

POXVIRUS VECTOR ENCODING RETROVIRUS (EG HIV) AND CYTOKINE

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

The present invention relates generally to a recombinant vector and its use in the treatment and/or prophylaxis of retroviral infections and the symptoms associated therewith. More particularly, the present invention provides a recombinant vector for
10 use in conjunction with anti-retroviral drug treatment (ARDT) to modulate viral load in a subject. The present invention specifically relates to a recombinant poxvirus vector expressing a retrovirus antigen and/or a modulatory factor and its use in conjunction with anti-HIV retroviral drug therapy in the treatment or prophylaxis of HIV infection, AIDS and AIDS-related disorders in a human subject. The vectors and methods of the
15 present invention are particularly useful in preventing, reducing or delaying viral rebound when retroviral therapy is interrupted.

20 DESCRIPTION OF THE PRIOR ART

Bibliographic details of the references in this specification are collected at the end of the description.

Reference to any prior art in the specification is not, and should not be taken as, an
25 acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Retroviruses are obligate intracellular parasites of vertebrate cells. Viral propagation of the enveloped RNA virus is via a double stranded DNA provirus intermediate which
30 integrates into the genomic DNA of a susceptible host cell and makes use of many host cell factors. This efficient system of infection and propagation makes eradication of the virus very difficult. It is estimated that HIV replication in an infected individual can

involve the production and clearance of 10 billion virions per day, each virion having a half-life of about six hours in the general circulation (Australian Society for HIV Medicine (ASHM)-2001 Australian Antiretroviral Guidelines).

- 5 All retrovirus genomes comprise three major coding domains: *gag*, which is responsible for matrix, capsid and nucleoprotein structures; *pol* which encodes RNA-dependent DNA polymerase, reverse transcriptase, and also integrase enzymes; and *env* which generates viral envelope proteins. In addition, all retroviruses also comprise the *pro* coding domain responsible for producing virion protease. A subset of retroviruses, termed "complex"
- 10 retroviruses, also comprise a range of regulatory factors which influence their own and host expression pathways.

The retrovirus family includes Lentiviruses such as Human immunodeficiency virus (HIV-1 and HIV-2), Simian immunodeficiency virus (SIV), Human T-cell leukaemia-bovine

15 leukaemia viral group such as Human T-cell leukaemia virus (HTLV), Feline leukaemia virus (FIV) and Spumaviruses as described in Vogt P.K. (Chapter 1: *Retroviruses: Coffin John M et al (eds), Cold Spring Harbour Laboratory Press, USA, 1997*).

- HIV is a particularly important complex retrovirus of humans as the causative agent of
- 20 Acquired Immune Deficiency Syndrome (AIDS) which remains a devastating and complex problem despite recent advances in anti-retroviral drug treatments.

HIV infects CD4+ immune cells and established HIV infections are characteristically associated with progressive immune system damage, opportunistic infections and wasting

25 syndromes. Commencement of anti-retroviral therapy is generally recommended at any stage of HIV infection when immune deficiency is present as determined by, for example, low levels of CD4+ cells. Reductions in plasma viral load in response to anti-retroviral treatment are associated with statistically significant improvements in survival and clinical outcome (Melors J.W. *et al, Science* 272:1167-1170, 1996). Complete eradication of HIV

30 in a subject is presently considered to be an unrealistic goal, and as viral levels may

increase or rebound if treatment is discontinued, infected individuals are *prima facie* committed to a life time of antiretroviral drug treatment.

There are a large range of anti-retroviral drug treatment regimens generally involving the
5 administration of combinations of anti-retroviral compounds (see for example ASHM-
2001 draft Australian antiretroviral guidelines, *supra*). In particular, limited clinical data
have indicated that triple therapy in the treatment of acute and advanced HIV infection
employing a nucleoside analogue combination and a non-nucleoside reverse transcriptase
inhibitor or protease inhibitor has a positive effect on surrogate markers of disease
10 progression and at least a short term clinical benefit.

The optimal regimens and timing for anti-retroviral treatment are unclear. The emergence
of drug resistant strains is a major problem contributing to drug treatment failure.
Compliance is also a major problem because anti-retroviral drug treatment regimens are
15 characteristically complex and require strict adherence in order to have any chance of
success. Current regimens often involve multiple dosings of up to four different active
agents. Each active agent typically has its own administration requirements, for example
administration before or after food. Similarly each agent will need to be administered in
specified quantities at specified periods, such that the patient will frequently be taking, for
20 example, one medication 4 times a day, another 3 times a day and another twice a day,
with one needing to be taken before food and one needing to be taken after food. In
addition the common side effects of anti-retroviral drug treatment include nausea,
vomiting, heart disease, diabetes and liver damage.

25 In view of the difficulties associated with anti-retroviral drug treatment there is an urgent
need for greater understanding of the host-retrovirus interaction and to identify effective
methods and reagents for controlling retroviral infections and improving current anti-
retroviral drug treatment regimens particularly to facilitate their long term efficacy. Also,
in view of the undesirable and often severe side-effects, there is a need for treatment
30 protocols which allow periods in which anti-retroviral drugs are not administered. As a

result of the onerous and intrusive treatment regimens, there is a demand from patients for protocols which allow them periods in which they do not take anti-retroviral drugs.

5 SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the
10 exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of sequence identifiers is
15 provided in Table 1. A sequence listing is provided after the claims.

The present invention is predicated, in part, on the development of a vector which expresses a retrovirus antigen and/or a host modulatory factor and which upon administration to a subject is capable of reducing, preventing or delaying viral rebound or
20 of reducing, preventing or delaying the increase or rate of increase in viral load in a subject. As there are significant disadvantages and difficulties with present anti-retroviral drug treatment regimens in terms of their efficacy, side effects and compliance, it is anticipated that the vectors of the present invention will find broad application in the treatment of retroviral infections in conjunction with anti-retroviral drug treatment.

25 In one aspect, the present vector is a poxvirus vector which expresses one or more retrovirus antigens and/or a cytokine and is administered in conjunction with anti-retroviral drug therapy. In some embodiments a retrovirus antigen and a cytokine are co-expressed by the poxivirus vector.

In one embodiment, the invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen or a functional homolog, derivative, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional homolog, derivative, part or analog thereof, for use in conjunction with anti-retroviral drug therapy to maintain or prolong a low retroviral load in a subject and to prevent, reduce or delay viral rebound during interruption of anti-retroviral drug treatment in a subject.

In some embodiments, the recombinant poxvirus vector comprises a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, derivative, part or analog thereof, for use in conjunction with anti-retroviral drug therapy wherein the antigen and the cytokine are co-expressed and are effective in reducing or alleviating one or more of the side effects of anti-retroviral drug therapy.

In other embodiments, the vector comprises a sequence of nucleotides encoding a retrovirus antigen or a functional homolog, derivative, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional homolog, derivative, part or analog thereof, for use in reducing or alleviating one or more side effects of anti-retroviral drug therapy.

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In particular embodiments, the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6. In some embodiments the cytokine is IFN γ .

In certain embodiments the IFN γ comprises the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least about 60% similarity thereto. In other embodiments IFN γ is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides encoding a functional homolog or derivative thereof having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

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In some embodiments, the retroviral antigen is encoded by a coding region selected from *gag*, *env*, *pol* and *pro* coding regions. In preferred embodiments, the retroviral antigen is encoded by *gag* and/or *pol* coding regions, wherein the *gag* and/or *pol* coding region of HIV being particularly preferred. In particular embodiments, the retroviral antigens encoded by *gag* and *pol* comprise the amino acid sequence set forth in SEQ ID NO: 2 and SEQ ID NO:4, respectively or a functional homolog, part or derivative thereof comprising a sequence of amino acids having at least 60% similarity thereto. In other embodiments, the *gag* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency, and wherein *pol* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 3 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency. The present invention is exemplified with respect to fowlpox virus vectors but extends to the use of other avipox virus vectors.

In some aspects, the present invention provides a method for treatment or prophylaxis of one or more symptoms of a retrovirus infection such as HIV infection, comprising the administration of a poxvirus vector encoding a retrovirus antigen and a cytokine, or a functional homolog, derivative, part or analog thereof, in conjunction with anti-retroviral drug therapy wherein said polypeptide and/or cytokine are expressed in a subject and are effective in maintaining a low viral load in a subject for a period of time, for example effectively preventing, reducing or delaying viral rebound during interruption of anti-retroviral drug treatment.

In other embodiments the method comprises administering to a subject a poxvirus vector encoding an antigen of the retrovirus or the retrovirus antigen and a cytokine, or a functional homolog, derivative, part or analog of the retrovirus antigen and/or the cytokine, in conjunction with anti-retroviral drug therapy wherein the antigen or the antigen and the

cytokine are expressed in the subject and are effective in maintaining or prolonging a low retroviral load in the subject for a period of time and are effective in preventing, reducing or delaying viral rebound during interruption of anti-retroviral drug treatment.

- 5 In another embodiment, the vector is administered to a subject exhibiting a low retroviral viral load as a result of anti-retroviral drug therapy. In other embodiments, the vector is administered to a subject exhibiting a low retroviral load prior to commencement of anti-retroviral drug therapy.
- 10 In particular embodiments, the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6. In some embodiments the cytokine is IFN γ .

