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(54) **Title:** NOVEL COMPOSITIONS

(57) **Abstract:** The present invention relates to particular immunogenic compositions comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a saponin and a lipopolysaccharide. Such compositions are substantially free of a NefTat related polypeptide, comprise between 10-40ug of a lipopolysaccharide and between 10-40ug of an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome or have a concentration of sodium chloride of 130 mM or lower.



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Novel compositions

FIELD OF THE INVENTION

5 The present invention relates to particular immunogenic compositions comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a saponin and a lipopolysaccharide. Methods for the preparation of such immunogenic compositions and related kits are also provided.

10 BACKGROUND OF THE INVENTION

HIV is the primary cause of acquired immune deficiency syndrome (AIDS) which is regarded as one of the world's major health problems. There were approximately 34 million people living with HIV in 2011 (WHO HIV/AIDS Fact sheet number 360, June 2013) and over 4 million
15 new infections are occurring every year. HIV has claimed more than 25 million lives over the past three decades. Although antiretroviral therapy is prolonging the lives of many people infected with HIV, to be effective antiretroviral therapy requires strict adherence to often complex multidrug administration regimes and does not cure the infection. New infections are greatly exceeding the number of people able to be treated through current global financial
20 efforts. There is a need for a vaccine to prevent new infections but the development of a safe and effective HIV vaccine poses a significant challenge.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is highly virulent and infective and is the cause of the majority of HIV infections globally, whilst HIV-2 has a lower
25 virulence and infectivity and is largely confined to West Africa (Gilbert et al., *Stat In Med* 22(4):573–593 (2003) and Reeves and Doms *J Gen Vir* 83:1253-1265 (2002)). There are many genetically distinct subtypes (also known as 'clades') of HIV-1 and the amino acid sequences of just the envelope glycoproteins (gp120 and gp41) can vary from 25–30% between subtypes (Kalish et al., *AIDS* 9:851-857 (1995)). The genetic diversity of HIV-1 along
30 with the high mutation rate are major obstacles for HIV-1 vaccine development.

Although extensive research throughout the world has been conducted to produce a vaccine, much work is still required.

The HIV envelope glycoprotein gp120 and other HIV-1 proteins

gp120 is the viral protein that is used for attachment to a host cell. This attachment is mediated by gp120 binding surface molecules of helper T cells and macrophages including one of the two chemokine receptors CCR-5 or CXCR-4. The gp120 protein is first expressed as a larger precursor molecule (gp160), which is then cleaved post-translationally to yield gp120 and gp41. The gp120 protein is retained on the surface of the virion, non-covalently associated with the gp41 molecule, which is inserted into the viral membrane. Three non-covalently associated envelope glycoprotein gp120 and gp41 heterodimers form the trimeric Env spike found on the surface of HIV-1.

Non-envelope proteins of HIV-1 have been described and include for example internal structural proteins such as other products of the *Env* gene (such as gp160 and gp41) the products of the *gag* and *pol* genes (such as MA, CA, SP1, NC, SP2 and P6; and RT, RNase H, IN and PR, respectively) and other non-structural proteins such as Rev, Nef, Vif, Vpr, Vpu and Tat (Greene et al., *New Eng J Med*, 324, 5, 308 et seq (1991) and Bryant et al. (Ed. Pizzo), *Pediatr Infect Dis J*, 11, 5, 390 et seq (1992)).

Previous work in the field

gp120 was among the first targets of HIV vaccine research and was considered to be useful as an antigenic component in vaccines intended to elicit cell-mediated immune responses. The gp120 protein contains epitopes that are recognized by cytotoxic T lymphocytes (CTL). These effector cells are able to eliminate virus-infected cells, and therefore constitute an antiviral immune mechanism. Some CTL epitopes appear to be relatively conserved among different HIV strains. However, current CTL-based vaccines do not protect against infection in animal models of HIV (Amara et al., *Science* 292(5514):69-74 (2001)). Furthermore, CTL-based vaccines studied to date in humans have not induced these intended responses in all recipients (Goepfert et al., *J Infect Dis* 192(7):1249-1259 (2005)). Such CTL-based vaccines were not able to protect against infection, nor impact viral load post infection (the "HVTN-505 study").

The gp120 protein is a major target of neutralising antibodies (Pantophlet et al., *Annu Rev Immunol* 24:739-769 (2006)) and neutralising antibodies were previously thought to be a potential correlate of protection (Bruck et al., *Vaccine* 12(12):1141-1148 (1994) and Plotkin, *Pediatr Infect Dis J* 20:63-75 (2001)).

One region of the gp120 protein in particular, the 'V3 loop', is targeted by neutralising antibodies. Antibodies present in immune sera of infected individuals bind to V3 loop peptides (Spenlehauer et al., *J Vir*, 72(12):9855-9864 (1998)). Accordingly, much research was focussed on the V3 loop of the gp120 protein. However, the V3 loop is unfortunately highly variable and highly strain specific (Vaine et al., *PLoS One*, 5(11):e13916, (2010), Jones et al., *J Infect Dis*, 179:558-566 (1999) and McCormack et al., *Vaccine* 18(13):1166-1177 (2000)).

It has been shown that vaccination with gp120 will not always induce neutralising antibodies against HIV and SHIV, and when neutralising antibodies are induced, these antibodies rarely confer protection against divergent viruses (Voss et al., *J Vir* 77(2):1049-1058 (2003), Plotkin, *Pediatr Infect Dis J* 20:63-75 (2001), Wren and Kent, *Hum Vacc* 7(4):466-473 (2011)).

Use of recombinant HIV-1 NefTat, gp120_{W61D} and SIV Nef proteins formulated with the AS02A adjuvant system (50ug of QS-21 and 50ug of 3D-MPL in an oil-in-water emulsion) was shown to protect against AIDS in a rhesus macaque SHIV animal model system when animals were challenged with SIV/HIV strain SHIV_{89.6p}. However, HIV-1 gp120_{W61D} formulated alone with AS02A did not provide protection. The lack of protection after immunisation with gp120_{W61D} formulated alone with AS02A was suggested to be related to the heterologous nature of the challenge virus (20.2% sequence difference for gp120), since HIV-1 gp120_{W61D} formulated in AS02A had previously been shown to induce sterile immunity against homologous SHIV_{W61D} challenge (Voss et al., *J Virol* 77(2):1049-1058 (2003) and Mooij et al., *AIDS* 12:F15-F22 (1998)).

NefTat and gp120_{W61D} formulated in AS02A has been administered to HIV seronegative individuals in a safety and immunogenicity study. However, the neutralising antibodies elicited by this vaccine had poor cross-subtype reactivity (Leroux-Roels et al., *Vaccine* 28:7016-7024 (2010) - the "PRO HIV-002 trial"). Furthermore, gp120 had a negative impact on antibody and T cell responses (geometric mean antibody titre and lymphocyte proliferation) when comparing administration of NefTat and gp120 versus NefTat alone (Goepfert et al., *Vaccine* 25:510-518 (2007) - the "HVTN-041" study). Accordingly, it was found that (i) a vaccine comprising gp120 with an adjuvant comprising QS-21 and 3D-MPL had poor cross-subtype reactivity and (ii) it appears that gp120 may have a detrimental impact on previously accepted potential markers of efficacy for HIV vaccines containing other antigens.

In light of the findings illustrated above, the use of gp120 as a vaccine antigen to elicit humoral responses (particularly when administered with an adjuvant comprising QS-21 and 3D-MPL) was thought to be of limited use for a broadly protective vaccine and, consequently, interest in this protein has reduced.

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The RV144 HIV-1 vaccine trial was the first to demonstrate evidence of protection against HIV-1 infection, with an estimated vaccine efficacy of 31.2% (Rerks-Ngarm et al, *N Engl J Med* 361:2209-2220 (2009)). The protocol consisted of four priming injections of ALVAC-HIV (vCP1521), and two booster injections of AIDSVAX B/E. ALVAC-HIV was administered at baseline (day 0), 4 weeks, 12 weeks and 24 weeks. AIDSVAX B/E was administered at weeks 12 and 24. ALVAC-HIV (vCP1521) is a recombinant canary pox viral vector containing gp120 from HIV-1 subtype E (CRF01_AE) strain 92TH023, linked to the transmembrane-anchoring portion of gp41 (carrying a deletion in the immunodominant region) from HIV-1 subtype B strain LAI. The vector also contained HIV_{LAI} gag and pol genes. AIDSVAX B/E is a preparation of recombinant HIV-1 subtype B MN gp120, CM244 subtype E A244 gp120 and an alum adjuvant.

In order to identify correlates of risk of HIV-1 infection in RV144, plasma specimens from RV144 study participants were analysed (Haynes et al., *N Engl J Med* 366:1275-1286 (2012)). Assays were performed on samples, obtained two weeks after final immunisation, from 41 vaccinees who became infected and 205 uninfected vaccinees. These assays examined the roles of T-cell, IgG antibody and IgA antibody responses in the modulation of infection risk. It was found that levels of antibodies specific for gp70-V1V2 (a scaffolded protein carrying the first and second variable regions of HIV-1 gp120 fused to murine leukemia virus gp70 – see Pinter et al., *Vaccine* 16(19):1803-1811 (1998)) were correlated with a lower risk of infection and the binding of plasma IgA antibodies to envelope proteins correlated a higher risk of infection. Furthermore, since this analysis was performed, it has been found that V2 antibodies induced in the RV144 trial cross-react with multiple HIV-1 subtypes (Zolla-Pazner et al., *PlosOne*, 8(1):e53629 (2013)). Accordingly, for the above reasons, it was concluded that vaccines which induce higher levels of V1V2 antibodies may have improved efficacy against HIV-1 infection.

To produce an HIV vaccine, it is therefore highly desirable to identify a composition capable of eliciting a high level of antibodies specific for the V1V2 region of gp120.

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To summarise, previous work in the field has demonstrated that:

- gp120 in AS02A adjuvant (QS-21 and 3D-MPL oil-in-water emulsion) did not provide protection against challenge with heterologous virus in a primate model,
- gp120 may have a detrimental impact on markers of vaccine efficacy and
- antibodies against the V1V2 region of gp120 are a correlate of HIV-1 protection.

Immunogenic compositions of the present invention may have one or more of the following advantages compared to compositions of the prior art:

- (i) achieve a stronger humoral immune response, for example a higher serum titre of antibodies binding the V1V2 region of gp120,
- (ii) achieve a stronger cellular immune response, for example proliferation of and cytokine release by polyfunctional T cells,
- (iii) achieve a broader humoral immune response, for example achieving a titre of antibodies binding the V1V2 region of gp120 in a larger proportion of vaccinees,
- (iv) achieve a broader cellular immune response, for example proliferation of and cytokine release by polyfunctional T cells in a larger proportion of vaccinees,
- (v) achieve a stronger humoral immune response against a particular subtype of HIV-1,
- (vi) achieve a stronger cellular immune response against a particular subtype of HIV-1,
- (vii) require fewer components,
- (viii) require non-live components,
- (ix) involve a more simple dosage regime,
- (x) be simpler to produce,
- (xi) be more easily stored,
- (xii) be of utility in the treatment or prevention of HIV-1 infection,
- (xiii) be of utility in the treatment or prevention of HIV-1 infection by a first HIV-1 subtype when the gp120 related polypeptide of the composition is derived from a second HIV-1 subtype,
- (xiv) achieve a more durable immune response, for example based on magnitude of response and/or responder rates,
- (xv) achieve a greater reduction in viral load,
- (xvi) induce a higher level of protection against infection.

SUMMARY OF THE INVENTION

The present inventors administered compositions containing either (a) gp120_{W61D} and AS01B or (b) gp120_{W61D}, AS01B and NefTat to mice and analysed the serological response of the mice.

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The present inventors found that, surprisingly, mice administered with gp120_{W61D} and AS01B (without NefTat) had a higher mean level of anti-V1V2 antibodies in their serum compared to mice administered with gp120_{W61D}, AS01B and NefTat.

- 10 The present inventors analysed serum from 30 subjects who had previously taken part in the "PRO HIV-002" trial and had been vaccinated with gp120, NefTat and AS01B adjuvant (QS-21 and 3D-MPL in liposomal form).

- 15 The present inventors found that, surprisingly, subjects from the PRO HIV-002 trial had a high level of anti-V1V2 antibodies in their serum, which showed high persistence and exceeded the level of anti-V1V2 antibodies in serum from subjects of the RV144 trial.

It may be expected that the claimed immunogenic compositions elicit a high level of anti-V1V2 antibodies, and have utility the in the prophylaxis of HIV infection.

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The present invention provides an immunogenic composition which is in the form of a human dose comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises between 10-40ug of a lipopolysaccharide and between 10-40ug of an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.

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Also provided is an immunogenic composition comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome wherein:

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- (i) the conductivity of the composition is 13 mS/cm or lower; and/or
- (ii) the concentration of salts in said composition is 130 mM or lower; and/or
- (iii) the concentration of sodium chloride in said composition is 130 mM or lower.

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Further provided is an immunogenic composition comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a lipopolysaccharide and an immunologically

active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome and wherein the composition is substantially free of a NefTat related polypeptide wherein the NefTat related polypeptide is a polypeptide consisting of SEQ ID NO: 4.

DESCRIPTION OF THE FIGURES

Figure 1: Mouse anti-V1V2 serology comparison

Figure 2: PRO HIV-002 and RV144 trial analysis: anti-V1V2 IgG comparison

Figure 3: PRO HIV-002 anti-V1V2 IgG responder rates

Figure 4: PRO HIV-002 anti-V1V2 IgG cross-subtype reactivity as compared to RV144

Figure 5: PRO HIV-002 anti-V1V2 IgG durability

DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1	gp120 _{W61D}
SEQ ID NO: 2	Nef
SEQ ID NO: 3	Tat
SEQ ID NO: 4	NefTat
SEQ ID NO: 5	gp120 _{ZM18}
SEQ ID NO: 6	polynucleotide sequence encoding gp120 _{ZM18}
SEQ ID NO: 7	native gp120 _{ZM18}
SEQ ID NO: 8	polynucleotide sequence encoding native gp120 _{ZM18}

DETAILED DESCRIPTION OF THE INVENTION

Polypeptides

As used herein, the term 'a gp120 related polypeptide' refers to a polypeptide comprising the V1V2 region of SEQ ID NO: 1 or to an immunogenic derivative or fragment of the V1V2 region of SEQ ID NO: 1, or to a polypeptide comprising the V1V2 region of SEQ ID NO: 5 or to an immunogenic derivative or fragment of the V1V2 region of SEQ ID NO: 5. Suitably, the gp120 related polypeptide refers to a polypeptide consisting of the V1V2 region of SEQ ID NO: 1 or to an immunogenic derivative or fragment of the V1V2 region of SEQ ID NO: 1, or to a polypeptide consisting of the V1V2 region of SEQ ID NO: 5 or to an immunogenic derivative or fragment of the V1V2 region of SEQ ID NO: 5.

The V1V2 region of a gp120 related polypeptide is the stretch of residues defined by two particular cysteine residues which form a disulphide bridge in the folded state of the polypeptide. The V1V2 region itself excludes these cysteine residues. An example of the V1V2 region in the case of the gp120_{W61D} polypeptide (SEQ ID NO: 1) is the stretch of residues from residue 90 to 184. The skilled person will appreciate that the position of these cysteine residues and therefore the position of the V1V2 region within a given gp120 related polypeptide can vary. This is illustrated by another gp120 related polypeptide, gp120_{ZM18} (SEQ ID NO: 5) wherein the V1V2 region is the stretch of residues from residue 90 to 172.

Suitably, the term 'a gp120 related polypeptide' refers to a polypeptide comprising SEQ ID NO: 1 or to an immunogenic derivative or fragment of SEQ ID NO: 1, or to a polypeptide comprising SEQ ID NO: 5 or to an immunogenic derivative or fragment of SEQ ID NO: 5. Suitably, the gp120 related polypeptide refers to a polypeptide consisting of SEQ ID NO: 1 or to an immunogenic derivative or fragment of SEQ ID NO: 1, or to a polypeptide consisting of SEQ ID NO: 5 or to an immunogenic derivative or fragment of SEQ ID NO: 5.

As used herein, the term "derivative" refers to a polypeptide that is modified relative to the reference sequence. Immunogenic derivatives are sufficiently similar to the reference sequence to remain capable of eliciting an immune response against the V1V2 region of the reference sequence. Suitably, immunogenic derivatives are sufficiently similar to the reference sequence to retain other key immunogenic properties of the reference sequence such as avoiding the introduction of immunodominant epitopes. A derivative may, for example, comprise a modified version of the reference sequence or alternatively may consist of a modified version of the reference sequence.

Suitably, the gp120 related polypeptide comprises or consists of a gp120 polypeptide derived from HIV-1 or HIV-2, suitably from groups M, N, O or P of HIV-1, suitably from subtype A, B, C, D, E, F, G, H, I, J or K of group M. Suitably, the gp120 related polypeptide comprises or consists of a gp120 polypeptide derived from HIV-1, suitably from group M, more suitably from subtype B. For example, the gp120 related polypeptide comprises or consists of the gp120 polypeptide from a HIV-1 or HIV-2, suitably from an M, N, O or P group HIV-1, suitably from a subtype A, B, C, D, E, F, G, H, I, J or K of group M HIV-1. Suitably, the gp120 related polypeptide comprises or consists of the gp120 polypeptide from an HIV-1, suitably from an HIV-1 group M, more suitably from an HIV-1 group M subtype B.

