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(54) **Title**: MULTIPLEXED CYTOKINE VACCINATION

(57) **Abstract**: A method is provided for treating, ameliorating or preventing a clinical condition, wherein a plurality of non-identical nucleic acid species encoding a distinct cytokine or hormone are provided to a population of cells, wherein only one or a few such nucleic acid species are provided per cell. A method is also provided, wherein an additional plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides is provided to said population of cells, such that each cell is provided with nucleic acid species encoding a distinct combination of a distinct cytokine or hormone and a distinct immunogenic polypeptide. Also provided are compositions and kits-of-parts comprising a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, and/or a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides for use as a medicament.

Multiplexed cytokine vaccination

Field of invention

The present invention relates to a method of treating, ameliorating or preventing a clinical condition, wherein a plurality of non-identical nucleic acid species encoding a distinct cytokine or hormone are provided to a population of cells, wherein only one or a few such nucleic acid species are provided per cell. A similar method is also provided, wherein an additional plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides is provided to said population of cells, such that each cell is provided with nucleic acid species encoding a distinct combination of a distinct cytokine or hormone and a distinct immunogenic polypeptide. Moreover, compositions and kits-of-parts are provided for practicing the methods of the present invention.

Background of invention

Immune-mediated diseases and immune-mediated transplant rejection collectively affect tens of millions of Americans and result in high medical and social costs. Over the past 40 years, advances in the treatment and prevention of transplant rejection have been achieved largely through the development of increasingly potent but globally immunosuppressive drugs. Systemic lucocorticosteroids, calcineurin inhibitors, antimetabolites, purine synthesis inhibitors, and panreactive mAbs have met with a degree of clinical success in treating acute transplant rejection.1 However, such therapies generally require life-long use, are costly and are associated with serious side effects, including nephrotoxicity, diabetes, and immunosuppression, exposing patients to heightened risks of infection and malignancy. Surprisingly, these agents have had only a modest effect on chronic graft rejection and long-term graft survival and have had limited utility in the management of autoimmune diseases and asthma and allergy. Hence a major goal of modern clinical immunology is to develop new strategies and treatments that will induce a state of immune tolerance by selectively blocking or eliminating pathogenic immune responses while maintaining protective immunity.

A variety of agents and approaches to induce or restore immune tolerance are now entering clinical trials and, if successful, will find applications in a range of clinical

scenarios, spanning allergy and autoimmunity in addition to transplantation. During immune development, the thymus molds the developing T-cell repertoire centrally by means of deletion of self-reactive clones. Small numbers of autoreactive T cells escape to the periphery, either through incomplete negative selection or because not all peripheral antigens are displayed within the thymus. These self-reactive T cells are inactivated at extrathymic sites, primarily the lymph nodes and spleen, when mature T cells encounter self-antigens. The processes that regulate peripheral tolerance (ie, clonal inactivation, clonal deletion, and cytokinedependent suppression and immune deviation) operate, to varying degrees, in the generation and maintenance of tolerance, although their relative contributions might vary depending on the nature of the antigen and the location in which tolerization occurs. The existence of multiple pathways presents a wide range of potential targets for intervention. Indeed, dozens of ligands, receptors, and signalling intermediates provide the structural underpinnings for a host of candidate drugs. This complexity also introduces many practical challenges because of the potential for functional redundancies in the targeted pathways and heterogeneity in their expression among different diseases and affected individuals.

Naive T cells require 2 distinct signals to become fully activated. Signal 1 is propagated on presentation of antigen to the T cell, initiating a signaling cascade involving a number of molecules, including the CD4 or CD8 coreceptors and their associated kinases. Professional antigen-presenting cells (APCs) deliver additional costimulatory signals, termed signal 2 that elicit robust and durable T-cell responses. Receptors act either directly in a costimulatory fashion or by upregulating other receptors and ligands needed for generation of signal 2, including CD28 itself. A major effect of costimulation is production of IL-2 and other cytokines required for T-cell proliferation and for arming differentiated T cells to take on effector functions. Once fully differentiated and armed for effector functions, neither CD4+ nor CD8+ T cells require costimulatory signals to respond.

For the purpose of immunotherapy, vectors expressing immunogenic peptides are known in the art. Traditionally, these immunogenic epitopes have been cloned to constitute a polypeptide consisting of several epitopes. In the present invention, the target cell is limited to only express a single epitope that are translated as a small peptide ready for loading onto the MHC presentation complex without further processing, thereby increasing the effectiveness of peptide vaccination.

Summary of invention

The present invention provides a new approach for treating, preventing and/or ameliorating a clinical disorder, in particular infectious disorders, cancer and/or autoimmune disorders.

In one aspect, the present invention relates to a method of treating, preventing or ameliorating a clinical condition, said method comprising

a. providing a population of cells,

b. bringing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof,

c. obtaining an immunogenic response of said population of cells,

wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species.

In another aspect, the present invention relates to a composition comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined in the present invention, and/or

b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides of the present invention, and/or

c. a plurality of non-identical vector species of the present invention, and/or

d. a plurality of non-identical retroviral particle species of the present invention, and/or

e. a population of gene gun particles of the present invention,

f. a plurality of any physical entity species comprising any component of any of a. to e.

for use as a medicament.

A third aspect relates to a kit-of-parts comprising a composition of the present invention, and at least one additional active ingredient for use as a medicament.

A fourth aspect relates to a method of reducing the risk of an human individual encountering a clinical condition, said method comprising administration of a composition of the present invention and/or a kit-of-parts of the present invention to said individual in an amount sufficient to generate a protective immune response.

In a fifth aspect the present invention relates to a method of immunizing an animal, said method comprising administration of a composition of the present invention and/or a kit-of-parts of the present invention to said individual in an amount sufficient to generate a protective immune response.

A sixth aspect relates to a method of monitoring immunization, said method comprising the steps of

a. providing a blood sample from an individual,

b. providing a composition of the present invention and/or a kit-of-parts of the present invention, and

c. determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding at least one species of said immunogenic polypeptides or part thereof

thereby determining whether an immune response has been raised in said individual.

A seventh aspect relates to a method of monitoring immunization, said method comprising the steps of

a. providing a blood sample from an individual,

b. providing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof to a population of blood cells, wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species

c. determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding at least one species of said immunogenic polypeptides or part thereof

thereby determining whether an immune response has been raised in said individual.

An eighth aspect of the present invention relates to use of a composition and/or a kit-of-parts according to the present invention for the manufacture of a medicament for the treatment, amelioration and/or prevention of a disorder of the present invention.

An ninth aspect relates to a composition and/or a kit-of-parts according to the present invention for the treatment, amelioration and/or prevention of a disorder of the present invention.

In a tenth aspect the present invention relates to a pharmaceutical composition for the treatment, amelioration and/or prevention of a disorder as defined in the present invention comprising a composition and/or a kit-of-parts according to the present invention.

Description of Drawings

Figure 1. The ELISpot Method

Figure 2. Schematic illustration of the vector CMVbipep. The vector is a circular DNA construct of approximately 5.800 bp. A chimeric Akv CMV-promoter is placed ahead of the cloning site used for MHC-1 peptides, eg. RGPGRAFVT, AMQMLKETI etc. The translation is always initiated using a startcodon encoding a methionine. An IRES-element is placed ahead of the second cloning site used for immunogenic stimuli, eg. IL-2, GM-csf etc.

Figure 3. illustrating the superior protection at viral challenge in monkeys immunized with live attenuated SIV. Numbers from Koff et al, 2006.

Figure 4. By pseudotyping of MLV-paticles (inner red core) with HIV envelope, it is possible to make retroviral particles that can infect CD4+ cells through the CD4 receptor and the CXCR4 co-receptor.

Figure 5 Schematic presentation of CMVBipep with cloning sites.

Figure 6. DNA-sequence from transduced cells shown above - the encoded peptide sequence shown below.

Figure 7. shows the IL-2 secretion cells transduced with CMVbipep-virus encoding RGPGRAFVT and IL-2. Background secretion of IL-2 from untransduced cells shown in yellow.

Figure 8. showing the GM-csf secretion cells transduced with CMVbipep-virus encoding RGPGRAFVT and GM-csf. Background secretion of GM-csf from untransduced and IL-2 transduced

Figure 9. showing the groups of mice that were immunized in the study

Figure 10. showing the results from the IFN-γ ELISPOT assay.

Figure 11. shows the results from the IL-2 ELISPOT assay

Detailed description of the invention

To increase the effectiveness of DNA vaccination the present invention provides to novel treatment strategies.

I): A vaccination strategy wherein a pool of cells are transduced/transfected with a variety of vectors each expressing a specific protein/polypeptide encoding a gene selected from a pool of cytokines but where any given cell only contains from one to a few species of each vectors. This strategy will be used to transducer/transfect infected cell and/or cancer cells either in vivo or ex vivo. If the cells are transduced ex vivo the cells will be reintroduced into the patient after transduction. The advantages of this new approach are that cancer cells and/or infected cells already before the treatment contain all the immunogenic peptides. By transfecting/transducing these with vector expressing cytokines the immune response can be augmented. By limiting the vectors used to transfect/transducer the individual cells to one or only a few vectors the immunesystem will only have to react to one specific cytokine in the vicinity of the transduced/transfected cell but the whole array of vector expressing cytokines can still be used in the patient resulting in better control of the individual pathways leading to immunrejection of the cancer/infected cells

II): A vaccination regime to prevent or treat infection/cancer wherein a pool of cells are transduced/transfected with a variety of vectors each expressing a specific protein/polypeptide encoding a gene selected from a pool of cytokines combined with expression of a specific peptide selected from a pool of peptides presented by MHC Class I or Class II molecules, but where any given cell only contains from one to a few species of each vectors. This type of vaccination strategy will be used to transduce target cells in vivo. Again the result is one cell expressing one immunogenic peptide and one cytokine. By limiting the vectors used to transduce the individual cells to one or only a few vectors the immunesystem will only have to react to one specific immunogenic polypeptide in combination with one specific cytokine in the vicinity of the transduced/transfected cell but the whole array of vector expressing cytokines can still

be used in the patient resulting in better control of the individual pathways leading to immunrejection.

Thus, it is a major objective of the present invention to provide an approach for treating, preventing and/or ameliorating a clinical disorder, in particular infectious disorders, cancer and/or autoimmune disorders. In the present invention, a plurality of non-identical nucleic acid species, each encoding a distinct cytokine or hormone or a functional homolog or part thereof are provided to a population of cells. Importantly, only one or a few nucleic acid species are taken up by each cell. Thus, in a population of treated cells, each individual cell comprise only one or a few nucleic acid species, and therefore only express one or a few cytokine or hormone species.
Moreover, compositions and kits-of-parts are provided for practicing the methods of the present invention.
HIV-1 polypeptides and nucleic acids encoding said polypeptides for production of a vaccine against HIV-1.
The present invention provides nucleic acids encoding immunogenic polypeptides or part thereof, as well as eukaryotic expression vectors comprising at least one said nucleic acid or part thereof. Also provided are biological entities such as eukaryotic cells, prokaryotic cells and/or viral particles, in particular retroviral particles, A kit-of-parts is also provided comprising said vaccine composition a second active ingredient, such as an immunostimulating composition, for example one or more interleukins and/or an antibiotic, such as amoxicillin, penicillin, acyclovir and/or vidarabine.
Specifically, the invention also provides for the use of the polypeptides, antigens, nucleic acids, vectors and biological entities for the manufacture of a medicament, and for methods of treating, preventing or ameliorating a clinical condition, The invention also provides methods for producing a vaccine and/or an antibody by administering a polypeptide, antigen, nucleic acid, vector and/or biological entity of the invention.

The components of the present invention can be used to induce an immune response against HIV, as well as to enhance the immune response in an immunized mammal relative to HIV antigen alone. Advantageously, the vaccine composition and/or components thereof of the invention can also induce HIV specific CD4 T helper cells and CD8+ T cells yielding potent Th1 immune responses against a broad spectrum of HIV epitopes, providing a strong HIV- specific cytotoxic T lymphocyte response. Thus, the vaccine compositions and components thereof of the invention are useful for

preventing HIV infection and/or slowing progression to AIDS in infected individuals. The compositions, components and methods can be used to elicit potent Th1 cellular and humoral immune responses specific for conserved HIV epitopes, elicit HIV- specific CD4 T helper cells, HIV-specific cytotoxic T lymphocyte activity, stimulate production of chemokines and cyotokines such as beta-chemokines, interferon-gamma, interleukin 2 (IL2), interleukin 7 (IL7), interleukin 15 (IL15), alpha-defensin, and the like, and increase memory cells. The vaccine compositions and components thereof can be administered via various routes of administration, and can be used to prevent maternal transmission of HIV, for vaccination of newborns, children and high-risk individuals, and for vaccination of infected individuals. The components of the present invention can also be used in combination with other HIV therapies, including antiretroviral therapy (ART) with various combinations of nuclease and protease inhibitors and agents to block viral entry, such as T20.

Terms and definitions

To facilitate understanding of the invention, a number of terms are defined below.

The term "a retroviral vector" comprises a retroviral vector capable of being transcribed into RNA, which can be packaged into a retroviral particle, reverse transcribed into double stranded DNA and inserted into the host genome by the retroviral enzymatic machinery. For translation of the cytokine, in a particular embodiment, an internal ribosome entry site (IRES) has been inserted upstream of the cytokine in the exemplified retroviral expression vector

The term "heterologous" is used hereinafter for any combination of nucleic acid sequences that is not normally found intimately associated in nature.

The terms “bicistronic” and “polycistronic” as used herein, relates to a transcript encoding a transcript, which comprise two or more open reading frames, respectively. In one embodiment the replication deficient vector of the present invention comprises one open reading frame, two open reading frames, three, four, five or six open reading frames.

The term "polynucleotide" or "nucleic acid” refers to a polymeric form of nucleotides at least 2 bases in length. By "isolated nucleic acid sequence" is meant a polynucleotide that is not immediately contiguous with either of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore

includes, for example, a recombinant DNA or RNA which is incorporated into a viral vector. The nucleotides of the invention can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double stranded forms of DNA.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

The term “polynucleotide(s)” generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single-and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein can also refer to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules.

As used herein, the term “polynucleotide” includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein.

It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia.

The term "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally

occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule. Unless otherwise specified, the amino acids in the present invention are designated by their conventional single letter code.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

A "fragment" or “part” in relation to polynucleotide, nucleic acid or polypeptide is a unique portion of said polynucleotide, nucleic acid or polypeptide, which is identical in sequence to but shorter in length than the parent sequence. For example, the term “fragment” or “part” in the present invention may refer to shorter sequences derived from cytokine or hormone polypeptides, as disclosed in the present invention. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide or amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

The term "Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

Methods of alignment of sequences for comparison are well-known in the art. Various programs and alignment algorithms are described and present a detailed consideration of sequence alignment methods and homology calculations, such as VECTOR NTI. The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage

identity (or similarity or homology); the higher the percentage, the more similar the two sequences will be.

The NCBI Basic Local Alignment Search Tool (BLAST) is available from several sources, including the National Center for Biotechnology Information (NBCI, Bethesda, Md.) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. It can be accessed at http://www.ncbi.nlm.nih.gov/BLAST/. A description of how to determine sequence identity using this program is available at http://www.ncbi.nlm.nih.gov/BLAST/blast_help.html.

Homologs of the disclosed polypeptides are typically characterised by possession of at least 94% sequence identity counted over the full length alignment with the disclosed amino acid sequence using the NCBI Basic Blast 2.0, gapped blastp with databases such as the nr or swissprot database. Alternatively, one may manually align the sequences and count the number of identical amino acids. This number divided by the total number of amino acids in your sequence multiplied by 100 results in the percent identity.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

The term "operably linked" refers to the situation in which a first nucleic acid sequence, amino acid sequence or ligand is placed in a functional relationship with a second nucleic acid sequence, amino acid sequence or ligand. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences or protein or ligands may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

The term "internal ribosome entry site" (IRES) defines a sequence motif which promotes attachment of ribosomes to that motif on internal mRNA sequences. Furthermore, all factors needed to efficiently start translation at the AUG-start-codon following said IRES attach to this sequence motive. Consequently, an mRNA containing a sequence motive of a translation control element, e. g. IRES, results in two translational products, one initiating from the 5'end of the mRNA and the other by an internal translation mechanism mediated by IRES. Accordingly, the insertion of a translational control element, such as IRES, operably linked to an ORF into a retroviral genome allows the translation of this additional ORF from a viral RNA transcript. Such RNA transcripts with the capacity to allow translation of two or more ORF are designated bi- or polycistronic RNA transcripts, respectively.

The term "treatment", as used anywhere herein comprises any type of therapy, which aims at terminating, preventing, ameliorating and/or reducing the susceptibility to a clinical condition as described herein. In a preferred embodiment, the term treatment relates to prophylactic treatment, i.e. a therapy to reduce the susceptibility of a clinical condition, a disorder or condition as defined herein.

Thus, "treatment," "treating," and the like, as used herein, refer to obtaining a desired pharmacologic and/or physiologic effect, covering any treatment of a pathological condition or disorder in a mammal, including a human. The effect may be prophylactic in terms of completely or partially preventing a disorder or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse affect attributable to the disorder. That is, "treatment" includes (1) preventing the disorder from occurring or recurring in a subject who may be predisposed to the disorder but has not yet been diagnosed as having it, (2) inhibiting the disorder, such as arresting its development, (3) stopping or terminating the disorder or at least symptoms associated therewith, so that the host no longer suffers from the disorder or its symptoms, such as causing regression of the disorder or its symptoms, for example, by restoring or repairing a lost, missing or defective function, or stimulating an inefficient process, or (4) relieving, alleviating, or ameliorating the disorder, or symptoms associated therewith, where ameliorating is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, such as inflammation, pain, and/or immune deficiency.

The terms "prevent," "preventing," and "prevention", as used herein, refer to a decrease in the occurrence of pathological cells and/or another disease phenotype in an animal. The prevention may be complete, e.g., the total absence of pathological cells and/or disease phenotype in a subject. The prevention may also be partial, such that the occurrence of pathological cells and/or disease phenotype in a subject is less than that which would have occurred without the present invention. Prevention also refers to reduced susceptibility to a clinical condition.

The terms “ameliorate”, “ameliorating” and “amelioration” , as used herein, specifically refers to an improvement of health status in an animal including a human being in relation to a clinical condition in response to a treatment according to the present invention. Thus, the terms encompass the situation, characterized by a decrease in any disease phenotype or disease symptom, including the occurrence of pathological cells in an animal in response to a method or treatment according to this invention.

A “replication-deficient retroviral vector” according to the present invention, is a vector, which does not comprise all essential genes for viral propagation. The vector may comprise one or more nucleic acid sequences encoding retroviral components.

A "pharmaceutically acceptable carrier," "pharmaceutically acceptable diluent," or "pharmaceutically acceptable excipient", or "pharmaceutically acceptable vehicle," used interchangeably herein, refer to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any conventional type. A pharmaceutically acceptable carrier is essentially non-toxic to recipients at the dosages and concentrations employed and are compatible with other ingredients of the formulation. For example, the carrier for a formulation containing polypeptides would not normally include oxidizing agents and other compounds that are known to be deleterious to polypeptides. Suitable carriers include, but are not limited to, water, dextrose, glycerol, saline, ethanol, and combinations thereof. The carrier can contain additional agents such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the formulation. Adjuvants of the invention include, but are not limited to Freunds's, Montanide ISA Adjuvants [Seppic, Paris, France], Ribi's Adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT), l Hunter's TiterMax (CytRx Corp., Norcross, GA), Aluminum Salt Adjuvants (Alhydrogel - Superfos of Denmark/Accurate Chemical and Scientific Co., Westbury, NY), Nitrocellulose-Adsorbed Protein, Encapsulated Antigens, and Gerbu Adjuvant (Gerbu Biotechnik GmbH, Gaiberg, Germany/C-C Biotech, Poway, CA). Topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylene monolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as anti-oxidants, humectants, viscosity stabilizers, and similar agents can be added as necessary. Percutaneous penetration enhancers such as Azone can also be included.

"Pharmaceutically acceptable salts" include the acid addition salts (formed with the free amino groups of the polypeptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, mandelic, oxalic, and tartaric. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, and histidine.

Compositions for oral administration can form solutions, suspensions, tablets, pills, capsules, sustained release formulations, oral rinses, or powders.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an "effective amount," that is, a dosage sufficient to produce the desired result or effect in association with a pharmaceutically acceptable carrier. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed, the host, and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host.

The term “epitope” means a protein determinant capable of specific binding to an antibody or a T-cell receptor. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

In the present invention, the term “species” is frequently used, for example in relation to nucleic acid species, vector species, polypeptide species, such as cytokine species and immunogenic polypeptide species. A species, for example, a nucleic acid species or an amino acid species relates to nucleic acid molecules or polypeptides with identical polynucleotide sequence or amino acid sequence, respectively. A species may comprise several identical molecules of, for example, polynuclotides or polypeptides; the term merely relates to an identical sequence composition between molecules of the same species. A nucleic acid species and nucleic acid sequence is used interchangeably; however a nucleic acid sequence nay comprise several nucleic acid species. For example, a nucleic acid vector may comprise one or more nucleic acid species, for example a distinct nucleic acid species encoding a cytokine and another nucleic acid species encoding an immunogenic polypeptide.

Different species of nucleic acid or polypeptide are intrinsically non-identical. Nevertheless, the term “non-identical” is used herein in relation to for example nucleic acid species, cytokine species and vector species, to stress the fact that the species differ with respect to polynucleotide sequence or amino acid sequence. Non-identical nucleic acid, polypeptide, such as cytokine molecules differ by at least one nucleotide or amino acid. However, in general non-identical species differ by at least 2 residues, such as at least 3, for example at least 4, for example at least 5 such as at least 10, for

example at least 15, such as at least 20, such as at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, for example at least 100, 200, 300, 400, 500, such as at least 1000 residues. The sequence identity between different species may be less than 98%, such as less than 95%, such as less than 90, for example less than 80%, less than 70, less than 60, less than 50%, 40%, 30%, 20%, foe example less than 10%, such as less than 5% for example 0%.

The term “distinct” as used herein in relation to species of for example nucleic acid or cytokine polypeptide is meant to indicate that said species is unique in relation to other species. For example, a nucleic acid species may encode a distinct species of cytokine or hormone, i.e. said nucleic acid species encodes a cytokine species, which is not encoded by other nucleic acid species. Due to the degeneracy of the genetic code, different nucleic acid species may encode the same polypeptide species, such as a cytokine species or an immunogenic polypeptide species. However, nucleic acid species encoding distinct cytokine species and/or immunogenic polypeptide species comprise a multiplicity of nucleic acid species, wherein each nucleic acid species encode a unique cytokine polypeptide species or a unique immunogenic polypeptide species or a unique combination of a cytokine polypeptide species or a unique immunogenic polypeptide species.

The term “plurality” is used herein in relation to for example nucleic acid species. As used herein, the term “plurality” relates to a multiplicity, i.e. more than one species, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, such as at least 30, for example at least 40, for example at least 50, such as at least 60 for example at least 70, 80, 90, for example at least 100, 200, 300, 400, for example at least 500 species.

The term “bringing into contact” as used herein is meant to comprise any method which is suitable for providing a nucleic acid to a cell, wherein said cell is brought into contact with said nucleic acid, and wherein said contact results in the cellular uptake of said nucleic acid. The nucleic acid species need not necessarily physically contact the cell. For example, the nucleic acid may be comprised in a physical entity such as a retroviral particle, a liposome or a nanoparticle. Moreover, the nucleic acid species may be comprised in a suitable vector, such as a nucleic acid vector as described elsewhere herein. A number of such methods are known to those of skill within the art. Nonlimiting examples include retroviral infection with retroviral particles, lipofection and/or gene gun administration.

The term “uptake” as used herein in relation to cells, which take up nucleic acid species in response to being contacted with said nucleic acid, as described above, merely denotes that the nucleic acid is internalized by said cell. The nucleic acid may exist as extrachromosomal nucleic acid, or be processed and integrated in the genome of the cell. Moreover, a polypeptide encoded by said nucleic acid may be expressed by the cell.

The term “population” is used herein for example in relation to a “population of cells”, or a “population of gene gun particles”. A population is a number of for example cells or gene gun particles, which are targeted or employed in a method, kit or composition of the present invention. A population may comprise one or more subpopluations. In the present invention, the term “subpopulations” are regarded as non-overlapping groups comprised in the larger population.

A “subset” is a group of elements, which are contained within another set of elements. For example a population may consist of two subset of subpopulation, i.e. each subset comprise a number of unique subpopulations, which are not comprised on the other subset of subpopulations.

“Immunomodulating polypeptide” according to the present invention include any polypeptide or peptide, which affect the immune response in an animal including a human being. The immunomodulating peptide may be an immunostimulating polypeptide, an immunodominant polypeptide and/or a genetic adjuvant. Specifically, the immunomodulating polypeptide may be a cytokine or hormone, or a functional homolog or part thereof.

As used herein, the expression "immunogenic" is used to describe an agent capable of eliciting at least one type of immune response directed against an HIV envelope polypeptide or fragment thereof. Thus, such an immune response may be any response, in particular a CTL response where CTLs are generated that are capable of recognising the HLA/polypeptide complex presented on cell surfaces resulting in cell lysis, i.e. the vaccine elicits the production in the vaccinated subject of effector T-cells having a cytotoxic effect against the host cells harbouring the retroviral vector or RNA thereof; as well as an antibody response giving rise to the production of anti-HIV antibodies.

Adjuvant: Any substance whose admixture with an administered immunogenic polypeptide/peptide/antigen/nucleic acid construct/biological entity increases or otherwise modifies the immune response to said determinant.

Antibody: Immunoglobulin molecules and active portions of immunoglobulin molecules. Antibodies are for example intact immunoglobulin molecules or fragments thereof retaining the immunologic activity.

Antigen: Any substance that can bind to a clonally distributed immune receptor (T-cell or B-cell receptor). Usually a peptide, polypeptide or a multimeric polypeptide. Antigens are preferably capable of eliciting an immune response.

APC: Antigen-presenting cell. An APC is a cell that displays foreign antigen complexed with MHC on its surface. T-cells may recognize this complex using their T-cell receptor (TCR). APCs fall into two categories: professional, (of which there are three types: Dendritic cells, macrophages and B-cells) or non-professional (does not constitutively express the Major histocompatibility complex proteins required for interaction with naive T cells; these are expressed only upon stimulation of the non-professional APC by certain cytokines such as IFN-γ).

Boost: To boost by a booster shot or dose is to give an additional dose of an immunizing agent, such as a vaccine, given at a time after the initial dose to sustain the immune response elicited by the previous dose of the same agent.

Cancer: Herein any preneoplastic or neoplastic disease, benign or malignant, where “neoplastic” refers to an abnormal proliferation of cells.

Carrier: Entity or compound to which for example antigens, polypeptides and/or biological entities are coupled to aid in the induction of an immune response.

Chimeric protein: A genetically engineered protein that is encoded by a nucleotide sequence made by a splicing together of two or more complete or partial genes or a series of (non)random nucleic acids.

Clinical condition: A condition that requires medical attention, herein especially conditions associated with the expression of HIV polypeptides such as envelope proteins. Examples of such conditions include: cancers and infections.

Complement: A complex series of blood proteins whose action “complements” the work of antibodies. Complement destroys bacteria, produces inflammation, and regulates immune reactions.

CTL: Cytotoxic T lymphocyte. A sub group of T-cells expressing CD8 along with the T-cell receptor and therefore able to respond to antigens presented by class I molecules.

Cytokine: Growth or differentiation modulator, used non-determinative herein, and should not limit the interpretation of the present invention and claims. In addition to the cytokines, adhesion or accessory molecules, or any combination thereof, may be employed alone or in combination with the cytokines.

Delivery vehicle: An entity whereby a nucleotide sequence or polypeptide or both can be transported from at least one media to another.

DC: Dendritic cell. (DCs) are immune cells and form part of the mammalian immune system. Their main function is to process antigen material and present it on the surface to other cells of the immune system, thus functioning as antigen-presenting cells (APCs).

HIV: As used herein, the term "HIV" refers to all forms, subtypes and variations of the HIV virus, and is synonymous with the older terms for HIV, such as HTLVIII and LAV. Various cell lines capable of propagating HIV or permanently infected with the HIV virus have been developed and deposited with the ATCC, including HuT 78 cells and the HuT 78 derivative H9, as well as those having accession numbers CCL 214, TIB 161, CRL 1552 and CRL 8543, which are described in U.S. Pat. No. 4,725,669 and Gallo, Scientific American 256:46 (1987).

MHC: Major histocompatibility complex, two main subclasses of MHC, Class I and Class II exist.

Pathogen: a specific causative agent of disease, especially a biological agent such as a virus, bacteria, prion or parasite that can cause disease to its host, also referred to as an infectious agent.

Peptide: Plurality of covalently linked amino acid residues defining a sequence and linked by amide bonds. The term is used analogously with oligopeptide and polypeptide. The natural and/or non-natural amino acids may be linked by peptide bonds or by non-peptide bonds. The term peptide also embraces post-translational modifications introduced by chemical or enzyme-catalyzed reactions, as are known in the art. The term can refer to a variant or fragment of a polypeptide.

Pharmaceutical carriers: also termed excipients, or stabilizers are non-toxic to the cell or individual being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-

forming counterions such as sodium; and/or nonionic surfactants such as TWEEN.TM., polyethylene glycol (PEG), and PLURONICS.TM.

Promoter: A binding site in a DNA chain at which RNA polymerase binds to initiate transcription of messenger RNA by one or more nearby structural genes.

Signal peptide: A short sequence of amino acids that determine the eventual location of a protein in the cell, also referred to as sorting peptide.

Surfactant: A surface active agent capable of reducing the surface tension of a liquid in which it is dissolved. A surfactant is a compound containing a polar group which is hydrophilic and a non polar group which is hydrophobic and often composed of a fatty chain.