In certain embodiments the IFN γ comprises the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least about 60% similarity thereto. In other

15 embodiments IFN γ is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides encoding a functional homolog or derivative thereof having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

- 20 In some embodiments, the retroviral antigen is encoded by a coding region selected from *gag*, *env*, *pol* and *pro* coding regions. In preferred embodiments, the retroviral antigen is encoded by *gag* and/or *pol* coding regions, wherein the *gag* and/or *pol* coding region of HIV being particularly preferred. In particular embodiments, the retroviral antigen encoded by *gag* and *pol* comprise the amino acid sequence set forth in SEQ ID
- 25 NO: 2 and SEQ ID NO:4, respectively or a functional homolog, part or derivative thereof comprising a sequence of amino acids having at least 60% similarity thereto. In other embodiments, the *gag* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which
- 30 hybridises thereto or to a complementary form thereof under conditions of medium stringency, and wherein *pol* is encoded by a sequence of nucleotides set forth in SEQ ID

NO: 3 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency. The present invention is exemplified with respect to fowlpox virus vectors but
5 extends to the use of other avipox virus vectors.

In other embodiment, the present invention provides a method for the treatment or prophylaxis of HIV/AIDS comprising administering to a subject a poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of
10 nucleotides encoding a cytokine, or a functional homolog, part, derivative or analogue thereof in conjunction with anti-retroviral drug therapy, wherein said method is effective in maintaining a low retroviral load in the subject or preventing, reducing or delaying viral rebound in the absence of anti-retroviral drug therapy.

15 In another embodiment, the method comprises administering to a subject a poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, part, derivative or analog of the antigen and/or the cytokine, in conjunction with anti-retroviral drug therapy, wherein said method is effective in maintaining a low retroviral load in the subject and preventing,
20 reducing or delaying retroviral rebound in the absence of anti-retroviral drug therapy.

In some embodiments, the retrovirus antigen is an HIV antigen.

Methods are also provided in one embodiment, for reducing or alleviating one or more side
25 effects of ARDT comprising administering the instant vectors for a time and under conditions to reduce or alleviate one or more of the said effects of ARDT. The vectors may be administered before and/or during ARDT and/or after withdrawal of ARDT. The present method facilitate *inter alia* a treatment program involving interrupted ARDT with a concomitant reduction or alleviation of its side effects.

In this embodiment, the method comprises administering to a subject exhibiting a retroviral infection a poxvirus vector comprising a sequence of nucleotides encoding an antigen of the retrovirus or a functional derivative, homolog, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express the antigen and the cytokine and to reduce or alleviate one or more side effects of anti-retroviral drug therapy in the subject.

Particularly, the present invention provides a method of reducing or alleviating one or more side effects of anti-retroviral drug therapy comprising administering to a subject a poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express the antigen and the cytokine and to reduce or alleviate one or more side effects of anti-retroviral drug therapy in the subject.

In some embodiments, the vector is administered to a subject exhibiting a low retroviral viral load as a result of anti-retroviral drug therapy. In other embodiments, the vector is administered to a subject exhibiting a low retroviral load prior to commencement of anti-retroviral drug therapy.

In particular embodiments, the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6. In some embodiments the cytokine is IFN γ .

In certain embodiments the IFN γ comprises the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least about 60% similarity thereto. In other embodiments IFN γ is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides encoding a functional homolog or derivative thereof having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

In some embodiments, the retroviral antigen is encoded by a coding region selected from *gag*, *env*, *pol* and *pro* coding regions. In preferred embodiments, the retroviral antigen is encoded by *gag* and/or *pol* coding regions, wherein the *gag* and/or *pol* coding region of HIV being particularly preferred. In particular embodiments, the retroviral antigen encoded by *gag* and *pol* comprise the amino acid sequence set forth in SEQ ID NO: 2 and SEQ ID NO:4, respectively or a functional homolog, part or derivative thereof comprising a sequence of amino acids having at least 60% similarity thereto. In other embodiments, the *gag* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency, and wherein *pol* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 3 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency. The present invention is exemplified with respect to fowlpox virus vectors but extends to the use of other avipox virus vectors.

In a related aspect, the present invention contemplates a use of a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in the treatment or prophylaxis of one or more symptoms of a retroviral infection such as HIV infection wherein the antigen and the cytokine are co-expressed in a subject and are effective in maintaining or prolonging a low retroviral load in the subject for a period of time and/or are effective in preventing, reducing or delaying viral rebound during interruption of anti-retroviral drug treatment.

The method also contemplates in some embodiments, a use of a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen or a functional derivative, homolog, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional derivative, homolog, part or analog thereof in the manufacture of a

medicament for use in maintaining or prolonging a low retroviral load in a subject for a period of time, and in preventing, reducing or delaying viral rebound during interruption of anti-retroviral drug treatment.

- 5 In some embodiments, the invention provides a use of a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and/or a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in reducing or alleviating one or more of the side effects of anti-retroviral drug therapy.

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In an exemplary embodiment, the vector is a fowlpox vector co-expressing gag/pol and IFN γ which effectively maintains a low viral vector load, or delays the increase in viral load during a period when antiretroviral drug treatment is interrupted. The present invention extends to pharmaceutical agents comprising the vectors of the present invention
15 and their use in a range of treatment regimens.

A summary of sequence identifiers used throughout the subject specification is provided in Table 1.

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TABLE 1
Summary of sequence identifiers

SEQUENCE ID NO:	DESCRIPTION
1	Nucleotide sequence encoding HIV gag
2	Amino acid sequence encoded by SEQ ID NO: 1
3	Nucleotide sequence encoding HIV pol
4	Amino acid sequence encoded by SEQ ID NO: 3
5	Nucleotide sequence encoding human IFN γ
6	Amino acid sequence encoded by SEQ ID NO: 5
7	Nucleotide sequence of insertion site of rFPV gag/pol IFN γ

BRIEF DESCRIPTION OF THE FIGURES

5 **Figure 1** is a graphical representation showing the mean viral load (non-log) over the 20 week period of the extension trial for each vector recipient group. Subject Group A (white line) received the full construct (FC) comprising recombinant FPV expressing HIV-1 gag/pol and interferon-gamma (IFN γ). Subject Group B (black line) received the partial construct (PC) comprising recombinant FPV expressing HIV-1 gag/pol. Subject Group C (grey line) received diluent alone (placebo).

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Figure 2 is a graphical representation showing the proportion of recipients in each recipient group whose viral load was low enough over the period of the study (in days) such that ARDT was not re-initiated. Subject Group A received the full construct (FC) comprising recombinant FPV expressing HIV-1 gag/pol and interferon-gamma (IFN γ).
15 Subject Group B received the partial construct (PC) comprising recombinant FPV expressing HIV-1 gag/pol. Subject Group C received diluent alone (placebo).

Figure 3 is an annotated representation of the nucleotide sequence (SEQ ID NO: 7) of the insertion site of the recombinant FPV gag/pol IFN γ employed in the Examples.

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DETAILED DESCRIPTION OF THE INVENTION

5 The present invention provides a vector which effectively modulates retroviral load in a subject. Specifically, the vector of the present invention maintains or prolongs a low viral load in a subject infected with a retroviral infection. In a preferred aspect the vector of the present invention is used in conjunction with anti-retroviral drug therapy and is useful in maintaining a low viral load before, after or between periods of drug therapy.

10 In one aspect, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and/or a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

15 In some embodiments, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and/or a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which expresses said sequences when used in conjunction with ARDT in the treatment or
20 prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

Reference herein to anti-retroviral drug treatment (ARDT) is used in its broadest context to include the use of one or more compounds, singly or in combination in regimens for retroviral, and in particular HIV retroviral treatment.

25 Anti-retroviral compounds act by a number of different mechanisms which selectively affect the virus. For example, protease inhibitors, reverse transcriptase inhibitors and ribonucleotide reduction inhibitors may be employed or compounds which inhibit viral adsorption, assembly, integration and transcription. As will be known to those skilled in
30 the art there are a large number of anti-retroviral compounds which may be administered. Examples of protease inhibitors include Indinavir and Nelfinavir. Reverse transcriptase

inhibitors include, for example, Zidovisdine, Stavudine and Didanosine. Examples of ribonucleotide reductase inhibitors include thiosemicarbazone derivatives.

5 The particular compounds and combinations used and the dosages and regimens will be determined by the administering practitioner and will depend *inter alia*, upon individual responses to the treatment.

10 In another aspect, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which co-expresses said constituents for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

15 As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to a "compound" includes a single compound, as well as two or more compounds; reference to "an active agent" includes a single active agent, as well as two or more active agents; and so forth.

20 The term "antigen" is used in its broadest context to include molecules comprising one or more epitopes against which an immune response is produced. The term however, also includes within its scope any polypeptide, including a protein or peptide. Antigenic portions may be identified using well known techniques, such as those set out in Paul, Fundamental Immunology, 3rd Ed., 243-247 (Raven Press, 1993) and references cited therein.

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The term "recombinant vector" is used herein in its broadest sense as a reference to constructs which are capable of vectoring or carrying nucleic acid molecules into a target cell for expression therein. The vectors of the present invention include viral vectors or similar constructs or derivatives thereof, plasmid vectors or naked nucleic acid molecules.

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Poxvirus vectors are particularly convenient vectors. As used herein reference to

"poxvirus" includes viruses selected from the group comprising avipox (eg, fowlpox, canarypox, pigeonpox) orthopox (eg, vaccinia) capripox (eg, sheep, goats) and suipox (eg, swinepox). Preferred poxvirus vectors are avipox or orthopox vectors. Avipox vectors are preferred vectors. A particularly preferred avipoxvirus vector is a fowlpox vector (FPV). Exemplary fowlpox vectors are FPV-M3 vectors as described in International Patent Publication No. WO 00/28003. The principles and procedures for generating and using recombinant poxvirus vectors are well known in the art. Briefly, homologous recombination between a donor recombination vector and a poxvirus within a host cell permits correct introduction of the desired sequences.