The skilled person will recognise that individual substitutions, deletions or additions to the gp120 related polypeptide which alter, add or delete a single amino acid or a small percentage of amino acids is an "immunogenic derivative" where the alteration(s) results in the substitution/deletion/addition of residues which do not substantially impact the immunogenic function. By "not substantially impact the immunogenic function" is meant at least 50%, suitably at least 75% and suitably at least 90% activity of the reference sequence in an assay of the level of antibodies binding the gp120 V1V2 region (e.g. ELISA) produced at a given time point after immunisation with the gp120 related polypeptide.

Conservative substitution tables providing functionally similar amino acids are well known in the art. In general, such conservative substitutions will fall within one of the amino-acid groupings specified below, though in some circumstances other substitutions may be possible without substantially affecting the immunogenic properties of the antigen. The following eight groups each contain amino acids that are typically conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
 - 2) Aspartic acid (D), Glutamic acid (E);
 - 3) Asparagine (N), Glutamine (Q);
 - 4) Arginine (R), Lysine (K);
 - 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
 - 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
 - 7) Serine (S), Threonine (T); and
 - 8) Cysteine (C), Methionine (M)
- (see, e.g., Creighton, Proteins 1984).

Suitably such substitutions do not occur in the region of an epitope, and do not therefore have a significant impact on the immunogenic properties of the antigen. Suitably an immunogenic derivative will contain substitutions of up to 20 residues (for example up to 5 residues) relative to the reference sequence.

Immunogenic derivatives may also include those wherein additional amino acids are inserted compared to the reference sequence. Suitably such insertions do not occur in the region of an epitope, and do not therefore have a significant impact on the immunogenic properties of the antigen. Suitably an immunogenic derivative will contain additions of up to 5 residues (for example 1 or 2 residues) at 0-5 locations (for example 0-2 locations) relative to the reference

sequence. One example of insertions includes a short stretch of histidine residues (e.g. 6 residues) to aid purification of the antigen in question.

Immunogenic derivatives include those wherein amino acids have been deleted compared to the reference sequence. Suitably such deletions do not occur in the region of an epitope, and do not therefore have a significant impact on the immunogenic properties of the antigen. Suitably an immunogenic derivative will contain deletions of up to 5 residues (for example 1 or 2 residues) at 0-5 locations (for example 0-2 locations) relative to the reference sequence.

Suitably the gp120 related polypeptide will comprise, such as consist of, an immunogenic derivative of the V1V2 region of SEQ ID NO: 1 having a small number of deletions, insertions and/or substitutions, such as a derivative of the V1V2 region of SEQ ID NO: 1 having deletions of up to 5 residues (for example 1 or 2 residues) at 0-5 locations (for example 0-2 locations), insertions of up to 5 residues (for example 1 or 2 residues) at 0-5 five locations (for example 0-2 locations) and substitutions of up to 20 residues (for example up to 5 residues).

Alternatively, the gp120 related polypeptide will comprise, such as consist of, an immunogenic derivative of the V1V2 region of SEQ ID NO: 5 having a small number of deletions, insertions and/or substitutions, such as a derivative of the V1V2 region of SEQ ID NO: 5 having deletions of up to 5 residues (for example 1 or 2 residues) at 0-5 locations (for example 0-2 locations), insertions of up to 5 residues (for example 1 or 2 residues) at 0-5 five locations (for example 0-2 locations) and substitutions of up to 20 residues (for example up to 5 residues).

Suitably the gp120 related polypeptide will comprise, such as consist of, an immunogenic derivative of SEQ ID NO: 1 having a small number of deletions, insertions and/or substitutions, such as a derivative of SEQ ID NO: 1 having deletions of up to 5 residues at 0-5 locations, insertions of up to 5 residues at 0-5 five locations and substitutions of up to 20 residues.

Alternatively, the gp120 related polypeptide will comprise, such as consist of, an immunogenic derivative of SEQ ID NO: 5 having a small number of deletions, insertions and/or substitutions, such as a derivative of SEQ ID NO: 5 having deletions of up to 5 residues at 0-5 locations, insertions of up to 5 residues at 0-5 five locations and substitutions of up to 20 residues.

Suitably, the gp120 related polypeptide comprises a polypeptide with at least 70% identity, more suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more

suitably at least 99% identity with the V1V2 region of SEQ ID NO: 1. Suitably the gp120 related polypeptide comprises the V1V2 region of SEQ ID NO: 1.

Suitably, the gp120 related polypeptide consists of a polypeptide with at least 70% identity, suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more suitably at least 99% identity with the V1V2 region of SEQ ID NO: 1. Suitably, the gp120 related polypeptide consists of the V1V2 region of SEQ ID NO: 1.

Alternatively, the gp120 related polypeptide comprises a polypeptide with at least 70% identity, more suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more suitably at least 99% identity with the V1V2 region of SEQ ID NO: 5. Suitably the gp120 related polypeptide comprises the V1V2 region of SEQ ID NO: 5.

Suitably, the gp120 related polypeptide consists of a polypeptide with at least 70% identity, suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more suitably at least 99% identity with the V1V2 region of SEQ ID NO: 5. Suitably the gp120 related polypeptide consists of the V1V2 region of SEQ ID NO: 5.

Suitably, the gp120 related polypeptide comprises a polypeptide with at least 70% identity, more suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more suitably at least 99% identity with SEQ ID NO: 1. Suitably, the gp120 related polypeptide comprises SEQ ID NO: 1.

Suitably, the gp120 related polypeptide consists of a polypeptide with at least 70% identity, suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more suitably at least 99% identity with SEQ ID NO: 1. Suitably, the gp120 related polypeptide consists of SEQ ID NO: 1.

Alternatively, the gp120 related polypeptide comprises a polypeptide with at least 70% identity, more suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more

suitably at least 99% identity with SEQ ID NO: 5. Suitably, the gp120 related polypeptide comprises SEQ ID NO: 5.

Suitably, the gp120 related polypeptide consists of a polypeptide with at least 70% identity, suitably at least 80% identity, more suitably at least 85% identity, more suitably at least 90% identity, more suitably at least 95% identity, more suitably at least 98% identity, more suitably at least 99% identity with SEQ ID NO: 5. Suitably, the gp120 related polypeptide consists of SEQ ID NO: 5.

The terms “identical” or “% identity,” in the context of two or more polypeptide sequences, refer to two or more sequences or sub-sequences that are the same or have a specified percentage of amino acid residues that are the same over a specified region, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least 300 amino acids in length, such as at least 400 amino acids or at least 500 amino acids. Most suitably, the comparison is performed over a window corresponding to the entire length of the reference sequence (as opposed to the derivative sequence).

For sequence comparison, one sequence acts as the reference sequence, to which the test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percentage sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A “comparison window”, as used herein, refers to a segment in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerised implementations of these algorithms (GAP, BESTFIT, FASTA, and

TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., *Curr Pro Mol Biol* (Ausubel et al., eds. 1995 supplement)).

5 An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (website at www.ncbi.nlm.nih.gov/). This
10 algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul et al., supra). These initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs containing
15 them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each
20 direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as
25 defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

30 The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two
35 nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of

the test nucleic acid to the reference nucleic acid is less than about 0.2, more suitably less than about 0.01, and most suitably less than about 0.001.

An "immunogenic fragment" will contain a contiguous sequence of amino acids from the gp120 related polypeptide of which it is a fragment. Suitably, the fragment contains at least 15 to 50 amino acids, at least 51 to 150, at least 300 amino acids, at least 350, at least 400, or at least 450 contiguous amino acids from SEQ ID NO: 1.

Alternatively the fragment contains at least 15 to 50 amino acids, at least 51 to 150 amino acids, at least 300, at least 350, at least 400, or at least 450 contiguous amino acids from SEQ ID NO: 5.

The immunogenic fragment will remain capable of eliciting an immune response against the V1V2 region of the reference sequence. By "remain capable of eliciting an immune response against the V1V2 region of the reference sequence" is meant at least 50%, suitably at least 75% and suitably at least 90% activity of the reference sequence in an assay of the level of antibodies binding the gp120 V1V2 region (e.g. ELISA) produced at a given time point after immunisation with the gp120 related polypeptide.

The gp120 related polypeptide may for example contain 2000 amino acid residues or fewer, such as 1500 amino acid residues or fewer, in particular 1000 amino acid residues or fewer, especially 800 amino acid residues or fewer.

Fusion proteins (also known as chimeric proteins) are proteins created through the covalent linkage of two or more polypeptide sequences which are not joined in nature, such as through a peptide linkage. For example, the gp120 related polypeptide may be provided in the form of a fusion protein also comprising a second HIV antigen.

The dose of a gp120 related polypeptide is suitably able to produce an adequate immune response in a human while having an acceptable reactogenicity profile.

Suitably the immunogenic composition comprises around 1 to 200 ug of gp120 related polypeptide, suitably between 1 to 100 ug, suitably between 2 to 50 ug, such as between 3 to 30 ug, in particular between 5 to 15 ug or between 16 to 25 ug, between 9 to 11 ug or between 19 to 21 ug, most suitably 10 ug or 20 ug.

Suitably, the immunogenic composition is provided in a volume which is suitable for administration to a human as a single dose. The volume which may be administered to a human is dependent on the method, route and/or location of administration. In one embodiment the human dose is between 0.1 and 1ml, more suitably between 0.3 and 0.75ml, such as between 0.45 and 0.55ml, in particular 0.5ml. Volumes of between 0.1 and 1ml are particularly suitable for administration through routes such as subcutaneous and in particular intramuscular delivery. In another embodiment the human dose is between 0.05 and 0.2ml, suitably between 0.075 and 0.15ml, in particular 0.1ml. Volumes of between 0.05 and 0.2ml are particularly suitable for administration through routes wherein a limited volume can be administered, such as intradermal delivery.

A gp120 polypeptide may be prepared by methods described in the art or methods analogous thereto.

As used herein, the term 'a Nef related polypeptide' refers to the polypeptide provided in SEQ ID NO: 2, or to an immunogenic derivative or fragment thereof.

As used herein, the term 'a Tat related polypeptide' refers to the polypeptide provided in SEQ ID NO: 3, or to an immunogenic derivative or fragment thereof.

As used herein, the term 'a NefTat related polypeptide' refers to the polypeptide provided in SEQ ID NO: 4, or to an immunogenic derivative or fragment thereof.

Suitably the Nef related polypeptide will comprise, such as consist of, an immunogenic derivative of SEQ ID NO: 2, optionally having a small number of deletions, insertions and/or substitutions. An example is a derivative of SEQ ID NO: 2 having deletions of up to 5 residues at 0-5 locations, insertions of up to 5 residues at 0-5 five locations and substitutions of up to 20 residues.

Suitably the Tat related polypeptide will comprise, such as consist of, an immunogenic derivative of SEQ ID NO: 3, optionally having a small number of deletions, insertions and/or substitutions. An example is a derivative of SEQ ID NO: 3 having deletions of up to 5 residues at 0-5 locations, insertions of up to 5 residues at 0-5 five locations and substitutions of up to 20 residues.

Suitably the NefTat related polypeptide will comprise, such as consist of, an immunogenic derivative of SEQ ID NO: 4, optionally having a small number of deletions, insertions and/or substitutions. An example is a derivative of SEQ ID NO: 4 having deletions of up to 5 residues at 0-5 locations, insertions of up to 5 residues at 0-5 five locations and substitutions of up to 20 residues.

Suitably, the Nef related polypeptide comprises SEQ ID NO: 2. Suitably the Nef related polypeptide comprises a polypeptide with at least 99% identity, suitably at least 98% identity, more suitably at least 95% identity, more suitably at least 90% identity, more suitably at least 85% identity, more suitably at least 80% identity, more suitably at least 75% identity, more suitably at least 70% identity with SEQ ID NO: 2.

Suitably, the Tat related polypeptide comprises SEQ ID NO: 3. Suitably the Tat related polypeptide comprises a polypeptide with at least 99% identity, suitably at least 98% identity, more suitably at least 95% identity, more suitably at least 90% identity, more suitably at least 85% identity, more suitably at least 80% identity, more suitably at least 75% identity, more suitably at least 70% identity with SEQ ID NO: 3.

Suitably, the NefTat related polypeptide comprises SEQ ID NO: 4. Suitably the NefTat related polypeptide comprises a polypeptide with at least 99% identity, suitably at least 98% identity, more suitably at least 95% identity, more suitably at least 90% identity, more suitably at least 85% identity, more suitably at least 80% identity, more suitably at least 75% identity, more suitably at least 70% identity with SEQ ID NO: 4.

Suitably, the Nef related polypeptide consists of SEQ ID NO: 2. Suitably the Nef related polypeptide consists of a polypeptide with at least 99% identity, suitably at least 98% identity, more suitably at least 95% identity, more suitably at least 90% identity, more suitably at least 85% identity, more suitably at least 80% identity, more suitably at least 75% identity, more suitably at least 70% identity with SEQ ID NO: 2.

Suitably, the Tat related polypeptide consists of SEQ ID NO: 3. Suitably the Tat related polypeptide consists of a polypeptide with at least 99% identity, suitably at least 98% identity, more suitably at least 95% identity, more suitably at least 90% identity, more suitably at least 85% identity, more suitably at least 80% identity, more suitably at least 75% identity, more suitably at least 70% identity with SEQ ID NO: 3.

Suitably, NefTat related polypeptide consists of SEQ ID NO: 4. Suitably the NefTat related polypeptide consists of a polypeptide with at least 99% identity, suitably at least 98% identity, more suitably at least 95% identity, more suitably at least 90% identity, more suitably at least 85% identity, more suitably at least 80% identity, more suitably at least 75% identity, more
5 suitably at least 70% identity with SEQ ID NO: 4.

In one embodiment a Nef related polypeptide is a NefTat related polypeptide. In another embodiment a Tat related polypeptide is a NefTat related polypeptide.

- 10 Particular derivatives of a Nef related polypeptide, Tat related polypeptide or NefTat related polypeptide include those with additional His residues at the N-terminus (e.g. a polyhistidine tag of six His residues, which may be used for nickel affinity purification).

- Suitably the compositions of the present invention are substantially free of a Nef related
15 polypeptide. Suitably, the compositions of the present invention contain a ratio of a Nef related polypeptide:gp120 related polypeptide of less than 1:20, suitably less than 1:25, suitably less than 1:50, suitably less than 1:100, suitably less than 1:200, suitably less than 1:500, more suitably less than 1:1000 by weight.

- 20 Suitably the compositions of the present invention contain a Nef related polypeptide at a level of less than 5ug, suitably less than 4ug, suitably less than 3ug, suitably less than 2ug, suitably less than 1ug, suitably less than 0.5ug, suitably less than 0.2ug, suitably less than 0.1ug, suitably less than 0.05ug, more suitably less than 0.01ug per human dose.

- 25 Most suitably the compositions of the present invention are free of a Nef related polypeptide.

- Suitably the compositions of the present invention are substantially free of a Tat related polypeptide. Suitably, the compositions of the present invention contain a ratio of a Tat related polypeptide:gp120 related polypeptide of less than 1:20, suitably less than 1:25, suitably less
30 than 1:50, suitably less than 1:100, suitably less than 1:200, suitably less than 1:500, more suitably less than 1:1000 by weight.

- Suitably the compositions of the present invention contain a Tat related polypeptide at a level of less than 5ug, suitably less than 4ug, suitably less than 3ug, suitably less than 2ug, suitably
35 less than 1ug, suitably less than 0.5ug, suitably less than 0.2ug, suitably less than 0.1ug, suitably less than 0.05ug, more suitably less than 0.01ug per human dose.

Most suitably the compositions of the present invention are free of a Tat related polypeptide.

Suitably the compositions of the present invention are substantially free of a NefTat related polypeptide. Suitably, the compositions of the present invention contain a ratio of a NefTat related polypeptide:gp120 related polypeptide of less than 1:20, suitably less than 1:25, suitably less than 1:50, suitably less than 1:100, suitably less than 1:200, suitably less than 1:500, more suitably less than 1:1000 by weight.

Suitably the compositions of the present invention contain a NefTat related polypeptide at a level of less than 5ug, suitably less than 4ug, suitably less than 3ug, suitably less than 2ug, suitably less than 1ug, suitably less than 0.5ug, suitably less than 0.2ug, suitably less than 0.1ug, suitably less than 0.05ug, more suitably less than 0.01ug per human dose.

Most suitably the compositions of the present invention are free of a NefTat related polypeptide.

The presence of further HIV proteins may increase the efficacy of the composition of the invention. Suitably the composition of the invention may comprise additional proteins which may be further products of the *Env* gene (such as gp160 and gp41 or a further gp120 related polypeptide) the products of the gag and pol genes (such as MA, CA, SP1, NC, SP2 and P6; and RT, RNase H, IN and PR, respectively) and, other non-structural proteins such as Rev, Vif, Vpr and Vpu (or immunogenic derivatives or fragments thereof).

