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Arg Ala385 390 395 400Ile Tyr Ala Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser Asn Ile 405 410 415Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly Val Pro 420 425 430Asn Asp Thr Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg Asn Asn 435 440 445Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys Pro Leu 450 455 460Gly Val Ala Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg Glu Lys465 470 475 480Arg Ala Val Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly Ala Ala 485 490 495Gly Ser Thr Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln Ala Arg 500 505 510Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu Arg Ala 515 520 525Ile Glu Ala Gln Gln His Met Leu Gln Leu Thr Val Trp Gly Ile Lys 530 535 540Gln Leu Gln Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln Asp Gln545 550 555 560Gln Leu Leu Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr 565 570 575Ala Val Pro Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr Asp Ile 580 585 590Trp Asp Asn Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly Asn Tyr 595 600 605Thr Gly Glu Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln Gln Glu 610 615 620Lys625929PRTArtificial Sequencesignal sequence 9Met Arg Val Arg Gly Met Leu Arg Asn Trp Gln Gln Trp Trp Ile Trp1 5 10 15Ser Ser Leu Gly Phe Trp Met Leu Met Ile Tyr Ser Val 20 251029PRTArtificial Sequencesignal sequence 10Met Arg Val Thr Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg Trp1 5 10 15Gly Thr Met Leu Leu Gly Ile Leu Met Ile Cys Ser Ala 20 251129PRTArtificial Sequencesignal sequence 11Met Arg Val Arg Gly Ile Gln Arg Asn Trp Pro Gln Trp Trp Ile Trp1 5 10 15Gly Ile Leu Gly Phe Trp Met Ile Ile Ile Cys Arg Val 20 251229PRTArtificial Sequencesignal sequence 12Met Arg Val Arg Gly Ile Gln Arg Asn Cys Gln His Leu Trp Arg Trp1 5 10 15Gly Thr Leu Ile Leu Gly Met Leu Met Ile Cys Ser Ala 20 251322PRTArtificial Sequencetransmembrane domain 13Ile Phe Ile Met Ile Val Gly Gly Leu Ile Gly Leu Arg Ile Ile Phe1 5 10 15Ala Val Leu Ser Ile Val 20147PRTArtificial Sequencetruncated cytoplasmic region 14Asn Arg Val Arg Gln Gly Tyr1 51532PRTArtificial SequenceGCN4 trimerization domain 15Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr1 5 10 15His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Val 20 25 301630PRTArtificial Sequencefoldon trimerization domain 16Gly Ser Gly Gly Tyr Ile Pro Glu Ala Pro Arg Asp Gly Gln Ala Tyr1 5 10 15Val Arg Lys Asp Gly Glu Trp Val Leu Leu Ser Thr Phe Leu 20 25 3017862PRTArtificial SequenceC4 sequence 17Met Arg Val Arg Gly Met Leu Arg Asn Trp Gln Gln Trp Trp Ile Trp1 5 10 15Ser Ser Leu Gly Phe Trp Met Leu Met Ile Tyr Ser Val Met Gly Asn 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Asp Ala Lys 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Ile Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp 100 105 110Ala Ser Leu Glu Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Asn Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr Tyr Asn Ile 130 135 140Ile His Asn Glu Thr Tyr Lys Glu Met Lys Asn Cys Ser Phe Asn Ala145 150 155 160Thr Thr Val Val Glu Asp

Arg Lys Gln Lys Val His Ala Leu Phe Tyr 165 170 175Arg Leu Asp Ile Val Pro Leu Asp Glu Asn Asn Ser Ser Glu Lys Ser 180 185 190Ser Glu Asn Ser Ser Glu Tyr Tyr Arg Leu Ile Asn Cys Asn Thr Ser 195 200 205Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp Pro Ile Pro Ile 210 215 220His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys Cys Asn Asn Lys225 230 235 240Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys 245 250 255Thr His Gly Ile Lys Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly 260 265 270Ser Leu Ala Glu Glu Glu Ile Ile Ile Arg Ser Glu Asn Leu Thr Asn 275 280 285Asn Ala Lys Thr Ile Ile Val His Leu Asn Glu Thr Val Asn Ile Thr 290 295 300Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro305 310 315 320Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln 325 330 335Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr Leu Gln Gly 340 345 350Val Lys Lys Lys Leu Ala Glu His Phe Pro Asn Lys Thr Ile Lys Phe 355 360 365Ala Pro His Ser Gly Gly Asp Leu Glu Ile Thr Thr His Thr Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Asn Leu Phe Asn Glu385 390 395 400Ser Asn Ile Glu Arg Asn Asp Ser Ile Ile Thr Leu Pro Cys Arg Ile 405 410 415Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Arg Ala Ile Tyr Ala 420 425 430Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser Asn Ile Thr Gly Leu 435 440 445Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly Val Pro Asn Asp Thr 450 455 460Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg Asn Asn Trp Arg Ser465 470 475 480Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys Pro Leu Gly Val Ala 485 490 495Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg Glu Lys Arg Ala Val 500 505 510Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly Ala Ala Gly Ser Thr 515 520 525Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln Ala Arg Gln Leu Leu 530 535 540Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu Arg Ala Ile Glu Ala545 550 555 560Gln Gln His Met Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln 565 570 575Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln Asp Gln Gln Leu Leu 580 585 590Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro 595 600 605Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr Asp Ile Trp Asp Asn 610 615 620Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly Asn Tyr Thr Gly Glu625 630 635 640Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu 645 650 655Lys Asp Leu Leu Ala Leu Asp Ser Trp Asn Asn Leu Trp Asn Trp Phe 660 665 670Ser Ile Ser Lys Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val 675 680 685Gly Gly Leu Ile Gly Leu Arg Ile Ile Phe Ala Val Leu Ser Ile Val 690 695 700Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Leu Gln Thr Leu Thr705 710 715 720Gln Asn Pro Gly Gly Leu Asp Arg Leu Gly Arg Ile Glu Glu Glu Gly 725 730 735Gly Glu Gln Asp Lys Asp Arg Ser Ile Arg Leu Val Asn Gly Phe Phe 740 745 750Ala Leu Phe Trp Asp Asp Leu Arg Ser Leu Cys Leu Phe Ser Tyr His 755 760 765Arg Leu Arg Asp Phe Ile Leu Ile Val Ala Arg Ala Val Glu Leu Leu 770 775 780Gly Arg Ser Ser Leu Arg Gly Leu Gln Arg Gly Trp Glu Ile Leu Lys785 790 795 800Tyr Leu Gly Ser Leu Leu Gln Tyr Trp Gly Leu Glu Leu Lys Lys Ser 805 810 815Ala Ile Asn Leu Leu Asp Thr Ile Ala Ile Ala Val Ala Glu Gly Thr 820 825 830Asp Arg Ile Ile Glu Leu Ile Gln Arg Ile Cys Arg Ala Ile Cys Asn 835 840 845Ile Pro Arg Arg Ile Arg Gln Gly Phe Glu Ala Ala Leu Gln 850 855 86018711PRTArtificial SequenceC4D7 sequence 18Met Arg Val Arg Gly Met Leu Arg Asn Trp Gln Gln Trp Trp Ile Trp1 5 10 15Ser Ser Leu Gly Phe Trp Met Leu Met Ile Tyr Ser Val Met Gly Asn 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Asp Ala Lys 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Ile Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp 100 105 110Ala Ser Leu Glu Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Asn Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr Tyr Asn Ile 130 135 140Ile 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Ala Glu His Phe Pro Asn Lys Thr Ile Lys Phe 355 360 365Ala Pro His Ser Gly Gly Asp Leu Glu Ile Thr Thr His Thr Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Asn Leu Phe Asn Glu385 390 395 400Ser Asn Ile Glu Arg Asn Asp Ser Ile Ile Thr Leu Pro Cys Arg Ile 405 410 415Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Arg Ala Ile Tyr Ala 420 425 430Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser Asn Ile Thr Gly Leu 435 440 445Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly Val Pro Asn Asp Thr 450 455 460Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg Asn Asn Trp Arg Ser465 470 475 480Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys Pro Leu Gly Val Ala 485 490 495Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg Glu Lys Arg Ala Val 500 505 510Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly Ala Ala Gly Ser Thr 515 520 525Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln Ala Arg Gln Leu Leu 530 535 540Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu Arg Ala Ile Glu Ala545 550 555 560Gln Gln His Met Leu Gln Leu Thr Val 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Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln 325 330 335Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr Leu Gln Gly 340 345 350Val Lys Lys Lys Leu Ala Glu His Phe Pro Asn Lys Thr Ile Lys Phe 355 360 365Ala Pro His Ser Gly Gly Asp Leu Glu Ile Thr Thr His Thr Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Asn Leu Phe Asn Glu385 390 395 400Ser Asn Ile Glu Arg Asn Asp Ser Ile Ile Thr Leu Pro Cys Arg Ile 405 410 415Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Arg Ala Ile Tyr Ala 420 425 430Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser Asn Ile Thr Gly Leu 435 440 445Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly Val Pro Asn Asp Thr 450 455 460Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg Asn Asn Trp Arg Ser465 470 475 480Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys Pro Leu Gly Val Ala 485 490 495Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg Glu Glu Arg Ala Val 500 505 510Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly Ala Ala Gly Ser Thr 515 520 525Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln Ala Arg Gln Leu Leu 530 535 540Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu Arg Ala Ile Glu Ala545 550 555 560Gln Gln His Met Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln 565 570 575Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln Asp Gln Gln Leu Leu 580 585 590Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro 595 600 605Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr Asp Ile Trp Asp Asn 610 615 620Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly Asn Tyr Thr Gly Glu625 630 635 640Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln Gln Glu Lys Met Lys 645 650 655Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile 660 665 670Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Val Gly Ser 675 680 685Gly Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg 690 695 700204050DNAArtificial Sequencenucleotide sequence encoding mos1GagPol 20atgggagcca gagccagcgt gctgtccgga ggggagctgg accgctggga gaagatcagg 60ctgaggcctg agggaagaa gaagtacagg ctgaagcaca tcgtgtgggc 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gtcccagaac cagcaggaaa agatgaagca gatcgaggac 1980aagatcgaag agattctgag caagatctac cacatcgaga acgagatcgc ccgcatcaag 2040aaactgatcg gcgaagtggg atccggcgct cccacaaagg ccaaaagacg ggtggtgcag 2100cgcgagaaac gc 2112281350PRTArtificial Sequencemos1GagPol mosaic antigen sequence 28Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp1 5 10 15Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Arg Leu Lys 20 25 30His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro 35 40 45Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu 50 55 60Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn65 70 75 80Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp 85 90 95Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys 100 105 110Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly Asn Ser Ser Gln Val 115 120 125Ser Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His 130 135 140Gln Ala Ile Ser Pro

Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu145 150 155 160Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser 165 170 175Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly 180 185 190Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu 195 200 205Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala 210 215 220Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr225 230 235 240Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile 245 250 255Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys 260 265 270Ile Val Arg Met Tyr Ser Pro Val Ser Ile Leu Asp Ile Arg Gln Gly 275 280 285Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu 290 295 300Arg Ala Glu Gln Ala Ser Gln Asp Val Lys Asn Trp Met Thr Glu Thr305 310 315 320Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala 325 330 335Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly 340 345 350Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser 355 360 365Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg 370 375 380Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His385 390 395 400Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys 405 410 415Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn 420 425 430Phe Leu Gly Lys Ile Trp Pro Ser Asn Lys Gly Arg Pro Gly Asn Phe 435 440 445Leu Gln Asn Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg 450 455 460Phe Gly Glu Glu Thr Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp465 470 475 480Lys Glu Met Tyr Pro Leu Ala Ser Leu Lys Ser Leu Phe Gly Asn Asp 485 490 495Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val 500 505 510Lys Leu Lys Pro Gly Met Asp Gly Pro Arg Val Lys Gln Trp Pro Leu 515 520 525Thr Glu Glu Lys Ile Lys Ala Leu Thr Ala Ile Cys Glu Glu Met Glu 530 535 540Lys Glu Gly Lys Ile Thr Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr545 550 555 560Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu 565 570 575Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val 580 585 590Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val 595 600 605Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu 610 615 620Gly Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Thr