HIV Vaccine Formulation

BACKGROUND OF THE INVENTION

[0001] 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.

[0002] 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.

[0003] Sequences encoding mosaic antigens have been cloned in vectors, for example, such as recombinant adenovirus serotype 26 (rAd26), and these recombinant vectors have been used in vaccines to generate immune responses against HIV (see e.g. Barouch et al, *supra*; and WO 2010/059732). Viral vectors expressing such mosaic HIV antigens have proven to be effective in eliciting an immune response against HIV infection.

[0004] Another therapeutic strategy that has been explored for inducing immune responses against HIV is the use of trimeric HIV envelope proteins as immunogens in vaccines, such as gp140. The native envelope spike on the surface of HIV is trimeric. Examples of trimeric envelope proteins include clade C gp140 protein, and a mosaic envelope trimer protein, such as those disclosed in WO 2014/042942 and WO 2014/107744.

[0010] Clade C gp140 protein has previously been described e.g. in WO 2010/042942 and in Nkolola et al. 2010, but there was no focus on any pharmaceutical formulation work in those disclosures. The protein was in phosphate-buffered saline (PBS) in some of the experiments in those disclosures. Mosaic gp140 has been described previously e.g. in WO 2014/107744 and in Nkolola et al 2014, but again there was no focus on any pharmaceutical

formulation work in those disclosures. The protein was in 25 mM Tris pH 7.5 and 150 mM NaCl in some of the experiments in those disclosures.

[0011] Trimeric HIV envelope proteins, such as gp140, are capable of inducing potent immune responses. Such envelope proteins can also be administered in combination with other HIV antigens, such as mosaic antigens, to provide enhanced immunity against HIV. However, the stability of the HIV envelope proteins as trimers is not optimal under conditions typically used for clinical and commercial manufacturing. The trimeric HIV envelope proteins are susceptible to both chemical and physical degradation. Moreover, many different factors, such as the buffer formulation, can affect the stability of proteins, and the effects are often unpredictable. For example, the use of HEPES buffer in protein formulations has been shown to result in generation of hydrogen peroxide when exposed to ambient light during the manufacturing process, which can impact the stability of the protein as well as other components in the formulation, such as surfactants. See, e.g., Baicu et al. *Cryobiology* (2002) 45(1) 33-48; Lepe-Zuniga et al. *J. Immunol. Methods* (1987) 103(1), 145; and Zigler et al. *In Vitro Cell. Dev. Biol.* (1985) 21(5), 282-287. It is desirable to have an HIV vaccine gp140 formulation that would be suitable for stability of different variants of gp140 protein, such as Clade C or mosaic gp140, and preferably with Aluminum Phosphate adjuvant as a single vial drug product (rather than being entirely dependent upon pharmacy mixing immediately prior to delivery of the vaccine), and in addition would enable drug product manufacturing meeting large late phase and commercial scale demands. It is generally unpredictable which combination of ingredients will result in a formulation that meets all these requirements.

[0012] Accordingly, there is a need in the art for improved formulations of HIV gp140 proteins with better stability under conditions used for clinical and commercial manufacturing in order to realize the full therapeutic potential of such trimeric envelope proteins. These formulations should also be compatible for use with additional HIV antigen(s), including vectors expressing HIV antigen(s), and/or adjuvants.

BRIEF SUMMARY OF THE INVENTION

[0005] The invention relates to immunogenic compositions of HIV gp140 proteins that have improved stability. The immunogenic compositions of the invention can be stored under refrigerated conditions for extended periods of time, and are more optimal for use in clinical and commercial manufacturing. The immunogenic compositions of the invention can also include an adjuvant. The invention also relates to methods of preparing the

immunogenic compositions, and methods of using the immunogenic compositions to induce an immune response against HIV.

[0006] In one general aspect, the invention relates to an immunogenic composition comprising, relative to the total volume of the composition:

a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;
b. 2% to 15% (w/v) sorbitol;
c. 0.01 to 0.05% (w/v) polysorbate 20; and
d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0.

[0010] Preferably, an immunogenic composition according to an embodiment of the invention comprises a stabilized trimeric HIV gp140 protein, such as an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[0011] In particular embodiments of the invention, the immunogenic composition comprises 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein.

[0012] In particular embodiments, an immunogenic composition according to an embodiment of the invention comprises 5% (w/v) to 12% (w/v) sorbitol.

[0013] In particular embodiments, the immunogenic composition comprises 0.02% (w/v) polysorbate 20.

[0014] In particular embodiments, the immunogenic composition comprises 10 mM histidine buffer at a pH of 6.5.

[0015] According to embodiments of the invention, the immunogenic composition can further comprise an adjuvant, such as aluminum phosphate adjuvant. In certain of such embodiments, aluminum phosphate may be present in the composition at a concentration of 0.7-5.0 mg/mL, e.g. 0.8-4.0 mg/mL, e.g. 0.85 mg/mL, 1 mg/mL, 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL, 3.5 mg/mL, 3.84 mg/mL, 4.0 mg/mL. In certain embodiments, aluminum phosphate is present in the immunogenic compositions at a concentration of 0.85 mg/mL. In certain embodiments, aluminum phosphate is present in the immunogenic compositions at a concentration of 3.84 mg/mL.

[0016] In a preferred embodiment of the invention, an immunogenic composition comprises, relative to a total volume of the composition,

a. 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2
b. 5% to 12% (w/v) sorbitol;
c. 0.02% (w/v) polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and

e. aluminum phosphate adjuvant, preferably at a concentration of 0.7-4.0 mg/mL.

[0017] In another general aspect, the invention relates to a method of preparing an immunogenic composition comprising admixing:

a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;
b. 2% to 15% (w/v) sorbitol;
c. 0.01 to 0.05% polysorbate 20; and
d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0;

to thereby obtain the immunogenic composition.

[0018] In certain embodiments, the immunogenic composition of the invention comprises aluminum phosphate adjuvant, preferably at a concentration of 0.7-4.0 mg/mL, and is stable upon storage at a temperature of 2-8°C, for at least one month, preferably at least 2, 3, 4, 5, 6 months, more preferably at least 7, 8, 9, 10, 11, 12 months, still more preferably at least 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months, most preferably at least 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36 months or longer, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months. In certain embodiments, the immunogenic composition of the invention comprises aluminum phosphate adjuvant, preferably at a concentration of 0.7-4.0 mg/mL, and is stable at a temperature of 25°C for at least 6 months, e.g. 6-12 months, or 6-24 months. In certain embodiments, the immunogenic composition of the invention comprises aluminum phosphate adjuvant, preferably at a concentration of 0.7-4.0 mg/mL, and is stable at a temperature of 40°C for at least 1 week, e.g. 1-12 weeks, e.g. at least 2 weeks, 3 weeks, 4 weeks, 1 month, e.g. 1-2 months, 1-3 months, or 3-6 months.

[0019] And in another general aspect, the invention relates to a method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof, comprising administering to the subject an effective amount of an immunogenic composition of the invention.

[0020] In a particular embodiment, a method of inducing an immune response against an HIV comprises administering to a subject in need thereof an immunogenic composition comprising, relative to the total volume of the composition,

a. 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2;
b. 5% to 12% (w/v) sorbitol;
c. 0.02% polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and
e. aluminum phosphate adjuvant, preferably at a concentration of 0.7-4.0 mg/mL.

[0021] In certain embodiments of the invention, a method of inducing an immune response against an HIV further comprises administering to the subject an effective amount of a second immunogenic composition comprising or encoding one or more additional HIV antigens, such as those comprising or encoding an amino acid sequence of SEQ ID NOs: 3-12. Preferably, the method comprises administering one or more vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens, such as those comprising an amino acid sequence of SEQ ID NOs: 3-12. Methods of inducing an immune response against an HIV can also comprise administering one or more MVA vectors encoding one or more HIV antigens, such as those comprising an amino acid sequence of SEQ ID NOs: 3-12. The one or more additional HIV antigens can also comprise SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575). In one embodiment, the antigen comprising SEQ ID NO: 11 comprises SEQ ID NO: 12.

[0022] In another general aspect, the invention relates to a method for preparing a long-term, storage stable immunogenic composition that comprises HIV gp140 protein, the method comprising:

(i) admixing the following components to create an immunogenic composition comprising these components in amounts relative to the total volume of the composition:

(a) 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;
(b) 2% to 15% (w/v) sorbitol;
(c) 0.01 to 0.05% (w/v) polysorbate 20; and
(d) 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0,
(e) water,
(f) aluminum phosphate adjuvant, preferably at a concentration of 0.7-4.0 mg/mL;

and

(ii) storing the composition at 2-8°C for at least one month, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months.

BRIEF DESCRIPTION OF THE FIGURES

[0023] 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.

