

Novel Method and CompositionsField of the invention

This invention relates to novel vaccine compositions and their use in the stimulation of immune responses in mammals, especially humans, and in particular for the prevention and treatment of infection by pathogens. In particular it relates to compositions capable of inducing CD4+ and CD8+ T-cell responses as well as antibody responses in subjects without recourse to complex prime-boost schedules.

Background to the invention

Inactivated whole organisms have been used in successful vaccination since the late nineteenth century. In more recent times, vaccines involving the administration of extracts, subunits, toxoids and capsular polysaccharides have been employed. Since genetic engineering techniques have been available, the use of recombinant proteins has been a favoured strategy, obviating many of the risks associated with use of purified proteins from natural sources.

Early vaccine approaches were based on the administration of proteins which stimulated some aspect of the immune response in vivo. Subsequently it was appreciated that immune responses could also be raised by administration of DNA which could be transcribed and translated by the host into an immunogenic protein.

The mammalian immune response has two key components: the humoral response and the cell-mediated response. The humoral response involves the generation of circulating antibodies which will bind to the antigen to which they are specific, thereby neutralising the antigen and favouring its subsequent clearance by a process involving other cells that are either cytotoxic or phagocytic. B-cells are responsible for generating antibodies (plasma B cells), as well as holding immunological humoral memory (memory B-cells), i.e. the ability to recognise an antigen some years after first exposure to it eg through vaccination. The cell mediated response involves the interplay of numerous different types of cells, among which are the T cells. T-cells are divided into a number of different subsets, mainly the CD4+ and CD8+ T cells.

Antigen-presenting cells (APC) such as macrophages and dendritic cells act as sentinels of the immune system, screening the body for foreign antigens. When extracellular foreign antigens are detected by APC, these antigens are phagocytosed (engulfed) inside the APC where they will be processed into smaller peptides. These peptides are subsequently presented on major histocompatibility complex class II (MHC II) molecules at the surface of the APC where they can be recognised by antigen-specific T lymphocytes expressing the CD4 surface molecules (CD4+ T cells). When CD4+ T cells recognise the antigen to which they are specific on MHCII molecules in the presence of additional adequate co-stimulatory signals, they become

activated and secrete an array of cytokines that subsequently activate the other arms of the immune system. In general, CD4+ T cells are classified into T helper 1 (Th1) or T helper 2 (Th2) subsets depending on the type of response they generate following antigen recognition. Upon recognition of a peptide-MHC II complex, Th1 CD4+ T cells secrete interleukins and cytokines
5 such as interferon gamma thereby activating macrophages to release toxic chemicals such as nitric oxide and reactive oxygen/nitrogen species. IL-2 and TNF-alpha are also commonly categorized as Th1 cytokines. In contrast, Th2 CD4+ T cells generally secrete interleukins such as IL-4, IL-5 or IL-13.

Other functions of the T helper CD4+ T cells include providing help to activate B cells to
10 produce and release antibodies. They can also participate to the activation of antigen-specific CD8+ T cells, the other major T cell subset beside CD4+ T cells.

CD8+ T cells recognize the peptide to which they are specific when it is presented on the surface of a host cell by major histocompatibility class I (MHC I) molecules in the presence of appropriate costimulatory signals. In order to be presented on MHC I molecules, a foreign
15 antigen need to directly access the inside of the cell (the cytosol or nucleus) such as it is the case when a virus or intracellular bacteria directly penetrate a host cell or after DNA vaccination. Inside the cell, the antigen is processed into small peptides that will be loaded onto MHC I molecules that are redirected to the surface of the cell. Upon activation CD8+ T cells secrete an array of cytokines such as interferon gamma that activates macrophages and other cells. In
20 particular, a subset of these CD8+ T cells secretes lytic and cytotoxic molecules (e.g. granzyme, perforin) upon activation. Such CD8+ T cells are referred to as cytotoxic T cells.

More recently, an alternative pathway of antigen presentation involving the loading of extracellular antigens or fragments thereof onto MHCI complexes has been described and called "cross-presentation".

25 The nature of the T-cell response is also influenced by the composition of the adjuvant used in a vaccine. For instance, adjuvants containing MPL & QS21 have been shown to activate Th1 CD4+ T cells to secrete IFN-gamma (Stewart et al. Vaccine, 2006, 24 (42-43):6483-92).

Whereas adjuvants are well known to have value in enhancing immune responses to
30 protein antigens, they have not generally been used in conjunction with DNA or DNA-based vector vaccination. There are several hypotheses as to why adjuvants have not been used in conjunction with DNA-vector based vaccines. Indeed, interferences between the adjuvant and the vector may have an impact on their stability. In addition, one might expect that adding an adjuvant to an attenuated vector could increase the reactogenicity induced by such product.
35 Finally, increasing the immunogenicity of a DNA-vector based vaccine may lead to an enhanced neutralizing immune response against the vector itself, thereby precluding any boosting effect of subsequent injections of the same vector-based vaccine. In fact, in a vaccination protocol

directed towards protection against *P. falciparum* infection, Jones et al (2001, J Infect Diseases 183, 303-312) have reported an adverse outcome after combining DNA, recombinant protein and adjuvant as a boosting composition following a prime by DNA. Indeed, the levels of parasitemia were significantly lower in a group in which the boosting composition contained protein and adjuvant only. It was concluded that use of the combination of DNA, recombinant protein and adjuvant in this protocol adversely affected the outcome on parasitemia as well as antibody responses.

On the other hand, there has been a report of enhancement of the efficacy of an adjuvanted DNA-based vector vaccine (Ganne et al. Vaccine (1994) 12(13) 1190-1196). In particular, the enhanced efficacy of a replication-defective adenovirus-vectored vaccine by the addition of oil adjuvants was correlated with higher antibody levels but the impact on CD4 and CD8 T cell responses was not reported.

The use of an apathogenic virus as an adjuvant has been disclosed in WO2007/016715. It was not mentioned that said virus could contain any heterologous polynucleotide.

It is generally thought that stimulation of both CD4+ and CD8+ cells are needed for optimal protective immunity, especially in certain diseases such as HIV infection/AIDS. In order to induce an optimal immune response either prophylactically or therapeutically, stimulation of both CD4+ and CD8+ cells is desirable. This is one of the main goal of "prime-boost" vaccination strategies in which the alternate administration of protein-based vaccines (inducing mostly CD4+T cells) with DNA-vector based vaccines, i.e. naked DNA, viral vectors or intracellular bacterial vectors such as listeria, (inducing mostly CD8+T cells) or vice versa most likely activates both CD4+ and CD8+ T cell responses.

However, although prime-boost vaccine strategies may generally give rise to a greater or more balanced response, the requirement to vaccinate on more than one occasion and certainly on more than two occasions can be burdensome or even unviable, especially in mass immunization programs for the developing world.

Furthermore, as already mentioned above, it is often not possible to boost the viral vector component because of immunity that may have been raised against the vector itself.

Thus the objects of the invention include one or more of the following: (a) to provide a complete vaccination protocol and a vaccine composition which stimulates the production of CD4+ and/or CD8+ cells and/or antibodies and in particular which obviates or mitigates the need for repeated immunizations; (b) to provide a vaccination protocol and a vaccine composition which better stimulates production of CD4+ cells and/or CD8+cells and/or antibodies relative to vaccine compositions containing an immunogenic polypeptide alone or a polynucleotide alone or relative to a conventional prime-boost protocol involving separate administration of immunogenic polypeptide and polynucleotide; (c) to provide a vaccine composition which stimulates or better stimulates Th1 responses; (d) to provide a vaccine

composition and vaccination protocol in which required doses of components, especially viral vectors, are minimised; and (e) more generally to provide a useful vaccine composition and vaccination protocol for treatment or prevention of diseases caused by pathogens. By "better stimulates" is meant that the intensity and/or persistence of the response is enhanced.

5

Summary of the invention

Thus according to the invention there is provided a method of raising an immune response against a pathogen which comprises administering (i) one or more first immunogenic polypeptides derived from said pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more first immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered concomitantly.

According to a specific aspect of the invention there is provided a vaccine composition comprising (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotide encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant.

There is also provided an immunogenic composition comprising (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant.

Said vaccines and immunogenic compositions suitably stimulate production of pathogen-specific CD4+ T-cells and/or CD8+ T-cells and/or antibodies.

By "pathogen-specific CD4+ T-cells and/or CD8+ T-cells and/or antibodies" is meant CD4+ T-cells and/or CD8+ T-cells and/or antibodies which specifically recognise the whole pathogen or a part (eg an immunogenic subunit) thereof. By "specifically recognise" is meant that the CD4+ T-cells and/or CD8+ T-cells and/or antibodies recognise in an immunospecific rather than a non-specific manner said pathogen (or part thereof).

There is also provided a method of stimulating an immune response in a mammal which comprises administering to a subject an immunologically effective amount of such a composition.

There is also provided use of such a composition in the manufacture of a medicament for stimulating an immune response in a mammal.

There is also provided such a composition for use in stimulating an immune response in a mammal.

There is also provided a method of stimulating the production of pathogen-specific CD4+ T-cells and/or CD8+ T-cells and/or antibodies in mammals which comprises administering

to said mammal (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more first immunogenic polypeptides, the one or more adenoviral
5 vectors and the adjuvant are administered concomitantly, for example by administering an immunologically effective amount of an aforesaid composition.

There is also provided use of aforesaid compositions in the manufacture of a medicament for stimulating the production of pathogen specific CD4+ and/or CD8+ cells and/or
10 antibodies in mammals.

For example, production of CD4+ T-cells or CD8+ T-cells or antibodies is stimulated.

Suitably production of 2 and especially 3 of CD4+ T-cells and/or CD8+ T-cells and/or
antibodies is stimulated.

Suitably production of CD8+ T-cells is stimulated. Suitably production of CD4+ and
CD8+ T-cells is stimulated. Suitably production of CD4+ and CD8+ T-cells and antibodies is
15 stimulated.

Alternatively suitably production of CD4+ T-cells is stimulated. Suitably production of
CD4+ and antibodies is stimulated.

Alternatively suitably production of antibodies is stimulated.

The methods of the invention are suitably intended to provide the steps adequate for a
20 complete method for raising an immune response (although the method may, if desired, be repeated). Therefore suitably the methods do not involve use of a priming dose of any immunogenic polypeptide or polynucleotide (e.g. in the form of a vector such as an adenoviral vector) encoding any immunogenic polypeptide.

For example there is provided a method of raising an immune response against a
25 pathogen which consists of (a) administering (i) one or more first immunogenic polypeptides derived from said pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more immunogenic polypeptide, the one or more adenoviral vector and the adjuvant are administered concomitantly; and (b)
30 optionally repeating the steps of (a).

The steps of the method may be repeated (e.g. repeated once) if a repeat gives rise to an improved immune response. An adequate response, at least as far as a T-cell response is concerned, may be obtained without any need for repetition.

There is also provided a method of raising an immune response against a pathogen
35 which comprises (a) administering (i) one or more first immunogenic polypeptides derived from said pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said

pathogen; and (iii) an adjuvant; wherein the one or more first immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered concomitantly; and wherein the method does not involve administering any priming dose of immunogenic polypeptide or polynucleotide encoding immunogenic polypeptide.

5 There is also provided a kit comprising (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; and in particular comprising (i) one or more first immunogenic polypeptides derived from a pathogen and an adjuvant; and (ii) one or more
10 second adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more immunogenic polypeptides derived from said pathogen; for use in a method according to the invention.

 Compositions and methods of the invention may be useful for the prevention of infection by pathogens in naïve subjects, or prevention of re-infection in subjects who have previously
15 been infected by pathogen or treatment of subjects who have been infected by pathogen.

Brief description of the figures

Figure 1 shows a graphical representation of the construction of plasmid p73i-Tgrn

Figures 2-8 show the results of experiments discussed in Example 1, specifically:

20 Figures 2a, 2b, 3a, 3b: CD4+ and CD8+ T-cell responses in response to restimulation by pools of peptides derived from p24, RT, Nef and p17 following various immunization protocols and at different timepoints;

Figure 4: antibody responses against F4;

Figures 5-8 antibody responses against F4 components p24, RT, p17 and Nef respectively;

25 Figure 9 shows the results of experiments discussed in Example 2, specifically:

CD4+ T-cell responses in response to restimulation by pools of peptides derived from p24 and RT following various immunization protocols;

Figures 10-12 show the results of experiments discussed in Example 3, specifically:

30 Figure 10 shows the lymphoproliferative response of rabbit PBMC against peptide pools covering the F4 sequence;

Figure 11 shows the timecourse of antibody responses against F4;

Figures 12a and 12b shows antibody responses (on day 77) against F4 components p24 and RT respectively;

Figure 13 shows the quantification of HIV-1-specific CD4 T cells;

35 Figure 14 shows distribution of the frequency of F4-specific CD4 T cells 7 days after two immunizations;

Figure 15 shows cytokine production of F4-specific CD4 T cells 7 days after two immunizations;

Figure 16 shows quantification of HIV-1-specific CD8 T cells;

Figure 17 shows cytokine production of F4-specific CD8 T cells 7 days after two immunizations;

5 Figure 18 shows quantification of CSP-specific CD4 T cells;

Figure 19 shows quantification of CSP-specific CD8 T cells;

Figure 20 shows quantification of CSP(N-term)-specific CD4 T cells;

Figure 21 shows quantification of CSP(C-term)-specific CD4 T cells;

Figure 22 shows quantification of CSP(N-term)-specific CD8 T cells;

10 Figure 23 shows quantification of CSP(C-term)-specific CD8 T cells;

Figure 24 shows quantification of CSP-specific antibody titers.

Summary of sequence listings

Amino acid or polynucleotide description	Sequence Identifier (SEQ ID No)
HIV Gag-RT-Nef ("GRN") (Clade B) (cDNA)	1
HIV Gag-RT-Nef ("GRN") (Clade B) (amino acid)	2
HIV Gag-RT-integrase-Nef ("GRIN") (Clade A) (cDNA)	3
HIV Gag-RT-integrase-Nef ("GRIN") (Clade A) (amino acid)	4
HIV gp140 (Clade A) (cDNA)	5
HIV gp140 (Clade A) (amino acid)	6
HIV gp120 (Clade B) (cDNA)	7
HIV gp120 (Clade B) (amino acid)	8
TB antigens fusion protein M72 (cDNA)	9
TB antigens fusion protein M72 (amino acid)	10
P. falciparum CS protein-derived antigen (cDNA)	11
P. falciparum CS protein-derived antigen (amino acid)	12

P. falciparum CS protein-derived fusion protein "RTS" (cDNA)	13
P. falciparum CS protein-derived fusion protein "RTS" (amino acid)	14
HIV p24-RT-Nef-p17 (cDNA)	15
HIV p24-RT-Nef-p17 (amino acid)	16

The above recited sequences may be employed as polypeptides or polynucleotides encoding polypeptides of use in exemplary aspects of the invention. Said polypeptides may consist of or comprise the above mentioned sequences. Initial Met residues are optional. N-terminal His residues (including His residues immediately following an initial Met, as in SEQ ID No 9) are optional or an N-terminal His tag of a different length may be employed (eg typically up to 6 His residues may be employed to facilitate isolation of the protein). Analogue proteins which have significant sequence identity eg greater than 80% eg greater than 90% eg greater than 95% eg greater than 99% sequence identity over the whole length of the reference sequence may be employed, especially when the analogue protein has a similar function and particularly when the analogue protein is similarly immunogenic. For example up to 20 eg up to 10 eg 1-5 substitutions (eg conservative substitutions) may be tolerated. Nucleic acids which differ from those recited above which encode the same proteins, or the aforementioned analogue proteins, may be employed. Sequence identity may be determined by conventional means eg using BLAST. In one specific variant of SEQ ID No 16 that may be mentioned, residue 398 is Ser and not Cys.

Detailed description of the invention.

As used herein the term "concomitantly" means wherein the one or more immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered within a period of no more than 12 hours eg within a period of no more than 1 hour, typically on one occasion e.g. in the course of the same visit to the health professional, for example the one or more immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered sequentially or simultaneously.

As used herein, the term "epitope" refers to an immunogenic amino acid sequence. An epitope may refer to an a minimum amino acid sequence of typically 6-8 amino acids which minimum sequence is immunogenic when removed from its natural context, for example when transplanted into a heterologous polypeptide. An epitope may also refer to that portion of a protein which is immunogenic, where the polypeptide containing the epitope is referred to as the antigen (or sometimes "polypeptide antigen"). A polypeptide or antigen may contain one or

more (eg 2 or 3 or more) distinct epitopes. The term "epitope" embraces B-cell and T-cell epitopes. The term "T-cell epitope" embraces CD4+ T-cell epitopes and CD8+ T-cell epitopes (sometimes also referred to as CTL epitopes).

5 The term "immunogenic polypeptide" refers to a polypeptide which is immunogenic, that is to say it is capable of eliciting an immune response in a mammal, and therefore contains one or more epitopes (eg T-cell and/or B-cell epitopes). Immunogenic polypeptides may contain one or more polypeptide antigens eg in an unnatural arrangement such as in a fusion protein.

10 Immunogenic polypeptides will typically be recombinant proteins produced eg by expression in a heterologous host such as a bacterial host, in yeast or in cultured mammalian cells.

The term "polypeptide derived from a pathogen" means a polypeptide which partially or wholly contains sequences (i.e. antigens) which occur naturally in pathogens or bear a high degree of sequence identity thereto (eg more than 95% identity over a stretch of at least 10 eg at least 20 amino acids).

15 Immunogenic polypeptides may contain one or more (eg 1, 2, 3 or 4) polypeptide antigens.

Unless otherwise specified, an "immune response" may be a cellular and/or a humoral response.

20 In one embodiment of the invention one or more of said one or more first immunogenic polypeptides is substantially the same as one or more of said one or more second immunogenic polypeptides. For example one of the at least one first immunogenic polypeptides and one of the at least one second immunogenic polypeptides may have an overall sequence identity of 90% or more eg 95% or more eg 98% or 99% or more over the length of one or other immunogenic polypeptides.

25 In another embodiment of the invention one or more of said one or more first immunogenic polypeptides contains at least one antigen which is substantially the same as an antigen contained in one or more of said one or more second immunogenic polypeptides. For example one of the at least one first immunogenic polypeptides and one of the at least one second immunogenic polypeptides may have an overall sequence identity of 90% or more eg 95% or more eg 98% or 99% or more over a stretch of 20 amino acids or more eg 40 amino acids or more eg 60 amino acids or more.

Suitably one or more first immunogenic polypeptides comprise at least one T cell epitope.

35 Suitably one or more second immunogenic polypeptides comprise at least one T cell epitope.

Suitably the one or more first immunogenic polypeptides comprise at least one B cell epitope.

Suitably the one or more second immunogenic polypeptides comprise at least one B cell epitope

In another embodiment of the invention one or more of said one or more first immunogenic polypeptides and one or more of said one or more second immunogenic polypeptides share one or more identical B-cell and/or T-cell epitopes. Suitably they share one or more identical amino acid sequences of length 10 amino acids or more eg 15 amino acids or more eg 25 amino acids or more.

In another embodiment of the invention, none of the one or more of said one or more first immunogenic polypeptides is substantially the same as or contains any antigen in common with one or more of said one or more second immunogenic polypeptides, for example they may have an overall sequence identity of less than 90% over a stretch of 20 amino acids or more eg 40 amino acids or more eg 60 amino acids or more.

Thus, they may not share any B-cell or T-cell epitopes. For example, they may not share any identical amino acid sequences of length 10 amino acids or more eg at 15 amino acids or more eg 25 amino acids or more.

In one specific embodiment of the invention a first immunogenic polypeptide and a second immunogenic polypeptide contain the same antigens in the same arrangement or in a different arrangement (eg in a different arrangement). By "different arrangement" is meant that they may be arranged in a different order and/or they may be divided. In another specific embodiment of the invention a first immunogenic polypeptide and a second immunogenic polypeptide are the same.

The composition according to the invention may contain one first immunogenic polypeptide as the only immunogenic polypeptide in the composition. Alternatively the composition according to the invention may contain more than one first immunogenic polypeptides eg 2 or 3 or 4 or more immunogenic polypeptides.

The composition according to the invention may comprise one adenoviral vector. Alternatively it may comprise more than one adenoviral vector eg 2 adenoviral vectors.

In compositions according to the invention a adenoviral vector may comprise a heterologous polynucleotide which encodes for one second immunogenic polypeptide or it may comprise more than one heterologous polynucleotide which together encode for more than one second immunogenic polypeptide under the control of more than one promoter.

As well as for prophylactic vaccination, the compositions of the invention may also be used in individuals that are already infected with pathogen, and result in improved immunological control of the established infection. This is of particular interest when the pathogen is HIV. In the case of HIV, this control is believed to be achieved by CD8-positive T cells that specifically recognize HIV-infected cells. Such CD8-positive T cell response is maintained by the presence of HIV-specific CD4-positive helper T cells. Therefore, the induction

of both types of immune response is particularly useful, and can be achieved by combining different vaccine compositions. A combination of an adjuvanted protein and a recombinant adenovirus is of particular interest. The HIV-infected patients that will benefit from the above-described vaccination are either in the primary infection, latency or terminal phase of HIV

5 infection at the time of vaccination. The patients may or may not undergo other therapeutic treatment interventions against pathogen (in the case of HIV - for example highly active antiretroviral therapy) at the time of vaccination.

Antigens

- 10 Antigens of use according to the invention are derived from pathogens. Pathogens include viruses, bacteria, protozoa and other parasitic organisms harmful to mammals including man.
- Suitable polypeptide antigens to be administered as polypeptide or polynucleotide encoding polypeptide according to the invention include antigens derived from HIV (eg HIV-1),
- 15 human herpes viruses (such as gH, gL gM gB gC gK gE or gD or derivatives thereof or Immediate Early protein such as ICP27 , ICP 47, ICP4, ICP36 from HSV1 or HSV2), cytomegalovirus, especially Human, (such as gB or derivatives thereof), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster Virus (such as gpl, II, III and IE63), or from a hepatitis virus such as hepatitis B virus (for example Hepatitis B Surface antigen, PreS1,
- 20 PreS2 and Surface env proteins, Hepatitis B core antigen or pol), hepatitis C virus (eg Core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A and B) and hepatitis E virus antigen, or from other viral pathogens, such as paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives thereof), or antigens from parainfluenza virus, measles virus, mumps virus, human papilloma viruses (for example HPV6, 11, 16, 18, eg L1, L2, E1, E2, E3, E4, E5,
- 25 E6, E7), flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus (such as haemagglutinin, nucleoprotein, NA, or M proteins, or combinations thereof), or antigens derived from bacterial pathogens such as *Neisseria spp*, including *N. gonorrhea* and *N. meningitidis*, eg, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *S. pyogenes* (for example M proteins or fragments thereof, C5A protease, *S. agalactiae*, *S. mutans*; *H. ducreyi*; *Moraxella spp*, including *M catarrhalis*, also known as *Branhamella catarrhalis* (for example high and low molecular weight adhesins and invasins); *Bordetella spp*, including *B. pertussis* (for example pertactin, pertussis toxin or derivatives thereof, filamentous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis* and *B. bronchiseptica*; *Mycobacterium spp.*, including *M. tuberculosis*, *M. bovis*,
- 30 *M. leprae*, *M. avium*, *M. paratuberculosis*, *M. smegmatis*; *Legionella spp*, including *L. pneumophila*; *Escherichia spp*, including enterotoxigenic *E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof),

enterohemorrhagic *E. coli*, enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio spp.*, including *V. cholera* (for example cholera toxin or derivatives thereof); *Shigella spp.*, including *S. sonnei*, *S. dysenteriae*, *S. flexnerii*; *Yersinia spp.*, including *Y. enterocolitica* (for example a Yop protein) , *Y. pestis*, *Y. pseudotuberculosis*; *Campylobacter* 5 *spp.*, including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli*; *Salmonella spp.*, including *S. typhi*, *S. paratyphi*, *S. choleraesuis*, *S. enteritidis*; *Listeria spp.*, including *L. monocytogenes*; *Helicobacter spp.*, including *H. pylori* (for example urease, catalase, vacuolating toxin); *Pseudomonas spp.*, including *P. aeruginosa*; *Staphylococcus spp.*, including *S. aureus*, *S. epidermidis*; *Enterococcus spp.*, including *E. faecalis*, *E. faecium*; *Clostridium* 10 *spp.*, including *C. tetani* (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof), *C. difficile* (for example clostridium toxins A or B and derivatives thereof); *Bacillus spp.*, including *B. anthracis* (for example botulinum toxin and derivatives thereof); *Corynebacterium spp.*, including *C. diphtheriae* (for example diphtheria toxin and derivatives thereof); *Borrelia spp.*, including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, 15 OspC, DbpA, DbpB), *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii*; *Ehrlichia spp.*, including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp.*, including *R. rickettsii*; *Chlamydia spp.*, including *C. trachomatis*, *C. pneumoniae*, *C. psittaci*; *Leptospira spp.*, including *L. interrogans*; *Treponema spp.*, including *T. pallidum* (for 20 example the rare outer membrane proteins), *T. denticola*, *T. hyodysenteriae*; or derived from parasites such as *Plasmodium spp.*, including *P. falciparum* and *P. vivax*; *Toxoplasma spp.*, including *T. gondii* (for example SAG2, SAG3, Tg34); *Entamoeba spp.*, including *E. histolytica*; *Babesia spp.*, including *B. microti*; *Trypanosoma spp.*, including *T. cruzi*; *Giardia spp.*, including *G. lamblia*; *Leishmania spp.*, including *L. major*; *Pneumocystis spp.*, including *P. carinii*; 25 *Trichomonas spp.*, including *T. vaginalis*; *Schistosoma spp.*, including *S. mansoni*, or derived from yeast such as *Candida spp.*, including *C. albicans*; *Cryptococcus spp.*, including *C. neoformans*.

Further bacterial antigens include antigens derived from *Streptococcus spp.*, including *S. pneumoniae* (PsaA, PspA, streptolysin, choline-binding proteins) and the protein antigen 30 Pneumolysin (Biochem Biophys Acta, 1989, 67, 1007; Rubins et al., Microbial Pathogenesis, 25, 337-342), and mutant detoxified derivatives thereof (WO 90/06951; WO 99/03884). Other bacterial antigens include antigens derived from *Haemophilus spp.*, including *H. influenzae type B* (for example PRP and conjugates thereof), *non typeable H. influenzae*, for example OMP26, high molecular weight adhesins, P5, P6, protein D and lipoprotein D, and fimbrin and fimbrin 35 derived peptides (US 5,843,464) or multiple copy variants or fusion proteins thereof.

In particular, the methods or compositions of the present invention may be used to protect against or treat viral disorders such as those caused by Hepatitis B virus, Hepatitis C

virus, Human papilloma virus, Human immunodeficiency virus (HIV), or Herpes simplex virus; bacterial diseases such as those caused by *Mycobacterium tuberculosis* (TB) or *Chlamydia sp*; and protozoal infections such as malaria.

It is to be recognised that these specific disease states, pathogens and antigens have been referred to by way of example only, and are not intended to be limiting upon the scope of the present invention.

TB antigens

The pathogen may, for example, be *Mycobacterium tuberculosis*.

Exemplary antigens derived from *M. tuberculosis* are for example alpha-crystallin (HspX), HBHA, Rv1753, Rv2386, Rv2707, Rv2557, Rv2558, RPFs: Rv0837c, Rv1884c, Rv2389c, Rv2450, Rv1009, aceA (Rv0467), ESAT6, Tb38-1, Ag85A, -B or -C, MPT 44, MPT59, MPT45, HSP10, HSP65, HSP70, HSP 75, HSP90, PPD 19kDa [Rv3763], PPD, 38kDa [Rv0934]), PstS1, (Rv0932), SodA (Rv3846), Rv2031c, 16kDa, Ra12, TbH9, Ra35, Tb38-1, Erd 14, DPV, MTI, MSL, DPPD, mTCC1, mTCC2, hTCC1 (WO 99/51748) and hTCC2, and especially Mtb32a, Ra35, Ra12, DPV, MSL, MTI, Tb38-1, mTCC1, TbH9 (Mtb39a), hTCC1, mTCC2 and DPPD. Antigens derived from *M. tuberculosis* also include fusion proteins and variants thereof where at least two, or for example, three polypeptides of *M. tuberculosis* are fused into a larger protein. Such fusions may comprise or consist of Ra12-TbH9-Ra35, Erd14-DPV-MTI, DPV-MTI-MSL, Erd14-DPV-MTI-MSL-mTCC2, Erd14-DPV-MTI-MSL, DPV-MTI-MSL-mTCC2, TbH9-DPV-MTI (WO 99/51748), Ra12-Tbh9-Ra35-Ag85B and Ra12-Tbh9-Ra35-mTCC2. A particular Ra12-Tbh9-Ra35 sequence that may be mentioned is defined by SEQ ID No 6 of WO2006/117240 together with variants in which Ser 704 of that sequence is mutated to other than serine, eg to Ala, and derivatives thereof incorporating an N-terminal His tag of an appropriate length (eg SEQ ID No 2 or 4 of WO2006/117240). See also SEQ ID No 10 which is a sequence containing an optional starting M and an optional N-terminal His-His tag (positions 2 and 3) and in which the Ala mutated relative to the wild-type Ser is at position 706.

Chlamydia antigens

The pathogen may, for example, be a *Chlamydia sp.* eg *C trachomatis*.

Exemplary antigens derived from *Chlamydia sp* eg *C trachomatis* are selected from CT858, CT 089, CT875, MOMP, CT622, PmpD, PmpG and fragments thereof, SWIB and immunogenic fragments of any one thereof (such as PmpDpd and PmpGpd) and combinations thereof. Preferred combinations of antigens include CT858, CT089 and CT875. Specific sequences and combinations that may be employed are described in WO2006/104890.

Plasmodium antigens

The pathogen may, for example be a parasite that causes malaria such as a *Plasmodium* sp. eg *P. falciparum* or *P. vivax*.

For example, antigens derived from *P. falciparum* include circumsporozoite protein (CS protein), PfEMP-1, Pfs 16 antigen, MSP-1, MSP-3, LSA-1, LSA-3, AMA-1 and TRAP. A particular hybrid antigen that may be mentioned is RTS. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P. falciparum* linked via four amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S. The structure of RTS and RTS,S is disclosed in WO 93/10152. TRAP antigens are described in WO 90/01496. Other *Plasmodium* antigens include *P. falciparum* EBA, GLURP, RAP1, RAP2, Sequestrin, Pf332, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs48/45, Pfs230 and their analogues in other *Plasmodium* spp. One embodiment of the present invention is a composition comprising RTS,S or CS protein or a fragment thereof such as the CS portion of RTS, S in combination with one or more further malarial antigens which may be selected for example from the group consisting of MSP-1, MSP-3, AMA-1, Pfs 16, LSA-1 or LSA-3. Possible antigens from *P. vivax* include circumsporozoite protein (CS protein) and Duffy antigen binding protein and immunogenic fragments thereof, such as PvRII (see eg WO02/12292).