Treg: Regulatory T cells / T lymphocytes

Vaccine: A substance or composition capable of inducing an immune response in an animal. Also referred to herein as an immunogenic composition. An immune response being an immune response (humoral/antibody and/or cellular) inducing memory in an organism, resulting in the infectious agent, being met by a secondary rather than a primary response, thus reducing its impact on the host organism. A vaccine of the present invention may be given as a prophylactic and/or therapeutic medicament. The composition may comprise one or more of the following: HIV-1 envelope polypeptide(s), antigen(s), nucleic acid(s), vector(s), biological entities, adjuvants and pharmaceutical carriers.

Vector: a genetically engineered nucleic acid construct. Typically comprising several elements such as genes or fragments of same, promoters, enhancers, terminators, polyA tails, linkers, polylinkers, operative linkers, multiple cloning sites (MCS), markers, STOP codons, other regulatory elements, internal ribosomal entry sites (IRES) or others. The present invention comprises expression vectors, such as eukaryotic or prokaryotic mammalian expression vectors, as well as vectors of retroviral origin.

Virosome: A virosome is a fusion between a virus and a liposome, for example a liposome with HIV-1 envelope polypeptides.

Population of cells

The present invention provides methods, composition, kits-of-parts and uses for targeting a population of cells with a plurality of non.identical nucleic acid species encoding a cytokine or hormone and/or a plurality of non.identical nucleic acid species encoding distinct species of immunogenic polypeptides, wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species

encoding a cytokine or hormone and/or at the most 5 non.identical nucleic acid species encoding distinct species of immunogenic polypeptides. In a preferred embodiment, the polypeptide(s) encoded by the nucleic acid species are expressed, after uptake in the respective cells. Expression of a cytokine or hormone and/or an immunogenic polypeptide of the present invention triggers an immunogenic response on said population of cells.

A population of cells according to the present invention may be derived from any animal, such as a mammal, preferably a human being. Moreover, the population of cells is derived from any tissue. In one embodiment, the population of cells is derived from blood cells. In another embodiment, the population of cells is derived from carcinogenic tissue cells, such as a tumor or melanoma. In yet another embodiment, the population of cells is derived from stem cells, for example hematopoietic stem cells.

Importantly, the term “population of cells” as used herein, refers exclusively to cells, which are brought into contact with a nucleic acid species of the present invention, and where said contact results in the nucleic acid species to be taken up by the respective cell of said population of cells. Cells, which do not take up a nucleic acid species of the present invention are not comprised in the term “population of cells” as used herein. Accordingly, the population of cells may exist in a mixture with other cells, which do not take up a nucleic acid species of the present invention, for example, the population of cells may be imbedded in a large group of cells, such as a tissue, a tumor, or a melanoma, of which only a subgroup of the cells take up a nucleic acid species of this invention, that subgroup being designated herein as “a population of cells”. The population of cells may consititute at least 1 percent of the treated tissue or group of cells, such as at least 2, at least 3, at least 4, at least 5 %, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of the treated tissue or group of cells. On the other hand, the population of cells may consititute at the most 1 percent of the treated tissue or group of cells, such as at the most 2, at the most 3, at the most 4, at the most 5 %, for example at the most 10 percent, such as at the most 15 percent, such as at the most 20 percent, such as at the most 30, for example at the most 40, such as at the most 50, for example at the most 60, such as at the most 70, for example at the most 80, such as at the most 90, for

example at the most 95, such as at the most 100 percent of the treated tissue or group of cells.

A population of cells may comprise one or more subpopluations. In the present invention, the term “subpopulations” are regarded as non-overlapping groups comprised in the larger population.

It is understood, the the nucleic acid species of the present invention may be provided to the population of cells in a manner, wherein on average at the most 1, 2, 3, 4, or 5 nucleic acid species are taken up by each individual cell. However, the number of nucleic acid acid species taken up by each cell may follow a certain statistical distribution depending one the method of transfection. For example by transfection or administration with gene gun, the number of nucleic acid species taken up by each cell may follow a normal distribution, wherein each individual cell of the population of cells on average take up at the most 1, 2, 3, 4, or 5 nucleic acid species, however, preferably at the most 1 nucleic acid species. The population of cells is for example divided into five subpopulation, wherein each subpopulation have taken up 1, 2, 3, 4, or 5 nucleic acid species.

Therefore, in a specific embodiment of the methods, compositions, kits-of-parts and uses of the present invention,

i) each individual cell of a first subpopulation of said population of cells takes up one and not more than one nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or one and not more than one additional nucleic acid species encoding said immunogenic polypeptide, and/or

ii) each individual cell of a second subpopulation of said population of cells takes up two and not more than two nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or two and not more than two additional nucleic acid species encoding said immunogenic polypeptide, and/or

iii) each individual cell of a third subpopulation of said population of cells takes up three and not more than three nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or three and not more than three additional nucleic acid species encoding said immunogenic polypeptide, and/or

iv) each individual cell of a fourth subpopulation of said population of cells takes up four and not more than four nucleic acid species encoding said cytokine or

hormone or functional homologue or part thereof and/or four and not more than four additional nucleic acid species encoding said immunogenic polypeptide, and/or

v) each individual cell of a fifth subpopulation of said population of cells takes up five and not more than five nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or five and not more than five additional nucleic acid species encoding said immunogenic polypeptide.

In one such embodiment, the cells of said first, second, third and fourth subpopulations together comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

In another embodiment , the cells of said first, second, and third subpopulations together comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

In yet another embodiment, the cells of said first and second subpopulations together comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

In a further embodiment, said first subpopulation comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

In another embodiment, the first subpopulation comprises at least 80 percent of said population of cells.

In a further embodiment, the second subpopulation comprise at least 10 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50,

for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as at least 98 percent of said population of cells.

In a further embodiment, the third subpopulation comprise at least 10 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as at least 98 percent of said population of cells.

In a further embodiment, the fourth subpopulation comprise at least 10 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as at least 98 percent of said population of cells.

In a further embodiment, the fifth subpopulation comprise at least 10 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as at least 98 percent of said population of cells.

In a further embodiment, said fifth subpopulation comprise at the most 1 percent, such as at the most 5 percent, for example at the most 10 percent, such as at the most 15 percent, such as at the most 20 percent, such as at the most 30, for example at the most 40, such as at the most 50, for example at the most 60, such as at the most 70, for example at the most 80, such as at the most 90, for example at the most 95, such as 100 percent of said population of cells.

In a further embodiment, the fourth subpopulation comprise at the most 10 percent, such as at the most 20 percent, such as at the most 30, for example at the most 40, such as at the most 50, for example at the most 60, such as at the most 70, for example at the most 80, such as at the most 90, for example at the most 95, such as at the most 98 percent of said population of cells.

In a further embodiment, the third subpopulation comprise at the most 10 percent, such as at the most 20 percent, such as at the most 30, for example at the most 40, such as at the most 50, for example at the most 60, such as at the most 70, for example at the most 80, such as at the most 90, for example at the most 95, such as at the most 98 percent of said population of cells.

In a further embodiment, the second subpopulation comprise at the most 10 percent, such as at the most 20 percent, such as at the most 30, for example at the most 40, such as at the most 50, for example at the most 60, such as at the most 70, for

example at the most 80, such as at the most 90, for example at the most 95, such as at the most 98 percent of said population of cells.

In a further embodiment, the first subpopulation comprise at the most 10 percent, such as at the most 20 percent, such as at the most 30, for example at the most 40, such as at the most 50, for example at the most 60, such as at the most 70, for example at the most 80, such as at the most 90, for example at the most 95, such as at the most 98 percent of said population of cells.

Cytokines or hormones

A primary aspect of the present invention relates to a method of treating, preventing or ameliorating a clinical condition, said method comprising a. providing a population of cells, b. bringing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof, c. obtaining an immunogenic response of said population of cells, wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species. Other aspects of the invention relates to compositions, kits-of-parts and uses, which comprise nucleic acid species encoding distinct species of cytokine or hormone, or a functional homolog or part thereof.

Cytokines are naturally existing polypeptides, which serve as messenger molecules within an animal, such as a human being. Cytokines act on specific cytokine receptors in the cells they affect. In particular, cytokines facilitate communication among immune system cells. Generally, Cytokines are smaller, water-soluble proteins and glycoproteins with a mass between 8 and 30 kDa.

However, in the present invention nucleic acid species encoding cytokines may be replaced by nucleic acid species encoding any immunomodulating polypeptide or peptide, such as an immunostimulating polypeptide, a genetic adjuvant, and/or hormone, and/or functional homologs and fragments of said cytokine, immunomodulating polypeptide, hormone or functional homolog.

In one embodiment, the cytokine or immunomodulating peptide species of the present invention are selected from the group consisting of IL-1β, IL-2, IL-2 receptor subunit

alpha, IL-4, IL-5, IL-6, IL-7, IL-8_, IL-10, IL-12, IL-15, IL-18, IL-21, GM-csf, TNF-α, IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereof. In another embodiment, the immunomodulating polypeptide is any one polypeptide selected from the group consisting of SEQ ID NO: 47 to 86 and/or fragments or functional homologes thereof. In a preferred embodiment, the cytokine species is selected from the group consisting of IL-2, IL-4, IL-5, IL-7, IL-12, GM-csf, and/or TNF-α. In a preferred embodiment, the cytokine is an artificial IL-12 (Chengyong Jiang et al., Infection And Immunity,1999).

In one embodiment, the nucleic acid species encode at least 2, such as at least 3, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, such as at least 15 species of cytokine or immunomodulating peptide species of the present invention are selected from the group consisting of IL-1β, IL-2, IL-2 receptor subunit alpha, IL-4, IL-5, IL-6, IL-7, IL-8_, IL-10, IL-12, IL-15, IL-18, IL-21, GM-csf, TNF-α, IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereof, for example selected from the group consisting of IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereof, or for example selected from the group consisting of IL-1β, IL-2, IL-2 receptor subunit alpha, IL-8_, IL-10, IL-12, IL-15, IL-18, IL-21, GM-csf, TNF-α, IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereofselected from the group consisting of IL-1β, IL-12, IL-15, IL-18, IL-21, GM-csf, TNF-α, IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereofselected from the group consisting of IL-10, IL-12, IL-15, IL-18, IL-21, GM-csf, TNF-α, IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereofselected from the group consisting of IL-1β, IL-2, IL-2 receptor subunit alpha, IL-4, IL-5, IL-6, IL-7, IFN-α, IFN-γ, and/or CCL5 and/or fragments or functional homologes thereof

In another embodiment, the cytokine or immunomodulating peptide species of the present invention are selected from the group consisting of Cholera toxin, C3d, Synthetic TLR9-agonsits, including: CpG 1826-ODN (TCCATGACGTTCCTGACGTT) CpG 2006/7909-ODN (TCGTCGTTTTGTCGTTTTGTCGTT). In one embodiment, the cytokine or immunomodulating peptide species of the present invention are selected from the group consisting of SEQ ID NO: 84, 85 and/or 86.

In a particular embodiment, the methods, vectors, kits-of-parts, compositions and uses of the present invention are suitable for treatment of HIV and/or AIDS. In these cases, the cytokine and/or immunomodulating polypeptides are selected from the group consisting of IL-1β, IL-2, IL-2 receptor subunit alpha, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-18, IL-15, IL-21, GM-csf, TNF-α, IFN- α, IFN-γ, CCL2, CCL3 and/or CCL5. In a preferred embodiment, the cytokine species is selected from the group consisting of IL-2, IL-4, IL-5, IL-7, IL-12, GM-csf, and/or TNF-α.

Immunogenic polypeptide species

In a primary aspect, the methods, vectors, kits-of-parts, compositions and uses of the present invention employ or comprise a plurality of non-identical nucleic acid species encoding an immunomodulating polypeptide, such as a cytokin or a hormone or functional homologs or parts thereof, wherein said nucleic acid species are provided to a population of cells, wherein each cell takes up one or a few, such as at the most 5, 4, 3, 2, or one nucleic acid species. It is understood that the immunomodulating agents may enhance an immune response in a cell, which already express an immunogenic peptide/epitope, for example in certain diseases such as cancer forms and/or HIV, see elsewhere herein.

However, in cells, which do not express specific epitopes, the immune response may be triggered by the methods, vectors, kits-of-parts, compositions and uses of the present invention, wherein additional nucleic acid species are introduced, said nucleic acid species encoding an immunogenic polypeptide. In this case, an animal including a human being may be subject to vaccination, i.e. prophylactic treatment by the methods, vectors, kits-of-parts, compositions and uses provided herein. Thus, in these embodiments, the plurality of nucleic acid species encoding an immunomodulating polypeptide is supplemented with a plurality of additional non-identical nucleic acid species, wherein each said additional non-identical nucleic acid species encodes a distinct species of immunogenic polypeptide, wherein each individual cell of said population of cells takes up at the most 5, such as 4, 3, 2, preferably 1 of said additional non-identical nucleic acid species.

In the plurality of nucleic acid species encoding immunomodulating polypeptide species and in the plurality of nucleic acid species encoding immunogenic polypeptide species, a distinct nucleic acid species encoding an immunomodulating polypeptide species and

a distinct nucleic acid species encoding an immunogenic polypeptide species may be located in the same nucleic acid sequence, wherein each nucleic acid sequence encodes a distinct combination of one immunomodulating polypeptide species and one immunogenic polypeptide species. That nucleic acid sequence may further be comprised in a nucleic acid vector, such as a eukaryotic or mammalian expression vector or a retroviral vector. Alternatively, the nucleic acid species encoding a distinct immunomodulating polypeptide species and the nucleic acid species encoding a distinct immunogenic polypeptide species is located in the different nucleic acid sequences, for example in different nucleic acid vector sequences.

The present invention, thus, provides a plurality of non-identical nucleic acid species, wherein each nucleic acid species encodes a distinct species of immunogenic polypeptide. The plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptide comprises at least 2 nucleic acid species encoding distinct immunogenic polypeptide species, such as at least 3, for example at least 4, such as at least 5 nucleic acid species, such as at least 10, such as at least 15 species, for example at least 20, such as at least 25 species, such as at least 30, such as at least 40 species, for example at least 50, such as at least 60 species such as at least 70, such as at least 80 species, for example at least 90, such as at least 100 species, for example at least 150 nucleic acid species, for example at least 200, such as at least 300 species such as at least 400, such as at least 500 species, for example at least 600, such as at least 700 species, for example at least 800 nucleic acid species. Moreover, the plurality of non-identical nucleic acid species encodes at least 2 nucleic acid species encoding distinct immunogenic polypeptide species, such as at least 3, for example at least 4, such as at least 5 nucleic acid species, such as at least 10, such as at least 15 species, for example at least 20, such as at least 25 species, such as at least 30, such as at least 40 species, for example at least 50, such as at least 60 species such as at least 70, such as at least 80 species, for example at least 90, such as at least 100 species, for example at least 150 nucleic acid species, for example at least 200, such as at least 300 species such as at least 400, such as at least 500 species, for example at least 600, such as at least 700 species, for example at least 800 distinct immunogenic polypeptide species.

In one embodiment, the immunogenic polypeptides of the methods, vectors, kits-of-parts, compositions and uses of the present invention consist of a consecutive

sequence of in the range of from 5 to 50 amino acids. However in another embodiment, the immunogenic polypeptides consists of a consecutive sequence of at least 5 amino acids, such as at least 20 amino acids, such as at least 30, such as at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 100 amino acids. In another embodiment, the immunogenic polypeptides consist of 8 to 10 or 18 to 25 consecutive amino acids.

The immunogenic polypeptides of the present invention preferably comprise at least 3, such as 4, 5, 6, 7, 8, 9, 10, 11, such as at least 12 consecutive amino acids. In one embodiment, the immunogenic polypeptide consists of at the most 50 amino acid residues, for example at the most 45 amino acid residues, such as at the most 40 amino acid residues, for example at the most 35 amino acid residues, such as at the most 30 amino acid residues, for example at the most 25 amino acid residues, such as 20 to 25 amino acid residues. In another embodiment, the immunogenic polypeptide consists of at the most 20 amino acid residues, for example at the most 19 amino acid residues, such as at the most 18 amino acid residues, for example at the most 17 amino acid residues, such as at the most 16 amino acid residues, for example at the most 15 amino acid residues, such as at the most 14 amino acid residues, for example at the most 13 amino acid residues, such as at the most 12 amino acid residues, for example at the most 11 amino acid residues, such as 8 to 10 amino acid residues.

In a most preferred embodiment, the immunogenic polypeptides are in the range of 7-12 consecutive amino acids such as consisting of 7, 8, 9, 10, 11, or 12 consecutive amino acid residues. In another preferred embodiment, the immunogenic polypeptides are in the range of 7-10 consecutive amino acids. In another preferred embodiment, the immunogenic polypeptides are in the range of 10-13 consecutive amino acids. In another preferred embodiment, translation of the immunogenic polypeptide is initiated using a startcodon encoding a methionine. Thus, any immunogenic polypeptide or nucleic acid sequences encoding said immunogenic polypeptide of the present invention is also claimed with a first redue, which as a methionine residue, or a nucleic acid sequence encoding a methionine residue as the first residue.

In a specific embodiment of the methods, vectors, kits-of-parts, compositions and uses of the present invention, the plurality of non-identical nucleic acid species encodes at least one, more preferably a plurality of distinct immunogenic polypeptide species

selected from the group consisting of the antigens set out in table 1a below. More specifically, the plurality of non-identical nucleic acid species encodes at least one, more preferably a plurality of distinct immunogenic polypeptide species comprising at least 3, such as at least 6, 7, 8, 9, 10, such as at least 11 consecutive amino acid residues selected from any region of the group consisting of the antigens set out in table 1a below.

In one embodiment, the plurality of nucleic acid species of the methods, vectors, kits-of-parts, compositions and uses of the present invention encode at least 2, such as at least 3, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, such as at least 15 species of cytokine or immunomodulating peptide species of the present invention are selected from the group consisting of the immunogenic polypeptides set out in any of tables 1a and 1b below.

Table 1a. *Unique human tumor Antigens for all cancers recognized by class I and class II HLA-restricted T cells*
(Source *The Journal of Immunology*, 2007, 178: 1975-1979.)

Ag	Abbreviation	Tumor	Reference
Class I HLA-restricted T cells			
Cyclin-dependent kinase 4	CDK-4/m[a]	Melanoma	22
Melanoma ubiquitous mutated 1, 2	MUM-1/2	Melanoma	18
Melanoma ubiquitous mutated 1, 2	MUM-1/2	Melanoma	23
Melanoma ubiquitous mutated 3	MUM-3 (*Helicase*)	Melanoma	24
β-Catenin-mutated	β-Catenin-m[a]	Melanoma	25
Myosin mutated	Myosin/m	Melanoma	26
Redox-perox mutated	Redox-perox/m	Melanoma	27
Melanoma Ag recognized by T cell-2	MART-2/m	Melanoma, lung small cancer cells	28
β-Actin-4 mutated	β-Actin/4/m[a]	Non-small cell lung cancer	29
Elongation factor 2	ELF2-M	Non-small cell lung cancer	30

Tumor draining lymph node	TDL	Non-small cell lung cancer	31
Caspase-8 mutated	CASP-8/m[a]	Head and neck	32
HLA-A2 mutated	HLA-A2-R17OJ	Renal cancer	33
Heat shock protein 70-2 mutated	HSP70-2/m[a]	Renal cancer	34
Cyclin-dependent kinase N2A	CDKN2A	Melanoma	42
Class II HLA-restricted T cells			
Cell division cycle 27	CDC27[a]	Melanoma	35
Triosephosphate isomerase	TPI	Melanoma	36
Low-density lipid receptor/GDP-L-fucose: β-D-galactosidase 2-α-L-fucosyltransferase	LDLR/FUT	Melanoma	37
Fibronectin mutated	Fibronectin/m	Melanoma	38
Receptor-type protein-tyrosine phosphatase K	RT-PTP-K/m[a]	Melanoma	39

[a] Proteins likely involved either in maintaining the neoplastic condition or in the progression and metastatization of cancer cells.

HIV immunogenic polypeptide species

In one embodiment, the nucleic acid species encoding immunogenic polypeptides of the methods, vectors, kits-of-parts, compositions and uses of the present invention are selected from any region of a gene involved in a clinical condition. In one preferred embodiment, said clinical condition is HIV, i.e. the plurality of nucleic acid species encoding immunogenic polypeptides of the methods, vectors, kits-of-parts, compositions and uses of the present invention are in one embodiment selected from any regions of a gene involved in HIV infection, and/or selected from any regions of a gene encoded by an HIV retrovirus, such as gag, pol, rev, or env.

In a specific embodiment, the plurality of immunogenic polypeptide species of the methods, vectors, kits-of-parts, compositions and uses of the present invention are selected from any regions of an HIV-1 envelope polypeptide from any HIV-1 subtype, for example from any regions of any polypeptide sequence selected from the group consisting of SEQ ID NO: 1-46, or functional homologs thereof, or polypeptide species having at least 70% identity, such as at least 75%, for example at least 80%, such as at

least 90% identity to any of said polypeptides. In a preferred embodiment, the immunogenic polypeptides of the methods, vectors, kits-of-parts, compositions and uses of the present invention consist of 9-11 consecutive amino acid residues or 11-12 consecutive amino acid residues selected from any region of an HIV-1 envelope polypeptide from any HIV-1 subtype, for example from any region of any polypeptide sequence selected from the group consisting of SEQ ID NO: 1-46, or functional homologs thereof, or polypeptides having at least 70% identity, such as at least 75%, for example at least 80%, such as at least 90% identity to any of said polypeptides. In a preferred embodiment, a methionine residue is inserted at the N-terminus of the immunogenic polypeptide derived from any of SEQ ID NO: 1-46, for initiating translation.

In a specific embodiment of the methods, vectors, kits-of-parts, compositions and uses of the present invention, the plurality of non-identical nucleic acid species encodes at least one, more preferably a plurality of distinct immunogenic polypeptide species selected from the group consisting SEQ ID NO: 95 to 1159, as set out in table 1b below. More specifically, the plurality of non-identical nucleic acid species encodes at least one, more preferably a plurality of distinct immunogenic polypeptide species comprising at least 3, such as at least 6, 7, 8, 9, 10, such as at least 11 consecutive amino acid residues selected from any region of the group of peptides consisting SEQ ID NO: 95 to 1159. The immunogenic polypeptides are also claimed without a methionine at the first position, i.e where the N-terminal methionine is deleted.

Table 1b. Immunogenic polypeptides for HIV treatment.

Epitope	Protein	HXB2 Location	Subtype	Species	SEQ ID NO:	HLA
MGARASVLSG	p17	1-10	CRF01_AE	human	95	A11.1
MASVLSGGEL	p17	5-13	B	human	96	
MASILRGGKLDK	p17	5-15	C	human	97	
MSVLSGGQLDR	p17	6-15	B	human	98	A11
MLSGGELDRWEK	p17	8-18		macaque	99	
MGELDRWEKI	p17	11-19	B	human	100	B*4002, B40
MELDRWEKIRL	p17	12-21	B, C	human	101	B63
MKIRLRPGGK	p17	18-26	A, B,	human,	102	A*0301,

			CRF01_AE	transgenic mouse		A03, A11, A3, A32, B08, B27, B62, B7, Cw3
MKIRLRPGGKK	p17	18-27	B, C, multiple	human	103	A*0301, A11, A3, B27
MKIRLRPGGKKKYKL	p17	18-31		human	104	A3, B62
MIRLRPGGKK	p17	19-27	B	human, scid-hu mouse	105	B*2705, B27, B2705
MIRLRPGGKKK	p17	19-28	multiple	human	106	A11, A3, B62
MRLRPGGKKK	p17	20-28	A, B, CRF02_AG, multiple	human	107	A*0301, A03, A11, A3, A30, A31, A68, B42, B62
MRPRPGGKKK	p17	20-28		human	108	A3
MRLRPGGKKKY	p17	20-29	B	human	109	A*0301, A3, A30, B42, B62
MRLRPGGKKHY	p17	20-29	C	human	110	A*3002
MRLRPGGKKKYK	p17	20-30		human	111	
MRLRPGGKKHYM	p17	20-30	C	human	112	
MRPGGKKHY	p17	22-29	A, C, D	human	113	B42, B7
MRPGGKKRYM	p17	22-30	C	human	114	B35, Cw*0602
MRPGGKKKYML	p17	22-31	A, C, D	human	115	B*0702, B*5801, B*8101
MRPGGKKRYKL	p17	22-31		human	116	B7
MRPGGKKKYKL	p17	22-31	B, D	human	117	Cw4
MGGKKKYKL	p17	24-31	A, B, CRF02_AG,	human	118	B*0801, B8

			D			
MGGKKKYRL	p17	24-31		human	119	B*0801, B8
MGGKKKYKLK	p17	24-32	B	human	120	B*0801, B8
MGGKKKYKLKHIV	p17	24-35		human	121	B8
MKRYMIKHLV	p17	27-35	C	human	122	Cw*0602
MHYMLKHIVW	p17	28-36	A, C	human	123	A*2301
MHYMLKHLVW	p17	28-36	C	human	124	A*2301, A*2402
MKYKLKHIVW	p17	28-36	B, CRF01_AE	human	125	A*2402, A23, A24
MHYMLNHIVW	p17	28-36	A, C, D	human	126	B*0702, B*5801, B*8101
MKYRLKHLVW	p17	28-36	A	human	127	Cw4
MHYMLKHLVWAS	p17	28-38	C	human	128	
MHLVWASREL	p17	33-41	C	human	129	Cw*0602, Cw*0804
MIVWASRELER	p17	34-43	B	human	130	A11
MLVWASRELERF	p17	34-44	C	human	131	A*3002, B*5703, B57
MLVWRELERF	p17	34-44		human	132	A30
MWASRELERF	p17	36-44	B, C	human	133	B*3501, B35, Cw*0602
MRFAVNPGLL	p17	43-51	B, C	human	134	B63
MGLLESSEGC	p17	49-57	B	human	135	A2
MQILEQLQPA	p17	59-67	B	human	136	A2
MQILEQLQPAL	p17	59-68	B	human	137	A2
MQLQPSLQTGS	p17	63-72	B	human	138	A2
MGSEELRSLY	p17	71-79	B	human	139	A*01, A*0101, A1
MGTEELRSLY	p17	71-79	A	human	140	A*0101
MEELRSLYNTV	p17	73-82	C	human	141	B*4006
MELRSLYNTV	p17	74-82	B,	human	142	A03, A32,

			CRF01_AE			B*0801, B08, B62, B8, Cw3
MELRSLYNTVA	p17	74-83	multiple	human	143	A2
MRSLYNTVATLY	p17	76-86	B, C	human	144	A*30, A*3002, A30, B57, B58, B63
MRLSYNTVATLY	p17	76-86		human	145	A*3002
MRSLYNTATLY	p17	76-86		human	146	A30
MKSLYNTVCV	p17	76-88		mouse	147	H2-Db
MSLYNTVATL	p17	77-85	A, B, C, CRF02_AG, D	human, mouse, transgenic mouse	148	A*02.01, A*0201, A*0202, A*0205, A*0214, A*68, A02, A2, B*1503
MSLFNTVATL	p17	77-85	A, B	human	149	A*0201, A2
MSLYNTIATL	p17	77-85	B, CRF01_AE	human	150	A*0201, A2
MSLYNTAVTL	p17	77-85		human	151	A2
MSLYNTVAVL	p17	77-85	B	human	152	A2
MSLYNTVATLY	p17	77-86	B, multiple	human	153	A2
MSLFNTVATLY	p17	77-86	C	human	154	
MLYNTVATL	p17	78-85	D	human	155	A24, C14, Cw14
MLYNTVATLY	p17	78-86	C	human	156	A*2902, A29, B*4403
MLFNTVATLY	p17	78-86	C	human	157	A*2902
MNTVATLYCV	p17	80-88		human, transgenic mouse	158	A2
MIATLWCVHQR	p17	82-91	CRF01_AE	human	159	A11

MATLYCVHQR	p17	83-91	B	human	160	A*11
MAVLYCVHQR	p17	83-91	B	human	161	A11
MTLYCVHQK	p17	84-91		human	162	A*1101
MTLYCVHQR	p17	84-91	B	human	163	A*1101, A11
MTLYCVHQKI	p17	84-92	B	human	164	A*1103
MTLYCVHQRI	p17	84-92		human	165	A11
MVLYCVHQRI	p17	84-92	B	human	166	A2
MLYCVHQKI	p17	85-92	D	human	167	A24
MYCVHAGIEVRD	p17	86-96	C	human	168	
MCVHQRIEIK	p17	87-95	B	human	169	A11
MHQRIEIKDTK	p17	89-98	B	human	170	A11
MRIDVKDTKEAL	p17	91-101		human	171	
MIEIKDTKEAL	p17	92-101	B	human	172	B*4001, B40, B60, B61
MIDIKDTKEAL	p17	92-101		human	173	B*4001
MEIKDTKEAL	p17	93-101	B, CRF01_AE	human	174	A03, A32, B08, B60, B62, B8, Cw3
MEVKDTKEAL	p17	93-101	B	human	175	B*0801, B8
MDVKDTKEAL	p17	93-101		human	176	
MKIEEEQNKSK	p17	103-112	B	human	177	A11
MAADTGNSSQ	p17	119-127	B	human	178	
MDTGHSNQVSQNY	p17	121-132	B	human	179	A33
MGNSSQVSQNY	p17	123-132	B	human	180	
MNSSKVSQNY	p17	124-132	B	human	181	B*3501, B35
MNSSQVSQNY	p17	124-132		human	182	B*3501
MPPSGKGGNY	p17	124-132	HIV-2	human	183	B35
MNSSQVSQNYP	p17-p24	124-1	B	human	184	
MGKKVSQNYPIV	p17-p24	125-3	C	human	185	
MQVSQNYPIV	p17-p24	127-3	B, D	human	186	A*6802, A68
MSQNYPIVQNIQ	p17-p24	129-7		macaque	187	