[0024] In the figures:

[0025] Figs. 1A and 1B show the results of SolvoVPE analysis of HIV gp140 protein formulations prepared with acetate buffer, phosphate buffer, and histidine buffer, and dynamic light scattering (DLS) analysis of HIV gp140 protein formulations prepared with sorbitol and sucrose; in particular:

Fig. 1A shows the results of the SolvoVPE analysis; the change in absorbance at 350 nm (turbidity) is plotted against the change in absorbance at 280 nm (concentration); data points indicate the change between samples analyzed at time 0 (T_0) and after stressing the samples at 40 °C for 24 hours (T_{24}); SolvoVPE analysis was performed as described in Example 1;

Fig. 1B shows the results of the DLS analysis; the change in radius (R_h) from time 0 (T_0) at 20°C to time 7 days ($T_{7\ days}$) at 70°C is plotted by sugar (sucrose vs. sorbitol); DLS analysis was performed as described in Example 1;

[0026] Figs. 2A-2F show the results of high-performance size exclusion chromatography (HP-SEC) analysis of HIV gp140 formulations as described in Example 2; the relative amount of hexamer, trimer and high/low molecular weight species of HIV clade C gp140 protein was determined by HP-SEC of samples analyzed at time 0 (T_0) and after stressing the samples at 40°C for 24 hours (T_{24}); "Tri +Hex" refers to the relative amount of trimer and hexamer species of HIV clade C gp140 protein; "LMW" refers to low molecular weight species, e.g., cleavage or degradation products, in particular:

Fig. 2A shows the relative amount (%) of hexamer and trimer species of HIV clade C gp140 protein in T_0 samples at different protein concentrations (0.2 mg/mL and 1.0 mg/mL);

Fig. 2B shows the relative amount (%) of hexamer and trimer species of HIV gp140 clade C protein in T_0 samples at different concentrations of surfactant (0.02% and 0.1%); the data shown is a combination of data collected from samples containing PS20 and samples containing PS80;

Fig. 2C shows the relative amount (%) of hexamer and trimer species of HIV clade C gp140 protein in T_{24} samples at different protein concentrations (0.2 mg/mL and 1.0 mg/mL);

Fig. 2D shows the relative amount (%) of hexamer and trimer species of HIV gp140 clade C protein in T_{24} samples at different concentrations of surfactant (0.02% and 0.1%); the data shown is a combination of data collected from samples containing PS20 and samples containing PS80;

Fig. 2E shows the relative amount (%) of low molecular weight species of HIV clade C gp140 protein based on surfactant type (PS20 versus PS80) in T_0 samples;

Fig. 2F shows the relative amount (%) of low molecular weight species of HIV gp140 clade C protein in T_{24} samples at different concentrations of surfactant;

[0027] Figs. 3A and 3B show the results of stability studies of HIV gp140 protein formulations according to embodiments of the invention; HIV mosaic gp140 protein formulations were subjected to multiple freeze-thaw cycles as described in Example 3;

Fig. 3A shows the protein concentration after one, three, and five freeze-thaw cycles as determined by measuring the absorbance at 280 nm; and

Fig. 3B shows the turbity of the tested formulations after one, three, and five freeze-thaw cycles as determined by measuring the absorbance at 350 nm; and

[0028] Figs. 4A-4C show the results of stability studies of HIV clade C gp140 protein formulations; HIV clade C gp140 protein formulations were stored at 25°C and 60% relative humidity (RH), and 40°C and 75% RH, both with and without aluminum phosphate adjuvant, and then tested using a reduced SDS analytical method as described in Example 4;

Fig. 4A shows the relative protein concentration (%) of HIV clade C gp140 formulations stored at 25°C and 60% RH without any aluminum phosphate adjuvant;

Fig. 4B shows the relative protein concentration (%) of HIV clade C gp140 formulations stored at 40°C and 75% RH without any aluminum phosphate adjuvant;

Fig. 4C shows the relative protein concentration (%) of HIV clade C gp140 formulations stored at 25°C and 60% RH with aluminum phosphate adjuvant; and

Fig. 4D shows the relative protein concentration (%) of HIV clade C gp140 formulations stored at 40°C and 75% RH with aluminum phosphate adjuvant.

DETAILED DESCRIPTION OF THE INVENTION

[0029] 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.

[0030] 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.

[0031] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term "about." Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0032] As used herein, "subject" means any animal, preferably a mammal, most preferably a human, to whom will be or has been administered an immunogenic composition according to embodiments of the invention. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0033] The invention generally relates to immunogenic compositions comprising HIV gp140 protein and optionally adjuvant, methods of preparing and storing such compositions, and methods of inducing an immune response against HIV in a subject with the immunogenic compositions, alone or in combination with one or more additional HIV antigens, which are preferably expressed by one or more vectors.

[0034] HIV Antigens

[0035] 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 to, but are not limited to, HIV-1 and HIV-2.

[0036] 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 O.

[0037] As used herein, the terms "HIV antigen," "HIV antigenic protein," "HIV antigenic polypeptide," 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 HIV antigen can be a protein of 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 HIV in a subject.

[0038] Preferably, an 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., vaccinating) a subject against a viral disease or infection, that protects the subject against the viral disease or infection. For example, the antigen can comprise a protein or fragments thereof from HIV, such as the HIV *gag, pol* and *env* gene products.

[0039] An HIV 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.

[0040] In certain embodiments, the HIV 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.

[0041] HIV antigens 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 HIV-1 Gag, Pol, and/or Env antigens. Mosaic HIV Gag, Pol, and/or Env antigens are mosaic antigens comprising multiple epitopes derived from one or more of the Gag, Pol, and/or Env polyprotein sequences of HIV. For example, a mosaic GagPol antigen comprises a mosaic Gag sequence and a mosaic Pol sequence.

[0042] 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 invention include, but are not limited to, mos1Env (SEQ ID NO: 3), mos2Env (SEQ ID NO: 4), mos1Pol (SEQ ID NO: 5), mos2Pol (SEQ ID NO: 6), mos1Gag (SEQ ID NO: 7), mos2Gag (SEQ ID NO: 8), and combinations thereof, for example mos1GagPol (SEQ ID NO: 9) and mos2GagPol (SEQ ID NO: 10). Other examples of mosaic HIV antigens include synthetic HIV Env proteins, which are non-naturally occurring HIV envelope proteins optimized to induce an immune response or provide an immunity against one or more naturally occurring HIV strains, such as that comprising SEQ ID NO: 11, or SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575), or in preferred embodiments, the antigen comprising SEQ ID NO: 11 comprises the amino acid sequence of SEQ ID NO: 12, all as described in PCT/EP2016/081159 (filed on 15 December 2016 in the name of Janssen Vaccines & Prevention B.V.), which is herein incorporated by reference in its entirety.

[0043] HIV gp140 Protein

[0044] As used herein, the term "HIV gp140 protein" refers to an uncleaved ectodomain of trimeric gp160 envelope protein, i.e., $(gp160)_3$. Embodiments of the invention relate to improved formulations of HIV gp140, preferably a trimer and/or hexamer of the gp140 subunits bound to the ectodomain of the gp41 subunits lacking the gp41 transmembrane and cytoplasmic segments. The HIV *env* gene encodes the precursor protein gp160, which is proteolytically cleaved into the two mature envelope glycoproteins gp120 and gp41. First, gp160 trimerizes to $(gp160)_3$ and then undergoes cleavage into the two noncovalently associated proteins gp120 and gp41 via a cleavage reaction mediated by a host cell protease,

furin, at a sequence highly conserved in retroviral envelope glycoprotein precursors. 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 non-covalently 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. HIV gp140 protein has been used as a surrogate for the native state of the cleaved, viral spike.

[0045] Expression of gp140 proteins has been described in several publications (e.g. Zhang et al., 2001; Sanders et al., 2002; Harris et al., 2011), and the protein can nowadays also be ordered from service providers, in different variants e.g. based on different HIV strains. A gp140 protein according to the invention can have a cleavage site mutation so that the gp120 domain and gp41 ectodomain are covalently linked, or alternatively the gp120 domain and gp41 ectodomain can be non-covalently linked (e.g. by a disulphur bridge as for instance in SOSIP variants). Gp140 proteins have been used in various vaccination experiments (e.g. Nkolola et al., 2010, 2014; Kovacs et al., 2012; Barouch et al., 2015; Sanders et al., 2015).

[0046] According to embodiments of the invention, the HIV gp140 protein can be a homotrimer (e.g., trimers comprising three identical polypeptide units), or a heterotrimer (e.g., trimers comprising three polypeptides that are not all identical). The HIV gp140 protein can also be a hexamer. Both trimer species and hexamer species of HIV gp140 protein have immunogenicity against HIV, or can induce immune responses against HIV *in vivo*. Preferably, the HIV gp140 protein is a trimer, and more preferably a homotrimer. An HIV gp140 protein can be a naturally occurring sequence, e.g., a sequence isolated from any HIV clade, such as clade A, clade B, clade C, etc., or a mosaic gp140 protein. A "mosaic gp140 protein" contains multiple epitopes derived from one or more gp140 sequences of one or more HIV clades.