The HIV gag gene encodes a precursor protein p55, which can assemble spontaneously into immature virus-like particles (VLPs). The precursor is proteolytically cleaved into the major structural proteins CA (capsid) and MA (matrix), and into several smaller proteins. Both the precursor protein p55 and its major derivatives CA and MA may be considered as appropriate vaccine antigens which may increase the efficacy of the composition of the invention. The precursor p55 and the capsid protein CA may be used as VLPs or as monomeric proteins. Suitably the composition of the present invention may comprise one or more of these proteins.

Suitably, the composition of the present invention may comprise a polynucleotide encoding a polypeptide described above including for example a gp120 related polypeptide. The polynucleotide may be in the form of plasmid DNA or in the form of a recombinant live vector.

In one embodiment, the composition of the present invention does not comprise additional HIV antigens. In a second embodiment of the invention the composition comprises 1-5 additional HIV antigens, such as 1 or 2 additional HIV antigens. Additional HIV antigens may be provided in the form of proteins or polynucleotides encoding proteins.

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Suitably, the immunogenic composition comprises a total of around 1 to 500 ug of antigenic material, suitably between 1 to 200 ug, such as between 5 to 100 ug, most suitably between 5 to 50 ug.

- 10 Suitably, the polypeptides and polynucleotides used in the present invention are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more suitably at least about 95% pure and most suitably at least about 99% pure.
- 15 A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of its natural environment.

Adjuvant

20 *Saponins*

- The immunogenic composition of the invention comprises an immunologically active saponin fraction ("a saponin") as an adjuvant or as a component of an adjuvant. A particularly suitable saponin for use in the present invention is Quil A and its derivatives. Quil A is a saponin
- 25 preparation isolated from the South American tree *Quillaja Saponaria* Molina and was first described by Dalsgaard et al. in 1974 ("Saponin adjuvants", *Archiv. für die gesamte Virusforschung*, Vol. 44, Springer Verlag, Berlin, p243-254) to have adjuvant activity. Purified fragments of Quil A have been isolated by HPLC which retain adjuvant activity without the toxicity associated with Quil A (US5604106), for example QS-7 and QS-21 (also known as
- 30 QA7 and QA21). QS-21 is a natural saponin derived from the bark of *Quillaja saponaria* Molina, which induces CD8⁺ cytotoxic T cells (CTLs), Th1 cells and a predominant IgG2a antibody response and is a preferred saponin in the context of the present invention.

- In a suitable form of the present invention, the saponin adjuvant within the immunogenic
- 35 composition is a derivative of *saponaria molina* quil A, suitably an immunologically active fraction of Quil A, such as QS-7 or QS-21, suitably QS-21. In one embodiment the

compositions of the invention contain the immunologically active saponin fraction in substantially pure form. Suitably the compositions of the invention contain QS-21 in substantially pure form, that is to say, the QS-21 is at least 90% pure, for example at least 95% pure, or at least 98% pure.

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In a specific embodiment, QS-21 is provided in a less reactogenic composition where it is quenched with an exogenous sterol, such as cholesterol for example. Several particular forms of less reactogenic compositions wherein the lytic activity of QS-21 is quenched with an exogenous cholesterol exist. In a specific embodiment, the saponin/sterol is in the form of a liposome structure (US6846489, Example 1). In this embodiment the liposomes suitably contain a neutral lipid, for example phosphatidylcholine, which is suitably non-crystalline at room temperature, for example egg yolk phosphatidylcholine, dioleoyl phosphatidylcholine (DOPC) or dilauryl phosphatidylcholine. The liposomes may also contain a charged lipid which increases the stability of the liposome-QS-21 structure for liposomes composed of saturated lipids. In these cases the amount of charged lipid is suitably 1-20% w/w, suitably 5-10%. The ratio of sterol to phospholipid is 1-50% (mol/mol), suitably 20-25%.

Suitable sterols include beta-sitosterol, stigmasterol, ergosterol, ergocalciferol and cholesterol. In one particular embodiment, the adjuvant composition comprises cholesterol as sterol. These sterols are well known in the art, for example cholesterol is disclosed in the Merck Index, 11th Edn., page 341, as a naturally occurring sterol found in animal fat.

The sterol according to the invention is taken to mean an exogenous sterol, i.e. a sterol which is not endogenous to the organism from which the antigenic preparation is taken but is added to the antigen preparation or subsequently at the moment of formulation. Typically, the sterol may be added during subsequent formulation of the antigen preparation with the saponin adjuvant, by using, for example, the saponin in its form wherein its lytic activity is quenched with the sterol. Suitably the exogenous sterol is associated to the saponin adjuvant as described in US6846489.

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Where the active saponin fraction is QS-21, the ratio of QS-21 : sterol will typically be in the order of 1:100 to 1:1 (w/w), suitably between 1:10 to 1:1 (w/w), and suitably 1:5 to 1:1 (w/w). Suitably excess sterol is present, the ratio of QS-21 : sterol being at least 1:2 (w/w). In one embodiment, the ratio of QS-21 : sterol is 1:5 (w/w).

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Other saponins which have been described in the literature include Escin, which has been described in the Merck index (12th ed: entry 3737) as a mixture of saponins occurring in the seed of the horse chestnut tree, Lat: *Aesculus hippocastanum*. Its isolation is described by chromatography and purification (Fiedler, *Arzneimittel-Forsch.* 4, 213 (1953)), and by ion-exchange resins (Erbring et al., US 3,238,190). Fractions of escin have been purified and shown to be biologically active (Yoshikawa M, et al. (*Chem Pharm Bull* (Tokyo) 1996 44(8):1454-1464)). Sapoalbin from *Gypsophilla struthium* (R. Vochten et al., 1968, *J Pharm Belg*, 42, 213-226) has also been described in relation to ISCOM production for example. Another useful saponin is those derived from the plant *Gyophilla struthium*.

Suitably, the total amount of saponin in the immunogenic composition of the present invention, particularly in a human dose of the immunogenic composition of the present invention. is between 1-100ug.

In one embodiment, there is provided an immunogenic composition comprising QS-21 at a level of around 50 ug, for example between 38-100 ug, suitably between 40-75 ug or between 45-60 ug, more suitably 49-51, most suitably 50 ug.

In a further embodiment, there is provided an immunogenic composition comprising QS-21 at a level of around 25 ug, for example between 10-37 ug, suitably between 15-30 ug or between 20-27 ug, more suitably 24-26, more suitably 25 ug.

In another embodiment, there is provided an immunogenic composition in a volume which is suitable for a human dose which human dose of the immunogenic composition comprises QS-21 at a level of around 50 ug, for example between 38-100 ug, suitably between 40-75 ug or between 45-60 ug, more suitably 49-51, most suitably 50 ug.

In another embodiment, there is provided an immunogenic composition in a volume which is suitable for a human dose which human dose of the immunogenic composition comprises QS-21 at a level of around 25 ug, for example between 10-37 ug, suitably between 15-30 ug or between 20-27 ug, more suitably 24-26, more suitably 25 ug.

The dose of QS-21 is suitably able to enhance an immune response to an antigen in a human. In particular a suitable QS-21 amount is that which improves the immunological potential of the composition compared to the unadjuvanted composition, or compared to the composition adjuvanted with another QS-21 amount, whilst being acceptable from a reactogenicity profile.

Lipopolysaccharide adjuvants

Lipopolysaccharides (LPS) are the major surface molecule of, and occur exclusively in, the external leaflet of the outer membrane of gram-negative bacteria. LPS impede destruction of bacteria by serum complements and phagocytic cells, and are involved in adherence for colonisation. LPS are a group of structurally related complex molecules of approximately 10,000 Daltons in size and consist of three covalently linked regions:

- (i) an O-specific polysaccharide chain (O-antigen) at the outer region
- (ii) a core oligosaccharide central region
- (iii) lipid A - the innermost region which serves as the hydrophobic anchor, it comprises glucosamine disaccharide units which carry long chain fatty acids.

The biological activities of LPS, such as lethal toxicity, pyrogenicity and adjuvanticity, have been shown to be related to the lipid A moiety. In contrast, immunogenicity is associated with the O-specific polysaccharide component (O-antigen). Both LPS and lipid A have long been known for their strong adjuvant effects, but the high toxicity of these molecules has precluded their use in vaccine formulations. Significant effort has therefore been made towards reducing the toxicity of LPS or lipid A while maintaining their adjuvanticity.

The *Salmonella minnesota* mutant R595 was isolated in 1966 from a culture of the parent (smooth) strain (Luderitz et al. 1966 *Ann N Y Acad Sci* 133:349-374). The colonies selected were screened for their susceptibility to lysis by a panel of phages, and only those colonies that displayed a narrow range of sensitivity (susceptible to one or two phages only) were selected for further study. This effort led to the isolation of a deep rough mutant strain which is defective in LPS biosynthesis and referred to as *S. minnesota* R595.

In comparison to other LPS, those produced by the mutant *S. minnesota* R595 have a relatively simple structure.

- (i) they contain no O-specific region - a characteristic which is responsible for the shift from the wild type smooth phenotype to the mutant rough phenotype and results in a loss of virulence
- (ii) the core region is very short - this characteristic increases the strain susceptibility to a variety of chemicals

(iii) the lipid A moiety is highly acylated with up to 7 fatty acids.

4'-monophosphoryl lipid A (MPL), which may be obtained by the acid hydrolysis of LPS extracted from a deep rough mutant strain of gram-negative bacteria, retains the adjuvant properties of LPS while demonstrating a toxicity which is reduced by a factor of more than 1000 (as measured by lethal dose in chick embryo eggs) (Johnson et al. 1987 *Rev Infect Dis* 9 Suppl:S512-S516). LPS is typically refluxed in mineral acid solutions of moderate strength (e.g. 0.1 M HCl) for a period of approximately 30 minutes. This process results in dephosphorylation at the 1 position, and decarboxylation at the 6' position, yielding MPL.

3-O-deacylated monophosphoryl lipid A (3D-MPL), which may be obtained by mild alkaline hydrolysis of MPL, has a further reduced toxicity while again maintaining adjuvanticity, see US4912094 (Ribi Immunochemicals). Alkaline hydrolysis is typically performed in organic solvent, such as a mixture of chloroform/methanol, by saturation with an aqueous solution of weak base, such as 0.5 M sodium carbonate at pH 10.5. Further information on the preparation of 3D-MPL is available in, for example, US4912094 (Corixa Corporation).

The composition further comprises an additional adjuvant which is a lipopolysaccharide, suitably a non-toxic derivative of lipid A, particularly monophosphoryl lipid A or more particularly 3-deacylated monophosphoryl lipid A (3D-MPL).

3D-MPL is sold under the name MPL by GlaxoSmithKline Biologicals N.A. and is referred throughout the document as MPL or 3D-MPL. see, for example, US4436727; US4877611; US4866034 and US4912094. 3D-MPL can be produced according to the methods disclosed in US4912094. Chemically it is a mixture of 3-deacylated monophosphoryl lipid A with 3, 4, 5 or 6 acylated chains. Suitably in the compositions of the present invention small particle 3D-MPL is used. Small particle 3D-MPL has a particle size such that it may be sterile-filtered through a 0.22µm filter. Such preparations are described in US5776468.

Suitably, the total amount of lipopolysaccharide in the immunogenic composition of the present invention, particularly in a human dose of the immunogenic composition of the present invention, is between 1-100µg.

In one embodiment, there is provided an immunogenic composition comprising 3D-MPL at a level of around 50 µg, for example between 38-100 µg, suitably between 40-75 µg or between 45-60 µg, more suitably 49-51, most suitably 50 µg.

In a further embodiment, there is provided an immunogenic composition comprising 3D-MPL at a level of around 25 ug, for example between 10-37 ug, suitably between 15-30 ug or between 20-27 ug, more suitably 24-26, more suitably 25 ug.

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In another embodiment, there is provided an immunogenic composition in a volume which is suitable for a human dose which human dose of the immunogenic composition comprises 3D-MPL at a level of around 50 ug, for example between 38-100 ug, suitably between 40-75 ug or between 45-60 ug, more suitably 49-51, most suitably 50 ug.

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In another embodiment, there is provided an immunogenic composition in a volume which is suitable for a human dose which human dose of the immunogenic composition comprises 3D-MPL at a level of around 25 ug, for example between 10-37 ug, suitably between 15-30 ug or between 20-27 ug, more suitably 24-26, more suitably 25 ug.

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Suitable compositions of the invention are those wherein liposomes are initially prepared without MPL (as described in US6846489), and MPL is then added, suitably as small particles of below 100 nm particles or particles that are susceptible to sterile filtration through a 0.22 um membrane. The MPL is therefore not contained within the vesicle membrane (known as MPL out). Compositions where the MPL is contained within the vesicle membrane (known as MPL in) also form an aspect of the invention. The antigen can be contained within the vesicle membrane or contained outside the vesicle membrane. Suitably soluble antigens are outside and hydrophobic or lipidated antigens are either contained inside or outside the membrane.

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The invention comprises both lipopolysaccharide and immunologically active saponin. In a specific embodiment of the invention, the lipopolysaccharide is 3D-MPL and the immunologically active saponin is QS-21. In an embodiment of the invention, the composition comprises a lipopolysaccharide and immunologically active saponin in a liposomal formulation. Suitably in one form of these embodiments, the composition comprises 3D-MPL and QS-21, with optionally a sterol which is suitably cholesterol.

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In a further embodiment of the invention, the adjuvant composition comprises in a liposomal formulation lipopolysaccharide and immunologically active saponin in combination with one or more further immunostimulants or adjuvants. Suitably in one form of this embodiment the lipopolysaccharide is 3D-MPL and the immunologically active saponin is QS-21.

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In a specific embodiment, QS-21 and 3D-MPL are present in a weight ratio of between 1:2 to 2:1. Suitably QS-21 and 3D-MPL are present in same final amount per human dose of the immunogenic composition. In one aspect of this embodiment, a human dose of immunogenic composition comprises a final level of 50 ug of 3D-MPL and 50 ug of QS-21. In another aspect, a human dose of immunogenic composition comprises a final level of 25 ug of 3D-MPL and 25 ug of QS-21. In a further embodiment, a human dose of immunogenic composition comprises a final level of 10ug each of MPL and QS-21.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, in US4235877. Conjugation of proteins to macromolecules is disclosed, for example, in US4372945 and in US4474757.

Salt concentration

Some antigens are sensitive to the presence of salts. Without being limited by theory, it is believed that these antigens are detrimentally impacted by a phenomenon known as "salting out" which may be defined as the precipitation of a protein from its solution by interaction with salts, such as sodium chloride. These antigens aggregate and precipitate at a concentration of sodium chloride as low as 150 mM. Consequently, the stability of immunogenic compositions comprising a gp120 related polypeptides may be improved by a reduction in the concentration of sodium chloride.

The immunogenic compositions of the invention will suitably be aqueous preparations.

Accordingly, the present invention provides an immunogenic composition comprising a gp120 related polypeptide, wherein the conductivity of the composition is 13 mS/cm or lower. In particular, the present invention provides immunogenic compositions wherein the conductivity of the immunogenic composition is 12 mS/cm or lower, for example 10 mS/cm or lower, 8 mS/cm or lower, 6 mS/cm or lower, 5 mS/cm or lower, 4 mS/cm or lower, or 3 mS/cm or lower. In a particular embodiment the conductivity of the immunogenic composition is 2.5 mS/cm or lower, such as 2.25 mS/cm or lower, or 2.0 mS/cm or lower. In a further specific embodiment the conductivity of the immunogenic composition is 1.5 to 2.5 mS/cm.

Additionally provided is an immunogenic composition comprising a gp120 related polypeptide, wherein the concentration of salts in said composition is 130 mM or lower. In particular, the

present invention provides immunogenic compositions wherein the concentration of salts in said composition is 100 mM or lower, for example 90 mM or lower, 80 mM or lower, 70 mM or lower, 60 mM or lower, 50 mM or lower, or 40 mM or lower. In a particular embodiment the concentration of salts in said composition is 35 mM or lower, such as 30 mM or lower, or 25 mM or lower. In a further specific embodiment the concentration of salts in said composition is 20 to 40 mM, such as 25 to 35 mM.

The present invention also provides an immunogenic composition comprising a gp120 related polypeptide, wherein the concentration of sodium chloride in said composition is 130 mM or lower. In particular, the present invention provides immunogenic compositions wherein the concentration of sodium chloride is 100 mM or lower, for example 90 mM or lower, 80 mM or lower, 70 mM or lower, 60 mM or lower, 50 mM or lower, 40 mM or lower, 30 mM or lower, 20 mM or lower or 15 mM or lower. In a particular embodiment the concentration of sodium chloride in the immunogenic composition is 10 mM or lower, such as 7.5 mM or lower. Suitably the concentration of sodium chloride in the immunogenic composition is at or below 5 mM. In a further specific embodiment, the immunogenic composition is essentially free of sodium chloride. By essentially free is meant that the concentration of sodium chloride is at or very near to zero mM (such as 3 mM or less, 2 mM or less or 1 mM or less).

Suitably, the concentration of CaCl_2 in the immunogenic compositions will be 40 mM or lower, 30 mM or lower, 20 mM or lower, 15 mM or lower or 10 mM or lower.