MNYPIVQNL	p17-p24	131-6		human	188	A*2402
MVQNLQGQMV	p24	3-11	B, C	human	189	B13
MGQMVHQAISP	p24	8-17	A, C, D	human	190	B*5802, B57
MGQMVHQAISPRTL	p24	8-20		human	191	Cw3
MGQMVHQAISPRTLN	p24	8-21	B, CRF01_AE	human	192	A03, A32, B08, B62, Cw3
MQMVHQAISPR	p24	9-18		human	193	A3 supertype
MVHQAISPR	p24	10-18		human	194	A3 supertype
MVHQAISPRTL	p24	11-20	C	human	195	B*1510
MVQHAISPRTLNAWV	p24	11-24		human	196	
MHQAISPRTL	p24	12-20		chimpanzee, human	197	B*1510, Patr-B2
MQAISPRTL	p24	13-20		human	198	Cw3
MQAISPRTLNAW	p24	13-23	B	human	199	A*2501, A25
MQAISPRTLNAV	p24	13-23		human	200	A25
MAISPRTLNAW	p24	14-23	B, C	human	201	B57, B63
MISPRTLNAW	p24	15-23	A, B, C, D	human	202	A*310102, A*6603, B*440302, B*5701, B*5703, B*58, B*5801, B57, B58, B63, Cw*040101, Cw*0602, Cw*07
MLSPRTLNAW	p24	15-23	A, C	human	203	B*5703, B*5801, B57, B58
MSPRTLNAWV	p24	16-24	B	chimpanzee, human	204	B*0702, B*8101, B07, B7

MRTLNAWVKV	p24	18-26		human, transgenic mouse	205	A2
MTLNAWVKW	p24	19-26		transgenic mouse	206	A*0201
MTLNAWVKVV	p24	19-27	B	human, transgenic mouse	207	A*02.01, A*0202, A2
MTLNAWVKVI	p24	19-27	B	human, transgenic mouse	208	A*0201, A2
MNAWVKIEEK	p24	21-30	B	human	209	
MNAWVKVVEEKA	p24	21-31		macaque	210	
MVKVIEEKAF	p24	24-32	C	human	211	B*1503
MVKVVEEKAF	p24	24-32	B, C	human	212	B*1503
MIEEKAFSPEV	p24	27-36	C	human	213	B*4006
MIEEKAFSPEVI	p24	27-37	C	human	214	B*4501
MEEKAFSPEV	p24	28-36	A, C	human	215	B*4415, B*4501
MEKAFSPEV	p24	29-36	C	human	216	Cw*0602
MEKAFSPEVIPM	p24	29-39		macaque	217	
MKAFSPEVI	p24	30-37	B	human	218	B*5703, B57
MKAFSPEVIPMF	p24	30-40	A, B, C, CRF02_AG, G	human	219	A*310102, A*6603, B*440302, B*5701, B*5703, B*5801, B57, B58, B63, B8, Cw*040101, Cw*07
MKGFNPEVIPMF	p24	30-40		human	220	B*5701
MAFSPEVIPMFT	p24	31-41	C	human	221	
MAFSPEVIPMFSALS	p24	31-44	B	human	222	

MFSPEVIPMF	p24	32-40	B, C	human	223	B57, B58, B63
MSPEVIPMF	p24	33-40	C	human	224	B35
MEVIPMFSAL	p24	35-43	A, B, C, CRF01_AE, D	human	225	A*2601, A*2603, A26
MEVIPMFTAL	p24	35-43	C	human	226	A*2601
MVIPMFSAL	p24	36-43	B	human	227	Cw*0102, Cw1, Cw2
MPMFTALSEGAT	p24	38-48	C	human	228	
MALSEGATPQDL	p24	42-52	C	human	229	
MLSEGATPQDL	p24	43-52	A, B	human	230	B*4403, B42, B44
MSEGATPQDL	p24	44-52	B, CRF02_AG	human	231	B*4001, B40, B44, B60, B61
MGATPQDLNTMLNTV	p24	46-59		human	232	
MATPQDLNTM	p24	47-55		human	233	B7
MATPQDLNMML	p24	47-56	A	human	234	B53
MCTPYDINQMLNC	p24	47-58	HIV-2	human	235	B58
MATPQDLNTMLNT	p24	47-58	B, D	human	236	B58
MTPQDLNTM	p24	48-55		human	237	B7
MTPQDLNTML	p24	48-56	A, B, C, D	human	238	B*0702, B*3910, B*4201, B*5301, B*8101, B07, B39, B42, B53, B7, B81, Cw*0802, Cw8
MTPQDLNMML	p24	48-56	A	human	239	B*4202, B*53C1, B53
MTPYDINQML	p24	48-56	HIV-2	human	240	B*5301, B53

MTPQDLNQML	p24	48-56		human	241	B53
MTPQDLNTML ?	p24	48-56		human	242	B81
MTPQDLNMMLN	p24	48-57	multiple	human	243	B7
MTPQDLNTMLN	p24	48-57	multiple	human	244	B14, B7
MTPQDLNQMLNTV	p24	48-59	B, G	human	245	B58
MPQDLNTMLN	p24	49-57	B	human	246	B14, Cw8
MDLNMMLNIV	p24	51-59	A	human	247	B14
MDLNTMLNTV	p24	51-59	B	chimpanzee, human	248	B*1402, B14, Cw8
MDLNTMLNTVG	p24	51-60	multiple	human	249	A2, B14
MNTMLNTVGGHQAAM	p24	53-66	C	human	250	
MGGHQAAMQMLK	p24	60-70	C	human	251	
MGHQAAMQML	p24	61-69	B, C	human	252	B*1510, B*3901, B38, B39
MGHQAAMQMLKE	p24	61-71	B	human	253	A2
MHQAAMQMLK	p24	62-70	B	human	254	A11, B52
MAMQMLKET	p24	65-72		mouse	255	H-2^{d}
MAMQMLKETI	p24	65-73	A, B, CRF02_AG	human, mouse	256	A2, H-2^{d}, H-2^{kd}, H-2D^{d}, H-2K^{d}, H-2d
MANQMLKDTI	p24	65-73	C	mouse	257	H-2^{d}
MAMQMLKDTI	p24	65-73	A, B, C	mouse	258	H-2K^{d}, H-2d
MAMQMLKETI?	p24	65-73		mouse	259	
MKETINEEAA	p24	70-78	B	human	260	B*04, B*4002
METINEEAAEW	p24	71-80	A, B, D	human	261	A*25, A*2501, A25, B*5301, B53, B58
MDTINEEAAEW	p24	71-80	A, B, C	human	262	B*5301, B*5801, B53
MEEAAEWDRV	p24	75-83	B	human	263	B40

MAAEWDRLHPVH	p24	77-87	C	human	264	
MAEWDRLHPV	p24	78-86	B, C	human	265	A2, B*04, B*4006, Cw*0602
MAEWDRVHPV	p24	78-86	B	human	266	B*4002, B40
MDRVHPVHAGPI	p24	81-91		macaque	267	
MRLHPVHAGPIA	p24	82-92	C	human	268	
MLHPVHAGPI	p24	83-91	B	human	269	
MVHPVHAGPIA	p24	83-92		human	270	B55
MHPVHAGPI	p24	84-91	D	human	271	B35
MHPVHAGPIA	p24	84-92	B, C	human	272	B*3910, B07, B35, B7
MHPVHAGPVA	p24	84-92	B	human	273	B7
MGPIAPGQM	p24	89-96	C, D	human	274	B35
MGQMREPRGSDI	p24	94-104	B, C	human	275	B13
MGQMREPRGSDIA	p24	94-105	C	human	276	
MTTSTLQEQI	p24	107-115	A, B, C	mouse	277	H-2K^{d}
MTSTLQEQIGW	p24	108-117	B, C, HIV-2	human	278	A*310102, A*6603, B*440302, B*5701, B*5703, B*58, B*5801, B27, B35, B57, B58, B63, B7, Cw*040101, Cw*07
MTSTLQEQIGN	p24	108-117	B	human	279	B*5701
MTSTLQEQIAW	p24	108-117	B, C	human	280	B*5701, B*5703, B*5801, B57
MTSTVEEQIQW	p24	108-117	HIV-2	human	281	B*5801

MTSTVEEQQIW	p24	108-117	HIV-2	human	282	B58
MTSTLQRQIGW	p24	108-117		human	283	
MTSNLQEQIGW	p24	108-117	B	human	284	
MTSTLQEQIGWF	p24	108-118	B	human	285	B*5801, B57
MSTLQEQIGW	p24	109-117	B, D	human	286	B*5701, B*5801, B57, B58
MSTLQEQIGWM	p24	109-118	B	human	287	A2
MTLQEQIGWM	p24	110-118		human	288	A2
MNLQEQIGWM	p24	110-118	B	human	289	A2
MNLQEQIGWMT	p24	110-119	B	human	290	A2
MWMTNNPPIPV	p24	117-126	B	human	291	A2
MTNNPPIPV	p24	118-126	B	human	292	A2, A2 supertype
MTSNPPIPV	p24	118-126	M	human, mouse	293	A*0201
MPPIPVGDIY	p24	122-130	B, C	human	294	B*3501, B*3502, B35
MPPVPVGDIY	p24	122-130	C	human	295	B35
MNPVPVGNIY	p24	122-130	HIV-2	human	296	B*3501
MPPIPVGEIY	p24	122-130	A, B, C, D	human	297	B35, B53, B7 supertype
MPPIPVGDIH	p24	122-130		human	298	
MPVGDIYKRWII	p24	125-135	C	human	299	
MVGEIYKRWIIGLNK	p24	126-140	B	human	300	
MGDIYKRWII	p24	127-135		human	301	B*0801
MGEIYKRWII	p24	127-135	A, B, C, CRF02_AG, D	human	302	B*0801, B8
MGEIYKRWIIL	p24	127-136	B	human	303	B*0801
MEIYKRWII	p24	128-135	B	human	304	B*0801, B8
MDIYKRWII	p24	128-135	B, C	human	305	B*0801, B8
MEIYRKWII	p24	128-135		human	306	B8
MEIYKRWII ?	p24	128-135		human	307	B8

MEIYKRWIIL	p24	128-136	B, D	human	308	A*2402, A2, A24
MIYKRWIIL	p24	129-136		human	309	A*2402
MIYKRWIILGL	p24	129-138	B, D	human	310	A*2402, A24, B27
MKRWIILGLN	p24	131-139		chimpanzee	311	Patr-B3
MKRWIILGLNK	p24	131-140	A, B, C, CRF01_AE, CRF02_AG, D	human, mouse	312	A2, B*2705, B27, B2705, B35, B57
MRRWIQLGLQK	p24	131-140		human	313	B*2703, B27
MKRWIILGGLNK	p24	131-140		human	314	B*2705
MKRWIIMGLNK	p24	131-140	B	human	315	B*2705, B27
MKRWIIMGNK	p24	131-140		human	316	B27
MKRWIILGNK	p24	131-140		human	317	B27
MKRWIIMGLHK	p24	131-140	B	human	318	B27
MKRWILLGLNK	p24	131-140	B	human	319	
MKRWIILGLNKIV	p24	131-142	B	human	320	B27
MKRWILGLNKIV	p24	131-142		human	321	B27
MKRWILGLNKIVRMY	p24	131-145		human	322	
MRWIILGLNK	p24	132-140	B	human	323	B27
MKWILGLNKIVRMY	p24	132-145		human	324	B27
MIILGLNKIV	p24	134-142	B	human	325	A2
MIILGLNKIVR	p24	134-143	B	human	326	A11, A3, A33
MILGLNKIV	p24	135-142	B, C, CRF01_AE	human	327	A*0201, A2, A3, B27
MILGLNKIVR	p24	135-143	B	human	328	A11, A3
MILGLNKIVRMY	p24	135-145	B	human	329	B7 supertype
MLGLNKIVRMY	p24	136-145	B	human	330	B62
MLGLNKIVRMYS	p24	136-146	C	human	331	B62
MGLNKIVRMY	p24	137-145	B	human	332	B*15, B*1501, B*62, B15,

						B1501, B27, B62
MGLNKVRMY	p24	137-145	B, CRF01_AE	human	333	B62
MVRMYSPVSI	p24	142-150	C	human	334	Cw*1801, Cw18
MRMYSPTSI	p24	143-150	B	human	335	B*5201, B52
MRMYSPVSIL	p24	143-151	C	human	336	A*0201
MRMYSPTSIL	p24	143-151	B, CRF01_AE, CRF02_AG	human	337	A2
MYSPTSIL	p24	144-151	B	human	338	A24
MSILDIRQGPK	p24	149-158	B	human	339	A11
MDIRQGPKEPFR	p24	152-162	B	human	340	B27
MEPFRDYVDRF	p24	159-168	B, C	human, mouse	341	A*0201, H-2^{d}, H-2L^{d}
MEPFRDYVDRFF	p24	159-169	A, C, D	human	342	B81
MPFRDYVDRFF	p24	160-169	C	human	343	
MFRDYVDRFF	p24	161-169	C	human	344	Cw*1801, Cw18
MFRDYVDRFYK	p24	161-170	B, D	human, macaque	345	B*1801, B18, B27
MFRDYVDRFFK	p24	161-170		human	346	
MFRDYVDRFYKTLRA	p24	161-174	B	human	347	
MRDYVDRFFKTL	p24	162-172	A	human	348	A*2402, A24
MRDYVDRFYKTL	p24	162-172	B, D	human, macaque	349	A26, B*4402, B44, B70
MDYVDRFYKT	p24	163-171	B	human	350	A*2402
MDYVDRFYKTL	p24	163-172		human	351	A24
MYVDRFYKTL	p24	164-172	B	human	352	A*0207, A2, B70
MYVDRFFKTL	p24	164-172	A, C, D	human	353	A*2601, A26,

						B*1503, B*1510, B15, B70, Cw*0303, Cw*0304
MYVDRFFKRL	p24	164-172		human	354	B*1510, B70
MYVDRFFKTL ?	p24	164-172		human	355	Cw0304
MVDRFYKLTRAEQAS	p24	165-178	B	human	356	A*33 motif
MVDRFYKTLRAEQAS	p24	165-178	B	human	357	
MDRFYKLTRA	p24	166-174	B	human	358	B14
MDRFFKTLRA	p24	166-174	C	human	359	B*1401, B*1402, B*1403, B14
MDRFYKTLRA	p24	166-174	B, D	human	360	B*1402, B14
MDRFWKTLRA	p24	166-174		human	361	B14
MDRFYKTLRAE	p24	166-175		human	362	B14
MDRFYKTRAE	p24	166-175		human	363	B14
MDRFYKTLRAEQ	p24	166-176	B	human	364	B*1402
MTLRAEQATQD	p24	171-180	C	human	365	Cw*0304
MRAEQASQEV	p24	173-181	B	human	366	Cw*0802, Cw08, Cw8
MRAEQASQEVK	p24	173-182	B	human	367	A11
MRAEQATQDVKN	p24	173-183	C	human	368	
MAEQASQDVKNW	p24	174-184	B, D	human	369	B*4402, B44
MAEQASQEVKNW	p24	174-184	B	human	370	B44
MAEQASADVKNW	p24	174-184	B	human	371	B44
MAEQASQEVKNWM	p24	174-185		human	372	Cw5
MEQASQEVKNWMT	p24	175-186		human	373	B44
MQASQEVKNW	p24	176-184	B, D	human	374	B*5301, B*5701, B*5801, B53, B57, B58, Cw04, Cw4

MQATQDVKNW	p24	176-184	C	human	375	B*5301, B*5801, B57
MQATQEVKNW	p24	176-184		human	376	B53
MQATQEVKNM	p24	176-184	A	human	377	B53
MQASGEVKNW	p24	176-184		human	378	Cw4
MQASKEVKNWV	p24	176-185		human	379	B57
MATQEVKNWM	p24	177-185		human	380	B53
MAAVKNWMTQTL	p24	179-189		mouse	381	H2-Db
MEVKNWMTETL	p24	180-189		human	382	B53
MVKNWMTETLL	p24	181-190	B	human	383	B8
MVKNWMTETLLV	p24	181-191	B	human	384	B*27 motif
MLVQNSNPDCK	p24	190-199	B	human	385	A11
MVQNSNPDCK	p24	191-199	B	human	386	A11
MNANPDCKTI	p24	193-201		human	387	B*5101, B51, B8
MNANPDCKTI?	p24	193-201		human	388	B51
MNANPDSKTI	p24	193-201		human	389	B51
MANPDCKTIL	p24	194-202		human	390	B7
MNPDCKTIL	p24	195-202	B, C	human	391	B*0801, B35
MNPDCKTILRAL	p24	195-205	C	human	392	B*3910
MPDCKTILKA	p24	196-204	B	human	393	
MDCKTILKAL	p24	197-205	B	human, humanized rabbit	394	B*0801, B8
MKALGPAATL	p24	203-211	B	human	395	
MALGPGASLEEM	p24	204-214	C	human	396	
MGPGATLEEM	p24	206-214	A, C, D	human	397	B*0702, B*5801, B*8101
MATLEEMMTA	p24	209-217	CRF01_AE	human, transgenic mouse	398	A*0206, A2
MEEMMTACQGVG	p24	212-222	C	human	399	
MEMMTACQGV	p24	213-221		human,	400	A*0201, A2

				transgenic mouse		
MACQGVGGPGHK	p24	217-227	B, CRF01_AE	human	401	A*11, A*1101, A*1103, A11
MCQGVGGPGHK	p24	218-227	B	human	402	A11
MGVGGPGHK	p24	220-227		human	403	A11
MGVGGPGHKAR	p24	220-229	B	human	404	A11
MVGGPGHKARVL	p24	221-231	B	human	405	B7
MGPGHKARVL	p24	223-231	B, C, D	human	406	B*0702, B*4201, B07, B35, B44, B7
MGPSHKARVL	p24	223-231	A, C, D	human	407	B*0702, B42, B7, B81
MGPGHKARVLA	p24-p2p7p1p6	223-1	multiple	human	408	B7
MRVLAEAMSQV	p24-p2p7p1p6	229-7	B	human	409	A2
MVLAEAMSQV	p24-p2p7p1p6	230-7	B	human, transgenic mouse	410	A*0201, A2, A2 supertype
MVLAEAMSQVT	p24-p2p7p1p6	230-8	B	human	411	A2
MAEAMSQVTNS	p2p7p1p6	1-10		human	412	B*4002, B*4501
MSQVTNSATI	p2p7p1p6	5-13	B	human	413	A2
MSQVTNPANI	p2p7p1p6	5-13		mouse	414	H-2D^{b}
MTNSANIMMQR	p2p7p1p6	8-17	B	human	415	
MKGPRRIVKC	p2p7p1p6	21-29	C	mouse	416	H-2d
MKGPKRIIKC	p2p7p1p6	21-29	C	mouse	417	H-2d
MSKRIVKCFNCG	p2p7p1p6	23-33	C	human	418	
MKTVKCFNCGR	p2p7p1p6	25-34	B	human	419	A11
MLARNCRAPRK	p2p7p1p6	38-47	multiple	human	420	A*3101, A3

MIAKNCRAPRKK	p2p7p1p6	38-48	C	human	421	
MCRAPRKKGC	p2p7p1p6	42-50	B	human	422	B14
MKAPRKKGCWK	p2p7p1p6	43-52	B	human	423	A11
MKCGKEGHQMK	p2p7p1p6	52-61	B	human	424	A11
MHQMKDCNERQAN	p2p7p1p6	58-69	B, G	human	425	A2
MDCTERQANFLG	p2p7p1p6	62-72	C	human	426	
MCTERQANFL	p2p7p1p6	63-71	B	human	427	B*61, B61
MTERQANFL	p2p7p1p6	64-71	B	human	428	B*04, B*4002
MERQANFLGKIW	p2p7p1p6	65-75	C	human	429	
MRQANFLGKI	p2p7p1p6	66-74	B, C	human	430	B13
MFLGKIWPS	p2p7p1p6	70-77	B	human, transgenic mouse	431	A2
MFLGKIWPSHK	p2p7p1p6	70-79	B, C	human	432	A*0201, A*0205, A2
MFLGKIWPSYK	p2p7p1p6	70-79	B	goat, human	433	A*0201, A02, A2
MKIWPSYKGR	p2p7p1p6	73-81		human	434	A*3101
MGNFLQSRPTAPPF	p2p7p1p6	83-97		human	435	A2
MFLQSRPEPTA	p2p7p1p6	85-94	B	human	436	A2
MEPTAPPEESF	p2p7p1p6	91-100	D	human	437	B35, B58
MTPSQKQEPI	p2p7p1p6	108-116	D	human	438	B35, B53
MSQKQEQIDK	p2p7p1p6	110-118	B	human	439	A11
MQEPIDKELY	p2p7p1p6	113-121	D	human	440	B44
MEPKDREPL	p2p7p1p6	114-123	C	human	441	B*0801
MEPIDKELYPL	p2p7p1p6	114-123	D	human	442	B35, B53
MKELYPLTSL	p2p7p1p6	118-126	B	human	443	B*4001, B40, B60
MLYPLASLRSL	p2p7p1p6	120-129	D	human	444	A24
MYPLASLRSL	p2p7p1p6	121-129	B, D	human	445	B53, B7
MYPLASLRSLF	p2p7p1p6	121-130	D	human	446	B35
MYPLTSLRSLF	p2p7p1p6	121-130	B	human	447	B7

MPLTSLKSLFGS	p2p7p1p6	122-132	C	human	448	
MLASLRSLF	p2p7p1p6	123-130	D	human	449	B58
MNSPTRREL	Gag_Pol_TF	24-31	B	human	450	Cw*0102
MAGAERQGTL	Gag_Pol_TF	44-52	C	mouse	451	H-2d
MFSFPQITLW	Gag_Pol_TF-Protease	54-6	B	human	452	
MITLWQRPLV	Protease	3-11	A, B, C, D	human, transgenic mouse	453	A*6802, A*7401, A19, A2, A28, A28 supertype, A74
MQRPLVTIKI	Protease	7-15		human	454	A*0101
MQRPLVTVKIG	Protease	7-16		human	455	B51
MVTILIGGQLK	Protease	11-20		human	456	A3 supertype
MTIKIGGQLK	Protease	12-20		human	457	A3 supertype
MLLDTGADDTV	Protease	23-32		transgenic mouse	458	A*0201
MLLDTGADDTVL	Protease	23-33		human	459	A2
MDTVLEEMNL	Protease	30-38	B	human	460	A*6802, A28, A68
MDTVLEDINL	Protease	30-38	A	human	461	A*6802
MDTVLEEWNL	Protease	30-38	D	human	462	A*6802
MEEINLPGKW	Protease	34-42	C	human	463	B44
MEEMNLPGRW	Protease	34-42	multiple	human	464	B*4402, B*4403, B18, B40, B44
MLPGRWKPKMI	Protease	38-47		human	465	Cw3
MWKPKMIGGI	Protease	42-50		human	466	Cw3
MKMIGGIGGF	Protease	45-53		human	467	B62
MKMIGGIGGFI	Protease	45-54	B	human, humanized mouse	468	A*0201, A2, A2 supertype,

						B62
MKVRQYDQIL	Protease	55-63		human	469	A2, Cw6
MVRQYDQIPIEI	Protease	56-66		human	470	B13
MRQYDQILIEI	Protease	57-66	B	human	471	B13
MRQYDQIPIEI	Protease	57-66		human	472	B13
MQYDQIPIEI	Protease	58-66		human	473	Cw*0401
MILIEICGHK	Protease	62-70		human	474	A3
MIEICGHKAIG	Protease	64-73		human	475	B18, B40, B44
MGKKAIGTVL	Protease	68-76	C	human	476	B*1503
MKAIGTVLV	Protease	70-77	B, C	human	477	B57, B58, B63
MVLVGPTPVNI	Protease	75-84	B	human, humanized mouse, transgenic mouse	478	A*0201
MLVGPTPVNI	Protease	76-84	B	human	479	A*0201, A02, A2, A2 supertype
MLVGPTPANI	Protease	76-84		human, macaque	480	A*0205
MLVGPTPVNII	Protease	76-85	C	human	481	A*0205
MVGPTPVNII	Protease	77-85	B	mouse	482	H-2D^{d}
MIGPTPVNII	Protease	77-85	C	mouse	483	H-2d
MPTPVNIIGRNL	Protease	79-89	B, C	human	484	B63
MTPVNIIGRNML	Protease	80-90	C	human	485	B*8101, B81
MTQIGCTLNF	Protease	91-99	B, C, CRF01_AE	human	486	B*1501, B*1503
MTQICGTLNF	Protease	91-99		human	487	B62, Cw3
MCTLNFPISPI	Protease-RT	95-5		human	488	A2 supertype
MTLNFPISPI	Protease-RT	96-5		human	489	A2 supertype
MSPIETVPVKL	RT	3-12	B, C, multiple	human	490	A2, B*8101, B61, B7, B8

MIETVPVKL	RT	5-12		human	491	B*4001, B40
MPGMDGPKVKQ	RT	14-23	multiple	human	492	A11
MGPKVKQWPL	RT	18-26	A, B, C, D	human	493	B*0801, B8
MGPKVKQWPLT	RT	18-27	multiple	human	494	B7, B8
MALVEICTEM	RT	33-41	B	human, humanized mouse, transgenic mouse	495	A*0201, A*19, A2, A3, B*3501, B44, Cw*07, Cw*16
MALVEICTEL	RT	33-41	B	human	496	A*0201
MALVEICTEMEK	RT	33-43		human	497	A*0301, A3
MALVICTEMEK	RT	33-43		human	498	A3
MTEMEKEGKI	RT	39-47		mouse	499	H-2K^{k}
MTEMEAEGKI	RT	39-47		mouse	500	
MEKEGKISKI	RT	42-50	B	human	501	B*5101, B51
MNTPVFAIKK	RT	57-65		human	502	A*0301, A*6801, A3 supertype
MNTPVFAIKKK	RT	57-66	B, multiple	baboon, chimpanzee, goat, human	503	A11, A68, B8
MKLVDFRELNK	RT	73-82	B, multiple	human	504	A*0301, A3
MGIPHPAGLK	RT	93-101	B, multiple	human	505	A*0301, A3
MGIPHPAGLKK	RT	93-102	CRF01_AE	human	506	A11
MTVLDVGDAY	RT	107-115	AG, B	human	507	B*3501, B35
MTVLDVGDIY	RT	107-115		human	508	B35
MVLDVGDAYFSV	RT	108-118	B	human, transgenic mouse	509	A*0201, A2
MDAYFSVPL	RT	113-120		human	510	A24, B*5101, B51
MFSVPLDEDF	RT	116-124	C	human	511	B*5702, B*5703, B35
MSVPLDESFRK	RT	117-126	CRF01_AE	human	512	A11

MVPLDEDFRKY	RT	118-127	B, C	human	513	B*3501, B35
MVPLDKDFRKY	RT	118-127		human	514	B*3501, B35
MKYTAFTIPSI	RT	126-135		human	515	A2
MYTAFTIPSI	RT	127-135	B, C	human	516	A*0205, A2, A2 supertype
MYTAFTIPSV	RT	127-135		human	517	A02, A2
MTAFTIPSI	RT	128-135	B	human	518	A*0201, A*0217, B*51, B*5101, B51
MNNETPGVRY	RT	136-144	B	human	519	B*1801
MNETPGIRYQY	RT	137-146	C	human	520	B18
MIRYQYNVL	RT	142-149	C	human	521	B*1401
MLPQGWKGSPAI	RT	149-159	C	human	522	B*3910
MQGWKGSPAI	RT	151-159		human	523	B*5101
MWKGSPAIFQSSMT	RT	153-165		human	524	B7
MSPAIFQSSM	RT	156-164	B, C	human	525	A*0702, B*0702, B*3501, B*4202, B*8101, B35, B7
MSPAIFQSSMT	RT	156-165	B, multiple	human	526	B35, B7
MAIFQSSMTK	RT	158-166	A, B, C, CRF01_AE, D, multiple	human, macaque	527	A*0301, A*1101, A*6801, A11, A3, A3 supertype, A33, A68
MSIFQSSMTK	RT	158-166		human	528	A11
MIFQSSMTKI	RT	159-167	A, B, C	mouse	529	H-2K^{d}, H-2d
MTKILEPFR	RT	164-172	B	human	530	A*0301, A3 supertype
MKQNPDIVIY	RT	173-181	B	human	531	A*3002,

						A30, B*1503
MAQNPEIVIY	RT	173-181	C	human	532	A*3002
MAQNPEIVIYQY	RT	173-183	C	human	533	A*3002
MNPEIVIYQY	RT	175-183	C	human	534	B18, B35
MNPDIVIYQY	RT	175-183	B	human	535	B*3501, B35
MHPDIVIYQY	RT	175-183	A, B	human, macaque	536	B*3501, B35
MNPDVILIQY	RT	175-183	HIV-2	human	537	B35
MNPDIVIYQYM	RT	175-184	B	human	538	B51
MVIYQYMDDL	RT	179-187	A, B, C, CRF01_AE, D	human, humanized mouse, macaque, mouse, transgenic mouse	539	A*0201, A*19, A2, B*3501, B44, Cw*07, Cw*16
MVIVQYMDDL	RT	179-187	B	human	540	A2
MVIYQYMMDL	RT	179-187	B	human	541	A*0202, A2
MVIYQYNDDL	RT	179-187		human	542	A2
MVIYQMDDL	RT	179-187		human	543	A2
MIYQYMDDLYV	RT	180-189	B	human	544	A*0201
MYQYMDDLYV	RT	181-189	B	human	545	A*0201, A2
MDLEIGQHRTK	RT	192-201		human	546	A3
MKIEELRQHL	RT	201-209		human	547	A2
MKIEELRQHLL	RT	201-210		human	548	B58
MIEELRQHLL	RT	202-210	B	human	549	B*4001, B40, B60, B61
MEELRQHLLR	RT	203-211		human	550	B44
MEELRQHLLRW	RT	203-212	B	human	551	B44
MELRQHLLRW	RT	204-212	B	human	552	A*2501
MRAHLLSWGF	RT	206-214	A, B, C	mouse	553	H-2K^{d}
MLLRWGLTTPDKK	RT	209-220	B	human, humanized	554	A*0201, A2

				mouse		
MLRWGFCTPDKK	RT	210-220		human	555	B57
MFTTPDKKHQK	RT	214-223	multiple	human	556	A11, A68
MTTPDKKHQKE	RT	215-224	multiple	human	557	A11
MPIVLPEKDSW	RT	243-252	B	human	558	B*5701, B57
MIVLPEKDSW	RT	244-252	B	human	559	B*5701, B*5801, B57
MIVPEKDSW	RT	245-252		human	560	B57
MKLVGKLNWA	RT	259-267		human	561	A2 supertype
MLVGKLNWASQIY	RT	260-271	B	human	562	B*1501, B15, B62
MKLNWASQIY	RT	263-271	B, C	human	563	A*3002, A30
MQIYPGIKVR	RT	269-277	B, C	human	564	A*0301, A3
MQIYAGIKVK	RT	269-277	B, CRF01_AE	human	565	A*1101, A11, A3
MQIYPGIKVK	RT	269-277	B	human	566	A3
MYPGIKVRQL	RT	271-279	B, C	human	567	B*4201, B42
MYPIGKVRQL	RT	271-279	C	human	568	B*4201, B*4202
MYPGIKVKQL	RT	271-279		human	569	B*4201
MIPLTEEAEL	RT	293-301	B	human	570	B*0702, B*1501, B*3501, B*5101, B*5301, B35, B51, B7
MEILKEPVGHV	RT	308-317	B	human	571	A*0201
MILKEPVHGV	RT	309-317	A, B, C, CRF02_AG, D	human, humanized mouse, macaque, mouse, transgenic mouse	572	A*0201, A*0202, A*0205, A02, A2, A2 supertype