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Reference to "modulates" includes down regulation of viral load, maintenance of viral load and a change in the rate of increase of viral load. Specifically, any change in viral load is usually but not exclusively determined over an appropriate period of time and is expressed in terms of change in average viral load over time of a subject or group of subjects.

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Accordingly, the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a modulatory factor, or a functional homolog, derivative, part or analog thereof, which co-expresses said constituents for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

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Reference to "treatment" and "prophylaxis" are to be considered in their broadest context. The term treatment includes partial and full recovery of HIV infection or of the clinical symptoms of AIDS. The term "prophylaxis" includes a delay in contracting an HIV infection or experiencing symptoms of HIV infection including the clinical symptoms of AIDS. Certain symptoms are shared between symptoms of an HIV infection, and the clinical symptoms of AIDS. As will be understood by one skilled in the art, examples of shared symptoms include a detectable viral load and reduced levels of CD4+ cells. Certain HIV infected individuals have a low viral load and fail to show the clinical symptoms of AIDS such as immunosuppression, wasting diseases or increased levels of opportunistic

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infections. Accordingly, the vectors of the present invention are used to treat the symptoms of HIV infection and clinical symptoms of AIDS and AIDS related disorders.

Although human subjects are primarily contemplated, reference to a "subject" should be understood to include mammals including primates (eg, humans, monkeys), livestock animals (eg, sheep, cows, horses, donkeys, goats, pigs), laboratory test animals (eg, mice, rats, ducks, dogs, guinea pigs, rabbits, hamsters), companion animals (eg, dogs, cats, birds), and captive wild animals (eg, kangaroos, deer, foxes). Preferably said subject is a mammal, more preferably a primate and even more preferably a human.

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The phrase "modulatory factor" is used herein include to those host factors which act as chemical messengers between cells to effect change in response to external or internal stimuli. Thus ligands for cellular receptors such as cytokines, growth factors and chemokines are contemplated together with their functional homologs, parts, derivatives and analogs. As will be understood by those skilled in the art, the activity of such a factor may also be achieved through the administration of a compound which acts as an agonist of said factor or as an antagonist of inhibitors of the said factor or by down stream effectors in the same pathway or network. Accordingly, the term modulatory factors includes reference to the host factor, its down stream effectors and agonists thereof.

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In a further embodiment, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, derivative, part or analog thereof, which co-expresses said sequences for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

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In a further embodiment, the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, derivative, part or analog thereof, which co-expresses said sequences for use in conjunction with ARDT in the

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treatment or prophylaxis of one or more symptoms associated with a retroviral infection in a subject.

5 In a particularly preferred embodiment, the modulatory factor of the present invention is selected from IFN γ , IL-12, IL-2, TNF and IL-6 and down stream effectors and agonists thereof. IFN γ is exemplified herein and IFN γ or its functional homologs, parts, derivatives and analogs are preferred.

10 In a preferred, aspect the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen and a sequence of nucleotides encoding IFN γ , or a functional homolog, derivative, part or analog thereof, which co-expresses said constituents for use in conjunction with ARDT in the treatment or prophylaxis of one or more symptoms of a retroviral infection in a subject.

15 Preferred retroviral antigens include those encoded by a coding regions selected from *gag*, *env*, *pol* and *pro* coding regions.

Particularly preferred antigens are those encoded by *gag* and/or *pol* coding regions. A *gag/pol* construct is also preferred.

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The present invention is particularly directed to the treatment of human retroviral infections such as HIV and preferably HIV-1.

25 In a particularly preferred embodiment the retroviral antigens are encoded by *gag* and *pol* coding regions derived from HIV and preferably HIV-1.

Accordingly, in another preferred aspect the present invention provides a recombinant poxvirus vector comprising a sequence of nucleotides encoding *gag* and/or *pol* antigens from HIV and a sequence of nucleotides encoding IFN γ , or a functional homologue,
30 derivative, part, or analogue thereof, which vector co-expresses said sequences for use in

conjunction with ARDT in the treatment or prophylaxis of one or more symptoms of an HIV infection or AIDS in a subject.

Accordingly, in another aspect the present invention provides a recombinant poxvirus
5 vector comprising a sequence of nucleotides encoding gag and pol antigens from HIV and
a sequence of nucleotides encoding IFN γ , or a functional homologue, derivative, part, or
analogue thereof, which vector co-expresses said sequences for use in conjunction with
ARDT in the treatment or prophylaxis of one or more symptoms of an HIV infection or
AIDS in a subject.

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Preferably said poxvirus is a fowlpox virus.

In a further embodiment, the gag antigen is encoded by a sequence of nucleotides set forth
in SEQ ID NO: 1 or a sequence of nucleotides having at least 60% similarity thereto after
15 optimal alignment or a sequence which hybridises thereto or to a complementary form
thereof under conditions of medium stringency.

In a further embodiment, the gag antigen is comprises a sequence of amino acids set forth
in SEQ ID NO: 2 or a sequence of amino acids having at least 60% similarity thereto after
20 optimal alignment.

In a further embodiment, the pol antigen is encoded by a sequence of nucleotides set forth
in SEQ ID NO: 3 or a sequence of nucleotides having at least 60% similarity thereto after
optimal alignment or a sequence which hybridises thereto or to a complementary form
25 thereof under conditions of medium stringency.

In a further embodiment, the pol antigen is comprises a sequence of amino acids set forth
in SEQ ID NO: 4 or a sequence of amino acids having at least 60% similarity thereto after
optimal alignment.

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In a further embodiment, the IFN γ antigen is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

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In a further embodiment, the IFN γ antigen comprises a sequence of amino acids set forth in SEQ ID NO: 6 or a sequence of amino acids having at least 60% similarity thereto after optimal alignment.

10 In a further embodiment, the vector is fowlpox vector comprising the nucleotide sequence set forth in SEQ ID NO: 7 or a sequence of nucleotides having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

15 SEQ ID NO: 7 and Figure 3 provide the sequence of the insertion site of rFPV gag/pol IFN γ representing the exemplified embodiments. As shown in Figure 1, subjects administered with this construct showed an approximately 10 fold reduction in average viral load over the period of the study and in the absence of ARDT. This resulted in at least a delay in the re-initiation of ARDT for most subjects.

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A "functional homolog" include species homologs whose function is conserved between species. Thus a functional homology of IFN γ retains its modulatory function. A functional homolog of pol, for example, retains its antigenic or biochemical function.

25 A "functional derivative" of an antigen or modulatory factor encompasses variants and portions or a part of a full length polypeptide, which retains the functional activity of the parent molecule. Such, active fragments include deletion mutants and small peptides, for example, of at least 10, preferably at least 20 and more preferably at least 30 contiguous amino acids, which exhibit the requisite activity. Peptides of this type may be obtained
30 through the application of standard recombinant nucleic acid techniques or synthesized using conventional liquid or solid phase synthesis as described in Chapter 9 entitled

"Peptide Synthesis" by Atherton and Shephard which is included in a publication entitled "Synthetic Vaccines" edited by Nicholson and published by Blackwell Scientific Publications.

- 5 The term "functional" means that the molecules retain or exceed the overall function of the parent. Accordingly, if in particular function is diminished in the derivative or homolog, this is compensated for new functions such as, for example, greater antigenicity, longevity, half-life, activity, avidity etc.
- 10 The term "variant" refers to nucleotide sequences displaying substantial sequence identity with a reference nucleotide sequences or polynucleotides that hybridize with a reference sequence under stringency conditions that are defined hereinafter. The terms "nucleotide sequence", "polynucleotide" and "nucleic acid molecule" may be used herein interchangeably and encompass polynucleotides in which one or more nucleotides have
- 15 been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference nucleotide sequence whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. The term "variant" also includes naturally-occurring allelic variants.

20

Functional derivatives of a target molecule include active portions of the target molecule whose modification in a subject ameliorates a disease or condition and which may be further modified to enhance this affect. A functional derivative of a target molecule in the form of a protein or peptide comprises a sequence of amino acids having at least 60%

25 similarity to the target molecule or portion thereof. A "portion" in peptide form may be as small as an epitope comprising less than 5 amino acids or as large as several hundred kilodaltons. The length of the polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues and preferably more than

30 about 35 residues.

When in nucleic acid form, a functional derivative comprises a sequence of nucleotides having at least 60% similarity to the target molecule after optimal alignment. A "portion" of a target nucleic acid molecule is defined as having a minimal size of at least about 10 nucleotides or preferably about 13 nucleotides or more preferably at least about 20 nucleotides and may have a minimal size of at least about 35 nucleotides. This definition includes all sizes in the range of 10-35 nucleotides including 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleotides as well as greater than 35 nucleotides including 50, 100, 300, 500, 600 nucleotides or nucleic acid molecules having any number of nucleotides within these values.

10

Functional derivatives of target molecules in nucleic acid form include nucleic acid molecules comprising a nucleotide sequence capable of hybridising to the target molecule or its complementary form under low stringency conditions.

15 Analogous contemplated herein include but are not limited to modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogs.

20 Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidation with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzoylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS);
25 acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal
30 and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitization, for example, to a corresponding amide.

5 Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline
10 pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with
15 tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.
20

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl
25 alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 2.