Asn Asn Glu625 630 635 640Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys 645 650 655Gly Ser Pro Ala Ile Phe Gln Cys Ser Met Thr Arg Ile Leu Glu Pro 660 665 670Phe Arg Ala Lys Asn Pro Glu Ile Val Ile Tyr Gln Tyr Met Ala Ala 675 680 685Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Ala Lys Ile 690 695 700Glu Glu Leu Arg Glu His Leu Leu Lys Trp Gly Phe Thr Thr Pro Asp705 710 715 720Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu 725 730 735His Pro Asp Lys Trp Thr Val Gln Pro Ile Gln Leu Pro Glu Lys Asp 740 745 750Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp 755 760 765Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu 770 775 780Leu Arg Gly Ala Lys Ala Leu Thr Asp Ile Val Pro Leu Thr Glu Glu785 790 795 800Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val 805 810 815His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln 820 825 830Lys Gln Gly His Asp Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe 835 840 845Lys Asn Leu Lys Thr Gly Lys Tyr Ala Lys Met Arg Thr Ala His Thr 850 855 860Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Ala Met Glu865 870 875 880Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Arg Leu Pro Ile Gln 885 890 895Lys Glu Thr Trp Glu Thr Trp Trp Thr Asp Tyr Trp Gln Ala Thr Trp 900 905 910Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp 915 920 925Tyr Gln Leu Glu Lys Asp Pro Ile Ala Gly Val Glu Thr Phe Tyr Val 930 935 940Ala Gly Ala Ala Asn Arg Glu Thr

Lys Leu Gly Lys Ala Gly Tyr Val945 950 955 960Thr Asp Arg Gly Arg Gln Lys Ile Val Ser Leu Thr Glu Thr Thr Asn 965 970 975Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly 980 985 990Ser Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile 995 1000 1005Gln Ala Gln Pro Asp Lys Ser Glu Ser Glu Leu Val Asn Gln Ile 1010 1015 1020Ile Glu Gln Leu Ile Lys Lys Glu Arg Val Tyr Leu Ser Trp Val 1025 1030 1035Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu 1040 1045 1050Val Ser Ser Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp 1055 1060 1065Lys Ala Gln Glu Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala 1070 1075 1080Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile 1085 1090 1095Val Ala Ser Cys Asp Gln Cys Gln Leu Lys Gly Glu Ala Met His 1100 1105 1110Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr 1115 1120 1125His Leu Glu Gly Lys Ile Ile Leu Val Ala Val His Val Ala Ser 1130 1135 1140Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu 1145 1150 1155Thr Ala Tyr Phe Ile Leu Lys Leu Ala Gly Arg Trp Pro Val Lys 1160 1165 1170Val Ile His Thr Ala Asn Gly Ser Asn Phe Thr Ser Ala Ala Val 1175 1180 1185Lys Ala Ala Cys Trp Trp Ala Gly Ile Gln Gln Glu Phe Gly Ile 1190 1195 1200Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys 1205 1210 1215Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His 1220 1225 1230Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys 1235 1240 1245Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Ile 1250 1255 1260Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln 1265 1270 1275Ile Ile Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg 1280 1285 1290Asp Pro Ile Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu 1295 1300 1305Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro 1310 1315 1320Arg Arg Lys Val Lys Ile Ile Lys Asp Tyr Gly Lys Gln Met Ala 1325 1330 1335Gly Ala Asp Cys Val Ala Gly Arg Gln Asp Glu Asp 1340 1345 1350291341PRTArtificial Sequencemos2GagPol mosaic antigen sequence 29Met Gly Ala Arg Ala Ser Ile Leu Arg Gly Gly Lys Leu Asp Lys Trp1 5 10 15Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys His Tyr Met Leu Lys 20 25 30His Leu Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Leu Asn Pro 35 40 45Gly Leu Leu Glu Thr Ser Glu Gly Cys Lys Gln Ile Ile Lys Gln Leu 50 55 60Gln Pro Ala Leu Gln Thr Gly Thr Glu Glu Leu Arg Ser Leu Phe Asn65 70 75 80Thr Val Ala Thr Leu Tyr Cys Val His Ala Glu Ile Glu Val Arg Asp 85 90 95Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Gln 100 105 110Gln Lys Thr Gln Gln Ala Lys Glu Ala Asp Gly Lys Val Ser Gln Asn 115 120 125Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Pro Ile 130 135 140Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala145 150 155 160Phe Ser Pro Glu Val Ile Pro Met Phe Thr Ala Leu Ser Glu Gly Ala 165 170 175Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln 180 185 190Ala Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu 195 200 205Trp Asp Arg Leu His Pro Val His Ala Gly Pro Val Ala Pro Gly Gln 210 215 220Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Asn Leu225 230 235 240Gln Glu Gln Ile Ala Trp Met Thr Ser Asn Pro Pro Ile Pro Val Gly 245 250 255Asp Ile Tyr Lys Arg Trp Ile Ile Leu Gly