Suitably, the concentration of MgSO_4 in the immunogenic compositions will be 80 mM or lower, 60 mM or lower, 40 mM or lower, 30 mM or lower, 20 mM or lower or 10 mM or lower

Suitably, the total concentration of NH_4^+ , Mg^{2+} and Ca^{2+} ions in the immunogenic compositions will be 80 mM or lower, 60 mM or lower, 40 mM or lower, 30 mM or lower, 20 mM or lower or 10 mM or lower.

It is well known that for parenteral administration solutions should have a pharmaceutically acceptable osmolality to avoid cell distortion or lysis. A pharmaceutically acceptable osmolality will generally mean that solutions will have an osmolality which is approximately isotonic or mildly hypertonic. Suitably the immunogenic compositions of the present invention will have an osmolality in the range of 250 to 750 mOsm/kg, for example, the osmolality may be in the range of 250 to 550 mOsm/kg, such as in the range of 280 to 500 mOsm/kg.

Osmolality may be measured according to techniques known in the art, such as by the use of a commercially available osmometer, for example the Advanced® Model 2020 available from Advanced Instruments Inc. (USA).

- 5 An "isotonicity agent" is a compound that is physiologically tolerated and imparts a suitable tonicity to a formulation to prevent the net flow of water across cell membranes that are in contact with the formulation.

10 In a particular embodiment there are provided immunogenic compositions further comprising a non-ionic tonicity agent. A non-ionic tonicity agent for use in an immunogenic composition will itself need to be pharmaceutically acceptable, e.g. suitable for use in humans, as well as being compatible with the gp120 related antigen and further compatible with other components such as the immunostimulant(s).

15 In one embodiment of the present invention, suitable non-ionic tonicity agents are polyols, sugars (in particular sucrose, fructose, dextrose or glucose) or amino acids such as glycine. In one embodiment the polyol is a sugar alcohol, especially a C3-6 sugar alcohol. Exemplary sugar alcohols include glycerol, erythritol, threitol, arabitol, xylitol, ribitol, sorbitol, mannitol, dulcitol and iditol. In a specific example of this embodiment, a suitable non-ionic tonicity agent
20 is sorbitol. The skilled person will recognise that an appropriate osmolality may be attained through the use of a mixture of different tonicity agents. In a particular embodiment of the invention the non-ionic tonicity agent in the compositions of the invention incorporates sucrose and/or sorbitol.

25 In one embodiment, a suitable concentration of polyol within the immunogenic composition is between about 2.5 and about 15% (w/v), in particular between about 2.5 and about 10% (w/v) for example between about 3 and about 7% (w/v), such as between about 4 and about 6% (w/v). In a specific example of this embodiment, the polyol is sorbitol.

30 In another embodiment, the immunogenic composition comprises sucrose and sorbitol. In such circumstances the immunogenic composition may suitably contain between about 2.5 and about 15% (w/v) of sucrose and between about 2.5 and about 15% (w/v) of sorbitol, in particular between about 2.5 and about 10% (w/v) of sucrose and between about 2.5 and about 10% (w/v) of sorbitol, for example, between about 3 and about 7% (w/v) of sucrose and
35 between about 3 and about 7% (w/v) of sorbitol, such as between about 4 and about 6% (w/v) of sucrose and between about 4 and about 6% (w/v) of sorbitol.

The pH of the immunogenic compositions should be suitable for parenteral administration. Typically the pH will be in the range of 6.0 to 9.0. Suitably the pH will be in the range 7.0 to 9.0, especially 7.25 to 8.75, such as 7.5 to 8.5, in particular pH 7.75 to 8.25. A pH of about 8.0 is of particular interest.

The pH may be controlled by the use of buffers, including for example Tris or phosphate buffers.

The conductivity of an immunogenic composition of the invention can be measured using techniques known in the art, for example using a dedicated conductivity meter or other instrument with the capability to measure conductivity. One suitable instrument is the Zetasizer Nano ZS from Malvern Instruments (UK).

The skilled person can readily test for the concentration of both sodium (Na^+) and chloride (Cl^-) ions using known techniques and kits. For example, sodium can be determined using a kit such as the Sodium Enzymatic Assay Kit (Catalogue Number: BQ011EAEL) from Biosupply. Chloride can be determined using a kit such as Chloride Enzymatic Assay Kit (Catalogue Number: BQ006EAEL) from Biosupply.

Immunogenic properties of the immunogenic composition of the present invention

In the present invention the immunogenic composition is suitably capable of inducing humoral response in a mammal, such as a human, administered with the immunogenic composition.

Humoral responses can be detected using an appropriate antibody-based assay. For example, the presence or absence in serum of an immunoglobulin G (IgG) antibody response to a gp120 related polypeptide can be analyzed using ELISA. The induction of humoral responses such as IgG antibodies, suitably IgG antibodies binding the V1V2 region of gp120, indicates the immunogenicity of the immunogenic compositions of the invention.

In a further embodiment, the immunogenic composition is capable of inducing an improved CD4 T-cell immune response.

By "improved CD4 T-cell immune response" is meant that a higher CD4 response is obtained in a mammal, such as a human, after administration of the adjuvanted immunogenic composition.

- 5 In particular but not exclusively, said 'improved CD4 T-cell immune response' is obtained in an immunologically unprimed patient, i.e. a patient who is seronegative to HIV.

The improved CD4 T-cell immune response (which may be provided by 'polyfunctional' T cells) may be assessed by measuring the number of cells producing any of the following immune
10 markers:

- CD4 T cells that express at least one immune marker
- cells producing at least two different immune markers (e.g. CD40L, IL-2 and/or IFN-gamma, TNF-alpha)
- cells producing at least CD40L and another immune marker (e.g. IL-2, TNF-gamma,
15 and/or IFN-gamma)
- cells producing at least IL-2 and another immune marker (e.g. CD40L, TNF-alpha and/or IFN-gamma)
- cells producing at least IFN-gamma and another immune marker (e.g. IL-2, TNF-alpha and/or CD40L)
- 20 • cells producing at least TNF-alpha and another immune marker (e.g. IL-2, CD40L and/or IFN-gamma)

There will be an improved CD4 T-cell immune response when cells producing any of the above immune markers are in a higher amount following administration. Typically at least one,
25 suitably two of the six conditions mentioned herein above will be fulfilled. In a particular embodiment, the cells producing all four immune markers will be present at a higher amount.

The improved CD4 T-cell immune response conferred by the gp120 composition of the present invention may be ideally obtained after one single administration.

30 In another embodiment, the administration of said immunogenic composition induces an improved B-memory cell response in a mammal, such as a human, administered with the immunogenic composition. An improved B-memory cell response is intended to mean an increased frequency of peripheral blood B lymphocytes capable of differentiation into antibody-secreting plasma cells upon antigen encounter as measured by stimulation of in-vitro
35 differentiation.

In a specific embodiment, the administration of said immunogenic composition induces at least two of the following responses: (i) an improved CD4 T-cell immune response, (ii) an improved B-memory cell response, (iii) an improved humoral response, against at least one of the component antigen(s) or antigenic composition compared to either immune response obtained with other compositions.

The magnitude of an immune response can also be expressed as the titre (or concentration) of antigen-specific antibodies induced by the immunogenic composition as determined by an appropriate serological test. The magnitude of a T cell response can be expressed as the frequency (or number) of antigen-specific cells induced by the immunogenic composition among the total population of T cells, which can be monitored by cytokine production.

Suitably the composition of the present invention elicits an immune response capable of cross-reactivity. Cross-reactivity is herein taken to mean the ability of immune responses induced by an immunogenic composition of the invention to recognize strains of HIV-1 from subtypes that are not represented in the immunogenic composition. For example, an immunogenic composition of the invention comprising a gp120 related polypeptide comprising from a strain of HIV-1 from subtype B is considered cross-reactive if the HIV-specific immune response, such as HIV-specific antibody or CD4+ T cell response (in particular an antibody response to the V1V2 loop of gp120), induced by the composition reacted with one or more different strains of HIV-1 not in the composition, for example, with a strain of HIV-1 from a subtype other than subtype B. Suitably, cross-reactivity will be in respect of an HIV-1 strain from a different sub-type, in particular in respect of an HIV-1 strain from a different group.

Suitably, the level of cross-reactivity observed is up to 10%, up to 15%, up to 20%, up to 25%, up to 30%, up to 35%, up to 40%, up to 45%, up to 50%, up to 55%, up to 60%, up to 65% up to 70%, up to 80%, up to 90% or up to 100% of antigen-specific cells induced by the immunogenic composition among the total population of T cells or titre (or concentration) of antigen-specific antibodies induced by the immunogenic composition.

When measuring cross-reactivity in terms of the percentage of responders to the strains of HIV-1 from different subtypes, the number or percentage of vaccinated individuals that show a positive response in an immunological assay after subsequent challenge can be measured. A responder can respond to one or more epitopes on an antigen. A responder can also respond

to one or more polypeptides in an immunogenic composition of the invention and/or to one or more antigens in an immunogenic composition of the invention.

Immunological assays such as serological tests that can be used to analyse the percentage of responders or the magnitude of an immune response are known in the art. Examples of such assays are known to a person skilled in the art.

Suitably, the level of cross-reactivity observed is up to 10%, up to 15%, up to 20%, up to 25%, up to 30%, up to 35%, up to 40%, up to 45%, up to 50%, up to 55%, up to 60%, up to 65% up to 70%, up to 80%, up to 90% or up to 100% of subjects in a sample are responders.

In an embodiment, the immunogenic composition of the invention is for use in eliciting high and long-lasting numbers of HIV-1-specific antibodies in an individual not infected with HIV.

In a further embodiment, the immunogenic composition of the invention is for use in eliciting high and long-lasting numbers of HIV-1-specific antibodies in an individual at risk of infection with an HIV-1 strain from one or more clades different from the one or more HIV-1 clades from which the gp120 related polypeptide in the immunogenic composition is derived.

In an embodiment, the immunogenic composition of the invention is for use in controlling or reducing viremia in an individual infected with HIV.

Suitably, after administration of the composition, the viral load of the subject remains below 100,000 copies/ml for at least four months after administration. In a further embodiment, the viral load of the subject remains below 100,000 copies/ml of serum for at least six months, at least twelve months, at least eighteen months, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years. In another embodiment, the subject maintains a viral load below 50,000 copies/ml, below 10,000 copies/ml, below 5000 copies/ml, below 1000 copies/ml, or below 500 copies/ml. Suitably, viral load is maintained or reduced for at least six months, at least twelve months, at least eighteen months, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years after administration of the composition.

Suitably, administration of the inventive composition results in a durable response. A durable response is for example the ability to detect, in the serum of an individual, IgG antibody

capable of binding to the V1V2 region of the gp120 related polypeptide of the composition at least 24 weeks, at least 48 weeks, at least 72 weeks, at least 96 weeks, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years after the only administration of the composition, or the first administration of the composition in a course of repeat administrations, to the individual. Suitably, antibody levels will be detected at a level of at least 5%, more suitably at least 10% and in particular at least 20% of the serum titre two weeks following the first administration. Suitably the antibody will be detectable in at least 50% of individuals, more suitably at least 60% of individuals and in particular at least 75%.

Suitably, a durable response is for example the ability to detect, in the serum of an individual, IgG antibody binding the V1V2 region of the gp120 related polypeptide of the composition at least 2 weeks, at least 6 months, at least 12 months, at least 18 months, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, or at least ten years after the final administration of the composition in a course of repeat administrations to the individual. Suitably, antibody levels will be detected at a level of at least 5%, more suitably at least 10% and in particular at least 20% of the serum titre two weeks following the final administration. Suitably the antibody will be detectable in at least 50% of individuals, more suitably at least 60% of individuals and in particular at least 75%.

Suitably, the present invention is capable of achieving a more durable immune response based on responder rates. Suitably, up to 10%, up to 15%, up to 20%, up to 25%, up to 30%, up to 35%, up to 40%, up to 45%, up to 50%, up to 55%, up to 60%, up to 65% up to 70%, up to 80%, up to 90% or up to 100% of vaccinated individuals mount an increased humoral response such as an increased serum level of IgG antibody binding the V1V2 region of the gp120 related polypeptide of the composition.

Vaccination means

The immunogenic compositions of the invention may be administered by any suitable delivery route, such as intradermal, mucosal e.g. intranasal, oral, intramuscular or subcutaneous. Other delivery routes are well known in the art. The intramuscular delivery route is preferred for the immunogenic composition.

Intradermal delivery is another suitable route. Any suitable device may be used for intradermal delivery, for example short needle devices such as those described in US4886499. Intradermal vaccines may also be administered by devices which limit the effective penetration length of a needle into the skin. Also suitable are jet injection devices which deliver liquid vaccines to the dermis via a liquid jet injector or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis. Jet injection devices are described for example in US5480381. Also suitable are ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis. Additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

Another suitable administration route is the subcutaneous route. Any suitable device may be used for subcutaneous delivery, for example classical needle. Suitably, a needle-free jet injector service is used, such as that published in US6623446. More suitably said device is pre-filled with the liquid vaccine formulation.

Alternatively the vaccine is administered intranasally. Typically, the vaccine is administered locally to the nasopharyngeal area, suitably without being inhaled into the lungs. It is desirable to use an intranasal delivery device which delivers the vaccine formulation to the nasopharyngeal area, without or substantially without it entering the lungs.

In a specific aspect of the present invention, the immunogenic composition may be given intramuscularly for the first administration, and a boosting composition may be administered through a different route, for example intradermal, subcutaneous or intranasal.

In one aspect of the invention a schedule for vaccination with the immunogenic composition may comprise the sequential ("prime-boost"), concomitant or simultaneous administration of the immunogenic composition and DNA encoding any of the above-mentioned proteins.

In one embodiment the schedule for vaccination with the immunogenic composition consists of three to five (for example four) administrations of the immunogenic composition to an individual over a period of four to eight (for example five to seven) months. Suitably, the schedule for vaccination with the immunogenic composition consists of four administrations of the immunogenic composition to an individual over a period of five to seven months.

Suitably, the gp120 related polypeptide of the invention may be substituted by a polynucleotide encoding the gp120 related polypeptide of the invention. Suitably, the polypeptide is codon-optimised.

- 5 The DNA may be delivered as plasmid DNA or in the form of a recombinant live vector, e.g. a poxvirus vector or any other suitable live vector such as retrovirus, lentivirus, adenovirus, adeno-associated virus and modified vaccinia ankara (MVA) virus. Suitably, an adenovirus is used. Alternatively a canary pox virus may be used.
- 10 The immunogenic composition may be injected once or several times followed by one or more DNA administrations. DNA may be used first for one or more administrations followed by one or more immunisations with the immunogenic composition. Alternatively, the immunogenic composition may be injected once or several times in conjunction with DNA administrations.

15 **Antiretrovirals**

- HIV is a retrovirus. The conversion of its RNA to DNA is accomplished through the action of the enzyme reverse transcriptase. Compounds that inhibit the function of reverse transcriptase inhibit replication of HIV in infected cells. Drugs incorporating such compounds
- 20 are useful in the prevention or treatment of HIV infection in humans and may be utilised in conjunction with a composition of the present invention.

- The composition of the present invention may be administered in conjunction with (i.e. before, during or after administration of) antiretroviral therapy (ART) such as nucleoside or non-
- 25 nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, entry inhibitors, maturation inhibitors, cellular inhibitors and integrase strand transfer inhibitors.

- Antiretroviral drugs include lamivudine and zidovudine, emtricitabine (FTC), zidovudine (ZDV), azidothymidine (AZT), lamivudine (3TC), zalcitabine, dideoxycytidine (ddC), tenofovir
- 30 disoproxil fumarate (TDF), didanosine (ddI), stavudine (d4T), abacavir sulfate (ABC), etravirine, delavirdine (DLV), efavirenz (EFV), nevirapine (NVP), amprenavir (APV), tipranavir (TPV), indinavir (IDV), saquinavir, saquinavir mesylate (SQV), lopinavir (LPV), ritonavir (RTV), fosamprenavir calcium (FOS-APV), ritonavir, RTV, darunavir, atazanavir sulfate (ATV), nelfinavir mesylate (NFV), enfuvirtide, T-20, maraviroc, dolutegravir and raltegravir. ART drugs
- 35 can also include antibodies, such as ibalizumab, targeting HIV proteins or cellular proteins associated with disease progression. Also included are immune-based therapies, such as IL-2,

IL-12 and alpha-epibromide. Each of these drugs can be administered alone or in combination with any other ART drug. Information about ART drugs and their administration can be found many pharmacopeia, such as the United States Pharmacopeia (USP) or accessed online, such as at www.aidsmeds.com (accessed 5 September 2013). Commercial names of these drugs and combinations of these drugs include Atripla (efavirenz, emtricitabine and tenofovir disoproxil fumarate), Complera (emtricitabine, rilpivirine, and tenofovir disoproxil fumarate), Stribild (elvitegravir, cobicistat, emtricitabine, tenofovir disoproxil fumarate), Combivir (lamivudine and zidovudine), Emtriva (emtricitabine, FTC), Epivir (lamivudine, 3TC), Epzicom (abacavir and lamivudine), Hivid (zalcitabine, dideoxycytidine, ddC), Retrovir (zidovudine, azidothymidine, AZT, ZDV), Trizivir (abacavir, zidovudine, and lamivudine), Truvada (tenofovir disoproxil fumarate and emtricitabine), Videx EC (enteric coated didanosine, ddl EC), Videx (didanosine, dideoxyinosine, ddl), Viread (tenofovir disoproxil fumarate, TDF), Zerit (stavudine, d4T), Ziagen (abacavir sulfate, ABC), rilpivirine, etravirine, Rescriptor (delavirdine, DLV), Sustiva (efavirenz, EFV), Viramune (nevirapine, NVP), Agenerase (amprenavir, APV), Aptivus (tipranavir, TPV), saquinavir, Invirase (saquinavir mesylate, SQV), Kaletra (lopinavir and ritonavir, LPV/RTV), Lexiva (Fosamprenavir Calcium, FOS-APV), Norvir (ritonavir, RTV), darunavir, Reyataz (atazanavir sulfate, ATV), Viracept (nelfinavir mesylate, NFV), Fuzeon (enfuvirtide, T-20), maraviroc, raltegravir, dolutegravir.