TABLE 2

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
			L-N-methylaspartic acid	Nmasp
10	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
	cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
25	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dom	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva

	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgabu
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- α -methyltyrosine	Dmtty	N-cyclodecylglycine	Ncdec
	D- α -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
30	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis

	D-N-methyllleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
5	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
10	D-N-methyltyrosine	Dnmtyr	N-methyla-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
15	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methy lasparagine	Masn
	L- α -methy laspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
20	L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- α -methyllleucine	Mleu	L- α -methyllysine	Mlys
	L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
	L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
25	L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
	L- α -methylserine	Mser	L- α -methylthreonine	Mthr
	L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
	L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
30	N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe

1-carboxy-1-(2,2-diphenyl-N-methylamino)cyclopropane

5 Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or
10 carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

15 These types of molecules may be important to stabilise vector constructs or their expressed products.

The terms "similarity" or "identity" as used herein include exact identity between
20 compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the
25 structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and amino acid sequence comparisons are made at the level of identity rather than similarity.

Terms used to describe sequence relationships between two or more polynucleotides or
30 polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence

identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as, for example, disclosed by Altschul *et al.* (*Nucl. Acids Res.* 25: 3389, 1997). A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.* ("Current Protocols in Molecular Biology" John Wiley & Sons Inc, 1994-1998, Chapter 15).

The terms "sequence similarity" and "sequence identity" as used herein refer to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched

positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS
5 computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation to sequence similarity.

10 Preferably, the percentage similarity between a particular sequence and a reference sequence (nucleotide or amino acid) is at least about 60% or at least about 70% or at least about 80% or at least about 90% or at least about 95% or above such as at least about 96%, 97%, 98%, 99% or greater. Percentage similarities or identities between 60% and 100% are also contemplated such as 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75,
15 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for
20 hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may be altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v
25 to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for
30 washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ (Marmur and Doty, *J. Mol. Biol.* 5: 109, 1962). However, the T_m of a duplex DNA decreases by 1°C

with every increase of 1% in the number of mismatch base pairs (Bonner and Laskey, *Eur. J. Biochem.* 46: 83, 1974). Formamide is optional in these hybridization conditions. Accordingly, particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC
5 buffer, 0.1% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C. High stringency conditions are particularly preferred.

The present invention contemplates expression of the nucleotide sequences encoding the
10 modulatory factor and/or the retroviral antigen in recipient cells. However, appropriate alternative means to deliver said agents to recipient cells may be practiced within the scope of the present invention. Thus, the modulatory factor may be administered in proteinaceous or other suitable and pharmaceutically acceptable chemical form optionally in conjunction with the vector of the present invention comprising a nucleotide sequence encoding a
15 retroviral antigen and/or said modulatory factor.

In another aspect the present invention provides a pharmaceutical composition comprising any one of the above-described vectors together with a pharmaceutically acceptable carrier and/or diluent for use in conjunction with ARDT in the treatment or prevention of a
20 retroviral infection.

The term pharmaceutical composition is used herein to refer to a chemical compound which induces a desired pharmacological and/or physiological effect. The term encompasses pharmaceutically acceptable and pharmacologically active ingredients of the
25 active agent and includes pharmaceutically acceptable and pharmacologically active salts, esters, amides, pro-forms, metabolites, analogues, etc. The term "compound" as used herein is not to be construed as a chemical molecule only but extends to peptides, polypeptides, and proteins as well as nucleic acid molecules and chemical analogues thereof.

By "pharmaceutically acceptable" excipient or diluent is meant a pharmaceutical vehicle comprised of material which is not biologically or otherwise undesirable, ie the material may be administered without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, colouring agents, wetting or emulsifying agents, buffering agents, preservatives, and the like.

In a preferred aspect said pharmaceutical composition is useful in conjunction with anti-retroviral drug treatment to modulate viral load in a subject.

10 In another aspect, the present invention contemplates a recombinant vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof, wherein upon administration to a subject carrying a low retroviral load, said antigen and/or cytokine is expressed in target cells and said low viral load is effectively maintained or
15 prolonged.

In another aspect, the present invention contemplates a recombinant vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analog thereof, wherein
20 upon administration to a subject carrying a low retroviral load, said nucleotide sequences are expressed in target cells and said low viral load is effectively maintained or prolonged.

Expression as used herein broadly is a reference to the production of a polypeptide from a nucleic acid molecule.

25

Viral load is measured in terms of the number of viral particles/ml of plasma and is a useful and direct measure of viral infection and a surrogate marker of efficacy in retroviral treatment regimens including drug treatments and immunisation protocols. In particular, anti-retroviral drug treatment is usually started in a patient when their viral load goes
30 above or is maintained above about 50 viral particles/ml of plasma for an appropriate period of time. One of the consequences of stopping or interrupting anti-retroviral drug

treatment is that the viral load may "rebound" to a level which is as high or higher than the level before treatment commenced. Such viral rebound when left untreated is associated with the progression in a subject to development of the symptoms of primary HIV infection or the clinical symptoms of AIDS, or a worsening thereof. Accordingly, another
5 useful measure of the efficacy of a treatment regimen in a subject is the time to development of detectable plasma viral loads or the time to re-initiation of anti-retroviral drug treatment. As absolute viral numbers as well as relative numbers are diagnostic it is also useful to consider the maximum viral load in a subject as well as the time-weighted change from a baseline value over a treatment period or during a post- or inter-treatment
10 period. The protocols used to measure and quantify plasma viral loads are well known in the art and typically employ RT-PCR.

Another measure of treatment success or clinical progression is the ratio of CD4:CD8 T-cells in a subject. Furthermore, the success of immunization strategies and a measure of
15 the immune status of a subject may be gauged by measuring CD8 T-cell responses and/or antibody responses to specific antigens. Methods of determining the cellular, virological and immunological status of a subject are well known in the art and are, for example, described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1998, and references cited therein.

20 A low viral load is an average figure and is preferably less than an average over time of about 50,000 copies/ml plasma. Preferably the average low viral load is less than about 40,000 copies/ml, more preferably less than about 30,000 copies/ml, still more preferably less than about 20,000 copies/ml, still more preferably less than about 10,000 copies/ml,
25 even still more preferably less than about 1000 copies/ml, or any number between these aforementioned figures or between 1000 and 0 or undetectable copies /ml such as between 1000 and 100 copies/ml, between 500 and 50 copies/ml, or between 750 and 80 copies/ml, etc. Most preferably a low viral load is below 50 copies/ml.

30 The delay in viral rebound or a delay in an increase in viral load is any time frame which is likely to convey clinical benefit and may be measured in days, weeks, months or years. As

exemplified herein, the average maximum viral load for subjects receiving the full construct (FC) was about 20,000 copies/ml and this was monitored over the 20 weeks of the study during withdrawal from anti-retroviral therapy.

- 5 Poxvirus vectors are particularly convenient vectors. Preferred poxvirus vectors are avipox or orthopox vectors which do not replicate efficiently in human subjects. A particularly preferred poxvirus vector is a fowlpox vector (FPV). Exemplary fowlpox vectors are FPV-M3 vectors as described in International Patent Publication No. WO 00/28003.

10

In a particularly preferred embodiment, the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6 and down stream effectors and agonists thereof. IFN γ is exemplified herein and IFN γ or its functional homologs, parts, derivatives and analogs are preferred.

- 15 Preferred retroviral antigens include those encoded by a coding regions selected from *gag*, *env*, *pol* and *pro* coding regions.

Particularly preferred antigens are those encoded by *gag* and/or *pol* coding regions. A *gag/pol* construct is also preferred.

20

The preferred retrovirus is HIV-1.

- In a further embodiment, the recombinant vector of the present invention is administered in conjunction with ARDT. By "in conjunction" is meant that the instant vector and ARDT are used together but not necessarily simultaneously in order to improve treatment efficacy. In accordance with the present invention treatment efficacy is improved by providing an alternative or additional treatment to ARDT wherein the deleterious side effects of ARDT are reduced. Specifically, administration of the instant vector permits a treatment protocol to be conducted in which anti-retroviral drugs may be taken
- 25
- 30 intermittently,

Accordingly, the present invention provides a recombinant vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof, wherein upon administration to a subject carrying a low retroviral load as a result of ARDT, said antigens
5 are expressed in target cells and said low viral load is effectively maintained or prolonged after or while ARDT is withdrawn.

For the avoidance of doubt, the instant vector may be administered before, during, after or between ARDT/s.

10

In a preferred aspect, the present invention provides a method of treatment or prophylaxis comprising the administration of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof in conjunction with ARDT wherein said
15 method is effective in maintaining a low retroviral load in a subject or reducing or delaying viral rebound in said subject.

In a preferred aspect, administration of the instant vector effectively prevents or treats one or more of the symptoms of HIV infection or AIDS.

20

In another aspect the present invention provides a method of treatment or prophylaxis comprising the administration of a vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analog thereof in conjunction with ARDT wherein said
25 method is effective in maintaining a low retroviral load in a subject or reducing or delaying viral rebound in a subject.

In a preferred aspect administration of the instant vectors effectively prevents or treats one or more of the symptoms of HIV infection or AIDS.

30

In a related aspect, the present invention provides a method of treatment or prophylaxis of AIDS comprising the administration of a vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine or a functional homolog, part, derivative or analogue thereof in conjunction with ARDT
5 wherein said method is effective in maintaining a low retroviral load in a subject or reducing or delaying viral rebound in the absence of ARDT.

To be "effective" an "effective amount" of the instant vector is administered. As used herein an effective amount mean a sufficient amount of the vector to provide the desired
10 therapeutic or physiological outcome. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount and frequency of administration required will vary from subject to subject, depending on the species, age and general clinical condition of the
15 subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

20 The molecules of the present invention can be formulated in pharmaceutic compositions which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing, Company, Easton, PA, U.S.A.). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may comprise,
25 in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. intravenous, oral, intrathecal, epineural or parenteral. Intramuscular
30 administration is preferred.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, 5 preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most 10 advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, International Patent Publication No. WO 96/11698.