MILKDPVHGV	RT	309-317	A	human	573	A2
MILRIPVHGV	RT	309-317	CRF01_AE	human	574	A2
MILKEPVHGVY	RT	309-318	A, B, D, multiple	human	575	A2, B*1501, B15, B62
MIKLEPVHGVY	RT	309-318		human	576	B15, B62
MVYYDPSKDL	RT	317-325	B, CRF02_AG	human	577	A24
MVYYDPSKDLI	RT	317-326	A, B, C	mouse	578	H-2K^d
MGQGQWTYQI	RT	333-341	B	human	579	B13
MQIYQEPFKNLK	RT	340-350	CRF01_AE	human	580	A11
MQIYQEPFKNLKTG	RT	340-352	B	human	581	A11
MIYQEPFKNL	RT	341-349	C	human	582	A*2301, A*2402
MIYQEPFKNLK	RT	341-350	B, C	human	583	A*1101, A11
MFKNLKTGKY	RT	346-354	B	human	584	
MRMRGAHTNDV	RT	356-365	B	human	585	A*3002
MRMRGAHTNDVK	RT	356-366	B	human	586	A*0301, A3
MRTRGAHTNDVK	RT	356-366	B	human	587	A3
MDVKQLTEVV	RT	364-372	A	human	588	A*6802, A28, B70
MKITTESIVIW	RT	374-383	B	human	589	B*5701, B57
MKIATESIVIW	RT	374-383		human	590	B57
MITTESIVIW	RT	375-383	B	human	591	B*5701, B*5801, B57
MIAMESIVIW	RT	375-383	C	human	592	B*5703, B*5801, B57
MPIQKETWETW	RT	392-401	B	human	593	A*3201, A32
MTWETWWTEYW	RT	397-406	B	human	594	B44
MQATWIPEWEF	RT	407-416	C	human	595	B*5702, B*5703, B57
MFVNTPPLV	RT	416-423		transgenic mouse	596	A2
MFVNTPPLVK	RT	416-424	CRF01_AE	human	597	A*1101, A11
MTPPLVKLWYQL	RT	419-429	C	human	598	B*8101

MPLVKLWYQL	RT	421-429		human, transgenic mouse	599	A*0201, A2
MEPIVGAETF	RT	432-440		human	600	B*3501, B35, B51
MEPIVGAETFY	RT	432-441	B	human, mouse	601	B*3501, B35, B51
MIVGAETFYVDGAAS	RT	434-447	B	human	602	A*6802
MGAETFYVDGA	RT	436-445	C	human	603	A*6802, B45
MGVETFYVDGA	RT	436-445		human	604	B45
MAETFYVDGA	RT	437-445	B	human	605	B*4501
MAETFYVDGAAN	RT	437-447	B	human	606	A28
METFYVDGAANR	RT	438-448	C	human	607	A26, A66
MRETKLGKAGY	RT	448-457		human	608	A29
METKLGKAGY	RT	449-457	B	human	609	A*2601, A26
MKLGKAGYVT	RT	451-459		human	610	A2
MALQDSGLEV	RT	485-493	B	human	611	A*0201, A2
MALQDSGSEVN	RT	485-494	B	transgenic mouse	612	A2
MEVNIVTDSQY	RT	492-501	B	human	613	A*2601
MVNIVTDSQYA	RT	493-502	B	human	614	
MIVTDSQYAL	RT	495-503		human	615	Cw*0802
MVTDSQYALGI	RT	496-505	B	human	616	B*1402, B*1503, B14, Cw8
MQPDKSESELV	RT	509-518	multiple	human	617	B7
MELVNQIIEQL	RT	516-525		human	618	A2
MQIIEQLIKK	RT	520-528	B, CRF01_AE, multiple	human	619	A*1101, A11, A3
MKVYLAWVPA	RT	530-538	B	human	620	A*0301, A3
MRVYLSWVPAHK	RT	530-540	C	human	621	A*0301
MYLAWVPAHK	RT	532-540		human, transgenic	622	A2, A3 supertype,

				mouse		B7
MSTGIRRVLFL	RT-Integrase	553-2	B	human	623	
MLFLDGIDKA	RT-Integrase	560-8		human	624	B81
MQEEHEKYHSNW	Integrase	9-19	B, C	human	625	B*4403
MRAMASDFNL	Integrase	20-28		human	626	A*0201, A2 supertype
MMASDFNLPPV	Integrase	22-31		human	627	A2 supertype
MLPPIVAKEI	Integrase	28-36	B, C	human	628	B*0705, B*4201, B*51, B*5101, B42, B51
MLPPVVAKEI	Integrase	28-36	B	human	629	B*5101, B51
MVASCDKCQL	Integrase	37-45	C	human	630	B*8101
MQVDCSPGI	Integrase	53-60	B	human	631	
MQLDCTHLEGK	Integrase	62-71	multiple	human	632	A3
MTHLEGKIIL	Integrase	66-74	C	human	633	B*1510
MGYIEAEVI	Integrase	82-89		human	634	A*2402
MYIEAEVIPA	Integrase	83-91	B	human	635	
MIPAETGQETA	Integrase	89-98	multiple	human	636	B56, B7
METGQETAY	Integrase	92-99	C	human	637	A*2601
METAYFILKL	Integrase	96-104	A	human	638	A*6802
MILKLAGRWPVK	Integrase	101-111	C	human	639	A*0301
MSTTVKAACW	Integrase	123-131		human	640	B57
MSTTVKAACWW	Integrase	123-132	B	human	641	B*5801, B57
MKAACWWAGI	Integrase	127-135		human	642	A2 supertype
MIQQEFGIPY	Integrase	135-143	C	human	643	B*1503
MKQEFGIPY	Integrase	136-143	B	human	644	B*1503
MELKKIIGQVR	Integrase	157-166	C	human	645	A*3301, A33
MQVRDQAEHL	Integrase	164-172	C	human	646	A*0205
MVRDQAEHL	Integrase	165-172	C	human	647	Cw*1801, Cw18
MHLKTAVQMAV	Integrase	171-180	multiple	human	648	A2

MKTAVQMAVF	Integrase	173-181	B	human	649	B*5701, B*5801, B57
MQMAVFIHNFK	Integrase	177-186		human	650	A3 supertype
MAVFIHNFK	Integrase	178-186		human	651	A3 supertype
MAVFIHNFKR	Integrase	179-187		human	652	A*0301, A3 supertype
MAVFIHNFKRK	Integrase	179-188	B, CRF01_AE, multiple	human	653	A*0301, A*11, A*1101, A11, A3, A68
MFKRKGGIGGY	Integrase	185-194	B, C	human	654	B*1503
MGERIVDII	Integrase	197-204	B	human	655	
MIIATDIQTK	Integrase	203-211	B	human	656	A*11, A1101
MTKIQNFRVYY	Integrase	218-227	B, C	human	657	B*1503
MKIQNFRYY	Integrase	219-226	C	human	658	A*3002
MKIQNFRVYY	Integrase	219-227	B, C	human	659	A*3002, A30
MKIQNFRVYYR	Integrase	219-228		human	660	A3 supertype
MLLWKGEGAV	Integrase	241-249	B	human, mouse, transgenic mouse	661	A*0201, A2
MLLWKGEGAW	Integrase	241-249		human	662	A*0201, A2
MVPRRKVKII	Integrase	260-268	C	human	663	B42
MVPRRKAKII	Integrase	260-268		human	664	B42
MRKAKIIRDY	Integrase	263-271	B, C	human	665	B*1503, B15
MRKAKIIKDY	Integrase	263-271	C	human	666	B*1503
MKIIRDYGK	Integrase	266-273	B	human	667	
MKIIKDYGKQM	Integrase	266-275	C	human	668	B*4201
MRIRTWKSLVK	Vif	17-26	B	human	669	A*0301, A3
MSLVKHHMYV	Vif	23-31		human, transgenic mouse	670	A2
MHMYISKKAK	Vif	28-36	B	human	671	A*0301, A3
MISKKAKGWF	Vif	31-39	B	human	672	B*5701,

						B*5801, B57
MVSKKAKGWI	Vif	31-39	B	human	673	B57
MHPRVSSEVHI	Vif	48-57	B	human	674	B*0702, B7
MHPKVSSEVHI	Vif	48-57	C	human	675	B*4201
MHPRISSEVHI	Vif	48-57	B	human	676	B7
MHPKISSEVHI	Vif	48-57	B	human	677	B7
MEVHIPLGEAR	Vif	54-63	C	human	678	A*6801
MIPLGDAKLII	Vif	57-66	B	human	679	B51
MDAKLIITTY	Vif	61-69	B	human	680	B*3501, B35
MDAKLVITTY	Vif	61-69		human	681	
MHTGERDWHL	Vif	73-81	B	human	682	B35
MWHLGHVSI	Vif	79-87		human	683	B*1510
MWHLGHGVSI	Vif	79-87	C	human	684	B*1510
MWHLGQGVSI	Vif	79-87		human	685	B*3801
MDLADQLIHLY	Vif	101-110	multiple	human	686	A2
MLADQLIHLHY	Vif	102-111	B	human	687	B*1801, B18
MHIVSPRCEY	Vif	127-135	B	human	688	A29
MALAALITPK	Vif	149-157		human, transgenic mouse	689	A2
MKIKPPLPSV	Vif	158-166		human, transgenic mouse	690	A2
MKTKPPLPSVKK	Vif	158-168	B	human	691	A*0301, A03, A11, A3
MRRKPPLPSIAK	Vif	158-168	B	human	692	A3
MRIKPPLPSVTK	Vif	158-168	B	human	693	A3
MKIKPPLPSVTK	Vif	158-168	B	human	694	A3
MKPPLPSVKKL	Vif	160-169	multiple	human	695	B7
MKLTEDRWNK	Vif	168-176	multiple	human	696	A3
MDQGPQREPY	Vpr	7-15	B	human	697	
MREPHNEWTL	Vpr	12-20	B	human	698	B*4002, B40

MTLEILEELKN	Vpr	19-28	B	human	699	A3?
MEAVRHFPRI	Vpr	29-37	B	human	700	B*5101, B51
MAVRHFPRIW	Vpr	30-38	B, C	human	701	B*5701, B*5801, B57, B63
MAVRHFRPIW	Vpr	30-38	B	human	702	
MVRHFPRIWL	Vpr	31-39		human	703	B27
MFPRPWLHGL	Vpr	34-42	C	human, transgenic mouse	704	A2, B*0702, B*4201, B*8101
MFPRIWLHGL	Vpr	34-42	B	human	705	B*0702, B*8101, B07, B7, B81
MFPRWLHGL	Vpr	34-42	C	human	706	B*0702, B*4201, B*8101
MFPRPWLHSL	Vpr	34-42	C	human	707	B*0702, B*4201, B*8101
MFPRTWLHGL	Vpr	34-42	B	human	708	B7
MSLGQHIYET	Vpr	41-49		human, transgenic mouse	709	A2
MIYETYGDTW	Vpr	46-54	B	human	710	A*2501
METYGDTWTGV	Vpr	48-57	C	human	711	A*6802
MDTWAGVEAIIR	Vpr	52-62		human	712	A*6801
MTWAVEAIIRI	Vpr	53-63		human	713	
MAIIRILQQL	Vpr	59-67	B	human	714	A*0201, A*19, A2, A2 supertype, B*3501, B44, Cw*07, Cw*16
MTWAGVEAIIRI	Vpr	59-67	B	human	715	A*11

MALIRILQQL	Vpr	59-67	B	human	716	A2
MRILQQLLFI	Vpr	62-70	B, M	human, humanized mouse, mouse	717	A*0201, A2, A2 supertype
MEPVDPRLEPW	Tat	2-11	B	human	718	B*5301, B53, B58
MEPVDPNLEPW	Tat	2-11	C	human	719	B*5301
MWPVDPRLEPW	Tat	2-11	B	human	720	
MPVDPRLEPW	Tat	3-11	B	human	721	A*2501
MKHPGSQPKTA	Tat	12-21	B	human	722	
MQPKTACTNC	Tat	17-25		mouse	723	H-2d
MQPKTACTTCY	Tat	17-26	B	human	724	B35
MPKTACTNCY	Tat	18-26	B	human	725	
MTACNNCYCK	Tat	20-28	multiple	human	726	A3
MTACNNCYCKK	Tat	20-29	multiple	human	727	A68
MNCYCKKCCY	Tat	24-32	B	human	728	A*2902
MNCYCKRCCF	Tat	24-32	B	human	729	A*2902
MNCYCKKCCF	Tat	24-32		human	730	
MCCFHCQVC	Tat	30-37	B	human	731	Cw*1203, Cw12
MCLHCQVCFI	Tat	31-39	B	human	732	A2
MFHCQVCFMTK	Tat	32-41	B	human	733	
MFHCQVCFITK	Tat	32-41		human	734	
MVCFTTKGLGI	Tat	36-45	B	human	735	B*15
MVCFMTKGLGI	Tat	36-45	B	human	736	B*1501
MVCFITKALGI	Tat	36-45		human	737	
MFQTKGLGISY	Tat	38-47	C	human	738	B*1503, B15
MTKGLGISY	Tat	39-47	B	human	739	B*1501
MITKALGISY	Tat	39-47		human	740	
MITKGLGISYGR	Tat	39-49	B	human	741	A*6801, A68
MTKGLGISY	Tat	40-47	B	human	742	B*1503
MTKALGISYGR	Tat	40-49	B	human	743	
MRKKRRQRRR	Tat	49-57		mouse	744	H-2^d

MAPQGHPNNQVSI	Tat	58-69	B	human	745	
MGPKESKKKVE	Tat	83-92		human	746	B58
MEELLKTVRL	Rev	10-18	B	human	747	
MLRAVRIIKI	Rev	13-21		human	748	B*5701, B*5703
MKAVRLIKFLY	Rev	14-23	B	human	749	B*5701, B*5801, B57, B63
MKAVRIKLFLY	Rev	14-23		human	750	B*5701, B*5801
MKAVRRLIKFLY	Rev	14-23	B	human	751	B*5701, B*5801
MKTGRLIKLLY	Rev	14-23	B	human	752	B57
MKTVRLIKFLY	Rev	14-23	B, C	human	753	B57, B58, B63
MTVRLIKFLY	Rev	15-23	B	human	754	A3
MKILYQSNPY	Rev	20-28	multiple	human	755	A3
MQIRTYSGWI	Rev	51-59	B	human	756	A2
MQIRSLSGWIL	Rev	51-60	B	human	757	
MIHSISERIL	Rev	52-60	C	human	758	B*1510
MISERILSTY	Rev	55-63	B	human	759	A*0101, A1
MSEWILSTHL	Rev	56-64	B	human	760	
MERILSTYLGR	Rev	57-66	B	human	761	A*0301, A3
MRILSTYLGR	Rev	58-66	B	human	762	A*0301
MYLGGSEEPV	Rev	63-71	B	human	763	A2
MRSAEPVPL	Rev	66-73		transgenic mouse	764	A2
MRPAEPVPLQL	Rev	66-75		human	765	B07, B7
MSAEPVPLQL	Rev	67-75	B	human	766	B14, Cw*0501, Cw5, Cw8
MPVPFQLPPL	Rev	70-78	B	human	767	A2
MLQLPPLERL	Rev	73-81	B	human	768	A2
MLQLPPIERL	Rev	73-81		human,	769	A2

				transgenic mouse		
MQLPPLERLT	Rev	74-82	B	human	770	A2
MLPPLERLTL	Rev	75-83	B	human	771	
MGMGSPQILV	Rev	96-104		human, transgenic mouse	772	A2
MQILGEPPTV	Rev	101-109	B	human	773	A2
MQVLGESPTVL	Rev	101-110	B	human	774	
MILVESPAVL	Rev	102-110	B	human, transgenic mouse	775	A2
MILGEPPTVL	Rev	102-110	B	human	776	A2
MPTVLESGTKE	Rev	107-116	multiple	human	777	A68
MLVILAIVALV	Vpu	4-13	multiple	human	778	B7
MLAIVALVVA	Vpu	7-15	B	human	779	
MVVAAIIAIV	Vpu	13-21		human	780	A2
MEYRKILRQR	Vpu	29-37	B	human	781	A*3303
MHAPWDVNDL	Vpu	74-82	B	human	782	Cw*0102
MRVKEKYQHL	gp160	2-10		human	783	B*0801, B8
MTQMNWPNLWK	gp160	6-12	CRF01_AE	human	784	A11
MAAENLWVTVYY	gp160	30-40		human	785	B44
MAENLVWTVYY	gp160	30-40		human	786	B44
MAENLWVTVY	gp160	31-39	B	human	787	B*1801, B*4403, B44
MAENLWVTVYY	gp160	31-40		human	788	B*4402, B44
MDNLWVTVYY	gp160	32-40	B, CRF01_AE	human	789	B44
MENLWVTVYY	gp160	32-40	B	human	790	
MKLWVTVYYGV	gp160	33-42	B	human	791	A*0201, A*19, A2, B*3501, B44, Cw*07, Cw*16

MNLWVTVYYGV	gp160	33-42	multiple	human	792	A02, A2
MLWVTVYYGV	gp160	34-42	B	human	793	A*0201
MVTVYYGVPV	gp160	36-44		human	794	A02, A2
MVTVYYGVPVWK	gp160	36-46		human	795	A*6801, A11
MVTVYYGVPVWR	gp160	36-46	CRF01_AE	human	796	A11
MTVYYGVPVW	gp160	37-45	A, B, C, D	human	797	A*11, A3, B*03
MTVYYGVPVWK	gp160	37-46	A, B, C, D, multiple	human, transgenic mouse	798	A*0301, A*6801, A11, A2, A3, A3 supertype, B18
MTVYYGVPVMK	gp160	37-46			799	A11, A3, A68
MVYYGVPVWKEA	gp160	38-48		human	800	Cw7
MVPVWKEATTT	gp160	42-51		human	801	B*5501, B55
MVPVWKEATTTL	gp160	42-52		human	802	B*3501, B35
MVPVWKEAKTTL	gp160	42-52	C	human	803	B*5301
MTTLFCASDAK	gp160	50-59		human	804	A3, A3 supertype
MTLFCASDAK	gp160	51-59		human	805	A*0301, A3, A3 supertype
MLFCASDAKAY	gp160	52-61	B	human	806	A*2402, A24
MLFCASCAKAY	gp160	52-61	B	human	807	B38
MKAYETEVHNVW	gp160	59-69	C	human	808	B*5801, B58
MYETEVHNVW	gp160	61-69	B	human	809	B*1801
MEVHNVWATHA	gp160	64-73	A, B, C, CRF01_AE, D	human	810	A*2603
MNIWATHACV	gp160	67-75		human	811	A2
MACVPTDPNP	gp160	73-81		human	812	
MVPTDPNPPEV	gp160	75-84		human	813	A2
MDPNPQEVVL	gp160	78-86	B	human	814	B*3501,

						B35, B51
MDPNPQEMVL	gp160	78-86	C	human	815	B35
MDPNPQEVAL	gp160	78-86		human	816	B35
MNVTENFNMW	gp160	88-96	B	human	817	A*2501
MVTEEFNMWK	gp160	89-97	A, B, C, D	human	818	
MVTENFNMWKN	gp160	89-98	multiple	human	819	A11, A68 supertype
MQMHEDIISL	gp160	103-111		human	820	A*0201
MHEDIISLW	gp160	104-112		human	821	B*3801
MHEDIISLWDQSLK	gp160	105-117		chimpanzee, human	822	A2
MIISLWDQSL	gp160	108-116	B	human	823	A*0201, A2.1
MISLWDQSLK	gp160	109-117	CRF01_AE	human	824	A11
MSLWDQSLKP	gp160	110-118	multiple	human	825	A03, A3
MKPCVKLTPLC	gp160	117-126	multiple	human	826	B7
MKLTPLCVTL	gp160	121-129	B	human, mouse, transgenic mouse	827	A*0201, A02, A2, A2 supertype
MKLTPLCVSL	gp160	121-129		human, transgenic mouse	828	A*0201, A2
MKTLPLCVTL	gp160	121-129		human	829	A2
MKNCSFNMTT	gp160	155-163	A, B, C, D	human	830	
MNCSFNISTSI	gp160	156-165		human	831	Cw8
MYRLINCNTSV	gp160	191-200	CRF01_AE	human	832	A2
MKLTSCNTSV	gp160	192-200		human	833	A2
MRLISCNTSV	gp160	192-200	B	human	834	A2
MTLTSCNTSV	gp160	192-200		human	835	A*0201, A2
MILRSCNTSV	gp160	192-200		human	836	A2
MSVITQACPK	gp160	199-207	B	human	837	A*11, A*1101
MKMTFEPIPIH	gp160	207-216	A	human	838	A29
MVSFEPIPIH	gp160	208-216	multiple	human	839	A3

MVSFEPIPIHY	gp160	208-217	B	human	840	A29
MVSFEPIPPHYCA	gp160	208-219	B	human	841	A2
MSFDPIPIHY	gp160	209-217	C	human	842	A29
MSFEPIPIHY	gp160	209-217	B, D	human, mouse	843	A*2902, A29
MYCAPAGFAIL	gp160	217-226	B	human	844	Cw*0102
MCTPAGYAILKC	gp160	218-228	CRF01_AE	human	845	A*0201, A*0206, A*0207
MGPCKNVSTVQ	gp160	237-246		human	846	B56
MCTNVSTVQC	gp160	239-247	B	human	847	A*0201, A*19, B*3501, B44, Cw*07, Cw*16, Cw8
MCKNVSTVQC	gp160	239-247	B	human	848	Cw*0802
MRPIVSTQLL	gp160	252-260		human	849	B*3501, B35
MKPVVSTQLLL	gp160	252-261	multiple	human	850	B07, B08, B7, B8
MRPVVSTQLLL	gp160	252-261	multiple	human	851	B7, B8
MNAKTIIVHL	gp160	280-288	CRF01_AE	human	852	Cw*0602
MAKTIIVHL	gp160	281-288	C	human	853	Cw*0602
MCTRPNNNTRK	gp160	296-305	B, CRF01_AE, multiple	human	854	A02, A03, A2, A3, A32, B08, B62, Cw3
MRPNNNTRKS	gp160	298-306	D	human	855	B7
MRPNNNTRKSI	gp160	298-307	B, C	human	856	B*0702, B*4201, B07, B7, B7?
MRPNNNTRRGI	gp160	298-307	B	human	857	B7
MRIQRGPGRAFVTIG	gp160	308-321	B	mouse	858	H-2 D^d
MRIQRGPGRAFVTIK	gp160	308-321		mouse	859	
MRIQRGPGRAFTIGK	gp160	308-322	B	mouse	860	
MIYIGPGRAF	gp160	309-317		human	861	A*2402

MITVGPGQVFY	gp160	309-318	CRF01_AE	human	862	A11
MHIGPGRAFY	gp160	310-318	B	human	863	A*3002, A2
MTLGPGRVLY	gp160	310-318	B	human	864	
MHIGPGRAFYTTKNI	gp160	310-323		mouse	865	H-2D^{d}
MIGPGRAFY	gp160	311-318		mouse	866	H-2D^{d}
MRGPGRAFVT	gp160	311-319	B	mouse	867	A2
MIGPGRAFHT	gp160	311-319	B	mouse	868	H-2 D^{dd}
MRGPGRAFYTT	gp160	311-320	B	human	869	A2
MRGPGRAFVTI	gp160	311-320	A, B, C	human, humanized mouse, macaque, mouse	870	A*0201, A2, D, H-2 D^{dd}, H-2d17, H-2d, H-2p, H-2u, H-2Dd, H-2Dd, H-2d
MMGPKRAFYAT	gp160	311-320		human	871	A2
MIGPGRARYAR	gp160	311-320		mouse	872	H-2D
MIGPGRAFYTT	gp160	311-320		mouse	873	H-2D^{d}
MRGPGRAFTVTI	gp160	311-320		mouse	874	H-2D^{d}
MIGPGRAFYAT	gp160	311-320		mouse	875	H-2D^{d}
MIGPGRAFYAR	gp160	311-320	B	mouse	876	H-2D^{d}
MGPGRAFVTI	gp160	312-320	B	mouse	877	H-2^{d}
MGRAFVTIGK	gp160	314-322	B	human	878	B*2705, B27
MEIIGDIRQAY	gp160	321-330	B	human	879	A*2501
MRVLKQVTEK	gp160	340-348	CRF01_AE	human	880	A11
MNTLKQIVIKL	gp160	340-349		chimpanzee	881	Patr-B14
MTLSQIVTKL	gp160	341-349	B	human	882	A*0201
MPSSGGDLEITTHSF	gp160	363-376	C	human	883	B18
MPEIVTHS	gp160	369-375		human	884	A2
MHSFNCGGEF	gp160	374-382	multiple	human	885	A3
MSFNCGGEFF	gp160	375-383	A, B, C	human	886	B*1516, B15, B1516, B63, Cw*0401, Cw*0407, Cw04, Cw4

MSFTCGGEFF	gp160	375-383		human	887	B15
MSFNCRGEFFY	gp160	375-384	C	human	888	A29
MSFNCGGEFFY	gp160	375-384	B	human	889	A29
MFNCGGEFF	gp160	376-383		human	890	Cw4
MFNCGGEFFY	gp160	376-384	B	human	891	A29, B8
MPNCGGEFFY	gp160	376-384		human	892	A29
MKNCGGEFFYCNS	gp160	376-387	B	human	893	A2
MNCGGEFFYCN	gp160	377-386	B	human	894	
MNSGGEFFYSNS	gp160	377-387		human	895	A2
MFYCNTTQLF	gp160	383-391	B	human	896	A*2402
MLPCRIKQII	gp160	416-424	B	human	897	B*5101, B51
MLPCRIKQFINMWQE	gp160	416-429		human	898	DR4 CD4+
MRIKQIINMW	gp160	419-427	B, CRF02_AG	human	899	A*3201, A32
MRIKQIINMW?	gp160	419-427		human	900	A29, A32
MRIKQFINMW	gp160	419-427	B	human	901	A32
MNMWQEVGKAM	gp160	425-434	multiple	human	902	A2
MYAPPIGGQI	gp160	434-443		mouse	903	H-2K^{d}
MYAPPISGQI	gp160	435-443		macaque	904	Mamu-A1
MRCSSNITGLL	gp160	444-453		human	905	B56
MYSENSSEYY	gp160	460-468	B, C	human	906	
MEVFRPGGGDM	gp160	466-475	B	human	907	A*2601
MNWRSELYKY	gp160	478-486	B	human	908	
MYKVVKIEPL	gp160	486-494	B	human	909	
MYRLGVGALI	gp160	511-519	C	human	910	Cw*1801, Cw18
MTMGAASITL	gp160	529-537		human	911	A2
MLLRAIEAQQHL	gp160	555-565	CRF01_AE	human	912	A11.1
MRAIEAQQM	gp160	557-564	C	human	913	Cw*0801
MRAIEAQQWQ	gp160	557-565		human	914	B*5101, B51
MRAIEAQQHL	gp160	557-565	A, B, C, D	human	915	B*1501, B*51, B*5101,

						B15, B51, B57, B58, B63, C*03, C*15, Cw*03, Cw*0304, Cw03, Cw12, Cw15
MRAIEAQQHM	gp160	557-565	C	human	916	Cw*0801, Cw8
MRAIEAQQHML	gp160	557-566	C	human	917	A69, B40
MLLQLTVWGI	gp160	565-573		human, transgenic mouse	918	A2, A2 supertype
MQTRVLAIERYL	gp160	577-587	C	human	919	B*5802
MVERYLKDQQL	gp160	583-592	B	human	920	A*0201, A*19, B*3501, B14, B44, Cw*07, Cw*16
MERYLKDQQL	gp160	584-592	A, B, C, D	human	921	A*0201, A*19, A*32, A32, B*1401, B*1402, B*3501, B14, B44, Cw*07, Cw*16
MERYLRDQQL	gp160	584-592	A, B, C, D	human	922	B14
MERYLKDQQLLG	gp160	584-594		human	923	
MRYLRDQQL	gp160	585-592		human	924	A*2402
MRYLKDQQL	gp160	585-592	B	human	925	B27
MRYLKDQQLL	gp160	585-593	B, C	human	926	A*23,