Leu Asn Lys Ile Val Arg 260 265 270Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu 275 280 285Pro Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala Glu 290 295 300Gln Ala Thr Gln Asp Val Lys Asn Trp Met Thr Asp Thr Leu Leu Val305 310 315 320Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Arg Ala Leu Gly Pro 325 330 335Gly Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly 340 345 350Pro Ser His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Thr Asn 355 360 365Ser Thr Ile Leu Met Gln Arg Ser Asn Phe Lys Gly Ser Lys Arg Ile 370 375 380Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn Cys385 390 395 400Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His 405 410 415Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile 420 425 430Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro 435 440 445Glu Pro Thr Ala Pro Pro Ala Glu Ser Phe Arg Phe Glu Glu Thr Thr 450 455 460Pro Ala Pro Lys Gln Glu Pro Lys Asp Arg Glu Pro Leu Thr Ser Leu465 470 475 480Arg Ser Leu Phe Gly Ser Asp Pro Leu Ser Gln Met Ala Pro Ile Ser 485 490 495Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro 500 505 510Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val 515 520 525Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly 530 535 540Pro Glu Asn Pro Tyr Asn Thr Pro Ile Phe Ala Ile Lys Lys Lys Asp545 550 555 560Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg 565 570 575Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly 580 585 590Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr 595 600 605Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr 610 615 620Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn625 630 635 640Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser 645 650 655Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val 660 665 670Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile 675 680 685Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg 690 695 700Trp Gly Phe Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe705 710 715 720Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro 725 730 735Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys 740 745 750Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly Ile Lys 755 760 765Val Lys Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu 770 775 780Val Val Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg785 790 795 800Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys 805 810 815Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr 820 825 830Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala 835 840 845Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala 850 855 860Val Gln Lys Ile Ala Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro865 870 875 880Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Ala Trp Trp Thr 885 890 895Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr 900 905 910Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val 915 920 925Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys 930 935 940Leu Gly Lys Ala Gly Tyr Val Thr Asp Arg Gly Arg Gln Lys Val Val945 950 955 960Ser Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile His 965 970 975Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser 980 985 990Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Lys Ser Glu Ser 995 1000 1005Glu Leu Val Ser Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys 1010 1015 1020Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn 1025 1030 1035Glu Gln Val Asp Lys Leu Val Ser Arg Gly Ile Arg Lys Val Leu 1040 1045 1050Phe Leu Asp Gly Ile Asp Lys Ala Gln Glu Glu His Glu Lys Tyr 1055 1060 1065His Ser Asn Trp Arg Ala Met Ala Ser Glu Phe Asn Leu Pro Pro 1070 1075 1080Ile Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu 1085 1090 1095Lys Gly Glu Ala Ile His Gly Gln Val Asp Cys Ser Pro Gly Ile 1100 1105 1110Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val 1115 1120 1125Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro 1130 1135 1140Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala 1145 1150 1155Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn 1160 1165 1170Phe Thr Ser Ala Thr Val Lys Ala Ala Cys Trp Trp Ala Gly Ile 1175 1180 1185Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val 1190 1195 1200Val Ala Ser Ile Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 