15 For parenteral administration, the compound may dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, 20 suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in a therapeutically effective amount. The actual amount administered and the rate and time-course of administration will depend on 25 the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc. is within the responsibility of general practitioners or specialists and typically takes account of the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in Remington's Pharmaceutical Sciences, *supra*.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic or if it would otherwise require too high a dosage or if it would not otherwise be able to enter the desired cells.

Cell based delivery system may be employed such as described in U.S. Patent No. 5,550,050 and International Patent Publication Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. The vector could be targeted to cells harbouring latent infection or expression of expression products could be limited to specific cells, stages of development or cell cycle stages. The cell based delivery system is designed to be implanted in a patient's body at the desired site. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. See, for example, European Patent Application No. 0 425 731A and International Patent Publication No. WO 90/07936.

In another aspect, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express said sequences and to reduce or alleviate one or more of the side effects of ARDT.

In a further aspect, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a vector comprising a sequence of nucleotides encoding a retroviral antigen and a sequence of nucleotides encoding a cytokine, or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express said sequences and to reduce or alleviate one or more of the side effects of ARDT.

In another aspect, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine, for a time and under conditions sufficient to co-express
5 said sequences and to reduce or alleviate one or more of the side effects of ARDT.

Preferably, said vector is a poxvirus vector. More preferably an avipox vector. Still more preferably a fowlpox vector.

10 In a further preferred embodiment, the cytokine is IFN- γ .

Preferably the retroviral antigen is *gag* and/or *pol*. Most preferably HIV *gag/pol* is employed.

15 In a further preferred embodiment, the present invention provides a method of reducing or alleviating one or more of the side effects of ARDT comprising the administration to a subject of a fowlpox vector comprising a sequence of nucleotides encoding HIV *gag/pol* and a sequence of nucleotides encoding IFN- γ or a functional derivative, homolog, part or analog thereof, for a time and under conditions sufficient to co-express said sequences and
20 to reduce or alleviate one or more side effect of ARDT.

The side effects of ARDT are numerous and are well known in the art and include, without limitation, nausea, vomiting, fever fat redistribution, heart disease, liver disease and insulin resistance. Treatment and prophylaxis regimens are tailored to the individual and include
25 priming and/or boosting with the vector before or during ARDT or after withdrawal ARDT and before or after re-initiation of ARDT. ARDT may be withdrawn for a period of time ranging from days to several months depending on the level and extent of side effects experienced by a recipient and the vector may be administered in prime and/or boost format during this period to maintain low level of viral load. By reducing viral load the
30 methods described herein are useful in increasing the time to restarting ARDT, preventing

further ARDT or allowing changes in the combination of anti-retroviral drugs which effectively also reduces the side effects of the treatments.

5 In a related aspect the present invention extends to the use of the subject vectors in the manufacture of a medicament for use in conjunction with ARDT in the treatment or prophylaxis of a retroviral infection and symptoms associated therewith.

10 In one aspect, the present invention broadly contemplates the use of a vector comprising a sequence of nucleotides encoding a retroviral antigen and/or a sequence of nucleotides encoding a cytokine or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in a method of reducing or alleviating one or more of the side effects of ARDT.

15 Preferably the subject has previously been treated with an anti-retroviral compound. The instant vectors may be administering before or during ARDT or after withdrawal of ARDT. When administered before or during ARDT, ARDT may subsequently be withdrawn and in accordance with the present invention, viral loads are maintained at a low level in the absence of ARDT.

20 In accordance with this aspect of the invention, preferably, said vector is a poxvirus vector. More preferably an avipox vector. Still more preferably a fowlpox vector.

25 In a further preferred embodiment the cytokine is IFN γ . Preferably the retroviral antigen is gag and/or pol. Most preferably HIV gag/pol are employed.

Accordingly, in a preferred embodiment, the present invention provides the use of a fowlpox vector comprising a sequence of nucleotides encoding HIV gag/pol and a sequence of nucleotides encoding IFN γ or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in a method of reducing or alleviating one or more of the side effects of ARDT.

30

Said medicament is conveniently in a format for administration as a priming dose and/or a boosting dose. A broad range of doses may be applicable. For example, a unit dose may comprise from about 1×10^6 PFU per ml to about 1×10^8 PFU per ml. Dosage regimens are adjusted to provide the optimum therapeutic dose and priming administrations may be administered daily, weekly or monthly or at other suitable time intervals or may be proportionately reduced as indicated by the exigencies of the situation. A preferred priming dose is 5×10^7 PFU per ml in one ml of diluent. Boosting doses may be the same as priming doses or they may be more or less concentrated as indicated by the exigencies of the situation. For other constructs, from about 0.1 μ g to 1 mg of vector may be administered per kilogram of body weight per day.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Randomised, Placebo-controlled, Phase I/IIa Evaluation of the Safety and Biological Activity of Avipox Virus Expressing HIV gag-pol and Interferon-gamma in HIV-1 Infected Subjects.

A clinical trial was conducted to establish the safety and immunogenicity of recombinant fowlpox virus vaccines (rFPV) expressing HIV gag-pol or co-expressing HIV gag/pol and human interferon-gamma (IFN γ) in HIV positive subjects taking combination anti-retroviral drug therapy (ART). A total of 34 patients completed the trial in which they received a series of injections and blood tests regularly over six months. Patients continued to take standard anti-retroviral therapies throughout the trial period. As announced on 17 February, 2003 (virax.com.au) the data for this trial indicated that neither construct elicited a specific immune response in trial participants receiving ART.

EXAMPLE 2

Safety, Biological Activity and Extension Study to Assess The Anti-retrovirological Properties of a Therapeutic HIV Vaccine Candidate Based on Recombinant Fowlpox Virus (rFPV).

A multicentre, randomised, double-blind, placebo-controlled trial recruited HIV-infected individuals treated with anti-retroviral therapy (ART) during primary HIV infection, who maintained control of virus replication (plasma viral load < 50 copies/mL) since initiation of ART. Subjects were randomised to one of three study arms: diluent alone (placebo), rFPV expressing HIV gag/pol (partial construct - PC) or rFPV expressing HIV gag/pol and IFN γ (full construct - FC). Vaccines were administered by intramuscular injection on day 0, week 4 and week 12 at a unit dose of 5×10^7 pfu/mL in 1.0mL of diluent. Follow-up continued over 52 weeks. Primary endpoints were mean change in CD8 $^{+}$ effector function

as determined by CTL response or ELISPOT assay from baseline to week 26 and increase in log viral load from baseline to week 52. Analyses of safety endpoints was according to treatment received. All analyses were performed using "intention to treat" methods.

5 In this trial, 35 eligible subjects were randomised (12 placebo, 11 PC-rFPV, 12 FC-rFPV). All but one subject (placebo group) received all three immunisations. All 35 subjects completed 52 weeks of follow-up. No significant toxicity or safety concerns were observed during the study. Episodes of detectable HIV viremia (eight episodes in five patients) were infrequent across the 52 weeks of study and there was no difference between vaccine
10 groups. There were no significant differences between the combined PC and FC groups with placebo patients for anti-HIV gag ELISPOT responses (time-weighted mean difference in change from baseline = -56 sfu/106 PBMC; $p = 0.062$), anti-HIV p55 lymphoproliferative responses (time-weighted mean difference in change from baseline = 4.4 SI; $p = 0.337$), anti-HIV gag lymphoproliferative responses (time-weighted mean
15 difference in change from baseline = 2.1 SI; $p = 0.778$). No additional anti-HIV antibody responses were observed during follow-up. Western Blot reactive anti-FPV antibodies were detected in all PC and FC recipients at week 6 and persisted for the duration of the study. Vaccine recipients generated long-lasting reactive anti-FPV antibodies soon after administration of candidate vaccines.

20

A pilot multicentre, double-blind, placebo-controlled 20-week extension of the study was conducted to examine the effect of immunisation with recombinant fowlpox virus vaccines (rFPV) on measures of HIV replication following cessation of combination antiretroviral therapy (ART). Previously enrolled individuals protocol were re-consented on day 0, prior
25 to receiving a boosting vaccination by intramuscular injection in accordance with their original randomised assignment: diluent alone (placebo), rFPV expressing HIV gag/pol (partial construct - PC) or rFPV expressing HIV gag/pol and interferon-gamma (full construct - FC). All ART was ceased one week following immunisation. Virological and immunological monitoring was monitored frequently for 20 weeks after immunisation. The
30 primary endpoint was time-weighted area under the curve change from plasma HIV-RNA VL (pVL) at baseline until reintroduction of ART. Secondary endpoints included log pVL

after cessation of ART (post-vaccination pVL set-point), kinetics and rate of pVL recrudescence, median time to reinitiation of ART, CD8+ T-cell responses to HIV antigens and CD4+/CD8+ T-cell count changes.