Thus in one suitable embodiment of the invention, the first and second immunogenic polypeptides are selected from antigens derived from *Plasmodium falciparum* and/or *Plasmodium vivax*.

For example, the first and/or second immunogenic polypeptides are selected from antigens derived from *Plasmodium falciparum* and/or *Plasmodium vivax* are selected from RTS (eg as RTS,S), circumsporozoite (CS) protein, MSP-1, MSP-3, AMA-1, LSA-1, LSA-3 and immunogenic derivatives thereof or immunogenic fragments thereof.

One specific derivative that may be mentioned is the hybrid protein known as RTS, especially when presented in the form of a mixed particle known as RTS,S.

An exemplary RTS sequence is shown in SEQ ID No 14.

An exemplary *P. falciparum* CS protein-derived antigen is shown in SEQ ID No 12. This particular sequence corresponds to the CSP sequence of *P. falciparum* (3D7 strain), which also contains a 19 aa insertion coming from 7G8 strain (81-100).

In one specific embodiment of the invention, a first immunogenic polypeptide is RTS,S and a second immunogenic polypeptide is the CS protein from *Plasmodium falciparum* or an immunogenic fragment thereof.

HPV antigens

The pathogen may, for example, be a Human Papilloma Virus.

Thus antigens of use in the present invention may, for example, be derived from the Human Papilloma Virus (HPV) considered to be responsible for genital warts (HPV 6 or HPV 11 and others), and/or the HPV viruses responsible for cervical cancer (HPV16, HPV18, HPV33, HPV51, HPV56, HPV31, HPV45, HPV58, HPV52 and others). In one embodiment the forms of genital wart prophylactic, or therapeutic, compositions comprise L1 particles or capsomers, and fusion proteins comprising one or more antigens selected from the HPV proteins E1, E2, E5 E6, E7, L1, and L2. In one embodiment the forms of fusion protein are: L2E7 as disclosed in WO96/26277, and proteinD (1/3)-E7 disclosed in PCT/EP98/05285.

A preferred HPV cervical infection or cancer, prophylaxis or therapeutic composition may comprise HPV 16 or 18 antigens. For example, L1 or L2 antigen monomers, or L1 or L2 antigens presented together as a virus like particle (VLP) or the L1 alone protein presented alone in a VLP or capsomer structure. Such antigens, virus like particles and capsomer are per se known. See for example WO94/00152, WO94/20137, WO94/05792, and WO93/02184.

Additional early proteins may be included alone or as fusion proteins such as E7, E2 or preferably E5 for example; particularly preferred embodiments of this includes a VLP comprising L1E7 fusion proteins (WO 96/11272). In one embodiment the HPV 16 antigens comprise the early proteins E6 or E7 in fusion with a protein D carrier to form Protein D - E6 or E7 fusions from HPV 16, or combinations thereof; or combinations of E6 or E7 with L2 (WO 96/26277).

Alternatively the HPV 16 or 18 early proteins E6 and E7, may be presented in a single molecule, preferably a Protein D- E6/E7 fusion. Such a composition may optionally provide either or both E6 and E7 proteins from HPV 18, preferably in the form of a Protein D - E6 or Protein D - E7 fusion protein or Protein D E6/E7 fusion protein. Additionally antigens from other HPV strains, preferably from strains HPV 31 or 33 may be employed.

HIV antigens

The pathogen may, for example, be HIV eg HIV-1.

Thus, antigens may be selected from HIV derived antigens, particularly HIV-1 derived antigens.

HIV Tat and Nef proteins are early proteins, that is, they are expressed early in infection and in the absence of structural protein.

The Nef gene encodes an early accessory HIV protein which has been shown to possess several activities. For example, the Nef protein is known to cause the removal of CD4, the HIV receptor, from the cell surface, although the biological importance of this function is debated. Additionally Nef interacts with the signal pathway of T cells and induces an active state, which in turn may promote more efficient gene expression. Some HIV isolates have

mutations or deletions in this region, which cause them not to encode functional protein and are severely compromised in their replication and pathogenesis in vivo.

The Gag gene is translated from the full-length RNA to yield a precursor polyprotein which is subsequently cleaved into 3 – 5 capsid proteins; the matrix protein p17, capsid protein p24 and nucleic acid binding protein (Fundamental Virology, Fields BN, Knipe DM and Howley M 1996 2. Fields Virology vol 2 1996).

The Gag gene gives rise to the 55-kilodalton (Kd) Gag precursor protein, also called p55, which is expressed from the unspliced viral mRNA. During translation, the N terminus of p55 is myristoylated, triggering its association with the cytoplasmic aspect of cell membranes.

The membrane-associated Gag polyprotein recruits two copies of the viral genomic RNA along with other viral and cellular proteins that triggers the budding of the viral particle from the surface of an infected cell. After budding, p55 is cleaved by the virally encoded protease (a product of the Pol gene) during the process of viral maturation into four smaller proteins designated MA (matrix [p17]), CA (capsid [p24]), NC (nucleocapsid [p9]), and p6.(4).

In addition to the 3 major Gag proteins (p17, p24 and p9), all Gag precursors contain several other regions, which are cleaved out and remain in the virion as peptides of various sizes. These proteins have different roles e.g. the p2 protein has a proposed role in regulating activity of the protease and contributes to the correct timing of proteolytic processing.

The MA polypeptide is derived from the N-terminal, myristoylated end of p55. Most MA molecules remain attached to the inner surface of the virion lipid bilayer, stabilizing the particle. A subset of MA is recruited inside the deeper layers of the virion where it becomes part of the complex which escorts the viral DNA to the nucleus. These MA molecules facilitate the nuclear transport of the viral genome because a karyophilic signal on MA is recognized by the cellular nuclear import machinery. This phenomenon allows HIV to infect non-dividing cells, an unusual property for a retrovirus.

The p24 (CA) protein forms the conical core of viral particles. Cyclophilin A has been demonstrated to interact with the p24 region of p55 leading to its incorporation into HIV particles. The interaction between Gag and cyclophilin A is essential because the disruption of this interaction by cyclosporine inhibits viral replication.

The NC region of Gag is responsible for specifically recognizing the so-called packaging signal of HIV. The packaging signal consists of four stem loop structures located near the 5' end of the viral RNA, and is sufficient to mediate the incorporation of a heterologous RNA into HIV-1 virions. NC binds to the packaging signal through interactions mediated by two zinc-finger motifs. NC also facilitates reverse transcription.

The p6 polypeptide region mediates interactions between p55 Gag and the accessory protein Vpr, leading to the incorporation of Vpr into assembling virions. The p6 region also

contains a so-called late domain which is required for the efficient release of budding virions from an infected cell.

The Pol gene encodes three proteins having the activities needed by the virus in early infection, reverse transcriptase RT, protease, and the integrase protein needed for integration of viral DNA into cellular DNA. The primary product of Pol is cleaved by the virion protease to yield the amino terminal RT peptide which contains activities necessary for DNA synthesis (RNA and DNA directed DNA polymerase, ribonuclease H) and carboxy terminal integrase protein. HIV RT is a heterodimer of full-length RT (p66) and a cleavage product (p51) lacking the carboxy terminal RNase H domain.

RT is one of the most highly conserved proteins encoded by the retroviral genome. Two major activities of RT are the DNA Pol and ribonuclease H. The DNA Pol activity of RT uses RNA and DNA as templates interchangeably and like all DNA polymerases known is unable to initiate DNA synthesis de novo, but requires a pre existing molecule to serve as a primer (RNA).

The RNase H activity inherent in all RT proteins plays the essential role early in replication of removing the RNA genome as DNA synthesis proceeds. It selectively degrades the RNA from all RNA - DNA hybrid molecules. Structurally the polymerase and ribo H occupy separate, non-overlapping domains within the Pol covering the amino two thirds of the Pol.

The p66 catalytic subunit is folded into 5 distinct subdomains. The amino terminal 23 of these have the portion with RT activity. Carboxy terminal to these is the RNase H domain.

After infection of the host cell, the retroviral RNA genome is copied into linear double stranded DNA by the reverse transcriptase that is present in the infecting particle. The integrase (reviewed in Skalka AM '99 Adv in Virus Res 52 271-273) recognises the ends of the viral DNA, trims them and accompanies the viral DNA to a host chromosomal site to catalyse integration. Many sites in the host DNA can be targets for integration. Although the integrase is sufficient to catalyse integration in vitro, it is not the only protein associated with the viral DNA in vivo - the large protein - viral DNA complex isolated from the infected cells has been denoted the pre integration complex. This facilitates the acquisition of the host cell genes by progeny viral genomes.

The integrase is made up of 3 distinct domains, the N terminal domain, the catalytic core and the C terminal domain. The catalytic core domain contains all of the requirements for the chemistry of polynucleotidyl transfer.

HIV-1 derived antigens for us in the invention may thus for example be selected from Gag (for example full length Gag), p17 (a portion of Gag), p24 (another portion of Gag), p41, p40, Pol (for example full length Pol), RT (a portion of Pol), p51 (a portion of RT), integrase (a portion of Pol), protease (a portion of Pol), Env, gp120, gp140 or gp160, gp41, Nef, Vif, Vpr, Vpu, Rev, Tat and immunogenic derivatives thereof and immunogenic fragments thereof, particularly Env, Gag, Nef and Pol and immunogenic derivatives thereof and immunogenic

fragments thereof including p17, p24, RT and integrase. HIV vaccines may comprise polypeptides and/or polynucleotides encoding polypeptides corresponding to multiple different HIV antigens for example 2 or 3 or 4 or more HIV antigens which may be selected from the above list. Several different antigens may, for example, be comprised in a single fusion protein.

- 5 More than one first immunogenic polypeptide and/or more than one second immunogenic polypeptide each of which is an HIV antigen or a fusion of more than one antigen may be employed.

For example an antigen may comprise Gag or an immunogenic derivative or immunogenic fragment thereof, fused to RT or an immunogenic derivative or immunogenic
10 fragment thereof, fused to Nef or an immunogenic derivative or immunogenic fragment thereof wherein the Gag portion of the fusion protein is present at the 5' terminus end of the polypeptide.

A Gag sequence of use according to the invention may exclude the Gag p6 polypeptide encoding sequence. A particular example of a Gag sequence for use in the invention
15 comprises p17 and/or p24 encoding sequences.

A RT sequence may contain a mutation to substantially inactivate any reverse transcriptase activity (see WO03/025003).

The RT gene is a component of the bigger *pol* gene in the HIV genome. It will be understood that the RT sequence employed according to the invention may be present in the
20 context of Pol, or a fragment of Pol corresponding at least to RT. Such fragments of Pol retain major CTL epitopes of Pol. In one specific example, RT is included as just the p51 or just the p66 fragment of RT.

The RT component of the fusion protein or composition according to the invention optionally comprises a mutation to remove a site which serves as an internal initiation site in
25 prokaryotic expression systems.

Optionally the Nef sequence for use in the invention is truncated to remove the sequence encoding the N terminal region i.e. removal of from 30 to 85 amino acids, for example from 60 to 85 amino acids, particularly the N terminal 65 amino acids (the latter truncation is referred to herein as trNef). Alternatively or additionally the Nef may be modified to remove the
30 myristylation site. For example the Gly 2 myristylation site may be removed by deletion or substitution. Alternatively or additionally the Nef may be modified to alter the dileucine motif of Leu 174 and Leu 175 by deletion or substitution of one or both leucines. The importance of the dileucine motif in CD4 downregulation is described e.g. in Bresnahan P.A. et al (1998) Current Biology, 8(22): 1235-8.

35 The Env antigen may be present in its full length as gp160 or truncated as gp140 or shorter (optionally with a suitable mutation to destroy the cleavage site motif between gp120 and gp41). The Env antigen may also be present in its naturally occurring processed form as

gp120 and gp41. These two derivatives of gp160 may be used individually or together as a combination. The aforementioned Env antigens may further exhibit deletions (in particular of variable loops) and truncations. Fragments of Env may be used as well.

An exemplary gp120 sequence is shown in SEQ ID No 8. An exemplary gp140
5 sequence is shown in SEQ ID No 6.

Immunogenic polypeptides according to the invention may comprise Gag, Pol, Env and Nef wherein at least 75%, or at least 90% or at least 95%, for example, 96% of the CTL epitopes of these native antigens are present.

In immunogenic polypeptides according to the invention which comprise p17/p24 Gag,
10 p66 RT, and truncated Nef as defined above, 96% of the CTL epitopes of the native Gag, Pol and Nef antigens are suitably present.

One embodiment of the invention provides an immunogenic polypeptide containing p17, p24 Gag, p66 RT, truncated Nef (devoid of nucleotides encoding terminal amino-acids 1-85 – "trNef") in the order Gag, RT, Nef. In polynucleotides encoding immunogenic polypeptides of
15 the invention, suitably the P24 Gag and P66 RT are codon optimized.

Specific polynucleotide constructs and corresponding polypeptide antigens according to the invention include:

1. p17, p24 (codon optimised) Gag - p66 RT (codon optimised) - truncated Nef;
2. truncated Nef - p66 RT (codon optimised) - p17, p24 (codon optimised) Gag;
- 20 3. truncated Nef - p17, p24 (codon optimised) Gag - p66 RT (codon optimised);
4. p66 RT (codon optimised) - p17, p24 (codon optimised) Gag - truncated Nef;
5. p66 RT (codon optimised) – truncated Nef - p17, p24 (codon optimised) Gag;
6. p17, p24 (codon optimised) Gag - truncated Nef - p66 RT (codon optimised).

An exemplary fusion is a fusion of Gag, RT and Nef particularly in the order Gag-RT-Nef
25 (see eg SEQ ID No 2). Another exemplary fusion is a fusion of p17, p24, RT and Nef particularly in the order p24-RT-Nef-p17 (see eg SEQ ID No 16, referred to elsewhere herein as "F4").

In another embodiment an immunogenic polypeptide contains Gag, RT, integrase and Nef, especially in the order Gag-RT-integrase-Nef (see eg SEQ ID No 4).

30 In other embodiments the HIV antigen may be a fusion polypeptide which comprises Nef or an immunogenic derivative thereof or an immunogenic fragment thereof, and p17 Gag and/or p24 Gag or immunogenic derivatives thereof or immunogenic fragments thereof, wherein when both p17 and p24 Gag are present there is at least one HIV antigen or immunogenic fragment between them.

35 For example, Nef is suitably full length Nef.

For example p17 Gag and p24 Gag are suitably full length p17 and p24 respectively.

In one embodiment an immunogenic polypeptide comprises both p17 and p24 Gag or immunogenic fragments thereof. In such a construct the p24 Gag component and p17 Gag component are separated by at least one further HIV antigen or immunogenic fragment, such as Nef and/or RT or immunogenic derivatives thereof or immunogenic fragments thereof. See
 5 WO2006/013106 for further details.

In fusion proteins which comprise p24 and RT, it may be preferable that the p24 precedes the RT in the construct because when the antigens are expressed alone in *E. coli* better expression of p24 than of RT is observed.

Some constructs according to the invention include the following:

- 10 1. p24 – RT – Nef – p17
2. p24 – RT* – Nef – p17
3. p24 – p51RT – Nef – p17
4. p24 – p51RT* - Nef – p17
5. p17 – p51RT – Nef
- 15 6. p17 – p51RT* - Nef
7. Nef – p17
8. Nef – p17 with linker
9. p17 – Nef
10. p17 – Nef with linker
- 20 * represents RT methionine₅₉₂ mutation to lysine

In another aspect the present invention provides a fusion protein of HIV antigens comprising at least four HIV antigens or immunogenic fragments, wherein the four antigens or fragments are or are derived from Nef, Pol and Gag. Preferably Gag is present as two separate components which are separated by at least one other antigen in the fusion. Preferably the Nef
 25 is full length Nef. Preferably the Pol is p66 or p51RT. Preferably the Gag is p17 and p24 Gag. Other preferred features and properties of the antigen components of the fusion in this aspect of the invention are as described herein.

Preferred embodiments of this aspect of the invention are the four component fusions as already listed above:

- 30 1. p24 – RT – Nef – p17
2. p24 – RT* – Nef – p17
3. p24 – p51RT – Nef – p17
4. p24 – p51RT* - Nef – p17

The immunogenic polypeptides of the present invention may have linker sequences
 35 present in between the sequences corresponding to particular antigens such as Gag, RT and Nef. Such linker sequences may be, for example, up to 20 amino acids in length. In a particular

example they may be from 1 to 10 amino acids, or from 1 to 6 amino acids, for example 4-6 amino acids.

Further description of such suitable HIV antigens can be found in WO03/025003.

HIV antigens of the present invention may be derived from any HIV clade, for example
5 clade A, clade B or clade C. For example the HIV antigens may be derived from clade A or B, especially B .

In one specific embodiment of the invention, a first immunogenic polypeptide is a polypeptide comprising Gag and/or Pol and/or Nef or a fragment or derivative of any of them (eg p24-RT-Nef-p17) . In one specific embodiment of the invention a second immunogenic
10 polypeptide is a polypeptide comprising Gap and/or Pol and/or Nef or a fragment or derivative of any of them (eg Gag-RT-Nef or Gag-RT-integrase-Nef).

Thus in one specific embodiment, a polypeptide comprising Gap and/or Pol and/or Nef or a fragment or derivative of any of them (eg p24-RT-Nef-p17) is a first immunogenic polypeptide and a polypeptide comprising Gap and/or Pol and/or Nef or a fragment or derivative
15 of any of them (eg Gag-RT-Nef or Gag-RT-integrase-Nef) is a second immunogenic polypeptide.

In another specific embodiment of the invention, a first immunogenic polypeptide is Env or a fragment or derivative thereof eg gp120, gp140 or gp160 (especially gp120). In one specific embodiment of the invention a second immunogenic polypeptide is a polypeptide
20 comprising Gag and/or Pol and/or Nef or a fragment or derivative of any of them (eg p24-RT-Nef-p17).

Thus in one specific embodiment, Env or a fragment or derivative thereof eg gp120, gp140 or gp160 (especially gp120) is a first immunogenic polypeptide and a polypeptide comprising Gag and/or Pol and/or Nef or a fragment or derivative of any of them (eg p24-RT-
25 Nef-p17) is a second immunogenic polypeptide.

In another specific embodiment of the invention, a first immunogenic polypeptide is a polypeptide comprising Gag and/or Pol and/or Nef or a fragment or derivative of any of them (eg p24-RT-Nef-p17). In one specific embodiment of the invention a second immunogenic polypeptide is Env or a fragment or derivative thereof eg gp120, gp140 or gp160 (especially
30 gp120).

Thus in one specific embodiment, a polypeptide comprising Gag and/or Pol and/or Nef or a fragment or derivative of any of them (eg p24-RT-Nef-p17) is a first immunogenic polypeptide and Env or a fragment or derivative thereof eg gp120, gp140 or gp160 (especially gp120) is a second immunogenic polypeptide.

35

Immunogenic derivatives and immunogenic fragments of antigens

The aforementioned antigens may be employed in the form of immunogenic derivatives or immunogenic fragments thereof rather than the whole antigen.

As used herein the term "immunogenic derivative" in relation to an antigen of native origin refers to an antigen that have been modified in a limited way relative to its native counterparts. For example it may include a point mutation which may change the properties of the protein eg by improving expression in prokaryotic systems or by removing undesirable activity, eg enzymatic activity. Immunogenic derivatives will however be sufficiently similar to the native antigens such that they retain their antigenic properties and remain capable of raising an immune response against the native antigen. Whether or not a given derivative raises such an immune response may be measured by a suitably immunological assay such as an ELISA (for antibody responses) or flow cytometry using suitable staining for cellular markers (for cellular responses).

Immunogenic fragments are fragments which encode at least one epitope, for example a CTL epitope, typically a peptide of at least 8 amino acids. Fragments of at least 8, for example 8-10 amino acids or up to 20, 50, 60, 70, 100, 150 or 200 amino acids in length are considered to fall within the scope of the invention as long as the polypeptide demonstrates antigenicity, that is to say that the major epitopes (eg CTL epitopes) are retained by the polypeptide.

Adenovirus

Adenoviral vectors of the present invention comprise one or more heterologous polynucleotides (DNA) which encode one or more immunogenic polypeptides.

Adenoviral vectors of use in the present invention may be derived from a range of mammalian hosts.

Adenoviruses (herein referred to as "Ad" or "Adv") have a characteristic morphology with an icosohedral capsid consisting of three major proteins, hexon (II), penton base (III) and a knobbed fibre (IV), along with a number of other minor proteins, VI, VIII, IX, IIIa and IVa2 (Russell W.C. 2000, Gen Viriol, 81:2573-2604). The virus genome is a linear, double-stranded DNA with a terminal protein attached covalently to the 5' termini, which have inverted terminal repeats (ITRs). The virus DNA is intimately associated with the highly basic protein VII and a small peptide termed mu. Another protein, V, is packaged with this DNA-protein complex and provides a structural link to the capsid via protein VI. The virus also contains a virus-encoded protease, which is necessary for processing of some of the structural proteins to produce mature infectious virus.

Over 100 distinct serotypes of adenovirus have been isolated which infect various mammalian species, 51 of which are of human origin. Thus one or more of the adenoviral vectors may be derived from a human adenovirus. Examples of such human-derived adenoviruses are Ad1, Ad2, Ad4, Ad5, Ad6, Ad11, Ad 24, Ad34, Ad35, particularly Ad5, Ad11

and Ad35. The human serotypes have been categorised into six subgenera (A-F) based on a number of biological, chemical, immunological and structural criteria.

Although Ad5-based vectors have been used extensively in a number of gene therapy trials, there may be limitations on the use of Ad5 and other group C adenoviral vectors due to preexisting immunity in the general population due to natural infection. Ad5 and other group C members tend to be among the most seroprevalent serotypes. Immunity to existing vectors may develop as a result of exposure to the vector during treatment. These types of preexisting or developed immunity to seroprevalent vectors may limit the effectiveness of gene therapy or vaccination efforts. Alternative adenovirus serotypes, thus constitute very important targets in the pursuit of gene delivery systems capable of evading the host immune response.

One such area of alternative serotypes are those derived from non human primates, especially chimpanzee adenoviruses. See US Patent 6,083,716 which describes the genome of two chimpanzee adenoviruses.

It has been shown that chimpanzee ("Pan" or "C") adenoviral vectors induce strong immune responses to transgene products as efficiently as human adenoviral vectors (Fitzgerald et al. J. Immunol. 170:1416).

Non human primate adenoviruses can be isolated from the mesenteric lymph nodes of chimpanzees. Chimpanzee adenoviruses are sufficiently similar to human adenovirus subtype C to allow replication of E1 deleted virus in HEK 293 cells. Yet chimpanzee adenoviruses are phylogenetically distinct from the more common human serotypes (Ad2 and Ad5). Pan 6 is less closely related to and is serologically distinct from Pans 5, 7 and 9.

Thus one or more of the adenoviral vectors may be derived from a non-human primate adenovirus eg a chimpanzee adenovirus such as one selected from serotypes Pan5, Pan6, Pan7 and Pan9.

Adenoviral vectors may also be derived from more than one adenovirus serotype, and each serotype may be from the same or different source. For example they may be derived from more than one human serotype and/or more than one non-human primate serotype. Methods for constructing chimeric adenoviral vectors are disclosed in WO2005/001103.

There are certain size restrictions associated with inserting heterologous DNA into adenoviruses. Human adenoviruses have the ability to package up to 105% of the wild type genome length (Bett et al 1993, J Virol 67 (10), 5911-21). The lower packaging limit for human adenoviruses has been shown to be 75% of the wild type genome length (Parks et al 1995, J Virol 71(4), 3293-8).

One example of adenoviruses of use in the present invention are adenoviruses which are distinct from prevalent naturally occurring serotypes in the human population such as Ad2 and Ad5. This avoids the induction of potent immune responses against the vector which limits

the efficacy of subsequent administrations of the same serotype by blocking vector uptake through neutralizing antibody and influencing toxicity.

Thus, the adenovirus may be an adenovirus which is not a prevalent naturally occurring human virus serotype. Adenoviruses isolated from animals have immunologically distinct capsid, hexon, penton and fibre components but are phylogenetically closely related. Specifically, the virus may be a non-human adenovirus, such as a simian adenovirus and in particular a chimpanzee adenovirus such as Pan 5, 6, 7 or 9. Examples of such strains are described in WO03/000283 and are available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and other sources. Desirable chimpanzee adenovirus strains are Pan 5 [ATCC VR-591], Pan 6 [ATCC VR-592], and Pan 7 [ATCC VR-593].

Use of chimpanzee adenoviruses is thought to be advantageous over use of human adenovirus serotypes because of the lack of pre-existing immunity, in particular the lack of cross-neutralising antibodies, to adenoviruses in the target population. Cross-reaction of the chimpanzee adenoviruses with pre-existing neutralizing antibody responses is only present in 2% of the target population compared with 35% in the case of certain candidate human adenovirus vectors. The chimpanzee adenoviruses are distinct from the more common human subtypes Ad2 and Ad5, but are more closely related to human Ad4 of subgroup E, which is not a prevalent subtype. Pan 6 is less closely related to Pan 5, 7 and 9.

The adenovirus of the invention may be replication defective. This means that it has a reduced ability to replicate in non-complementing cells, compared to the wild type virus. This may be brought about by mutating the virus e.g. by deleting a gene involved in replication, for example deletion of the E1a, E1b, E3 or E4 gene.

The adenoviral vectors in accordance with the present invention may be derived from replication defective adenovirus comprising a functional E1 deletion. Thus the adenoviral vectors according to the invention may be replication defective due to the absence of the ability to express adenoviral E1a and E1b, i.e., are functionally deleted in E1a and E1b. The recombinant adenoviruses may also bear functional deletions in other genes [see WO 03/000283] for example, deletions in E3 or E4 genes. The adenovirus delayed early gene E3 may be eliminated from the adenovirus sequence which forms part of the recombinant virus. The function of E3 is not necessary to the production of the recombinant adenovirus particle. Thus, it is unnecessary to replace the function of this gene product in order to package a recombinant adenovirus useful in the invention. In one particular embodiment the recombinant adenoviruses have functionally deleted E1 and E3 genes. The construction of such vectors is described in Roy et al., Human Gene Therapy 15:519-530, 2004.

Recombinant adenoviruses may also be constructed having a functional deletion of the E4 gene, although it may be desirable to retain the E4 ORF6 function. Adenovirus vectors

according to the invention may also contain a deletion in the delayed early gene E2a. Deletions may also be made in any of the late genes L1 through to L5 of the adenovirus genome. Similarly deletions in the intermediate genes IX and IVa may be useful.

Other deletions may be made in the other structural or non-structural adenovirus genes.

5 The above deletions may be used individually, i.e. an adenovirus sequence for use in the present invention may contain deletions of E1 only. Alternatively, deletions of entire genes or portions thereof effective to destroy their biological activity may be used in any combination. For example in one exemplary vector, the adenovirus sequences may have deletions of the E1 genes and the E4 gene, or of the E1, E2a and E3 genes, or of the E1 and E3 genes (such as
10 functional deletions in E1a and E1b, and a deletion of at least part of E3), or of the E1, E2a and E4 genes, with or without deletion of E3 and so on. Such deletions may be partial or full deletions of these genes and may be used in combination with other mutations, such as temperature sensitive mutations to achieve a desired result.

The adenoviral vectors can be produced on any suitable cell line in which the virus is
15 capable of replication. In particular, complementing cell lines which provide the factors missing from the viral vector that result in its impaired replication characteristics (such as E1 and/or E4) can be used. Without limitation, such a cell line may be HeLa [ATCC Accession No. CCL 2], A549 [ATCC Accession No. CCL 185], HEK 293, KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells, among others. These cell lines are all available from the
20 American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209. Other suitable parent cell lines may be obtained from other sources, such as PER.C6® cells, as represented by the cells deposited under ECACC no. 96022940 at the European Collection of Animal Cell Cultures (ECACC) at the Centre for Applied Microbiology and Research (CAMR, UK) or Her 96 cells (Crucell).

25 The polynucleotide sequences which encode immunogenic polypeptides may be codon optimised for mammalian cells. Such codon-optimisation is described in detail in WO05/025614. Codon optimization for certain HIV sequences is further described in WO 03/025003.

In one embodiment of the present invention the polynucleotide constructs comprise an
30 N-terminal leader sequence. The signal sequence, transmembrane domain and cytoplasmic domain are individually all optionally present or deleted. In one embodiment of the present invention all these regions are present but modified.

A promoter for use in the adenoviral vector according to the invention may be the promoter from HCMV IE gene, for example wherein the 5' untranslated region of the HCMV IE
35 gene comprising exon 1 is included and intron A is completely or partially excluded as described in WO 02/36792.

When several antigens are fused into a fusion protein, such protein would be encoded by a polynucleotide under the control of a single promoter.

In an alternative embodiment of the invention, several antigens may be expressed separately through individual promoters, each of said promoters may be the same or different.

5 In yet another embodiment of the invention some of the antigens may form a fusion, linked to a first promoter and other antigen(s) may be linked to a second promoter, which may be the same or different from the first promoter.

Thus, the adenoviral vector may comprise one or more expression cassettes each of which encode one antigen under the control of one promoter. Alternatively or additionally it may
10 comprise one or more expression cassettes each of which encode more than one antigen under the control of one promoter, which antigens are thereby expressed as a fusion. Each expression cassette may be present in more than one locus in the adenoviral vector.

The polynucleotide or polynucleotides encoding immunogenic polypeptides to be expressed may be inserted into any of the adenovirus deleted regions, for example into the E1
15 deleted region.

Although two or more polynucleotides encoding immunogenic polypeptides may be linked as a fusion, the resulting protein may be expressed as a fusion protein, or it may be expressed as separate protein products, or it may be expressed as a fusion protein and then subsequently broken down into smaller subunits.

20

Adjuvant

Adjuvants are described in general in Vaccine Design – the Subunit and Adjuvant Approach eg Powell and Newman, Plenum Press, New York, 1995.

Suitable adjuvants include an aluminium salt such as aluminium hydroxide or aluminium
25 phosphate, but may also be a salt of calcium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes.