						A*2301, A*2402, A23, A24
MRYLRDQQLL	gp160	585-593		human	927	A*2402
MRYLRDQQLLGI	gp160	585-595		human	928	A*2402
MYLKDQQLL	gp160	586-593	A, B, CRF01_AE	human, macaque	929	A*2402, A24, B*0801, B8
MYLRDQQLL	gp160	586-593		human	930	A24
MYLRDQQLLGIWGC	gp160	586-598		human	931	Cw7
MTAVPWNASW	gp160	606-614	B	human	932	B*3501, B35
MWLWYIKIFI	gp160	678-686	B	human	933	A*0201, A*19, A2, B*3501, B44, Cw*07, Cw*16
MWYIKIFIMI	gp160	680-688		human	934	A*2402
MYIKIFIMIV	gp160	681-689		human, transgenic mouse	935	A2
MFIMIVGGLV	gp160	685-693	B	human	936	A*0201
MVFAVLSIVNR	gp160	698-707	B	human	937	A*3303
MAVLSVVNRV	gp160	700-708		human	938	A2
MEIIFDIRQAY	gp160	703-712		human	939	A*2501
MIVNRNRQGY	gp160	704-712	B	human	940	A*3002, A30
MIVNRVRQGY	gp160	704-712		human	941	A*3002
MYSPLSLQTL	gp160	712-720	B	human	942	Cw*0102
MRLVNGSLAL	gp160	747-755		human	943	A2
MRLVSGFLAL	gp160	747-755	CRF01_AE	human	944	A2
MVSGFLALAW	gp160	749-757	A, B, C, D	human	945	
MLRSLFLFS	gp160	760-767		human	946	A*2301
MSYRRLRDLL	gp160	767-775		human	947	A*2402
MSYHRLRDFI	gp160	767-775	A	mouse	948	H-2K^{d}
MSYHRLRDLLLIVTR	gp160	767-780	B	human	949	A31

MHRLRDLLLI	gp160	769-777		human	950	
MRLRDLLLIV	gp160	770-778		human	951	A*0201
MRLRDLLLIVTR	gp160	770-780	B, C	human	952	A*0301, A*3101, A03, A3, A31
MIVTRIVELL	gp160	777-785	B	human	953	A*6802
MGRRGWEALK	gp160	786-794	B	human	954	B*2705, B27
MGRRGWEALKY	gp160	786-795	B	human	955	B*2705, B27, B2705
MRRGWEVLKY	gp160	787-795		human	956	A*0101, A1
MKYCWNLLQY	gp160	794-802	B	human	957	A*3002, A30
MKYCWNLLQY ?	gp160	794-802		human	958	A*3002
MLLQYWSQEL	gp160	799-807	B	human	959	A*0201
MQELKNSAVSL	gp160	805-814	B	human	960	B*4001, B60, B61
MSLLNATAIAV	gp160	813-822	B, CRF01_AE	human, mouse	961	A2
MSLLNATDIAV	gp160	813-822	B	human, mouse	962	A*0201, A2, A68
MLLNATAIAV	gp160	814-822	B	human	963	A*0201, A*19, A2, B*3501, B44, Cw*07, Cw*16
MLLDTIAIAV	gp160	814-822	C	human	964	A*0205
MLLNATDIAV	gp160	814-822	B	human	965	A2
MVAEGTDRVIEI	gp160	822-832		human	966	
MEGTDRIVIEI	gp160	824-832	B	human	967	
MRVIEVLQRA	gp160	828-836	B	human	968	A*0201, A2
MKVIEVAQGA	gp160	828-836	CRF01_AE	human	969	A2
MEVAQRAYR	gp160	831-838	B	human	970	A*3303
MVQRTCRAIL	gp160	833-841	B	human	971	
MRAYRAILHI	gp160	835-843		human	972	B*5101, B51

MIPRRIRQGF	gp160	843-851	A	human	973	B7
MIPRRIRQGL	gp160	843-851	A, B, C, D	human	974	B*0702, B07, B7
MIPRRRIRQGL	gp160	843-851		human	975	B7
MRRIRQGLERILL	gp160	845-856	B	human	976	A30, B7, B8
MRIRQGLERA	gp160	846-854	B	human	977	A*0205, A2
MRQGLERALL	gp160	848-856		human	978	B8
MQGLERALL	gp160	849-856	B	human	979	B8
MGKWSKSSIV	Nef	3-11	C	human	980	A2
MWSKSSIIGW	Nef	5-13	B	human	981	A*2501
MVEWPAVRERM	Nef	11-20	B	human	982	
MWPTVRERM	Nef	13-20	B	human	983	B*0801, B8
MWPAIRERM	Nef	13-20	C	human	984	B*0801
MAVRERMRRT	Nef	15-23	C	human	985	B7, B8
MTVRERMRRA	Nef	15-23	B	human	986	
MRMRRAEPAA	Nef	19-27	B	human	987	B62
MRAEPAADGV	Nef	22-30	C	human	988	A*6901
MRTEPAAEGF	Nef	22-30	C	human	989	A1
MGVGAVSRDL	Nef	29-37	B	human	990	A2
MGVGAASQDL	Nef	29-37	C	human	991	A*0205, B7
MGVGAASRDL	Nef	29-37	C	human	992	A*6901
MLEKHGAITS	Nef	37-45		human	993	B*4001, B4001, B50
MALTSSNTAA	Nef	42-50		human, humanized mouse, mouse	994	A*0201, A2
MALTSSNTDT	Nef	42-50	C	human	995	A*0201
MTAATNADCA	Nef	48-56	B	mouse	996	H-2^{b}
MATNADCAWL	Nef	50-58		human	997	A2
MNADCAWLRA	Nef	52-60	C	human	998	A1
MLQAQEEEDV	Nef	58-66	A	human	999	
MGEVGFPVRPQV	Nef	64-74	C	human	1000	B45
MVGFPVRPQV	Nef	66-74	C	human	1001	A*0201

MVGFPVTPQVPLRMT	Nef	66-80		human	1002	A1, B8
MFPVKPQVPL	Nef	68-76	B	human	1003	B35, B7
MFPVTPQVPL	Nef	68-76	B	human	1004	B*0702, B35, B7, B7 supertype
MFPVRPQVPL	Nef	68-76	B, C	human	1005	B*0702, B*3501, B35, B7, Cw*0401
MFPVRPQVPLR	Nef	68-77	B	human	1006	B7
MFPVTPQVPLR	Nef	68-77	B	human	1007	B*0702, B7
MFPVTPQVPLRMTY	Nef	68-81	B	human	1008	
MTPQVPLRPM	Nef	71-79	B	human	1009	B*0702, B07, B35, B7, B7 supertype
MRPQVPLRPM	Nef	71-79	C	human	1010	B*0702, B*4201, B*4202, B*8101, B42, B7, B81, Cw*0401
MRPQVPLRPM ?	Nef	71-79		human	1011	B81
MRPQVPLRPMTY	Nef	71-81	A, B, D	human, macaque	1012	B*3501, B35, B51
MTPQVPLRPMTY	Nef	71-81	B	human	1013	B35, B7, B7 supertype
MPQVPLRPMTY	Nef	72-81	B	human	1014	B35, B51
MQVPLRPMTYK	Nef	73-82	A, B, C, CRF01_AE, CRF02_AG, D	human, macaque	1015	A*0301, A*11, A*1101, A03, A11, A2, A3, A3 supertype,

						A30, B27, B35, B8
MQVPLRRMTYK	Nef	73-82		human	1016	A3
MSVPLRPMTYK	Nef	73-82	B	human	1017	B35, Cw4
MQVPLRPMTYKA	Nef	73-83	B	human	1018	A3
MVPLRPMTY	Nef	74-81	A, B, C, D, HIV-2	human, macaque	1019	A3, B*3501, B*4201, B*4202, B35
MVPLRPMTYK	Nef	74-82		human	1020	A11
MPLRPMTYK	Nef	75-82	B	human	1021	A*1101
MLRPMTKGAF	Nef	76-85	C	human	1022	B*2702, B*2705
MRPMTYKGAL	Nef	77-85	B	human	1023	B*07-super, B7
MRPMTYKAAL	Nef	77-85	B	human	1024	B*0702, B7
MRPMTYKAAV	Nef	77-85	B, CRF02_AG	human	1025	B7
MPMTKGAFDL	Nef	78-87	C	human	1026	A2
MTYKAALDL	Nef	79-87		human, humanized mouse, mouse	1027	A*0201, A2
MTYKAAVDL	Nef	79-87	B, C	human	1028	B63
MTYKGALDL	Nef	79-87	B	human	1029	
MTYKGAVDL	Nef	79-87	A	human	1030	
MTYKAAVDL	Nef	80-87	B, CRF02_AG	human	1031	A24
MKGAFDLSFF	Nef	82-90	C	human	1032	B57
MKAAVDLSHF	Nef	82-90	B, C	human	1033	B*5801, B57
MKAAFDLSFF	Nef	82-90	C	human	1034	B*5801, B57
MKAALDLSHFL	Nef	82-91	B	human	1035	A2
MKAAVDLSHFL	Nef	82-91	B	human	1036	Cw*0802, Cw08, Cw8
MAAVDLSHF	Nef	83-90	B, CRF01_AE	human	1037	B62

MAAVDLSHFL	Nef	83-91	B, CRF01_AE, CRF02_AG	human	1038	A2, A68, B*62, B60, B62, C*03, Cw*0802, Cw8
MAALDLSHFL	Nef	83-91		human, humanized mouse, mouse	1039	A*0201, A2, Cw3
MGAFDLSFFL	Nef	83-91	C	human	1040	A*0201, A*0205, A2, B7, B8, Cw*0602
MGAFDLSFFL ?	Nef	83-91		human	1041	A*0205
MAALDMSHFL	Nef	83-91		human	1042	Cw3
MGAVDLSHFL	Nef	83-91	A	human	1043	
MGALDLSHFL	Nef	83-91	B	human	1044	
MAAVDLSHFLK	Nef	83-92	B	human	1045	A*11, A11
MGAFDLSFFLK	Nef	83-92	CRF01_AE	human	1046	A11
MAAVDLSHFLKEK	Nef	83-94		human	1047	A11
MALDLSHFL	Nef	84-91	B	human	1048	A2
MAVDLSHFL	Nef	84-91	B, CRF02_AG	human	1049	A2, B62
MAVDLSHFLK	Nef	84-92	A, B, CRF01_AE, CRF02_AG, D, F	human, macaque	1050	A*0301, A*11, A*1101, A03, A11, A3
MAFDLSFFLK	Nef	84-92	C	human	1051	A1
MDLSHFLKEK	Nef	86-94	A, B, CRF01_AE, D, F	human, macaque	1052	A*0301, A11, A3
MSHFLKEKGGLEGL	Nef	88-100	C	human	1053	
MFFLKEKGGL	Nef	89-97	C	human	1054	Cw*0401
MFLKEKGGL	Nef	90-97	A, B, C, CRF02_AG,	human, macaque	1055	A2, A3, B*0801, B8

			D			
MFLKEKGGL ?	Nef	90-97		human	1056	B8
MFLKEKGGLE	Nef	90-98	C	human	1057	B8
MFLKEKGGLEGL	Nef	90-100	B	human	1058	A2
MKEKGGLEGL	Nef	92-100	B	human	1059	B*04, B*4001, B*4002, B40, B60, B61
MKEKGGLEGLVYSQK	Nef	92-105	B	human	1060	
MEKGGLEGLIHSQRR	Nef	93-106		human	1061	A1, B8
MGLEGLIYSK	Nef	96-104	C	human	1062	A1
MIYSQKRQDI	Nef	101-109	B	human	1063	
MHSQRRQDIL	Nef	102-110	C	human	1064	A24, B37, Cw*0401, Cw*0602
MYSKKRQEIL	Nef	102-110	C	human	1065	B8
MHSQRRQDILDLWIY	Nef	102-115	B	human	1066	B7
MQRRQDILDL	Nef	104-112	C	human	1067	B37, Cw*0602
MKRQEILDLW	Nef	105-113	C	human	1068	B*2702, B*2705
MRRQDILDLWI	Nef	105-114	B	human	1069	B*2705, B27
MRRQDILDLWVY	Nef	105-115		human	1070	B18, C*07
MKRQDILDLWVY	Nef	105-115	B	human	1071	Cw*07
MKRQDILDLWIY	Nef	105-115	C	human	1072	Cw*0701, Cw*0702
MKRQEILDLWVY	Nef	105-115	C	human	1073	Cw*0701, Cw*0702, Cw7
MRRQDILDLWIY	Nef	105-115	B	human	1074	Cw07, Cw7
MKKQEILDLWVY	Nef	105-115	C	human	1075	Cw7
MRQDILDLWI	Nef	106-114	C	human	1076	A24, B13, B37,

						Cw*0602
MRQDILDLWV	Nef	106-114	B, C	human	1077	B13
MRQDILDLWIY	Nef	106-115	B	human	1078	B*0702, B7
MRQDILDLWVY	Nef	106-115	B	human	1079	Cw7
MQDILDLWIY	Nef	107-115	C	human	1080	B18, B37, Cw*0602
MDILDLWIY	Nef	108-115	B	human	1081	Cw*0701, Cw7
MILDLWVYHT	Nef	109-117	C	human	1082	A*0201, A*0205
MILDLWIYHT	Nef	109-117	C	human	1083	A3
MWIYHTQGYF	Nef	113-121	C	human	1084	A1, B35, Cw*0401, Cw*0602
MWIYHTQGYFPDWQ	Nef	113-125		human	1085	B17
MVYHTQGYFPDWQNY	Nef	114-127		human	1086	
MYHTQGYFPDWQ	Nef	115-125		human	1087	B17
MHTQGYFPDW	Nef	116-124	B, C, CRF02_AG	human	1088	B*5801, B57, B58
MHTQGYFPDWQ	Nef	116-125	B, C	human	1089	B*5701, B57, B63
MTQGYFPDWQNY	Nef	117-127	B	human	1090	B*1501, B15, B62
MTQGYFPDWQNYT	Nef	117-128		human	1091	B17, B37
MQGYFPDWQNY	Nef	118-127	B	human	1092	B62
MGYFPDWQNY	Nef	119-127	B, CRF02_AG	human	1093	A24
MYFPDWQNY	Nef	120-127	C	human	1094	A*3002, A29, B*5801, B57
MYFPDWQNYT	Nef	120-128	B, C	human	1095	A01, A1, A29, B*3701, B*5701,

						B37, B57, B63, Cw6
MFFPDWKNYT	Nef	120-128		human	1096	B15, B51
MYFPDWQDYT	Nef	120-128	B	human	1097	B57
MFPDWQNYT	Nef	121-128	B, CRF01_AE	human	1098	A1
MNYTPGPGVRY	Nef	126-135	B	human	1099	A24
MNYTPGPGVRYPLT	Nef	126-138		human	1100	B7
MYTPGPGVRY	Nef	127-135	C	human	1101	A1
MYTPGPGIRY	Nef	127-135	B, C	human	1102	B57, B58, B63
MTPGPGVRY	Nef	128-135	B	human	1103	B*0702, B7 supertype
MTPGPGVRYP	Nef	128-136	B	human	1104	B7
MTPGPGVRYPL	Nef	128-137	B, C	human, macaque	1105	B*0702, B*3501, B*4201, B*8101, B07, B35, B7, B7 supertype
MTPGPGTRYPL	Nef	128-137	B	human	1106	B35
MTPGPGIRYPL	Nef	128-137		human	1107	
MPGVRYPLTF	Nef	130-139	B	human	1108	B35
MGPGVRYPLTFGWCY	Nef	130-143	B	human	1109	B57
MGPGIRYPLTFGWCF	Nef	130-143		human	1110	B57
MGIRYPLTFGWCFK	Nef	132-144		human	1111	
MTRYPLTFGW	Nef	133-141		human	1112	A33
MVRYPLTFGW	Nef	133-141	C	human	1113	B*2702, B*2705
MRYPLTFGW	Nef	134-141	B, C	human	1114	A*2301, A*2402, A24, A33, B27

MRYPLTFGWCF	Nef	134-143	B	human	1115	A*2402, A24
MRYPLTFGWCY	Nef	134-143	B, CRF01_AE, CRF02_AG	human	1116	A24, B35
MRYPLTFGWCYK	Nef	134-144	B	human	1117	B18
MYPLTFGWCF	Nef	135-143	B, C, D	human	1118	A2, B*1801, B*5301, B18, B35, B53, Cw*0401
MYPLTFGWCY	Nef	135-143	B, CRF02_AG	human	1119	B*0702, B*1801, B*5301, B18, B35, B49, B53, B7, B7 supertype
MYLPTFGWCY	Nef	135-143	B	human	1120	B49
MPLTFGWCYK	Nef	136-144	B	human	1121	A3
MPLTFGWCYKL	Nef	136-145	B, CRF01_AE	human	1122	A*0201, A2
MPLTFGWCFKL	Nef	136-145		human, transgenic mouse	1123	A*0201, A2
MPLTFGWCYKLV	Nef	136-146	B	human	1124	A*0201
MLTFGWCFKL	Nef	137-145	A, B, C	human, mouse	1125	A*0201, A*0205, A2, A2 supertype, B*1517, B57, B63
MLTFGWCYKL	Nef	137-145	B	human	1126	A2
MLTFGWCYKLV	Nef	137-146	C	human	1127	A*6901
MLTFGWCFKLV	Nef	137-146	B	human	1128	A02, A2, A2 supertype

MTFGWCFKLV	Nef	138-146	C	human	1129	Cw*0401
MWCFKLVPV	Nef	141-148		human, transgenic mouse	1130	A2
MKLVPVDPEL	Nef	144-152	B	human	1131	
MNEGENNCLL	Nef	157-165	C	human	1132	A*0205, B7
MHPVSLHGMDDPE	Nef	166-177		human	1133	B35
MDPEKEVLQWK	Nef	175-184	B	human	1134	B7
MHREVLKWKF	Nef	177-185	C	human	1135	B*2702, B*2705
MVLKWKFDSQ	Nef	180-188	C	human	1136	B8
MVLEWRFDSRL	Nef	180-189	B, CRF01_AE	human, mouse	1137	A*0201, A2
MVLMWQFDSRL	Nef	180-189	B	transgenic mouse	1138	A*0201
MLEWRFDSRL	Nef	181-189		human	1139	A2
MLKWKFDSQL	Nef	181-189	B	human	1140	A2, Cw*0602
MLMWKFDSRL	Nef	181-189	B	human	1141	
MEWRFDSRL	Nef	182-189	B	human	1142	B8
MWRFDSRLAF	Nef	183-191	B	human	1143	B*15, B*1503, B15
MWKFDSRLAF	Nef	183-191	B, C	human	1144	B*1503
MWRFDSRLAFH	Nef	183-192	B	human	1145	A1
MRFDSRLAF	Nef	184-191	B, CRF01_AE	human	1146	A1
MDSRLAFHH	Nef	186-193	B	human	1147	B35
MDSRLAFHHM	Nef	186-194	B	human	1148	A24
MDSRLAFHHV	Nef	186-194	B	human	1149	B51
MRLAFHHVAR	Nef	188-196	B	human	1150	A11, A3, B52
MRLAFHHVARELHPE	Nef	188-201	B	human	1151	B35, Cw4
MLAFHHVAREL	Nef	189-198	C	human	1152	A*6901
MAFHHVAREL	Nef	190-198	B,	human	1153	A*0201,

			CRF01_AE			A*0202, A2, A24, B52
MALKHRAYEL	Nef	190-198	A	human, macaque	1154	A*0201, A2
MALHHVAREL	Nef	190-198	B	human	1155	A2
MAFHHVAREK	Nef	190-198	B	human	1156	A3, B51
MAHRHMAREL	Nef	190-198	C	human	1157	Cw*0602
MARELHPEY	Nef	195-202	B, CRF02_AG	human	1158	A*01, A1
MARELHPEFY	Nef	195-203	C	human	1159	B*2702, B*2705

In a specific embodiment, the plurality of nucleic acid species encoding immunogenic polypeptide species according to the present invention comprise nucleic acid species encoding at least one immunogenic polypeptide selected from the group consisting of polypeptide sequences RGPGRAFVT, AMQMLKETI, MRGPGRAFVT, MAMQMLKETI, and/or a part comprising at least 7 amino acids thereof

MHC

In general, the methods, vectors, kits-of-parts, compositions and uses of the present invention may be employed for providing any immunogenic polypeptide, which are recognized by the major histocompatability complex (MHC). Thus, the plurality of nucleic acid species of the methods, vectors, kits-of-parts, compositions and uses of the present invention comprise any nucleic acid species encoding any immunogenic polypeptide, which is recognized by the major histocompatability complex (MHC).

There are two types of MHC molecules; MHC class I molecules and MHC class II molecules. MHC class I molecules are recognized by CD8 T-cells, which are the principal effector cells of the adaptive immune response. MHC class II molecules are mainly expressed on the surface of antigen presenting cells (APCs), the most important of which appears to be the dendritic cells. APCs stimulate naïve T-cells, as well as other cells in the immune system. They stimulate both CD8 T-cells and CD4 T-cells.

In one embodiment, the immunogenic polypeptide species of the present invention are MHC Class I-restricted peptide fragments consisting of 7-10 consecutive amino acids

from any immunogenic polypeptide disclosed herein, for example any peptide selected from the group consisting of SEQ ID NO: 95 to 1159. Specifically, said MHC Class I-restricted peptide fragments are characterized by having at least one of several features, one of which is the ability to bind to the Class I HLA molecule to which it is restricted at an affinity as measured by the amount of the peptide that is capable of half maximal recovery of the Class I HLA molecule (C50 value) which is at the most 50 µM as determined by for example an assembly binding assay, based on stabilization of the HLA molecule after loading of peptide to a peptide transporter deficient cell line. Subsequently, correctly folded stable HLA heavy chains are immunoprecipitated using conformation dependent antibodies and the peptide binding is quantitated. The peptides of this embodiment comprises (or more preferably consists of) at the most 200, preferably at the most 100, more preferably at the most 50, yet more preferably at the most 25, even more preferably at the most 20, yet even more preferably at the most 15, such as at the most 10, for example in the range of 8 to 10 contiguous amino acids of any of SEQ ID NO 1-46 and/or 95-1159, or a functional homolog thereof.

This assay provides a simple means of screening candidate peptides for their ability to bind to a given HLA allele molecule at the above affinity. In preferred embodiments, the immunogenic polypeptide of the invention in one having a C50 value, which is at the most 30 µM, such as a C50 value, which is at the most 20 µM including C50 values of at the most 10 µM, at the most 5 µM and/or at the most 2 µM.

Translation of the MHC restricted immunogenic polypeptide is initiated using a startcodon encoding a methionine. Thus, any immunogenic polypeptide or nucleic acid sequences encoding said immunogenic polypeptide, wherein a methionine residue, or a nucleic acid sequence encoding a methionine residue is inserted at the N-terminus of an immunogenic polypeptide provided herein, is also within the scope of the present invention.

In a preferred of the present invention, the immunogenic polypeptides are no longer than 8 to 10 amino acid residues are provided. Polypeptides longer than 8 to 10 amino acids are processed by the proteasome to a shorter length for binding to HLA molecules. Thus, when administering a polypeptide longer than 8 to 10 amino acid residues long, the “long” polypeptide / protein / protein fragment / variant is processed into a series of smaller peptides in the cytosol by the proteasome. Thus, by providing a

longer polypeptide that may be processed by the proteasome into a variety of different shorter peptides, multiple HLA classes may be targeted with one immunogenic polypeptides. By providing a smaller immunogenic polypeptide of 8 to 10 amino acids according to the present invention, only one particular HLA class is targeted. In a preferred embodiment of the present invention, only one HLA class is targeted per cell, i.e. only one immunogenic polypeptide is restricted by a HLA class protein per cell.

In another preferred embodiment, the immunogenic polypeptide species of the present invention are MHC Class II-restricted peptide fragments comprising at least 9 consecutive amino acid residues selected from any region of any immunogenic polypeptide disclosed herein, for example any peptide selected from the group consisting of SEQ ID NO: 95 to 1159. Specifically, said MHC Class II-restricted peptide fragments are characterized by having at least one of several features described herein below. The peptides of this embodiment comprises (or more preferably consists of) between 4 and 120, preferably between 8 and 100, more preferably between 10 and 75, yet more preferably between 12 and 60, even more preferably between 15 and 40, such as between 18 and 25 contiguous amino acids of any of SEQ ID NO 1-46 and/or 95-1159, or a functional homolog thereof. In one embodiment, the peptide comprises a methionine residue at the N-terminus, the example the peptide is any peptide selected from the group consisting of SEQ ID NO: 95-1159, wherein a methionine residue has been inserted as the N-terminal amino acid.

In particular embodiments the present invention provides a plurality of non-identical nucleic acid species, each nucleic acid species encoding distinct immunogenic polypeptide species, which is an MHC Class I-restricted peptide or an MHC class II-restricted peptide having at least one of the following characteristics:
(i) capable of eliciting INF-γ -producing cells in a PBL population of a cancer patient at a frequency of at least 1 per 10^4 PBLs as determined by an ELISPOT assay, and/or
(ii) capable of in situ detection in an individual of cytotoxic T-lymphocytes (CTLs) that are reactive with the immunogenic peptide epitope,
(iii) capable of inducing the growth in vitro of T-cells specific for any polypeptide selected from the group consisting of SEQ ID NO: 1-46 and/or 95-1159
(iv) capable of inducing the growth in vitro of T-cells specific for an infectious disorder, an autoimmune disorder and/or any cancer form as described elsewhere herein.

More preferred peptides according to the present invention are peptides capable of raising a specific T-cell response as determined by an ELISPOT assay, for example the ELISPOT assay described in Example 1 herein below. Some polypeptides although they do not bind MHC class I or class II with high affinity, may still give rise to a T-cell response as determined by ELISPOT. Other peptides capable of binding MHC class I or class II with high affinity also give rise to a T-cell response as determined by ELISPOT. Both kinds of immunogenic polypeptides are preferred peptides according to the invention.

Hence, preferred immunogenic polypeptides according to the present invention are peptides capable of raising a specific T-cell response as measured by an ELISPOT assay, wherein more than 50 peptide specific spots per 10^8 cells, more preferably per 10^7, even more preferably per 10^6, yet more preferably per 10^5 cells, such as per 10^4 cells are measured.

Most preferred immunogenic polypeptides according to the present invention are peptides that are capable of eliciting a cellular immune response in an individual suffering from a clinical condition selected from the group consisting of infectious disorders, autoimmune disorder and/or cancer, as specified elsewhere herein, most preferably HIV, AIDS, hepatitis C and/or any cancer form.

Other preferred immunogenic polypeptides according to the present invention are peptides that are capable of eliciting a cellular immune response in an individual suffering from a clinical condition characterized by the expression of a polypeptide selected from the group consisting of SEQ ID NO: 1-46 and/or 95-1159, the clinical condition preferably being a cancer or infection, and most preferably a cancer.

As described above, the HLA system represents the human major histocompatibility (MHC) system. Generally, MHC systems control a range of characteristics: transplantation antigens, thymus dependent immune responses, certain complement factors and predisposition for certain diseases. More specifically, the MHC codes for three different types of molecules, i.e. Class I, II and III molecules, which determine the more general characteristics of the MHC. Of these molecules, the Class I molecules are so-called HLA-A, HLA-B and HLA-C molecules that are presented on the surface of most nucleated cells and thrombocytes.

The immunogenic polypeptides of the present invention are characterized by their ability to bind to (being restricted by) a particular MHC Class I HLA molecule. Thus, in one embodiment an immunogenic polypeptide is one which is restricted by a MHC Class I HLA-A molecule including HLA-A1, HLA-A2, HLA-A3, HLA-A9, HLA-A10, HLA-A11, HLA-Aw19, HLA-A23(9), HLA-A24(9), HLA-A25(10), HLA-A26(10), HLA-A28, HLA-A29(w19), HLA-A30(w19), HLA-A31(w19), HLA-A32(w19), HLA-Aw33(w19), HLA-Aw34(10), HLA-Aw36, HLA-Aw43, HLA-Aw66(10), HLA-Aw68(28), HLA-A69(28). More simple designations are also used throughout the literature, where only the primary numeric designation is used, e.g. HLA-A19 or HLA-A24 instead of HLA-Aw19 and HLA-A24(49), respectively. In specific embodiments, the peptide of the invention is restricted by a MHC Class I HLA species selected from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11 and HLA-A24. In specific embodiment, the peptide of the invention is restricted by a MHC Class I HLA species HLA-A2 or HLA-A3.

In further useful embodiments, an immunogenic polypeptide of the invention is a peptide, which is restricted by a MHC Class I HLA-B molecule including any of the following: HLA-B5, HLA-B7, HLA-B8, HLA-B12, HLA-B13, HLA-B14, HLA-B15, HLA-B16, HLA-B17, HLA-B18, HLA-B21, HLA-Bw22, HLA-B27, HLA-B35, HLA-B37, HLA-B38, HLA-B39, HLA-B40, HLA-Bw41, HLA-Bw42, HLA-B44, HLA-B45, HLA-Bw46 and HLA-Bw47. In specific embodiments of the invention, the MHC Class I HLA-B species to which the peptide of the invention is capable of binding is selected from HLA-B7, HLA-B35, HLA-B44, HLA-B8, HLA-B15, HLA-B27 and HLA-B51.

In further useful embodiments, an immunogenic polypeptide of the invention is a peptide, which is restricted by a MHC Class I HLA-C molecule including but not limited to any of the following: HLA-Cw1, HLA-Cw2, HLA-Cw3, HLA-Cw4, HLA-Cw5, HLA-Cw6, HLA-Cw7 and HLA-Cw1.

In further useful embodiments, an immunogenic polypeptide of the invention is a peptide, which is restricted by a MHC Class II HLA molecule including but not limited to any of the following: HLA-DPA-1, HLA-DPB-1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB and all alleles in these groups and HLA-DM, HLA-DO.

The selection of peptides potentially having the ability to bind to a particular HLA molecule can be made by the alignment of known sequences that bind to a given particular HLA molecule to thereby reveal the predominance of a few related amino acids at particular positions in the peptides. Such predominant amino acid residues are also referred to herein as "anchor residues" or "anchor residue motifs". By following such a relatively simple procedure based on known sequence data that can be found in accessible databases, immunogenic polypeptides can be derived from a target polypeptide, for example HIV-1 envelope such as any of SEQ ID NO: 1-59, which are likely to bind to a specific HLA molecule. Representative examples of such analyses for a range of HLA molecules are given in the below table 2.