- 5 Twenty-five (71%) of the original study cohort consented to participate (placebo = 7; PC = 8; FC = 10). Antiretroviral therapy was re-introduced in 7 patients (placebo = 3; PC = 3; FC = 1). Immunisations were well-tolerated. One patient (PC group) experienced a transient grade 3 thrombocytopenia that resolved without treatment. The time weighted mean change from baseline pVL over 20 weeks was 1.80 (0.72), 1.78 (0.91) and 0.96
10 (0.91) for placebo, PC and FC respectively ($p = 0.253$, when comparing FC and PC recipients to placebo). The time-weighted mean change from baseline CD4+ cell count was -90.7 (210.1), 2.05 (166.3) and 3.45 (160.9) for placebo, PC and FC respectively ($p = 0.238$, when comparing FC and PC recipients to placebo). All patients had at least one detectable pVL (>50 copies/mL) during follow-up. FC and PC recipients compared to
15 placebo had similar times to detectable pVL (hazard ratio 1.21, 95% CI 0.40 – 2.97, $p = 0.682$). Time to reinitiation of ART was not statistically significantly different in FC and PC recipients compared to placebo (hazard ratio = 2.08, 95% CI 0.49 – 9.31, $p = 0.338$).

Recipients of the Full construct (FC) rFPV immunization experienced a log reduction in pVL
20 compared to recipients of the PC rFPV or placebo. Specifically, the average maximum viral loads for each of the groups was as follows: placebo group-67173 copies/ml; partial construct group-68841; and full construct group-18897 (see Figure 1). Unexpectedly therefore, notwithstanding the lack of any demonstrable immune response in the early part of the trial, in the absence of ARDT, administration of the vector resulted in an approximately 10 fold
25 reduction in average viral load and therapeutic effect over the 20 week period of the study. As specified above, retroviral therapy was re-introduced in a total of seven patients, the seven comprising three from the placebo group, three from the group receiving the partial construct and only one from the largest group receiving the full construct as shown in Figure 2.

- 30 The nucleotide sequence of the insertion site of the vector of rFPV gag/pol IFN γ trialed in this study is set forth in Figure 3 and is represented in SEQ ID NO: 7.

Those skilled in the art will appreciate that the invention disclosed herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also
5 includes all steps, features, compositions referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more steps or features.

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Claims

1. A use of a poxvirus vector encoding an antigen of a retrovirus or a retrovirus antigen and a cytokine, or a functional homolog, derivative, part or analog of the retrovirus antigen and/or the cytokine, in the manufacture of a medicament for use in conjunction with interrupted anti-retroviral drug therapy to reduce or alleviate one or more side effects of anti-retroviral drug therapy wherein the antigen or the antigen and the cytokine are expressed in a subject and are effective in maintaining or prolonging a low retroviral load in the subject for a period of time and are effective in preventing, reducing or delaying viral rebound during interruption of anti-retroviral drug treatment.
2. The use of claim 1, wherein the retrovirus is HIV.
3. The use of claim 1 or 2, wherein the vector is administered to a subject exhibiting a low retroviral viral load as a result of anti-retroviral drug therapy.
4. The use of claim 1 or 2, wherein the vector is administered to a subject exhibiting a low retroviral load prior to commencement of anti-retroviral drug therapy.
5. The use of claim 1, 2, 3 or 4, wherein the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6.
6. The use of claim 5, wherein the cytokine is IFN γ .
7. The use of any one of claims 1 to 6, wherein the retrovirus antigen is encoded by a coding region selected from *gag*, *env*, *pol* and *pro* coding regions.
8. The use of claim 7, wherein the retrovirus antigen is encoded by *gag* and/or *pol* coding regions.

9. The use of claim 8, wherein the retrovirus antigen is encoded by *gag* and *pol* coding regions of HIV.
10. The use of any one of claims 1 to 9, wherein the poxvirus vector is an avipox virus vector.
11. The use of claim 10, wherein the avipox virus vector is a fowlpox virus vector.
12. A use of a poxvirus vector comprising a sequence of nucleotides encoding an HIV antigen and a sequence of nucleotides encoding a cytokine, or a functional homolog, part, derivative or analog of the antigen and/or the cytokine, in the manufacture of a medicament for use in conjunction with interrupted anti-HIV drug therapy to reduce or alleviate one or more side effects of anti-HIV drug therapy, maintain a low HIV load in a subject, and prevent, reduce or delay HIV viral rebound in the absence of anti-retroviral drug therapy.
13. The use of claim 12, wherein the vector is administered to a subject exhibiting a low retroviral viral load as a result of anti-retroviral drug therapy.
14. The use of claim 12, wherein the vector is administered to a subject exhibiting a low retroviral load prior to commencement of anti-retroviral drug therapy.
15. The use of claim 12, 13 or 14, wherein the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6.
16. The use of claim 15, wherein the cytokine is IFN γ .
17. The use of claim 16, wherein IFN γ comprises the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least about 60% similarity thereto.

18. The use of claim 16, wherein IFN γ is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides encoding a functional homolog, part, derivative or analog thereof having at least 60% similarity thereto, or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.
19. The use of any one of claims 12 to 18, wherein the retrovirus antigen is encoded by a coding region selected from *gag*, *env*, *pol* and *pro* coding regions.
20. The use of claim 19, wherein the retrovirus antigen is encoded by *gag* and/or *pol* coding regions.
21. The use of claim 20, wherein the retrovirus antigen is encoded by *gag* and *pol* coding regions of HIV.
22. The use of claim 21, wherein the retrovirus antigens encoded by *gag* and *pol* comprise the amino acid sequence set forth in SEQ ID NO: 2 or a functional homolog, part or derivative thereof or a sequence of amino acids having at least 60% similarity thereto, and SEQ ID NO: 4 or a functional homolog, part or derivative thereof, or a sequence of amino acids having at least 60% similarity thereto, respectively.
23. The use of claim 21, wherein the retrovirus antigen encoded by *gag* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency, and wherein the retrovirus antigen encoded by *pol* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 3 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a

complementary form thereof under conditions of medium stringency.

24. The use of any one of claims 12 to 23, wherein the poxvirus vector is an avipox virus vector.
25. The use of claim 24, wherein the avipox virus vector is a fowlpox virus vector.
26. The use of claim 26, wherein the insertion site in the fowlpox vector comprises the sequence of nucleotides set forth in SEQ ID NO: 7.
27. A use of a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen or a functional derivative, homolog, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional derivative, homolog, part or analog thereof in the manufacture of a medicament for use in conjunction with interrupted anti-retroviral drug treatment to maintain or prolong a low retroviral load in a subject for a period of time, and to prevent, reduce or delay viral rebound during interruption of anti-retroviral drug treatment.
28. A use of a recombinant vector comprising a sequence of nucleotides encoding a retrovirus antigen or a functional derivative, homolog, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional derivative, homolog, part or analog thereof, in the manufacture of a medicament for use in reducing or alleviating one or more side effects of anti-retroviral drug therapy.
29. A use according to claim 27 or 28, wherein the retrovirus is HIV.
30. A recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen or a functional homolog, derivative, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional homolog, derivative, part or analog thereof, when used in conjunction with interrupted anti-retroviral drug therapy to maintain or prolong a low retroviral load in a subject and to

prevent, reduce or delay viral rebound during interruption of anti-retroviral drug treatment in a subject.

31. A recombinant poxvirus vector comprising a sequence of nucleotides encoding a retrovirus antigen or a functional homolog, derivative, part or analog thereof, and a sequence of nucleotides encoding a cytokine or a functional homolog, derivative, part or analog thereof, when used for reducing or alleviating one or more side effects of anti-retroviral drug therapy.
32. The recombinant poxvirus vector of claim 31, when used for maintaining or prolonging a low retroviral load in the subject during anti-retroviral treatment interruption and for reducing or alleviating one or more side effects of anti-retroviral drug therapy.
33. The recombinant poxvirus vector of claims 30, 31 or 32, wherein the retrovirus is HIV.
34. The recombinant vector of claims 30, 31, 32 or 33, wherein the cytokine is selected from IFN γ , IL-12, IL-2, TNF and IL-6.
35. The recombinant vector of claim 34, wherein the cytokine is IFN γ .
36. The recombinant vector of claim 35, wherein the IFN γ comprises the amino acid sequence set forth in SEQ ID NO: 6 or an amino acid sequence having at least about 60% similarity thereto.
37. The recombinant vector of claim 35, wherein IFN γ is encoded by a sequence of nucleotides set forth in SEQ ID NO: 5 or a sequence of nucleotides encoding a functional homolog or derivative thereof having at least 60% similarity thereto or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.

38. The recombinant vector of any one of claims 30 to 37, wherein the retrovirus antigen is encoded by a coding region selected from *gag*, *env*, *pol* and *pro* coding regions.
39. The recombinant vector of claim 38, wherein the retrovirus antigen is encoded by *gag* and/or *pol* coding regions.
40. The recombinant vector of claim 39, wherein the retrovirus antigen is encoded by *gag* and *pol* coding regions of HIV.
41. The recombinant vector of claim 40, wherein the retrovirus antigens encoded by *gag* and *pol* comprise the amino acid sequence set forth in SEQ ID NO: 2 or a functional homolog, part or derivative thereof or a sequence of amino acids having at least 60% similarity thereto, and SEQ ID NO: 4 or a functional homolog, part or derivative thereof or a sequence of amino acids having at least 60% similarity thereto, respectively.
42. The recombinant vector of claim 40, wherein the retrovirus antigen encoded by *gag* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 1 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency, and wherein the retrovirus antigen encoded by *pol* is encoded by a sequence of nucleotides set forth in SEQ ID NO: 3 or a sequence of nucleotides encoding a functional homolog, part or derivative thereof having at least 60% similarity thereto after optimal alignment or a sequence which hybridises thereto or to a complementary form thereof under conditions of medium stringency.
43. The recombinant vector of any one of claims 30 to 42, wherein the poxvirus vector is an avipox virus vector.