1205 1210 1215Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val 1220 1225 1230Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Glu Tyr Ser 1235 1240 1245Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Ser Asp Ile Gln Thr 1250 1255 1260Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val 1265 1270 1275Tyr Tyr Arg Asp Ser Arg Asp Pro Leu Trp Lys Gly Pro Ala Lys 1280 1285 1290Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser 1295 1300 1305Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp 1310 1315 1320Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln 1325 1330 1335Asp Glu Asp 134030709PRTArtificial Sequencesc1 sequence 30Met Arg Val Arg Gly Ile Gln Arg Asn Trp Pro Gln Trp Trp Ile Trp1 5 10 15Gly Ile Leu Gly Phe Trp Met Ile Ile Ile Cys Arg Val Met Gly Asn 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Lys 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Met Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Arg Leu Trp Asp 100 105 110Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Glu Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr Tyr Asn Ile 130 135 140Ile His Asn Glu Thr Tyr Lys Glu Met Lys Asn Cys Ser Phe Asn Ala145 150 155 160Thr Thr Val Val Glu Asp Arg Lys Gln Lys Val His Ala Leu Phe Tyr 165 170 175Arg Leu Asp Ile Val Pro Leu Asp Glu Asn Asn Ser Ser Glu Lys Ser 180 185 190Ser Glu Asn Ser Ser Ser Glu Tyr Tyr Arg Leu Ile Asn Cys Asn Thr Ser 195 200 205Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp Pro Ile Pro Ile 210 215 220His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys Cys Asn Asn Lys225 230 235 240Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys 245 250 255Thr His Gly Ile Lys Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly 260 265 270Ser Leu Ala Glu Glu Glu Ile Ile Ile Arg Ser Glu Asn Leu Thr Asn 275 280 285Asn Ala Lys Thr Ile Ile Val His Leu Asn Glu Thr Val Asn Ile Thr 290 295 300Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro305 310 315 320Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln 325 330 335Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr Leu Gln Gly 340 345 350Val Lys Lys Lys Leu Ala Glu His Phe Pro Asn Lys Thr Ile Asn Phe 355 360 365Thr Ser Ser Ser Gly Gly Asp Leu Glu Ile Thr Thr His Ser Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Gly Leu Phe Asn Gly385 390 395 400Thr Tyr Met Pro Asn Gly Thr Asn Ser Asn Ser Ser Ser Asn Ile Thr 405 410 415Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly 420 425 430Arg Ala Met Tyr Ala Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser 435 440 445Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly

Ser Asn Asn Gly 450 455 460Val Pro Asn Asp Thr Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg465 470 475 480Asn Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys 485 490 495Pro Leu Gly Val Ala Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg 500 505 510Glu Glu Arg Ala Val Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly 515 520 525Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln 530 535 540Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu545 550 555 560Arg Ala Ile Glu Ala Gln Gln His Met Leu Gln Leu Thr Val Trp Gly 565 570 575Ile Lys Gln Leu Gln Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln 580 585 590Asp Gln Gln Leu Leu Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys 595 600 605Thr Thr Ala Val Pro Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr 610 615 620Asp Ile Trp Asp Asn Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly625 630 635 640Asn Tyr Thr Gly Glu Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln 645 650 655Gln Glu Lys Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser 660 665 670Lys Ile Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile 675 680 685Gly Glu Val Gly Ser Gly Ala Pro Thr Lys Ala Lys Arg Arg Val Val 690 695