In the formulation of the invention it is preferred that the adjuvant composition preferentially induces a Th1 response. However it will be understood that other responses, including other
30 humoral responses, are not excluded.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either Th1 or Th2 - type cytokine responses. Traditionally the best indicators of the Th1:Th2 balance of the immune response after a vaccination or infection includes direct measurement of the production of Th1 or Th2 cytokines by T lymphocytes *in vitro* after restimulation with antigen,
35 and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a Th1-type adjuvant is one which stimulates isolated T-cell populations to produce high levels of Th1-type cytokines *in vivo* (as measured in the serum) or *ex vivo* (cytokines that

are measured when the cells are re-stimulated with antigen *in vitro*), and induces antigen specific immunoglobulin responses associated with Th1-type isotype.

Preferred Th1-type immunostimulants which may be formulated to produce adjuvants suitable for use in the present invention include and are not restricted to the following:

- 5 The Toll like receptor (TLR) 4 ligands, especially an agonist such as a lipid A derivative particularly monophosphoryl lipid A or more particularly 3 Deacylated monophosphoryl lipid A (3 D – MPL).

3 D –MPL is sold under the trademark MPL® by GlaxoSmithKline and primarily promotes CD4+ T cell responses characterized by the production of IFN- γ (Th1 cells i.e. CD4 T helper cells with a type-1 phenotype). It can be produced according to the methods disclosed in GB 2 220 211 A. Chemically it is a mixture of 3-deacylated monophosphoryl lipid A with 3, 4, 5 or 6 acylated chains. Preferably in the compositions of the present invention small particle 3 D-MPL is used. Small particle 3 D -MPL has a particle size such that it may be sterile-filtered through a 0.22 μ m filter. Such preparations are described in International Patent Application No. WO94/21292. Synthetic derivatives of lipid A are known and thought to be TLR 4 agonists including, but not limited to:

OM174 (2-deoxy-6-o-[2-deoxy-2-[(R)-3-dodecanoyloxytetra-decanoylamino]-4-o-phosphono- β -D-glucopyranosyl]-2-[(R)-3-hydroxytetradecanoylamino]- α -D-glucopyranosyl)dihydrogenphosphate), (WO 95/14026)

20 **OM 294 DP** (3S, 9 R) –3--[(R)-dodecanoyloxytetradecanoylamino]-4-oxo-5-aza-9(R)-[(R)-3-hydroxytetradecanoylamino]decan-1,10-diol,1,10-bis(dihydrogenophosphate) (WO99 /64301 and WO 00/0462)

25 **OM 197 MP-Ac DP** (3S-, 9R) -3-[(R) -dodecanoyloxytetradecanoylamino]-4-oxo-5-aza-9-[(R)-3-hydroxytetradecanoylamino]decan-1,10-diol,1 -dihydrogenophosphate 10-(6-aminohexanoate) (WO 01/46127)

30 Other TLR4 ligands which may be used are alkyl Glucosaminide phosphates (AGPs) such as those disclosed in WO9850399 or US6303347 (processes for preparation of AGPs are also disclosed), or pharmaceutically acceptable salts of AGPs as disclosed in US6764840. Some AGPs are TLR4 agonists, and some are TLR4 antagonists. Both are thought to be useful as adjuvants.

35 Saponins are also preferred Th1 immunostimulants in accordance with the invention. Saponins are well known adjuvants and are taught in: Lacaille-Dubois, M and Wagner H. (1996. A review of the biological and pharmacological activities of saponins. *Phytomedicine* vol 2 pp 363-386). For example, Quil A (derived from the bark of the South American tree *Quillaja Saponaria* Molina), and fractions thereof, are described in US 5,057,540 and "Saponins as vaccine adjuvants", Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279 B1. The haemolytic saponins QS21 and QS17 (HPLC purified fractions of Quil A)

have been described as potent systemic adjuvants, and the method of their production is disclosed in US Patent No. 5,057,540 and EP 0 362 279 B1. Also described in these references is the use of QS7 (a non-haemolytic fraction of Quil-A) which acts as a potent adjuvant for systemic vaccines. Use of QS21 is further described in Kensil *et al.* (1991. J. Immunology vol 146, 431-437). Combinations of QS21 and polysorbate or cyclodextrin are also known (WO 99/10008). Particulate adjuvant systems comprising fractions of QuilA, such as QS21 and QS7 are described in WO 96/33739 and WO 96/11711. One such system is known as an Iscom and may contain one or more saponins.

The adjuvant of the present invention may in particular comprises a Toll like receptor (TLR) 4 ligand, especially 3D-MPL, in combination with a saponin.

Other suitable adjuvants include TLR 9 ligands (agonists). Thus another preferred immunostimulant is an immunostimulatory oligonucleotide containing unmethylated CpG dinucleotides ("CpG"). CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. CpG is known in the art as being an adjuvant when administered by both systemic and mucosal routes (WO 96/02555, EP 468520, Davis *et al.*, *J.Immunol.*, 1998, 160(2):870-876; McCluskie and Davis, *J.Immunol.*, 1998, 161(9):4463-6). Historically, it was observed that the DNA fraction of BCG could exert an anti-tumour effect. In further studies, synthetic oligonucleotides derived from BCG gene sequences were shown to be capable of inducing immunostimulatory effects (both in vitro and in vivo). The authors of these studies concluded that certain palindromic sequences, including a central CG motif, carried this activity. The central role of the CG motif in immunostimulation was later elucidated in a publication by Krieg, *Nature* 374, p546 1995. Detailed analysis has shown that the CG motif has to be in a certain sequence context, and that such sequences are common in bacterial DNA but are rare in vertebrate DNA. The immunostimulatory sequence is often: Purine, Purine, C, G, pyrimidine, pyrimidine; wherein the CG motif is not methylated, but other unmethylated CpG sequences are known to be immunostimulatory and may be used in the present invention.

In certain combinations of the six nucleotides a palindromic sequence is present. Several of these motifs, either as repeats of one motif or a combination of different motifs, can be present in the same oligonucleotide. The presence of one or more of these immunostimulatory sequences containing oligonucleotides can activate various immune subsets, including natural killer cells (which produce interferon γ and have cytolytic activity) and macrophages (Wooldrige *et al* Vol 89 (no. 8), 1977). Other unmethylated CpG containing sequences not having this consensus sequence have also now been shown to be immunomodulatory.

CpG when formulated into vaccines, is generally administered in free solution together with free antigen (WO 96/02555; McCluskie and Davis, *supra*) or covalently conjugated to an antigen (WO 98/16247), or formulated with a carrier such as aluminium hydroxide ((Hepatitis

surface antigen) Davis *et al. supra* ; Brazolot-Millan *et al.*, *Proc.Natl.Acad.Sci.*, USA, 1998, 95(26), 15553-8).

Other TLR9 agonists of potential interest include immunostimulatory CpG motif containing oligonucleotides and YpG motif containing oligonucleotides (Idera).

5 Such immunostimulants as described above may be formulated together with carriers, such as for example liposomes, oil in water emulsions, and or metallic salts, including aluminium salts (such as aluminium hydroxide). For example, 3D-MPL may be formulated with aluminium hydroxide (EP 0 689 454) or oil in water emulsions (WO 95/17210); QS21 may be advantageously formulated with cholesterol containing liposomes (WO 96/33739), oil in water
10 emulsions (WO 95/17210) or alum (WO 98/15287); CpG may be formulated with alum (Davis *et al. supra* ; Brazolot-Millan *supra*) or with other cationic carriers.

Combinations of immunostimulants are also preferred, in particular a combination of a monophosphoryl lipid A and a saponin derivative (WO 94/00153; WO 95/17210; WO 96/33739; WO 98/56414; WO 99/12565; WO 99/11241), more particularly the combination of QS21 and
15 3D-MPL as disclosed in WO 94/00153. Alternatively, a combination of CpG plus a saponin such as QS21 also forms a potent adjuvant for use in the present invention. Alternatively the saponin may be formulated in a liposome or in an Iscorn and combined with an immunostimulatory oligonucleotide.

Thus, suitable adjuvant systems include, for example, a combination of monophosphoryl
20 lipid A, preferably 3D-MPL, together with an aluminium salt (eg as described in WO00/23105).

An enhanced system involves the combination of a monophosphoryl lipid A and a saponin derivative particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched in cholesterol containing liposomes (DQ) as disclosed in WO 96/33739. This combination may additionally
25 comprise an immunostimulatory oligonucleotide.

Thus an example adjuvant comprises QS21 and/or MPL and/or CpG.

A particularly potent adjuvant formulation involving QS21, 3D-MPL & tocopherol in an oil in water emulsion is described in WO 95/17210 and is another preferred formulation for use in the invention.

30 Another preferred formulation comprises a CpG oligonucleotide alone or together with an aluminium salt.

In a further aspect of the present invention there is provided a method of manufacture of a vaccine formulation as herein described, wherein the method comprises admixing one or more first immunogenic polypeptides according to the invention with a suitable adjuvant.

35 Particularly preferred adjuvants for use in the formulations according to the invention are as follows:

i) 3D-MPL + QS21 in a liposome (see eg Adjuvant B below)

- ii) Alum + 3D-MPL
- iii) Alum + QS21 in a liposome + 3D-MPL
- iv) Alum + CpG
- v) 3D-MPL + QS21 + oil in water emulsion
- 5 vi) CpG
- vii) 3D-MPL + QS21 (eg in a liposome) + CpG
- viii) QS21+ CpG.

Preferably, the adjuvant is presented in the form of a liposome, ISCOM or an oil-in-water emulsion. In one example embodiment of the invention the adjuvant comprises an oil-in-water emulsion. In another example embodiment of the invention the adjuvant comprises liposomes.

Suitably the adjuvant component does not contain any virus. Thus suitably, compositions for use according to the invention do not contain any virus other than the one or more more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from a pathogen.

Compositions, dosage and administration

In methods of the invention, the immunogenic polypeptide(s), the adenoviral vector(s) and the adjuvant are administered concomitantly.

Typically the adjuvant will be co-formulated with an immunogenic polypeptide. Suitably the adjuvant will also be co-formulated with any other immunogenic polypeptide to be administered.

Thus in one embodiment of the invention there is provided a method of raising an immune response which comprises administering (i) one or more first immunogenic polypeptides co-formulated with an adjuvant; and (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides; wherein one or more first immunogenic polypeptides and adjuvant, and one or more adenoviral vectors are administered concomitantly.

By "co-formulated" is meant that the first immunogenic polypeptide and the adjuvant are contained within the same composition eg a pharmaceutical composition.

Typically the adenoviral vector is contained in a composition eg a pharmaceutical composition.

Alternatively, the one or more first immunogenic polypeptides, the one or more adenoviral vectors and an adjuvant are co-formulated.

Thus, there are provided compositions according to the invention which comprise one or more immunogenic polypeptides, one or more adenoviral vectors, and an adjuvant.

Compositions and methods according to the invention may involve use of more than one immunogenic polypeptide and/or more than one adenoviral vector. Use of multiple antigens is

especially advantageous in raising protective immune responses to certain pathogens, such as HIV, *M. tuberculosis* and *Plasmodium sp.* Compositions according to the invention may comprise more than one adjuvant.

Compositions and methods employed according to the invention may typically comprise
5 a carrier eg an aqueous buffered carrier. Protective components such as sugars may be included.

Compositions should be administered in sufficient amounts to transduce the target cells and to provide sufficient levels of gene transfer and expression and to permit pathogen-specific immune responses to develop thereby to provide a prophylactic or therapeutic benefit without
10 undue adverse or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the retina and other intraocular delivery methods, direct delivery to the liver, inhalation, intranasal, intravenous, intramuscular, intratracheal, subcutaneous, intradermal, epidermal, rectal, oral and other parenteral routes of
15 administration. Routes of administration may be combined, if desired, or adjusted depending upon the gene product or the condition. The route of administration primarily will depend on the nature of the condition being treated. Most suitably the route is intramuscular, intradermal or epidermal.

Preferred tissues to target are muscle, skin and mucous membranes. Skin and mucous
20 membranes are the physiological sites where most infectious antigens are normally encountered.

When the first immunogenic polypeptide, adjuvant and adenoviral vector are not co-formulated, the different formulations (eg polypeptide/adjuvant and adenoviral vector formulations) may be administered by the same route of administration or by different routes of
25 administration.

Dosages of compositions in the methods will depend primarily on factors such as the condition being treated, the age, weight and health of the subject, and may thus vary among subjects. For example, a therapeutically effective adult human or veterinary dosage is generally in the range of from about 100 μ L to about 100 mL of a carrier containing concentrations of from
30 about 1×10^6 to about 1×10^{15} particles, about 1×10^{11} to 1×10^{13} particles, or about 1×10^9 to 1×10^{12} particles of virus together with around 1-1000ug, or about 2-100ug eg around 4-40ug immunogenic polypeptide. Dosages will range depending upon the size of the animal and the route of administration. For example, a suitable human or veterinary dosage (for about an 80 kg animal) for intramuscular injection is in the range of about 1×10^9 to about 5×10^{12} virus
35 particles and 4-40 ug protein per mL, for a single site. One of skill in the art may adjust these doses, depending on the route of administration, and the therapeutic or vaccinal application for which the composition is employed.

The amount of adjuvant will depend on the nature of the adjuvant and the immunogenic polypeptide, the condition being treated and the age, weight and health of the subject. Typically for human administration an amount of adjuvant of 1-100ug eg 10-50 ug per dose may be suitable.

5 Suitably an adequate immune response is achieved by a single concomitant administration of the composition or compositions of the invention in methods of the invention. However if the immune response is further enhanced by administration of a further dose of first immunogenic polypeptide, adjuvant and adenoviral vector on a second or subsequent occasion (for example after a month or two months) then such a protocol is embraced by the invention.

10 We have found that good pathogen-specific CD4+ and/or CD8+ T-cell responses may typically be raised after a single concomitant administration of the composition or compositions of the invention in methods of the invention. However we have found that good pathogen-specific antibody responses may require a second or further concomitant administration of the composition or compositions of the invention.

15 The components of the invention may be combined or formulated with any suitable pharmaceutical excipient such as water, buffers and the like.

Examples

Adjuvant preparations

20 **1) The preparation of oil in water emulsion followed the protocol as set forth in WO 95/17210.**

The emulsion contains: 42.72 mg/ml squalene, 47.44 mg/ml tocopherol, 19.4 mg/ml Tween 80. The resulting oil droplets have a size of approximately 180 nm

Tween 80 was dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS.

25 To provide 100 ml two fold concentrate, emulsion 5g of DL alpha tocopherol and 5ml of squalene were vortexed until mixed thoroughly. 90ml of PBS/Tween solution was added and mixed thoroughly. The resulting emulsion was then passed through a syringe and finally microfluidised by using an M110S microfluidics machine. The resulting oil droplets have a size of approximately 180 nm

30 **2) Preparation of oil in water emulsion with QS21 and MPL**

Sterile bulk emulsion was added to PBS to reach a final concentration of 500 µl of emulsion per ml (v/v). 3 D-MPL was then added. QS21 was then added. Between each addition of component, the intermediate product was stirred for 5 minutes. Fifteen minutes later, the pH was checked and adjusted if necessary to 6.8 +/- 0.1 with NaOH or HCl. The final concentration of 3D-MPL and QS21 was 100 µg per ml for each.

3) Preparation of liposomal MPL

A mixture of lipid (such as phosphatidylcholine either from egg-yolk or synthetic) and cholesterol and 3 D-MPL in organic solvent, was dried down under vacuum (or alternatively under a stream of inert gas). An aqueous solution (such as phosphate buffered saline) was then added, and the vessel agitated until all the lipid was in suspension. This suspension was then microfluidised until the liposome size was reduced to about 100 nm, and then sterile filtered through a 0.2 µm filter. Extrusion or sonication could replace this step.

Typically the cholesterol: phosphatidylcholine ratio was 1:4 (w/w), and the aqueous solution was added to give a final cholesterol concentration of 10 mg/ml.

The final concentration of MPL is 2 mg/ml.

The liposomes have a size of approximately 100 nm and are referred to as SUV (for small unilamellar vesicles). The liposomes by themselves are stable over time and have no fusogenic capacity.

4) Preparation of Adjuvant B ("adj B")

Sterile bulk of SUV was added to PBS. PBS composition was Na₂HPO₄: 9 mM; KH₂PO₄: 48 mM; NaCl: 100 mM pH 6.1. QS21 in aqueous solution was added to the SUV. The final concentration of 3D-MPL and QS21 was 100 µg per ml for each. This mixture is referred as **Adjuvant B**. Between each addition of component, the intermediate product was stirred for 5 minutes. The pH was checked and adjusted if necessary to 6.1 +/- 0.1 with NaOH or HCl.

Preparation of p24-RT-Nef-P17 protein ("F4")

F4 was prepared as described in WO2006/013106 Example 1, codon-optimised method.

Preparation of Chimp adenovirus Pan7 containing Gag-RT-Nef transgene ("Pan7GRN")

Construction of Gag, RT, Nef plasmid.

Plasmid p73i-Tgrn

The full sequence of the Tgrn plasmid insert is given in SEQ ID No 1 and the plasmid construction shown graphically in Fig 1. This contains p17 p24 (codon optimised) Gag, p66 RT (codon optimised and inactivated) and truncated Nef.

The plasmid P73i-Tgrn was prepared as described in WO03/025003 Examples 1 - 13.

Construction of the E1/E3 deleted Pan 7 Adenovirus

The E1/E3 deleted Pan 7 Adenovirus was prepared as described in WO2006/120034 Example 1.

Other serotypes of vectors can be constructed in a similar way. A full description of the construction of E1, E3 and E4 deletions in this and other Pan Adenovirus serotypes is given in WO03/0046124. Further information is also available in Human Gene Therapy 15:519-530.

Insertion of Gag, RT, Nef sequence into Adenovirus

Using plasmid P73i-Tgrn, the GRN expression cassette was inserted into E1/E3 deleted Pan 7 adenovirus to produce C7-GRNc as described in WO2006/120034 Example 3. C7-GRNc is the Pan7GRN adenovirus component used in the examples set out herein.

5

Example 1

Immunogenicity study in mice immunised with adenovirus component (Pan7GRN) and protein component (F4/adjuvant B) separately or with both adenovirus and protein components co-formulated together

10

The mouse strain used was CB6F1 and 3 mice were used per timepoint. For immunisation with F4/adjuvant B (P), 1/10 of the human dose was injected i.e. 9 ug of F4 protein in 50uL of adjuvant B. For immunisation with Pan7GRN (A), 10×10^8 virus particles in 50uL of saline (0.9% NaCl water for injection solution) was used. The Pan7GRN chimp adenovirus carries the genes coding for Gag (G), RT (R) and Nef (N).

15

The vaccination schedule was as follows:

Group	Day 0	Day 21	Day 42	Day 63
1	-	-	F4/adj B	F4/adj B
2			Pan7GRN	Pan7GRN
3	F4/ adj B	F4/adj B	Pan7GRN	Pan7GRN
4	Pan7GRN	Pan7GRN	F4/adj B	F4/adj B
5	-	-	-	F4/adj B/ Pan7GRN
6	-	-	F4/adj B/ Pan7GRN	F4/adj B/ Pan7GRN
7	-	-	adj B	adj B
8	-	-	-	-

Thus it can be seen that in groups 1 and 2, the mice were immunized with 2 injections of protein (PP) or adenovirus (AA), respectively. Mice from groups 3 and 4, received a conventional prime-boost schedule: protein then adenovirus (PPAA) or the other way round (AAPP) whereas in groups 5 and 6, the mice received one or two injections of a combination (combo) of protein and adenovirus together according to the invention. Mice from group 7 only received adjuvant control whereas mice from group 6 were naive.

25

The following read-outs were performed:

Antibody responses (ELISA performed on the sera from each individual animals from each group):

-antibody response against F4 (Figure 4)

-antibody response against F4 components p24, RT, Nef and p17 (Figure 5-8)

5

Cellular responses (Figures 2-3):

-measured by flow cytometry following surface and intracellular cytokine staining after overnight restimulation of spleen cells with pools of peptides of p24, RT, Nef or p17. The spleen cells of 3 mice per timepoint and per group were pooled for the analysis.

10

For groups 1 and 2, samples were taken for measurement 21 days after the corresponding final immunisation. For the remaining groups, measurements were taken 21days, 56 days and 112 days after the corresponding final immunisation.

15 Results:

The results are shown in Figures 2-8.

The X axis labels correspond as follows:

PP – Group 1 animals following second immunisation

AA - Group 2 animals following second immunisation

20 PPAA - Group 3 animals following fourth immunisation

AAPP - Group 4 animals following fourth immunisation

Combo - Group 5 animals following immunisation

Combo x 2 - Group 6 animals following second immunisation

The measurement timepoints (21, 56 or 112 days post last immunisation) are indicated in parentheses.

25

Cellular responses (Figure 2-3):

At the timepoints analysed, the data show that CD4+ T-cell responses were observed mainly against p24, RT and Nef.

30

As shown in Figures 2a and 2b (left panels), 21 days post last immunisation, the highest CD4+ T-cell responses are observed with two immunisations of adenovirus followed by two immunisations of protein/adjuvant (Group 4 animals). One injection of the combination of adenovirus/protein/adjuvant induces higher CD4+ T-cell levels than two injections of protein/adjuvant following restimulation with p24, RT or Nef peptides.

35

For restimulation by RT and Nef, two immunisations with the combination of adenovirus/protein/adjuvant induces a CD4+ T-cell response slightly higher than with one

immunisation with the combination, whereas the responses with one or two immunisations were identical for p24.

At the timepoints analysed, the CD8+ T-cell responses are mainly observed against the p24 and
5 RT peptides, and no significant numbers of CD8+ T-cells specific for Nef or p17 were detected.
As shown in Figures 2a and 2b (right panels), 21 days post last immunisation CD8+ T-cell
responses were similar after one or two immunisations with the combination of
adenovirus/protein/adjuvant. The CD8 response against p24 observed in groups immunised
either (i) twice with adenovirus or (ii) twice with adenovirus followed by twice with protein or (iii)
10 once or twice with the combination of adenovirus/protein/adjuvant were comparable to each
other and slightly lower than the one from the group immunised twice with protein followed by
twice with adenovirus. The CD8 response against RT observed in groups immunised once or
twice with the combination of adenovirus/protein/adjuvant were comparable and slightly lower to
the one from the groups immunised either (i) twice with adenovirus or (ii) twice with adenovirus
15 followed by twice with protein or (iii) twice with protein followed by twice with adenovirus.

The CD4 and CD8 T cell responses were also analysed at later timepoints (56 and 112 days
post last immunisation), when persistence of the responses can be determined (Figures 3a and
3b). The CD4 responses (Fig 3a and 3b, left panels) are mainly observed against p24, RT and
20 Nef. At these timepoints, the highest CD4 responses are observed in the animals immunised
twice with adenovirus followed by twice with protein. The CD4 response in mice immunised
once or twice with the combination of adenovirus/protein/adjuvant were comparable to each
other and generally higher than the response observed in groups immunised twice with protein
followed by twice with adenovirus.

25 At the later timepoints, the CD8 response against p24 is the highest in the group immunised
once with the combination of adenovirus/protein/adjuvant (Fig 3b, right panel). It is comparable
to the one from animals immunised twice with protein followed by twice with adenovirus and
slightly higher than the one from the animals immunised either (i) twice with the combination of
adenovirus/protein/adjuvant or (ii) twice with adenovirus followed by twice with protein. The
30 latter two are comparable between each other. The CD8 response against RT is the highest
and similar in groups immunised (i) twice with the combination of adenovirus/protein/adjuvant or
(ii) twice with adenovirus followed by twice with protein. The CD8 response against RT from
groups immunised (i) twice with the combination of adenovirus/protein/adjuvant or (ii) twice with
protein followed by twice with adenovirus was slightly lower but comparable between each other
35 (Figure 3). As shown in figure 3a (right panel), no significant numbers of CD8+ T-cells specific
for Nef or p17 were detected.

Antibody responses:

As shown in Figures 4 to 8, the antibody responses detected are mainly directed against p24 (Fig 5), RT (Fig 6) and Nef (Fig 8). The anti-F4 (Fig 4) response generally mimics the response observed against each of the p24, RT or Nef components and can be characterized as follows:

- 5 - Low to no antibody response is detected in groups immunised (i) twice with adenovirus or (ii) once with the combination of adenovirus/protein/adjuvant;
- The highest antibody responses usually detected in group immunised twice with the protein at 21 days post immunisation. However, it is also in this group that the highest variability between individuals is observed. In addition, for the anti-Nef serology, the
- 10 group immunised twice with adenovirus followed by twice with protein appears to display the highest response, when compared to the other groups;
- The response observed in groups immunised (i)) twice with the combination of adenovirus/protein/adjuvant or (ii) twice with protein followed by twice with adenovirus or
- (iii) twice with adenovirus followed by twice with protein are comparable, peak at 21 days
- 15 post last immunisation and then slightly decrease over time.

Antibody responses against p17 (Fig 7) were very low to undetectable in all groups.

Conclusion:

Globally, the highest antigen-specific cell-mediated immune response is observed in the AAPP treatment group after 4 immunisations. However, when comparing groups after 2 immunisations

20 (i.e. AA, PP and 2xcombo groups), the induction of both antigen-specific CD4 and CD8 T cell responses is only observed in the group immunised twice with the protein/adenovirus/adjuvant combination. In addition, similar levels of CD4 and CD8 T cell responses can be reached after a single injection of the protein/adenovirus/adjuvant combination. Moreover, in terms of

25 persistence, the antigen-specific T cell responses observed 112 days after the 2nd immunisation with the protein/adenovirus/adjuvant combination are comparable to the ones observed 112 days after the 4th immunisations in the AAPP treatment group. Finally, it appears that 2 immunisations with the protein/adenovirus/adjuvant combination are needed to obtain an antibody response comparable to the one obtained in the group immunised twice with the

30 adjuvanted protein, group that provided the highest antibody responses in general.

Example 2

Immunogenicity study in mice immunised with Pan7GRN adenovirus and F4 protein/adjuvant B co-formulated together

35

The mouse strain used was CB6F1 with 9 mice per group. Mice were immunized once with a co-formulation of the F4 protein (1/10 of the human dose was injected i.e. 9 ug) together with 10

$\times 10^8$ virus particles of Pan7GRN, in 50uL of adjuvant B or a dilution of the latter (1/2, 1/4 or 1/10). The CD4 and CD8 cellular responses against a pool of either Nef, p17, p24 or RT peptides were determined 21 days post immunization (3 pools of 3 spleens for each group).

5 The following read-out was performed:

Cellular responses (Figure 9):

-measured by flow cytometry following surface and intracellular cytokine staining after overnight restimulation of spleen cells with pools of peptides of p24, RT, Nef or p17. The spleen cells were pooled (3 pools of 3 spleens per group) for the analysis.

10

Results:

The results shown in Figure 9 represent the cellular responses observed after restimulation with a pool of p24 or RT peptides.

The X axis labels correspond as follows:

15 Adj B – Mice immunised with $9\mu\text{gF4}/10^8\text{vpPan7GRN/}$ non-diluted adjuvant B

1/2 Adj B – Mice immunised with $9\mu\text{gF4}/10^8\text{vpPan7GRN/}$ adjuvant B diluted 1/2

1/4 Adj B – Mice immunised with $9\mu\text{gF4}/10^8\text{vpPan7GRN/}$ adjuvant B diluted 1/4

1/10 Adj B – Mice immunised with $9\mu\text{gF4}/10^8\text{vpPan7GRN/}$ adjuvant B diluted 1/10

Naïve – Naïve mice (no immunisation)

20

The results indicate that CD4 (Figure 9, left panel) and CD8 (Figure 9, right panel) responses are mainly observed against p24 and RT, with the CD8 T cell response specific to RT being lower than the one specific to p24. In addition, the results indicate that the CD4 responses against p24 and RT at 21 days post-immunisations in the groups immunised with the non-

25 diluted adjuvant B or a 1/2 dilution of it are similar. These CD4 responses tend to decrease

when the adjuvant is diluted 1/4. When the adjuvant B is diluted at 1/10, the CD4 responses observed are similar to the ones from groups immunised with the 1/4 dilution of the adjuvant B.

The anti-CD8 responses against p24 are comparable whether the adjuvant is diluted 1/2 or not.

However, the response decreases when the adjuvant B is diluted 1/4 and even more so if it is

30 diluted 1/10. In contrast, such trends are not seen for the anti-RT CD8 responses where there is not a real dose range effect of the dose of adjuvant used.

Conclusion:

CD4+ cells and CD8+ cells against F4 components were induced by a single administration of a
35 composition containing an immunogenic polypeptide, an adenoviral vector containing a heterologous polynucleotide encoding an immunogenic polypeptide and an adjuvant, even when the latter was diluted. The impact of adjuvant dilution differed depending on the antigen-

specific CD4 or CD8 responses of interest. In particular the highest responses observed were against p24 and the anti-p24 CD4 and CD8 T cell responses show a dose range effect correlating with the dose of adjuvant used in the combination vaccine. While the same effect can be observed for the anti-RT CD4 T cell response, the dose range effect of the dose of adjuvant used in the combo is less clear for the anti-RT CD8 T cell response. Finally, if we consider the global antigen-specific CD4 and CD8 T cell responses and sum the responses against the 4 antigens, a dose range can be observed.

Example 3:

- 10 Immunogenicity study in New Zealand white rabbits immunised with Pan7GRN or F4/adjuvant B sequentially or with both adenovirus and protein components co-formulated together
- For immunisation with F4/adjuvant B, the human dose was injected i.e. 90 ug of F4 protein in 500uL of adjuvant B. For immunisation with Pan7GRN, 10×10^{10} or 10×10^{12} virus particles in 500uL of saline were used. For the immunization with both adenovirus and protein components
- 15 co-formulated together, 90µg of F4 protein, 10×10^{11} virus particles of Pan7 GRN in 500uL of adjuvant B were used.

The vaccination schedule was as follows:

Group	Day 0	Day 14	Day 126
1	F4/ adj B	F4/ adj B	F4/adj B
2	Pan7GRN 10^{10}		Pan7GRN 10^{10}
3	Pan7GRN 10^{12}		Pan7GRN 10^{12}
4	F4/adj B/ Pan7GRN 10^{11}	F4/adj B/ Pan7GRN 10^{11}	F4/adj B/ Pan7GRN 10^{11}

- 20 There were 3 rabbits per group except for group 1 which included only 2 rabbits.

The following read-outs were performed:

Antibody responses (ELISA performed on the sera from each individual animals from each group):

- 25 -antibody response against F4
-antibody response against F4 components p24, RT, Nef and p17

Lymphoproliferative responses:

- 30 The lymphoproliferation was determined by the uptake of tritiated thymidine by peripheral blood mononuclear cells (isolated from whole blood after a density gradient) restimulated in vitro with pools of Nef, p17, p24 and/or RT peptides for 88 hours in the presence of tritiated thymidine for the last 16 hours of the incubation.