[0047] Preferably, an HIV gp140 protein is a stabilized trimeric gp140 protein. A "stabilized trimeric gp140 protein" is a gp140 protein that can have or can be modified to include a polypeptide sequence, such as a trimerization domain, that increases the stability of the trimeric structure, or it can be a gp140 protein that is modified to contain mutations (as compared to natural gp140 sequences) that stabilize a trimeric structure, such as SOSIP and/or other mutations. Examples of trimerization domains include, but are not limited to,

the T4-fibritin "foldon" trimerization domain, e.g., that having the amino acid sequence of GSGGYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 13); the coiled-coil trimerization domain derived from GCN4, e.g., that having the amino acid sequence of MKQIEDKIEEILSKIYHIENEIARIKKLIGEV (SEQ ID NO: 14); and the catalytic subunit of *E. coli* aspartate transcarbamoylase as a trimer tag. Such trimerization domains can be used to support stable trimer formation (see e.g. WO 2010/042942, Nkolola et al. 2010, WO 2014/107744, and Nkolola et al. 2014, for stabilized trimers of gp140 proteins). A stabilized trimeric gp140 protein for use in the invention can also include cleavage site mutations to enhance stability, e.g., in the furin cleavage sites, and/or so-called SOSIP mutations (see, e.g. Sanders et al., 2002, 2015). A stabilized trimeric gp140 protein can be derived from a gp140 protein isolated from any HIV clade, e.g., clade A, clade B, clade C, etc. A stabilized trimeric gp140 protein can also be a stabilized mosaic gp140 protein.

[0048] Exemplary HIV gp140 proteins that can be used in the invention include HIV clade C gp140 protein (SEQ ID NO: 1), HIV mosaic gp140 protein (SEQ ID NO: 2), and combinations thereof. Both the HIV clade C gp140 protein (SEQ ID NO: 1) and HIV mosaic gp140 protein (SEQ ID NO: 2) are stabilized trimeric gp140 proteins comprising a T4-fibritin "foldon" trimerization domain.

[0049] Immunogenic Compositions

[0050] In a first aspect, the invention relates to an immunogenic composition comprising an HIV gp140 protein. An "immunogenic composition" as used herein refers to a composition capable of inducing an immune response in a subject who has been or will be administered the composition. An immunogenic composition can be a vaccine. A "vaccine" refers to a composition that can provide protective immunity or a protective immune response to a subject, or that can be used to vaccinate a subject. According to embodiments of the invention, any HIV gp140 protein known in the art in view of the present disclosure can be used in an immunogenic composition of the invention. An immunogenic composition can comprise one or more HIV gp140 proteins, such as one, two, or three HIV gp140 proteins.

[0051] Preferably, the immunogenic composition of the invention comprises a stabilized trimeric gp140 protein. In one preferred embodiment, an HIV gp140 protein is an HIV clade C gp140 protein, such as that comprising the amino acid sequence of SEQ ID NO: 1. In another preferred embodiment, an HIV gp140 protein is a HIV mosaic gp140 protein, such as that comprising the amino acid sequence of SEQ ID NO: 2.

[0052] In other embodiments, an immunogenic composition comprises both an HIV clade C gp140 protein and an HIV mosaic gp140 protein, such as those comprising SEQ ID NO: 1

and SEQ ID NO: 2. For example, an immunogenic composition can comprise a mixture of an HIV clade C gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 and an HIV mosaic gp140 protein comprising the amino acid sequence of SEQ ID NO: 2, e.g., in a 1:1 ratio.

[0053] The immunogenic compositions of the invention also comprise water, preferably water for injection. It is added to the composition in sufficient quantity (q.s.), depending on the volume of the composition being prepared.

[0054] According to embodiments of the invention, an immunogenic composition comprises 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein, such as 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, or 5 mg/mL. In particular embodiments of the invention, an immunogenic composition comprises 0.2 mg/mL or 1 mg/mL of an HIV gp140 protein, such as those comprising SEQ ID NO: 1 or SEQ ID NO: 2. In other particular embodiments of the invention, an immunogenic composition comprises a 0.05 mg/mL to 5 mg/mL of a mixture of HIV gp140 proteins, such as those comprising SEQ ID NO: 1 and SEQ ID NO: 2, e.g., in a 1:1 ratio.

[0055] Immunogenic compositions of the invention further comprise sorbitol (sugar), polysorbate 20 (surfactant), and histidine buffer. The inventors surprisingly discovered that the stability of the HIV gp140 protein in a composition comprising histidine had improved stability as compared to that in compositions comprising other amino acids. The inventors also surprisingly discovered that inclusion of polysorbate 20 in the composition further improved the stability of the HIV gp140 protein in the composition. Typically, buffers used for protein formulations contain a combination of acetate and sorbitol, or a combination of histidine and sucrose (see e.g., Uchiyama, *Biochimica Biophysica Acta* (2014) 1844, 2041-2052). Histidine and sorbitol are not usually used in combination in a buffer for protein formulations. To the best of the knowledge of the inventors, the combination of histidine and sorbitol with polysorbate 20 (surfactant) has not been used in any commercial protein drug formulations. Therefore, it was surprising to see that the combination of histidine and sorbitol improved the stability of the HIV gp140 protein.

[0056] According to embodiments of the invention, the concentration of sorbitol can be in a range of 2% to 15% (w/v), such as 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14% or 15% (w/v). In one preferred embodiment, the concentration of sorbitol is 5% (w/v). In another preferred embodiment, the concentration of sorbitol is 12% (w/v).

[0057] According to embodiments of the invention, the concentration of polysorbate 20 can be in a range of 0.01% to 0.05% (w/v), such as 0.01%, 0.02%, 0.03%, 0.04%, or 0.05% (w/v). In one preferred embodiment, the concentration of polysorbate 20 is 0.02% (w/v).

[0058] According to embodiments of the invention, the concentration of histidine buffer is in a range of 5 mM to 20 mM, such as 5 mM, 10 mM, 15 mM or 20 mM, and is preferably 10 mM. The pH of the histidine buffer is in a range of 5.5 to 7.0, such as 5.5, 6.0, 6.5, or 7.0, and is preferably 6.5. In one preferred embodiment, the concentration of the histidine buffer is 10 mM and the pH of the histidine buffer is 6.5. Any pH value described herein, is to be understood as being modified in all instances by the term "about," which, when used with reference to a pH value includes ± 0.5 of the recited pH value. Unless specified otherwise, all pH values refer to the pH of the histidine buffer itself that is included in an immunogenic composition of the invention.

[0059] In certain embodiments of the invention, an immunogenic composition further comprises an adjuvant. 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, or enhance an immune response. For example, an adjuvant can be used to enhance an immune response to an HIV gp140 protein and/or an immunogenic composition of the invention when administered alone or further in combination with one or more adenovirus vectors encoding one or more 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, Iscomatrix, 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. aluminum hydroxide, and/or aluminum phosphate; an example of an aluminum phosphate adjuvant is AdjuPhos, a sterilized aluminum phosphate wet gel suspension), Adjuplex, and MF59. Preferably, the adjuvant is an aluminum phosphate adjuvant.

[0060] According to embodiments of the invention, an aluminum phosphate adjuvant can be included in an immunogenic composition at concentrations of between about 0.7 and 5.0 mg/mL, for instance 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1 mg/ml, e.g. at a fixed concentration of 0.85 mg/mL, or for instance 1.5, 2.0, 2.5, 3.0, 3.5, 3.6, 3.7, 3.8, 3.85, 3.9, 3.95, 4.0, 4.5, or 5.0 mg/mL, e.g. at a fixed concentration of 3.84 mg/mL. One goal of the invention was to provide long-term stable formulations with aluminum phosphate adjuvant present together with the gp140 protein immunogen. It was surprisingly found that the liquid formulations of the invention that comprise HIV gp140, histidine buffer, sorbitol, and polysorbate 20, are

stable for at least 6 months at 2-8°C. These formulations were also found to be stable for at least 6 months at elevated temperatures of 25°C. In addition, these formulations were even found to be stable for at least 2 weeks, 1 month, or even up to 3 months at 40°C, as measured by reduced SDS PAGE.

[0061] In an exemplary embodiment of the invention, an immunogenic composition comprises, relative to the total volume of the composition:

a. 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2;
b. 5% to 12% (w/v) sorbitol;
c. 0.02% (w/v) polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and
e. 0.7 to 4.0 mg/mL (e.g. 0.85 mg/mL or 3.84 mg/mL) aluminum phosphate adjuvant.

[0062] Immunogenic 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 for instance can include subcutaneous injection, intramuscular injection, or intradermal injection. Immunogenic compositions of the invention can also be formulated for other routes of administration, e.g. transmucosal, rectal, sublingual administration, oral, or intranasal. Preferably, an immunogenic composition is formulated for intramuscular injection.

[0063] Immunogenic compositions of the invention are advantageous in that the HIV gp140 protein can be stably stored in liquid form at refrigerated temperature, for instance 2°C to 8°C, for extended periods of time, such as about 2 years. In certain embodiments, the immunogenic compositions include an adjuvant, for instance aluminum phosphate adjuvant. The immunogenic compositions containing adjuvant are also compatible with storage in liquid form under refrigerated conditions for extended periods of time. The immunogenic compositions of the invention are also compatible with storage in lyophilized form. However, the compositions of the invention are thus preferably liquid formulations, meaning that they are in liquid form at the preferred storage temperature, i.e. at 2-8°C. Typically the liquid formulations or compositions according to the invention are aqueous suspensions, meaning that not all protein and/or particulate material such as aluminum phosphate may be entirely dissolved. In such cases it is advised to mix the composition before use. It is preferred to

store the compositions of the invention that comprise aluminum phosphate adjuvant above the freezing point of water, most preferably at 2-8°C, e.g. e.g. 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, or 8°C. Advantages are that no resource-intensive and costly lyophilization and associated re-dissolving before use is needed, no bed-side mixing and separate storage of the aluminum phosphate adjuvant is needed but rather the formulations can be stored 'ready-for-use'. In addition, storage at refrigerated but not frozen conditions makes that the compositions can be used more easily in resource-limited settings e.g. where no freezing capacity is available. Moreover, the observed maintained stability at elevated temperatures (e.g. 25°C, and even 40°C) of the compositions of the invention indicates that inadvertent temperature excursions, e.g. when temporarily the composition is exposed to room temperature even in warm climates, should not immediately be detrimental to the vaccine composition of the invention.