700Gln Arg Glu Lys Arg70531867PRTArtificial SequenceC1 sequence 31Met Arg Val Arg Gly Ile Gln Arg Asn Trp Pro Gln Trp Trp Ile Trp1 5 10 15Gly Ile Leu Gly Phe Trp Met Ile Ile Ile Cys Arg Val Met Gly Asn 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Lys 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Met Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Arg Leu Trp Asp 100 105 110Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Glu Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr Tyr Asn Ile 130 135 140Ile His Asn Glu Thr Tyr Lys Glu Met Lys Asn Cys Ser Phe Asn Ala145 150 155 160Thr Thr Val Val Glu Asp Arg Lys Gln Lys Val His Ala Leu Phe Tyr 165 170 175Arg Leu Asp Ile Val Pro Leu Asp Glu Asn Asn Ser Ser Glu Lys Ser 180 185 190Ser Glu Asn Ser Ser Glu Tyr Tyr Arg Leu Ile Asn Cys Asn Thr Ser 195 200 205Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp Pro Ile Pro Ile 210 215 220His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys Cys Asn Asn Lys225 230 235 240Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys 245 250 255Thr His Gly Ile Lys Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly 260 265 270Ser Leu Ala Glu Glu Glu Ile Ile Ile Arg Ser Glu Asn Leu Thr Asn 275 280 285Asn Ala Lys Thr Ile Ile Val His Leu Asn Glu Thr Val Asn Ile Thr 290 295 300Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro305 310 315 320Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln 325 330 335Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr Leu Gln Gly 340 345 350Val Lys Lys Lys Leu Ala Glu His Phe Pro Asn Lys Thr Ile Asn Phe 355 360 365Thr Ser Ser Ser Gly Gly Asp Leu Glu Ile Thr Thr His Ser Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Gly Leu Phe Asn Gly385 390 395 400Thr Tyr Met Pro Asn Gly Thr Asn Ser Asn Ser Ser Ser Asn Ile Thr 405 410 415Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly 420 425 430Arg Ala Met Tyr Ala Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser 435 440 445Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly 450 455 460Val Pro Asn Asp Thr Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg465 470 475 480Asn Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys 485 490 495Pro Leu Gly Val Ala Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg 500 505 510Glu Lys Arg Ala Val Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly 515 520 525Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln 530 535 540Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu545 550 555 560Arg Ala Ile Glu Ala Gln Gln His Met Leu Gln Leu Thr Val Trp Gly 565 570 575Ile Lys Gln Leu Gln Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln 580 585 590Asp Gln Gln Leu Leu Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys 595 600 605Thr Thr Ala Val Pro Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr 610 615 620Asp Ile Trp Asp Asn Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly625 630 635 640Asn Tyr Thr Gly Glu Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln 645 650 655Gln Glu Lys Asn Glu Lys Asp Leu Leu Ala Leu Asp Ser Trp Lys Asn 660 665 670Leu Trp Asn Trp Phe Asp Ile Thr Asn Trp Leu Trp Tyr Ile Lys Ile 675 680 685Phe Ile Met Ile Val Gly Gly Leu Ile Gly Leu Arg Ile Ile Phe Ala 690 695 700Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser705 710 715 720Leu Gln Thr Leu Thr Gln Asn Pro Gly Gly Leu Asp Arg Leu Gly Arg 725 730 735Ile Glu Glu Glu Gly Gly Glu Gln Asp Lys Asp Arg Ser Ile Arg Leu 740 745 750Val Asn Gly Phe Phe Ala Leu Phe Trp Asp Asp Leu Arg Ser Leu Cys 755 760 765Leu Phe Ser Tyr His Arg Leu Arg Asp Phe Ile Leu Ile Val Ala Arg 770 775 780Ala Val Glu Leu Leu Gly Arg Ser Ser Leu Arg Gly Leu Gln Arg Gly785 790 795 800Trp Glu Ile Leu Lys Tyr Leu Gly Ser Leu Leu Gln Tyr Trp Gly Leu 805 810 815Glu Leu Lys Lys Ser Ala Ile Asn Leu Leu Asp Thr Ile Ala Ile Ala 820 825 830Val Ala Glu Gly Thr Asp Arg Ile Ile Glu Leu Ile Gln Arg Ile Cys 835 840 845Arg Ala Ile Cys Asn Ile Pro Arg Arg Ile Arg Gln Gly Phe Glu Ala 850 855 860Ala Leu Gln86532716PRTArtificial SequenceC1D7 sequence 32Met Arg Val Arg Gly Ile Gln Arg Asn Trp Pro Gln Trp Trp Ile Trp1 5 10 15Gly Ile Leu Gly Phe Trp Met Ile Ile Ile Cys Arg Val Met Gly Asn 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Lys 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Met Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Arg Leu Trp Asp 100 105 110Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Glu Cys Arg Asn Val Arg Asn Val Ser Ser Asn Gly Thr Tyr Asn Ile 130 135 140Ile His Asn Glu Thr Tyr Lys Glu Met Lys Asn Cys Ser Phe Asn Ala145 150 155 160Thr Thr Val Val Glu Asp Arg Lys Gln Lys Val His Ala Leu Phe Tyr 165 170 175Arg Leu Asp Ile Val Pro Leu Asp Glu Asn Asn Ser Ser Glu Lys Ser 180 185 190Ser Glu Asn Ser Ser Glu Tyr Tyr Arg Leu Ile Asn Cys Asn Thr Ser 195 200 205Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp Pro Ile Pro Ile 210 215 220His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys Cys Asn Asn Lys225 230 235 240Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val Ser Thr Val Gln Cys 245 250 255Thr His Gly Ile Lys Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly 260 265 270Ser Leu Ala Glu Glu Glu Ile Ile Ile Arg Ser Glu Asn Leu Thr Asn 275 280 285Asn Ala Lys Thr Ile Ile Val His Leu Asn Glu Thr Val Asn Ile Thr 290 295 300Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro305 310 315 320Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln 325 330 335Ala His Cys Asn Leu Ser Arg Asp Gly Trp Asn Lys Thr Leu Gln Gly 340 345 350Val Lys Lys Lys Leu Ala Glu His Phe