Results:

Lymphoproliferative response:

As shown in Figure 10, the highest lymphoproliferative responses are observed in the group immunised twice with protein. The lymphoproliferative response from animals immunised twice with the combination of adenovirus/protein/adjuvant was observed in all rabbits from the group. It actually peaked after one injection and could be further recalled (at similar levels than after the 1st injection) following a third injection of the combination of adenovirus/protein/adjuvant, suggesting that the first two injections did not induce a neutralizing response that would inhibit any response to a further similar injection. In its intensity, the proliferative response observed in rabbits immunised with the combination of adenovirus/protein/adjuvant was comparable to the one observed in animals immunised once or twice with 10^{12} viral particles of adenovirus and appeared higher than the one from animals immunised once or twice with 10^{10} viral particles of adenovirus. Altogether, this suggests that using the combination of adenovirus/protein/adjuvant could decrease the dose of adenovirus to be used. Finally, after a third injection of the combination of adenovirus/protein/adjuvant, the response observed in group 4 was similar to the one from animals immunised 3 times with the protein (group 1).

Serology:

As shown in Figure 11, the kinetic of the anti-F4 antibody response observed in the animals immunised twice with the combination of adenovirus/protein/adjuvant is similar to the one from animals immunised twice with the protein: it is already detected at 7 days post-2nd injection and then decrease over time. However, in terms of intensity, the anti-F4 response of animals immunised twice with the combination of adenovirus/protein/adjuvant remains higher at later timepoints (21 and 63 days post-2nd immunisation) when compared to the anti-F4 response from animals immunised twice with the protein. No anti-F4 antibody response is observed in rabbits immunised once with 10^{10} viral particles of adenovirus. In rabbits immunised once with 10^{12} viral particles of adenovirus, an anti-F4 response is only detected at 21 and 63 days post-immunisation. In that group, the high variability of the response observed at the 63 day post-immunisation timepoint (d77) results from a single animal (out of the 3) displaying higher titers against the different F4 components, especially p24 and RT as shown in Figures 12a and 12b respectively. The anti-F4 antibody response is mainly composed of antibodies targeting p24 and RT and to a much lesser extent Nef and p17.

Conclusion:

Lymphoproliferative and antibody responses could be induced in rabbits after two injections of a composition containing an immunogenic polypeptide, an adenoviral vector containing a heterologous polynucleotide encoding an immunogenic polypeptide and an adjuvant. In

addition, we have evidence that a lymphoproliferative response can be recalled after a third injection of such composition. Finally, the best antibody response (in intensity and persistence) is observed with the adenovirus/protein/adjuvant combination.

5 **Example 4**

Immunogenicity of F4 (codon optimized)/adjuvant B and C7-GRN when administrated as a combination in CB6F1 mice.

10 **Experimental design**

CB6F1 mice were immunized twice (days 0 and 21) with different combinations listed below. F4co/ adjuvant B was used at 9µg F4co/animal in 50µl AdjuvantB (1/10 human dose) and the C7-GRN virus at 10⁸ viral particles/animal. F4co in Example 4 is F4 prepared as described in
 15 WO2006/013106 Example 1, codon-optimised method.

Combinations

20 C7-GRN
 C7-GRN/ adjuvant B
 C7-GRN/F4co
 C7-GRN/F4co/ adjuvant B
 F4co
 F4co/ adjuvant B
 25 adjuvant B
 C7 empty
 C7empty/ adjuvant B
 C7empty/F4co
 C7empty/F4co/ adjuvant B

30

Schedule of immunizations and immune response analysis

Immunisations were carried out at day 0 and day 21. Intracellular cytokine staining (ICS) was carried out at 21 days, 28 days (7 days post immunisation 2), 42 days (21 days post
 35 immunisation 2), and 77 days (56 days post immunisation 2).

Results

HIV-specific CD4 T cell responses

5 The results are shown in the following figures:

Figure 13. Quantification of HIV-1-specific CD4 T cells. The % of CD3 CD4 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at four time-points. Peripheral blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then
10 overnight) with a pool of peptides covering F4 sequence and the cytokine production was measured by ICS. Each value is the geometric mean of 5 pools of 3 mice.

Figure 14. Distribution of the frequency of F4-specific CD4 T cells 7 days after two immunizations. The frequency of F4-specific circulating CD4 T cells at 7 days after two
15 immunizations is represented for each protocol. Each dot represents the value obtained for one pool of 3 mice.

Figure 15. Cytokine production of F4-specific CD4 T cells 7 days after two immunizations. The % of F4-specific CD4 T cells secreting IL-2 and/or IFN- γ is represented for 5 pools of 3 mice.
20 Results for the immunization with F4co/ adjuvant B (A), F4co/ adjuvant B /C7 empty (B) and F4co/ adjuvant B /C7-GRN (C) are presented.

The frequency of F4-specific circulating CD4 T cells reaches 2.82% 21 days after two immunizations with the F4co/ adjuvant B combination and declines to 0.91% 56 days post-immunization (Figure 13). Two doses of the C7-GRN virus alone result in 0.52% of F4-specific
25 circulating CD4 T cells 21 days post last immunization and the presence of the adjuvant adjuvant B does not alter this response.

The presence of the empty vector C7 or the recombinant C7-GRN virus in addition of the F4co/ adjuvant B mix does not increase nor interfere with the frequency of F4-specific CD4 T cell
30 response (3.58% and 2.82% respectively, 21 days post-last immunization). Even if no statistical analysis has been performed, the population distribution suggests that the intensity of the F4-specific CD4 T cell responses is not different between the three protocols F4co/ adjuvant B, F4co/ adjuvant B /C7 empty and F4co/ adjuvant B /C7-GRN (Figure 14).

35 As expected, administration of the F4co without adjuvant B does not induce significant F4-specific CD4 T cells.

The profile of cytokine production shows that after immunization with F4co/ adjuvant B, the F4-specific CD4 T cells secrete both IFN- γ and IL-2. Addition of C7empty or C7-GRN in the immunization protocol does not alter this profile.

- 5 As a result, these data suggest that the greatest F4-specific CD4 T cell response is obtained after immunization with the F4co/ adjuvant B combination and that the presence of the C7-GRN virus does not improve nor alter this response.

Antigen-specific CD8 T cell responses

10

The results are shown in the following figures

- 15 Figure 16. Quantification of HIV-1-specific CD8 T cells. The % of CD3 CD8 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at four time-points. Peripheral blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of Brefeldin then overnight) with a pool of peptides covering F4 and the cytokine production was measured by ICS. Each value is the geometric mean of 5 pools of 3 mice.

- 20 Figure 17. Cytokine production of F4-specific CD8 T cells 7 days after two immunizations. The % of F4-specific CD8 T cells secreting IL-2 and/or IFN- γ is represented for 5 pools of 3 mice. Results for the immunization with C7-GRN (**A**), C7-GRN/ adjuvant B (**B**) and C7-GRN+F4co/ adjuvant B (**C**) are presented.

- 25 After one injection, the recombinant vector C7-GRN induces a high frequency of F4-specific circulating CD8 T cells (9,70% of total CD8 T cells, 21 days post-immunization) (Figure 4). A second injection does not boost the F4-specific CD8 T cell response. The F4co/ adjuvant B combination induces low to undetectable F4-specific CD8 T cells and adding this combination to the C7-GRN does not improve or impair the F4-specific CD8 T cell response.

- 30 The F4-specific CD8 T cell response is delayed when the adjuvant B is added to the C7-GRN, but reaches the same level as with the C7-GRN alone or the C7-GRN/F4co/ adjuvant B combination at 21 days post-second immunization.

- 35 The F4-specific CD8 T cells mainly secrete IFN- γ whether the C7-GRN vector is injected alone or in combination with F4co/ adjuvant B (Figure 17).

Interestingly, the F4-specific CD8 T cell response persists up to 56 days post-last immunization without declining, suggesting that the C7 vector elicits high and persistent CD8 T cells.

Conclusions

- 5 The F4co/adjuvant B vaccine induces a high frequency of poly-functional HIV-specific CD4 T cells but no HIV-specific CD8 T cells in CB6F1 mice. In the same animal model, the recombinant adenovirus C7 expressing Gag, RT and Nef (Ad C7-GRN) induces a high antigen-specific CD8 T cell response and low to undetectable antigen-specific CD4 T cells. A combination of F4/ adjuvant B and Ad C7-GRN elicits both antigen-specific CD4 and CD8 T
10 cells at the same time. A combination of three components, F4co, adjuvantB and C7-GRN elicits the highest levels of both antigen specific CD4 and CD8 T cells at the same time. Combining F4/ adjuvant B and Ad C7-GRN has an additive effect concerning the intensity of both arms of the cellular immune response. The effect of the antigen-specific CD4 T cell response on the functionality of antigen-specific CD8 T cell response remains to be determined
15 in this model.

Example 5

- 20 **Immunogenicity of the chimpadenovirus C7 expressing CS2 construct of CSP protein from *Plasmodium falciparum* (C7-CS2) when administered alone**

Experimental design:

- CB6F1 mice were immunized once intramuscularly with a dose range (10^{10} , 10^9 & 10^8 viral
25 particles) of the C7 chimpadenovirus expressing the CSP malaria antigen and the CSP-specific (C-term and N-term) CD4 and CD8 T cell responses were determined 21, 28 and 35 days post-injection by ICS (Intra-cellular Cytokine Staining).

CSP-specific CD4 T cell responses

30

The results are shown in the following figures:

- Figure 18. Quantification of CSP-specific CD4 T cells. The % of CD4 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at three time-points. Peripheral
35 blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then overnight) with a pool of peptides covering CSP N-term or CSP C-term sequences and the cytokine production was measured by ICS. The responses to the C-term and N-term peptide pools were added up and each value is the average of 5 pools of 4 mice.

Figure 19. Quantification of CSP-specific CD8 T cells. The % of CD8 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at three time-points. Peripheral blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then overnight) with a pool of peptides covering CSP N-term or CSP C-term sequences and the cytokine production was measured by ICS. The responses to the C-term and N-term peptide pools were added up and each value is the average of 5 pools of 4 mice.

These results indicate that both 10^{10} and 10^9 doses of C7-CS2 elicit similar levels of CSP-specific CD4 T cell responses (peak 0.5%) and similar levels of CSP-specific CD8 T cell responses (peak 8%). The dose of 10^{10} of C7-CS2 was chosen in subsequent experiments where the immunogenicity of C7-CS2 in combination with RTS,S was tested (see below).

Example 6

Immunogenicity of C7-CS2 and RTS,S when administered as a combination in CB6F1 mice

Experimental design:

CB6F1 mice were immunized three times intramuscularly (day 0, 14 & 28) with either a combination of the malaria vaccine candidate RTS,S (5 μ g) in 50 μ l of Adjuvant B (referred as P-P in the figures below) or a combination of RTS,S (5 μ g) and C7-CS2(10^{10} viral particles) in 50 μ l of Adjuvant B (referred as C-C-C in the figures below). The CSP-specific (C-term and N-term) CD4 and CD8 T cell responses were determined at the following time-points:

- 7 days post 2 immunizations

- 7, 21, 35 and 49 days post 3 immunizations

CSP-specific T cell responses were determined by ICS (Intra-cellular Cytokine Staining).

The CSP-specific antibody responses in the sera from immunized animals were also determined by ELISA at 14 and 42 days post- 3rd immunization.

CSP-specific CD4 T cell responses

The results are shown in the following figures:

Figure 20. Quantification of CSP(N-term)-specific CD4 T cells. The % of CD4 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at five time-points. Peripheral

blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then overnight) with a pool of peptides covering the CSP N-term sequence and the cytokine production (IFN γ and/or IL-2) was measured by ICS. Each value is the average of 4 pools of 7 mice.

5

Figure 21. Quantification of CSP(C-term)-specific CD4 T cells. The % of CD4 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at five time-points. Peripheral blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then overnight) with a pool of peptides covering the CSP C-term sequence and the cytokine production (IFN γ and/or IL-2) was measured by ICS. Each value is the average of 4 pools of 7 mice.

10

These results indicate that mice immunized with 3 injections of the combination [RTS,S + C7-CS2 10^{10} + Adjuvant B] display higher antigen-specific CD4 T cell responses (both against the C-term and N-term part of CSP) than the mice immunized with 3 injections of RTS,S+Adjuvant B.

15

CSP-specific CD8 T cell responses

20

The results are shown in the following figures:

Figure 22. Quantification of CSP(N-term)-specific CD8 T cells. The % of CD8 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at five time-points. Peripheral blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then overnight) with a pool of peptides covering the CSP N-term sequence and the cytokine production (IFN γ and/or IL-2) was measured by ICS. Each value is the average of 4 pools of 7 mice.

25

Figure 23. Quantification of CSP(C-term)-specific CD8 T cells. The % of CD8 T cells secreting IFN- γ and/or IL-2 is represented for each protocol of immunization at five time-points. Peripheral blood lymphocytes (PBLs) were stimulated *ex vivo* (2 hours before addition of the Brefeldin then overnight) with a pool of peptides covering the CSP C-term sequence and the cytokine production (IFN γ and/or IL-2) was measured by ICS. Each value is the average of 4 pools of 7 mice.

30

35

These results indicate that mice immunized with 3 injections of the combination [RTS,S + C7-CS2 10^{10} + Adjuvant B] display higher antigen-specific CD8 T cell responses (both against the

C-term and N-term part of CSP) than the mice immunized with 3 injections of RTS,S+Adjuvant B.

CSP-specific antibody responses

5

The results are shown in the following figure:

Figure 24. Quantification of CSP-specific antibody titers. The sera from the mice were collected at 14 and 42 days post 3rd immunization. The anti-CSP antibody titers were measured in each of these individual sera by ELISA. The data shown is the geometric mean antibody titers $\pm$ 95% confidence interval.

10

These results indicate that mice immunized with 3 injections of the combination [RTS,S + C7-CS2 10^{10} + Adjuvant B] display similar CSP-specific antibody titers than the mice immunized with 3 injections of RTS,S+Adjuvant B.

15

Conclusions

The RTS,S/adjuvant B vaccine induces a high frequency of CSP C-term-specific CD4 T cells but no CSP N-term specific CD4 T cells. In addition, the RTS,S/adjuvant B vaccine induces low to undetectable CSP C& N-term specific CD8 T cells. In the same animal model, the recombinant adenovirus C7 expressing CSP induces high CSP(C-term and N-term)-specific CD8 T cell responses and lower CSP(C-term and N-term)-specific CD4 T cell responses. A combination of RTS,S/ adjuvant B and Ad C7-CS2 elicits high levels of both CSP(C-term and N-term)-specific CD4 and CD8 T cells at the same time. Combining RTS,S/ adjuvant B and Ad C7-CS2 has an additive effect concerning the intensity of both arms of the T cell response. Finally, the combination of RTS,S/ adjuvant B and Ad C7-CS2 elicits high levels of CSP-specific antibody responses that are comparable to the ones induced by RTS,S/adjuvant B.

25

30

SEQUENCES

SEQ ID No 1:

	1	atgggtgccc	gagcttcggt	actgtctggt	ggagagctgg	acagatggga
5	51	gaaaattagg	ctgcgcccgg	gaggcaaaaa	gaaatacaag	ctcaagcata
	101	tcgtgtgggc	ctcgagggag	cttgaacggt	ttgccgtgaa	cccaggcctg
	151	ctggaaacat	ctgagggatg	tcgccagatc	ctggggcaat	tgacgccatc
	201	cctccagacc	gggagtgaag	agctgaggtc	cttgataaac	acagtggcta
	251	ccctctactg	cgtacaccag	aggatcgaga	ttaaggatac	caaggaggcc
10	301	ttggacaaaa	ttgaggagga	gcaaaacaag	agcaagaaga	aggcccagca
	351	ggcagctgct	gacactgggc	atagcaacca	ggtatcacag	aactatccta
	401	ttgtccaaaa	cattcagggc	cagatggttc	atcaggccat	cagcccccg
	451	acgtcctaatg	cctgggtgaa	ggttgcgaa	gagaaggcct	tttctcctga
	501	ggttatcccc	atgttctccg	ctttgagtga	ggggggccact	cctcaggacc
15	551	tcaatacaat	gcttaatacc	gtgggcggcc	atcaggccgc	catgcaaatg
	601	ttgaaggaga	ctatcaacga	ggaggcagcc	gagtgaggaca	gagtgcattc
	651	cgtccacgct	ggcccacatc	cgcccggaca	gatgcgggag	cctcgcggct
	701	ctgacattgc	cggcaccacc	tctacactgc	aagagcaaat	cggatggatg
	751	accaacaatc	ctcccattcc	agttggagaa	atctataaac	ggtggatcat
20	801	cctgggcctg	aacaagatcg	tcgcgatgta	ctctccgaca	tccatccttg
	851	acattagaca	gggacccaaa	gagcctttta	gggattacgt	cgaccggttt
	901	tataagaccc	tgcgagcaga	gcaggcctct	caggaggtca	aaaactggat
	951	gacggagaca	ctcctggtac	agaacgctaa	ccccgactgc	aaaacaatct
	1001	tgaaggcact	aggcccggct	gccaccctgg	aagagatgat	gaccgcctgt
25	1051	cagggagtag	gcggacccgg	acacaaagcc	agagtgttga	tgggccccat
	1101	cagtcccatc	gagaccgtgc	cgtgtaagct	gaaacccggg	atggacggcc
	1151	ccaaggtcaa	gcagtggcca	ctcaccgagg	agaagatcaa	ggcctggtg
	1201	gagatctgca	ccgagatgga	gaaagagggc	aagatcagca	agatcgggcc
	1251	ggagaaccca	tacaacaccc	ccgtgtttgc	catcaagaag	aaggacagca
30	1301	ccaagtggcg	caagctgggtg	gatttccggg	agctgaataa	gcggacccag
	1351	gatttctggg	aggtccagct	gggcatcccc	catccggccg	gcctgaagaa
	1401	gaagaagagc	gtgaccgtgc	tggacgtggg	cgacgcttac	ttcagcgtcc
	1451	ctctggacga	ggacttttaga	aagtacaccg	cctttaccat	cccatctatc
	1501	aacaacgaga	cccctggcat	cagatatcag	tacaacgtcc	tccccagggg
35	1551	ctggaagggc	tctcccgcga	ttttccagag	ctccatgacc	aagatcctgg
	1601	agccgtttcg	gaagcagaac	cccgatatcg	tcattctacca	gtacatggac
	1651	gacctgtacg	tgggctctga	cctggaaatc	gggcagcatc	gcacgaagat
	1701	tgaggagctg	aggcagcatc	tgctgagatg	gggcctgacc	actccggaca
	1751	agaagacatc	gaaggagccg	ccattcctga	agatggggcta	cgagctccat
40	1801	cccgacaagt	ggaccgtgca	gcctatcgtc	ctccccgaga	aggacagctg
	1851	gaccgtgaac	gacatccaga	agctgggtggg	caagctcaac	tgggctagcc
	1901	agatctatcc	cgggatcaag	gtgcgccagc	tctgcaagct	gctgcgcggc
	1951	accaaggccc	tgaccgaggt	gattcccctc	acggaggaag	ccgagctcga
	2001	gctggctgag	aaccgggaga	tccctgaagg	gcccgtgcac	ggcgtgtact
45	2051	atgaccctc	caaggacctg	atcgccgaaa	tccagaagca	gggccagggg
	2101	cagtggacat	accagattta	ccaggagcct	ttcaagaacc	tcaagaccgg
	2151	caagtacgcc	cgcatgaggg	gcgcccacac	caacgatgtc	aagcagctga
	2201	ccgaggccgt	ccagaagatc	acgaccgagt	ccatcgtgat	ctgggggaaag
	2251	acacccaagt	tcaagctgcc	tatccagaag	gagacctggg	agacgtgggtg
50	2301	gaccgaatat	tggcaggcca	cctggattcc	cgagtgggag	ttcgtgaata
	2351	cacctcctct	ggtgaagctg	tggtagcagc	tcgagaagga	gcccacgtg
	2401	ggcgcgagga	cattctacgt	ggacggcgcg	gccaaccgcg	aaacaaagct
	2451	cgggaaggcc	gggtacgtca	ccaaccgggg	ccgccagaag	gtcgtcaccc
	2501	tgaccgacac	caccaaccag	aagacggagc	tgaggcccat	ctatctcgct
55	2551	ctccaggact	ccggcctgga	ggtgaacatc	gtgacggaca	gccagtacgc
	2601	gctgggcatt	attcaggccc	agccggacca	gtccgagagc	gaactggtga
	2651	gctgggcatt	cgagcagctg	atcaagaaaag	agaaggctca	cctcgcttgg
	2701	gtcccggccc	ataagggtcat	tggcggcaac	gagcaggtcg	acaagctgggt
	2751	gagtgcgggg	attagaaagg	tgctgatggg	gggttttcca	gtcacacctc
60	2801	aggtaccttt	aagaccaatg	acttacaagg	cagctgtaga	tcttagccac
	2851	tttttaaaag	aaaagggggg	actggaaggg	ctaattcact	cccaaagaag

5	2901	acaagatatac	cttgatctgt	ggatctacca	cacacaaggc	tacttccttg
	2951	attggcagaa	ctacacacca	gggccagggg	tcagatatcc	actgaccttt
	3001	ggatggtgct	acaagctagt	accagttgag	ccagataagg	tagaagaggc
	3051	caataaagga	gagaacacca	gcttggtaca	ccctgtgagc	ctgcatggga
	3101	tggatgaccc	ggagagagaa	gtgttagagt	ggaggtttga	cagccgccta
	3151	gcatttcatac	acgtggcccg	agagctgcat	cgggagtact	tcaagaactg
	3201	ctga				
10	SEQ ID No 2:					
	1	MGARASVLSG	GELDRWEKIR	LRPGGKKKYK	LKHIVWASRE	LERFAVNPGL
	51	LETSEGCRIQI	LGQLQPSLQT	GSEELRSLYN	TVATLYCVHQ	RIEIKDTKEA
	101	LDKIBEEQNK	SKKKAQQAAA	DTGHSNQVSQ	NYPIVQNIQG	QMVHQAIQSPR
15	151	TLNAWVKVVE	EKAFSPEVIP	MFSALSEGAT	PQDLNMTLNT	VGGHQAAMQM
	201	LKETINEEAA	EWDRVHPVHA	GPIAPGQMRE	PRGSDIAGTT	STLQEQIGWM
	251	TNNPPIPVGE	IYKRWIILGL	NKIVRMYSP	SILDIRQGP	EPFRDYVDRF
	301	YKTLRAEQAS	QEVKNWMTET	LLVQNPANPDC	KTILKALGPA	ATLEEMMTAC
	351	QGVGGPGHKA	RVLMPISPI	ETVPVKLKPG	MDGPKVKQWP	LTEEKIKALV
	401	EICTEMEKEG	KISKIGPENP	YNTPVFAIKK	KDSTKWRKLV	DFRELNKRTQ
20	451	DFWEVQLGIP	HPAGLKKKKS	VTVLDVGDAY	FSVPLDEDFR	KYTAFTIPSI
	501	NNETPGIRYQ	YNVLPQGWKG	SPAIFQSSMT	KILEPFRKQN	PDIVIYQYMD
	551	DLYVGSLEI	GQHRTKIEEL	RQHLLRWGLT	TPDKKHQKEP	PFLKMGYELH
	601	PDKWTVQPIV	LPEKDSWTVN	DIQKLVGKLN	WASQIYPGIK	VRQLCKLLRG
25	651	TKALTEVIPL	TEEALELAE	NREILKEPVH	GVYYDPSKDL	IAEIQKQGGG
	701	QWTYQIYQEP	FKNLKTGKYA	RMRGAHTNDV	KQLTEAVQKI	TTESIVIWGK
	751	TPKFKLPIQK	ETWETWWTEY	WQATWIPEWE	FVNTPLVLKL	WYQLEKEPIV
	801	GAETFYVDGA	ANRETKLGKA	GYVTNRGRQK	VVTLTDTTNQ	KTELQAIYLA
	851	LQDSGLEVNI	VTDSQYALGI	IQAQPDQSES	ELVNQIIEQL	IKKEKVYLA
	901	VPAHKGIGGN	EQVDKLVSA	IRKVLVGF	VTPQVPLRPM	TYKAAVDLSH
30	951	FLKEKGGLEG	LIHSQRRQDI	LDLWIYHTQG	YFPDWQNYTP	GPGVRYPLTF
	1001	GCYKLVPE	PDKVEEANKG	ENTSLHPVS	LHGMDPERE	VLEWRFSRL
	1051	AFHHVARELH	PEYFKNC			
35	SEQ ID No 3:					
	1	atggccgcca	gagccagcat	cctgagcggg	ggcaagctgg	acgcctggga
	51	gaagatcaga	ctgaggcctg	gcggcaagaa	gaagtaccgg	ctgaagcacc
	101	tgggtgtggg	cagcagagag	ctggatcgct	tcgccctgaa	tcctagcctg
40	151	ctggagacca	ccgagggctg	ccagcagatc	atgaaccagc	tgcagccgc
	201	cgtgaaaaacc	ggcaccgagg	agatcaagag	cctgttcaac	accgtggcca
	251	ccctgtactg	cgtgcaccag	cggatcgacg	tgaaggatac	caaggaggcc
	301	ctggacaaga	tcgaggagat	ccagaacaag	agcaagcaga	aaaccagca
	351	ggccgctgcc	gacaccggcg	acagcagcaa	agtgagccag	aactaccca
45	401	tcattccagaa	tgcccagggc	cagatgatcc	accagaacct	gagccccaga
	451	accctgaatg	cctgggtgaa	agtgatcgag	gaaaaggcct	tcagccccga
	501	agtgatecct	atgttcagcg	ccctgagcga	gggcgccacc	ccccaggacc
	551	tgaacgtgat	gctgaacatt	gtgggcggac	accaggccgc	catgcagatg
	601	ctgaaggaca	ccatcaatga	ggaggccgcc	gagtgggaca	gactgcaccc
	651	cgtgcaggcc	ggacccatcc	cccctggcca	gatcagagag	cccagaggca
50	701	gcgacatcgc	cggcaccacc	tccaccctc	aagaacagct	gcagtggatg
	751	accggcaacc	ctcccatccc	tgtgggcaac	atctacaagc	ggtggatcat
	801	cctgggcctg	aacaagattg	tgcggtatga	cagccccctg	tccatcctgg
	851	atatcaagca	gggccccaa	gagcccttca	gagactacgt	ggaccggttc
55	901	ttcaaggccc	tgagagccga	gcaggccacc	caggacgtga	agggctggat
	951	gaccgagacc	ctgctggtgc	agaacgcca	ccccgactgc	aagagcatcc
	1001	tgaaggccct	gggcagcggc	gccacactgg	aggagatgat	gaccgcctgc
	1051	cagggagtg	gcggaccgg	ccacaaggcc	agagtgtg	ccgaggccat
	1101	gagccaggcc	cagcagacca	acatcatgat	gcagcggggc	aacttcagag
	1151	gccagaagcg	gatcaagtgc	ttcaactgcg	gcaaggagg	ccacctggcc
60	1201	agaaactgca	gagccccag	gaagaagggc	tgctggaagt	gtggcaagga
	1251	agggcaccag	atgaaggact	gcaccgagag	gcaggccaat	ttcctgggca

	1301	agattttggcc	tagcagcaag	ggcagacccg	gcaattttccc	ccagagcaga
	1351	cccagagccca	ccgcccctcc	cgccgagctg	ttcggcatgg	gcgagggcat
	1401	cgccagcctg	cccaagcagg	agcagaagga	cagagagcag	gtgccccccc
5	1451	tggtgtccct	gaagtccctg	ttcggcaacg	atcctctgag	ccagggatcc
	1501	cccatcagcc	ccatcgagac	cgtgcccgtg	accctgaagc	ccggcatgga
	1551	tggcccca	gtgaaacagt	ggcccctgac	cgaggagaag	attaaggccc
	1601	tgaccgaaat	ctgtaccgag	atggagaagg	agggcaagat	cagcaagatc
	1651	ggccccgaga	acccctacaa	cacccccatc	ttcgccatca	agaagaagga
10	1701	cagcaccaag	tggcggaac	tggtggactt	ccgggagctg	aacaagagga
	1751	cccaggactt	ctgggaagtg	cagctgggca	tccccaccc	tgccggcctg
	1801	aagaagaaga	agtccgtgac	agtgtggat	gtgggagacg	cctacttcag
	1851	cgtgcccctg	gacgagaact	tcaggaagta	caccgccttc	accatcccc
	1901	gcaccaacaa	cgagaccccc	ggagtgaagat	accagtacaa	cgtgctgcct
	1951	cagggtcggg	agggcagccc	cgccatcttc	cagagcagca	tgaccaagat
15	2001	cctggagccc	ttccggagca	agaaccccga	gatcatcatc	taccagtaca
	2051	tggccgccc	gtatgtgggc	agcgatctgg	agatcggcca	gcacaggacc
	2101	aagatcgaag	agctgagggc	ccacctgctg	agctggggct	tcaccacccc
	2151	cgataaaga	caccagaagg	agccccctt	cctgtggatg	ggctacgagc
20	2201	tgacccccga	taagtggacc	gtgcagccca	tcctgctgcc	cgataaggag
	2251	agctggaccg	tgaacgacat	ccagaaactg	gtgggcaagc	tgaattgggc
	2301	cagccaaatc	tacgccggca	ttaaagtga	gcagctgtgc	aggctgctga
	2351	gaggcgccaa	agccctgaca	gacatcgtga	cactgacaga	ggaggccgag
	2401	ctggagctgg	ccgagaacag	ggagatcctg	aaggacccc	tgacggcgt
	2451	gtactacgac	cccagcaagg	acctggtggc	cgagattcag	aagcagggcc
25	2501	aggaccagt	gacctaccaa	atctaccagg	agcctttcaa	gaacctgaaa
	2551	accgggaagt	acgccaggaa	gagaagcgcc	cacaccaacg	atgtgaggca
	2601	gctggccgaa	gtggtgcaga	aagtggctat	ggagagcatc	gtgatctggg
	2651	gcaagacccc	caagtccaag	ctgcccctcc	agaaggagac	ctgggaaacc
30	2701	tggtggactg	actactggca	ggccacctgg	attcctgagt	gggagttcgt
	2751	gaacaccccc	cctctggtga	agctgtggta	tcagctggag	aaggacccca
	2801	tcctgggcgc	cgagaccttc	tacgtggacg	gagccgcca	tagagagacc
	2851	aagctgggca	aggccggcta	cgtgaccgac	agaggcagac	agaaagtggg
	2901	gtctctgacc	gagacaacca	accagaaaac	cgagctgcac	gccatcctgc
	2951	tggccctgca	ggacagcgcc	agcgaagtga	acatcgtgac	cgactcccag
35	3001	tacgccctgg	gcatcattca	ggcccagccc	gatagaagcg	agagcgagct
	3051	ggtgaaccag	atcatcgaga	agctgatcgg	caaggacaaa	atctacctga
	3101	gctgggtgcc	cgcccacaa	ggcatcggcg	gcaacgagca	ggtggacaa
	3151	ctggtgtcca	gcggcatccg	gaaagtgtcg	tttctggacg	gcatcgacaa
40	3201	ggcccaggag	gaccacgaga	gataccacag	caactggcgg	acaatggcca
	3251	gcgacttcaa	cctgcctccc	atcgtggcca	aggagatcgt	ggccagctgc
	3301	gataagtgtc	agctgaagg	cgaggccatg	cacggccagg	tggactgcag
	3351	ccctggcatc	tggcagctgg	cctgcaccca	cctggagggc	aaagtgattc
	3401	tggtggccgt	gcacgtggcc	agcggctaca	tcgaggccga	agtgattccc
45	3451	gccgagaccg	gccaggagac	cgccctacttc	ctgctgaagc	tggccggcag
	3501	atggcccgtg	aaagtgggtc	acaccgcca	cggcagcaac	ttcacctctg
	3551	ccgccgtgaa	ggccgcctgt	tggtgggcca	atatccagca	ggagttcggc
	3601	atccccctaca	acctcagag	ccagggcgtg	gtggccagca	tgaacaagga
	3651	gctgaagaag	atcatcggcc	aggtgagggg	ccaggccgag	cacctgaaaa
50	3701	cagccgtgca	gatggccgtg	ttcatccaca	acttcaagcg	gaaggcgggc
	3751	attggcggt	acagcgccgg	agagcggtatc	atcgacatca	tcgccaccga
	3801	tatccagacc	aaggaactgc	agaagcagat	caccaagatt	cagaacttca
	3851	gagtgtacta	ccgggacagc	agggacccca	tctggaagg	ccctgccaag
	3901	ctgctgtgga	agggcggaag	cgccgtgggtg	atccaggaca	acagcgacat
55	3951	caaagtgggtg	ccccggagga	aggccaagat	tctgcgggac	tacggcaaac
	4001	agatggccgg	cgatgactgc	gtggccggca	ggcaggatga	ggacagatct
	4051	atggggcgga	agtgggtccaa	gggcagcatt	gtgggctggc	ccgagatccg
	4101	ggagagaatg	agaagagccc	ctgcgcgcgc	tcctggagtg	ggcgccgtgt
	4151	ctcaggatct	ggataagcac	ggcgccatca	ccagcagcaa	catcaacaac
	4201	cccagctgtg	tgtggctgga	ggcccaggaa	gaggaggaag	tgggcttccc
60	4251	tgtgagaccc	caggtgcccc	tgagacccat	gacctacaag	ggcgcccttcg
	4301	acctgagcca	cttcctgaag	gagaagggcg	gcctggacgg	cctgatctac