[0064] The compositions of the invention, surprisingly including the ones comprising aluminum phosphate, are stable upon storage at a temperature of 2-8°C for at least one day, one week, two weeks, one month, preferably at least 2, 3, 4, 5, 6 months. More preferably these compositions are stable under these conditions for at least 7, 8, 9, 10, 11, 12 months, still more preferably at least 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months, most preferably at least 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36 months or longer, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months. The invention in certain embodiments thus provides compositions according to the invention, which are stable when stored at 2-8°C for at least one month, at least three months, at least 6 months. Preferably such compositions are stable for at least 12 months, at least 18 months, at least 24 months, at least 30 months, at least 36 months. In certain embodiments, the invention provides using the immunogenic compositions of the invention for vaccinating a subject, preferably a human subject, after the immunogenic compositions have been stored at 2-8°C for at least one day, at least one week, at least two weeks, at least three weeks, at least one month, at least two months, at least three months, at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27. 28. 29, 30, 31, 32, 33, 34, 35, 36 months.

[0065] For the purpose of the present invention, an immunogenic composition that comprises HIV gp140 protein and that after having been stored for at least one week, preferably at least one month, at a given temperature does not show more than 25%, preferably not more than 10%, of degradation of said gp140 protein on a reduced SDS PAGE gel, is considered a long-term storage stable immunogenic composition at said temperature. An immunogenic composition that comprises HIV gp140 protein is considered "stable" according to the present invention under certain conditions (e.g. 2-8°C) for a specified time

(e.g. 6 months), if under these conditions after said specified time said gp140 protein does not show more than 25%, preferably less than 20%, preferably less than 15%, preferably less than 10%, most preferably less than 5%, of degradation (compared to initial measurement at t=0) on a reduced SDS PAGE gel. Degradation is visible as additional bands below the gp140 band of desired molecular weight. Alternative assays such as ELISA can also be used to measure stability, and in certain embodiments, a composition that is stable as defined above, also does not show more than 50%, preferably less than 25%, degradation (reduction as compared to initial signal at t=0) in an ELISA assay.

[0066] Methods of Preparing an Immunogenic Composition

[0067] The invention also relates to a method of preparing an immunogenic composition of the invention. According to embodiments of the invention, a method of preparing an immunogenic composition comprises admixing an HIV gp140 protein, sorbitol, polysorbate 20, and histidine buffer in the appropriate concentration ranges. One of ordinary skill in the art will be familiar with conventional techniques used to prepare such compositions.

[0068] For example, immunogenic compositions can be prepared by mixing histidine buffer, sorbitol, and polysorbate 20 at the desired concentrations. Then, the HIV gp140 protein can be added. The pH of the composition can be adjusted before or after addition of the HIV gp140 protein. As another illustrative example, first a buffer solution containing histidine and sorbitol can be prepared. The HIV gp140 protein is prepared in buffer at the desired concentration, and then added to the buffer solution containing histidine and sorbitol, followed by addition of Polysorbate at the target concentration. Adjuvant can be added last to obtain the final composition. The invention also provides methods for preparing a long-term, storage stable immunogenic composition that comprises HIV gp140 protein. In certain embodiments, such methods comprise: (i) providing an immunogenic composition according to the invention (i.e. comprising 0.05-5 mg/mL HIV gp140 protein, 2-15% (w/v) sorbitol, 0.01-0.05% polysorbate 20, 5-20 mM histine buffer pH 5.5-7.0, water, and preferably 0.7-4.0 mg/mL aluminum phosphate), and (ii) storing said composition at 2-8°C for at least one month, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months, e.g. 18-30 months. In certain embodiments, such methods comprise:

(i) admixing the following components to create an immunogenic composition comprising these components in amounts relative to the total volume of the composition:

(a) 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;

(b) 2% to 15% (w/v) sorbitol;

(c) 0.01 to 0.05% (w/v) polysorbate 20;

(d) 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0,

(e) water (preferably water for injection),

(f) aluminum phosphate adjuvant, preferably at a concentration of 0.7-4 mg/mL;

and

(ii) storing the composition at 2-8°C for at least one month, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months, e.g. 18-30 months.

Methods of Inducing an Immune Response

[0069] The invention also relates to methods of inducing an immune response against human immunodeficiency virus (HIV) in a subject in need thereof with an immunogenic composition of the invention. The immune response can be against one or more HIV clades. The methods described herein also include administering an immunogenic composition of the invention in combination with one or more additional HIV antigens that are preferably expressed from one or more vectors, such as adenovirus vectors or MVA vectors, including methods of priming and boosting an immune response.

[0070] In one general aspect, a method of inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof comprises administering to the subject an effective amount of an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention. Any of the immunogenic compositions described herein can be used in a method of inducing an immune response against HIV in a subject. Preferably, the composition comprises a stabilized trimeric HIV gp140 protein, such as an HIV clade C gp140 protein comprising the amino acid sequence of SEQ ID NO: 1, or an HIV mosaic gp140 protein comprising the amino acid sequence of SEQ ID NO: 2, or a mixture thereof. The composition can further comprise an adjuvant, such as aluminum phosphate adjuvant. An exemplary embodiment of an immunogenic composition for use in the methods of the invention comprises, relative to the total volume of the composition,

a. 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2;
b. 5% to 12% (w/v) sorbitol;
c. 0.02% (w/v) polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and

e. 0.7 to 4.0 mg/mL (e.g. 0.85 mg/mL or 3.84 mg/mL) aluminum phosphate adjuvant.

[0071] 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 an 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 an HIV infection, for therapeutic purposes. Preferably, the methods of the invention are for prophylactic purposes, such as for providing protective immunity. The immune response can be a cellular immune response and/or a humoral immune response.

[0072] 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.

[0073] Typically, administration of immunogenic compositions according to embodiments of the invention will have a prophylactic aim to generate an immune response against an HIV antigen before infection or development of symptoms. In other embodiments, the immunogenic compositions can be administered for post-exposure prophylactics. Immunogenic compositions of the invention can also be administered to a non-human mammal, such as for experimental purposes.

[0074] As used herein, "an 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 effective amount means an amount sufficient to induce an immune response in a subject in need thereof. In certain embodiments, an 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. In certain embodiments, an effective amount means an amount sufficient to enhance an immune response in a subject in need thereof. For example, when used in combination with one or more other components or immunogenic compositions capable of effecting an immune response, such as in a prime-boost regimen, an effective amount can be

an amount sufficient to enhance the immune response induced by the one or more other components or immunogenic compositions.

[0075] An 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, e.g., whether inducing immune response or providing protective immunity; and the particular disease, e.g., viral infection, for which immunity is desired. An effective amount can readily be determined by one of ordinary skill in the art in view of the present disclosure. As general guidance, when used with reference to an HIV gp140 protein an effective amount can range from, e.g. about 0.3 to about 3000 microgram (μg), e.g. 1-1000 μg, e.g. 10-500 μg, e.g. about 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 μg.

[0076] An effective amount of an immunogenic composition 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 and/or injectables), wherein the administration of the multiple compositions (e.g., tablets, capsules and/or injectables) collectively provides a subject with the immunogenically effective amount. It is also possible to administer an effective amount of an immunogenic composition to a subject, and subsequently administer another dose of an effective amount of an immungenic composition to the same subject, in a so-called prime-boost regimen, as described in greater detail below.

[0077] According to embodiments of the invention, an immunogenic composition can be administered to a subject by any means known in the art including, but not limited to, oral (enteral) administration and parenteral injections. The parenteral injections could for instance include subcutaneous injection, intramuscular injection, or intradermal injection. Preferably, the immunogenic composition is administered by intramuscular injection.

[0078] It is also possible to administer immunogenic compositions of the invention together with one or more additional HIV antigens, or one or more vectors, such as adenovirus vectors, encoding one or more additional HIV antigens. As used herein, the terms "co-delivery," "co-administration," "administered together with," or "administered in combination with" refer to simultaneous administration of two or more components, such as an immunogenic composition comprising an HIV gp140 protein, or multiple viral expression vectors, such as adenovirus 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, or they can be administered in a single composition at the same time. Non-limiting examples of

administration of immunogenic compositions of gp140 protein with one or more additional HIV antigens encoded by vectors such as adenoviral vectors, are provided in WO 2016/049287, the disclosure of which is herein incorporated by reference in its entirety.

[0079] Thus, in certain embodiments of the invention, a method of inducing an immune response further comprises administering to the subject an effective amount of a second immunogenic composition comprising one or more HIV antigens or one or more vectors encoding the HIV antigens. Any HIV antigen known to those skilled in the art in view of the present disclosure can be used, such as HIV Nef, Gag, Env, or Pol antigens or any antigenic portion or epitope or combination thereof. Mosaic HIV antigens can also be used. Exemplary HIV antigens include, but are not limited to, mosaic Env, Gag, and/or Pol antigens and combinations thereof, such as those comprising the amino acid sequences of SEQ ID NOs: 3-12, or SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575). The additional HIV antigens can for instance be administered to the subject as isolated proteins or polypeptides or as vectors encoding these proteins or polypeptides.