Table 2

HLA allele	Position 1	Position 2	Position 3	Position 5	Position 6	Position 7	C-terminal
HLA-A1		T,S	D,E			L	Y
HLA-A2		L, M			V		L,V
HLA-A3		L,V,M	F,Y				K, Y, F
HLA-A11		V,I,F,Y	M,L,F,Y,I				K, R
HLA-A23		I,Y					W,I
HLA-A24		Y		I,V	F		I,L,F
HLA-A25		M,A,T	I				W
HLA-A26	E,D	V,T,I,L,F			I,L,V		Y,F
HLA-A28	E,D	V,A,L					A,R
HLA-A29		E					Y,L
HLA-A30		Y,L,F,V					Y
HLA-A31			L,M,F,Y				R
HLA-A32		I,L					W
HLA-A33		Y,I,L,V					R
HLA-A34		V,L					R
HLA-A66	E,D	T,V					R,K
HLA-A68	E,D	T,V					R,K
HLA-A69		V,T,A					V,L
HLA-A74		T					V,L
HLA-B5		A,P	F,Y				I,L
HLA-B7	*	P					L,F
HLA-B8			K	K,R			L
HLA-B14		R,K					L,V

HLA-B15 (B62)		Q,L,K,P, H,V,I,M,S ,T				F,Y,W
HLA-B17						L,V
HLA-B27		R				Y, K,F,L
HLA-B35		P				I, L, M, Y
HLA-B37		D,E				I,L,M
HLA-B38		H	D,E			F,L
HLA-B39		R,H				L,F
HLA-B40 (B60,61)		E	F,I,V			L,V,A,W, M,T,R
HLA-B42		L,P				Y,L
HLA-B44		E				F,Y,W
HLA-B46		M,I,L,V				Y,F
HLA-B48		Q,K				L
HLA-B51		A,P,G				F,Y,I,V
HLA-B52		Q	F,Y			I,V
HLA-B53		P				W,F,L
HLA-B54		P				
HLA-B55		P				A,V
HLA-B56		P				A,V
HLA-B57		A,T,S				F,W,Y
HLA-B58		A,T,S				F,W,Y
HLA-B67		P				L
HLA-B73		R				P
HLA-Cw1		A,L				L
HLA-Cw2		A,L				F,Y
HLA-Cw3		A,L				L,M
HLA-Cw4		Y,P,F				L,M,F,Y
HLA-Cw6						L,I,V,Y
HLA-Cw6		Y				L,Y,F
HLA-Cw8		Y				L,I,
HLA-Cw16		A,L				L,V

* In one embodiment there is no specific anchor residue for this position, however in a preferred embodiment the anchor residue is R or A.

Thus, as an example, nonapeptides potentially having the ability to bind to HLA-A3 would have one of the following sequences: X-L-Y-X-X-X-X-X-K, X-L-Y-X-X-X-X-X-Y;

X-L-Y-X-X-X-X-X-F or X-V-Y-X-X-X-X-X-K (X indicating any amino acid residue). In a similar manner, sequences potentially having the ability to bind to any other HLA molecule can be designed. It will be appreciated that the person of ordinary skill in the art will be able to identify further "anchor residue motifs" for a given HLA molecule.

The immunogenic polypeptide species of the invention may have a sequence which is a native sequence of HIV envelope, such as defined by any of SEQ ID NO: 1-46. However, immunogenic polypeptide having a higher affinity to any given HLA molecule may be derived from such a native sequence by modifying the sequence by substituting, deleting or adding at least one amino acid residue, e.g. on the basis of the procedure described above, whereby anchor residue motifs in respect of the given HLA molecule are identified.

Thus, in useful embodiments, the immunogenic polypeptides of the invention include peptides, the sequences of which comprise, for each of the specific HLA alleles listed in the table, any of the amino acid residues as indicated in table 2 above.

Thus, the immunogenic polypeptides of the invention may be any of the above-mentioned peptides comprising contiguous sequences from any of SEQ ID NO: 1-46 and/or 59-1159, wherein in the range of 1 to 10, preferably in the range of 1 to 5, more preferably in the range of 1 to 3, even more preferably in the range of 1 to 2, yet more preferably 1 amino acid has been exchanged for another amino acid, preferably in a manner so that the peptide comprises one or more, preferably all anchor residues of a given HLA-A specific peptide as indicated in table 2 above.

Examples of preferable HLA species, to which preferred immunogenic polypeptides of the present invention are restricted include: a MHC Class I HLA species selected from the group consisting of HLA-A1, HLA-A2, HLA-A3, HLA-A11 and HLA-A24, more preferably the peptide is restricted by HLA-A3 or HLA-A2. Alternatively, a preferred HLA species includes MHC Class I HLA-B species selected from the group consisting of HLA-B7, HLA -B35, HLA -B44, HLA-B8, HLA-B15, HLA-B27 and HLA-B51.

An approach to identifying immunogenic polypeptides of the invention includes the following steps: selecting a particular HLA molecule, e.g. one occurring at a high rate in a given population, carrying out an alignment analysis as described above to identify

"anchor residue motifs" in the target protein, for example HIV-1 envelope, isolating or constructing peptides of a suitable size that comprise one or more of the identified anchor residues and testing the resulting peptides for the capability of the peptides to elicit INF-γ -producing cells in a PBL population of a cancer patient at a frequency of at least 1 per 10^4 PBLs as determined by an ELISPOT assay as described in Example 1.

A significant feature of the immunogenic polypeptides of the invention is the capability to recognize or elicit INF-γ -producing responder T cells, i.e. cytotoxic T cells (CTLs) that specifically recognize the particular peptide in a PBL population, on an APC or tumor / neoplastic cells of an individual suffering from a clinical condition of the present invention, such as an infectious disorder, an autoimmune disease and/or any cancer form as described elsewhere herein (target cells). This activity is readily determined by subjecting PBLs, APCs or target cell (e.g. tumor cells) from an individual to an ELISPOT assay. Prior to the assay, it may be advantageous to stimulate the cells to be assayed by contacting the cells with the immunogenic polypeptide, or the plurality of such peptides to be tested. Preferably, the peptide is capable of eliciting or recognizing INF-γ -producing T cells at a frequency of at least 1 per 10^4 PBLs as determined by an ELISPOT assay as used herein. More preferably the frequency is at least 5 per 10^4 PBLs, most preferably at least 10 per 10^4 PBLs, such as at least 50 or 100 per 10^4 PBLs.

Expression of peptides for MHC presentation

Multiple immunogenic peptides compete for a limited number of class I molecules. Those with the lowest affinity are unlikely to bind MHC-I and are subsequently degraded. Notably, immunodominant peptides are not always those with the highest affinity for a given MHC-I molecule, as shown in murine models, an observation that has been extended to EBV, and HIV epitopes. Another factor involved in epitope hierarchy is the amount and kinetics of protein production. This has been shown for several viruses such as lymphocytic choriomeningitis virus (LCMV), EBV, and vaccinia virus, in which earlier proteins are expressed, processed, and presented first, and may also be true for HIV since the most immunodominant responses are located in early accessory proteins such as Nef and highly expressed proteins like Gag. Nevertheless, within Gag and Nef lie subdominant epitopes, suggesting that other cellular factors are involved. TCR repertoire and epitope affinity for the TCR also play a role in immunodominance . In addition to the above factors, the efficiency of antigen

processing has been shown to influence viral epitope hierarchy in animal models. The processing of epitopes from ubiquitinylated proteins or from defective ribosomal translation products relies on sequential cleavages, mainly by the proteasome and other amino- or endopeptidases in the cytosol and in the ER. Poorly processed epitopes or epitopes with a low affinity for transporter associated with antigen processing (TAP) will be less likely to enter the ER and be loaded onto the MHC. The importance of antigen-processing efficiency on epitope hierarchy is indirectly demonstrated by the effect of mutations impairing epitope production and presentation. Artificial mutations in flanking regions of mouse viral epitopes as well as naturally occurring mutations flanking HIV or HCV epitopes have been shown to impair their processing and presentation and to lead to CTL escape. Mice deficient in an ER aminopeptidase (ERAAP/ERAP1) or in immunoproteasome subunits involved in epitope trimming present a different pattern of viral and cellular CTL immunodominant responses than WT mice, strongly supporting the role of finely tuned antigen-processing activities in establishing immunodominance.

Expression of peptides for MHC class I presentation are more efficient when expressed as small peptides that can be loaded directly without any further trimming of the polypeptide and it is more efficient to produce the peptides endogenous than it is to add the peptides exogenous. Therefore, the immunogenic polypeptide of the present invention are preferably within the range of small peptides which can be loaded directly without any further trimming of the polypeptide

Vectors

As set out elsewhere herein, the present invention relates to a plurality of non-identical nucleic acid species, wherein each of said nucleic acid encodes a distinct cytokine or hormone, or a functional homologue or part thereof and/or a plurality of non-identical nucleic acid species, wherein each of said nucleic acid encodes a distinct immunogenic polypeptide as defined herein. Thus, in one embodiment of the methods, composition, kits-of-parts and/or uses of the present invention, the non-identical nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof is comprised in a nucleic acid expression vector species, and/or each said additional non-identical nucleic acid species encoding a distinct species of said immunogenic polypeptide is comprised in a nucleic acid expression vector species. In as preferred embodiment, each expression vector species comprises one and not more

than one nucleic acid encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof and/or one and not more than one nucleic acid encoding a distinct species of said immunogenic polypeptide.

In one aspect, the present invention provides nucleic acid vectors, which comprise nucleic acid sequences encoding immunomodulating polypeptide (cytokine or hormone) and/or immunogenic polypeptides as defined elsewhere herein. Specifically, the present invention provides a plurality of non-identical nucleic acid vector species, wherein wherein each of said non-identical nucleic acid vector species comprises a distinct nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and/or a distinct nucleic acid species encoding a distinct species of immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids.

Thus in one embodiment, the present invention provides a plurality of non-identical nucleic acid vector species, wherein wherein each of said nucleic acid vector species comprises a distinct nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof.

In another embodiment, the present invention provides a plurality of non-identical nucleic acid vector species, wherein wherein each of said nucleic acid vector species comprises a distinct combination of a nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and a distinct nucleic acid species encoding a distinct species of immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids.

Importantly, the plurality of nucleic acid vectors of the present invention are provided to a population of cell by a method of transfectionas defined elsewhere herein, wherein each individual cell of said population of cells takes up at the most 5, such as 4, 3, 2, or 1 of said non-identical nucleic acid vector species. The plurality of nucleic acid vector species is on one embodiment provided by gene gun as described herein.

A nucleic acid acid vector of the present invention comprises any suitable eukaryotic expression vector or retroviral vector as defined herein below. Numerous vectors are available and the skilled person will be able to select a useful vector for the specific

purpose. The vector may, for example, be in the form of a plasmid, cosmid, viral particle or artificial chromosome. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures, for example, DNA may be inserted into an appropriate restriction endonuclease site(s) using techniques well known in the art. Apart from the nucleic acid sequence according to the invention, the vector may furthermore comprise one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. The vector may also comprise additional sequences, such as enhancers, poly-A tails, linkers, polylinkers, operative linkers, multiple cloning sites (MCS), STOP codons, internal ribosomal entry sites (IRES) and host homologous sequences for integration or other defined elements. Methods for engineering nucleic acid constructs are well known in the art (see, e.g., Molecular Cloning: A Laboratory Manual, Sambrook et al., eds., Cold Spring Harbor Laboratory, 2nd Edition, Cold Spring Harbor, N.Y., 1989). The vector is preferably an expression vector, comprising the nucleic acid operably linked to a regulatory nucleic acid sequence directing expression thereof in a suitable cell. Within the scope of the present invention said regulatory nucleic acid sequence should in general be capable of directing expression in a mammalian cell, preferably a human cell, more preferably in an antigen presenting cell or a T-cell.

The nucleic acid vector species may comprise at least one intron, which will facilitate the transport from the nucleus to the cytoplasma of the vector encoded RNA, for example in packaging cells. In another embodiment, the vector is capable of expressing RNA in the cytoplasm by cytoplasmic transcription, which can be translated into envelope polypeptide. The vector is also, in one embodiment, capable of expressing high levels of vector encoded RNA, which is transported to the cytoplasma to be translated into envelope polypeptide as encoded in the vector. Thus, in one embodiment the vector species of the present invention is transcribed in the nucleus, thereby producing high levels of transcript, which after transport to the cytoplasm can be translated into polypeptide. The vector of the present invention may be transfected into a packaging cell which is capable of producing viral particles comprising said lentiviral envelope polypeptide.

The nucleic acid vector species of the present invention may comprise at least one additional nucleic acid sequence, in addition to a nucleic acid encoding an immunomodulating polypeptide and/or a nucleic acid species encoding an

immunogenic polypeptide. For example, the vector species may comprise a reporter gene. Thus, in one embodiment, the at least one additional nucleic acid sequence of a vector of the present invention encodes a reporter gene. In the present context the term “reporter gene” refers to any reporter gene that can be used to evaluate whether a host cell harbours the vector, provirus, retroviral particle, composition and/or kits-of-parts of the present invention. A number of reporter genes and systems for detection exist which will be appreciated by a person skilled in the art. For example the reporter gene of the present invention is selected from the group consisting of the enhanced green fluorescent protein (eGFP), lac Z, dsRed, enhanced yellow fluorescent protein (eYFP), enhanced cyan fluorescent protein (eCFP), enhanced blue fluorescent protein (eBFP) and the human alpha-1-antitrypsin (hAAT). It is understood that any of the enhanced green fluorescent protein (eGFP), lac Z, dsRed, enhanced yellow fluorescent protein (eYFP), enhanced cyan fluorescent protein (eCFP), enhanced blue fluorescent protein (eBFP) or the human alpha-1-antitrypsin (hAAT) there are also claimed in separate embodiments. In a preferred embodiment the eGFP gene is used.

In another embodiment of the present invention, the vector species of the present invention comprise a suicide gene and/or a selection gene encoding a selective marker. The selection gene of the present invention may be any gene suitable for example for selecting cells harbouring the vector constructs of the present invention. Typically the selection gene is a gene that confers resistance to antibiotics or drugs. Examples of such selection genes are the puromycin resistance gene (Puro), the tetracycline resistance gene, the streptomycin resistance gene, the hygromycin B resistance gene (Hygro), the zeocin resistance gene (zeo), the neomycin resistance gene (neo),and the blasticidin resistance gene (Bst). Therefore, the selection gene of the present invention is selected from the group consisting of puromycin resistance gene (Puro), the tetracycline resistance gene, the streptomycin resistance gene, the hygromycin B resistance gene (Hygro), the zeocin resistance gene (zeo), the neomycin resistance gene (neo) and the blasticidin resistance gene (Bst). In a preferred embodiment the selection gene is selected from the group consisting of puromycin resistance gene (Puro), the hygromycin B resistance gene (Hygro), the zeocin resistance gene (zeo), the neomycin resistance gene (neo) and the blasticidin resistance gene (Bst). It is appreciated that the resistance gene is selected from any of puromycin resistance gene (Puro), the tetracycline resistance gene, the streptomycin resistance gene, the hygromycin B resistance gene (Hygro), the zeocin resistance

gene (zeo), the neomycin resistance gene (neo) or the blasticidin resistance gene (Bst). In a preferred embodiment the resistance gene is the neomycin resistance gene. In another preferred embodiment, the selective marker is neomycin phosphotransferase II.

The suicide gene and/or a selection gene encoding a selective marker according to the present invention is in another embodiment Herpes simplex virus thymidine kinase (HSV-TK).

In a specific embodiment, the genes of the nucleic acids and/or vectors of the present invention are under the control of a constitutive promoter, however in another embodiment, the promoter is non-constitutive and may be activated.

The nucleic acid vectors of the present invention comprising a distinct combination of nucleic acid species encoding, for example, a cytokine and an immunogenic polypeptide are bicistronic or multicistronic. Therefore, in one embodiment, the nucleic vectors of the present invention further comprise at least one internal ribosomal entry site. Thus, the vector according to the present invention comprises, in one embodiment, two additional nucleic acid sequences, three, four, five or six additional nucleic acid sequences and at least two IRES elements, three, four, five or six IRES elements. In one embodiment, the at least one IRES are of different origin. Specifically, the nucleic acid sequence encoding an immunomodulating polypeptide of the present invention or fragment thereof may be preceded by an IRES. Similarly, any of the immunogenic polypeptides may be preceded by an IRES. An IRES preceding a nucleic acid sequence results in the translation of said sequence under the control of the IRES. In one embodiment of the present invention the immunomodulating polypeptide (e.g. cytokine) or fragment thereof is translated under the control of IRES. In another embodiment, the immunogenic polypeptide is translated under the control of IRES.

Thus, in one embodiment of the methods, compositions, kits-of-parts and/or uses of the present invention, the cytokine or hormone, or a functional homologue or part thereof, and/or said immunogenic polypeptide is translated from the vector transcript by means of an internal ribosomal entry site (IRES).

The IRES elements according to the present invention comprise any suitable element, which supports translation of an open reading frame. For example, the IRES may be derived from picornaviridae, retroviridae or retrotransposons, mammalia or combinations thereof. In a specific embodiment, the IRES is selected from the IRES elements of encephalomyocarditis (ECMV) or another picornavirus. Specifically, the IRES according to the present invention can be derived from the IRES element of eIF4G.

When an IRES element is comprise in the vector, multiple nucleic acid species may be expressed by the same promoter. However as an alternative to the inclusion of IRES elements in the vector, the nucleic acid species encoding a distinct cytokine or hormone, or a functional homologue or part thereof, and the nucleic acid species encoding said immunogenic polypeptide are, in one embodiment, expressed by different promoters of the nucleic acid vector.

Moreover, the nucleic acid species encoding the cytokine or hormone, or a functional homologue or part thereof, and the nucleic acid species encoding said immunogenic polypeptide in one embodiment comprise identical 3'-untranslated region (UTR), i.e. the nucleic acid sequence of the 3'-UTR of the two nucleic acid species are the same. The 3'-UTR comprise different cis-acting elements involved in transcriptional and translational regulation as well as mRNa/mRNP metabolism, for example polyadenylation signal and/or 3'-processing elements. In another embodiment, nucleic acid species of the vector comprise different 3'-untranslated region.

In a specific embodiment of the methods, compositions, kits-of-parts and/or uses of the present invention, the nucleic acid species encoding an immunomodulating polypeptide (e.g. cytokine) or part thereof, and said nucleic acid species encoding said immunogenic polypeptide are produced by different splicing of the same vector transcript species.

In a preferred embodiment, the present invention relates to a plurality of non-identical nucleic acid vector species, wherein each species comprises a distinct nucleic acid species encoding an immunomodulating polypeptide, such as a cytokine or hormone selected from the group consisting of SEQ ID NO: 1-46 and/or a functional homolog and/or part thereof. In another embodiment, the present invention relates to a plurality

of non-identical nucleic acid vector species, wherein each species comprises a distinct nucleic acid species encoding an immunogenic polypeptide selected from the group consisting of SEQ ID NO: 95-1159. In yet another embodiment, the present invention relates to a plurality of non-identical nucleic acid vector species, wherein each species comprises a distinct combination of one distinct nucleic acid species encoding an immunomodulating polypeptide, such as a cytokine or hormone selected from the group consisting of SEQ ID NO: 1-46, a functional homolog and/or part thereof and one distinct nucleic acid species encoding an immunogenic polypeptide selected from the group consisting of SEQ ID NO: 95-1159.

In one embodiment, the vector species comprises an intron. The intron is deleted by splicing, thus, facilitating the transport from the nucleus to the cytoplasm of said vector encoded RNA in packaging cells. As an alternative to an intron, the vectors of the present invention may also comprise a constitutive transport element (CTE). The CTE facilitates the transport of the vector encoded RNA from the nucleus to the cytoplasm of the semipackaging cell. In another approach, the vector may comprise a Rev responsive element (RRE). The presence of an RRE in the RNA transcript facilitates its transport from the nucleus to the cytoplasma of the semipackaging cell, when REV/REX is coexpressed. In one specific embodiment, the vector is transcribed in the cytoplasm, thereby producing high levels of transcript, which can be translated into cytokine polypeptide or immunogenic polypeptide species.

Eukaryotic expression vector

The nucleic acid vector species of the present invention may be any eukaryotic expression vector, for example a mammalian expression vector, a yeast vector, or a protozoan vector. The vector is any suitable eukaryotic expression vector, preferably a a mammalian expression vector or a human expression vector.

The nucleic acid species as set out herein encoding an immunomodulating polypeptide species (e.g. a cytokine) and/or an immunogenic polypeptide may be comprised in separate vectors. Alternatively, one vector may comprise a distinct combination of a nucleic acid species encoding an immunomodulating polypeptide (e.g. a cytokine) and a nucleic acid species encoding an immunogenic polypeptide. Thus, in one embodiment, the nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and said additional nucleic acid

species encoding a distinct species of said immunogenic polypeptide are comprised in separate nucleic acid expression vectors species, and in another embodiment, nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and said additional nucleic acid species encoding a distinct species of said immunogenic polypeptide are comprised in the same nucleic acid expression vector species.

The design of the vectors of the present invention allows the vectors to be used for vaccination purposes, e.g. as componentc of a pharmaceutical composition, such as a vaccine composition. Thus, in one embodiment, upon transfection into a population of cells, the vectors as defined by the present invention are capable of inducing an immunogenic response in a host animal. For example, said immunogenic response is an antibody response and/or cytotoxic T Lymphocyte (CTL) response. In another embodiment, the immunogenic response is a CTL response, wherein said vector is integrated into the genome of a host cell.

Retroviral vector

The plurality of non-identical nucleic acid species, wherein each of said nucleic acid encodes a distinct immunomodulating polypeptide (e.g. cytokine) or part thereof and the plurality of non-identical nucleic acid species, wherein each of said nucleic acid encodes a distinct immunogenic polypeptide as defined herein are in one preferred embodiment comprised in a plurality retroviral vector species. Each retroviral vector species of said plurality of retroviral vector species comprise either a distinct nucleic acid species encoding a distinct immunomodulating polypeptide (e.g. cytokine) or part thereof, or a specific combination of one nucleic acid species encoding said distinct immunomodulating polypeptide (e.g. cytokine) or part thereof and a nucleic acid encoding a distinct immunogenic polypeptide as defined herein.

Thus, in one embodiment of the methods, composition, kits-of-parts and/or uses of the present invention, the non-identical nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof is comprised in a retroviral vector species, and/or each said additional non-identical nucleic acid species encoding a distinct species of said immunogenic polypeptide is comprised in a retroviral vector species. In a preferred embodiment, each retroviral vector species comprises one and not more than one nucleic acid encoding a distinct species of

cytokine or hormone, or a functional homologue or part thereof and/or one and not more than one nucleic acid encoding a distinct species of said immunogenic polypeptide.

In a preferred embodiment, the methods, composition, kits-of-parts and/or uses of the present invention relates to a plurality of retroviral vector species, wherein each vector species comprise a distinct combination of a nucleic acid species encoding an immunomodulating polypeptide (e.g. a cytokine) and a nucleic acid species encoding an immunogenic polypeptide. In another preferred embodiment, the methods, composition, kits-of-parts and/or uses of the present invention relates to a plurality of retroviral vector species, wherein each vector species comprise a distinct nucleic acid species encoding an immunomodulating polypeptide (e.g. a cytokine).

Thus in one embodiment, the nucleic acid species encoding said cytokine or hormone, or a functional homologue or part thereof, and/or said nucleic acid species encoding said immunogenic polypeptide are comprised in a retroviral vector species, or a retroviral vector transcript species, for example a retroviral expression vector.

The retroviral vector is in one embodiment a non-integrating retroviral vector.

In a specific embodiment, the retroviral vector is selected from the group consisting of SEQ ID NO: 87-94.

In one embodiment, the retroviral vector species of the present invention is comprised in a retroviral particle, in a liposome, a nanoparticle or any other physical or biological entity as described herein.

The retroviral vector can be derived from any species of retroviridae. In one embodiment, the retroviral vector is derived from Orthoretrovirinae, comprising Alpharetrovirus, Betaretrovirus, and Gammaretrovirus. In a specific embodiment, the retroviral vector is derived from Avian carcinoma Mill Hill virus 2, Avian leukosis virus, Avian myeloblastosis virus, Avian myelocytomatosis virus 29, Avian sarcoma virus CT10, Fujinami sarcoma virus, Rous sarcoma virus, UR2 sarcoma virus or Y73 sarcoma virus. Each of the alphaviruses specified above is intended to be an individual

embodiment. Consequently, a retroviral vector according to the present invention derived from each of them is claimed individually.

In another specific embodiment, the retroviral vector is derived from Jaagsiekte sheep retrovirus, Langur virus, Mason-Pfizer monkey virus, Mouse mammary tumor virus or Squirrel monkey retrovirus. Each of the betaviruses specified herein is intended to be an individual embodiment. Consequently, a retroviral vector according to the present invention derived from each of them may be claimed individually.

In another embodiment the retroviral vector according to the present invention is derived from a gammaretrovirus, including avian, and mammalian gammaretroviruses, for example murine retroviruses, such as murine leukaemia viruses and other related viruses. The vectors of the present invention are preferably derived from gamma-retroviruses, for example Murine Leukemia Virus (MLV), Moloney Murine Leukemia Virus (MoMLV), or Akv MLV.

The retroviral vector is in one embodiment derived from Chick syncytial virus, Feline leukemia virus, Finkel-Biskis-Jinkins murine sarcoma virus, Gardner-Arnstein feline sarcoma virus, Gibbon ape leukemia virus, Guinea pig type-C oncovirus, Hardy-Zuckerman feline sarcoma virus, Harvey murine sarcoma virus, Kirsten murine sarcoma virus, Moloney murine sarcoma virus, Murine leukemia virus (MLV), Porcine type-C oncovirus, Reticuloendotheliosis virus, Snyder-Theilen feline sarcoma virus, Trager duck spleen necrosis virus, Viper retrovirus or Woolly monkey sarcoma virus. Each of the gammaviruses specified herein is intended to be an individual embodiment. Consequently, a retroviral vector according to the present invention derived from each of them may be claimed individually. In a particularly preferred embodiment, the retroviral vector is derived from Murine Leukemia Virus (MLV) or Moloney Murine Leukemia Virus (MoMLV) or Akv MLV.

In a specific embodiment, the retroviral vector is derived from Avian (Reticuloendotheliosis) virus group such as Chick syncytial virus, Reticuloendotheliosis virus, Avian spleen necrosis virus , Spleen necrosis virus, Mammalian virus group , Murine endogenous retrovirus , Murine leukemia-related retroviruses , Epicrionops marmoratus retrovirus , Ichthyophis kohtaoensis retrovirus , Osteolaemus tetraspis retrovirus , Sericulus bakeri retrovirus , Terdus iliacus retrovirus , Tomistoma schlegelii

retrovirus , Viper berus retrovirus , Xenotropic MuLV-related virus , Monodelphis sp. retrovirus, Replication competent viruses , Feline leukemia virus , Gibbon ape leukemia virus (GALV) , Murine leukemia virus , Porcine type-C oncovirus , Replication defective viruses , Abelson murine leukemia virus , Gardner-Arnstein feline sarcoma virus , Hardy-Zuckerman feline sarcoma virus , Harvey murine sarcoma virus , Kirsten murine sarcoma virus , Moloney murine sarcoma virus , Murine osteosarcoma virus , Snyder-Theilen feline sarcoma virus , Spleen focus-forming virus , Woolly monkey sarcoma virus, unclassified Gammaretrovirus , Baboon endogenous virus , Baboon endogenous virus strain M7, Feline endogenous virus , Feline endogenous virus ECE1 , Feline endogenous virus RD114, Koala retrovirus , Macaca mulatta type C retrovirus , Macaca endogenous retrovirus, MLV-related retrovirus , Rat leukemia virus , Rat sarcoma virus , RD114 retrovirus , Recombinant M-MuLV/RaLV retrovirus, Murine endogenous retrovirus , Murine leukemia-related retroviruses , Epicrionops marmoratus retrovirus , Ichthyophis kohtaoensis retrovirus , Osteolaemus tetraspis retrovirus , Sericulus bakeri retrovirus , Terdus iliacus retrovirus , Tomistoma schlegelii retrovirus , Viper berus retrovirus , Xenotropic MuLV-related virus , Xenotropic MuLV-related virus VP35 , Xenotropic MuLV-related virus VP42 , Xenotropic MuLV-related virus VP62, Monodelphis sp. retrovirus, Replication competent viruses , Feline leukemia virus , Feline leukemia provirus (clone CFE-16) , Feline leukemia provirus (clone CFE-6) , Feline leukemia provirus ftt , Feline leukemia virus strain A/Glasgow-1 , Feline leukemia virus strain B/lambda-B1 , Feline leukemia virus strain C/FA27 , Feline leukemia virus strain C/FS246 , Feline leukemia virus strain C/Sarma , Feline sarcoma virus , Gardner-Arnstein feline leukemia oncovirus B, Gibbon ape leukemia virus (GALV) , Simian sarcoma-associated virus, Murine leukemia virus , AKR (endogenous) murine leukemia virus , Friend murine leukemia virus , Moloney murine leukemia virus , Murine leukemia virus isolates , unclassified Murine leukemia virus , Porcine type-C oncovirus , Porcine endogenous retrovirus , Porcine endogenous type C retrovirus, Replication defective viruses , Abelson murine leukemia virus , Gardner-Arnstein feline sarcoma virus , Hardy-Zuckerman feline sarcoma virus , Feline sarcoma virus (STRAIN HARDY-ZUCKERMAN 2) , Feline sarcoma virus (STRAIN HARDY-ZUCKERMAN 4), Harvey murine sarcoma virus , Kirsten murine sarcoma virus , Moloney murine sarcoma virus , Cas-NS-1 murine sarcoma virus , FBJ murine osteosarcoma virus , Moloney murine sarcoma virus (STRAIN HT-1) , Moloney murine sarcoma virus (STRAIN M1) , Moloney murine sarcoma virus (strain TS110) , Murine sarcoma virus 3611 , Myeloproliferative sarcoma virus , NS.C58 murine sarcoma virus, Murine

osteosarcoma virus , FBR murine osteosarcoma virus, Snyder-Theilen feline sarcoma virus , Spleen focus-forming virus , Friend spleen focus-forming virus , Rauscher spleen focus-forming virus or Woolly monkey sarcoma virus. Each of the gammaviruses mentioned above is intended to be an individual embodiment. Consequently, a retroviral vector according to the present invention derived from each of them is claimed individually.