51

5	4351	agccggaagc	ggcaggagat	cctggatctg	tgggtgtacc	acaccaggg
	4401	ctacttcccc	gactggcaga	attacacccc	tggccctgga	gtgcggtatc
	4451	ccctgacctt	cggctggtgc	ttcaagctgg	tgcctatgga	gcccagacgaa
	4501	gtggagaagg	ccacagaggg	cgagaacaac	agcctgctgc	accctatctg
	4551	ccagcacggc	atggacgatg	aggagcggga	agtgctgatc	tgggaagtctg
	4601	acagcaggct	ggccctgaag	cacagagccc	aggaactgca	cccagagttc
	4651	tacaaggact	gctga			
10	SEQ ID No 4:					
	1	MAARASILSG	GKLDWEKIR	LRPGGKKKYR	LKHLVWASRE	LDRFALNPSL
	51	LETTEGCQOI	MNQLQPAVKT	GTEEIKSLFN	TVATLYCVHQ	RIDVKDTKEA
	101	LDKIBEIQNK	SKQKTQAAA	DTGDSSKVSQ	NYPIIQNAQG	QMIHQNLSPR
	151	TLNAWVKVIE	EKAFSPEVIP	MFSALSEGAT	PQDLNVMLNI	VGGHQAAMQM
15	201	LKDTINEEAA	EWDRLHPVQA	GPIPPGQIRE	PRGSDIAGTT	STPQEQQLQWM
	251	TGNPPIPVGN	IYKRWIILGL	NKIVRMYSVP	SILDIKQGP	EPFRDYVDRF
	301	FKALRAEQAT	QDVKGWMTET	LLVQANANPDC	KSILKALGSG	ATLEEMMTAC
	351	QGVGGPGHKA	RVLAEAMSQA	QQTNIMMQRG	NFRGQKRIKC	FNCKKEGHLA
	401	RNCRAPRKKK	CWCKGKEGHQ	MKDCITERQAN	FLGKIWPSSK	GRPGNFPQSR
20	451	PEPTAPPAEL	FGMGEIASL	PKQEQKDREQ	VPPLVSLKSL	FGNDPLSQGS
	501	PISPIETVPV	TLKPGMDGPK	VKQWPLTEEK	IKALTEICTE	MEKEGKISKI
	551	GPENPYNTPI	FAIKKKDSTK	WRKLVDREL	NKRTQDFWEV	QLGIPHPAGL
	601	KKKKSVTVLD	VGDAYFSVPL	DENFRKYTAF	TIPSTNNETP	GVMRYQNVLP
	651	QGWKGSPAIF	QSSMTKILEP	FRSKNPEIII	YQYMAALYVG	SDLEIGQHRT
25	701	KIEELRAHLL	SWGFTTPDKK	HQKEPPFLWM	GYELHPDKWT	VQPIMLPDKE
	751	SWTVNDIQKL	VGKLNWASQI	YAGIKVKQLC	RLLRGAKALT	DIVTLTEEAE
	801	LELAENREIL	KDPVHGVYYD	PSKDLVAEIQ	KQGQDQWTYQ	IYQEPFKNLK
	851	TGKYARKRSA	HTNDVRQLAE	VVQKVAMESI	VIWGTTPKFK	LPIQKETWET
	901	WWMYDYOATW	IPWEFVNTP	PLVKLWYQLE	KDPILGAETF	YVDGAANRET
30	951	KLKAGYVTD	RGRQKVSLT	ETTNQKTELH	AILLALQDSG	SEVNIIVTDSQ
	1001	YALGIIQAQP	DRSESELVQ	IEKLIGKDK	IYLSWVPAHK	GIGGNEQVQDK
	1051	LVSSGIRKVL	FLDGIDKAQE	DHERYHSNWR	TMASDFNLPP	IVAKEIVASC
	1101	DKCQLKGEAM	HGQVDCSPGI	WQLACTHLEG	KVILVAVHVA	SGYIEAEVIP
	1151	AETGQETAYF	LLKLGRWPV	KVVHTANGSN	FTSAAVKAAC	WWANIQQEFQ
35	1201	IPYNPQSQGV	VASMNKELKK	IIGQVRDQAE	HLKTAVQMAV	FIHNFKRKGG
	1251	IGGYSAGERI	IDIIATDIQT	KELQKQITKI	QNFRVYYRDS	RDPIWKGPAK
	1301	LLWKGEAVV	IQDNSDIKVV	PRRKAKILRD	YGKQMGAGDDC	VAGRQDEDRS
	1351	MGGKWSKSGI	VGWPEIRERM	RRAPAAAPGV	GAVSQDLKDH	GAITSSNINN
	1401	PCVWLEAQE	EEEVGFVVRP	QVPLRPMTYK	GAFDLSHFLK	EKGGLDGLIY
40	1451	SRKRQEILD	WVYHTQGYFP	DWQNYTPGPG	VRYPLTFGWC	FKLVPMPEDE
	1501	VEKATEGENN	SLLHPICQHG	MDDEEREVL	WKFDRLALK	HRAQELHPEF
	1551	YKDC				
45	SEQ ID No 5:					
	1	atgaggggtga	tggagatcca	gcggaactgc	cagcacctgc	tgagatgggg
	51	catcatgatc	ctgggcatga	ttatcatctg	cagcaccgcc	gacaacctgt
	101	gggtgaccgt	gtactacggc	gtgcctgtgt	ggagagatgc	cgagaccacc
	151	ctgtttctgcg	ccagcgacgc	caaggcctac	agcaccgaga	agcacaatgt
50	201	gtgggcccacc	cagcctgctg	tgccctaccga	tcccaaccct	caggagatcc
	251	ccctggacaa	cgtgaccgag	gagttcaaca	tgtggaagaa	caacatggtg
	301	gaccagatgc	acgaggacat	catcagcctg	tgggaccaga	gcctgaagcc
	351	ctgcgtgcag	ctgaccccc	tgtgcgtgac	cctgaactgc	agcaacgcca
	401	gagtgaaacgc	caccttcaac	tccaccgagg	acaggaggagg	catgaagaac
55	451	tgcagcttca	acatgaccac	cgagctgcgg	gataagaagc	agcaggtgta
	501	cagcctgttc	taccggctgg	acatcgagaa	gatcaacagc	agcaacaaca
	551	acagcgagta	ccggctggtg	aactgcaata	ccagcgccat	caccagggcc
	601	tgcccataagg	tgaccttcga	gcccatacccc	atccactact	gcgcccctgc
	651	cggtctcgcc	atcctgaagt	gcaacgacac	cgagttcaat	ggcaccggcc
60	701	cctgcaagaa	tgtgagcacc	gtgcagtgca	cccacggcat	caagcccgtg
	751	gtgtccaccc	agctgctgct	gaacggcagc	ctggccgaga	gagaagtgcg

5 801 gatcaggagc gagaacatcg ccaacaacgc caagaacatc atcgtgcagt
 851 tcgccagccc cgtgaagatc aactgcatcc ggcccaacaa caatacccgg
 901 aagagctaca gaatcggccc tggccagacc ttctacgcca ccgacattgt
 951 gggcgacatc agacaggccc actgcaacgt gtccaggacc gactggaaca
 1001 acaccctgag actggtggcc aaccagctgc ggaagtactt cagcaacaag
 1051 accatcatct tcaccaacag cagcggcgga gacctggaga tcaccaccca
 1101 cagcttcaat tgtggcgggc agttcttcta ctgcaacacc tccggcctgt
 1151 tcaatagcac ctggaccacc aacaacatgc aggagtccaa cgacaccagc
 1201 aacggcacca tcaccctgcc ctgccggatc aagcagatca tccggatgtg
 10 1251 gcagcgctg ggcaggcca tgtacgcccc tcccatcgag ggcgtgattc
 1301 gctgcgagag caacatcacc ggctgatcc tgaccagaga tggcggcaac
 1351 aacaattccg ccaacgagac cttcagacct ggcggcgagg atatccggga
 1401 caactggcgg agcgagctgt acaagtacaa ggtggtgaag atcgagcccc
 1451 tgggcgtggc ccccaccaga gccaaagaaa gagtgggtga gcgggagaag
 15 1501 agagccgtgg gcatcggcgc cgtgtttctg ggcttctctg gagccgcggc
 1551 atctacaatg ggagccgcca gcatcacctt gaccgtgcag gccagacagc
 1601 tgctgagcgg catcgtgcag cagcagagca atctgctgag agccatcgag
 1651 gccagcagc agctgctgaa gctgacagtg tggggcatca agcagctgca
 1701 gcccagggtg ctggccgtgg agagatacct gagggaccag cagctcctgg
 20 1751 gcatctgggg ctgcagcggc aagctgatct gcaccaccaa cgtgccctgg
 1801 aatagcagct ggagcaacaa gagctacgac gacatctggc agaacatgac
 1851 ctggctgcag tgggacaagg agatcagcaa ctacaccgac atcatctaca
 1901 gcctgatcga ggagagccag aaccagcagg agaagaacga gcaggatctg
 1951 ctggccctgg acaagtgggc caacctgtgg aactggttcg acatcagcaa
 25 2001 gtggctgtgg tacatcagat cttga

SEQ ID No 6:

30 1 MRVMEIQRNC QHLLRWGIMI LGMIIICSTA DNLWVTVYYG VPVWRDAETT
 51 LFCASDAKAY STEKHNWAT HACVPTDPNP QEIPLDNVTE EFNMWKNNMV
 101 DQMHEIDIISL WDQSLKPCVQ LTPLCVTLNC SNARVNATFN STEDREGMKN
 151 CSFNMTELR DKKQQVYSLF YRLDIEKINS SNNNSEYRLV NCNTSAITQA
 201 CPKVTFEPIP IHYCAPAGFA ILKCNDFEFN GTGPCKNVST VQCTHGIKPV
 251 VSTQLLLNGS LAEREVRIRS ENIANNKNI IVQFASPVKI NCIRPNNNTR
 35 301 KSYRIGPGQT FYATDIVGDI RQAHCNVSRT DWNNTLRLVA NQLRKYFSNK
 351 TIIFTNSSGG DLEITTHSFN CGGEFFYCNT SGLFNSTWTT NNMQESNDTS
 401 NGTITLPCRI KQIIRMWQRV GQAMYAPPIE GVIRCESNIT GLILTRDGGN
 451 NNSANETFRP GGGDIRDNWR SELYKYKVVK IEPLGVAFTR AKRRVVEREK
 501 RAVGIGAVFL GFLGAAGSTM GAASITLTVQ ARQLLSGIVQ QQSNNLLRAIE
 40 551 AQQQLLKLTV WGIKQLQARV LAVERYLRDQ QLLGIWGCSG KLICTTNVPW
 601 NSSWSNKSYS DIWQNMWLQ WDKEISNYTD IIYSLIEESQ NQOEKNEQDL
 651 LALDKWANLW NWFDISKWLW YIRS

45 SEQ ID No 7:

atgaaagtga aggagaccag gaagaattat cagcacttgt ggagatgggg 50
 caccatgctc cttgggatgt tgatgatctg tagtgctgca gaacaattgt 100
 gggtcacagt ctattatggg gtacctgtgt ggaaagaagc aactaccact 150
 ctattctgtg catcagatgc taaagcatat gatacagagg tacataatgt 200
 50 ttgggccaca catgcctgtg taccacaga ccccaaccca caagaagtag 250
 tattgggaaa tgtgacagaa tattttaaca tgtggaaaaa taacatggta 300
 gaccagatgc atgaggatat aatcagttta tgggatcaaa gcttgaagcc 350
 atgtgtaaaa ttaaccccac tctgtgttac tttagattgc gatgatgtga 400
 ataccactaa tagtactact accactagta atggttggac aggagaaata 450
 55 aggaaaggag aaataaaaaa ctgctctttt aatatcacca caagcataag 500
 agataagggt caaaaagaat atgcactttt ttataacctt gatgtagtag 550
 caatagatga tgataatgct actaccaaaa ataaaactac tagaaacttt 600
 aggttgatac attgtaactc ctcagtcagt acacaggcct gtccaaagggt 650
 atcatttgaa ccaattccca tacattattg tgccccggct ggttttgaga 700
 60 ttctgaagtg taacaataag acgtttgatg gaaaaggact atgtacaaat 750
 gtcagcacag tacaatgtac acatggaatt aggccagtag tgtcaactca 800

53

	actgctgtta	aatggcagtc	tagcagaaga	agaggtagta	attagatctg	850
	acaattttcat	ggacaatact	aaaaccataa	tagtacagct	gaatgaatct	900
	gtagcaatta	attgtacaag	acccaacaac	aatacaagaa	aaggatataca	950
	tataggacca	gggagagcct	tttatgcagc	aagaaaaata	ataggagata	1000
5	taagacaagc	acattgtaac	cttagtagag	cacaatggaa	taacacttta	1050
	aaacagatag	ttataaaatt	aagagaacac	tttgggaata	aaacaataaa	1100
	atttaaatcaa	tcctcaggag	gggacccaga	aattgtaagg	catagtttta	1150
	attgtggagg	ggaatttttc	tactgtgata	caacacaact	gtttaatagt	1200
	acttggaaatg	gtactgaagg	aaataacact	gaaggaaata	gcacaatcac	1250
10	actcccatgt	agaataaaaac	aaattataaa	catgtggcag	gaagtaggaa	1300
	aagcaatgta	tgccccctccc	atcggaggac	aaattagatg	ttcatcaaat	1350
	attacagggc	tgctattaac	aagagatggt	ggtaccgaag	ggaatgggac	1400
	agagaatgag	acagagatct	tcagacctgg	aggaggagat	atgagggaca	1450
	attggagaag	tgaattatat	aaatataaag	tagtaaaagt	tgaaccacta	1500
15	ggagtagcac	ccaccagggc	aaagagaaga	gtggtgcaga	gataa	1545

SEQ ID No 8:

	MKVKETRKNY	QHLWRWGTM	LGMLMICSAA	EQLWVTVYYG	VPVWKEATTT	50
20	LFCASDAKAY	DTEVHNWAT	HACVPTDNP	QEVVLGNVTE	YFNMWKNNMV	100
	DQMHEDIISL	WDQSLKPCVK	LTPLCVTLDC	DDVNTTNSTT	TTSNGWTGEI	150
	RKGEIKNCSF	NITTSIRDKV	QKEYALFYNL	DVVPIDDDNA	TTKNKTTRNF	200
	RLIHCNSSVM	TQACPKVSFE	PIPIHYCAPA	GFAILKCNNK	TFDGLGLCTN	250
	VSTVQCTHGI	RPVVSTQLLL	NGSLAEEVV	IRSDNFMDNT	KTIIVQLNES	300
25	VAINCTRPN	NTRKGIHGP	GRAFYAARKI	IGDIRQAHCN	LSRAQWNNTL	350
	KQIVIKLREH	FGNKTIFNQ	SSGGDPEIVR	HSFNCGGEFF	YCDTTQLFNS	400
	TWNGTEGNNT	EGNSTITLPC	RIKQIINMWQ	EVGKAMYAPP	IGGQIRCSSN	450
	ITGLLLTRDG	GTEGNGTENE	TEIFRPGGGD	MRDNWRSELY	KYKVVKVEPL	500
30	GVAPTRAKRR	VVQR				514

SEQ ID No 9:

	atgcatcaca	cggccgcgtc	cgataacttc	cagctgtccc	aggggtgggca	gggattcgcc	60
	attccgatcg	ggcaggcgat	ggcgatcgcg	ggccagatcc	gatcggggtg	ggggtcaccc	120
35	accgttcata	tcgggcctac	cgccttcctc	ggcttggggtg	ttgtcgacaa	caacggcaac	180
	ggcgacagag	tccaacgcgt	ggtcgggagc	gctccggcgg	caagtctcgg	catctccacc	240
	ggcgacgtga	tcaccgcggt	cgacggcgct	ccgatcaact	cggccaccgc	gatggcggac	300
	gcgcttaacg	ggcatcatcc	cggtgacgtc	atctcggtga	cctggcaaac	caagtccggc	360
	ggcacgcgta	cagggaacgt	gacattggcc	gagggacccc	cggccgaatt	catggtggat	420
40	ttcggggcgt	taccaccgga	gatcaactcc	gcgaggatgt	acgcggcccc	gggttcggcc	480
	tcgctgggtg	ccgcggctca	gatgtgggac	agcgtggcga	gtgacctgtt	ttcgcccgcg	540
	tcggcgcttc	agtcggtggt	ctggggtctg	acggtggggt	cgtggatagg	ttcgtcggcg	600
	ggtctgatgg	tggcggcggc	ctcgccgtat	gtggcggtga	tgagcgtcac	cgcggggcag	660
	gccgagctga	ccgcgcgcca	ggtccgggtt	gctgcggcgg	cctacgagac	ggcgtatggg	720
45	ctgacgggtc	ccccgcgggt	gatcgccgag	aaccgtgctg	aactgatgat	tctgatagcg	780
	accaacctct	tggggcaaaa	caccccgggc	atcgcggtca	acgaggccga	atacggcgag	840
	atgtggggccc	aagacgcgcg	cgcgatgttt	ggctacgcgc	cggcgacggc	gacggcgacg	900
	gcgacgttgc	tgccgttcga	ggaggcgccg	gagatgacca	gcgcgggtgg	gctcctcgag	960
	caggccgcgc	cggtcgagga	ggcctccgac	accgccgcgg	cgaaccagtt	gatgaacaat	1020
50	gtgccccagg	cgctgcaaca	gctggcccag	cccacgcagg	gcaccacgcc	ttcttccaag	1080
	ctgggtggcc	tgtggaagac	ggtctcgccg	catcggtcgc	cgatcagcaa	catggtgtcg	1140
	atggccaaca	accacatgtc	gatgaccaac	tcgggtgtgt	cgatgaccaa	caccttgagc	1200
	tcgatgttga	agggctttgc	tcgggcggcg	gccgcccagg	ccgtgcaaac	cgcggcgcaa	1260
	aacgggggtcc	ggcgcatgag	ctcgctgggc	agctcgctgg	gttcttcggg	tctgggcggg	1320
55	gggggtggcc	ccaacttggg	tcgggcggcc	tcggtcgggt	cgttgctcgg	gccgcaggcc	1380
	tgggcgcgcg	ccaaccaggc	agtcaccccg	gcggcgcgcg	cgctgccgct	gaccagcctg	1440
	accagcgccg	cggaaaagag	gcccgggcag	atgctgggcg	ggctgcccgt	ggggcgagatg	1500
	ggcgccaggg	cggttggtgg	gctcagtggt	gtgctgcgtg	ttccgcgcgc	accttatgtg	1560
	atgccgcatt	ctccggcagc	cggcgatatc	gccccgcggg	ccttgctcgca	ggaccggttc	1620
60	gccgacttcc	ccgcgctgcc	cctcgaccgc	tcgcgcatgg	tcgcccaggt	ggggccacag	1680
	gtggtcaaca	tcaacaccaa	actgggctac	aacaacgcgc	tgggcgcgcg	gaccggcatc	1740

	gtcatcgatc	ccaacgggtgt	cgtgctgacc	aacaaccacg	tgatcgcggg	cgccaccgac	1800
	atcaatgctg	tcagcgctcg	ctccggccaa	acctacggcg	tcgatgtggt	cgggtatgac	1860
	cgcacccagg	atgtcgcggt	gctgcagctg	cgcggtgccg	gtggcctgcc	gtcggcgggc	1920
5	atcgggtggc	gcgtcgcggt	tggtgagccc	gtcgctcgca	tgggcaacag	cgggtgggcg	1980
	ggcggaaacg	cccgtgcggg	gcctggcagg	gtggtcgcg	tcggccaaac	cgtgcaggcg	2040
	tcggattcgc	tgaccgggtg	cgaagagaca	ttgaacgggt	tgatccagtt	cgatgccgcg	2100
	atccagcccc	gtgatgcggg	cgggcccgtc	gtcaacggcc	taggacaggt	ggtcggtatg	2160
	aacacggccg	cgctcctag					2178

10

SEQ ID No 10:

	MHHTAASDNF	QLSQGGQGFA	IPIGQAMAIA	GQIRSGGSP	TVHIGPTAFL	GLGVVDNNGN	60
	GARVQRVVG	APAASLGIST	GDVITAVDGA	PINSATAMAD	ALNGHHPGDV	ISVTWQTKSG	120
	GTRTGNVTLA	EGPPAEFMVD	FGALPPEINS	ARMYAGPGSA	SLVAAAQMW	SVASDLFSAA	180
15	SAFQSVVWGL	TVGSWIGSSA	GLMVAAASPY	VAWMSVTAGQ	AELTAAQVRV	AAAAYETAYG	240
	LTVPPPVIIE	NRAELMILIA	TNLLGQNTPA	IAVNEAEYGE	MWAQDAAAMF	GYAAATATAT	300
	ATLLPFEEAP	EMTSAGLLE	QAAVEEASD	TAAANQLMNN	VPQALQQLAQ	PTQGTTPSSK	360
	LGGLWKTVP	HRSPISNMVS	MANNHMSMTN	SGVSMTNTLS	SMLKGFAPAA	AAQAVQTAAQ	420
	NGVRAMSSLG	SSLGSSGLGG	GVAANLGRAA	SVGSLSVPA	WAAANQAVTP	AARALPLTSL	480
20	TSAAERGPQ	MLGGLPVGQM	GARAGGGLSG	VLRVPPRPYV	MPHSPAAGDI	APPALSQDRF	540
	ADFPALPLDP	SAMVAQVGPQ	VVNINTKLG	NNAVGAGTGI	VIDPNGVVL	NNHVIAGATD	600
	INAFSVGSQ	TYGVDVVG	RTQDVAVLQ	RGAGGLPSAA	IGGGVAVGEP	VVAMGNSGGQ	660
	GGTPRAVPGR	VVALGQTVQA	SDSLTGAEET	LNGLIQFDA	IQPGDAGGPV	VNGLGQVVGM	720
25	NTAAS						725

25

SEQ ID No 11:

	atgatgagaa	aacttgccat	cctcagcgtc	agctctttcc	tggtcgtgga	50
	ggccctcttc	caggagtatc	agtgtctacg	aagcagcagc	aatacaaggg	100
30	tcctgaacga	gctcaactat	gacaacgctg	gaacgaacct	gtataacgag	150
	ctggagatga	actactatgg	caagcaggag	aactggtata	gcctgaagaa	200
	gaacagcccg	tccctggg	agaacgacga	cggcaacaac	aacaacggcg	250
	acaacggcag	ggagggcaaa	gatgaggaca	agagggacgg	gaacaacgag	300
	gataacgaga	agctgcggaa	gccaagcac	aagaaactca	agcagcccg	350
35	cgacgggaac	ccggacccca	atgcaaatcc	caacgtcgac	ccaaacgcaa	400
	accctaactg	ggaccccaac	gccaatccca	acgtcgatcc	taatgccaat	450
	ccaaatgcc	accctaactg	aaatccta	gcaaacccca	acgccaatcc	500
	taacgccaac	ccaaatgcc	acccaaacgc	taaccccaac	gctaaccctaa	550
	atgcaaatcc	caatgctaac	ccaaacgtgg	accctaacgc	taaccccaac	600
40	gcaaacccta	acgccaatcc	taacgcaaac	cccaatgcaa	acccaaacgc	650
	aaatcccaac	gtaaccccta	acgcaaac	caacgccaac	cctaatagcc	700
	accccaatgc	taaccccaac	gccaatccca	acgcaaatcc	aaacgccaac	750
	ccaaatgcaa	accccaacgc	taatcccaac	gccaacccaa	acgccaatcc	800
	taacaagaac	aatcagggca	acgggcagg	ccataacatg	ccgaacgacc	850
45	ctaatacgga	tgtggacgag	aacgccaacg	ccaacagcgc	cgtgaagaac	900
	aacaacaacg	aggagccctc	cgacaagcac	atcaaggaat	acctaagcaa	950
	gatccagaac	agtctgagca	ccgagtgggc	ccccctgctc	gtgacctg	1000
	gcaacggcat	ccaggtgagg	atcaagcccc	gtcccgccaa	caagcccaag	1050
	gacgagctgg	actacgcca	cgacatcgag	aagaagatct	gcaagatgga	1100
50	gaaatgcagct	ctgtgttcaac	gtcgtgaa	ctccgccatc	ggcctgtga	1149

50

SEQ ID No 12:

	MMRKLAILSV	SSFLFVEALF	QEYQCYGSSS	NTRVLNELNY	DNAGTNLYNE	50
55	LEMNYYGKQE	NWYSLKKNR	SLGENDDGNN	NNGDNGREGK	DEDKRDGNNE	100
	DNEKLRKPKH	KKLKQPADGN	PDPNANPNVD	PNANPNVDPN	ANPNVDPNAN	150
	PNANPNANPN	ANPNANPNAN	PNANPNANPN	ANPNANPNAN	PNVDPNANPN	200
	ANPNANPNAN	PNANPNANPN	ANPNANPNAN	PNANPNANPN	ANPNANPNAN	250
	PNANPNANPN	ANPNANPNKN	NQNGQGHNM	PNDPNRNVD	NANANSVKN	300
60	NNNEEPSDKH	IKEYLNKIQN	SLSTEWSPCS	VTCNGIQVR	IKPGSANKPK	350
	DELDYANDIE	KKICKMEKCS	SVFNVVNSAI	GL		382

SEQ ID No 13:

	atgatggctc	ccgatcctaa	tgcaaatcca	aatgcaaacc	caaacgcaaa	50
	ccccaatgca	aatcctaata	caaaccocaa	tgcaaatcct	aatgcaaata	100
5	ctaatagcaa	tccaaatgca	aatccaaatg	caaaccocaa	cgcaaacccc	150
	aatgcaaata	ctaatagcaa	tccaaatgca	aatccaaatg	caaaccocaa	200
	tgcaaaccca	aatgcaaacc	ccaatgcaaa	tccataataa	aacaatacaag	250
	gtaataggaca	aggtcacaat	atgccaaatg	acccaaaccg	aaatgtagat	300
	gaaaatgcta	atgccaaacag	tgctgtaaaa	aataataata	acgaagaacc	350
10	aagtataag	cacataaaaag	aatattttaa	caaaatacaa	aattctcttt	400
	caactgaatg	gtcccatgt	agtgtactt	gtggaaatgg	tattcaagtt	450
	agaataaagc	ctggctctgc	taataaacct	aaagacgaat	tagattatgc	500
	aaatgatatt	gaaaaaaaaa	tttgtaaaat	ggaaaaatgt	tccagtgtgt	550
	ttaatgtcgt	aaatagttca	ataggattag	ggcctgtgac	gaacatggag	600
15	aacatcacat	caggattcct	aggaccctg	ctcgtgttac	aggcgggggt	650
	tttcttgttg	acaagaatcc	tcacaatacc	gcagagtcta	gactcgtggg	700
	ggacttctct	caattttcta	gggggatcac	ccgtgtgtct	tggccaaaat	750
	tcgcagctcc	caacctccaa	tcactcacca	acctcctgtc	ctccaatttg	800
	tcctgggttat	cgctggatgt	gtctgcggcg	ttttatcata	ttcctcttca	850
20	tcctgctgct	atgcctcatc	ttcttattgg	ttcttctgga	ttatcaaggt	900
	atgttgcccg	tttgcctctc	aattccagga	tcaacaacaa	ccaatacggg	950
	accatgcaaa	acctgcacga	ctcctgtctc	aggcaactct	atgtttccct	1000
	catgttgctg	tacaaaacct	acggatggaa	attgcacctg	tattcccatc	1050
	ccatcgtcct	gggctttcgc	aaaataccta	tgggagtggg	cctcagtcgg	1100
25	tttctcttgg	ctcagtttac	tagtgccatt	tggtcagtg	ttcgtagggc	1150
	tttccccac	tggttggtt	tcagctatat	ggatgatgtg	gtattggggg	1200
	ccaagtctgt	acagcatcgt	gagtcctttt	ataccgctgt	taccaatttt	1250
	cttttgtctc	tgggtataca	tttaa			1275