[0080] Preferably, in the method comprising administering to the subject an effective amount of a second immunogenic composition, the second immunogenic composition comprises one or more vectors encoding one or more HIV antigens.

[0081] Any vector known to those skilled in the art in view of the present disclosure can be used. Preferably, the vector is an adenovirus vector, more preferably an adenovirus 26 vector. The preparation of recombinant adenoviral vectors is well known in the art. Preparation of recombinant adenovirus 26 (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. Other adenovirus vectors that can be used in combination with immunogenic compositions of the invention include those described in WO 2016/049287 and PCT Application No. PCT/EP2016/081159, the disclosures of which are herein incorporated by reference in their entirety.

[0082] According to embodiments of the invention, adenovirus vectors can comprise one HIV antigen, or more than one HIV antigen, such as two, three, or four or more HIV antigens. Immunogenic compositions can comprise one or more adenovirus vectors, such as two, three, four or more HIV antigens, encoding one or more different HIV antigens. Also according to embodiments of the invention, a second composition can comprise one adenovirus vector, or more than one adenovirus vector, such as two, three, four or more adenovirus vectors. If a second composition comprises more than one adenovirus vector, the adenovirus vectors can encode the same or different HIV antigens.

[0083] Adenovirus vectors encoding one or more HIV antigens for use in the methods of the invention comprise nucleic acid encoding an HIV antigen that 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). Examples of suitable promoters 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.

[0084] As general guidance, an "effective amount" when used with reference to adenovirus vectors, can range from about 10^8 viral particles to about 10^{12} viral particles, for example 10^8, 10^9, 10^{10}, 10^{11}, or 10^{12} viral particles. The preparation and use of immunogenic compositions comprising adenovirus vectors, such as adenovirus 26 vectors, 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.

[0085] For instance recombinant adenovirus vector can 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, and 2.5% glycerol. Another useful adenovirus formulation buffer suitable for administration to humans is 20 mM Tris, 2 mM $MgCl_2$, 25 mM NaCl, 10% (w/v) sucrose, and 0.2% (w/v) polysorbate-80. 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. Other buffers can be used, and several examples of suitable formulations for the storage and for pharmaceutical administration of purified vectors are known.

[0086] Administration of immunogenic compositions comprising the one or more additional HIV antigens or one or more adenovirus vectors encoding the one or more additional HIV antigens 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.

[0087] In one embodiment, an immunogenic composition comprising a gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 3.

[0088] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 4.

[0089] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 5.

[0090] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 6.

[0091] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 7.

[0092] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 8.

[0093] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 9.

[0094] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 10.

[0095] In one embodiment, an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention is used in combination with an adenovirus vector, preferably an adenovirus 26 vector, encoding a HIV antigen comprising the amino acid sequence of SEQ ID NO: 11, SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575), or SEQ ID NO: 12.

[0096] Upon administration of adenovirus vectors encoding one or more HIV antigens, the adenovirus vector expresses the encoded HIV antigens, such that the HIV antigens are presented to the immune system of the subject, thereby inducing the required response to produce immunity, or induce an immune response. An immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention can be administered together with the one or more adenovirus vectors to prime the immune response, and/or subsequent to administration of the one or more adenovirus vectors to boost the immune response.

[0097] Thus, in other embodiments of the invention, a method of inducing an immune response against an HIV in a subject in need thereof comprises (i) administering to the subject an effective amount of an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention, and preferably further comprising an adjuvant, and (ii) administering to the subject an effective amount of a second immunogenic composition comprising one or more adenovirus vectors encoding one or more HIV antigens. Steps (i) and (ii) are conducted in either order, with one of the steps for priming immunization and the other for boosting immunization. Optionally, the method can further comprise administering one or more Modified Vaccinia Ankara (MVA) vectors encoding one

or more HIV antigens. MVA vectors can encode any HIV antigen described herein. Preferably, the MVA vectors encode one or more HIV antigens selected from the group consisting of SEQ ID NOs: 3-12, or SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575).

[0098] Examples of adenovirus vectors, MVA vectors, and prime-boost regimens that can be used in combination with an immunogenic composition comprising an HIV gp140 protein according to an embodiment of the invention include those described in WO 2016/049287, the disclosure of which is herein incorporated by reference in its entirety.

[0099] For example, in one embodiment of the disclosed methods, one or more adenovirus vectors encoding one or more HIV antigens are used to prime the immune response. An immunogenic composition comprising an HIV gp140 protein according to the invention can be used together with the one or more adenovirus 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 4-14 weeks, e.g. 10-14 weeks after the initial priming administration. The immunogenic composition comprising an HIV gp140 protein optionally together with one or more additional adenovirus or MVA vectors encoding one or more additional HIV antigens can be used to boost the immune response. A boosting immunization can also be administered once or multiple times, for example, first at about 22-26 weeks after the initial priming administration, followed by another boosting administration at about 46-50 weeks after the initial priming administration. The immune response induced by the immunization is monitored.

[00100] In other general aspects, the invention relates to vaccine combinations for inducing an immune response against a human immunodeficiency virus (HIV) in a subject in need thereof. According to embodiments of the invention, the vaccine combination comprises an immunogenic composition comprising an HIV gp140 protein, such as that comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2; one or more adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens, such as an HIV antigen comprising an amino acid sequence selected from the group consisting of SEQ ID NO:s 3 to 12, and SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to

Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575) and optionally one or more MVA vectors encoding one or more HIV antigens, such as an HIV antigen comprising an amino acid sequence selected from the group consisting of SEQ ID NO:s 3 to 12, and SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575).

[00101] According to embodiments of the invention, the vaccine combinations can be used in any of the methods described herein for inducing an immune response against an HIV in a subject in need thereof, including for priming and boosting an immune response.

EMBODIMENTS

[00102] Embodiment 1 is an immunogenic composition comprising, relative to the total volume of the composition:

a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;
b. 2% to 15% (w/v) sorbitol;
c. 0.01 to 0.05% (w/v) polysorbate 20; and
d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0.

[00103] Embodiment 2 is the immunogenic composition of embodiment 1, wherein the concentration of the HIV gp140 is 0.2 mg/mL.

[00104] Embodiment 3 is the immunogenic composition of embodiment 1, wherein the concentration of the HIV gp140 is 1 mg/mL.

[00105] Embodiment 4 is the immunogenic composition of any of embodiments 1 to 3, wherein the concentration of sorbitol is 5% (w/v).

[00106] Embodiment 5 is the immunogenic composition of any of embodiments 1 to 3, wherein the concentration of sorbitol is 12% (w/v).

[00107] Embodiment 6 is the immunogenic composition of any of embodiments 1-5, wherein the concentration of polysorbate 20 is 0.02% (w/v).

[00108] Embodiment 7 is the immunogenic composition of any of embodiments 1-6, wherein the concentration of the histidine buffer is 10 mM, and the pH of the histidine buffer is 6.5.

[00109] Embodiment 8 is the immunogenic composition of any of embodiments 1-7, further comprising aluminum phosphate adjuvant, for instance at a concentration of 0.7-1.0 mg/mL, preferably at a concentration of 0.85 mg/mL.

[00110] Embodiment 9 is the immunogenic composition of any of embodiments 1-8, wherein the HIV gp140 protein comprises the amino acid sequence of SEQ ID NO: 1.

[00111] Embodiment 10 is the immunogenic composition of any of embodiments 1-8, wherein the HIV gp140 protein comprises the amino acid sequence of SEQ ID NO: 2.

[00112] Embodiment 11 is an immunogenic composition comprising, relative to the total volume of the composition,

a. 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2;
b. 5% to 12% (w/v) sorbitol;
c. 0.02% (w/v) polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and
e. aluminum phosphate adjuvant, e.g. at a concentration of 0.7-4.0 mg/mL, e.g. 0.85 mg/mL or 3.84 mg/mL.

[00113] Embodiment 12 is the immunogenic composition of embodiment 11, comprising 0.2 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 and 5% (w/v) sorbitol.

[00114] Embodiment 13 is the immunogenic composition of embodiment 11, comprising 0.2 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 2 and 5% (w/v) sorbitol.

[00115] Embodiment 14 is the immunogenic composition of embodiment 11, comprising 0.2 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 and 12% (w/v) sorbitol.

[00116] Embodiment 15 is the immunogenic composition of embodiment 11, comprising 0.2 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 2 and 12% (w/v) sorbitol.

[00117] Embodiment 16 is the immunogenic composition of embodiment 11, comprising 1.0 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 and 5% (w/v) sorbitol.

[00118] Embodiment 17 is the immunogenic composition of embodiment 11, comprising 1.0 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 2 and 5% (w/v) sorbitol.

[00119] Embodiment 18 is the immunogenic composition of embodiment 11, comprising 1.0 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 and 12% (w/v) sorbitol.

[00120] Embodiment 19 is the immunogenic composition of embodiment 11, comprising 1.0 mg/mL HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 2 and 12% (w/v) sorbitol.