Pro Asn Lys Thr Ile Asn Phe 355 360 365Thr Ser Ser Ser Gly Gly Asp Leu Glu Ile Thr Thr His Ser Phe Asn 370 375 380Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser Gly Leu Phe Asn Gly385 390 395 400Thr Tyr Met Pro Asn Gly Thr Asn Ser Asn Ser Ser Ser Asn Ile Thr 405 410 415Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly 420 425 430Arg Ala Met Tyr Ala Pro Pro Ile Ala Gly Asn Ile Thr Cys Arg Ser 435 440 445Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ser Asn Asn Gly 450 455 460Val Pro Asn Asp Thr Glu Thr Phe Arg Pro Gly Gly Gly Asp Met Arg465 470 475 480Asn Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Glu Val Lys 485 490 495Pro Leu Gly Val Ala Pro Thr Glu Ala Lys Arg Arg Val Val Glu Arg 500 505 510Glu Lys Arg Ala Val Gly Ile Gly Ala Val Phe Leu Gly Ile Leu Gly 515 520 525Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Ile Thr Leu Thr Val Gln 530 535 540Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Ser Asn Leu Leu545 550 555 560Arg Ala Ile Glu Ala Gln Gln His Met Leu Gln Leu Thr Val Trp Gly 565 570 575Ile Lys Gln Leu Gln Thr Arg Val Leu Ala Ile Glu Arg Tyr Leu Gln 580 585 590Asp Gln Gln Leu Leu Gly Leu Trp Gly Cys Ser Gly Lys Leu Ile Cys 595 600 605Thr Thr Ala Val Pro Trp Asn Thr Ser Trp Ser Asn Lys Ser Gln Thr 610 615 620Asp Ile Trp Asp Asn Met Thr Trp Met Gln Trp Asp Lys Glu Ile Gly625 630 635 640Asn Tyr Thr Gly Glu Ile Tyr Arg Leu Leu Glu Glu Ser Gln Asn Gln 645 650 655Gln Glu Lys Asn Glu Lys Asp Leu Leu Ala Leu Asp Ser Trp Lys Asn 660 665 670Leu Trp Asn Trp Phe Asp Ile Thr Asn Trp Leu Trp Tyr Ile Lys Ile 675 680 685Phe Ile Met Ile Val Gly Gly Leu Ile Gly Leu Arg Ile Ile Phe Ala 690 695 700Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly Tyr705 710 715332127DNAArtificial Sequencenucleotide sequence encoding sC1 33atgagagtgc ggggcattca gagaaactgg ccccagtggt ggatctgggg catcctgggc 60ttttggatga tcattatctg ccgcgtgatg ggcaacctgt gggtcaccgt gtactacggc 120gtgcccgtgt ggaaagaggc caagaccacc ctgttctgcg ccagcgacgc caaggcctac 180gagaaagagg tgcacaacgt ctgggccacc cacgcctgtg 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cggcctgcgg 2100atcatctttg ccgtgctgag catcgtgaac cgcgtgcggc agggctac 214836724PRTArtificial Sequencemosaic Env trimer sequence 36Met Arg Val Arg Gly Ile Gln Arg Asn Cys Gln His Leu Trp Arg Trp1 5 10 15Gly Thr Leu Ile Leu Gly Met Leu Met Ile Cys Ser Ala Ala Gly Lys 20 25 30Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr 35 40 45Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val 50 55 60His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro65 70 75 80Gln Glu Val Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys 85 90 95Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp 100 105 110Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu 115 120 125Asn Cys Thr Asp Asp Val Arg Asn Val Thr Asn Asn Ala Thr Asn Thr 130 135 140Asn Ser Ser Trp Gly Glu Pro Met Glu Lys Gly Glu Ile Lys Asn Cys145 150 155 160Ser Phe Asn Ile Thr Thr Ser Ile Arg Asn Lys Val Gln Lys Gln Tyr 165 170 175Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asp Ser 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Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu 610 615 620Asp Lys Ile Trp Asn Asn Met Thr Trp Met Glu Trp Glu Arg Glu Ile625 630 635 640Asn Asn Tyr Thr Ser Leu Ile Tyr Thr Leu Ile Glu Glu Ser Gln Asn 645 650 655Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala 660 665 670Ser Leu Trp Asn Trp Phe Asp Ile Ser Asn Trp Leu Trp Tyr Ile Lys 675 680 685Ser Arg Ile Glu Gly Arg Gly Ser Gly Gly Tyr Ile Pro Glu Ala Pro 690 695 700Arg Asp Gly Gln Ala Tyr Val Arg Lys Asp Gly Glu Trp Val Leu Leu705 710 715 720Ser Thr Phe Leu3728PRTArtificial SequenceResidues 655-682 of SEQ ID NO18 37Asn Glu Lys Asp Leu Leu Ala Leu Asp Ser Trp Asn Asn Leu Trp Asn1 5 10 15Trp Phe Ser Ile Ser Lys Trp Leu Trp Tyr Ile Lys 20 25384056DNAArtificial Sequencenucleotide sequence of Mos1Gag-Pol antigen 38atgggagcta gagccagtgt gctgagcggc ggagagctgg atagatggga gaaaattcgg 60ctgcggccag gcggcaaaaa gaagtatcgc ctgaaacata ttgtgtgggc ctctcgcgag 120ctggaaagat ttgctgtgaa tcctggactg ctcgagacat ctgaaggctg ccgccagatc 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