44. The recombinant vector of claim 43, wherein the avipox virus vector is a fowlpox virus vector.
45. The recombinant vector of claim 44, wherein the insertion site in the fowlpox vector comprises the sequence of nucleotides set forth in SEQ ID NO: 7.

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Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp
 1 5 10 15
 Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys
 20 25 30
 His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro
 35 40 45
 Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu
 50 55 60
 Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn
 65 70 75 80

Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Asp Val Lys Asp
 85 90 95

Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys
 100 105 110

Lys Lys Ala Gln Gln Ala Ala Ala Ala Ala Gly Thr Gly Asn Ser Ser
 115 120 125

Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met
 130 135 140

Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val
 145 150 155 160

Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala
 165 170 175

Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr
 180 185 190

Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn
 195 200 205

Glu Glu Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro
 210 215 220

Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly
 225 230 235 240

Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro
 245 250 255

Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu
 260 265 270

Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg
 275 280 285

Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys
 290 295 300

Thr Leu Arg Ala Glu Gln Ala Ser Gln Asp Val Lys Asn Trp Met Thr

305		310		315		320
Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu						
		325		330		335
Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys						
		340		345		350
Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala						
		355		360		365
Met Ser Gln Val Thr Asn Pro Ala Asn Ile Met Met Gln Arg Gly Asn						
		370		375		380
Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Glu						
		385		390		395
Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp						
		405		410		415
Arg Cys Gly Arg Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln						
		420		425		430
Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser Tyr						
		435		440		

<210> 3
 <211> 3012
 <212> DNA
 <213> Human immunodeficiency virus

<220>
 <221> CDS
 <222> (1)..(3012)
 <223>

<400>	3	
ttt ttt agg gaa gat ctg gcc ttc cta caa ggg aag gcc agg gaa ttt		48
Phe Phe Arg Glu Asp Leu Ala Phe Leu Gln Gly Lys Ala Arg Glu Phe		
1 5 10 15		
tct tca gag cag acc aga gcc aac agc ccc acc aga aga gag ctt cag		96
Ser Ser Glu Gln Thr Arg Ala Asn Ser Pro Thr Arg Arg Glu Leu Gln		
20 25 30		
gtt tgg gga gga gaa aac aac tcc ctc tca gaa gca gga gcc gat aga		144
Val Trp Gly Gly Glu Asn Asn Ser Leu Ser Glu Ala Gly Ala Asp Arg		

35						40				45						
caa gga act gta tcc ttt aac ttc cct cag atc act ctt tgg caa cga	Gln Gly Thr Val Ser Phe Asn Phe Pro Gln Ile Thr Leu Trp Gln Arg	50	55	60		192										
ccc ctc gtc aca ata agg ata ggg ggg caa cta aag gaa gct cta tta	Pro Leu Val Thr Ile Arg Ile Gly Gly Gln Leu Lys Glu Ala Leu Leu	65	70	75	80	240										
gat aca gga gca gat gat aca gta tta gaa gaa atg aat ttg cca gga	Asp Thr Gly Ala Asp Asp Thr Val Leu Glu Glu Met Asn Leu Pro Gly		85	90	95	288										
aaa tgg aaa cca aaa atg ata ggg gga att gga ggt ttt atc aaa gta	Lys Trp Lys Pro Lys Met Ile Gly Gly Ile Gly Gly Phe Ile Lys Val		100	105	110	336										
aga cag tac gat cag ata cct gta gaa atc tgt gga cat aaa gct ata	Arg Gln Tyr Asp Gln Ile Pro Val Glu Ile Cys Gly His Lys Ala Ile		115	120	125	384										
ggt aca gta tta gta gga cct aca cct gtc aac ata att gga aga aat	Gly Thr Val Leu Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn		130	135	140	432										
ctg ttg act cag att ggt tgt act tta aat ttc ccc att agt cct att	Leu Leu Thr Gln Ile Gly Cys Thr Leu Asn Phe Pro Ile Ser Pro Ile		145	150	155	480										
gaa act gta cca gta aaa tta aag cca gga atg gat ggc cca aaa gtt	Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val		165	170	175	528										
aag caa tgg cca ttg aca gaa gaa aaa ata aaa gca tta gta gag ata	Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile		180	185	190	576										
tgt aca gaa atg gaa aag gaa ggg aaa att tca aaa att ggg cct gaa	Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu		195	200	205	624										
aat cca tac aat act cca gta ttt gct ata aag aaa aaa gac agt act	Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr		210	215	220	672										
aaa tgg aga aaa cta gta gat ttc aga gaa ctt aat aaa aga act caa	Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln		225	230	235	720										
gac ttc tgg gaa gtt cag tta gga ata cca cac ccc gca ggg tta aaa	Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys		245	250	255	768										
aag aaa aaa tca gta aca gta ttg gat gtg ggt gat gca tac ttt tca	Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser		260	265	270	816										

gtt ccc tta gat aaa gac ttt aga aag tat act gca ttt acc ata cct Val Pro Leu Asp Lys Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro 275 280 285	864
agt ata aac aat gag aca cca ggg att aga tat cag tac aat gtg ctg Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu 290 295 300	912
cca cag gga tgg aaa gga tca cca gca ata ttc caa agt agc atg aca Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr 305 310 315 320	960
aaa atc tta gag cct ttt aga aaa cag aat cca gac ata gtt atc tat Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr 325 330 335	1008
caa tac atg gat gat ttg tat gta gga tct gac tta gaa ata ggg cag Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln 340 345 350	1056
cat aga aca aaa ata gag gaa ctg aga cag cat ctg ttg agg tgg gga His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly 355 360 365	1104
ttt acc aca cca gac aaa aaa cat cag aaa gaa cct cca ttc ctt tgg Phe Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 370 375 380	1152
atg ggt tat gaa ctc cat cct gat aaa tgg aca gta cag cct ata atg Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Met 385 390 395 400	1200
ctg cca gaa aaa gac agc tgg act gtc aat gac ata cag aag tta gtg Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val 405 410 415	1248
gga aaa ttg aat tgg gca agt cag att tat gca ggg att aaa gta aag Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly Ile Lys Val Lys 420 425 430	1296
cag tta tgt aaa ctc ctt aga gga acc aaa gca cta aca gaa gta ata Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 435 440 445	1344
cca cta aca gaa gaa gca gag cta gaa ctg gca gaa aac agg gag att Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile 450 455 460	1392
cta aaa gaa cca gta cat gaa gta tat tat gac cca tca aaa gac tta Leu Lys Glu Pro Val His Glu Val Tyr Tyr Asp Pro Ser Lys Asp Leu 465 470 475 480	1440
gta gca gaa ata cag aag cag ggg caa ggc caa tgg aca tat caa att Val Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile 485 490 495	1488

tat caa gag cca ttt aaa aat ctg aaa aca gga aag tat gca agg atg Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met 500 505 510	1536
agg ggt gcc cac act aat gat gta aaa cag tta aca gag gca gtg caa Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln 515 520 525	1584
aaa gta tcc aca gaa agc ata gta ata tgg gga aag att cct aaa ttt Lys Val Ser Thr Glu Ser Ile Val Ile Trp Gly Lys Ile Pro Lys Phe 530 535 540	1632
aaa cta ccc ata caa aag gaa aca tgg gaa gca tgg tgg atg gag tat Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Ala Trp Trp Met Glu Tyr 545 550 555 560	1680
tgg caa gct acc tgg att cct gag tgg gag ttt gtc aat acc cct ccc Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro 565 570 575	1728
tta gtg aaa tta tgg tac cag tta gag aaa gaa ccc ata gta gga gca Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala 580 585 590	1776
gaa act ttc tat gta gat ggg gca gct aat agg gag act aaa tta gga Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly 595 600 605	1824
aaa gca gga tat gtt act gac aga gga aga caa aaa gtt gtc tcc ata Lys Ala Gly Tyr Val Thr Asp Arg Gly Arg Gln Lys Val Val Ser Ile 610 615 620	1872
gct gac aca aca aat cag aag act gaa tta caa gca att cat cta gct Ala Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile His Leu Ala 625 630 635 640	1920
ttg cag gat tgg gga tta gaa gta aac ata gta aca gac tca caa tat Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr 645 650 655	1968
gca tta gga atc att caa gca caa cca gat aag agt gaa tca gag tta Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Lys Ser Glu Ser Glu Leu 660 665 670	2016
gtc agt caa ata ata gag cag tta ata aaa aag gaa aag gtc tac ctg Val Ser Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu 675 680 685	2064
gca tgg gta cca gca cac aaa gga att gga gga aat gaa caa gta gat Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 690 695 700	2112
aaa tta gtc agt gct gga atc agg aaa gta cta ttt ttg aat gga ata Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asn Gly Ile 705 710 715 720	2160
gat aag gcc caa gaa gaa cat gag aaa tat cac agt aat tgg aga gca	2208