[00121] Embodiment 20 is an immunogenic composition according to any of embodiments 1 to 19 formulated for intramuscular injection.

[00122] Embodiment 21 is a method of preparing an immunogenic composition comprising admixing:

a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;
b. 2% to 15% (w/v) sorbitol;
c. 0.01 to 0.05% polysorbate 20; and
d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0;

to thereby obtain the immunogenic composition.

[00123] Embodiment 22 is a method of inducing an immune response against an HIV in a subject in need thereof, comprising administering to the subject an effective amount of an immunogenic composition comprising, relative to the total volume of the composition,

a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein;
b. 2% to 15% (w/v) sorbitol;
c. 0.01 to 0.05% polysorbate 20; and
d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0.

[00124] Embodiment 23 is the method of embodiment 22, wherein the immunogenic composition comprises, relative to the total volume of the composition,

a. 0.2 mg/mL to 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2;

b. 5% to 12% (w/v) sorbitol;
c. 0.02% polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and
e. aluminum phosphate adjuvant, e.g. at a concentration of 0.7-4.0 mg/mL, e.g. 0.85 mg/mL or 3.84 mg/mL.

[00125] Embodiment 24 is a method of inducing an immune response against an HIV in a subject in need thereof, comprising administering to the subject an effective amount of an immunogenic composition according to any one of embodiments 1 to 20, or to any one of embodiments 36-37.

[00126] Embodiment 25 is the method of any one of embodiments 22 to 24, further comprising administering to the subject an effective amount of one or more adenovirus vectors, preferably adenovirus 26 vectors, encoding one or more HIV antigens.

[00127] Embodiment 26 is the method of any one of embodiments 22 to 25 further comprising administering to the subject an effective amount of one or more MVA vectors encoding one or more HIV antigens.

[00128] Embodiment 27 is the method of embodiment 25 or embodiment 26, wherein the one or more HIV antigens comprise an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 to 12, and SEQ ID NO: 11 having one or more mutations selected from the group consisting of (i) I529P (Ile to Pro at position 529), (ii) K480E (Lys to Glu at position 480), and (iii) a combination of EK479-480RRRR (i.e. replacing GluLys at position 479 and 480 by four consecutive Arg residues), I529P (Ile to Pro at position 529), A471C (Ala to Cys at position 471) and T575C (Thr to Cys at position 575).

[00129] Embodiment 28 is an immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 36-37, which is stable at 2-8°C for at least six months.

[00130] Embodiment 29 is an immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 36-37, which is stable at 2-8°C for at least 12 months, at least 18 months, at least 24 months, at least 30 months, at least 36 months.

[00131] Embodiment 30 is an immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 36-37, which is stable at 2-8°C for 6-72 months.

[00132] Embodiment 31 is an immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 36-37, which is stable at 25°C for at least six months.

[00133] Embodiment 32 is an immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 36-37, which is stable at 40°C for at least one week, preferably at least two weeks.

[00134] Embodiment 33 is a method for storing an immunogenic composition comprising HIV gp140 protein, the method comprising providing a immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 28-32, or to any one of embodiments 36-37, and storing said composition at 2-8°C for at least one day, e.g. at least one week, e.g. at least one month, e.g. 1 day-72 months, e.g. 6-48 months, e.g. 12-36 months, e.g. 18-30 months.

[00135] Embodiment 34 is a method for preparing a long-term, storage stable immunogenic composition that comprises HIV gp140 protein, the method comprising: (i) providing an immunogenic composition that comprises 0.05-5 mg/mL HIV gp140 protein, 2-15% (w/v) sorbitol, 0.01-0.05% polysorbate 20, 5-20 mM histine buffer pH 5.5-7.0, water, and preferably 0.7-4.0 mg/mL aluminum phosphate), and (ii) storing said composition at 2-8°C for at least one week, e.g. at least one month, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months, e.g. 18-30 months.

[00136] Embodiment 35 is use of an immunogenic composition according to any one of embodiments 1-20, or to any one of embodiments 28-32, or to any one of embodiments 36-37, for administering to a subject, preferably a human subject, to induce an immune response against HIV, wherein the immunogenic composition prior to said administering has been stored at 2-8°C for at least one day, e.g. at least one week, e.g. at least two weeks, e.g. at least three weeks, e.g. at least one month, e.g. at least two months, e.g. at least three months, e.g. at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27. 28. 29, 30, 31, 32, 33, 34, 35, 36 months.

[00137] Embodiment 36 is the immunogenic composition of any one of embodiments 1-9, wherein the HIV gp140 protein comprises a mixture of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO:1 and an HIV gp140 protein comprising the amino acid sequence of SEQ NO: 2.

[00138] Embodiment 37 is the immunogenic composition of embodiment 36, wherein the HIV gp140 protein comprising the amino acid sequence of SEQ ID NO:1 and the HIV gp140

protein comprising the amino acid sequence of SEQ NO: 2 are present in the mixture at a 1:1 ratio.

EXAMPLES

[00139] **Example 1: Formulation Development Study for HIV Clade C gp140 Protein**

[00140] There are numerous possibilities for each component that can be included in a protein formulation, e.g., buffer, sugar, pH value, surfactant etc. For example, different possibilities for buffers can include phosphate, acetate, HEPES, Tris, MOPS, etc.; different possibilities for amino acids can include histidine, arginine, lysine, alanine, etc.; different possibilities for sugars can include sucrose, sorbitol, glycerol, mannitol, trehalose, etc.; and different possibilities for surfactant can include polysorbate 20, polysorbate 80, Tween-20, Tween-80, etc. There are also many other types of excipients, and numerous possibilities for each, that can further be included in a protein formulation, such as osmolytes (e.g., glycine, proline, glycerol, urea, etc.), salts (e.g., sodium chloride, potassium chloride, sodium sulfate, etc.), carbohydrates (e.g., lactose), proteins and polymers (e.g., HSA, gelatin, PVP, PLGA, PEG, etc.), chelators and antioxidants (e.g., EDTA, DTPA, ethanol, etc.), preservatives (e.g., benzyl alcohol, m-cresol, phenol, etc.), etc. See, e.g., Kamerzell et al. Advanced Drug Delivery Reviews (2011) 63, 1118-1159. Accordingly, there are many different theoretical combinations of components that could be used to identify a formulation. However, the most suitable formulation is dependent upon the particular protein in the formulation.

[00141] The inventors therefore set out to find improved formulations that could meet the complex requirements in the unpredictable art of protein formulation, in particular, an improved HIV gp140 formulation that enables drug product manufacturing meeting large late phase and commercial scale demands, and includes aluminum phosphate adjuvant and gp140 protein to be stored as drug product in single vials at refrigerated temperature, and prevents instability associated with certain components present in currently used formulations. The formulations were designed for storage in liquid form, but with the potential to be stored in lyophilized form and reconstituted in liquid prior to injection.

[00142] Formulation screening studies were designed to evaluate different buffers (histidine, phosphate, and acetate), buffer strengths (10 mM to 50 mM), sugars (sucrose and sorbitol from 2% to 12% w/v), surfactants (polysorbate 20 and polysorbate 80 from 0.02% to 1% w/v), and pH values from 4.5 to 7.5 on the stability of HIV clade C gp140 protein formulations. The parameters were varied as shown in Table 1.

[00143] **Table 1**: HIV clade C gp140 protein formulation study design

Buffer	Histidine (His)	Phosphate (Pho)	Acetate (Ace)
pH	5.5-6.5	6.5-7.5	4.5-5.5
Buffer Strength	10 mM, 20 mM, or 50 mM		
Sugar	Sucrose or sorbitol at 2% or 12% (w/v)		
Surfactant	Polysorbate 20 (PS20) or polysorbate 80 (PS80) at 0.02%, 0.05%, or 0.10% (w/v)		

[00144] The formulations were prepared by adding the buffer components at the pH value to be tested, followed by addition of sugar and surfactant. The pH was adjusted as necessary. Then, the clade C gp140 protein (SEQ ID NO: 1) was added to a concentration of 1 mg/mL or 0.2 mg/mL.

[00145] Formulation stability was analyzed using a SolvoVPE system to evaluate concentration and turbidity. Samples of each formulation were analyzed at time 0 (T_0), and then after stressing at 40°C for 24 hours (T_{24}). Formulation stability was also analyzed using dynamic light scattering (DLS).

[00146] SolvoVPE Analysis: A SolvoVPE (C Technologies, Inc.; Bridgeport, NJ, USA) was used to determine protein concentration by measuring the UV absorbance of samples of each formulation at 280 nm and 350 nm. The change in turbidity was determined from the difference in absorbance at 350 nm between T_0 and T_{24} measurements. For desired formulations, any change in turbidity should preferably be as small as possible. Increases in turbidity indicate that the protein is precipitating out of solution, and that the formulation is thus less stable. The results of the SolvoVPE analysis are shown in Fig. 1.

[00147] The results of the SolvoVPE analysis show that the acetate buffer formulations had the highest increase in turbidity (average change of +0.339), whereas phosphate buffer formulations showed a moderate increase (average change of +0.177), and histidine buffer formulations shows almost no change in turbidity (average change of -0.010). Since a large change in turbidity is undesirable, the turbidity data indicate that histidine buffer is the most optimal buffer of those tested for improving the stability of HIV gp140 protein.