In one embodiment, the immunogenic composition is administered to a patient who is also taking antiretrovirals. In a further embodiment, the immunogenic composition is administered to a patient who has previously taken antiretrovirals. In a further embodiment, the immunogenic composition is administered to a patient who is not taking and has not previously taken antiretrovirals.

In one embodiment, the immunogenic composition is administered to a patient having a CD4 count of 200 or more, suitably 500 or more, most suitably between 500-1700 cells per cubic millimetre of blood. In a further embodiment, the immunogenic composition is administered to a patient having a nadir CD4 count of 200 or more, more suitably 500 or more cells per cubic millimetre of blood.

In one embodiment the immunogenic composition is administered to a subject, such as a human, who is not infected with HIV. In one embodiment the immunogenic composition is administered to a subject, such as a human, who is infected with HIV.

In one aspect of the invention, there is provided a gp120 related protein for use in the manufacture of an immunogenic composition as described herein. Such as for the prophylaxis of HIV infection. Alternatively, the composition may be intended for the treatment of HIV infection.

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In a further aspect there is provided a method for the prophylaxis of HIV infection by administration of an immunogenic composition as described herein to a mammal, such as a human. Such administration may reduce the risk of HIV infection and/or the severity of HIV infection.

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In a further aspect there is provided a method for the treatment of HIV infection by administration of an immunogenic composition as described herein to a mammal infected with HIV, such as a human. Such administration may reduce the severity of HIV infection.

15 In a further aspect there is provided a method for reducing the risk of HIV transmission from an HIV-infected individual to a partner of said HIV-infected individual, by administration of an immunogenic composition as described herein to the HIV-infected individual.

20 There is also provided an immunogenic composition as described herein for use in the prophylaxis of HIV infection.

There is also provided an immunogenic composition as described herein for use in the treatment of HIV infection.

25 The invention is illustrated by way of the following clauses:

Clause 1. An immunogenic composition comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome and wherein the composition is substantially free of a NefTat related polypeptide wherein the NefTat related polypeptide is a polypeptide consisting of SEQ ID NO: 4.

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Clause 2. An immunogenic composition which is in the form of a human dose comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises between 10-40ug of a lipopolysaccharide and between 10-40ug of an

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immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.

Clause 3. An immunogenic composition comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome wherein:

- (i) the conductivity of the composition is 13 mS/cm or lower; and/or
- (ii) the concentration of salts in said composition is 130 mM or lower; and/or
- (iii) the concentration of sodium chloride in said composition is 130 mM or lower.

Clause 4. The immunogenic composition according to either clause 2 or 3, which is substantially free of a NefTat related polypeptide wherein the NefTat related polypeptide is a polypeptide consisting of SEQ ID NO: 4.

Clause 5. The immunogenic composition according to either clause 1 or 4, which contains a ratio of NefTat related polypeptide:gp120 related polypeptide of less than 1:20.

Clause 6. The immunogenic composition according to either clause 1 or 4, which contains less than 1ug of NefTat related polypeptide.

Clause 7. The immunogenic composition according to either clause 5 or 6 wherein the composition is free of NefTat related polypeptide.

Clause 8. The immunogenic composition according to any one of clause 1 or 4 to 7 wherein the NefTat related polypeptide comprises a polypeptide with at least 99% identity with SEQ ID NO: 4.

Clause 9. The immunogenic composition according to clause 8 wherein the NefTat related polypeptide comprises a polypeptide with at least 90% identity with SEQ ID NO: 4.

Clause 10. The immunogenic composition according to clause 9 wherein the NefTat related polypeptide comprises a polypeptide with at least 80% identity with SEQ ID NO: 4.

Clause 11. The immunogenic composition according to clause 10 wherein the NefTat related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 4.

Clause 12. The immunogenic composition according to either clause 1 or 4, which contains a ratio of Nef related polypeptide:gp120 related polypeptide of less than 1:20.

Clause 13. The immunogenic composition according to either clause 1 or 4, which contains less than 1ug of Nef related polypeptide.

- Clause 14. The immunogenic composition according to either clause 12 or 13 wherein the composition is free of Nef related polypeptide.
- 5 Clause 15. The immunogenic composition according to any one of clauses 1, 4 or 12 to 14 wherein the Nef related polypeptide comprises a polypeptide with at least 99% identity with SEQ ID NO: 2.
- Clause 16. The immunogenic composition according to clause 15 wherein the Nef related polypeptide comprises a polypeptide with at least 90% identity with SEQ ID NO: 2.
- 10 Clause 17. The immunogenic composition according to clause 16 wherein the Nef related polypeptide comprises a polypeptide with at least 80% identity with SEQ ID NO: 2.
- Clause 18. The immunogenic composition according to clause 17 wherein the Nef related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 2.
- 15 Clause 19. The immunogenic composition according to either clause 1 or 4, which contains a ratio of Tat related polypeptide:gp120 related polypeptide of less than 1:20.
- Clause 20. The immunogenic composition according to either clause 1 or 4, which contains less than 1ug of Tat related polypeptide.
- 20 Clause 21. The immunogenic composition according to either clause 19 or 20 wherein the composition is free of Tat related polypeptide.
- Clause 22. The immunogenic composition according to any one of clauses 1, 4 or 19 to 21 wherein the Tat related polypeptide comprises a polypeptide with at least 99% identity with SEQ ID NO: 3.
- 25 Clause 23. The immunogenic composition according to clause 22 wherein the Tat related polypeptide comprises a polypeptide with at least 90% identity with SEQ ID NO: 3.
- Clause 24. The immunogenic composition according to clause 23 wherein the Tat related polypeptide comprises a polypeptide with at least 80% identity with SEQ ID NO: 3.
- 30 Clause 25. The immunogenic composition according to clause 24 wherein the Tat related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 3.
- Clause 26. The immunogenic composition according to any one of clauses 1 or 3 to 25 wherein the lipopolysaccharide is present at a level of between 10-100ug.
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- Clause 27. The immunogenic composition according to clause 26 wherein the lipopolysaccharide is present at a level of between 15-80ug.
- Clause 28. The immunogenic composition according to clause 27 wherein the lipopolysaccharide is present at a level of between 20-65ug.
- 5 Clause 29. The immunogenic composition according to clause 28 wherein the lipopolysaccharide is present at a level of between 30-60ug.
- Clause 30. The immunogenic composition according to clause 29 wherein the lipopolysaccharide is present at a level of between 45-55ug.
- Clause 31. The immunogenic composition according to clause 30 wherein the lipopolysaccharide is present at a level of 50ug.
- 10 Clause 32. The immunogenic composition according to any one of clauses 1 or 3 to 31 wherein the saponin is present at a level of between 10-100ug.
- Clause 33. The immunogenic composition according to clause 32 wherein the saponin is present at a level of between 15-80ug.
- 15 Clause 34. The immunogenic composition according to clause 33 wherein the saponin is present at a level of between 20-65ug.
- Clause 35. The immunogenic composition according to clause 34 wherein the saponin is present at a level of between 30-60ug.
- Clause 36. The immunogenic composition according to clause 35 wherein the saponin is present at a level of between 45-55ug.
- 20 Clause 37. The immunogenic composition according to clause 36 wherein the saponin is present at a level of 50ug.
- Clause 38. The immunogenic composition according to any one of clauses 1 to 25 wherein the lipopolysaccharide is present at a level of between 15-35ug.
- 25 Clause 39. The immunogenic composition according to clause 38 wherein the lipopolysaccharide is present at a level of between 20-30ug.
- Clause 40. The immunogenic composition according to clause 39 wherein the lipopolysaccharide is present at a level of 25ug.
- Clause 41. The immunogenic composition according to any one of clauses 1 to 31 wherein the saponin is present at a level of between 15-35ug.
- 30 Clause 42. The immunogenic composition according to clause 41 wherein the saponin is present at a level of between 20-30ug.
- Clause 43. The immunogenic composition according to clause 42 wherein the saponin is present at a level of 25ug.
- 35 Clause 44. The immunogenic composition according to any one of clauses 1 to 43 wherein the lipopolysaccharide is 3D-MPL.

- Clause 45. The immunogenic composition according to any one of clauses 1 to 44 wherein the saponin is QS-21.
- Clause 46. The immunogenic composition according to any one of clauses 1, 2 or 4 to 45 wherein:
5 (i) the conductivity of the composition is 13 mS/cm or lower; and/or
(ii) the concentration of salts in said composition is 130 mM or lower; and/or
(iii) the concentration of sodium chloride in said composition is 130 mM or lower.
- Clause 47. The immunogenic composition according to clause 46 wherein the conductivity
10 of the composition is 13 mS/cm or lower.
- Clause 48. The immunogenic composition according to clause 47 wherein the conductivity of the composition is 6 mS/cm or lower.
- Clause 49. The immunogenic composition according to clause 48 wherein the conductivity of the composition is 1.5 to 2.5 mS/cm.
- 15 Clause 50. The immunogenic composition according to clause 46 wherein the concentration of salts in said composition is 130 mM or lower.
- Clause 51. The immunogenic composition according to clause 50 wherein the concentration of salts in said composition is 60 mM or lower.
- Clause 52. The immunogenic composition according to clause 51 wherein the
20 concentration of salts in said composition is 20 to 40 mM.
- Clause 53. The immunogenic composition according to clause 46 wherein the concentration of sodium chloride in said composition is 130 mM or lower.
- Clause 54. The immunogenic composition according to clause 53 wherein the concentration of sodium chloride in said composition is 60 mM or lower.
- 25 Clause 55. The immunogenic composition according to clause 54 wherein the concentration of sodium chloride in said composition is 10 mM or lower.
- Clause 56. The immunogenic composition according any one of clauses 1 to 55, wherein the concentration of CaCl_2 in the immunogenic composition is 30 mM or lower.
- Clause 57. The immunogenic composition according any one of clauses 1 to 56, wherein
30 the concentration of MgSO_4 in the immunogenic composition is 60 mM or lower.
- Clause 58. The immunogenic composition according any one of clauses 1 to 57, total concentration of NH_4^+ , Mg^{2+} and Ca^{2+} ions is 40 mM or lower.
- Clause 59. The immunogenic composition according to any one of clauses 1 to 58 which is an aqueous solution.
- 35 Clause 60. The immunogenic composition according to any one of clauses 1 to 59 which is a single human dose.

- Clause 61. The immunogenic composition according to any one of clauses 1 to 60 wherein the human dose is between 0.1 and 1ml.
- Clause 62. The immunogenic composition according to clause 61 wherein the human dose is between 0.3 and 0.75ml.
- 5 Clause 63. The immunogenic composition according to clause 62 wherein the human dose is 0.5ml.
- Clause 64. The immunogenic composition according to any one of clauses 1 to 60 wherein the human dose is between 0.05 and 0.2ml.
- Clause 65. The immunogenic composition according to any one of clauses 1 to 64 wherein
10 the osmolality is in the range of 250 to 750 mOsm/kg.
- Clause 66. The immunogenic composition according to clause 65 wherein the osmolality is in the range of 250 to 550 mOsm/kg.
- Clause 67. The immunogenic composition according to any one of clauses 1 to 66, comprising a non-ionic tonicity agent.
- 15 Clause 68. The immunogenic composition according to clause 67, wherein the non-ionic tonicity agent is a polyol.
- Clause 69. The immunogenic composition according to clause 68, wherein the polyol is sorbitol.
- Clause 70. The immunogenic composition according to clause 69, wherein the
20 concentration of sorbitol is between about 4 and about 6% (w/v).
- Clause 71. The immunogenic composition according to any one of clauses 1 to 70, wherein the concentration of sucrose is between about 4 and about 6% (w/v).
- Clause 72. The immunogenic composition according to any one of clauses 1 to 71 wherein the pH is in the range of 6.0 to 9.0.
- 25 Clause 73. The immunogenic composition according to any one of clauses 1 to 72 wherein the composition comprises the gp120 related polypeptide at a level of around 1 to 100 ug.
- Clause 74. The immunogenic composition according to clause 73 wherein the composition comprises the gp120 related polypeptide at a level of 3 to 30 ug.
- 30 Clause 75. The immunogenic composition according to clause 74 wherein the composition comprises the gp120 related polypeptide at a level of 5 to 15 ug.
- Clause 76. The immunogenic composition according to clause 74 wherein the composition comprises the gp120 related polypeptide at a level of 16 to 25 ug.
- Clause 77. The immunogenic composition according to any one of clauses 1 to 76 wherein
35 the composition comprises 1-5 additional HIV antigens.

- Clause 78. The immunogenic composition according to clause 77 wherein the composition comprises additional HIV antigens selected from the list consisting of gp160, gp41, MA, CA, SP1, NC, SP2, P6, RT, RNase H, IN, PR, Rev, Vif, Vpr and Vpu.
- 5 Clause 79. The immunogenic composition according to any one of clauses 1 to 78 wherein the composition comprises a total of around 1 to 500 ug of antigenic material.
- Clause 80. The composition according to any one of clauses 1 to 79 wherein the gp120 related polypeptide comprises a gp120 polypeptide derived from HIV-1.
- Clause 81. The composition according to clause 80 wherein the gp120 related polypeptide comprises a gp120 polypeptide derived from HIV-1 group M.
- 10 Clause 82. The composition according to clause 81 wherein the gp120 related polypeptide comprises a gp120 polypeptide derived from HIV-1 group M subtype C.
- Clause 83. The composition according to clause 82 wherein the gp120 related polypeptide comprises a gp120 polypeptide derived from HIV-1 group M subtype B.
- Clause 84. The composition according to any one of clauses 1 to 81 and 83 wherein the
15 gp120 related polypeptide comprises a polypeptide with at least 70% identity with the V1V2 region of SEQ ID NO: 1.
- Clause 85. The composition according to clause 84 wherein the gp120 related polypeptide comprises a polypeptide with at least 80% identity with the V1V2 region of SEQ ID NO: 1.
- 20 Clause 86. The composition according to clause 85 wherein the gp120 related polypeptide comprises a polypeptide with at least 90% identity with the V1V2 region of SEQ ID NO: 1.
- Clause 87. The composition according to clause 86 wherein the gp120 related polypeptide comprises a polypeptide with at least 95% identity with the V1V2 region of SEQ
25 ID NO: 1.
- Clause 88. The composition according to clause 87 wherein the gp120 related polypeptide comprises a polypeptide with at least 98% identity with the V1V2 region of SEQ ID NO: 1.
- Clause 89. The composition according to clause 88 wherein the gp120 related polypeptide
30 comprises a polypeptide with at least 99% identity with the V1V2 region of SEQ ID NO: 1.
- Clause 90. The composition according to clause 89 wherein the gp120 related polypeptide comprises the V1V2 region of SEQ ID NO: 1.
- Clause 91. The composition according to any one of clauses 1 to 81 and 83 to 90 wherein
35 the gp120 related polypeptide consists of a polypeptide with at least 70% identity with the V1V2 region of SEQ ID NO: 1.

- Clause 92. The composition according to clause 91 wherein the gp120 related polypeptide consists of a polypeptide with at least 80% identity with the V1V2 region of SEQ ID NO: 1.
- 5 Clause 93. The composition according to clause 92 wherein the gp120 related polypeptide consists of a polypeptide with at least 90% identity with the V1V2 region of SEQ ID NO: 1.
- Clause 94. The composition according to clause 93 wherein the gp120 related polypeptide consists of a polypeptide with at least 95% identity with the V1V2 region of SEQ ID NO: 1.
- 10 Clause 95. The composition according to clause 94 wherein the gp120 related polypeptide consists of a polypeptide with at least 98% identity with the V1V2 region of SEQ ID NO: 1.
- Clause 96. The composition according to clause 95 wherein the gp120 related polypeptide consists of a polypeptide with at least 99% identity with the V1V2 region of SEQ ID NO: 1.
- 15 Clause 97. The composition according to clause 96 wherein the gp120 related polypeptide consists of the V1V2 region of SEQ ID NO: 1.
- Clause 98. The composition according to any one of clauses 1 to 81 and 83 to 97 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 1.
- 20 Clause 99. The composition according to clause 98 wherein the gp120 related polypeptide comprises a polypeptide with at least 80% identity with SEQ ID NO: 1.
- Clause 100. The composition according to clause 99 wherein the gp120 related polypeptide comprises a polypeptide with at least 90% identity with SEQ ID NO: 1.
- 25 Clause 101. The composition according to clause 100 wherein the gp120 related polypeptide comprises a polypeptide with at least 95% identity with SEQ ID NO: 1.
- Clause 102. The composition according to clause 101 wherein the gp120 related polypeptide comprises a polypeptide with at least 98% identity with SEQ ID NO: 1.
- 30 Clause 103. The composition according to clause 102 wherein the gp120 related polypeptide comprises a polypeptide with at least 99% identity with SEQ ID NO: 1.
- Clause 104. The composition according to clause 103 wherein the gp120 related polypeptide comprises SEQ ID NO: 1.
- 35 Clause 105. The composition according to any one of clauses 1 to 81 and 83 to 104 wherein the gp120 related polypeptide consists of a polypeptide with at least 70% identity with SEQ ID NO: 1.