30

SEQ ID No 14:

	MMA PDNPANP	NANPNANPNA	NPANPNANP	NANPNANPNA	NPANPNANP	50
	NANPNANPNA	NPANPNANP	NANPNANPNK	NNQNGNGQHN	MPNDPNRNV	100
	ENANANSVAVK	NNNNEEPSDK	HIKEYLNKIQ	NSLSTEWSPC	SVTCNGIQV	150
35	RIKPGSANKP	KDEL DYANDI	EKKICKMEKC	SSVFN VNSS	IGLGPVTNME	200
	NITSGFLGPL	LVLQAGFFLL	TRILTIPQSL	DSWWTSLNFL	GGSPVCLGQN	250
	SQSPSTNHSP	TSCPPICPGY	RWMCLRRFII	FLFILLLCLI	FLLVLLDYQG	300
	MLPVCPLIPG	STTTNTGPCK	TCTTPAQGNS	MFPSCCCTKP	TDGNCTCIPI	350
	PSSWAFAYL	WEWASVRFSW	LSLLVPFVQW	FVGLSPTVWL	SAIWMWYWG	400
40	PSLYSIVSPF	IPLLPIFFCL	WVYI			424

SEQ ID No 15:

	atggtcattg	ttcagaacat	acagggccaa	atggtcacc	aggcaattag	50
	tccgcgaact	cttaatgcat	gggtgaaggt	cgtggaggaa	aaggcattct	100
45	ccccggaggt	cattccgatg	tttctgcgc	tatctgagg	cgcaacgcgg	150
	caagacctta	ataccatgct	taacacggta	ggcgggcacc	aagccgctat	200
	gcaaatagcta	aaagagacta	taaacgaaga	ggccgccgaa	tgggacgag	250
	tgcacccggg	gcacgcgggc	ccaattgcac	caggccagat	gcgcgagccg	300
	cgcgggtctg	atattgcagg	aactacgtct	acccttcagg	agcagattgg	350
50	gtggatgact	aacaatccac	caatcccggt	cggagagatc	tataagaggt	400
	ggatcatact	gggactaaac	aagatagtcc	gcatgtattc	tccgacttct	450
	atactggata	tacgccaaag	cccaaaggag	ccgttcagg	actatgtcga	500
	ccgattctat	aagacccttc	gcgcagagca	ggcatcccag	gaggtcaaaa	550
	attggatgac	agaaactctt	ttggtgcaga	atgcgaatcc	ggattgtaaa	600
55	acaattttaa	aggctctagg	accggccgca	acgctagaag	agatgatgac	650
	ggcttgtcag	ggagtcgggt	gaccggggca	taaagccgc	gtcttacaca	700
	tgggcccgat	atctccgata	gaaacagttt	cgggtcaagct	taaaccagg	750
	atggatgggc	caaagggtcaa	gcagtggccg	ctaaccggaag	agaagattaa	800
	ggcgtctcga	gagatttgta	ctgaaatgga	gaaggaaggc	aagataagca	850
60	agatcggggc	agagaacccg	tacaatacac	cgggtatttgc	aataaagaaa	900

	aaggattcaa	caaaatggcg	aaagcttgta	gatttttaggg	aactaaacaa	950
	gcgaacccaa	gacttttggg	aagtccaact	agggatccca	catccagccg	1000
	gtctaaagaa	gaagaaatcg	gtcacagtcc	tggatgtagg	agacgcata	1050
	tttagtgtag	cgcttgatga	ggacttccga	aagtatactg	cgtttactat	1100
5	accgagcata	aacaatgaaa	cgccaggcat	tcgctatcag	tacaacgtgc	1150
	tcccgcaggg	ctggaagggg	tctccggcga	tatttcagag	ctgtatgaca	1200
	aaaatacttg	aaccattccg	aaagcagaat	ccggatattg	taatttacca	1250
	atacatggac	gatctctatg	tgggctcgga	tctagaaatt	gggcagcatc	1300
	gcactaagat	tgaggaactg	aggcaacatc	tgcttcgatg	gggcctcact	1350
10	actcccgaca	agaagcacca	gaaggagccg	ccgttcctaa	agatgggcta	1400
	cgagcttcat	ccggacaagt	ggacagtaca	gccgatagtg	ctgcccga	1450
	aggattcttg	gaccgtaaat	gatattcaga	aactagtcgg	caagcttaac	1500
	tgggcctctc	agattttacc	aggcattaag	gtccgacagc	tttgcaagct	1550
	actgagggga	actaaggctc	taacagaggt	catcccat	acggaggaag	1600
15	cagagcttga	gctggcagag	aatcgcgaaa	ttcttaagga	gccggtgcac	1650
	gggggtatact	acgacccctc	caaggacctt	atagccgaga	tccagaagca	1700
	ggggcagggc	caatggacgt	accagatata	tcaagaaccg	tttaagaatc	1750
	tgaagacttg	gaagtacgag	cgcatgcgag	gggctcat	taatgatgta	1800
	aagcaactta	gggaagcagt	acaaaagatt	actactgagt	ctattgtgat	1850
20	atggggcaag	accccaaagt	tcaagctgcc	catacagaag	gaaacatggg	1900
	aaacatggtg	gactgaatat	tggcaagcta	cctggattcc	agaatgggaa	1950
	tttgtcaaca	cgccgccact	tggttaagctt	tggtaccagc	ttgaaaagga	2000
	gccgatagta	ggggcagaga	ccttctatgt	cgatggcgcc	gcgaatcgcg	2050
	aaacgaagct	aggcaaggcg	ggatacgtga	ctaatagggg	ccgccaaaag	2100
25	gtcgtaaccc	ttacggatac	caccaatcag	aagactgaac	tacaagcgat	2150
	ttaccttgca	cttcaggata	gtggcctaga	ggtcaacata	gtcacggact	2200
	ctcaatatgc	gcttggcatt	attcaagcgc	agccagatca	aagcgaaagc	2250
	gagcttgtaa	accaaataat	agaacagctt	ataaagaaag	agaaggtata	2300
	tctggcctgg	gtccccgcctc	acaagggaat	tggcggcaat	gagcaagtgg	2350
30	acaagctagt	cagcgctggg	attcgcaagg	ttcttgcgat	ggggggtaag	2400
	tggctctaagt	ctagcgtagt	cggctggccg	acagtcgcg	agcgcatgcg	2450
	acgcgccgaa	ccagccgcag	atggcgtggg	ggcagcgtct	agggatctgg	2500
	agaagcacgg	ggctataact	tccagtaaca	cggcggcgac	gaacgccgca	2550
	tgcgcagtgt	tagaagccca	agaagaggaa	gaagtagggt	ttccggtaac	2600
35	tccccagggtg	ccgttaaggc	cgatgacct	taaggcagcg	gtggatcttt	2650
	ctcacttcct	taaggagaaa	ggggggctgg	agggttaat	tcacagccag	2700
	aggcgacagg	atattcttga	tctgtggatt	taccataccc	aggggtactt	2750
	tccggactgg	cagaattaca	ccccggggcc	aggcgtgcgc	tatcccctga	2800
	ctttcggttg	gtgctacaaa	ctagtcccag	tggaaaccga	caaggtcgaa	2850
40	gaggctaata	agggcgagaa	cacttctctt	cttcacccgg	taagcctgca	2900
	cgggatggat	gaccagaaac	gagagggtct	agaatggagg	ttcgactctc	2950
	gacttgctgt	ccatcacgta	gcacgcgagc	tgcatccaga	atatttcaag	3000
	aactgccgcc	caatgggcgc	cagggccagt	gtacttagtg	gcggagaact	3050
	agatcgatgg	gaaaagatac	gcctacgccc	ggggggcaag	aagaagtaca	3100
45	agcttaagca	cattgtgttg	gcctctcgcg	aacttgagcg	attcgcagtg	3150
	aatccaggcc	tgcttgagac	gagtgaaggc	tgtaggcaaa	ttctggggca	3200
	gctacagccg	agcctacaga	ctggcagcga	ggagcttcgt	agtctttata	3250
	ataccgtcgc	gactctctac	tgcgttcac	aacgaattga	aataaaggat	3300
	actaaagagg	cccttgataa	aattgaggag	gaacagaata	agtcgaaaaa	3350
50	gaaggcccag	caggccgcgc	ccgacaccgg	gcacagcaac	caggtgtccc	3400
	aaaactacta	a				3411

SEQ ID No 16:

	MVIVQNIQQQ	MVHQAIISPT	LNAWVKVVEE	KAFSPVIVPM	FSALSEGATP	50
55	QDLNITMLNTV	GGHQAAQML	KETINEBAAE	WDRVHPVHAG	PIAPGQMRP	100
	RGSDIAGTTS	TLQEQIGWMT	NNPPIPVGEI	YKRWIILGLN	KIVRMYSPST	150
	ILDIRQGPKE	PFRDYVDRFY	KTLEAEQASQ	EVKNWMTETL	LVQANPDCK	200
	TILKALGPAA	TLEEMMTACQ	GVGGPGHKAR	VLHMGPIPI	ETVSVKLKPG	250
	MDGPKVKQWP	LTEEKIKALV	EICTEMEKEG	KISKIGPENP	YNTPVFAIKK	300
60	KDSTKWRKLV	DFRELNKRTQ	DFWEVQLGIP	HPAGLKKKKS	VTVLVDGDAY	350

	FSVPLDEDFR	KYTAFTIPSI	NNETPGIRYQ	YNVLPQGWK	SPAIFQSCMT	400
	KILEPFRKQN	PDIVIYQYMD	DLYVGSDEI	GQHRTKIEEL	RQHLLRWGLT	450
	TPDKKHQKEP	PFLKMGYELH	PKWTVQPIV	LPEKDSWTVN	DIQKLVGKLN	500
	WASQIYPGIK	VRQLCKLLRG	TKALTEVIPL	TEEAELAE	NREILKEPVH	550
5	GVIYDPSKDL	IAEIQKQGQ	QWTYQIYQEP	FKNLKTGKYA	RMRGAHTNDV	600
	KQLTEAVQKI	TTESIVIWGK	TPKFKLPIQK	ETWETWWTEY	WQATWIPEWE	650
	FVNTPLVLK	WYQLEKEPIV	GAETFYVDGA	ANRETKLGKA	GYVTNRGRQK	700
	VVTLTDTTNQ	KTELQAIYLA	LQDSGLEVNI	VTDSQYALGI	IQAQPDQSES	750
	ELVNQIIEQL	IKKEKVYLAW	VPAHKGIGGN	EQVDKLVSAG	IRKVLAMGGK	800
10	WSKSSVVGWP	TVRERMRAE	PAADGVGAAS	RDLEKHGAIT	SSNTAATNAA	850
	CAWLEAQEEE	EVGFVPVTPQV	PLRPMTYKAA	VDLSHFLKEK	GGLEGLIHSQ	900
	RRQDILDWI	YHTQGYFPDW	QNYTPGPGVR	YPLTFGW CYK	LVPVEPKVE	950
	EANKGENTSL	LHPVSLHGMD	DPEREVLEWR	FDSRLAFHHV	ARELHPEYFK	1000
	NCRPMGARAS	VLSGGELDRW	EKIRLRPGGK	KKYKLKHIVW	ASRELERFAV	1050
15	NPGLLETSEG	CRQILGQLQP	SLQTGSEELR	SLYNTVATLY	CVHQRIEIKD	1100
	TKEALDKIEE	EONKSKKKAQ	QAAADTGHSN	QVSQNY		1136

SEQUENCE LISTING

5 <110> Glaxosmithkline Biologicals
 Glaxosmithkline Biologicals
 <120> Novel method and composition
 <130> V62209
 10 <160> 16
 <170> PatentIn version 3.5
 15 <210> 1
 <211> 3204
 <212> DNA
 <213> HIV
 20 <400> 1
 atgggtgccc gagcttcggt actgtctggt ggagagctgg acagatggga gaaaattagg 60
 ctgcgcccgg gaggcaaaaa gaaatacaag ctcaagcata tcgtgtgggc ctcgagggag 120
 25 cttgaacggt ttgccgtgaa cccaggcctg ctggaaacat ctgagggatg tcgccagatc 180
 ctggggcaat tgcagccatc cctccagacc gggagtgaag agctgaggtc cttgtataac 240
 acagtggcta ccctctactg cgtacaccag aggatcgaga ttaaggatac caaggaggcc 300
 30 ttggacaaaa ttgaggagga gcaaaacaag agcaagaaga aggcccagca ggcagctgct 360
 gacactgggc atagcaacca ggtatcacag aactatccta ttgtccaaaa cattcagggc 420
 35 cagatgggtc atcaggccat cagcccccg acgctcaatg cctgggtgaa ggttgctgaa 480
 gagaaggcct tttctcctga ggttatcccc atgttctcgc ctttgagtga gggggccact 540
 cctcaggacc tcaatacaat gcttaatacc gtgggcggcc atcaggccgc catgcaaatg 600
 40 ttgaaggaga ctatcaacga ggaggcagcc gagtgggaca gagtgcattc cgtccacgct 660
 ggcccaatcg cgcccggaac gatgcgggag cctcgcggct ctgacattgc cggcaccacc 720
 45 tctacactgc aagagcaaat cggtatggatg accaacaatc ctcccatccc agttggagaa 780
 atctataaac ggttgatcat cctgggcctg aacaagatcg tgcgatgta ctctccgaca 840
 tccatccttg acattagaca gggacccaaa gagcctttta gggattacgt cgaccggttt 900
 50 tataagacct tgcgagcaga gcaggcctct caggagggtca aaaactggat gacggagaca 960
 ctcttggtac agaacgctaa ccccgactgc aaaacaatct tgaaggcact agggccggct 1020
 55 gccaccctgg aagagatgat gaccgcctgt cagggagtag gcggacccgg acacaaagcc 1080
 agagtgttga tgggccccat cagtcccatc gagaccgtgc cggatgaagct gaaaccggg 1140
 atggacggcc ccaaggtcaa gcagtggcca ctaccgagg agaagatcaa ggccttggtg 1200
 60 gagatctgca ccgagatgga gaaagagggc aagatcagca agatcgggac ggagaaccca 1260
 tacaacaccc ccgtgtttgc catcaagaag aaggacagca ccaagtggcg caagctggtg 1320
 65 gatttcgggg agctgaataa gcggacccag gatttctggg aggtccagct gggcatcccc 1380
 catccggccg gcctgaagaa gaagaagagc gtgaccgtgc tggacgtggg cgacgcttac 1440
 ttcagcgtcc ctctggacga ggactttaga aagtacaccg cctttaccat cccatctatc 1500

5 aacaacgaga cccctggcat cagatatcag tacaacgtcc tccccaggg ctggaagggc 1560
 tctcccgcca ttttccagag ctccatgacc aagatcctgg agccgtttcg gaagcagaac 1620
 cccgatatcg tcatctacca gtacatggac gacctgtacg tgggctctga cctggaaatc 1680
 gggcagcatc gcacgaagat tgaggagctg aggcagcatc tgctgagatg gggcctgacc 1740
 10 actccggaca agaagcatca gaaggagccg ccattcctga agatgggcta cgagctccat 1800
 cccgacaagt ggaccgtgca gcctatcgtc ctccccgaga aggacagctg gaccgtgaac 1860
 15 gacatccaga agctggtggg caagctcaac tgggctagcc agatctatcc cgggatcaag 1920
 gtgcgccagc tctgcaagct gctgcgcggc accaaggccc tgaccgaggt gattccccctc 1980
 acggaggaag ccgagctcga gctggctgag aaccgggaga tcctgaagga gcccgtagc 2040
 20 ggcgtgtact atgacccctc caaggacctg atcgccgaaa tccagaagca gggccagggg 2100
 cagtggacat accagattta ccaggagcct ttcaagaacc tcaagaccgg caagtacgcc 2160
 25 cgcattgagg ggcgccacac caacgatgtc aagcagctga ccgaggccgt ccagaagatc 2220
 acgaccgagt ccacgtgat ctgggggaag acaccaagt tcaagctgcc tatccagaag 2280
 gagacctggg agacgtggtg gaccgaatat tggcaggcca cctggattcc cgagtgggag 2340
 30 ttcgtgaata cacctcctct ggtgaagctg tggtagcagc tcgagaagga gcccatcgtg 2400
 ggcgcggaga cattctacgt ggacggcgcg gccaaaccgg aaacaaagct cgggaaggcc 2460
 35 gggtagctca ccaaccgggg ccgccagaag gtcgtcacc tgaccgacac caccaaccag 2520
 aagacggagc tgcaggccat ctatctcgct ctccaggact ccggcctgga ggtgaacatc 2580
 gtgacggaca gccagtacgc gctgggcatt attcaggccc agccggacca gtccgagagc 2640
 40 gaactggtga accagattat cgagcagctg atcaagaaag agaaggtcta cctcgccctg 2700
 gtcccgcccc ataaggcat tggcggaac gagcaggtcg acaagctggt gagtgcgggg 2760
 45 attagaaagg tgctgatggt gggttttcca gtcacacctc aggtaccttt aagaccaatg 2820
 acttacaagg cagctgtaga tcttagccac tttttaaaag aaaagggggg actggaaggg 2880
 ctaattcact cccaaagaag acaagatatc ctgtgatctg ggatctacca cacacaaggc 2940
 50 tacttcctg attggcagaa ctacacacca gggccagggg tcagatatcc actgaccttt 3000
 ggatggtgct acaagctagt accagttgag ccagataagg tagaagaggc caataaagga 3060
 55 gagaacacca gcttgttaca ccctgtgagc ctgcatggga tggatgacct ggagagagaa 3120
 gtgttagagt ggaggtttga cagccgccta gcatttcac acgtggcccg agagctgcat 3180
 ccggagtact tcaagaactg ctga 3204
 60
 <210> 2
 <211> 1067
 <212> PRT
 <213> HIV
 65
 <400> 2

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp

60

	1		5		10		15									
5	Glu	Lys	Ile	Arg	Leu	Arg	Pro	Gly	Gly	Lys	Lys	Lys	Tyr	Lys	Leu	Lys
			20					25					30			
10	His	Ile	Val	Trp	Ala	Ser	Arg	Glu	Leu	Glu	Arg	Phe	Ala	Val	Asn	Pro
		35						40				45				
15	Gly	Leu	Leu	Glu	Thr	Ser	Glu	Gly	Cys	Arg	Gln	Ile	Leu	Gly	Gln	Leu
	50						55				60					
20	Gln	Pro	Ser	Leu	Gln	Thr	Gly	Ser	Glu	Glu	Leu	Arg	Ser	Leu	Tyr	Asn
	65					70					75				80	
25	Thr	Val	Ala	Thr	Leu	Tyr	Cys	Val	His	Gln	Arg	Ile	Glu	Ile	Lys	Asp
				85						90					95	
30	Thr	Lys	Glu	Ala	Leu	Asp	Lys	Ile	Glu	Glu	Glu	Gln	Asn	Lys	Ser	Lys
			100						105				110			
35	Lys	Lys	Ala	Gln	Gln	Ala	Ala	Ala	Asp	Thr	Gly	His	Ser	Asn	Gln	Val
		115						120				125				
40	Ser	Gln	Asn	Tyr	Pro	Ile	Val	Gln	Asn	Ile	Gln	Gly	Gln	Met	Val	His
	130						135				140					
45	Gln	Ala	Ile	Ser	Pro	Arg	Thr	Leu	Asn	Ala	Trp	Val	Lys	Val	Val	Glu
	145					150				155					160	
50	Glu	Lys	Ala	Phe	Ser	Pro	Glu	Val	Ile	Pro	Met	Phe	Ser	Ala	Leu	Ser
				165						170					175	
55	Glu	Gly	Ala	Thr	Pro	Gln	Asp	Leu	Asn	Thr	Met	Leu	Asn	Thr	Val	Gly
			180					185					190			
60	Gly	His	Gln	Ala	Ala	Met	Gln	Met	Leu	Lys	Glu	Thr	Ile	Asn	Glu	Glu
		195					200					205				
65	Ala	Ala	Glu	Trp	Asp	Arg	Val	His	Pro	Val	His	Ala	Gly	Pro	Ile	Ala
	210						215				220					
70	Pro	Gly	Gln	Met	Arg	Glu	Pro	Arg	Gly	Ser	Asp	Ile	Ala	Gly	Thr	Thr
	225					230					235				240	
75	Ser	Thr	Leu	Gln	Glu	Gln	Ile	Gly	Trp	Met	Thr	Asn	Asn	Pro	Pro	Ile
				245						250				255		
80	Pro	Val	Gly	Glu	Ile	Tyr	Lys	Arg	Trp	Ile	Ile	Leu	Gly	Leu	Asn	Lys
			260						265				270			
85	Ile	Val	Arg	Met	Tyr	Ser	Pro	Thr	Ser	Ile	Leu	Asp	Ile	Arg	Gln	Gly

61

	275		280		285														
5	Pro	Lys	Glu	Pro	Phe	Arg	Asp	Tyr	Val	Asp	Arg	Phe	Tyr	Lys	Thr	Leu			
	290						295					300							
10	Arg	Ala	Glu	Gln	Ala	Ser	Gln	Glu	Val	Lys	Asn	Trp	Met	Thr	Glu	Thr			
	305					310					315					320			
15	Leu	Leu	Val	Gln	Asn	Ala	Asn	Pro	Asp	Cys	Lys	Thr	Ile	Leu	Lys	Ala			
					325					330					335				
20	Leu	Gly	Pro	Ala	Ala	Thr	Leu	Glu	Glu	Met	Met	Thr	Ala	Cys	Gln	Gly			
				340					345					350					
25	Val	Gly	Gly	Pro	Gly	His	Lys	Ala	Arg	Val	Leu	Met	Gly	Pro	Ile	Ser			
			355					360					365						
30	Pro	Ile	Glu	Thr	Val	Pro	Val	Lys	Leu	Lys	Pro	Gly	Met	Asp	Gly	Pro			
	370						375					380							
35	Lys	Val	Lys	Gln	Trp	Pro	Leu	Thr	Glu	Glu	Lys	Ile	Lys	Ala	Leu	Val			
	385					390					395					400			
40	Glu	Ile	Cys	Thr	Glu	Met	Glu	Lys	Glu	Gly	Lys	Ile	Ser	Lys	Ile	Gly			
					405					410					415				
45	Pro	Glu	Asn	Pro	Tyr	Asn	Thr	Pro	Val	Phe	Ala	Ile	Lys	Lys	Lys	Asp			
				420					425					430					
50	Ser	Thr	Lys	Trp	Arg	Lys	Leu	Val	Asp	Phe	Arg	Glu	Leu	Asn	Lys	Arg			
			435					440					445						
55	Thr	Gln	Asp	Phe	Trp	Glu	Val	Gln	Leu	Gly	Ile	Pro	His	Pro	Ala	Gly			
	450						455					460							
60	Leu	Lys	Lys	Lys	Lys	Ser	Val	Thr	Val	Leu	Asp	Val	Gly	Asp	Ala	Tyr			
	465					470					475					480			
65	Phe	Ser	Val	Pro	Leu	Asp	Glu	Asp	Phe	Arg	Lys	Tyr	Thr	Ala	Phe	Thr			
					485					490					495				
70	Ile	Pro	Ser	Ile	Asn	Asn	Glu	Thr	Pro	Gly	Ile	Arg	Tyr	Gln	Tyr	Asn			
				500					505					510					
75	Val	Leu	Pro	Gln	Gly	Trp	Lys	Gly	Ser	Pro	Ala	Ile	Phe	Gln	Ser	Ser			
			515					520					525						
80	Met	Thr	Lys	Ile	Leu	Glu	Pro	Phe	Arg	Lys	Gln	Asn	Pro	Asp	Ile	Val			
	530						535					540							
85	Ile	Tyr	Gln	Tyr	Met	Asp	Asp	Leu	Tyr	Val	Gly	Ser	Asp	Leu	Glu	Ile			

	545					550						555					560
5	Gly	Gln	His	Arg	Thr	Lys	Ile	Glu	Glu	Leu	Arg	Gln	His	Leu	Leu	Arg	
					565					570					575		
10	Trp	Gly	Leu	Thr	Thr	Pro	Asp	Lys	Lys	His	Gln	Lys	Glu	Pro	Pro	Phe	
				580					585					590			
15	Leu	Lys	Met	Gly	Tyr	Glu	Leu	His	Pro	Asp	Lys	Trp	Thr	Val	Gln	Pro	
			595					600					605				
20	Ile	Val	Leu	Pro	Glu	Lys	Asp	Ser	Trp	Thr	Val	Asn	Asp	Ile	Gln	Lys	
	610						615					620					
25	Leu	Val	Gly	Lys	Leu	Asn	Trp	Ala	Ser	Gln	Ile	Tyr	Pro	Gly	Ile	Lys	
	625					630					635					640	
30	Val	Arg	Gln	Leu	Cys	Lys	Leu	Leu	Arg	Gly	Thr	Lys	Ala	Leu	Thr	Glu	
					645					650					655		
35	Val	Ile	Pro	Leu	Thr	Glu	Glu	Ala	Glu	Leu	Glu	Leu	Ala	Glu	Asn	Arg	
				660					665					670			
40	Glu	Ile	Leu	Lys	Glu	Pro	Val	His	Gly	Val	Tyr	Tyr	Asp	Pro	Ser	Lys	
		675						680					685				
45	Asp	Leu	Ile	Ala	Glu	Ile	Gln	Lys	Gln	Gly	Gln	Gly	Gln	Trp	Thr	Tyr	
	690						695					700					
50	Gln	Ile	Tyr	Gln	Glu	Pro	Phe	Lys	Asn	Leu	Lys	Thr	Gly	Lys	Tyr	Ala	
	705					710					715					720	
55	Arg	Met	Arg	Gly	Ala	His	Thr	Asn	Asp	Val	Lys	Gln	Leu	Thr	Glu	Ala	
					725					730					735		
60	Val	Gln	Lys	Ile	Thr	Thr	Glu	Ser	Ile	Val	Ile	Trp	Gly	Lys	Thr	Pro	
				740					745					750			
65	Lys	Phe	Lys	Leu	Pro	Ile	Gln	Lys	Glu	Thr	Trp	Glu	Thr	Trp	Trp	Thr	
		755						760					765				
70	Glu	Tyr	Trp	Gln	Ala	Thr	Trp	Ile	Pro	Glu	Trp	Glu	Phe	Val	Asn	Thr	
	770						775					780					
75	Pro	Pro	Leu	Val	Lys	Leu	Trp	Tyr	Gln	Leu	Glu	Lys	Glu	Pro	Ile	Val	
	785					790					795					800	
80	Gly	Ala	Glu	Thr	Phe	Tyr	Val	Asp	Gly	Ala	Ala	Asn	Arg	Glu	Thr	Lys	
					805					810					815		
85	Leu	Gly	Lys	Ala	Gly	Tyr	Val	Thr	Asn	Arg	Gly	Arg	Gln	Lys	Val	Val	

63

	820	825	830
5	Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr 835 840 845		
10	Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser 850 855 860		
15	Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser 865 870 875 880		
20	Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val 885 890 895		
25	Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln 900 905 910		
30	Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Met Val Gly 915 920 925		
35	Phe Pro Val Thr Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Ala 930 935 940		
40	Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly 945 950 955 960		
45	Leu Ile His Ser Gln Arg Arg Gln Asp Ile Leu Asp Leu Trp Ile Tyr 965 970 975		
50	His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro 980 985 990		
55	Gly Val Arg Tyr Pro Leu Thr Phe Gly Trp Cys Tyr Lys Leu Val Pro 995 1000 1005		
60	Val Glu Pro Asp Lys Val Glu Glu Ala Asn Lys Gly Glu Asn Thr 1010 1015 1020		
65	Ser Leu Leu His Pro Val Ser Leu His Gly Met Asp Asp Pro Glu 1025 1030 1035		
	Arg Glu Val Leu Glu Trp Arg Phe Asp Ser Arg Leu Ala Phe His 1040 1045 1050		
	His Val Ala Arg Glu Leu His Pro Glu Tyr Phe Lys Asn Cys 1055 1060 1065		
	<210> 3		
	<211> 4665		
	<212> DNA		
	<213> HIV		