[00148] Dynamic Light Scattering (DLS) Analysis: DLS was used to evaluate the colloidal stability, and specifically whether there was any protein aggregation in the protein formulations. The change in radius (R_h) from T_0 at 20°C to $T_{7\ days}$ at 70°C was measured. More specifically, the R_h was measured for the initial sample at 20°C. Then, the sample was heated to 70°C, and held at a temperature of 70°C for 7 days before measuring the final R_h. The difference in the initial R_h value and the final R_h values is plotted in Fig. 1B. A large

change in R_h indicates that there is protein aggregation, and that the formulation is thus less desirable.

[00149] The results of the DLS analysis show that formulations containing sorbitol had a lower change in R_h as compared to formulations containing sucrose. Since a larger change in R_h is undesirable, the DLS data indicate that sorbitol is the most optimal sugar of those tested for improving the stability of HIV gp140 protein.

[00150] The results of the above described studies also indicated that the sugar, sugar concentration, surfactant, surfactant concentration and protein concentration had an effect on the stability, so these parameters were further investigated as described in Example 2 below.

[00151] **Example 2: Effect of Sugar, Surfactant, and Protein Concentration on Stability of HIV Clade C gp140 Formulations**

[00152] HIV gp140 protein immunogenic formulations were prepared in a 10 mM histidine buffer, pH 6.0 ± 0.5, with the following parameters being varied: sugar (sorbitol and sucrose), sugar concentration (2% and 12% w/v), polysorbate (PS20 and PS80), polysorbate concentration (0.02% and 0.1% w/v), and protein concentration (0.2 mg/mL and 1.0 mg/mL). The formulations were prepared by adding 10 mM histidine buffer, pH 6.0 ± 0.5, followed by addition of sugar and surfactant. The pH was adjusted as necessary. HIV gp140 clade C protein (SEQ ID NO: 1) was added to the desired concentration. The formulations were then analyzed by High-Performance Size Exclusion Chromatography (HP-SEC), as described below.

[00153] The formulations prepared and tested are shown below in Table 2.

[00154] **Table 2**: HIV gp140 protein formulations

Formulation	Sugar	Sugar Concentration (% w/v)	Surfactant	Surfactant Concentration (% w/v)	Protein concentration (mg/mL)
1	sorbitol	12	PS20	0.02	0.2
2	sorbitol	12	PS80	0.1	0.2
3	sorbitol	2	PS20	0.1	0.2
4	sorbitol	2	PS20	0.1	0.2
5	sorbitol	2	PS80	0.02	1
6	sorbitol	12	PS80	0.02	1
7	sorbitol	12	PS20	0.02	1
8	sorbitol	2	PS80	0.1	1
9	sorbitol	12	PS80	0.1	1
10	sucrose	12	PS80	0.02	0.2
11	sucrose	2	PS20	0.02	0.2
12	sucrose	12	PS80	0.1	0.2
13	sucrose	2	PS80	0.02	0.2
14	sucrose	2	PS80	0.1	1
15	sucrose	2	PS20	0.02	1
16	sucrose	2	PS20	0.1	1
17	sucrose	12	PS20	0.1	1
18	sucrose	12	PS20	0.1	1

[00155] High-Performance Size Exclusion Chromatography (HP-SEC) Analysis: HP-SEC was used to analyze the amount of hexamer, trimer, and high/low molecular weight species in formulation samples by monitoring the absorbance at 280 nm. The HIV gp140 protein exists predominantly as a trimer. Some HIV gp140 protein hexamer species is also observed. Although the trimer species is the desired species, immunogenicity against HIV is observed for both the trimer species and the hexamer species. High molecular weight (HMW) and low molecular weight (LMW) species in the formulations are undesired. In particular, the presence of LMW species indicates cleavage and/or degradation of the gp140 protein. HMW species may be caused by a number of factors, such as aggregation of the gp140 protein. Samples of each formulation were analyzed at time 0 (T_0), and then stressed at 40°C for 24 hours (T_{24}). Formulations containing 1 mg/mL clade C gp140 protein were diluted two-fold before injection into the HP-SEC system, and formulations containing 0.2 mg/mL clade C gp140 protein were not diluted prior to injection.

[00156] The results of the HP-SEC analysis are shown below in Table 3, and in Figs. 2A-2F. The results indicate that amount of trimer and hexamer for both T_0 and T_{24} samples depends on the concentration of surfactant and HIV gp140 protein. Formulations having a

lower surfactant concentration (0.02% w/v) had a higher observed value of trimer +hexamer species of the HIV gp140 protein, with less high/low molecular weight species observed (see Figs. 2B, 2D, and 2F), both before and after sample stressing. Formulations having a higher HIV gp140 protein concentration (1.0 mg/mL) also had a higher observed value of trimer +hexamer species of the HIV gp140 protein, with less high/low molecular weight species observed (see Figs. 2B and 2D), both before and after sample stressing (see Figs. 2A and 2C). Formulations containing polysorbate 20, as opposed to polysorbate 80, also had a lower amount of lower molecular weight species present (see Fig. 2E).

[00157] **Table 3**: Results of HP-SEC Analysis.

Formulation	High Molecular Weight Species (%)		Hexamer (%)		Trimer (%)		Low Molecular Weight Species (%)	
	T_0	T_{24}	T_0	T_{24}	T_0	T_{24}	T_0	T_{24}
1	0.57	0	10.69	10.13	88.05	89.87	0	0
2	0.22	0	8.48	9.55	80.08	85.39	11.22	5.06
3	0.2	0	9.15	9.51	84.47	85.77	0	4.72
4	0	0	9.26	9.56	84.63	86.71	0	3.73
5	0	0	10.72	12.27	88.41	87.73	0.86	0
6	0	0	10.78	11.6	88.44	88.4	0.78	0
7	0	0	11.08	11.7	88.6	88.3	0.32	0
8	0	0	10.46	11.67	87.33	88.33	2.21	0
9	0	0	10.6	11.57	86.91	88.43	2.48	0
10	0	0	9.79	10.02	88.17	89.98	2.05	0
11	0	0	10.52	10.54	88.75	89.46	0	0
12	0	0	8.9	9.23	79.79	83.51	11.31	7.26
13	0	0	9.74	10.58	87.58	89.42	2.68	0
14	0	0	10.37	11.82	87.38	87.98	2.25	0.2
15	0	0	10.91	12.15	88.77	87.85	0.32	0
16	0	0	10.57	11.42	87.71	87.72	1.72	0.86
17	0	0	10.63	11.45	88.51	88.43	0	0.12
18	0	0	10.74	11.54	88.28	88.46	0	0

[00158] The data shown in Table 3 indicates that the combination of sorbitol and histidine buffer is preferable to the combination of histidine buffer and sucrose. For examples, the formulations containing histidine buffer and sorbitol, such as formulations 1, 4, and 7, showed the least change between T_0 and T_{24} in the amount of hexamer and trimer species as compared to that observed for the formulations containing histidine buffer and sucrose. This result was surprising because histidine buffer and sorbitol are not typically used in combination for protein formulations, whereas histidine buffer and sucrose are often used in

combination. It was thus unexpected that the most optimal formulation comprised histidine buffer (pH 6.0 ± 0.5) and sorbitol, rather than histidine buffer and sucrose. The above study also indicates that including polysorbate 20 as a surfactant (see, e.g., Fig. 2E) at a concentration of 0.2% (w/v) (see, e.g., Figs. 2B, 2D, and 2F) provides a formulation in which the stability of the HIV gp140 protein is further improved.

[00159] Advantages of the formulations identified herein over the one that is currently used for gp140 drug product in clinical trials, include that they use existing qualified compendial grade excipients that are readily available for large scale manufacturing and do not suffer from known issues that might negatively influence stability in the long term, and they enable storage of stable bulk drug substance and antigen drug product. Moreover, these formulations enable storage and stability of adjuvanted drug product as a single vial drug product at refrigerated temperature. These formulations are suitable for storage in liquid form, but also have the potential to be stored in lyophilized form and then reconstituted in liquid prior to injection, which is yet another advantage.

[00160] **Example 3: Stability Studies of HIV Mosaic gp140 Protein Formulations**

[00161] Two formulations containing HIV mosaic gp140 protein (SEQ ID NO: 2) were examined for stability in a freeze-thaw and temperature cycling study. The formulations tested are shown in Table 4.

[00162] **Table 4**: HIV Mosaic gp140 Protein Formulations

Formulation	Protein	Buffer	Sugar	Surfactant
F1	1.0 mg/mL	10 mM histidine, pH 6.5	12% sorbitol	0.02% PS20
F2	1.0 mg/mL	10 mM histidine, pH 6.5	2% sorbitol	0.02% PS20

[00163] The formulations were subjected to multiple freeze-thaw cycles. One freeze-thaw cycle was conducted by freezing at -80°C or -40°C for 24 hours, followed by thawing at -2°C to 8°C for 24 hours. Samples were analyzed at the end of 1, 3, and 5 cycles of freeze-thaw by measuring the absorbance at 280 nm (concentration) and 350 nm (turbidity). The results are shown in Fig. 3A and Fig. 3B.