- Clause 106. The composition according to clause 105 wherein the gp120 related polypeptide consists of a polypeptide with at least 80% identity with SEQ ID NO: 1.
- Clause 107. The composition according to clause 106 wherein the gp120 related polypeptide consists of a polypeptide with at least 90% identity with SEQ ID NO: 1.
- 5 Clause 108. The composition according to clause 107 wherein the gp120 related polypeptide consists of a polypeptide with at least 95% identity with SEQ ID NO: 1.
- Clause 109. The composition according to clause 108 wherein the gp120 related polypeptide consists of a polypeptide with at least 98% identity with SEQ ID NO: 1.
- 10 Clause 110. The composition according to clause 109 wherein the gp120 related polypeptide consists of a polypeptide with at least 99% identity with SEQ ID NO: 1.
- Clause 111. The composition according to clause 110 wherein the gp120 related polypeptide consists of SEQ ID NO: 1.
- 15 Clause 112. The composition according to any one of clauses 1 to 82 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with the V1V2 region of SEQ ID NO: 5.
- Clause 113. The composition according to clause 112 wherein the gp120 related polypeptide comprises a polypeptide with at least 80% identity with the V1V2 region of SEQ ID NO: 5.
- 20 Clause 114. The composition according to clause 113 wherein the gp120 related polypeptide comprises a polypeptide with at least 90% identity with the V1V2 region of SEQ ID NO: 5.
- Clause 115. The composition according to clause 114 wherein the gp120 related polypeptide comprises a polypeptide with at least 95% identity with the V1V2 region of SEQ ID NO: 5.
- 25 Clause 116. The composition according to clause 115 wherein the gp120 related polypeptide comprises a polypeptide with at least 98% identity with the V1V2 region of SEQ ID NO: 5.
- 30 Clause 117. The composition according to clause 116 wherein the gp120 related polypeptide comprises a polypeptide with at least 99% identity with the V1V2 region of SEQ ID NO: 5.
- Clause 118. The composition according to clause 117 wherein the gp120 related polypeptide comprises the V1V2 region of SEQ ID NO: 5.
- 35 Clause 119. The composition according to any one of clauses 1 to 82 and 112 to 118 wherein the gp120 related polypeptide consists of a polypeptide with at least 70% identity with the V1V2 region of SEQ ID NO: 5.

- Clause 120. The composition according to clause 119 wherein the gp120 related polypeptide consists of a polypeptide with at least 80% identity with the V1V2 region of SEQ ID NO: 5.
- 5 Clause 121. The composition according to clause 120 wherein the gp120 related polypeptide consists of a polypeptide with at least 90% identity with the V1V2 region of SEQ ID NO: 5.
- Clause 122. The composition according to clause 121 wherein the gp120 related polypeptide consists of a polypeptide with at least 95% identity with the V1V2 region of SEQ ID NO: 5.
- 10 Clause 123. The composition according to clause 122 wherein the gp120 related polypeptide consists of a polypeptide with at least 98% identity with the V1V2 region of SEQ ID NO: 5.
- Clause 124. The composition according to clause 123 wherein the gp120 related polypeptide consists of a polypeptide with at least 99% identity with the V1V2 region of SEQ ID NO: 5.
- 15 Clause 125. The composition according to clause 124 wherein the gp120 related polypeptide consists of the V1V2 region of SEQ ID NO: 5.
- Clause 126. The composition according to any one of clauses 1 to 82 and 112 to 125 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 5.
- 20 Clause 127. The composition according to clause 126 wherein the gp120 related polypeptide comprises a polypeptide with at least 80% identity with SEQ ID NO: 5.
- Clause 128. The composition according to clause 127 wherein the gp120 related polypeptide comprises a polypeptide with at least 90% identity with SEQ ID NO: 5.
- 25 Clause 129. The composition according to clause 128 wherein the gp120 related polypeptide comprises a polypeptide with at least 95% identity with SEQ ID NO: 5.
- Clause 130. The composition according to clause 129 wherein the gp120 related polypeptide comprises a polypeptide with at least 98% identity with SEQ ID NO: 5.
- Clause 131. The composition according to clause 130 wherein the gp120 related polypeptide comprises a polypeptide with at least 99% identity with SEQ ID NO: 5.
- 30 Clause 132. The composition according to clause 131 wherein the gp120 related polypeptide comprises SEQ ID NO: 5.
- Clause 133. The composition according to any one of clauses 1 to 82 and 112 to 132 wherein the gp120 related polypeptide consists of a polypeptide with at least 70% identity with SEQ ID NO: 5.
- 35

- Clause 134. The composition according to clause 133 wherein the gp120 related polypeptide consists of a polypeptide with at least 80% identity with SEQ ID NO: 5.
- Clause 135. The composition according to clause 134 wherein the gp120 related polypeptide consists of a polypeptide with at least 90% identity with SEQ ID NO: 5.
- 5 Clause 136. The composition according to clause 135 wherein the gp120 related polypeptide consists of a polypeptide with at least 95% identity with SEQ ID NO: 5.
- Clause 137. The composition according to clause 136 wherein the gp120 related polypeptide consists of a polypeptide with at least 98% identity with SEQ ID NO: 5.
- Clause 138. The composition according to clause 137 wherein the gp120 related polypeptide
10 consists of a polypeptide with at least 99% identity with SEQ ID NO: 5.
- Clause 139. The composition according to clause 138 wherein the gp120 related polypeptide consists of SEQ ID NO: 5.
- Clause 140. The immunogenic composition according to any one of clauses 1 to 139
15 wherein the composition further comprises a sterol, wherein the ratio of saponin:sterol is from 1:1 to 1:100 w/w.
- Clause 141. The immunogenic composition according to clause 140 wherein the ratio of saponin:sterol is from 1:1 to 1:5 w/w.
- Clause 142. The immunogenic composition according to either clause 140 or 141 wherein said sterol is cholesterol.
- 20 Clause 143. The immunogenic composition according to any one of clauses 1 to 142, which is provided in a multi-dose presentation.
- Clause 144. The immunogenic composition according to any one of clauses 1 to 143, which is provided in presentation containing an overage of 1-50% to allow for wastage during administration.
- 25 Clause 145. The immunogenic composition according to any one of clauses 1 to 144, for use as a medicament.
- Clause 146. The immunogenic composition according to clause 145, for use in the treatment or prevention of HIV-1 from group M, N, O or P.
- Clause 147. The immunogenic composition according to clause 146, for use in the treatment
30 or prevention of HIV-1 group M subtype A, B, C, D, E, F, G, H, I, J or K.
- Clause 148. The immunogenic composition according to any one of clauses 1 to 147, wherein the gp120 related polypeptide is derived from a first HIV-1 subtype, for use in the treatment or prevention of HIV-1 infection by a second HIV-1 subtype.
- 35 Clause 149. The immunogenic composition according to any one of clauses 1 to 148, wherein the gp120 related polypeptide is derived from a first HIV-1 subtype, for

use in the treatment or prevention of HIV-1 infection by a second HIV-1 subtype, wherein the first and second HIV-1 subtypes have different native gp120 polypeptide sequences.

Clause 150. The immunogenic composition according to clause 149, wherein the first HIV-1 subtype is selected from the list consisting of A, B, C, D, E, F, G, H, I, J or K; of HIV-1 group M.

Clause 151. The immunogenic composition of any one of clauses 1 to 150 for use in inducing a humoral immune response against HIV-1 strains from one or more subtypes different from the HIV-1 subtype from which the gp120 related polypeptide of the composition is derived.

Clause 152. The immunogenic composition according to any one of clauses 1 to 151 for use in eliciting antibodies against the V1V2 loop of HIV-1 gp120.

Clause 153. A method of treatment or prophylaxis of HIV-1 infection comprising the step of administering a composition according to any one of clauses 1 to 152 to an individual.

Clause 154. The method of treatment or prophylaxis of HIV-1 infection according to clause 153 further comprising concomitant administration of an antiretroviral drug.

Clause 155. The method of treatment or prophylaxis of HIV-1 infection according to either clause 153 or 154 wherein IgG antibody capable of binding to the V1V2 region of the gp120 related polypeptide of the composition is detectable in the serum of an individual at least 24 weeks after the only administration of the composition or the first administration of the composition in a course of repeat administrations to the individual.

Clause 156. The method of treatment or prophylaxis of HIV-1 infection according to clause 155 wherein the IgG antibody is detectable at least 48 weeks after the only administration of the composition or the first administration of the composition in a course of repeat administrations to the individual.

Clause 157. The method of treatment or prophylaxis of HIV-1 infection according to clause 156 wherein the IgG antibody is detectable at least 72 weeks after the only administration of the composition or the first administration of the composition in a course of repeat administrations to the individual.

Clause 158. The method of treatment or prophylaxis of HIV-1 infection according to clause 157 wherein the IgG antibody is detectable at least 96 weeks after the only administration of the composition or the first administration of the composition in a course of repeat administrations to the individual.

- 5 Clause 159. The method of treatment or prophylaxis of HIV-1 infection according to either clause 153 or 154 wherein IgG antibody capable of binding to the V1V2 region of the gp120 related polypeptide of the composition is detectable in the serum of an individual at least 2 weeks after the final administration of the composition in a course of repeat administrations to the individual.
- Clause 160. The method of treatment or prophylaxis of HIV-1 infection according to clause 159 wherein the IgG antibody is detectable at least 6 months after the final administration of the composition in a course of repeat administrations to the individual.
- 10 Clause 161. The method of treatment or prophylaxis of HIV-1 infection according to clause 160 wherein the IgG antibody is detectable at least 12 months after the final administration of the composition in a course of repeat administrations to the individual.
- 15 Clause 162. The method of treatment or prophylaxis of HIV-1 infection according to clause 161 wherein the IgG antibody is detectable at least 18 months after the final administration of the composition in a course of repeat administrations to the individual.
- Clause 163. The method of treatment or prophylaxis of HIV-1 infection according to any one of clauses 155 to 158 wherein antibody levels are detectable at at least 5% of the serum titre two weeks following the only administration.
- 20 Clause 164. The method of treatment or prophylaxis of HIV-1 infection according to any one of clauses 155 to 162 wherein antibody levels are detectable at at least 5% of the serum titre two weeks following the first administration.
- Clause 165. The method of treatment or prophylaxis of HIV-1 infection according to any one of clauses 159 to 162 wherein antibody levels are detectable at at least 5% of the serum titre two weeks following the final administration.
- 25 Clause 166. The method of treatment or prophylaxis according to any one of clauses 155 to 165 wherein the antibody will be detectable in at least 50% of individuals administered with the composition.
- 30 Clause 167. The method of treatment or prophylaxis according to clause 166 wherein the antibody will be detectable in at least 80% of individuals administered with the composition.
- 35 Clause 168. The method of treatment or prophylaxis according to any one of clauses 153 to 167 wherein a polynucleotide encoding a gp120 related polypeptide is administered to the individual and subsequently the composition according to any one of clauses 1 to 152 is administered to the individual.

- Clause 169. The method of treatment or prophylaxis according to any one of clauses 153 to 167 wherein the composition according to any one of clauses 1 to 152 is administered to the individual and subsequently a polynucleotide encoding a gp120 related polypeptide is administered to the individual.
- 5 Clause 170. A method of reducing the risk of HIV transmission from an HIV-infected individual to a partner of said HIV-infected individual comprising the step of administering the immunogenic composition of any one of clauses 1 to 152 to the HIV-infected individual.
- 10 Clause 171. A method of making the composition according to any one of clauses 1 to 152, comprising adding a gp120 derived polypeptide to a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.
- 15 Clause 172. A kit for making an immunogenic composition according to any one of clauses 1 to 152, comprising a first container and a second container, wherein the first container comprises a gp120 derived polypeptide and the second container comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.
- 20 Clause 173. The kit according to clause 172, wherein the second container comprises an aqueous solution.
- Clause 174. A viral vector comprising a polynucleotide encoding a polypeptide comprising the gp120_{W61D} polypeptide of SEQ ID No: 1.
- Clause 175. The viral vector of clause 174 comprising a polynucleotide encoding a polypeptide consisting of the gp120_{W61D} polypeptide of SEQ ID No: 1.
- 25 Clause 176. A viral vector comprising a polynucleotide encoding a polypeptide comprising the gp120_{ZM18} polypeptide of SEQ ID NO: 5.
- Clause 177. The viral vector of clause 176 comprising a polynucleotide encoding a polypeptide consisting of the gp120_{ZM18} polypeptide of SEQ ID NO: 5.
- 30 Clause 178. The viral vector of any one of clauses 174 to 177 which is an adenovirus, canary pox virus or MVA virus.

The present invention will now be further described by means of the following non-limiting examples.

EXAMPLES

Example 1: Serology comparison

5 Method

A first composition containing 2ug gp120_{W61D} and 50ul AS01B and a second composition containing 2ug gp120_{W61D}, 50ul AS01B and 2ug NefTat were prepared. At three timepoints (days 0, 14 and 28), the first composition was administered intramuscularly (IM) to a first group
10 of 25 CB6F1 mice and at the same three timepoints the second composition was administered IM to a second group of 25 CB6F1 mice.

The titre of anti-V1V2 antibodies in the sera of the mice in each group was compared at 14 days after the second administration and at 14 days after the third administration. To evaluate
15 the quantity of anti-V1V2 IgG antibodies present in each serum sample, the HIV-1 subtype B case A2 gp120 V1V2 region scaffolded onto a murine gp70 protein was used in an Igtot ELISA binding antibody assay. During the development of the assay, the CH58 (the anti-V2 monoclonal antibody used in the RV144 trial) was used as a positive control to detect successful binding of serum Igtot antibodies to scaffolded V1V2. In addition, gp70 protein (not
20 bound to a V1V2 region) was used as a negative control to assess specificity during development of the assay.

Results

25 On both timepoints tested, a trend for a higher anti-V1V2 antibody response was observed in serum samples from mice which had been administered with the composition which did not contain NefTat as compared to serum samples from mice which had been administered with the composition containing NefTat. This was particularly notable at 14 days after the third administration (see Figure 1).

30

Example 2: PRO HIV-002 and RV144 trial analysis - anti-V1V2 IgG comparison

Background: The PRO-HIV-002 trial

35 This was a study aimed to determine the safety and immunogenicity of a gp120/NefTat candidate HIV-1 vaccine formulated with one of three different adjuvant systems (AS02A,

AS02V and AS01B) in healthy HIV-seronegative adults. See Leroux-Roels et al., *Vaccine* 28:7016-7024 (2010) for further details.

Background: The RV144 trial

This was a study aimed to evaluate the efficacy of four priming injections of a recombinant canarypox vector vaccine (ALVAC-HIV [vCP1521]) plus two booster injections of a recombinant gp120 subunit vaccine (AIDSVAX B/E) at preventing HIV-1 infection in healthy adults. See Rerks-Ngarm et al, *N Engl J Med* 361:2209-2220 (2009) for further details.

Anti-V1V2 IgG comparison

Method

Serum samples were obtained from 30 subjects from the PRO-HIV-002 trial who had been vaccinated with 20ug gp120_{W61D}, 20ug NefTat and AS01B (liposome-based, 50ug MPL and 50ug QS-21) at 0, 1, 3 and 6 months, according to the trial protocol. The serum had been collected at 9 different timepoints. These timepoints were baseline (the first of the four vaccinations, day 0), day 42 (2 weeks after the second vaccination), day 84, day 98 (2 weeks after the third vaccination), day 168, day 182 (2 weeks after the fourth vaccination), day 336, day 504 and day 672 (18 months after the fourth vaccination).

To evaluate the quantity of anti-V1V2 IgG antibodies present in each serum sample, the HIV-1 subtype B case A2 gp120 V1V2 region scaffolded onto a murine gp70 protein was used in an IgG multiplex binding antibody assay, alongside a gp70 control and ConSgp140 (group M consensus gp140). CH58 (the anti-V2 monoclonal antibody used in the RV144 trial) was used to detect successful binding of serum IgG antibodies to scaffolded V1V2. Samples were run at a dilution of 1:100.

The levels of anti-V1V2 clade B antibodies in the serum samples from the 30 subjects of the PRO-HIV-002 trial were compared to those of 205 vaccinees in the case-control group who remained uninfected in the RV144 trial.