	<400> 3	
	atggccgcca gagccagcat cctgagcggg ggcaagctgg acgcctggga gaagatcaga	60
5	ctgaggcctg gcggcaagaa gaagtaccgg ctgaagcacc tgggtgtggc cagcagagag	120
	ctggatcgct tcgccctgaa tcctagcctg ctggagacca ccgagggctg ccagcagatc	180
	atgaaccagc tgcagcccg cgtgaaaacc ggcaccgagg agatcaagag cctgttcaac	240
10	accgtggcca ccctgtactg cgtgcaccag cggatcgacg tgaaggatac caaggaggcc	300
	ctggacaaga tcgaggagat ccagaacaag agcaagcaga aaacccagca ggccgctgcc	360
15	gacaccggcg acagcagcaa agtgagccag aactacccca tcatccagaa tgcccagggc	420
	cagatgatcc accagaacct gagccccaga accctgaatg cctgggtgaa agtgatcgag	480
	gaaaaggcct tcagccccga agtgatccct atgttcagcg cctgagcga gggcgccacc	540
20	ccccaggacc tgaacgtgat gctgaacatt gtgggcggac accaggccgc catgcagatg	600
	ctgaaggaca ccatcaatga ggaggccgcc gagtgggaca gactgcaccc cgtgcaggcc	660
25	ggacccatcc ccctggcca gatcagagag ccagaggca gcgacatcg cggcaccacc	720
	tccacccctc aagaacagct gcagtggatg accggcaacc ctcccatccc tgtgggcaac	780
	atctacaagc ggtggatcat cctgggcctg aacaagattg tgcggatgta cagcccctg	840
30	tccatcctgg atatcaagca gggccccaag gagcccttca gagactacgt ggaccggttc	900
	ttcaaggccc tgagagccga gcaggccacc caggacgtga agggetggat gaccgagacc	960
35	ctgctggtgc agaacgcca ccccgactgc aagagcatcc tgaaggccct gggcagcggc	1020
	gccacactgg aggagatgat gaccgcctgc caggagtggt gcggacccgg ccacaaggcc	1080
	agagtgtctg ccgaggccat gagccaggcc cagcagacca acatcatgat gcagcggggc	1140
40	aacttcagag gccagaagcg gatcaagtgc ttcaactgcg gcaaggaggg ccacctggcc	1200
	agaaactgca gagccccag gaagaagggc tgctggaagt gtggcaagga agggcaccag	1260
45	atgaaggact gcaccgagag gcaggccaat ttcctgggca agatttgcc tagcagcaag	1320
	ggcagacccg gcaatttccc ccagagcaga cccgagccca ccgcccctcc cgcagagctg	1380
	ttcgcatggt gcgagggcat cgcagcctg cccaagcagg agcagaagga cagagagcag	1440
50	gtgccccccc tgggtgtccct gaagtccctg ttcggcaacg atcctctgag ccagggatcc	1500
	cccatcagcc ccctcgagac cgtgccctg accctgaagc ccggcatgga tggccccaaa	1560
55	gtgaaacagt ggcccctgac cgaggagaag attaaggccc tgaccgaaat ctgtaccgag	1620
	atggagaagg agggcaagat cagcaagatc ggccccgaga acccctacaa ccccccatc	1680
	ttcgccatca agaagaagga cagcaccaag tggcggaac tgggtggactt ccgggagctg	1740
60	aacaagagga cccaggactt ctgggaagtg cagctgggca tccccaccc tgccggcctg	1800
	aagaagaaga agtccgtgac agtgctggat gtgggcgacg cctacttcag cgtgcccctg	1860
65	gacgagaact tcaggaagta caccgccttc accatcccca gcaccaacaa cgagaccccc	1920
	ggagtggagat accagtacaa cgtgctgcct cagggtgga agggcagccc cgccatcttc	1980
	cagagcagca tgaccaagat cctggagccc ttccggagca agaaccccga gatcatcatc	2040

	taccagtaca	tggccgccct	gtatgtgggc	agcgatctgg	agatcggcca	gcacaggacc	2100
5	aagatcgaag	agctgagggc	ccacctgctg	agctggggct	tcaccacccc	cgataagaag	2160
	caccagaagg	agcccccttt	cctgtggatg	ggctacgagc	tgcaccccca	taagtggacc	2220
	gtgcagccca	tcatgctgcc	cgataaggag	agctggaccg	tgaacgacat	ccagaaactg	2280
10	gtgggcaagc	tgaattgggc	cagccaaatc	tacgccggca	ttaaagtga	gcagctgtgc	2340
	aggctgctga	gagggcggca	agccctgaca	gacatcgtga	cactgacaga	ggaggccgag	2400
15	ctggagctgg	ccgagaacag	ggagatcctg	aaggaccccc	tgcacggcgt	gtactacgac	2460
	cccagcaagg	acctgggtggc	cgagattcag	aagcagggcc	aggaccagtg	gacctaccaa	2520
	atctaccagg	agcctttcaa	gaacctgaaa	accgggaagt	acgccaggaa	gagaagcgcc	2580
20	cacaccaacg	atgtgaggca	gctggccgaa	gtggtgcaga	aagtggctat	ggagagcatc	2640
	gtgatctggg	gcaagacccc	caagttcaag	ctgcccatcc	agaaggagac	ctgggaaacc	2700
25	tggtggatgg	actactggca	ggccacctgg	attcctgagt	gggagttcgt	gaacaccccc	2760
	cctctggtga	agctgtggta	tcagctggag	aaggaccccc	tcctggggcg	cgagaccttc	2820
	tacgtggacg	gagccggcaa	tagagagacc	aagctgggca	aggccggcta	cgtgaccgac	2880
30	agaggcagac	agaaagtggg	gtctctgacc	gagacaacca	accagaaaac	cgagctgcac	2940
	gccatcctgc	tggccctgca	ggacagcggc	agcgaagtga	acatcgtgac	cgactcccag	3000
35	tacgccctgg	gcatcattca	ggccagccc	gatagaagcg	agagcgagct	ggtgaaccag	3060
	atcatcgaga	agctgatcgg	caaggacaaa	atctacctga	gctgggtgcc	cgcccacaag	3120
	ggcatcggcg	gcaacgagca	ggtggacaag	ctggtgtcca	gcggcatccg	gaaagtgctg	3180
40	tttctggacg	gcatcgacaa	ggcccaggag	gaccacgaga	gataccacag	caactggcgg	3240
	acaatggcca	gcgacttcaa	cctgcctccc	atcgtggcca	aggagatcgt	ggccagctgc	3300
45	gataagtgtc	agctgaaggg	cgaggccatg	cacggccagg	tggactgcag	ccctggcatc	3360
	tggcagctgg	cctgcaccca	cctggagggc	aaagtgattc	tggtggccgt	gcacgtggcc	3420
	agcggctaca	tcgaggccga	agtgattccc	gccgagaccg	gccaggagac	cgccctacttc	3480
50	ctgctgaagc	tggccggcag	atggcccgtg	aaagtgtgtc	acaccgcca	cggcagcaac	3540
	ttcacctctg	ccgccgtgaa	ggccgcctgt	tggtgggcca	atatccagca	ggagtccggc	3600
55	atccccata	accctcagag	ccagggcgtg	gtggccagca	tgaacaagga	gctgaagaag	3660
	atcatcggcc	aggtgagggg	ccaggccgag	cacctgaaaa	cagccgtgca	gatggccgtg	3720
	ttcatccaca	acttcaagcg	gaagggcggc	attggcggct	acagcgccgg	agagcggatc	3780
60	atcgacatca	tcgccaccga	tatccagacc	aaggaactgc	agaagcagat	caccaagatt	3840
	cagaacttca	gagtgtacta	ccgggacagc	agggaccccc	tctggaaggg	ccctgccaa	3900
65	ctgctgtgga	agggcgaaag	cgccgtggtg	atccaggaca	acagcgacat	caaagtgggtg	3960
	ccccggagga	aggccaagat	tctgcggggc	tacggcaaac	agatggccgg	cgatgactgc	4020
	gtggccggca	ggcaggatga	ggacagatct	atgggcggca	agtgttccaa	gggcagcatt	4080

gtgggctggc ccgagatccg ggagagaatg agaagagccc ctgccgccgc tcctggagtg 4140
 5 ggcgccgtgt ctccagatct ggataagcac ggcgccatca ccagcagcaa catcaacaac 4200
 cccagctgtg tgtggctgga ggcccaggaa gaggaggaag tgggcttccc tgtgagaccc 4260
 caggtgcccc tgagacccat gacctacaag ggcgccttcg acctgagcca cttcctgaag 4320
 10 gagaagggcg gcctggacgg cctgatctac agccggaagc ggcaggagat cctggatctg 4380
 tgggtgtacc acaccagggt ctacttcccc gactggcaga attacacccc tggccctgga 4440
 15 gtgcggtatc ccctgacctt cggctggtgc ttcaagctgg tgccatgga gcccgacgaa 4500
 gtggagaagg ccacagaggg cgagaacaac agcctgctgc accctatctg ccagcacggc 4560
 atggacgatg aggagcggga agtgcctgac tggaagtctg acagcaggct ggccctgaag 4620
 20 cacagagccc aggaactgca cccagagttc tacaaggact gctga 4665

 <210> 4
 25 <211> 1554
 <212> PRT
 <213> HIV
 <400> 4
 30 Met Ala Ala Arg Ala Ser Ile Leu Ser Gly Gly Lys Leu Asp Ala Trp
 1 5 10 15
 35 Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Arg Leu Lys
 20 25 30
 40 His Leu Val Trp Ala Ser Arg Glu Leu Asp Arg Phe Ala Leu Asn Pro
 35 40 45
 45 Ser Leu Leu Glu Thr Thr Glu Gly Cys Gln Gln Ile Met Asn Gln Leu
 50 55 60
 50 Gln Pro Ala Val Lys Thr Gly Thr Glu Glu Ile Lys Ser Leu Phe Asn
 65 70 75 80
 55 Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Asp Val Lys Asp
 85 90 95
 60 Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Ile Gln Asn Lys Ser Lys
 100 105 110
 Gln Lys Thr Gln Gln Ala Ala Ala Asp Thr Gly Asp Ser Ser Lys Val
 115 120 125
 65 Ser Gln Asn Tyr Pro Ile Ile Gln Asn Ala Gln Gly Gln Met Ile His
 130 135 140
 Gln Asn Leu Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu
 145 150 155 160

	Glu	Lys	Ala	Phe	Ser	Pro	Glu	Val	Ile	Pro	Met	Phe	Ser	Ala	Leu	Ser
					165					170					175	
5																
	Glu	Gly	Ala	Thr	Pro	Gln	Asp	Leu	Asn	Val	Met	Leu	Asn	Ile	Val	Gly
				180					185					190		
10																
	Gly	His	Gln	Ala	Ala	Met	Gln	Met	Leu	Lys	Asp	Thr	Ile	Asn	Glu	Glu
			195					200					205			
15																
	Ala	Ala	Glu	Trp	Asp	Arg	Leu	His	Pro	Val	Gln	Ala	Gly	Pro	Ile	Pro
		210					215					220				
20																
	Pro	Gly	Gln	Ile	Arg	Glu	Pro	Arg	Gly	Ser	Asp	Ile	Ala	Gly	Thr	Thr
	225					230					235					240
25																
	Ser	Thr	Pro	Gln	Glu	Gln	Leu	Gln	Trp	Met	Thr	Gly	Asn	Pro	Pro	Ile
				245						250					255	
30																
	Pro	Val	Gly	Asn	Ile	Tyr	Lys	Arg	Trp	Ile	Ile	Leu	Gly	Leu	Asn	Lys
				260					265					270		
35																
	Ile	Val	Arg	Met	Tyr	Ser	Pro	Val	Ser	Ile	Leu	Asp	Ile	Lys	Gln	Gly
			275					280					285			
40																
	Pro	Lys	Glu	Pro	Phe	Arg	Asp	Tyr	Val	Asp	Arg	Phe	Phe	Lys	Ala	Leu
		290					295					300				
45																
	Arg	Ala	Glu	Gln	Ala	Thr	Gln	Asp	Val	Lys	Gly	Trp	Met	Thr	Glu	Thr
	305					310					315					320
50																
	Leu	Leu	Val	Gln	Asn	Ala	Asn	Pro	Asp	Cys	Lys	Ser	Ile	Leu	Lys	Ala
				325						330					335	
55																
	Leu	Gly	Ser	Gly	Ala	Thr	Leu	Glu	Glu	Met	Met	Thr	Ala	Cys	Gln	Gly
				340					345					350		
60																
	Val	Gly	Gly	Pro	Gly	His	Lys	Ala	Arg	Val	Leu	Ala	Glu	Ala	Met	Ser
			355					360					365			
65																
	Gln	Ala	Gln	Gln	Thr	Asn	Ile	Met	Met	Gln	Arg	Gly	Asn	Phe	Arg	Gly
		370					375					380				
70																
	Gln	Lys	Arg	Ile	Lys	Cys	Phe	Asn	Cys	Gly	Lys	Glu	Gly	His	Leu	Ala
	385					390					395					400
75																
	Arg	Asn	Cys	Arg	Ala	Pro	Arg	Lys	Lys	Gly	Cys					

68

Gly Lys Ile Trp Pro Ser Ser Lys Gly Arg Pro Gly Asn Phe Pro Gln
 435 440 445
 5
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Ala Glu Leu Phe Gly Met Gly
 450 455 460
 10
 Glu Gly Ile Ala Ser Leu Pro Lys Gln Glu Gln Lys Asp Arg Glu Gln
 465 470 475 480
 15
 Val Pro Pro Leu Val Ser Leu Lys Ser Leu Phe Gly Asn Asp Pro Leu
 485 490 495
 20
 Ser Gln Gly Ser Pro Ile Ser Pro Ile Glu Thr Val Pro Val Thr Leu
 500 505 510
 Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu
 515 520 525
 25
 Glu Lys Ile Lys Ala Leu Thr Glu Ile Cys Thr Glu Met Glu Lys Glu
 530 535 540
 30
 Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Ile
 545 550 555 560
 35
 Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp
 565 570 575
 40
 Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu
 580 585 590
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val
 595 600 605
 45
 Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asn Phe
 610 615 620
 50
 Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Thr Asn Asn Glu Thr Pro
 625 630 635 640
 55
 Gly Val Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser
 645 650 655
 60
 Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg
 660 665 670
 Ser Lys Asn Pro Glu Ile Ile Ile Tyr Gln Tyr Met Ala Ala Leu Tyr
 675 680 685
 65
 Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu
 690 695 700

69

Leu Arg Ala His Leu Leu Ser Trp Gly Phe Thr Thr Pro Asp Lys Lys
 705 710 715 720
 5
 His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro
 725 730 735
 10
 Asp Lys Trp Thr Val Gln Pro Ile Met Leu Pro Asp Lys Glu Ser Trp
 740 745 750
 15
 Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser
 755 760 765
 20
 Gln Ile Tyr Ala Gly Ile Lys Val Lys Gln Leu Cys Arg Leu Leu Arg
 770 775 780
 Gly Ala Lys Ala Leu Thr Asp Ile Val Thr Leu Thr Glu Glu Ala Glu
 785 790 795 800
 25
 Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Asp Pro Val His Gly
 805 810 815
 30
 Val Tyr Tyr Asp Pro Ser Lys Asp Leu Val Ala Glu Ile Gln Lys Gln
 820 825 830
 35
 Gly Gln Asp Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn
 835 840 845
 40
 Leu Lys Thr Gly Lys Tyr Ala Arg Lys Arg Ser Ala His Thr Asn Asp
 850 855 860
 Val Arg Gln Leu Ala Glu Val Val Gln Lys Val Ala Met Glu Ser Ile
 865 870 875 880
 45
 Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu
 885 890 895
 50
 Thr Trp Glu Thr Trp Trp Met Asp Tyr Trp Gln Ala Thr Trp Ile Pro
 900 905 910
 55
 Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln
 915 920 925
 60
 Leu Glu Lys Asp Pro Ile Leu Gly Ala Glu Thr Phe Tyr Val Asp Gly
 930 935 940
 Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asp
 945 950 955 960
 65
 Arg Gly Arg Gln Lys Val Val Ser Leu Thr Glu Thr Thr Asn Gln Lys
 965 970 975

70

Thr Glu Leu His Ala Ile Leu Leu Ala Leu Gln Asp Ser Gly Ser Glu
 980 985 990

5

Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala
 995 1000 1005

10

Gln Pro Asp Arg Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu
 1010 1015 1020

15

Lys Leu Ile Gly Lys Asp Lys Ile Tyr Leu Ser Trp Val Pro Ala
 1025 1030 1035

20

His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser
 1040 1045 1050

Ser Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala
 1055 1060 1065

25

Gln Glu Asp His Glu Arg Tyr His Ser Asn Trp Arg Thr Met Ala
 1070 1075 1080

30

Ser Asp Phe Asn Leu Pro Pro Ile Val Ala Lys Glu Ile Val Ala
 1085 1090 1095

35

Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 1100 1105 1110

40

Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu
 1115 1120 1125

Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr
 1130 1135 1140

45

Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala
 1145 1150 1155

50

Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Val Val
 1160 1165 1170

55

His Thr Ala Asn Gly Ser Asn Phe Thr Ser Ala Ala Val Lys Ala
 1175 1180 1185

60

Ala Cys Trp Trp Ala Asn Ile Gln Gln Glu Phe Gly Ile Pro Tyr
 1190 1195 1200

Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 1205 1210 1215

65

Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys
 1220 1225 1230

Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys
 1235 1240 1245
 5
 Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Ile Asp Ile
 1250 1255 1260
 10
 Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr
 1265 1270 1275
 15
 Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asp Pro
 1280 1285 1290
 20
 Ile Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala
 1295 1300 1305
 Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg
 1310 1315 1320
 25
 Lys Ala Lys Ile Leu Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp
 1325 1330 1335
 30
 Asp Cys Val Ala Gly Arg Gln Asp Glu Asp Arg Ser Met Gly Gly
 1340 1345 1350
 35
 Lys Trp Ser Lys Gly Ser Ile Val Gly Trp Pro Glu Ile Arg Glu
 1355 1360 1365
 40
 Arg Met Arg Arg Ala Pro Ala Ala Ala Pro Gly Val Gly Ala Val
 1370 1375 1380
 Ser Gln Asp Leu Asp Lys His Gly Ala Ile Thr Ser Ser Asn Ile
 1385 1390 1395
 45
 Asn Asn Pro Ser Cys Val Trp Leu Glu Ala Gln Glu Glu Glu
 1400 1405 1410
 50
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr
 1415 1420 1425
 55
 Tyr Lys Gly Ala Phe Asp Leu Ser His Phe Leu Lys Glu Lys Gly
 1430 1435 1440
 60
 Gly Leu Asp Gly Leu Ile Tyr Ser Arg Lys Arg Gln Glu Ile Leu
 1445 1450 1455
 Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln
 1460 1465 1470
 65
 Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe Gly
 1475 1480 1485

Ala Thr Glu Gly Glu Asn Asn Ser Leu Leu His Pro Ile Cys Gln
1505 1510 1515

His Gly Met Asp Asp Glu Glu Arg Glu Val Leu Ile Trp Lys Phe
1520 1525 1530

Asp Ser Arg Leu Ala Leu Lys His Arg Ala Gln Glu Leu His Pro
1535 1540 1545

<210>	5
<211>	2025
<212>	DNA
<213>	HIV

<400>	5						
atgaggggtga	tggagatcca	gcggaactgc	cagcacctgc	tgagatgggg	catcatgatc		60
ctgggcatga	ttatcatctg	cagcaccgcc	gacaacctgt	gggtgacCGT	gtactacggc		120
gtgccttgtt	ggagagatgc	cgagaccacc	ctgttctgcg	ccagcgagcg	caaggcctac		180
agcaccgaga	agcacaatgt	gtggggccacc	cacgcctgcg	tgcctaccga	tcccaaacct		240
caggagatcc	ccttgacaa	cgtgaccgag	gagttcaaca	tgtggaagaa	caacatggtg		300
gaccagatgc	acgaggacat	catcagcctg	tgggaccaga	gcctgaagcc	ctgcgtgcag		360
ctgaccccc	tgtgcgtgac	cctgaactgc	agcaacGCCA	gagtgaacgc	caccttcaac		420
tccaccgagg	acagggaggg	catgaagaac	tgcagcttca	acatgaccac	cgagctgcgg		480
gataagaagc	agcaggtgta	cagcctgttc	taccggctgg	acatcgagaa	gatcaacagc		540
agcaacaaca	acagcgagta	ccggctggtg	aactgcaata	ccagcgccat	caccagggcc		600
tgcctaagg	tgaccttcga	gcccatcccc	atccactact	gcgcccctgc	cggtctcgcc		660
atcctgaagt	gcaacgacac	cgagttcaat	ggcaccggcc	cctgcaagaa	tgtgagcacc		720
gtgcagtgca	cccacggcat	caagcccgtg	gtgtccaccc	agctgctgct	gaacggcagc		780
ctggccgaga	gagaagtgcg	gatcaggagc	gagaacatcg	ccaacaacgc	caagaacatc		840
atcgtgcagt	tgcCagccc	cgtgaagatc	aactgcatcc	ggcccaacaa	caatacccgg		900
aagagetaca	gaatcgggcc	tggccagacc	ttctacgcca	ccgacattgt	gggcgacatc		960
agacaggccc	actgcaacgt	gtccaggacc	gactggaaca	acaccctgag	actggtggcc		1020
aaccagctgc	ggaagtactt	cagcaacaag	accatcatct	tcaccaacag	cagcggcgga		1080
gacctggaga	tcaccaccca	cagcttcaat	tgtggcgggc	agtctcttcta	ctgcaacacc		1140
tccggcctgt	tcaatagcac	ctggaccacc	aacaacatgc	aggagtccaa	cgacaccagc		1200
aacggcacca	tcaccctgCC	ctgccggatc	aagcagatca	tccg gatgtg	gcagcgcgtg		1260

	ggccaggcca tgtacgcccc tcccatcgag ggcgtgattc gctgcgagag caacatcacc	1320
5	ggcctgatcc tgaccagaga tggcggcaac aacaattccg ccaacgagac cttcagacct	1380
	ggcggcggag atatccggga caactggcgg agcgagctgt acaagtacaa ggtggtgaag	1440
	atcgagcccc tgggcgtggc ccccaccaga gccaagagaa gagtggtgga gcgggagaag	1500
10	agagccgtgg gcatcggcgc cgtgtttctg ggcttcctgg gagccgccgg atctacaatg	1560
	ggagccgcca gcatcaccct gaccgtgcag gccagacagc tgctgagcgg catcgtgcag	1620
15	cagcagagca atctgctgag agccatcgag gcccagcagc agctgctgaa gctgacagt	1680
	tggggcatca agcagctgca ggccagggtg ctggccgtgg agagatacct gagggaccag	1740
	cagctcctgg gcatctgggg ctgcagcggc aagctgatct gcaccaccaa cgtgccctgg	1800
20	aatagcagct ggagcaacaa gagctacgac gacatctggc agaacatgac ctggctgcag	1860
	tgggacaagg agatcagcaa ctacaccgac atcatctaca gcctgatcga ggagagccag	1920
25	aaccagcagg agaagaacga gcaggatctg ctggccctgg acaagtgggc caacctgtgg	1980
	aactggttcg acatcagcaa gtggctgtgg tacatcagat cttga	2025
30	<210> 6 <211> 674 <212> PRT <213> HIV	
35	<400> 6	
	Met Arg Val Met Glu Ile Gln Arg Asn Cys Gln His Leu Leu Arg Trp	
	1 5 10 15	
40	Gly Ile Met Ile Leu Gly Met Ile Ile Ile Cys Ser Thr Ala Asp Asn	
	20 25 30	
45	Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Arg Asp Ala Glu	
	35 40 45	
50	Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Ser Thr Glu Lys	
	50 55 60	
55	His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro	
	65 70 75 80	
60	Gln Glu Ile Pro Leu Asp Asn Val Thr Glu Glu Phe Asn Met Trp Lys	
	85 90 95	
65	Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp	
	100 105 110	
	Gln Ser Leu Lys Pro Cys Val Gln Leu Thr Pro Leu Cys Val Thr Leu	
	115 120 125	
	Asn Cys Ser Asn Ala Arg Val Asn Ala Thr Phe Asn Ser Thr Glu Asp	

74

	130		135		140												
5	Arg 145	Glu	Gly	Met	Lys	Asn 150	Cys	Ser	Phe	Asn 155	Met	Thr	Thr	Glu	Leu	Arg 160	
10	Asp	Lys	Lys	Gln	Gln 165	Val	Tyr	Ser	Leu	Phe 170	Tyr	Arg	Leu	Asp	Ile 175	Glu	
15	Lys	Ile	Asn	Ser	Ser 180	Asn	Asn	Asn	Ser	Glu	Tyr	Arg	Leu	Val	Asn 190	Cys	
20	Asn	Thr	Ser	Ala	Ile 195	Thr	Gln	Ala	Cys 200	Pro	Lys	Val	Thr	Phe	Glu	Pro	
25	Ile	Pro	Ile	His	Tyr	Cys 215	Ala	Pro	Ala	Gly	Phe	Ala 220	Ile	Leu	Lys	Cys	
30	Asn	Asp	Thr	Glu	Phe	Asn 230	Gly	Thr	Gly	Pro	Cys 235	Lys	Asn	Val	Ser	Thr 240	
35	Val	Gln	Cys	Thr	His 245	Gly	Ile	Lys	Pro	Val	Val 250	Ser	Thr	Gln	Leu 255	Leu	
40	Leu	Asn	Gly	Ser	Leu 260	Ala	Glu	Arg	Glu	Val 265	Arg	Ile	Arg	Ser	Glu	Asn 270	
45	Ile	Ala	Asn	Asn	Ala 275	Lys	Asn	Ile	Ile	Val 280	Gln	Phe	Ala 285	Ser	Pro	Val	
50	Lys	Ile	Asn	Cys	Ile 290	Arg	Pro	Asn	Asn	Asn 295	Thr	Arg 300	Lys	Ser	Tyr	Arg	
55	Ile	Gly	Pro	Gly	Gln 305	Thr	Phe	Tyr	Ala	Thr 310	Asp	Ile 315	Val	Gly	Asp	Ile 320	
60	Arg	Gln	Ala	His	Cys 325	Asn	Val	Ser	Arg	Thr 330	Asp	Trp	Asn	Asn	Thr 335	Leu	
65	Arg	Leu	Val	Ala	Asn 340	Gln	Leu	Arg	Lys	Tyr 345	Phe	Ser	Asn	Lys 350	Thr	Ile	
70	Ile	Phe	Thr	Asn	Ser 355	Ser	Gly	Gly	Asp	Leu 360	Glu	Ile	Thr 365	Thr	His	Ser	
75	Phe	Asn	Cys	Gly	Gly 370	Glu	Phe	Phe	Tyr	Cys 375	Asn	Thr 380	Ser	Gly	Leu	Phe	
80	Asn	Ser	Thr	Trp	Thr 385	Thr	Asn	Asn	Met	Gln 390	Glu	Ser 395	Asn	Asp	Thr	Ser 400	
85	Asn	Gly	Thr	Ile	Thr	Leu	Pro	Cys	Arg	Ile	Lys	Gln	Ile	Ile	Arg	Met	

[illegible]

5	<210>	7																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		</
---	-------	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	----

77

Met Lys Val Lys Glu Thr Arg Lys Asn Tyr Gln His Leu Trp Arg Trp
 1 5 10 15
 5 Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Ala Glu Gln
 20 25 30
 10 Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr
 35 40 45
 15 Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
 50 55 60
 20 His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
 65 70 75 80
 Gln Glu Val Val Leu Gly Asn Val Thr Glu Tyr Phe Asn Met Trp Lys
 85 90 95
 25 Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp
 100 105 110
 30 Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu
 115 120 125
 35 Asp Cys Asp Asp Val Asn Thr Thr Asn Ser Thr Thr Thr Thr Ser Asn
 130 135 140
 40 Gly Trp Thr Gly Glu Ile Arg Lys Gly Glu Ile Lys Asn Cys Ser Phe
 145 150 155 160
 Asn Ile Thr Thr Ser Ile Arg Asp Lys Val Gln Lys Glu Tyr Ala Leu
 165 170 175
 45 Phe Tyr Asn Leu Asp Val Val Pro Ile Asp Asp Asp Asn Ala Thr Thr
 180 185 190
 50 Lys Asn Lys Thr Thr Arg Asn Phe Arg Leu Ile His Cys Asn Ser Ser
 195 200 205
 55 Val Met Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile
 210 215 220
 60 His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn Lys
 225 230 235 240
 Thr Phe Asp Gly Lys Gly Leu Cys Thr Asn Val Ser Thr Val Gln Cys
 245 250 255
 65 Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly
 260 265 270

78

Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Asp Asn Phe Met Asp
 275 280 285
 5
 Asn Thr Lys Thr Ile Ile Val Gln Leu Asn Glu Ser Val Ala Ile Asn
 290 295 300
 10
 Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Gly Ile His Ile Gly Pro
 305 310 315 320
 15
 Gly Arg Ala Phe Tyr Ala Ala Arg Lys Ile Ile Gly Asp Ile Arg Gln
 325 330 335
 20
 Ala His Cys Asn Leu Ser Arg Ala Gln Trp Asn Asn Thr Leu Lys Gln
 340 345 350
 Ile Val Ile Lys Leu Arg Glu His Phe Gly Asn Lys Thr Ile Lys Phe
 355 360 365
 25
 Asn Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Arg His Ser Phe Asn
 370 375 380
 30
 Cys Gly Gly Glu Phe Phe Tyr Cys Asp Thr Thr Gln Leu Phe Asn Ser
 385 390 395 400
 35
 Thr Trp Asn Gly Thr Glu Gly Asn Asn Thr Glu Gly Asn Ser Thr Ile
 405 410 415
 40
 Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val
 420 425 430
 Gly Lys Ala Met Tyr Ala Pro Pro Ile Gly Gly Gln Ile Arg Cys Ser
 435 440 445
 45
 Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Thr Glu Gly
 450 455 460
 50
 Asn Gly Thr Glu Asn Glu Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp
 465 470 475 480
 55
 Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys
 485 490 495
 60
 Val Glu Pro Leu Gly Val Ala Pro Thr Arg Ala Lys Arg Arg Val Val
 500 505 510
 Gln Arg
 65
 <210> 9
 <211> 2178
 <212> DNA

<213> Mycobacterium tuberculosis

<400> 9

5	atgcatcaca cggccgcgtc cgataacttc cagctgtccc aggggtgggca gggattcgcc	60
	attccgatcg ggcaggcgat ggcgatcgcg ggccagatcc gatcgggtgg ggggtcaccc	120
	accgttcata tcgggcctac cgccttcctc ggcttgggtg ttgtcgacaa caacggcaac	180
10	ggcgcacgag tccaacgcgt ggtcgggagc gctccggcgg caagtctcgg catctccacc	240
	ggcgacgtga tcaccgcggt cgacggcgct ccgatcaact cggccaccgc gatggcggac	300
15	gcgcttaacg ggcacatccc cggtgacgtc atctcggtga cctggcaaac caagtcgggc	360
	ggcacgcgta cagggaacgt gacattggcc gagggacccc cggccgaatt catggtggat	420
	ttcggggcgt taccaccgga gatcaactcc gcgaggatgt acgcggcccc gggttcggcc	480
20	tcgctggtgg ccgcggctca gatgtgggac agcgtggcga gtgacctgtt ttcggccgcg	540
	tcggcgtttc agtcggtggt ctggggtctg acggtggggt cgtggatagg ttcgtcggcg	600
25	ggtctgatgg tggcggcggc ctgcgcgtat gtggcggtga tgagcgtcac cgcggggcag	660
	gccgagctga ccgccgcccc ggtccggggt gctgcggcgg cctacgagac ggcgtatggg	720
	ctgacgggtg ccccgccggt gatcgccgag aacctgtctg aactgatgat tctgatagcg	780
30	accaaacctct tggggcaaaa cccccggcg atcgcggtca acgaggccga atacggcgag	840
	atgtgggccc aagacgcgcg cgcgatgttt ggctacgcg cggcgacggc gacggcgcg	900
35	gcgacgttgc tgccgttcga ggaggcgccg gagatgacca gcgcgggtgg gctcctcgag	960
	caggccgcgg cggtcgagga ggctccgac accgccgcgg cgaaccagtt gatgaacaat	1020
	gtgccccagg cgctgcaaca gctggcccag ccacgcagg gcaccacgcc ttcttccaag	1080
40	ctgggtggcc tgtggaagac ggtctcgccg catcggtcgc cgatcagcaa catggtgtcg	1140
	atggccaaca accacatgtc gatgaccaac tcgggtgtgt cgatgaccaa caccttgagc	1200
45	tcgatgttga agggctttgc tccggcgcg cccgccagg ccgtgcaaac cgcggcgcaa	1260
	aacgggggtcc gggcgatgag ctgcgtgggc agctcgctgg gttcttcggg tctgggcgggt	1320
	ggggtggccg ccaacttggg tcgggcggcc tcggtcggtt cgttgctcgt gccgcaggcc	1380
50	tgggcgcggg ccaaccaggc agtcaccccg gcggcgcggg cgctgccgct gaccagcctg	1440
	accagcgccg cggaaagagg gcccgggcag atgctgggcg ggctgccggt ggggcagatg	1500
55	ggcgccaggg ccggtggtgg gctcagtgg gtgctgcgtg ttccgcccg accctatgtg	1560
	atgccgcatt ctccggcagc cggcgatatc gcccgcggc ccttgctcga ggaccggttc	1620
	gccgaacttc ccgcgtgcc cctcgaccgg tccgcgatgg tcgcccaagt ggggccacag	1680
60	gtggtcaaca tcaacaccaa actgggctac aacaacgcgg tgggcgcggg gaccggcatc	1740
	gtcatcgatc ccaacggtgt cgtgctgacc aacaaccacg tgatcgcggg cggcaccgac	1800
65	atcaatgcgt tcagcgtcgg ctccggccaa acctacggcg tcgatgtggt cgggtatgac	1860
	cgcacccagg atgtcgcggt gctgcagctg cgcggtgccg gtggcctgcc gtcggcgggc	1920
	atcgggtggcg gcgtcgcggt tggtagcccc gtcgtcgcga tgggcaacag cgggtgggcag	1980