[00164] The results show that the absorbance at 350 nm and 280 nm was largely unchanged for both formulations F1 and F2 after multiple freeze-thaw cycles, indicating that the concentration and turbidity of the formulation were relatively unaffected. This

demonstrates that the HIV gp140 protein in the formulations is stable to freeze-thaw.

[00165] **Example 4: Long Term Stability Study of HIV gp140 protein formulations**

[00166] The stability of HIV gp140 protein compositions in histidine buffer, both with and without adjuvant according to embodiments of the invention was compared to the stability of an HIV gp140 protein composition formulated in HEPES buffer, both with and without adjuvant. The compositions tested are shown in Table 5. All formulations contained 0.2 mg/mL HIV clade C gp140 protein (SEQ ID NO: 1).

[00167] **Table 5**: HIV gp140 protein formulations for long term stability study.

Formulation	Buffer Composition	Adjuvant?
1	20 mM HEPES,pH 6.5 90 m,M NaCl 4% (w/v) sucrose 0.02% polysorbate 80	No adjuvant
2	10 mM histidine buffer, pH 6.5 12% (w/v) sorbitol 0.02% (w/v) polysorbate 20	No adjuvant
3	10 mM histidine buffer, pH 6.5 2% (w/v) sorbitol 0.02% (w/v) polysorbate 20	No adjuvant
4	10 mM histidine buffer, pH 6.5 5% (w/v) sorbitol 0.02% (w/v) polysorbate 20	No adjuvant
5	20 mM HEPES,pH 6.5 90 m,M NaCl 4% (w/v) sucrose 0.02% polysorbate 80	+ aluminum phosphate adjuvant (0.85 mg/mL)
6	10 mM histidine buffer, pH 6.5 12% (w/v) sorbitol 0.02% (w/v) polysorbate 20	+ aluminum phosphate adjuvant (0.85 mg/mL)
7	10 mM histidine buffer, pH 6.5 2% (w/v) sorbitol 0.02% (w/v) polysorbate 20	+ aluminum phosphate adjuvant (0.85 mg/mL)
8	10 mM histidine buffer, pH 6.5 5% (w/v) sorbitol 0.02% (w/v) polysorbate 20	+ aluminum phosphate adjuvant (0.85 mg/mL)

[00168] Compositions were stored at 2°C to 8°C; 25°C and 60% relative humidity (RH); and 40°C and 75% RH. The study is ongoing, and samples are tested after storage for 2 weeks, 1, 2, 3, 6, 9, 12, 18, 24, 30, and 36 months.

[00169] Samples were tested by reduced SDS after storage for up to three months. Under reducing conditions, as the SDS-PAGE was performed, the trimeric and hexameric forms were reduced to a monomeric form that showed up a single band on the gel. Any degradation of the protein to lower molecular weight species resulted in a change (decrease) in the monomeric form of the protein observed on the gel. The data for samples stored at 25°C and 60% RH, and at 40°C and 75% RH are shown in Figs. 4A-4D. The data show that all formulations were stable under storage at 25°C and 60% RH both with and without aluminum phosphate adjuvant for up to three months (Figs. 4A and 4C). However, formulations containing histidine buffer and sorbitol were more stable than the formulation containing HEPES and sucrose (Figs. 4B and 4D) when stored at 40°C and 75% RH, both with and without aluminum phosphate adjuvant. In addition, after six months storage at 25°C the histidine formulated material with 12% sorbitol, without aluminum phosphate adjuvant, showed better stability compared to the HEPES buffered formulation as measured by SDS-PAGE (smaller change in % compared to initial measurement; data not shown). Moreover, gp140 that was formulated with histidine buffer, sorbitol and aluminum phosphate was observed to be stable at 25°C for at least six months (data not shown). Also when formulated with histidine buffer and sorbitol, at higher concentrations of aluminum phosphate adjuvant (3.84 mg/mL), the HIV gp140 protein was observed to be stable at 25°C for at least six months and at 40°C for at least three months as measured by reduced SDS PAGE (less than 25% reduction, data not shown; while measurements using ELISA showed stability (less than 50% reduction) at 40°C for at least two weeks, data not shown). These data further indicate that HIV gp140 protein formulations containing histidine buffer and sorbitol have enhanced stability, and can thus be stored as an adjuvanted drug product in a single vial at elevated temperatures, which is an advantage as it is often difficult to formulate adjuvanted protein that is stable to storage at elevated temperatures. This means that the drug product with adjuvant can be stored in a single vial at a manufacturing or a fill-and-finish site, and does not require separate storage with mixing in a pharmacy or at bed-side just prior to administration. Apart from being economically beneficial, this is a huge advantage especially for an HIV vaccine product that is intended to be also used in many places where capacity and facilities may be limited. It is also an advantage of limiting the number of operations to be performed with the drug product on site where a large number of subjects are to be vaccinated in a single campaign. Thus, the formulations of the invention surprisingly improve the practical properties for use of the final vaccine, especially in resource-limited settings.

[00170] A combination of two HIV gp140 proteins, one having SEQ ID NO: 1 and one having SEQ ID NO: 2 is mixed (total protein concentration 0.2-1.0 mg/mL, e.g. 0.1 mg/mL for each protein resulting in a total concentration of gp140 protein of 0.2 mg/mL) and tested in the preferred formulation, i.e. 2% to 15% (w/v) sorbitol; 0.01 to 0.05% (w/v), e.g. 0.02%, polysorbate 20; and 5 to 20 mM, e.g. 10 mM, histidine buffer at a pH of 5.5 to 7.0, e.g. pH 6.5, and preferably aluminum phosphate, e.g. 0.7-4 mg/mL, e.g. 0.85 or 3.84 mg/mL, using methods as described above. This composition is also expected to be stable for at least six months at 2-8°C, like the individual proteins in this formulation as indicated above.

[00171] It is understood that the examples and embodiments described herein are for illustrative purposes only, and that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the invention as defined by the appended claims.

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Claims

1. An immunogenic composition comprising, relative to the total volume of the composition:

   a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein or of a mixture of at least two HIV gp140 proteins;
   b. 2% to 15% (w/v) sorbitol;
   c. 0.01 to 0.05% (w/v) polysorbate 20; and
   d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0.

2. The immunogenic composition of claim 1, wherein the concentration of the HIV gp140 protein or of the mixture of HIV gp140 proteins is 0.2 mg/mL.

3. The immunogenic composition of claim 1, wherein the concentration of the HIV gp140 protein or of the mixture of HIV gp140 proteins is 1 mg/mL.

4. The immunogenic composition of any one of claims 1-3, wherein the concentration of sorbitol is 5% (w/v).

5. The immunogenic composition of any one of claims 1-3, wherein the concentration of sorbitol is 12% (w/v).

6. The immunogenic composition of any one of claims 1-5, wherein the concentration of polysorbate 20 is 0.02% (w/v).

7. The immunogenic composition of any one of claims 1-6, wherein the concentration of the histidine buffer is 10 mM, and the pH of the histidine buffer is 6.5.

8. The immunogenic composition of any one of claims 1-7, further comprising aluminum phosphate adjuvant, e.g. at 0.7-4.0 mg/mL, e.g. at 0.85 mg/mL or 3.84 mg/mL.

9. The immunogenic composition of any one of claims 1-8, wherein the HIV gp140 protein comprises the amino acid sequence of SEQ ID NO: 1.

10. The immunogenic composition of any one of claims 1-8, wherein the HIV gp140 protein comprises the amino acid sequence of SEQ ID NO: 2.

11. The immunogenic composition of any one of claims 1-8, comprising a mixture of an HIV gp140 protein comprising the amino acid *sequence* of SEQ ID NO:1 and an HIV gp140 protein comprising the amino acid sequence of SEQ NO: 2.

12. An immunogenic composition comprising, relative to the total volume of the composition,

a. 0.2 mg/mL or 1 mg/mL of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2 or of a mixture of an HIV gp140 protein comprising the amino acid sequence of SEQ ID NO:1 and an HIV gp140 protein comprising the amino acid sequence of SEQ NO: 2;
b. 5% or 12% (w/v) sorbitol;
c. 0.02% (w/v) polysorbate 20;
d. 10 mM histidine buffer at a pH of 6.5; and
e. aluminum phosphate adjuvant, e.g. 0.85 mg/mL or 3.84 mg/mL.

13. A method of preparing an immunogenic composition comprising admixing:

a. 0.05 mg/mL to 5 mg/mL of an HIV gp140 protein or of at least two HIV gp140 proteins;
b. 2% to 15% (w/v) sorbitol;
c. 0.01 to 0.05% polysorbate 20; and
d. 5 to 20 mM histidine buffer at a pH of 5.5 to 7.0;

to thereby obtain the immunogenic composition.

14. The method according to claim 13, further comprising admixing:
e.g. 0.7-4.0 mg/mL aluminum phosphate adjuvant.

15. The immunogenic composition of any one of claims 1-12, which is stable upon storage at 2-8°C for at least six months.

16. A method for preparing a long-term, storage stable immunogenic composition that comprises HIV gp140 protein, the method comprising: (i) providing an immunogenic composition according to any one of claims 1-12 or 15, and (ii) storing said composition at 2-8°C for at least one week, e.g. at least one month, e.g. 1-72 months, e.g. 6-48 months, e.g. 12-36 months, e.g. 18-30 months.