In another embodiment, the retroviral vector is derived from Bovine immunodeficiency virus, Caprine arthritis encephalitis virus, Equine infectious anemia virus, Feline immunodeficiency virus, Human immunodeficiency virus 1, Human immunodeficiency virus 2, Puma lentivirus, Simian immunodeficiency virus, Visna/maedi virus and/or hepatitis C.

The retroviral vectors and genomes of biological entities, of the present invention, in one embodiment, further comprise a constitutive transport element (CTE) that is characterised in that it serves as a signal of nuclear export of unspliced viral RNAs. The CTE of the present invention may be selected from the CTEs listed below in table 3.

Table 3. CTE elements

Element	Origin	Ref
CTE	Mason-Pfizer Monkey Virus	Bray et al 1994 PNAS
RTE	Rodent intracisternal type A particle element (IAPE)	Wodrich et al 2001 J Virol
CTE	Simian type D retrovirus	Tabernero et al 1996 J Virol
WPRE	Woodchuck Hepatitis virus	Zufferey et al 1999 J Virol
RTEm26	Rodent intracisternal type A particle element (IAPE)	Smulevitch et al 2005 J Virol
DR	Rous Sarcoma Virus	Paca et al 2000 J Virol

In a particular embodiment the CTE is derived from for Mason–Pfizer monkey virus, in another preferred embodiment the CTE is derived from the Woodchuck Hepatitis virus, for example the Woodchuck Hepatitis virus posttranscriptional regulatory element (WPRE).

Moreover, the retroviral vector may comprise a Rev responsive element (RRE). The presence of an RRE in the RNA transcript facilitates its transport from the nucleus to the cytoplasma, for example in a semipackaging cell, when REV/REX is coexpressed. In one specific embodiment, the vector is transcribed in the cytoplasm, thereby

producing high levels of transcript, which can be translated into envelope polypeptide. This envelope polypeptide may then be incorporated into viral particles in a packaging/producer cell.

In a specific embodiment of the present invention, the retroviral vector species comprises an IRES, wherein said IRES is located in the 3'-LTR or the 5'-LTR, or in a region flanked by the 3'-LTR and the 5'-LTR. In particular, said IRES may be located in the R region of both the 5'-LTR and/or 3'-LTR. In another embodiment, the IRES is located in the U3 region of the 3'-Long Terminal Repeat or the U5 region of the 5'-LTR. In yet another specific embodiment of the present invention, said IRES is located in the U3 region between the inverted repeats and the transcription regulatory elements.

Physical entity

The scope of the present invention extents to a plurality of any physical entity species, wherein said each said physical entity species comprise distinct:

a. non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined elsewhere herein, and/or
b. non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined elsewhere herein, and/or
c. non-identical vector species as defined elsewhere herein, and/or
d. non-identical retroviral particle species as defined elsewhere herein, and/or
e. gene gun particles as defined elsewhere herein.

The physical entity may be any biological entity and include for example a virual particle, a liposome, a nanoparticle, a virosome, a protzoa, an enterobacteria, any gene gun particle (any particle which can be used by a gene gun), such as a gold particle, and/or a nanoparticle or a liposome with synthetic envelope polypeptides.

In a primary aspect, the present invention provides a plurality of non-identical biological entity species, such as a eukaryotic cell, a prokaryotic cell, a viral particle, and/or a retroviral particle, wherein each said non-identical biological entity species comprises a distinct nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and/or a distinct species of an immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids. Cytokine

or hormone species, or functional homologs or parts thereof as well as immunogenic polypeptides are defined elsewhere herein. In a preferred embodiment of the plurality of physical entities of the present invention, each physical entity comprise at least one nucleic acid species encoding an immunogenic polypeptide species selected from the group consisting of SEQ ID NO: 95-1159. In another embodiment, the nucleic acid species enocode an immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids selected from any region of an HIV-1 envelope polypeptide selected from the group consisting of SEQ ID NO: 1-46.

In a preferred embodiment, the plurality of physical entity species of the present invention is a plurality biological entity species. In one example, the biological entity is a retroviral particle and/or provirus. However, in another preferred embodiment the biological entity is a prokaryotic cell and/or a eukaryotic cell, such as a mammalian cell. In yet another embodiment the biological entity is a liposome, a nanoparticle, a virosome, a protzoa, and/or enterobacteria. Specifically, the biological entity may be prokaryotic and/or eukaryotic cells in the intestinal tract and/or other parts of the digestive system. Moreover, the biological entity may be a nanoparticle or a liposome with synthetic envelope polypeptides.

Thus, in one embodiment, the present invention provides a method of treating, preventing or ameliorating a clinical condition, said method comprising

a. providing a population of cells,
b. bringing a plurality non-identical physical entity species into contact with said population of cells, wherein said contact results in uptake of said physical entity species in said cells, and wherein each of said non-identical physical entity species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof, and
c. obtaining an immunogenic response of said population of cells,

wherein each individual cell of said population of cells take up at the most 5, such as 4, 3, 2, and preferably 1 non-identical physical entity species.

In a preferred embodiment the immunogenic polypeptide is expressed on the surface of the physical entity of the present invention.

Specifically, the immunogenic polypeptide of said physical entity comprises gal-alfa1-3Galbeta1-4GlcNAc-R epitopes. The physical entity of the present invention is capable of mediating fusion of said physical entity and cells expressing receptors for HIV. In another embodiment, the physical entity of the present invention is capable of infecting a CD4 positive cell. Moreover, the physical entity is in one embodiment capable of inducing an immunogenic response in a host animal. In particular, said immunogenic response may be directed towards said physical entity in said host animal. The host animal may be any animal, however, preferably a mammal, more preferably a human being. However, in one preferred embodiment, the biological entity of the present invention is capable of infecting mammalian cells, such as preferably human cells. In the present context, the term “human cells” comprise any sort of human cell including mutated human cells pathogenic cells as well as any type of human stem cell.

In a specific embodiment, the physical entity is a nanoparticle, for example a nanoparticle with synthetic envelope peptides, a liposome, for example a virosome or any combination thereof. Thus, in a specific embodiment, the present invention provides a plurality of non-identical liposome species, wherein each said liposome species comprise a distinct nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and/or a distinct species of an immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids. Cytokine or hormone species, or functional homologs or parts thereof as well as immunogenic polypeptides are defined elsewhere herein.

In a specific embodiment, physical entity, such as a biological entity of the present invention, which express a distinct cytokine or hormone polypeptide or a functional homolog or part thereof and/or an immunogenic polypeptide as defined herein are encapsulated, wherein the capsules have a porous capsule wall which is permeable to said retroviral particles and/or said biological entity.

Retroviral particles

The distinct nucleic acid species of the plurality of non-identical nucleic acid species encoding distinct species of cytokines of hormones and/or immunogenic polypeptides is in one embodiment comprised in a retroviral vector, as described elsewhere herein. Thus, the present invention also encompass a plurality of non-identical retroviral

vectors, wherein each retroviral vector species comprise a distinct nucleic acid species encoding a distinct species of a cytokine or hormone and/or a distinct species of a immunogenic polypeptides. That plurality of non-identical retroviral vectors is in one embodiment packaged into a plurality of non-identical retroviral particles, wherein each species of retroviral particle comprise a distinct nucleic acid encoding a distinct species of cytokines of hormones, and/or a distinct species of immunogenic polypeptide, as defined elsewhere herein.

Thus, the plurality of nucleic acid species according to the present invention is in one embodiment provided to the population of cells by retroviral infection, by bringing said plurality of retroviral particles into contact with said population of cells.

A main aspect of the present invention is to provide a plurality of retroviral particles, which is fusogenic, i.e. the fusogenic particles are infectious and mediate fusion with target cells.

In a preferred embodiment, the retroviral particles comprise a plurality of nucleic acid species encoding a cytokine or hormone or a functional homolog or part thereof, wherein at least one cytokine or hormone is selected from the group consisting of SEQ ID NO: 47-86 and/or a functional homolog thereof. In another embodiment, the plurality of non-identical nucleic acid species encoding a distinct cytokine or hormone or a functional homolog or part thereof are as defined elsewhere herein.

In another preferred embodiment, the retroviral particles comprise a plurality of nucleic acid species encoding an immunogenic polypeptide, wherein at least one of said peptide species is selected from the group consisting of SEQ ID NO: 1-46 and/or 95-1159 and/or any part thereof, preferably consisting of 7-9, or 10-11 amino acid residues thereof. In another embodiment, the plurality of non-identical nucleic acid species encoding a distinct immunogenic polypeptide are as defined elsewhere herein.

The retroviral particle of the present invention is derived from any retrovirus, including any of the retroviruses, wherefrom the retroviral vectors of the present invention may be derived, as describes above. In a preferred embodiment, the retroviral particle is a gamma-retroviral particle. In a specific embodiment, the retroviral particle is a murine leukaemia virus.

In one embodiment, the retroviral particles of the present invention are fusogenic and/or infectious.

The retroviral particle may be s pseudotyped particle, such as a pseudotyped MLV particle. The particle may infect a target population of cells by any suitable receptor. In one example, the plurality retroviral particles of the present invention infect the population of cells by means of the CD4-CXCR4/CCR5 receptor pathway. Thus, in one embodiment, present invention relates to any component of the present invention including a vector, a nucleic acid, a physical entity, such as a retroviral particle, a composition, and/or a kit-of-parts described herein comprising a retroviral envelope polypeptide, capable of mediating infection of a cell, by use of the CD4-CXCR4/CCR5 receptor pathway.

Producer cells

The retroviral vector of the present invention is in one embodiment constructed as a replication-defective vector based on any of the retroviruses mentioned elsewhere herein. A replication-defective retroviral vector is characterised in that, one or more genes essential for virus replication, packaging of viral RNA and/or formation of infective particle, have been deleted from the retroviral vector. Thus, to reconstitute the viral life cycle and generate viral particles comprising such replication defective vectors a specialised producer cell (packaging cell) providing the deleted genes is needed. Such producer cell are constructed by transducing a cell with DNA constructs encoding the genetic information of the retroviral proteins, which are essential for packaging a retroviral vector genome and generating viral particles.

Thus, producer cells according to the present invention refers to cells that express trans-acting virus-encoded components necessary for the packaging of retroviral RNA genome or derivatives thereof. Packaging cells are also referred to as producer cells, and the terms are used interchangeably and synonymously herein.

Packaging/producer cells are often produced by transfecting cells with genetic information and/or genes essential for retroviral particle formation. The culture of packaging cells is subsequently supertransfected with the vector DNA. However, the genetic information and/or genes essential for retroviral particle formation and the

vector DNA may also be introduced in a single round of transfection. Supertransfection here describes another or a second transfection event, namely the transfection of the packaging cell with the vector. The resulting supertransfected packaging cell will subsequently produce infectious viral particles comprising the vector RNA genome. Said particles, which will be released from the packaging cell, can be isolated. It should be noted that only supertransfected packaging cells produce infectious viral particles. Accordingly, the transduction efficiency directly correlates with the amount of infectious viral particles produced.

In the presence of a retroviral vector genome (the process known as transduction) a producer cell will generate infectious viral particles which comprise the retroviral genome derived from the vector. The viral particles produced in this manner will be released and can infect another producer cell. Such producer cells are also designated as packaging cells. In one embodiment, the retroviral particle of the present invention is obtained by transfection of a producer cell with a retroviral vector or part thereof, or an RNA or part thereof according to the present invention.

In one aspect, the present invention relates to a producer cell comprising a vector as provided by the present invention. According to one embodiment of the present invention, one of said nucleic acid sequences encoding said cytokine or hormone polypeptide and/or an immunogenic polypeptide is under translational control of a heterologous IRES.

In another embodiment, the retroviral particle according to the present invention comprises vesicular stomatitis virus envelope (VSV-G), the amphotropic murine leukemia virus envelope, Mutated SL3-2 envelope, Xenotropic murine leukaemia virus envelope, 10A1 virus envelope, Hepatitis virus C envelope, gibbon ape leukaemia virus. Human T-cell lymphotropic virus, or envelopes of endogenous human retroviruses. In one embodiment, the retroviral particle comprises the G glycoprotein of the vesicular stomatitis virus envelope (VSV-G).

The retroviral particles are preferably capable of infecting animal cells, such as mammalian cells, preferably human cells. In a specific embodiment, the retroviral particles are capable of infecting stem cells. In another specific embodiment, the retroviral particles are capable of infecting a CD4 positive cell. Moreover, the retroviral

particles of the present invention are capable of inducing an immunogenic response in a host animal, preferably a human being.

In a specific embodiment, the retroviral packaging cells or semipackaging cells producing retroviral particles are encapsulated. Similarly, any other physical entity, such as a biological entity of the present invention, which express a cytokine or hormone polypeptide or a functional homolog or part thereof and/or an immunogenic polypeptide as defined herein are encapsulated, wherein the capsules have a porous capsule wall which is permeable to said retroviral particles and/or said biological entity.

In a particular embodiment of the present invention, the producer cell does not comprise lentiviral tat or rev, for example HIV-1 tat or rev. Also the rex gene originating from HTLV is not present in the host cell.

Target cells – population of cells

A primary aspect of the present invention relates to a method of treating, preventing or ameliorating a clinical condition, wherein a plurality of non-identical nucleic acid species is provided to a population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof, and wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species.

In one embodiment, a plurality of additional non-identical nucleic acid species is also provided to said population of cell, wherein each said additional non-identical nucleic acid species encodes a distinct species of immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids, wherein each individual cell of said population of cells takes up at the most 5 of said additional non-identical nucleic acid species. Examples of cytokine, hormones and immunogenic polypeptide are provided elsewhere herein.

Thus, the primary aspect if the present invention relates to the transduction of the cells of the population of target cells, wherein only one or a few nucleic acid species or vector species of the present invention are transferred to each cell of the targeted population. In one embodiment, only one or a few nucleic acid species encoding a cytokine or hormone or part thereof as defined herein are provided to each cell. However, in another embodiment, one or a few nucleic acid species encoding a distinct

cytokine or hormone species or part thereof as defined herein and one or a few nucleic acid species encoding an immunogenic polypeptide are provided to each cell. The nucleic acid species are in one embodiment comprised in a nucleic acid vector as defined elsewhere herein, such as a mammalian expression vector or a retroviral vector.

It is understood, that in the present invention, preferably one or a few nucleic acid species are transferred to each cell. However, depending on the means of administration, the number of nucleic acid species provided per cell and/or taken up per cell may follow a statistical distribution, such as a normal distribution. It is therefore comprehended that in the present invention, when the term “one or a few” nucleic acid species per cell is to be interpreted as an average measure.

Thus, in one embodiment,

i) each individual cell of a first subpopulation of said population of cells takes up one and not more than one nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or one and not more than one additional nucleic acid species encoding said immunogenic polypeptide, and/or

ii) each individual cell of a second subpopulation of said population of cells takes up two and not more than two nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or two and not more than two additional nucleic acid species encoding said immunogenic polypeptide, and/or

iii) each individual cell of a third subpopulation of said population of cells takes up three and not more than three nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or three and not more than three additional nucleic acid species encoding said immunogenic polypeptide, and/or

iv) each individual cell of a fourth subpopulation of said population of cells takes up four and not more than four nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or four and not more than four additional nucleic acid species encoding said immunogenic polypeptide, and/or

v) each individual cell of a fifth subpopulation of said population of cells takes up five and not more than five nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or five and not more than five additional nucleic acid species encoding said immunogenic polypeptide.

In one embodiment, the cells of said first, second, third and fourth subpopulations comprise at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.
In another embodiment, the cells of said first, second, and third subpopulations comprise at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.
In another embodiment, the cells of said first and second subpopulations comprise at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.
In yet another embodiment, the first subpopulation comprise at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.
In a further embodiment, the first subpopulation comprises at least 80 percent of said population of cells.
In another embodiment, the second subpopulation comprise at least 10 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as at least 98 percent of said population of cells.

The population of cells of the present invention comprise any cells, which may be targeted for treatment of an infectious disorder, an autoimmune disorder and/or a cancer form as described elsewhere herein. The population of cells are derived from any tissue, animal tissue or human tissue. In a preferred embodiment, the targeted cells are human cells, for example a population of cancer cells.

The population of target cells are in one embodiment animal cells, such as mammalian cells, preferably human cells. In a specific embodiment, the target cells are stem cells. In another specific embodiment, the target cells are CD4 positive cells. In one embodiment, the host animal is a human being.

The population of cells are may be removed from a tissues or from the animal or human body. However, in another embodiment, the population of cells are treated in situ, i.e. without removal from the animal or human body.

One embodiment of the present invention relates to any component of the present invention including a vector, a nucleic acid, a physical entity, such as a retroviral particle, a composition, and/or a kit-of-parts described herein comprising a retroviral envelope polypeptide, capable of mediating infection of a cell, by use of the CD4-CXCR4/CCR5 receptor pathway. Thus, embodiments of the present invention comprise any components of the present invention comprising an HIV- envelope polypeptide as defined herein, capable of mediating infection of a CD4-positive cell, and/or a CXCR4/CCR5-positive cell.

CD4 (cluster of differentiation 4) is a glycoprotein that is found primarily on the surface of helper T cells, as well as regulatory T cells and dendritic cells. CD4 is a member of the immunoglobulin superfamily. It has four immunoglobulin domains (D1 to D4) that are exposed on the extracellular surface of the cell: D1 and D3 resemble immunoglobulin variable (IgV) domains, while D2 and D4 resemble immunoglobulin constant (IgC) domains. On T cells, CD4 is the co-receptor for the T cell receptor (TCR) and recruits the tyrosine kinase lck.

CD4 is also a primary receptor used by HIV-1 to gain entry into host T cells. The HIV-1 envelope gp120 protein attaches to CD4, creating a shift in the conformation of the viral gp120 protein, which allows HIV-1 to bind to two other cell surface receptors on the host cell (the chemokine receptors CCR5 and CXCR4). Following another change in shape of a different HIV-1 envelope protein (gp41), HIV inserts a fusion peptide into the host T cell that allows the outer membrane of the virus to fuse with the T-cell membrane. HIV infection leads to a progressive reduction in the number of T cells possessing CD4 receptors and, therefore, the CD4 count is used as an indicator to help physicians decide when to begin treatment in HIV-infected patients.

The terms “CD4-positive cell” or “CXCR4/CCR5-positive cell” as used herein, relates to any cells expressing CD4 receptor or CXCR4/CCR5 receptor, respectively. The CD4 receptor and/or CXCR/CCR5 receptor may be accessible from the extracellular space at the cellular membrane.

Disorders

The present invention provides methods, kits-of-parts, compositions, and uses for treating, preventing or ameliorating a clinical condition. In one broad aspect, the present invention relates to a method of treating, preventing or ameliorating a clinical condition, said method comprising

a. providing a population of cells,
b. bringing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof,
c. obtaining an immunogenic response of said population of cells, wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species.

A clinical condition of the present invention comprises any infectious disorder, autoimmune disorder, and/or cancer form. The clinical conditions of the present invention comprise any kind of clinical condition giving rise to an immune response, including inflammation, and therefore include infectious diseases, chronic infections, autoimmune conditions and allergic inflammations. Thus, infections, such as infectious diseases, chronic infections, autoimmune conditions, allergic inflammations and cancer are all clinical conditions of relevance for the present invention. The clinical conditions of most relevance for the present invention are presented in turn hereunder as Infectious disorders, autoimmune disorders/allergy, and cancer.

Infections

An infectious disorder may be caused by a virus, and viral diseases against which the methods, kits-of-parts, composition and uses of the present invention may be provided, for example in the treatment of HIV, AIDS, AIDS Related Complex (ARC); thus it is an

object of the present invention to administer a component if the present invention as the treatment of or as part of the treatment of these viral infections.

In one embodiment, the methods, kits-of-parts, compositions and uses of the present invention are employed in the treatment of an infectious disorder, wherein said infectious disorder according to the present invention is selected from the group consisting of AIDS, Anal Warts, Anthrax, Bronchitis, Bug-borne Diseases, Campylobacter, Cellulitis, Chickenpox, Chlamydia Infections, Common Cold, Crab Lice, Cryptosporidiosis, Cytomegalovirus Infections, Dengue, Diphtheria, E. Coli Infections, Ehrlichiosis, Fever, Flea Bites, Foodborne Diseases, Fungal Infections, Gonorrhea, Hepatitis, Hepatitis C, Herpes Simplex, Herpes Zoster, HPV, Impetigo, Infectious Diseases (General), Influenza, Legionnaire's Disease, Leprosy, Lyme Disease, Malaria, Measles, Meningitis, MRSA, Mumps, Norwalk Virus Infections, Parasitic Diseases, Pelvic Inflammatory Disease, Pfiesteria piscicida, PID, Pneumonia, Polio and Post-Polio Syndrome, Rubella, Salmonella Infections, Scabies, Severe Acute Respiratory Syndrome, Sexually Transmitted Diseases, Shigella Infections, Shingles (Herpes Zoster), Sinusitis, Smallpox, Staphylococcal Infections, STD, Streptococcal Infections, Syphilis, Tetanus, Tick Bites, Trichomoniasis, Tuberculosis, Viral Infections, and/or West Nile Virus.

In one embodiment, clinical condition of the methods, compositions, kits-of-parts and/or uses of the present invention is an infectious disorder selected from the group consisting of AIDS, Anthrax, Campylobacter, Chlamydia Infections, Common Cold, Cytomegalovirus Infections, Dengue, Diphtheria, E. Coli Infections, Gonorrhea, Hepatitis C, Herpes Simplex, Herpes Zoster, HPV, Influenza, Legionnaire's Disease, Leprosy, Lyme Disease, Malaria, Measles, Norwalk Virus Infections, Salmonella Infections, Scabies, Shigella Infections, Syphilis, Tuberculosis, and/or West Nile Virus.

In a preferred embodiment, the clinical condition is hepatitis C. In another preferred embodiment, the clinical condition is HIV, such as HIV-1 or HIV-2, AIDS and/or AIDS related complex (ARC).

Infectious Disease Combination Treatment

It is further provided for that a treatment of any infectious disease by the methods, kits-of-parts, compositions and/or uses according to the present invention may be given in

conjunction with a further (second) active ingredient or in combination with a further treatment such as antibiotic treatment, chemotherapy, treatment with immunostimulating substances, treatment using dendritic cells, antiviral agents anti parasitic agents and so forth.

Examples of a second active ingredient that may be used in the treatment of an infectious disease in combination with the vaccine of the present invention include, and are not limited to antibiotics. The term antibiotics herein refers to substances with anti-bacterial, anti-fungal, anti-viral and/or anti-parasitical activity; examples of relevance to the present invention include, but are not limited to: Amikacin, Gentamycin, Kanamycin, Neomycin, Netilmicin, Paromomycin, Streptomycin, Tobramycin, Ertapenem, Imipenem, Meropenem, Chloramphenicol, Fluoroquinolones, Ciprofloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Norfloxacin, Ofloxacin, Sparfloxacin, Trovafloxacin, Glycopeptides, Vancomycin, Lincosamides, Clindamycin, Macrolides / Ketolides, Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Cefadroxil, Cefazolin, Cephalexin, Cephalothin, Cephapirin, Cephradine, Cefaclor, Cefamandole, Cefonicid, Cefotetan, Cefoxitin, Cefprozil, Cefuroxime, Loracarbef, Cefdinir, Cefditoren, Cefixime, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, Ceftriaxone, Cefepime, Monobactams, Aztreonam, Nitroimidazoles, Metronidazole, Oxazolidinones, Linezolid, Penicillins, Amoxicillin, Amoxicillin / Clavulanate, Ampicillin, Sulbactam, Bacampicillin, Carbenicillin, Cloxacillin, Dicloxacillin, Methicillin, Mezlocillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin, Piperacillin / Tazobactam, Ticarcillin, Ticarcillin / Clavulanate, Streptogramins, Quinupristin, Dalfopristin, Sulfonamide / Sulfamethoxazole, Trimethoprim, Tetracyclines, Demeclocycline, Doxycycline, Minocycline, Tetracycline, Azole antifungals Clotrimazole Fluconazole, Itraconazole, Ketoconazole, Miconazole, Voriconazole, Amphotericin B, Nystatin, Echinocandin, Caspofungin, Micafungin, Ciclopirox, Flucytosine, Griseofulvin, and Terbinafine. Of further relevance are antivirals such as Vidarabine, Acyclovir, Gancyclovir and Valcyte (valganciclovir), Nucleoside-analog reverse transcriptase inhibitors (NRTI): AZT (Zidovudine), ddI (Didanosine), ddC (Zalcitabine), d4T (Stavudine), 3TC (Lamivudine), Non-nucleoside reverse transcriptase inhibitors (NNRTI): Nevirapine, Delavirdine, Protease Inhibitors: Saquinavir, Ritonavir, Indinavir, Nelfinavir, Ribavirin, Amantadine / Rimantadine, Relenza and Tamiflu, Pleconaril, Interferons

In an embodiment, the present invention regards the methods, kits-of-parts, compositions and/or uses according to the present invention comprise a plurality of non-identical nucleic acid species as defined elsewhere herein, for the treatment of an infectious disorder, such as HIV, AIDS and/or ARC in combination with at least one antibiotic. Preferably, the methods, kits-of-parts, compositions and/or uses of the present invention are used for the treatment of chronic infections e.g. HIV, AIDS and/or ARC and therefore is used in combination with any of the above listed antibiotics such as anti-viral agents.

Autoimmune disorder, including allergy:
In one embodiment, the methods, kits-of-parts, compositions and uses of the present invention are employed in the treatment of a clinical condition, wherein said clinical condition is an autoimmune disorder. An autoimmune disorder in the context of the present invention includes any autoimmune condition and allergic inflammation, including autoimmune diseases, airborne allergy, and/or food allergy. Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

In one embodiment, the methods, kits-of-parts, compositions and uses of the present invention are employed in the treatment of a clinical condition, wherein said clinical condition is an autoimmune disease selected from the group consisting of Rheumatoid Arthritis (RA), Type 1 diabetes, Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE), Inflammatory Bowel Disease (IBD), and/or Autoimmune Thyroid Disease (ATD).

In another embodiment, the methods, kits-of-parts, compositions and uses of the present invention are employed in the treatment of a clinical condition, wherein said clinical condition is a food allergy selected from the group consisting of Food Allergy: Egg Allergy, Milk Allergy, Peanut Allergy, and/or Soy Allergy.

In another embodiment, the methods, kits-of-parts, compositions and uses of the present invention are employed in the treatment of a clinical condition, wherein said clinical condition is an airborne allergy selected from the group consisting of Pollen Allergy, Mold Allergy, Dust Mite Allergy, and/or Animal Allergy.