80

5 ggcggaacgc cccgtgcggt gcctggcagg gtggtcgcgc tcggccaaac cgtgcaggcg 2040
 tcggattcgc tgaccggtgc cgaagagaca ttgaacgggt tgatccagtt cgatgccgcg 2100
 atccagcccc gtgatgcggg cgggcccgtc gtcaacggcc taggacaggt ggtcggtatg 2160
 aacacggccg cgtcctag 2178
 10 <210> 10
 <211> 725
 <212> PRT
 <213> Mycobacterium tuberculosis
 15 <400> 10
 Met His His Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly
 20 1 5 10 15
 Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln
 20 25 30
 25 Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala
 35 40 45
 30 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
 50 55 60
 35 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
 65 70 75 80
 40 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
 85 90 95
 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
 100 105 110
 45 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
 115 120 125
 50 Leu Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu
 130 135 140
 55 Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala
 145 150 155 160
 Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu
 165 170 175
 60 Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val
 180 185 190
 65 Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser
 195 200 205

81

5 Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr
 210 215 220
 Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly
 225 230 235 240
 10 Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met
 245 250 255
 15 Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala
 260 265 270
 Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala
 275 280 285
 20 Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu
 290 295 300
 25 Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu
 305 310 315 320
 30 Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln
 325 330 335
 35 Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr
 340 345 350
 40 Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val
 355 360 365
 Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn
 370 375 380
 45 His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser
 385 390 395 400
 50 Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Gln Ala Val Gln
 405 410 415
 55 Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser
 420 425 430
 60 Leu Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg
 435 440 445
 Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala
 450 455 460
 65 Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu
 465 470 475 480

82

Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro
 485 490 495
 5
 Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu
 500 505 510
 10
 Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly
 515 520 525
 15
 Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro
 530 535 540
 20
 Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln
 545 550 555 560
 Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala
 565 570 575
 25
 Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn
 580 585 590
 30
 His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser
 595 600 605
 35
 Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp
 610 615 620
 40
 Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala
 625 630 635 640
 Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn
 645 650 655
 45
 Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val
 660 665 670
 50
 Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu
 675 680 685
 55
 Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly
 690 695 700
 60
 Asp Ala Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met
 705 710 715 720
 Asn Thr Ala Ala Ser
 725
 65
 <210> 11
 <211> 1149
 <212> DNA

<213> Plasmodium falciparum

<400> 11

5 atgatgagaa aacttgccat cctcagcgtc agctctttcc tgttcgtgga ggccctcttc 60
 caggagtatc agtgctacgg aagcagcagc aatacaaggg tcttgaacga gctcaactat 120
 gacaacgctg gaacgaacct gtataacgag ctggagatga actactatgg caagcaggag 180
 10 aactgggtata gcctgaagaa gaacagccgg tccctgggcg agaacgacga cggcaacaac 240
 aacaacggcg acaacggcag ggagggcaaa gatgaggaca agagggacgg gaacaacgag 300
 15 gataacgaga agctgcggaa gccaagcac aagaaactca agcagcccg cgcgagggaac 360
 ccggacccca atgcaaattc caacgtcgac ccaaacgcaa accctaacgt ggaccccaac 420
 gccaatccca acgtcgatcc taatgccaat ccaaattgcca accctaacgc aaatcctaatt 480
 20 gcaaaccacca acgccaattc taacgccaac ccaaattgcca acccaaacgc taaccaccaac 540
 gctaaccacca atgcaaattc caatgctaac ccaaacgtgg accctaacgc taaccaccaac 600
 25 gcaaacccta acgccaattc taacgcaaac ccaattgcaa acccaaacgc aaatcccaac 660
 gctaacccta acgcaaaccc caacgccaac cctaattgcaa accccaatgc taaccaccaac 720
 gccaatccca acgcaaatcc aaacgccaac ccaaattgcaa accccaacgc taatcccaac 780
 30 gccaaaccaca acgccaattc taacaagaac aatcagggca acgggcaggg ccataacatg 840
 ccgaacgacc ctaatcgga tgtggacgag aacgccaacg ccaacagcgc cgtgaagaac 900
 35 aacaacaacg aggagccctc cgacaagcac atcaaggaat acctgaacaa gatccagaac 960
 agtctgagca ccgagtggtc cccctgctcc gtgacctgag gcaacggcat ccagggtgagg 1020
 atcaagcccg gctccgcca caagcccaag gacgagctgg actacgcca cgacatcgag 1080
 40 aagaagatct gcaagatgga gaaatgcagc tctgtgttca acgtcgtgaa ctccgccatc 1140
 ggctctgtga 1149

45 <210> 12
 <211> 382
 <212> PRT
 <213> Plasmodium falciparum

50 <400> 12

Met Met Arg Lys Leu Ala Ile Leu Ser Val Ser Ser Phe Leu Phe Val
 1 5 10 15

55 Glu Ala Leu Phe Gln Glu Tyr Gln Cys Tyr Gly Ser Ser Ser Asn Thr
 20 25 30

60 Arg Val Leu Asn Glu Leu Asn Tyr Asp Asn Ala Gly Thr Asn Leu Tyr
 35 40 45

65 Asn Glu Leu Glu Met Asn Tyr Tyr Gly Lys Gln Glu Asn Trp Tyr Ser
 50 55 60

Leu Lys Lys Asn Ser Arg Ser Leu Gly Glu Asn Asp Asp Gly Asn Asn

84

	65				70					75					80	
5	Asn	Asn	Gly	Asp	Asn	Gly	Arg	Glu	Gly	Lys	Asp	Glu	Asp	Lys	Arg	Asp
					85					90					95	
10	Gly	Asn	Asn	Glu	Asp	Asn	Glu	Lys	Leu	Arg	Lys	Pro	Lys	His	Lys	Lys
				100					105					110		
15	Leu	Lys	Gln	Pro	Ala	Asp	Gly	Asn	Pro	Asp	Pro	Asn	Ala	Asn	Pro	Asn
			115					120					125			
20	Val	Asp	Pro	Asn	Ala	Asn	Pro	Asn	Val	Asp	Pro	Asn	Ala	Asn	Pro	Asn
			130				135					140				
25	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn
				165					170					175		
30	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn
				180					185					190		
35	Val	Asp	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn
			195					200					205			
40	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn
	225				230					235					240	
45	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn
				245					250					255		
50	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Ala	Asn	Pro	Asn	Lys	Asn	Asn	Gln
				260					265					270		
55	Gly	Asn	Gly	Gln	Gly	His	Asn	Met	Pro	Asn	Asp	Pro	Asn	Arg	Asn	Val
			275					280					285			
60	Asp	Glu	Asn	Ala	Asn	Ala	Asn	Ser	Ala	Val	Lys	Asn	Asn	Asn	Asn	Glu
	290						295					300				
65	Glu	Pro	Ser	Asp	Lys	His	Ile	Lys	Glu	Tyr	Leu	Asn	Lys	Ile	Gln	Asn
	305					310					315					320
65	Ser	Leu	Ser	Thr	Glu	Trp	Ser	Pro	Cys	Ser	Val	Thr	Cys	Gly	Asn	Gly
				325						330					335	
	Ile	Gln	Val	Arg	Ile	Lys	Pro	Gly	Ser	Ala	Asn	Lys	Pro	Lys	Asp	Glu

85

340

345

350

5	Leu Asp Tyr Ala Asn Asp Ile Glu Lys Lys Ile Cys Lys Met Glu Lys 355 360 365	
10	Cys Ser Ser Val Phe Asn Val Val Asn Ser Ala Ile Gly Leu 370 375 380	
15	<210> 13 <211> 1275 <212> DNA <213> Plasmodium falciparum	
20	<400> 13 atgatggctc ccgatcctaa tgcaaatcca aatgcaaacc caaacgcaaa ccccaatgca aatcctaata ccaaccccaa tgcaaatcct aatgcaaacc ctaatgccaa tccaaatgca aatccaaatg ccaaccccaa cgcacacccc aatgcaaacc ctaatgccaa tccaaatgca aatccaaatg ccaaccccaa tgcaaaccca aatgcaaacc ccaatgcaaa tcctaataaa aacaatcaag gtaatggaca aggtcacaaat atgccaaatg acccaaacccg aaatgtagat gaaaaatgcta atgccaacag tgctgtaaaa aataataata acgaagaacc aagtataag cacataaaag aatatttaaa caaaatacaa aattctcttt caactgaatg gtcccatgt agtgtacttt gtggaaatgg tattcaagtt agaataaagc ctggctctgc taataaacct aaagacgaat tagattatgc aaatgatatt gaaaaaaaaa ttgtataaat ggaaaaatgt tccagtgtgt ttaatgtcgt aaatagttca ataggattag ggctgtgac gaacatggag aacatcacat caggattcct aggcacctg ctctgtttac aggcgggggt tttctgttg acaagaatcc tcacaatacc gcagagtcta gactcgtggg ggacttctct caattttcta gggggatcac ccgtgtgtct tggccaaaat tcgcagtcac caacctcaa tcaactacca acctcctgtc ctccaatttg tctgtgttat cgtgtgatgt gtctgcggcg tttatcata ttcctcttca tctgtgtgt atgcctcacc ttcttatttg ttcttctgga ttatcaaggt atgttgcccc tttgtcctct aattccagga tcaacaacaa ccaatacggg accatgcaaa acctgcacga ctctgtctca aggcaactct atgtttccct catgttgctg tacaaaacct acggatggaa attgcacctg tattcccatc ccatcgtcct gggttttcgc aaaataccta tgaggatggg cctcagtcct tttctcttgg ctctgtttac tagtgccatt tgttcagtgg ttcgtagggc tttccccac tggttggtt tcagctatat ggatgatgtg gtattggggg ccaagtctgt acagcatcgt ggtcccttt ataccgtgt taccaatttt ctttctctc tggtatatac tttaa	60 120 180 240 300 360 420 480 540 600 660 720 780 840 900 960 1020 1080 1140 1200 1260 1275
65	<210> 14 <211> 424 <212> PRT <213> Plasmodium falciparum <400> 14	

86

5 Met Met Ala Pro Asp Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
 1 5 10 15
 Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
 20 25 30
 10 Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
 35 40 45
 15 Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala
 50 55 60
 20 Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro Asn Lys
 65 70 75 80
 Asn Asn Gln Gly Asn Gly Gln Gly His Asn Met Pro Asn Asp Pro Asn
 85 90 95
 25 Arg Asn Val Asp Glu Asn Ala Asn Ala Asn Ser Ala Val Lys Asn Asn
 100 105 110
 30 Asn Asn Glu Glu Pro Ser Asp Lys His Ile Lys Glu Tyr Leu Asn Lys
 115 120 125
 35 Ile Gln Asn Ser Leu Ser Thr Glu Trp Ser Pro Cys Ser Val Thr Cys
 130 135 140
 40 Gly Asn Gly Ile Gln Val Arg Ile Lys Pro Gly Ser Ala Asn Lys Pro
 145 150 155 160
 Lys Asp Glu Leu Asp Tyr Ala Asn Asp Ile Glu Lys Lys Ile Cys Lys
 165 170 175
 45 Met Glu Lys Cys Ser Ser Val Phe Asn Val Val Asn Ser Ser Ile Gly
 180 185 190
 50 Leu Gly Pro Val Thr Asn Met Glu Asn Ile Thr Ser Gly Phe Leu Gly
 195 200 205
 55 Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu
 210 215 220
 60 Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu
 225 230 235 240
 Gly Gly Ser Pro Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser
 245 250 255
 65 Asn His Ser Pro Thr Ser Cys Pro Pro Ile Cys Pro Gly Tyr Arg Trp
 260 265 270

87

Met Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys
 275 280 285
 5 Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro Val
 290 295 300
 10 Cys Pro Leu Ile Pro Gly Ser Thr Thr Thr Asn Thr Gly Pro Cys Lys
 305 310 315 320
 15 Thr Cys Thr Thr Pro Ala Gln Gly Asn Ser Met Phe Pro Ser Cys Cys
 325 330 335
 20 Cys Thr Lys Pro Thr Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser
 340 345 350
 25 Ser Trp Ala Phe Ala Lys Tyr Leu Trp Glu Trp Ala Ser Val Arg Phe
 355 360 365
 30 Ser Pro Thr Val Trp Leu Ser Ala Ile Trp Met Met Trp Tyr Trp Gly
 385 390 395 400
 35 Pro Ser Leu Tyr Ser Ile Val Ser Pro Phe Ile Pro Leu Leu Pro Ile
 405 410 415
 40 Phe Phe Cys Leu Trp Val Tyr Ile
 420
 <210> 15
 <211> 3411
 <212> DNA
 45 <213> HIV
 <400> 15
 atggtcattg ttcagaacat acagggccaa atggtccacc aggcaattag tccgcgaact 60
 50 cttaatgcat ggggtgaaggc cgtggaggaa aaggcattct ccccgagggt cattccgatg 120
 ttttctgcgc tatctgaggg cgcaacgccg caagacctta ataccatgct taacacggta 180
 55 ggcgggcacc aagccgctat gcaaagtcta aaagagacta taaacgaaga ggccgccgaa 240
 tgggatcgag tgcacccggc gcacgccggc ccaattgcac caggccagat gcgcgagccg 300
 cgcggggtctg atattgcagg aactacgtct acccttcagg agcagattgg gtggatgact 360
 60 aacaatccac caatcccggc cggagagatc tataagaggc ggatcatact gggactaaac 420
 aagatagtcc gcatgtattc tccgacttct atactggata tacgccaagg cccaaaggag 480
 65 ccgttcaggc actatgtcga ccgattctat aagacccttc gcgcagagca ggcattccag 540
 gaggtcaaaa attggatgac agaaactctt ttggtgcaga atgcgaatcc ggattgtaaa 600
 acaattttaa aggctctagg accggccgca acgctagaag agatgatgac ggcttgtcag 660

	ggagtcggtg gaccggggca taaagcccg	gtcttacaca tgggcccgat atctccgata	720
5	gaaacagttt cgggtcaagct taaaccagg	atggatggtc caaagggtcaa gcagtggccg	780
	ctaacggaag agaagattaa ggcgctcgta	gagatttgta ctgaaatgga gaaggaaggc	840
	aagataagca agatcggggc agagaacccg	tacaatacac cgggtatttgc aataaagaaa	900
10	aaggattcaa caaaatggcg aaagcttgta	gatttttaggg aactaaacaa gcgaacccaa	960
	gacttttggg aagtccaact agggatccca	catccagccg gtctaaagaa gaagaaatcg	1020
15	gtcacagtcc tggatgtagg agacgcata	tttagtgtag cgcttgatga ggacttccga	1080
	aagtatactg cgtttactat accgagcata	aacaatgaaa cgccaggcat tcgctatcag	1140
	tacaacgtgc tcccgaggcg ctggaaggcg	tctccggcga tatttcagag ctgtatgaca	1200
20	aaaataactg aaccattccg aaagcagaat	cgggatattg taatttacca atacatggac	1260
	gatctctatg tgggctcgga tctagaaatt	gggcagcctc gcaactaagat tgaggaactg	1320
25	aggcaacatc tgcttcgatg gggcctcact	actcccgaca agaagcacca gaaggagccg	1380
	ccgttcctaa agatgggcta cgagcttcat	ccggacaagt ggacagtaca gccgatagtg	1440
	ctgcccgaag aggattcttg gaccgtaaat	gatattcaga aactagtcgg caagcttaac	1500
30	tgggcctctc agatttaccc aggcattaag	gtccgacagc tttgcaagct actgagggga	1560
	actaaggctc taacagaggc catccatta	acggaggaag cagagcttga gctggcagag	1620
35	aatcgcaaaa ttcttaagga gccggtgcac	gggtataact acgacccctc caaggacctt	1680
	atagccgaga tccagaagca ggggcaggcg	caatggacgt accagatata tcaagaaccg	1740
	tttaagaatc tgaagactgg gaagtacgcg	cgcattcgag gggctcatalc taatgatgta	1800
40	aagcaactta cggaagcagt acaaaagatt	actactgagt ctattgtgat atggggcaag	1860
	accccaaagt tcaagctgcc catacagaag	gaaacatggg aaacatggtg gactgaatat	1920
45	tggcaagcta cctggattcc agaattgggaa	tttgtcaaca cgccgccact tgtaagctt	1980
	tggtagcagc ttgaaaagga gccgatagta	ggggcagaga ccttctatgt cgatggcgcc	2040
	gcgaatcgcg aaacgaagct aggcaggcg	ggatacgtga ctaatagggg ccgccccaaag	2100
50	gtcgtaacct ttacggatac caccatcag	aagactgaac tacaagcgat ttaccttgca	2160
	cttcaggata gtggcctaga ggtaacata	gtcacggact ctcaatatgc gcttggcatt	2220
55	attcaagcgc agccagatca aagcgaaagc	gagcttgtaa accaaataat agaacagctt	2280
	ataaagaaag agaaggatata tctggcctgg	gtccccgctc acaagggaat tggcggcaat	2340
	gagcaagtgg acaagctagt cagcgctggg	attcgcaagg ttcttgcgat ggggggtaag	2400
60	tgggtetaagt ctacgtagt cggctggccg	acagtcgctg agcgcatgcg acgcgccgaa	2460
	ccagccgcag atggcgtggg ggcagcgtct	agggatctgg agaagcacgg ggctataact	2520
65	tccagtaaca cggcgccgac gaacgccgca	tgcgcarggt tagaagccca agaagaggaa	2580
	gaagtagggg ttccggtaac tccccagggt	ccgttaaggc cgatgacctc taaggcagcg	2640
	gtggatcttt ctcaacttct taaggagaaa	ggggggctgg agggcttaat tcacagccag	2700

	agggcgacagg atattcttga tctgtggatt taccataccc aggggtactt tccggactgg	2760
5	cagaattaca ccccgggggcc aggcgtgccc tatccctga ctttcgggtg gtgctacaaa	2820
	ctagtcccag tggaaccoga caaggtegaa gaggctaata agggcgagaa cacttctctt	2880
	cttccaccgg taagcctgca cgggatggat gaccagaac gagaggttct agaatggagg	2940
10	ttcgactctc gacttgcggt ccatcacgta gcacgcgagc tgcattcaga atatttcaag	3000
	aactgccgcc caatggggcgc cagggccagt gtacttagtg gcggagaact agatcgatgg	3060
15	gaaaagatac gcctacgccc ggggggcaag aagaagtaca agcttaagca cattgtgtgg	3120
	gcctctcgcg aacttgagcg attcgagtg aatccaggcc tgcttgagac gagtgaaggc	3180
	tgtaggcaaa ttctggggca gctacagccg agcctacaga ctggcagcga ggagcttcgt	3240
20	agtctttata ataccgtcgc gactctctac tgcgttcac aacgaattga aataaaggat	3300
	actaaagagg cccttgataa aattgaggag gaacagaata agtcgaaaaa gaaggcccag	3360
25	caggccgccc ccgacaccgg gcacagcaac caggtgtccc aaaactacta a	3411
	<210> 16	
	<211> 1136	
30	<212> PRT	
	<213> HIV	
	<400> 16	
35	Met Val Ile Val Gln Asn Ile Gln Gly Gln Met Val His Gln Ala Ile	
	1 5 10 15	
	Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala	
40	20 25 30	
	Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala	
	35 40 45	
45	Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln	
	50 55 60	
50	Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu	
	65 70 75 80	
55	Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln	
	85 90 95	
	Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu	
60	100 105 110	
	Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly	
	115 120 125	
65	Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg	
	130 135 140	

90

Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu
 145 150 155 160
 5
 Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu
 165 170 175
 10
 Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val
 180 185 190
 15
 Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro
 195 200 205
 20
 Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly
 210 215 220
 Pro Gly His Lys Ala Arg Val Leu His Met Gly Pro Ile Ser Pro Ile
 225 230 235 240
 25
 Glu Thr Val Ser Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 245 250 255
 30
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 260 265 270
 35
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 275 280 285
 40
 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 290 295 300
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 305 310 315 320
 45
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 325 330 335
 50
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 340 345 350
 55
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 355 360 365
 60
 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 370 375 380
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Cys Met Thr
 385 390 395 400
 65
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 405 410 415

Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 420 425 430
 5
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 435 440 445
 10
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Lys
 450 455 460
 15
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 465 470 475 480
 20
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 485 490 495
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 500 505 510
 25
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 515 520 525
 30
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 530 535 540
 35
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 545 550 555 560
 40
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 565 570 575
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 580 585 590
 45
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 595 600 605
 50
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 610 615 620
 55
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 625 630 635 640
 60
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 645 650 655
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 660 665 670
 65
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 675 680 685

92

Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 690 695 700

5

Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
 705 710 715 720

10

Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 725 730 735

15

Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 740 745 750

20

Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 755 760 765

25

Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 770 775 780

30

Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Ala Met Gly Gly Lys
 785 790 795 800

35

Trp Ser Lys Ser Ser Val Val Gly Trp Pro Thr Val Arg Glu Arg Met
 805 810 815

40

Arg Arg Ala Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp
 820 825 830

45

Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn
 835 840 845

50

Ala Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Glu Val Gly Phe
 850 855 860

55

Pro Val Thr Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Ala Ala
 865 870 875 880

60

Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu
 885 890 895

65

Ile His Ser Gln Arg Arg Gln Asp Ile Leu Asp Leu Trp Ile Tyr His
 900 905 910

Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly
 915 920 925

Val Arg Tyr Pro Leu Thr Phe Gly Trp Cys Tyr Lys Leu Val Pro Val
 930 935 940

Glu Pro Asp Lys Val Glu Glu Ala Asn Lys Gly Glu Asn Thr Ser Leu
 945 950 955 960

93

Leu His Pro Val Ser Leu His Gly Met Asp Asp Pro Glu Arg Glu Val
 965 970 975
 5
 Leu Glu Trp Arg Phe Asp Ser Arg Leu Ala Phe His His Val Ala Arg
 980 985 990
 10
 Glu Leu His Pro Glu Tyr Phe Lys Asn Cys Arg Pro Met Gly Ala Arg
 995 1000 1005
 15
 Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp Glu Lys Ile
 1010 1015 1020
 20
 Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile
 1025 1030 1035
 Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly
 1040 1045 1050
 25
 Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu
 1055 1060 1065
 30
 Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr
 1070 1075 1080
 35
 Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile
 1085 1090 1095
 40
 Lys Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn
 1100 1105 1110
 Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly His
 1115 1120 1125
 45
 Ser Asn Gln Val Ser Gln Asn Tyr
 1130 1135
 50
 55

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

5

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 but not to the exclusion of any other integer, step, group of integers or group of steps.

10

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:

15

Claims

1. A method of raising an immune response against a pathogen which comprises administering (i) one or more first immunogenic polypeptides derived from said pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more first immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered concomitantly.
2. A method of raising an immune response against a pathogen which comprises administering (i) one or more first immunogenic polypeptides derived from said pathogen co-formulated with an adjuvant; and (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; wherein one or more immunogenic polypeptides and adjuvant, and one or more adenoviral vectors are administered concomitantly.
3. A method of stimulating the production of pathogen-specific CD4+ and/or CD8+ T-cells and/or antibodies in mammals which comprises administering to said mammal (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more first immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered concomitantly, for example by administering an immunologically effective amount of an aforeseaid composition.
4. A method of raising an immune response against a pathogen which consists of (a) administering (i) one or more first immunogenic polypeptides derived from said pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more immunogenic polypeptide, the one or more adenoviral vector and the adjuvant are administered concomitantly; and (b) optionally repeating the steps of (a).
5. A method of raising an immune response against a pathogen which comprises administering (i) one or more first immunogenic polypeptides derived from said pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant; wherein the one or more first immunogenic polypeptides, the one or more adenoviral vectors and the adjuvant are administered concomitantly; and wherein the method does not involve administering any priming dose of immunogenic polypeptide or polynucleotide encoding immunogenic polypeptide.

6. A method according to any one of claims 1 to 5 wherein one or more immunogenic polypeptides, one or more adenoviral vectors and an adjuvant are co-formulated.

7. A method according to any one of claims 1 to 6 wherein production of pathogen specific CD4+ T-cells and CD8+ T-cells and antibodies is stimulated.

5 8. A vaccine composition comprising (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotide encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant.

9. A method or vaccine composition according to any one of claims 1 to 8 wherein one or
10 more of said one or more first immunogenic polypeptides is substantially the same as one or more of said one or more second immunogenic polypeptides.

10. A method or vaccine composition according to any one of claims 1 to 8 wherein one or more of said one or more first immunogenic polypeptides contains at least one antigen which is substantially the same as an antigen contained in one or more of said one or more second
15 immunogenic polypeptides.

11. A method or vaccine composition according to any one of claims 1 to 10 wherein one or more the first immunogenic polypeptides comprises at least one T cell epitope.

12. A method or vaccine composition according to any one of claims 1 to 11 wherein the one or more first immunogenic polypeptide comprises at least one B cell epitope.

20 13. A method or vaccine composition according to any one of claims 1 to 12 wherein one or more of said one or more first immunogenic polypeptides and one or more of said one or more second immunogenic polypeptides share one or more identical B-cell and/or T-cell epitopes.

14. A method or vaccine composition according to any one of claims 1 to 8 wherein none of the one or more of said one or more first immunogenic polypeptides is substantially the same as
25 or contains any antigen in common with one or more of said one or more second immunogenic polypeptides.

15. A method or vaccine composition according to any one of claims 1 to 14 wherein one or more of the adenoviral vectors is derived from a human adenovirus.

16. A method or vaccine composition according to claim 15 wherein the human adenovirus serotype is selected from Ad1, Ad2, Ad4, Ad5, Ad6, Ad11, Ad 24, Ad34 and Ad35.
30

17. A method or vaccine composition according to any one of claims 1 to 14 wherein one or more of the adenoviral vectors is derived from a non-human primate adenovirus.

18. A method or vaccine composition according to claim 17 wherein the non-human primate adenovirus serotype is selected from chimpanzee adenovirus serotypes Pan5, Pan6, Pan7 and
35 Pan9.

19. A method or vaccine composition according to any one of claims 1 to 18 wherein the pathogen is HIV.

20. A method or vaccine composition according to claim 19 wherein the immunogenic polypeptides contain HIV derived antigens which are selected from Env, Nef, Gag, and Pol and immunogenic derivatives thereof and immunogenic fragments thereof.
21. A method or vaccine composition according to claim 20 wherein a first immunogenic polypeptide is p24-RT-Nef-p17.
22. A method or vaccine composition according to claim 20 or claim 21 wherein a second immunogenic polypeptide is Gag-RT-Nef.
23. A method or vaccine composition according to any one of claims 1 to 18 wherein the pathogen is *Plasmodium falciparum* and/or *Plasmodium vivax*.
24. A method or vaccine composition according to claim 23 wherein the immunogenic polypeptides contain antigens derived from *Plasmodium falciparum* and/or *Plasmodium vivax* which are selected from circumsporozoite (CS) protein, MSP-1, MSP-3, AMA-1, LSA-1, LSA-3 and immunogenic derivatives thereof or immunogenic fragments thereof.
25. A method or vaccine composition according to claim 24 wherein a/the immunogenic polypeptide is the hybrid protein RTS.
26. A method or vaccine composition according to claim 25 wherein RTS is presented in the form of a mixed particle known as RTS,S.
27. A method or vaccine composition according to any one of claims 24 to 26 wherein a/the immunogenic polypeptide encoded by a polynucleotide is the CS protein from *Plasmodium falciparum* or immunogenic fragment thereof.
28. A method or vaccine composition according to any one of claims 1 to 18 wherein the pathogen is *Mycobacterium tuberculosis*.
29. A method or vaccine composition according to any one of claims 1 to 28 wherein the adjuvant comprises a preferential stimulator of Th1 responses.
30. A method or vaccine composition according to claim 29 wherein the adjuvant comprises QS21 and/or 3D-MPL and/or CpG.
31. A method or vaccine composition according to claim 30 wherein the adjuvant comprises QS21 and 3D-MPL.
32. A method or vaccine composition according to any one of claims 1 to 31 wherein the adjuvant contains an oil-in-water emulsion.
33. A method or vaccine composition according to any one of claims 1 to 31 wherein the adjuvant contains liposomes.
34. A method of stimulating an immune response in a mammal which comprises administering to a subject an immunologically effective amount of a vaccine composition according to any one of claims 8 to 33.
35. Use of a vaccine composition according to any one of claim 8 to 33 in the manufacture of a medicament for stimulating an immune response in a mammal.

36. A vaccine composition according to any one of claims 8 to 33 for use in stimulating an immune response in a mammal.

37. A kit comprising (i) one or more first immunogenic polypeptides derived from a pathogen; (ii) one or more adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more second immunogenic polypeptides derived from said pathogen; and (iii) an adjuvant.

38. A kit comprising (i) one or more first immunogenic polypeptides derived from a pathogen and an adjuvant; and (ii) one or more second adenoviral vectors comprising one or more heterologous polynucleotides encoding one or more immunogenic polypeptides derived from said pathogen.

39. A method, or vaccine, or kit, or use according to any preceding claim wherein the first immunogenic polypeptide comprises p24-RT-Nef-p17, the adjuvant comprises 3D-MPL and QS21 in a liposome such as adjuvant B herein, and the adenoviral vector comprises a chimpanzee adenovirus serotype Pan7 vector comprising a polynucleotide encoding the immunogenic polypeptide Gag-RT-Nef, optionally codon optimised.

40. A method, or vaccine, or kit, or use according to any preceding claim wherein one, or two, or all of the polypeptide, adenoviral vector and adjuvant components are combined with a pharmaceutically acceptable excipient.

20

25