Results

The serum samples from the PRO-HIV-002 trial subjects contained a higher level of anti-V1V2 clade B antibodies than the serum samples from the RV144 trial subjects at 2 weeks after the last vaccination in both trials (see Figure 2). The serum samples from the PRO-HIV-002 trial subjects showed a 100% response rate (average: 80.7ug/ml, median 62.2 ug/ml) compared to
5 serum samples from the RV144 trial subjects showing a 64% response rate (average: 4.2 ug/ml, median 1.6 ug/ml).

It was found that anti-V1V2 clade B antibodies in the serum samples from the PRO-HIV-002 trial subjects persisted (87% responders) at 18 months after the fourth priming injection of
10 gp120/NefTat/AS01B (see Figure 2 and Figure 3). In the RV144 trial, responses to the gp120 V2 loop was reduced 6 months after the final vaccination (Excler et al., Poster TUP231 - IAS 2013 – Kuala Lumpur, Malaysia).

The serum samples from the PRO-HIV-002 trial subjects all contained anti-V1V2 antibodies
15 after the third injection of gp120/NefTat/AS01B (see Figure 3).

The serum samples from the PRO-HIV-002 trial subjects had a different reactivity profile against varying subtypes of HIV-1 compared to serum samples from the RV144 trial subjects 2 weeks after the last vaccination in both trials (see Figure 4).
20

All references referred to in this application, including patent and patent applications, are
25 incorporated herein by reference to the fullest extent possible.

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps
30 but not to the exclusion of any other integer, step, group of integers or group of steps.

The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take

the form of product, composition, process, or use claims and may include, by way of example and without limitation, the following claims.

SEQUENCE LISTING**SEQ ID NO: 1 – gp120_{W61D}**

5 AEQLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLGNVTEYFNMWKNM
 VDQMHEDIISLWDQSLKPCVKLTPLCVTLDCDDVNTTNSTTTTSNGWTGEIRKGEIKNCSFNITTSIRDK
 VQKEYALFYNLDVVPIDDDNATTKNKTTNRNFRLIHCNSSVMTQACPKVSFEPIPIHYCAPAGFAILKCNN
 KTFDGKGLCTNVSTVQCTHGIRPVVSTQLLNGSLAEEEVVIRSDNFMDNTKTIIVQLNESVAINCTRPN
 10 NNTRKGIHIGPGRFYAARKIIGDIRQAHCNLSRAQWNNTLKQIVIKLREHFGNKTIFKNQSSGGDPEIV
 RHSFNCGGEFFYCDTTQLFNSTWNGTEGNTEGNSTITLPCRIKQIINMWQEVGKAMYAPPIGGQIRCSS
 NITGLLLTRDGGTEGNGTENETEIFRPGGDMRDNRSELYKYKVVKVEPLGVAPTRAKRRVVQR

V1V2 region underlined.

SEQ ID NO: 2 – Nef

15 MGGKWSKSSVVGWPTVRRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFPV
 TPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFG
 WCYKLVPEPDKVEEANKGENTSLHLPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNC

20

SEQ ID NO: 3 – Tat

EPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSK
 QPTSQSRGDPTGPKE

SEQ ID NO: 4 – NefTat

25 MGGKWSKSSVVGWPTVRRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQEEEEVGFPV
 TPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWYHTQGYFPDWQNYTPGPGVRYPLTFG
 WCYKLVPEPDKVEEANKGENTSLHLPVSLHGMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSE
 30 PVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQ
PTSQSRGDPTGPKE

Nef portion: amino acids 1-206

Tat portion: amino acids 209-293 (underlined)

Box: additional linking amino acids introduced in fusion protein

35

SEQ ID NO: 5 – gp120_{ZM18}

GDNLWVTVYYGVPVWKEAKTTLFCASDAKAYEREVHNWATHACVPTDPNPQEIIVLGNVTENFNMWKNDM
 VDQMHEDIIRLWDQSLKPCVKLTPLCVTLECGNVNVTHENSTKGEMKNCSFNATTELKDKKQRVYALFYK
 40 LDIVPLNENNNSSSEDSSEYRLINCNTSAITQACPKVTLDPIPIHYCAPAGYAILKCNNKTFNGTGCHNV
STVQCTHGIIKPVVSTQLLNGSLAEEEEIIIRSENLTNNAKTIIVHLNESVEIVCTRPSNNTRKSIRIGPG
QAFYATGGIIGNIRQAHCNISKENWNKTLQKVGKKLAEHFPNKTIKFDQHSGGDLEITTHSFNCRGEFFY
CNTSNLNFNSTYKPNDTNSTYNPNDTITLPCRIKQIINMWQGVGQAMYAPPIAGNITCKSNITGLLLTRDG
GSNDTTNTETFRPGGDMRDNRSELYKYKVEIKTLGIAPTAARVVETR

45

V1V2 region underlined.

SEQ ID NO: 6 – polynucleotide sequence encoding gp120_{ZM18}

ggagacaacttgtgggtcacagtctattatgggggtacctgtgtggaaagaagcaaaaactactttattct
 gtgcatcagatgctaaagcatatgagagagaagtgcataatgtctgggctacacatgcctgtgtacccac
 agaccccaaccacagaataagttttgggaaatgtaacagaaaattttaacatgtggaaaaatgacatg
 5 gtggatcagatgcatgaggatataatcaggttatgggatcaaagccttaaagccatgtgtaaagttgaccc
 cactctgtgtcacttttagaatgtggaaatgttaatgttaccatgagaatagcacgaagggggaaatgaa
 aaattgctctttcaatgcaaccacagaactaaaagataaaaaacagagagtgtatgcacttttttataaa
 cttgatatagtaccacttaatgagaataacaactctagtgaggactctagtgagtatagattaataaatt
 gtaataacctcagccataacacaagcctgtccaaagggtcacttttgacccaatttcctatacattattgtgc
 10 tccagctggatatgcgattctaaagtgtataataagacattcaatgggacaggaccatgccataatgtc
 agcacagtacaatgtacacacggaatcaagccagtggatcaactcaactactgttaaattggtagcctag
 cagaagaagagataataattaggtctgaaaatctaacaacaatgccaaaacaataatagtacatcttaa
 tgaatctgtagaattgtgtgtacaagaccagcaataatacaagaaaaagtataaggataggaccagga
 caagcattctatgcaacaggtggcataataggaaacataagacaagcacattgtaacattagtaaagaga
 15 actggaataaaaactttacaaaaggtaggaaaaaaattagcagagcacttcctaataaaaacaataaaatt
 tgaccaacactcaggaggggacctagaaattacaacacatagctttaattgtagaggagaatttttctat
 tgcaatacatcaaacctgtttaatagtacatataagcctaattgatacaaatagtacatataatcctaattg
 atacaatcacactcccattgcagaataaaaacaaattataaacatgtggcagggggtaggacaagcaatgta
 tgccctcccatgacaggaaacataacatgtaaatcaaatatcacaggactactattgacacgggatgga
 20 ggggtcaaatgataccacaaacacagagacattcagacctggaggaggagatatgagggacaattggagaa
 gtgaactatataaatataaagtggtagaaattaaaacattgggcatagcacccactgcggcaaaaaggag
 agtgggtggagacgagataa

SEQ ID NO: 7 – native gp120_{ZM18}

25 GDNLWVTVYYGVPVWKEAKTTLFCASDAKAYEREVHNVWATHACVPTDPNPQEIVLGNVTENFNMWKNDM
 VDQMHEDIIRLWDQSLKPCVKLTPLCVTLECGNVNVTHENSTKGEMKNCSFNATTELKDKKQRVYALFYK
 LDIVPLNENNNSSSEDSSEYRLINCNTSAITQACPKVTLDPPIPIHYCAPAGYAILKCNKTFNGTGPCHNV
 STVQCTHGIKPVVSTQLLLNGSLAEEEEIIIRSENLTNNAKTIIVHLNESVEIVCTRPSNNTRKSIRIGPG
 QAFYATGGIIGNIRQAHCNISKENWNKTLQKVGKKLAEHFPNKTIKFDQHSGGDLEITTHSFNCRGEFFY
 30 CNTSNLNFNSTYKPNDTNSTYNPNDTITLPCRIKQIINMWQGVGQAMYAPPIAGNITCKSNITGLLLTRDG
 GSNDTTNTETFRPGGGDMRDNRSELYKYKVVEIKPLGIAPTAARVETR

Native gp120_{ZM18} polypeptide sequence contains underlined proline instead of threonine.

35

SEQ ID NO: 8 – polynucleotide sequence encoding native gp120_{ZM18}

ggagacaacttgtgggtcacagtctattatgggggtacctgtgtggaaagaagcaaaaactactttattct
 gtgcatcagatgctaaagcatatgagagagaagtgcataatgtctgggctacacatgcctgtgtacccac
 agaccccaaccacagaataagttttgggaaatgtaacagaaaattttaacatgtggaaaaatgacatg
 40 gtggatcagatgcatgaggatataatcaggttatgggatcaaagccttaaagccatgtgtaaagttgaccc
 cactctgtgtcacttttagaatgtggaaatgttaatgttaccatgagaatagcacgaagggggaaatgaa
 aaattgctctttcaatgcaaccacagaactaaaagataaaaaacagagagtgtatgcacttttttataaaa
 cttgatatagtaccacttaatgagaataacaactctagtgaggactctagtgagtatagattaataaatt
 gtaataacctcagccataacacaagcctgtccaaagggtcacttttggaccaatttccctatacattattgtgc
 45 tccagctggatatgcgattctaaagtgtataataagacattcaatgggacaggaccatgccataatgtc
 agcacagtacaatgtacacacggaatcaagccagtggatcaactcaactactgttaaattggtagcctag
 cagaagaagagataataattaggtctgaaaatctaacaacaatgccaaaacaataatagtacatcttaa
 tgaatctgtagaattgtgtgtacaagaccagcaataatacaagaaaaagtataaggataggaccagga
 caagcattctatgcaacaggtggcataataggaaacataagacaagcacattgtaacattagtaaagaga
 50 actggaataaaaactttacaaaaggtaggaaaaaaattagcagagcacttcctaataaaaacaataaaatt
 tgaccaacactcaggaggggacctagaaattacaacacatagctttaattgtagaggagaatttttctat
 tgcaatacatcaaacctgtttaatagtacatataagcctaattgatacaaatagtacatataatcctaattg

5 atacaatcacactcccatgcagaataaaacaaattataaacatgtggcaggggtaggacaagcaatgta
tgccctccattgcaggaaacataacatgtaaatcaaatatcacaggactactattgacacgggatgga
gggtcaaattgataccacaaacacagagacattcagacctggaggaggagatatgagggacaattggagaa
gtgaactatataaatataaagtggtagaaattaaccattgggcatagcacccactgcggcaaaaaggag
agtggaggagacgagataa

To produce the gp120_{ZM18} polynucleotide sequence from the native gp120_{ZM18} polynucleotide sequence:

- a) one silent point mutation is introduced (C instead of T at position 597 (underlined)).
- 10 b) one non-silent point mutation A instead of C is introduced, at position 1426 (underlined) modifying the CCA native codon (proline) for the ACA codon (threonine).

CLAIMS

1. An immunogenic composition comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome and wherein the composition is substantially free of a NefTat related polypeptide wherein the NefTat related polypeptide is a polypeptide consisting of SEQ ID NO: 4.
2. An immunogenic composition which is in the form of a human dose comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises between 10-40ug of a lipopolysaccharide and between 10-40ug of an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.
3. An immunogenic composition comprising a gp120 related polypeptide and an adjuvant, wherein the adjuvant comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome wherein:
 - (i) the conductivity of the composition is 13 mS/cm or lower; and/or
 - (ii) the concentration of salts in said composition is 130 mM or lower; and/or
 - (iii) the concentration of sodium chloride in said composition is 130 mM or lower.
4. The immunogenic composition according to either claim 2 or 3, which is substantially free of a NefTat related polypeptide wherein the NefTat related polypeptide is a polypeptide consisting of SEQ ID NO: 4.
5. The immunogenic composition according to any one of claims 1, 3 or 4 wherein the lipopolysaccharide is present at a level of between 10-100ug.
6. The immunogenic composition according to any one of claims 1 or 3 to 5 wherein the saponin is present at a level of between 10-100ug.
7. The immunogenic composition according to any one of claims 1 to 6 wherein the lipopolysaccharide is 3D-MPL.

8. The immunogenic composition according to any one of claims 1 to 7 wherein the saponin is QS-21.
9. The immunogenic composition according to any one of claims 1, 2 or 4 to 8 wherein:
 - (i) the conductivity of the composition is 13 mS/cm or lower; and/or
 - (ii) the concentration of salts in said composition is 130 mM or lower; and/or
 - (iii) the concentration of sodium chloride in said composition is 130 mM or lower.
10. The immunogenic composition according to any one of claims 1 to 9 which is an aqueous solution.
11. The immunogenic composition according to any one of claims 1 to 10 which is a single human dose.
12. The immunogenic composition according to any one of claims 1 to 11 wherein the human dose is between 0.1 and 1ml.
13. The immunogenic composition according to any one of claims 1 to 12, comprising a non-ionic tonicity agent.
14. The immunogenic composition according to any one of claims 1 to 13 wherein the composition comprises the gp120 related polypeptide at a level of around 1 to 100 ug.
15. The immunogenic composition according to any one of claims 1 to 14 wherein the composition comprises 1-5 additional HIV antigens.
16. The composition according to any one of claims 1 to 15 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with the V1V2 region of SEQ ID NO: 1.
17. The composition according to any one of claims 1 to 16 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 1.
18. The composition according to any one of claims 1 to 15 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with the V1V2 region of SEQ ID NO: 5.

19. The composition according to any one of claims 1 to 16 wherein the gp120 related polypeptide comprises a polypeptide with at least 70% identity with SEQ ID NO: 5.
- 5 20. The immunogenic composition according to any one of claims 1 to 19 wherein the composition further comprises a sterol, wherein the ratio of saponin:sterol is from 1:1 to 1:100 w/w.
- 10 21. The immunogenic composition according to any one of claims 1 to 20, for use in the treatment or prevention of HIV-1 from group M, N, O or P.
22. The immunogenic composition according to any one of claims 1 to 20, for use in the treatment or prevention of HIV-1 group M subtype A, B, C, D, E, F, G, H, I, J or K.
- 15 23. The immunogenic composition according to any one of claims 1 to 22, wherein the gp120 related polypeptide is derived from a first HIV-1 subtype, for use in the treatment or prevention of HIV-1 infection by a second HIV-1 subtype, wherein the first and second HIV-1 subtypes have different native gp120 polypeptide sequences.
- 20 24. The immunogenic composition according to any one of claims 1 to 23 for use in eliciting antibodies against the V1V2 loop of HIV-1 gp120.
- 25 25. The immunogenic composition according to any one of claims 1 to 23 for use in the treatment or prophylaxis of HIV infection wherein a polynucleotide encoding a gp120 related polypeptide is administered to the individual and subsequently the composition according to any one of claims 1 to 24 is administered to the individual.
- 30 26. The immunogenic composition according to any one of claims 1 to 23 for use in the treatment or prophylaxis of HIV infection wherein the composition according to any one of claims 1 to 23 is administered to the individual and subsequently a polynucleotide encoding a gp120 related polypeptide is administered to the individual.
- 35 27. A method of treatment or prophylaxis of HIV-1 infection comprising the step of administering a composition according to any one of claims 1 to 26 to an individual.

28. A method of reducing the risk of HIV transmission from an HIV-infected individual to a partner of said HIV-infected individual comprising the step of administering the immunogenic composition of any one of claims 1 to 26 to the HIV-infected individual.
- 5 29. A method of making the composition according to any one of claims 1 to 26, comprising adding a gp120 derived polypeptide to a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.
- 10 30. A kit for making an immunogenic composition according to any one of claims 1 to 26, comprising a first container and a second container, wherein the first container comprises a gp120 derived polypeptide and the second container comprises a lipopolysaccharide and an immunologically active saponin fraction derived from the bark of Quillaja Saponaria Molina presented in the form of a liposome.
- 15 31. A viral vector comprising a polynucleotide encoding a polypeptide comprising the gp120_{W61D} polypeptide of SEQ ID No: 1.
- 20 32. A viral vector comprising a polynucleotide encoding a polypeptide comprising the gp120_{ZM18} polypeptide of SEQ ID NO: 5.