Asp	Lys	Ala	Gln	Glu	Glu	His	Glu	Lys	Tyr	His	Ser	Asn	Trp	Arg	Ala	
			725						730					735		
atg	gct	agt	gat	ttt	aac	ctg	cca	cct	gta	gta	gca	aaa	gaa	ata	gta	2256
Met	Ala	Ser	Asp	Phe	Asn	Leu	Pro	Pro	Val	Val	Ala	Lys	Glu	Ile	Val	
			740					745					750			
gcc	agc	tgt	gat	aaa	tgt	cag	cta	aaa	gga	gaa	gcc	atg	cat	gga	caa	2304
Ala	Ser	Cys	Asp	Lys	Cys	Gln	Leu	Lys	Gly	Glu	Ala	Met	His	Gly	Gln	
			755				760					765				
gta	gac	tgt	agt	cca	gga	ata	tgg	caa	cta	gat	tgt	aca	cat	cta	gaa	2352
Val	Asp	Cys	Ser	Pro	Gly	Ile	Trp	Gln	Leu	Asp	Cys	Thr	His	Leu	Glu	
	770					775						780				
gga	aaa	att	atc	ctg	gta	gca	gtt	cat	gta	gcc	agt	gga	tat	ata	gaa	2400
Gly	Lys	Ile	Ile	Leu	Val	Ala	Val	His	Val	Ala	Ser	Gly	Tyr	Ile	Glu	
785					790					795					800	
gca	gaa	gtt	att	cca	gca	gag	aca	ggg	cag	gaa	aca	gca	tat	ttt	ctc	2448
Ala	Glu	Val	Ile	Pro	Ala	Glu	Thr	Gly	Gln	Glu	Thr	Ala	Tyr	Phe	Leu	
				805					810					815		
tta	aaa	tta	gca	gga	aga	tgg	cca	gta	aaa	aca	ata	cat	aca	gac	aat	2496
Leu	Lys	Leu	Ala	Gly	Arg	Trp	Pro	Val	Lys	Thr	Ile	His	Thr	Asp	Asn	
			820					825						830		
ggc	agc	aat	ttc	acc	agt	act	acg	gtt	aag	gcc	gcc	tgt	tgg	tgg	gca	2544
Gly	Ser	Asn	Phe	Thr	Ser	Thr	Thr	Val	Lys	Ala	Ala	Cys	Trp	Trp	Ala	
		835					840					845				
ggg	atc	aag	cag	gaa	ttt	ggc	att	ccc	tac	aat	ccc	caa	agt	caa	gga	2592
Gly	Ile	Lys	Gln	Glu	Phe	Gly	Ile	Pro	Tyr	Asn	Pro	Gln	Ser	Gln	Gly	
	850					855					860					
gta	gta	gaa	tct	atg	aat	aat	gaa	tta	aag	aaa	att	ata	gga	cag	gta	2640
Val	Val	Glu	Ser	Met	Asn	Asn	Glu	Leu	Lys	Lys	Ile	Ile	Gly	Gln	Val	
865					870					875					880	
aga	gat	cag	gct	gaa	cac	ctt	aag	aca	gca	gta	caa	atg	gca	gta	ttc	2688
Arg	Asp	Gln	Ala	Glu	His	Leu	Lys	Thr	Ala	Val	Gln	Met	Ala	Val	Phe	
				885					890					895		
atc	cac	aat	ttt	aaa	aga	aaa	ggg	ggg	att	ggg	gga	tac	agt	gca	ggg	2736
Ile	His	Asn	Phe	Lys	Arg	Lys	Gly	Gly	Ile	Gly	Gly	Tyr	Ser	Ala	Gly	
		900						905					910			
gaa	aga	ata	gta	gac	ata	ata	gca	aca	gac	ata	caa	act	aaa	gaa	cta	2784
Glu	Arg	Ile	Val	Asp	Ile	Ile	Ala	Thr	Asp	Ile	Gln	Thr	Lys	Glu	Leu	
		915					920					925				
caa	aag	caa	att	aca	aaa	att	caa	aat	ttt	cgg	gtt	tat	tac	agg	gac	2832
Gln	Lys	Gln	Ile	Thr	Lys	Ile	Gln	Asn	Phe	Arg	Val	Tyr	Tyr	Arg	Asp	
		930				935					940					
aac	aaa	gat	ccc	ctt	tgg	aaa	gga	cca	gca	aag	ott	ctc	tgg	aaa	ggt	2880
Asn	Lys	Asp	Pro	Leu	Trp	Lys	Gly	Pro	Ala	Lys	Leu	Leu	Trp	Lys	Gly	

945	950	955	960	
gaa ggg gca gta gta ata caa gat aat agt gac ata aaa gta gtg cca				2928
Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro				
	965	970	975	
aga aga aaa gca aaa atc att agg gat tat gga aaa cag atg gca ggt				2976
Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly				
	980	985	990	
gat gat tgt gtg gca agt aga cag gat gag gat tag				3012
Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp				
	995	1000		

<210> 4
 <211> 1003
 <212> PRT
 <213> Human immunodeficiency virus

<400> 4

Phe Phe Arg Glu Asp Leu Ala Phe Leu Gln Gly Lys Ala Arg Glu Phe				
1	5	10	15	
Ser Ser Glu Gln Thr Arg Ala Asn Ser Pro Thr Arg Arg Glu Leu Gln				
	20	25	30	
Val Trp Gly Gly Glu Asn Asn Ser Leu Ser Glu Ala Gly Ala Asp Arg				
	35	40	45	
Gln Gly Thr Val Ser Phe Asn Phe Pro Gln Ile Thr Leu Trp Gln Arg				
	50	55	60	
Pro Leu Val Thr Ile Arg Ile Gly Gly Gln Leu Lys Glu Ala Leu Leu				
	65	70	75	80
Asp Thr Gly Ala Asp Asp Thr Val Leu Glu Glu Met Asn Leu Pro Gly				
	85	90	95	
Lys Trp Lys Pro Lys Met Ile Gly Gly Ile Gly Gly Phe Ile Lys Val				
	100	105	110	
Arg Gln Tyr Asp Gln Ile Pro Val Glu Ile Cys Gly His Lys Ala Ile				
	115	120	125	
Gly Thr Val Leu Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn				
	130	135	140	

Leu Leu Thr Gln Ile Gly Cys Thr Leu Asn Phe Pro Ile Ser Pro Ile
 145 150 155 160

Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 165 170 175

Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 180 185 190

Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 195 200 205

Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 210 215 220

Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 225 230 235 240

Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 245 250 255

Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 260 265 270

Val Pro Leu Asp Lys Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 275 280 285

Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 290 295 300

Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
 305 310 315 320

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 325 330 335

Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 340 345 350

His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 355 360 365

Phe Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 370 375 380

Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Met
 385 390 395 400

Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 405 410 415

Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly Ile Lys Val Lys
 420 425 430

Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 435 440 445

Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 450 455 460

Leu Lys Glu Pro Val His Glu Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 465 470 475 480

Val Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 485 490 495

Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 500 505 510

Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 515 520 525

Lys Val Ser Thr Glu Ser Ile Val Ile Trp Gly Lys Ile Pro Lys Phe
 530 535 540

Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Ala Trp Trp Met Glu Tyr
 545 550 555 560

Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 565 570 575

Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 580 585 590

Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 595 600 605

Lys Ala Gly Tyr Val Thr Asp Arg Gly Arg Gln Lys Val Val Ser Ile
 610 615 620

Ala Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile His Leu Ala
 625 630 635 640

Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 645 650 655

Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Lys Ser Glu Ser Glu Leu
 660 665 670

Val Ser Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 675 680 685

Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 690 695 700

Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asn Gly Ile
 705 710 715 720

Asp Lys Ala Gln Glu Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 725 730 735

Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 740 745 750

Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 755 760 765

Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
 770 775 780

Gly Lys Ile Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 785 790 795 800

Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 805 810 815

Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn

820	825	830
Gly Ser Asn Phe Thr Ser Thr Thr Val Lys Ala Ala Cys Trp Trp Ala		
835	840	845
Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly		
850	855	860
Val Val Glu Ser Met Asn Asn Glu Leu Lys Lys Ile Ile Gly Gln Val		
865	870	875
Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe		
885	890	895
Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly		
900	905	910
Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu		
915	920	925
Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp		
930	935	940
Asn Lys Asp Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly		
945	950	955
Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro		
965	970	975
Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly		
980	985	990
Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp		
995	1000	

<210> 5
 <211> 501
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(501)
 <223>

<400> 5
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 ggt tct ctt ggc tgt tac tgc cag gac cca tat gta aaa gaa gca gaa 96
 Gly Ser Leu Gly Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu
 20 25 30
 aac ctt aag aaa tat ttt aat gca ggt cat tca gat gta gcg gat aat 144
 Asn Leu Lys Lys Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn
 35 40 45
 gga act ctt ttc tta ggc att ttg aag aat tgg aaa gag gag agt gac 192
 Gly Thr Leu Phe Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp
 50 55 60
 aga aaa ata atg cag agc caa att gtc tcc ttt tac ttc aaa ctt ttt 240
 Arg Lys Ile Met Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe
 65 70 75 80
 aaa aac ttt aaa gat gac cag agc atc caa aag agt gtg gag acc atc 288
 Lys Asn Phe Lys Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile
 85 90 95
 aag gaa gac atg aat gtc aag ttt ttc aat agc aac aaa aag aaa cga 336
 Lys Glu Asp Met Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg
 100 105 110
 gat gac ttc gaa aag ctg act aat tat tcg gta act gac ttg aat gtc 384
 Asp Asp Phe Glu Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val
 115 120 125
 caa cgc aaa gca ata cat gaa ctc atc caa gtg atg gct gaa ctg tcg 432
 Gln Arg Lys Ala Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser
 130 135 140
 cca gca gct aaa aca ggg aag cga aaa agg agt cag atg ctg ttt cga 480
 Pro Ala Ala Lys Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Arg
 145 150 155 160
 ggt cga aga gca tcc cag taa 501
 Gly Arg Arg Ala Ser Gln
 165

<210> 6
 <211> 166
 <212> PRT
 <213> Homo sapiens

<400> 6
 Met Lys Tyr Thr Ser Tyr Ile Leu Ala Phe Gln Leu Cys Ile Val Leu
 1 5 10 15

Gly Ser Leu Gly Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu
 20 25 30

Asn Leu Lys Lys Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn
 35 40 45

Gly Thr Leu Phe Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp
 50 55 60

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