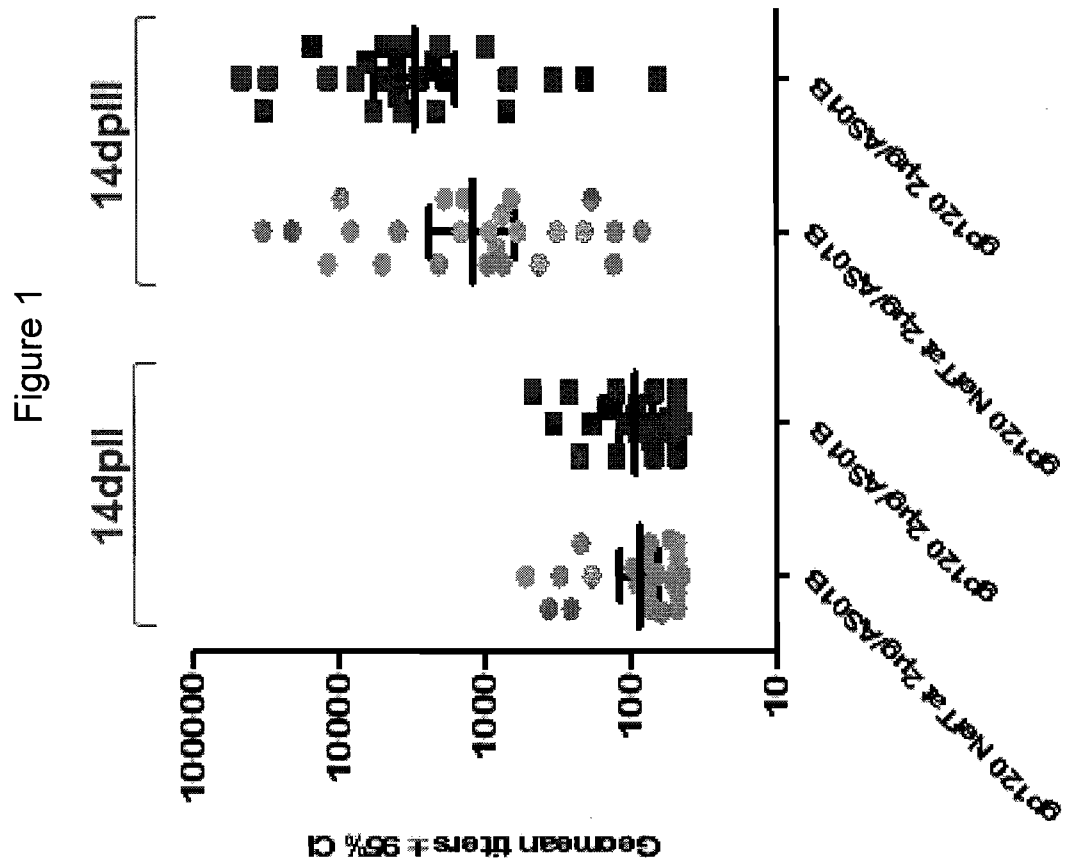


Figure 2

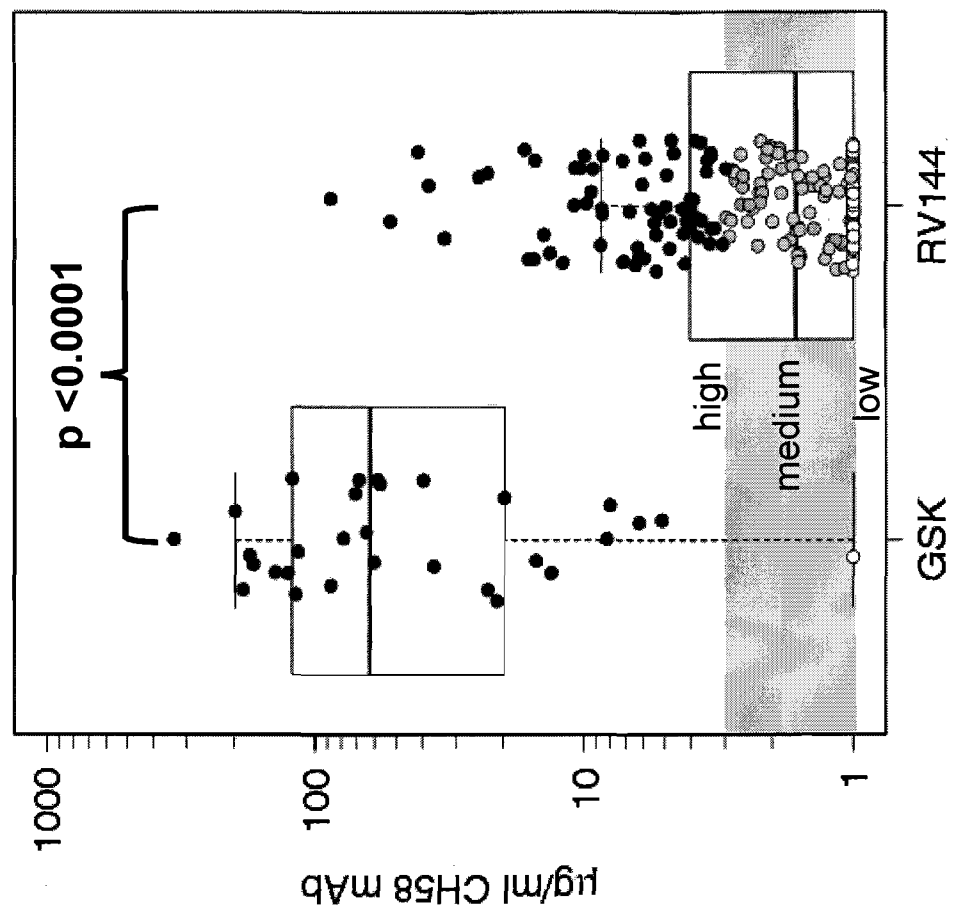


Figure 3

	Day 0 PRE			Day 42 2W PII			Day 84 2M PII			Day 98 2W PIII			Day 168 3M PIII		
	n	N	%	n	N	%	n	N	%	n	N	%	n	N	%
gp120	0	30	0	29	30	97	27	30	90	30	30	100	28	30	93
ConSgp140	0	29	0	30	30	100	29	30	97	26	26	100	29	30	97
gp70 V1V2	0	29	0	21	30	70	20	30	67	26	26	100	26	30	87

	Day 182 2W PIV			Day 336 6M PIV			Day 504 12M PIV			Day 672 18M PIV		
	n	N	%	n	N	%	n	N	%	n	N	%
gp120	29	30	97	29	30	97	29	30	97	28	30	93
ConSgp140	30	30	100	30	30	100	28	30	93	29	30	97
gp70 V1V2	30	30	100	30	30	100	27	30	90	26	30	87

Immunization

n: # responders
N: # tested
%: responders

Figure 4

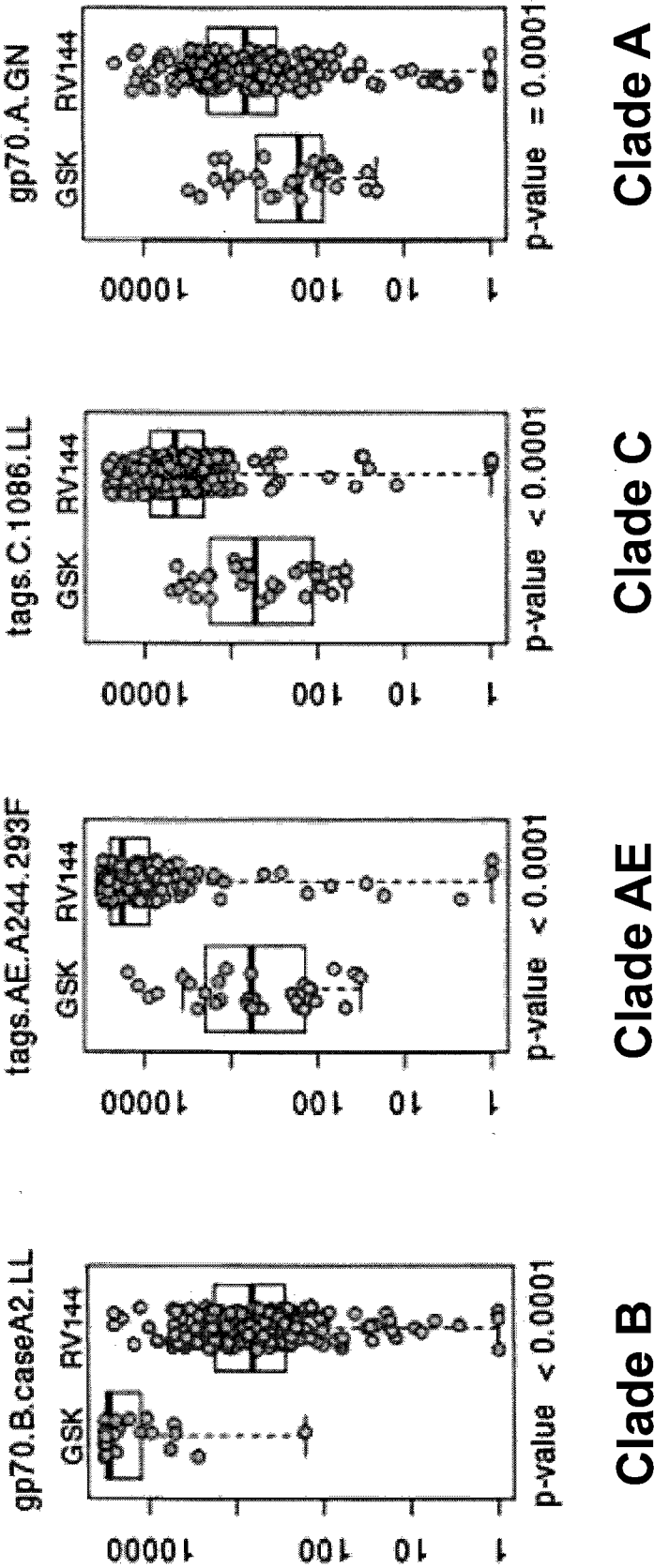
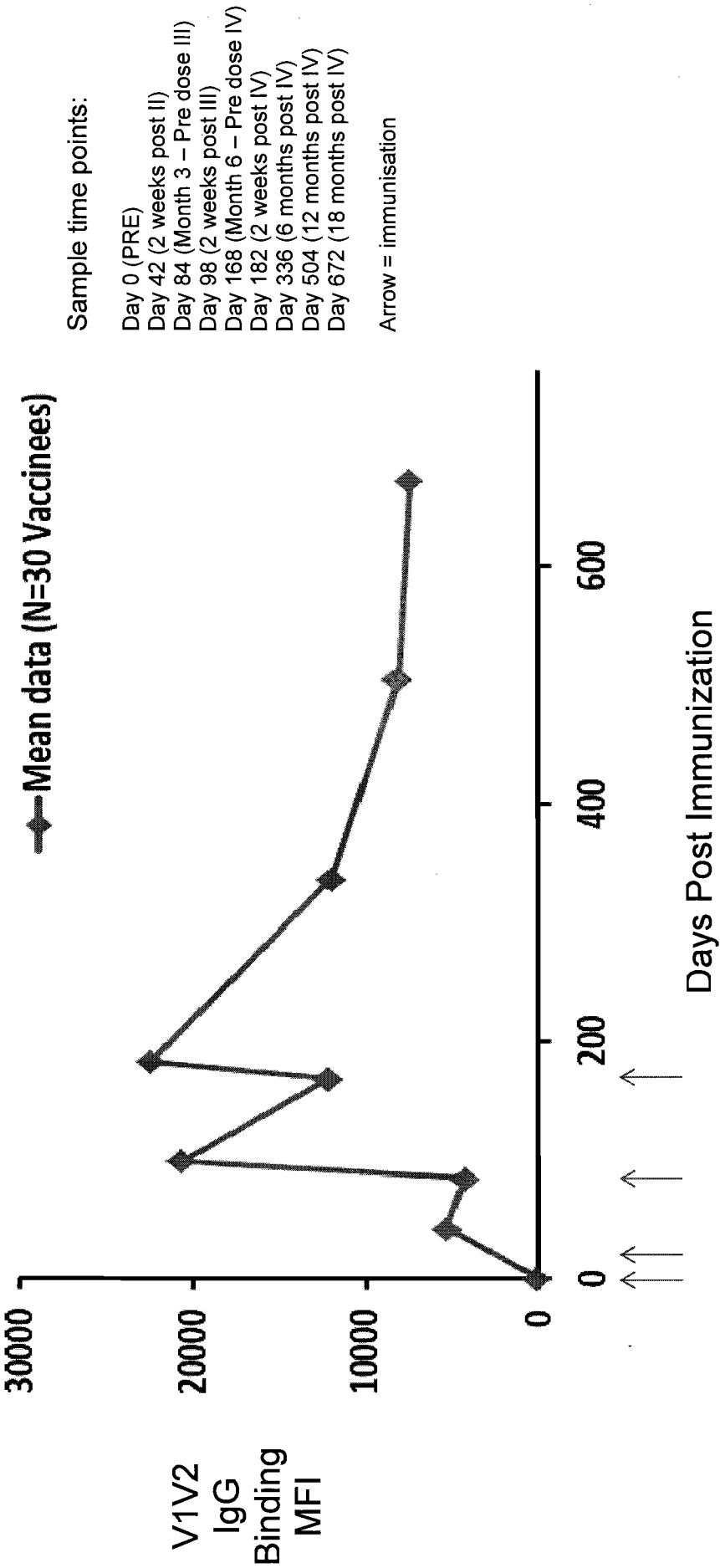


Figure 5



INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2013/073365

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. (means)
- ☐ on paper
- ☒ in electronic form
- b. (time)
- ☒ in the international application as filed
- ☐ together with the international application in electronic form
- ☐ subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/073365

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/21 C07K14/16
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MCCORMACK S ET AL: "A phase I trial in HIV negative healthy volunteers evaluating the effect of potent adjuvants on immunogenicity of a recombinant gp120W61D derived from dual tropic R5X4 HIV-1ACH320", VACCINE, vol. 18, no. 13, January 2000 (2000-01), pages 1166-1177, XP002718159, ISSN: 0264-410X	1,5-13, 16-19, 21-24, 27-30
Y	the whole document ----- -/--	1-30



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 December 2013

Date of mailing of the international search report

14/01/2014

Name and mailing address of the ISA/

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Authorized officer

Merlos, Ana Maria

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/073365

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI Y ET AL: "Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary isolate trimeric and monomeric envelope glycoproteins in selected adjuvants", JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 80, no. 3, 1 February 2006 (2006-02-01), pages 1414-1426, XP009115093, ISSN: 0022-538X, DOI: 10.1128/JVI.80.3.1414 1426.2006	1-10, 13-15, 21-24, 27,29,30
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Y	page 12, right-hand column, paragraphs 2, 3; figures 1, 4 page 13, left-hand column, paragraph 2	1-30

X	BEDDOWS SIMON ET AL: "Comparison of the antibody repertoire generated in healthy volunteers following immunization with a monomeric recombinant gp120 construct derived from a CCR5/CXCR4-using human immunodeficiency virus type 1 isolate with sera from naturally infected individuals", JOURNAL OF VIROLOGY, vol. 73, no. 2, February 1999 (1999-02), pages 1740-1745, XP002718160, ISSN: 0022-538X	1,4,8, 10,16, 17,24, 27,29
Y	the whole document	1-30

Y	LEROUX-ROELS I ET AL: "Strong and persistent CD4<+> T-cell response in healthy adults immunized with a candidate HIV-1 vaccine containing gp120, Nef and Tat antigens formulated in three Adjuvant Systems", VACCINE, ELSEVIER LTD, GB, vol. 28, no. 43, 8 October 2010 (2010-10-08), pages 7016-7024, XP027392051, ISSN: 0264-410X [retrieved on 2010-08-20] the whole document	1-30

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/073365

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EVA VAN BRAECKEL ET AL: "An adjuvanted polyprotein HIV-1 vaccine induces polyfunctional cross-reactive CD4+ T cell responses in seronegative volunteers", CLINICAL INFECTIOUS DISEASES, THE UNIVERSITY OF CHICAGO PRESS, CHICAGO, IL, US, vol. 52, no. 4, 15 February 2011 (2011-02-15), pages 522-531, XP002639982, ISSN: 1058-4838, DOI: 10.1093/CID/CIQ160 [retrieved on 2011-01-05] the whole document	1-30
X	----- WO 2010/023260 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]; PASTEUR INSTITUT [FR]; FEVRIER MICHELE) 4 March 2010 (2010-03-04)	31
Y	page 9, last paragraph page 7, last paragraph - page 8, last paragraph page 21, line 12 - line 15 page 22, line 19 - line 26 page 26, paragraph 3 - page 30, paragraph 1	25,26
X	----- WO 2005/035555 A1 (US GOV HEALTH & HUMAN SERV [US]; MOSS BERNARD [US]; CENTER ROB J [US]) 21 April 2005 (2005-04-21) page 10, line 14 - line 28	32
Y	----- VANDEPAPELIERE ET AL: "Vaccine Adjuvant Systems containing monophosphoryl lipid A and QS21 induce strong and persistent humoral and T cell responses against hepatitis B surface antigen in healthy adult volunteers", VACCINE, ELSEVIER LTD, GB, vol. 26, no. 10, 14 January 2008 (2008-01-14), pages 1375-1386, XP022492632, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2007.12.038 page 1377, paragraph 4 page 1382, right-hand column, paragraph 2 - page 1383, left-hand column, paragraph 3 page 1383, right-hand column, paragraph 4 page 1384, left-hand column, paragraph 4	1-30
A	----- WO 2011/117408 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]; BOURGUIGNON PATRICIA [BE]; KOUTSOUKOS) 29 September 2011 (2011-09-29) the whole document	1-32

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/073365

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