Cancer forms

The methods, compositions, kit-of-parts and uses of the present invention may be used for treating, preventing and/or ameliorating any cancer form. In one embodiment, the cancer form is selected from the group consisting of Acute Lymphoblastic Leukemia - Adult, Acute Lymphoblastic Leukemia - Childhood, Acute Myeloid Leukemia - Adult, Acute Myeloid Leukemia - Childhood, Adrenocortical Carcinoma, Adrenocortical Carcinoma - Childhood, AIDS-Related Cancers, AIDS-Related Lymphoma, Anal Cancer, Appendix Cancer, Astrocytoma - Childhood Cerebellar, Astrocytoma - Childhood Cerebral, Basal Cell Carcinoma - Skin Cancer (Nonmelanoma), Bile Duct Cancer - Extrahepatic, Bladder Cancer, Bladder Cancer - Childhood, Bone Cancer - Osteosarcoma and Malignant Fibrous Histiocytoma, Brain Stem Glioma - Childhood, Brain Tumor - Adult, Brain Tumor - Brain Stem Glioma - Childhood, Brain Tumor - Central Nervous System Embryonal Tumors - Childhood, Brain Tumor - Cerebellar Astrocytoma - Childhood, Brain Tumor - Cerebral Astrocytoma/Malignant Glioma - Childhood, Brain Tumor - Ependymoblastoma - Childhood, Brain Tumor - Ependymoma - Childhood, Brain Tumor - Medulloblastoma - Childhood, Brain Tumor - Medulloepithelioma - Childhood, Brain Tumor - Pineal Parenchymal Tumors of Intermediate Differentiation - Childhood , Brain Tumor - Supratentorial Primitive Neuroectodermal Tumors and Pineoblastoma - Childhood, Brain Tumor - Visual Pathway and Hypothalamic Glioma - Childhood, Brain and Spinal Cord Tumors - Childhood (Other), Breast Cancer, Breast Cancer and Pregnancy, Breast Cancer - Childhood, Breast Cancer - Male, Bronchial Tumors - Childhood, Burkitt Lymphoma, Carcinoid Tumor - Childhood, Carcinoid Tumor,Gastrointestinal, Carcinoma of Unknown Primary, Central Nervous System Embryonal Tumors - Childhood , Central Nervous System Lymphoma - Primary, Cerebellar Astrocytoma - Childhood, Cerebral

Astrocytoma/Malignant Glioma - Childhood, Cervical Cancer, Cervical Cancer - Childhood, Childhood Cancers, Chordoma - Childhood, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer - Childhood, Cutaneous T-Cell Lymphoma - see Mycosis Fungoides and Sézary Syndrome, Embryonal Tumors - Central Nervous System - Childhood , Endometrial Cancer, Ependymoblastoma - Childhood , Ependymoma - Childhood, Esophageal Cancer, Esophageal Cancer - Childhood, Ewing Family of Tumors, Extracranial Germ Cell Tumor - Childhood, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer - Intraocular Melanoma, Eye Cancer - Retinoblastoma, Gallbladder Cancer, Gastric (Stomach) Cancer, Gastric (Stomach) Cancer - Childhood, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST), Gastrointestinal Stromal Cell Tumor - Childhood, Germ Cell Tumor - Extracranial - Childhood, Germ Cell Tumor - Extragonadal, Germ Cell Tumor - Ovarian, Gestational Trophoblastic Tumor, Glioma - Adult, Glioma - Childhood Brain Stem, Glioma - Childhood Cerebral Astrocytoma, Glioma - Childhood Visual Pathway and Hypothalamic, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular (Liver) Cancer - Adult (Primary), Hepatocellular (Liver) Cancer - Childhood (Primary), Hodgkin Lymphoma - Adult, Hodgkin Lymphoma - Childhood, Hypopharyngeal Cancer, Hypothalamic and Visual Pathway Glioma - Childhood, Intraocular Melanoma, Islet Cell Tumors (Endocrine Pancreas), Kaposi Sarcoma, Kidney (Renal Cell) Cancer, Kidney Cancer - Childhood, Laryngeal Cancer, Laryngeal Cancer - Childhood, Leukemia - Acute Lymphoblastic - Adult, Leukemia - Acute Lymphoblastic - Childhood, Leukemia - Acute Myeloid - Adult, Leukemia - Acute Myeloid - Childhood, Leukemia - Chronic Lymphocytic, Leukemia - Chronic Myelogenous, Leukemia - Hairy Cell, Lip and Oral Cavity Cancer, Liver Cancer - Adult (Primary), Liver Cancer - Childhood (Primary), Lung Cancer - Non-Small Cell, Lung Cancer - Small Cell, Lymphoma - AIDS-Related, Lymphoma - Burkitt, Lymphoma - Cutaneous T-Cell - see Mycosis Fungoides and Sézary Syndrome, Lymphoma - Hodgkin - Adult, Lymphoma - Hodgkin - Childhood, Lymphoma - Non-Hodgkin - Adult, Lymphoma - Non-Hodgkin - Childhood, Lymphoma - Primary Central Nervous System, Macroglobulinemia - Waldenström, Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Medulloblastoma - Childhood, Medulloepithelioma - Childhood , Melanoma, Melanoma - Intraocular (Eye), Merkel Cell Carcinoma, Mesothelioma - Adult Malignant, Mesothelioma - Childhood, Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Multiple Endocrine Neoplasia Syndrome - Childhood, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis

Fungoides, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Diseases, Myelogenous Leukemia - Chronic, Myeloid Leukemia - Adult Acute, Myeloid Leukemia - Childhood Acute, Myeloma - Multiple, Myeloproliferative Disorders - Chronic, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Nasopharyngeal Cancer - Childhood, Neuroblastoma, Non-Hodgkin Lymphoma - Adult, Non-Hodgkin Lymphoma - Childhood, Non-Small Cell Lung Cancer, Oral Cancer - Childhood, Oral Cavity Cancer - Lip and, Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma of Bone, Ovarian Cancer - Childhood, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Pancreatic Cancer - Childhood, Pancreatic Cancer - Islet Cell Tumors, Papillomatosis - Childhood, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pheochromocytoma, Pineal Parenchymal Tumors of Intermediate Differentiation - Childhood , Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors - Childhood, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Pleuropulmonary Blastoma, Pregnancy and Breast Cancer, Primary Central Nervous System Lymphoma, Prostate Cancer, Rectal Cancer, Renal Cell (Kidney) Cancer, Renal Cell (Kidney) Cancer - Childhood, Renal Pelvis and Ureter - Transitional Cell Cancer, Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Retinoblastoma, Rhabdomyosarcoma - Childhood, Salivary Gland Cancer, Salivary Gland Cancer - Childhood, Sarcoma - Ewing Family of Tumors, Sarcoma - Kaposi, Sarcoma - Soft Tissue - Adult, Sarcoma - Soft Tissue - Childhood, Sarcoma - Uterine, Sézary Syndrome, Skin Cancer (Nonmelanoma), Skin Cancer - Childhood, Skin Cancer (Melanoma), malignant melanoma, Skin Carcinoma - Merkel Cell, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma - Adult, Soft Tissue Sarcoma - Childhood, Squamous Cell Carcinoma - see Skin Cancer (Nonmelanoma), Squamous Neck Cancer with Occult Primary - Metastatic, Stomach (Gastric) Cancer, Stomach (Gastric) Cancer - Childhood, Supratentorial Primitive Neuroectodermal Tumors - Childhood, T-Cell Lymphoma - Cutaneous - see Mycosis Fungoides and Sézary Syndrome, Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thymoma and Thymic Carcinoma - Childhood, Thyroid Cancer, Thyroid Cancer - Childhood, Transitional Cell Cancer of the Renal Pelvis and Ureter, Trophoblastic Tumor - Gestational, Unknown Primary Site - Carcinoma of - Adult, Unknown Primary Site - Cancer of - Childhood, Unusual Cancers of Childhood, Ureter and Renal Pelvis - Transitional Cell Cancer, Urethral Cancer, Uterine Cancer - Endometrial, Uterine Sarcoma, Vaginal Cancer, Vaginal Cancer - Childhood, Visual

Pathway and Hypothalamic Glioma - Childhood, Vulvar Cancer, Waldenström Macroglobulinemia, Wilms Tumor and/or Women's Cancers.

In a preferred embodiment, the cancer form is skin cancer, or malignant melanoma.

Composition

The present invention also pertains to a composition, such as a pharmaceutical composition, for example a vaccine composition for treating, preventing and/or ameliorating a clinical conditions as defined herein, for example an infectious disorder, an autoimmune disorder and/or cancer; in other words the composition of the present invention is in a preferred embodiment a pharmaceutical composition or a vaccine composition, and is accordingly referred to as such. However, the vaccine composition of the present invention may also be referred to as a pharmaceutical composition, and the term “composition” incorporates both a pharmaceutical composition and a vaccine composition. The compositions of the present invention may be “traditional” vaccine compositions comprising antigens such as proteins, polypeptides and/or nucleic acid molecules. They may also be in the form of compositions comprising cells, such as modified cells originating from the individual and later processed, or to compositions comprising complex molecules such as antibodies or TCRs. In particular, the vaccine compositions of the present invention may comprise viral particles such as retroviral particle as defined elsewhere herein. All compositions and/or vaccine compositions of the present invention are claimed for use as a medicament.

Generally, a vaccine is a substance or composition capable of inducing an immune response in an individual. The composition may comprise one or more of the following: an “active component” such as an antigen(s) (e.g. protein, polypeptides, peptides, nucleic acids and the like), nucleic acid constructs comprising one or more antigens amongst other elements, cells, (e.g. loaded APC, T cells for adoptive transfer aso.), complex molecules (Antibodies, TCRs and MHC complexes and more), carriers, adjuvants and pharmaceutical carriers. The various components of a vaccine composition according to the present invention are disclosed in more detail elsewhere herein.

In a broadest aspect, the present invention relates to a composition, such as a vaccine composition comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined elsewhere herein, and/or
b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined elsewhere herein, and/or
c. a plurality of non-identical vector species as defined elsewhere herein, and/or
d. a plurality of non-identical retroviral particle species as defined elsewhere herein, and/or
e. a population of gene gun particles as defined elsewhere herein, and/or
f. a plurality of any physical entity species comprising any component of any of a. to e, and/or
g. an adjuvant and/or a carrier.

The present invention also in one aspect, relates to a method of producing a vaccine composition of the present invention as defined elsewhere herein, comprising combining

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined elsewhere herein, and/or
b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined elsewhere herein, and/or
c. a plurality of non-identical vector species as defined elsewhere herein, and/or
d. a plurality of non-identical retroviral particle species as defined elsewhere herein, and/or
e. a population of gene gun particles as defined elsewhere herein, and/or
f. a plurality of any physical entity species comprising any component of any of a. to e, and/or
g. an adjuvant and/or a carrier

In one embodiment, the cytokine or hormone, or a functional homolog or part thereof comprise any such protein, as defined elsewhere herein, including any cytokine selected from the group consisting of SEQ ID NO: 47 to 94.

In one embodiment, the immunogenic polypeptide species of the composition above consists of a consecutive sequence of in the range of from 7-10 amino acids. However

in another embodiment, the immunogenic polypeptide species of the composition above consists of a consecutive sequence of at least 10 amino acids, such as at least 20 amino acids, such as at least 30, such as at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 100 amino acids. In another embodiment, the immunogenic polypeptide species of the composition above consists of 7 to 10 consecutive amino acids or 10-12 consecutive amino acid residues of a polypeptide as defined herein, for example any peptide selected from the group consisting of SEQ ID NO: 95-1159. In another embodiment, the immunogenic polypeptide species of the composition above consists of 7 to 10 consecutive amino acids or 10-12 consecutive amino acid residues selected from any region of a polypeptide selected from the group consisting of SEQ ID NO: 1-46.

The composition of the invention is capable of eliciting an immune response against any peptide derived from a polypeptide involved in a clinical condition of the present invention. In one embodiment, composition of the invention is capable of eliciting an immune response against an HIV-1 envelope polypeptide, for example a peptide consisting of 7 to 10 consecutive amino acids or 10-12 consecutive amino acid residues selected from any region of a polypeptide selected from the group consisting of SEQ ID NO: 1-46, and/or any peptide selected from the group consisting of SEQ ID NO: 95-1159, and/or a functional homolog thereof having at least 70% identity to any such immunogenic polypeptide, when said composition is administered to an individual. In another embodiment, the composition of the invention is capable of eliciting an immune response against an antigen presenting cell expressing such an immunogenic polypeptide of the present invention, for example and/or against a physical entity of the present invention, when administered to an individual. In one embodiment the individual is infected with HIV. The composition of the invention is in one embodiment capable of eliciting a cellular immune response in the individual.
For example, the composition is capable of eliciting the production in a vaccinated individual of effector T-cells having a cytotoxic effect against HIV-1 infected cells in a subject. In another embodiment, the vaccine composition is capable of eliciting the production in a vaccinated individual of regulatory T-cells having a cytotoxic effect against cells expressing HIV-1 envelope polypeptide or part thereof, and/or antigen presenting cells expressing HIV-1 envelope or part thereof. In another embodiment, the

composition of the present invention is capable of initiating an antibody response in an individual and/or a physical entity, such as a biological entity.

In a particular embodiment the composition is to be given against infection with HIV, in particular HIV-1. The present invention therefore also pertains to a vaccine composition which is administered to an animal including a human being, in which the vaccine is capable of eliciting an immune response against a disease caused by a lentivirus, in particular HIV-1. Thus, a vaccine composition of the present invention is capable of eliciting a clinical response in a subject, wherein the clinical response is characterised by a reduced susceptibility, resistance, stabilisation, remission or curing/recovery of an HIV infection and/or AIDS.

One embodiment combines any one of the components of the compositions of the present invention with various at least one adjuvant and/or immunomodulating peptide to produce a vaccine composition, wherein said components include a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined elsewhere herein, and/or a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined elsewhere herein, and/or a plurality of non-identical vector species as defined elsewhere herein, and/or a plurality of non-identical retroviral particle species as defined elsewhere herein, and/or a population of gene gun particles as defined elsewhere herein, and/or a plurality of any physical entity species according to the present invention.

Examples of adjuvants and immunomodulating peptides are described elsewhere herein. Adjuvants, broadly defined, are substances which promote immune responses. Frequently, the adjuvant of choice is Freund's complete or incomplete adjuvant, or killed *B. pertussis* organisms, used e.g. in combination with alum precipitated antigen. A general discussion of adjuvants is provided in Goding, Monoclonal Antibodies: Principles & Practice (2nd edition, 1986) at pages 61-63. Goding notes, however, that when the antigen of interest is of low molecular weight, or is poorly immunogenic, coupling to an immunogenic carrier is recommended. Examples of such carrier molecules include keyhole limpet haemocyanin, bovine serum albumin, ovalbumin and fowl immunoglobulin. Various saponin extracts have also been suggested to be useful as adjuvants in immunogenic compositions. Recently, it has been proposed to use

granulocyte-macrophage colony stimulating factor (GM-CSF), a well known cytokine, as an adjuvant (WO 97/28816).

The vaccine compositions according to the invention preferably comprise an adjuvant and/or a carrier and/or at least one immunomodulating peptide. Examples of useful adjuvants and carriers are given elsewhere herein. Thus, the biological components of the present invention, such as the plurality of nucleic acid species, vector species or retroviral particle species present in the composition can be associated with a carrier such as e.g. a protein or an antigen-presenting cell to a T cell.

In particular the use of adjuvants is desired when the immunogenic agents of the present invention are used to boost the immune response due to the ability of the biological entities of the present invention, such as retroviral particles are able to infect, integrate and display immunogenic polypeptides on the surface of a host cell, thereby boosting or enhancing the immune response, in particular the CTL response as discussed elsewhere herein.

Adjuvants are any substance whose admixture into the vaccine composition increases or otherwise modifies the immune response of the biological entity coated with the HIV-1 envelope polypeptide or fragment thereof as defined elsewhere herein. Carriers are scaffold structures, for example a polypeptide or a polysaccharide, to which the plurality of nucleic acid species, vector species, retroviral particle species, and/or physical entity species coated with the immunogenic polypeptide is capable of being associated.

Desirable functionalities of adjuvants capable of being used in accordance with the present invention are listed in the below table 4.

Table 4. Modes of adjuvant action

Action	Adjuvant type	Benefit
1. Immunomodulation	Generally small molecules or proteins which modify the cytokine network	Upregulation of immune response. Selection of Th1 or Th2
2. Presentation	Generally amphipathic molecules or complexes which interact with	Increased neutralizing antibody response. Greater duration of

	immunogen in its native conformation	response
3. CTL induction	• Particles which can bind or enclose immunogen and which can fuse with or disrupt cell membranes	Cytosolic processing of protein yielding correct class 1 restricted peptides
	• w/o emulsions for direct attachment of peptide to cell surface MHC-1	Simple process if promiscuous peptide(s) known
4. Targeting	• Particulate adjuvants which bind immunogen. Adjuvants which saturate Kupffer cells	Efficient use of adjuvant and immunogen
	• Carbohydrate adjuvants which target lectin receptors on macrophages and DCs	As above. May also determine type of response if targeting selective
5. Depot Generation	• w/o emulsion for short term Microspheres or nanospheres for long term	Efficiency Potential for single-dose vaccine

Source: Cox, J.C., and Coulter, A.R. (1997). Vaccine *15*, 248-56.

A vaccine composition according to the present invention may comprise more than one different adjuvant. Furthermore, the invention encompasses a therapeutic composition further comprising any adjuvant substance including any of the above or combinations thereof. It is also contemplated that the composition of the present invention and the adjuvant can be administered separately in any appropriate sequence.

A carrier may be present independently of an adjuvant. In particular, the inclusion of a carrier is relevant in connection with using a physical entity of the present invention to boost the immune response due to the ability of the retroviral particles of the present invention to infect, integrate and display the immunogenic polypeptide on the surface of a host cell, thereby boosting or enhancing the immune response, in particular the CTL response as discussed elsewhere herein.

The present invention encompasses a pharmaceutical composition, such as a vaccine composition further comprising at least one adjuvant and/or carrier including any

combination thereof. It is also contemplated that the composition of the present invention and the adjuvant, carrier and/or any combination thereof can be administered separately in any appropriate sequence.

The pharmaceutical compositions may be prepared and administered using any conventional protocol known by a person skilled in the art. It will be appreciated by the person skilled in the art that the protocol may be easily adapted to any of the vaccine compositions described herein.

In on embodiment of the invention, the vaccine compositions of the invention are useful for the prophylaxis of HIV infection or for treatment of HIV infection in a human being, where the human being is receiving treatment for the infection. In a further embodiment, the compositions, pharmaceutical compositions, vaccines and vaccine compositions of the invention are suitable for the treatment, amelioration and/or prevention of a lentiviral infection, such as HIV infection, including HIV-1 infection, and AIDS.

The choice of components in the vaccine composition of the invention will depend on parameters determinable by the person of skill in the art. The composition of the invention comprise combination of two or more HIV-1 envelope polypeptides, antigens, nucleic acids, mammalian vectors and/or physical entities. Thus, as examples, the vaccine composition may contain any combination of those components of the present invention. Also, the composition may comprise a combination of a peptide restricted by a HLA-A molecule and a peptide restricted by a HLA-B molecule, e.g. including those HLA-A and HLA-B molecules that correspond to the prevalence of HLA phenotypes in the target population, such as e.g. HLA-A2 and HLA-B35. Additionally, the composition may comprise a peptide restricted by an HLA-C molecule.

In the case of peptide-based vaccines, epitopes can be administered in an 'MHC-ready' form, which enables presentation through exogenous loading independently of antigen uptake and processing by host antigen-presenting cells. The peptides of the present invention comprise both peptides in a short 'MHC-ready' form and in a longer form requiring processing by the proteasome thus providing a more complex vaccine composition that can target multiple tumor antigens. This approach allows for the

targeting of different HLA groups by the vaccine, and thus a higher likelihood of the vaccine functioning in diverse populations.

Adjuvants

A composition according to the present invention may comprise one or more than one adjuvant. Immunostimulatory adjuvants augment antigen-specific immune responses by physical localization and improved presentation of antigen, and by provocation of inflammatory or innate immune responses (Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. Immunol Cell Biol 2004;82(5):488-96). A key feature in the innate immune system is its capability to detect foreign organisms using a set of cell receptors termed pattern-recognition receptors (PRR). One family of PRRs are Toll-Like Receptors, such as Toll-Like Receptor 9 (TLR9). TLR9 detects unmethylated CpG dinucleotides, which are relative common in the genomes of most bacteria and DNA viruses.

Use of CpG oligodeoxynucleotides (ODNs) as adjuvants has been tested in several vaccine trials. Cooper (2005) used CpG 7909 as an adjuvant to a hepatitis B vaccination schedule in HIV patients and after 12 months seroprotective titres were found in 100% of subjects in the CpG group compared to 63% in the control group ($p=0.008$). In a recent study immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis appeared to offer long-term clinical efficacy in the treatment of ragweed allergic rhinitis (Creticos PS, Schroeder JT and Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006;355(14):1445-55). TLR9-receptor agonists are also currently being evaluated as adjuvant to novel malaria vaccine candidates but they are also being used in a number of cancer trials.

Some of the shortcomings of regular vaccination are:

- need for several boosts to achieve protection
- delay in rise of protective antibodies
- prevalence of vaccine non-responders (as outlined above - this is particularly a problem for immune-compromised individuals)
- cost of antigen and vaccine production which is a very significant limitation in the development of new conjugated pneumococcal vaccines.

- poorly protective antibodies with low affinity – this has been observed in a number of trials with pneumococcal vaccines in HIV-infected individuals.
- Fall in antibody titre over time.

These shortcomings can be overcome by the effects of TLR9-agonists:

- Reduce number of vaccinations required to achieve seroprotection (this was demonstrated in the Engerix and CpG7909 trial (Cooper CL, Davis HL and Angel JB, et al. CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in antiretroviral-treated HIV-infected adults. AIDS 2005;19(14):1473-9))
- Accelerate seroconversion, possibly permitting post-exposure vaccination
- Reduce non-responders rate
- Reduce amount of antigen required
- Increase antibody avidity and protective activity
- More sustained antibody levels

Thus in one embodiment, the nucleic acids, vectors, methods, physical entities, retroviral particles, uses, compositions, vaccines, vaccine compositions and kits-of-parts according to the present invention comprise an adjuvant and/or a nucleic acid sequence encoding an adjuvant.

In one embodiment, the adjuvant of the present invention is an immunostimulatory adjuvant. Examples of such adjuvants are toll-like receptor agonists, such as agonists for TLR9, including CpG ODNs.

TLR agonist may in one embodiment be utilized as a mean of attracting and activating antigen presenting cells (APC; primarily Monocyte/macrophages and dendritic cells). This allows to selectively targeting the infection of pseudotyped MLV particles to the activated APC and thus promoting immunological cross-talk.

In yet another embodiment, the components of the present invention, including the nucleic acids, vectors, methods, physical entities, retroviral particles, uses, compositions, vaccines, vaccine compositions and kits-of-parts, comprises at least one immunomodulating peptide and/or at least one nucleic acid sequence encoding an immunomodulating peptide. For example, the at least one additional nucleic acid

sequence of a vector of the present invention encodes an immunomodulating polypeptide. Specifically, said immunomodulating peptide may be an immunostimulating polypeptide, an immunodominant polypeptide and/or a genetic adjuvant. Such embodiments include components of the present invention including vectors, nucleic acids, retroviral particles, compositions, and kit-of-parts comprising at least one cytokine and/or hormone, and/or at least one nucleic acid sequence encoding a cytokine and/or a hormone. Examples of cytokines include without restriction Interleukin-2 (IL-2), Interleukin-4 (IL-4) , Interleukin-10 (IL-10) , Granulocyte-macrophage colony stimulating factor (GM-CSF), Vascular endothelial growth factor (VEGF), Interleukin-12 (IL-12) , Fibroblast growth factor (FGF), Interleukin-7 (IL-7) , Interleukin-6 (IL-6) , Tumor Necrosis Factor-alpha (TNF-a) , Tumor Necrosis Factor-beta (TNF-b), Lymphotactin, Interferon-alpha (IFN-a), Interferon-beta (IFN-b), Interferon-gamma (IFN-g), Tumor Necrosis Factor (TNF), Interleukin-15 (IL-15) , Interleukin-5 (IL-5) , Interleukin-13 (IL-13) , Interleukin-1a (IL-1alfa) , Interleukin-1b (IL-1beta) , Interleukin-18 (IL-18), MCP-1, MIP-1a, MIP-1b, RANTES, TCA-3, CD80, CD86, CD40L, CCL3, CCL4, CCL5, Lymphocyte Chemotactic Factor (LCF), Erythropoietin (EPO), Prothymosin-alpha, Thymopoietin, Thymosin-alpha-1. Each of the cytokines mentioned herein is intended to be an individual embodiment. Consequently, a vector, method, provirus, use, composition, vaccine, vaccine composition and/or kit comprising at least one heterologous nucleic acid sequence encoding a cytokine polypeptide or a fragment thereof derived from each of them are claimed individually.

Immunomodulating polypeptides may also be expressed by the vector by including at least one nucleic acid sequence encoding an immunomodulating polypeptide. A number of immunomodulating peptides are known to the person skilled in the art. The immunomodulating polypeptides may be immunostimulatory adjuvants, immunostimulatory cytokines or immunostimulant polypeptides other than cytokines. Non-limiting examples of immunomodulating polypeptides according to the present invention are shown in table 5a, table 5b and table 5c.

Table 5a. List of preferred immunostimulatory adjuvants which can be used as immunostimulatory adjuvants according to the present invention.

Protein and peptides	
Alpha-1,3-galactosyltransferase	J. Virol. 2006 Jul;80(14):6943-51. Increased immunogenicity of human

	immunodeficiency virus gp120 engineered to express Galalpha1-3Galbeta1-4GlcNAc-R epitopes. Abdel-Motal U, Wang S, Lu S, Wigglesworth K, Galili U.
bacillus Calmette-Guérin (BCG)	Cancer Immunol. Immunother. 2002 Nov;51(10):521-31. Epub 2002 Sep 20. Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy. Dredge K, Marriott JB, Todryk SM, Dalgleish AG.
Mycobacterium vaccae (SRL 172)	Cancer Immunol. Immunother. 2002 Nov;51(10):521-31. Epub 2002 Sep 20. Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy. Dredge K, Marriott JB, Todryk SM, Dalgleish AG.
Flagellin (ligand to TLR5)	Hum. Gene Ther. 2006 Nov;17(11):1051-61. DNA vaccines: recent developments and future possibilities. Liu MA, Wahren B, Karlsson Hedestam GB.
Antrax proteins	Hum. Gene Ther. 2006 Nov;17(11):1051-61. DNA vaccines: recent developments and future possibilities. Liu MA, Wahren B, Karlsson Hedestam GB.

Table 5b. List of immunostimulatory cytokines, which can be used as immunostimulatory adjuvants according to the present invention. Genetic adjuvants other than cytokines and conventional adjuvants.

Category	Classification	Name	References
Genetic adjuvants	Costimulatory molecules	CD80	[22,23]
		CD86	[22,23]
		CD154 (CD40L)	[25,26]
	Chemokines	TCA-3	[24]
		RANTES	[34]
		MIP1-α	[33]
	Complement	C3d	[28]
	Heat shock protein	Hsp70	[29]
	Apoptosis inducer	Fas	[27]
		Caspase	[30,32]
	Transcriptional factors	IRFs	[31]
Conventional adjuvants	Mineral salts	Aluminum phosphate	
		Aluminum hydroxide	[38]
	Bacteria-derived adjuvants	Monophosphoryl lipid A	[41,43]
		Cholera toxin	[44]
		Muramyl peptides	
	Lipid particles	Cationic liposomes	[35–37]
		Mannan-coated liposomes	[39]
	Emulsifier-based adjuvants	QS-21	[42]
	Synthetic adjuvants	Ubenimex	[40]

(From Methods. 2003 Nov;31(3):243-54. Adjuvant formulations and delivery systems for DNA vaccines. Sasaki S, Takeshita F, Xin KQ, Ishii N, Okuda K.)

Table 5c. List of immunostimulatory cytokines, which can be used as immunostimulatory adjuvants according to the present invention. (Vaccine. 2001 Mar 21;19(17-19):2647-56. Genetic adjuvants for DNA vaccines. Scheerlinck JY.)

Cytokines	Ag/model[a]	Species[b], route[c]	Effects[d]					Reference
			Protection	Ab/IgG	CTL	IgG1/IgG2a	DTH	
MCP-1	BAE/No Ag	R, i.m.	↑	↑[e]	n.d.	n.d.	n.d.	[51,52]
MIP-1α	BAE/No Ag	R, i.m.	↑	↑[e]	n.d.	n.d.	n.d.	[51,52]
MIP-1α	HBV-1	M, i.m.	n.d.	n.d.	↑	↑	n.d.	[55]
MIP-1α	HBV-1	M, i.m./i.n.	n.d.	↑	↑	↓	↑	[56]
MIP-1β	BAE/No Ag	R, i.m.	↑	↑[e]	n.d.	n.d.	n.d.	[51]
RANTES	HBV-1	M, i.m.	n.d.	n.d.	↑	n.d.	n.d.	[55]
RANTES	BAE/No Ag	R, i.m.	=	↑[e]	n.d.	n.d.	n.d.	[51]
TCA-3	HBV-1	M, i.m.	n.d.	↓	↑	↓	↓	[57]
CD80	HSV-2	M, i.m.	=	=	n.d.	=	=	[54]
CD80	HSV-2	M, i.d.	↑	=	n.d.	=	↑	[54]
CD80	HBV-1	M, i.m.	n.d.	=	=	n.d.	=	[58,59]
CD80[f]	Influenza	M, i.m.	n.d.	n.d.	=	n.d.	n.d.	[39]
CD86	HSV-2	M, i.m./i.d.	=	=	n.d.	=	=	[54]
CD86	HBV-1	M, i.m.	n.d.	=	↑	=	↑	[58,60,53,59]
CD86	HBV-1	C, i.m.	n.d.	n.d.	↑	n.d.	n.d.	[60]
CD86[f]	Influenza	M, i.m.	n.d.	n.d.	↑	n.d.	n.d.	[39]
CD40L	β-gal	M, i.m.	↑	↑	↑	↓	n.d.	[46]

Adjuvants could for example be selected from the group consisting of: $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, silica, alum, $Al(OH)_3$, $Ca_3(PO_4)_2$, kaolin, carbon, aluminum hydroxide, muramyl dipeptides, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-DMP), N-acetyl-nornuramyl-L-alanyl-D-isoglutamine (CGP 11687, also referred to as nor-MDP), N-acetylmuramyul-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'2'-dipalmitoyl-sn -glycero-3-hydroxphosphoryloxy)-ethylamine (CGP 19835A, also referred to as MTP-PE), RIBI (MPL+TDM+CWS) in a 2% squalene/Tween-80.RTM. emulsion, lipopolysaccharides and its various derivatives, including lipid A, Freund's Complete Adjuvant (FCA), Freund´s Incomplete Adjuvants, Merck Adjuvant 65, polynucleotides (for example, poly IC and poly AU acids), wax D from Mycobacterium, tuberculosis, substances found in Corynebacterium parvum, Bordetella pertussis, and members of the genus Brucella, Titermax, ISCOMS, Quil A, ALUN (see US 58767 and 5,554,372), Lipid A derivatives, choleratoxin derivatives, HSP derivatives, LPS derivatives, synthetic peptide matrixes or GMDP, Interleukin 1, Interleukin 2, Montanide ISA-51 and QS-21. Preferred adjuvants to be used with the invention include oil/surfactant based adjuvants such as Montanide adjuvants (available from Seppic, Belgium), preferably Montanide ISA-51. Other preferred adjuvants are bacterial DNA based adjuvants, such as adjuvants including CpG oligonucleotide sequences. Yet other preferred adjuvants

are viral dsRNA based adjuvants, such as poly I:C. Imidazochinilines are yet another example of preferred adjuvants. The most preferred adjuvants are adjuvants suitable for human use.

Montanide adjuvants (all available from Seppic, Belgium), may be selected from the group consisting of Montanide ISA-51, Montanide ISA-50, Montanide ISA-70, Montanide ISA-206, Montanide ISA-25, Montanide ISA-720, Montanide ISA-708, Montanide ISA-763A, Montanide ISA-207, Montanide ISA-264, Montanide ISA-27, Montanide ISA-35, Montanide ISA 51F, Montanide ISA 016D and Montanide IMS, preferably from the group consisting of Montanide ISA-51, Montanide IMS and Montanide ISA-720, more preferably from the group consisting of Montanide ISA-51. Montanide ISA-51 (Seppic, Inc.) is oil/surfactant based adjuvants in which different surfactants are combined with a non-metabolizable mineral oil, a metabolizable oil, or a mixture of the two. They are prepared for use as an emulsion with an aqueous solution comprising HIV-1 envelope polypeptide or peptide fragment thereof. The surfactant is mannide oleate. QS-21 (Antigenics; Aquila Biopharmaceuticals, Framingham, MA) is a highly purified, water-soluble saponin that handles as an aqueous solution. QS-21 and Montanide ISA-51 adjuvants can be provided in sterile, single-use vials.

The well-known cytokine GM-CSF is another preferred adjuvant of the present invention. GM-CSF has been used as an adjuvant for a decade and may preferably be GM-CSF as described in WO 97/28816.

In a preferred embodiment, the vaccine composition adjuvant is selected from the group consisting of bacterial DNA based adjuvants, oil/surfactant based adjuvants, viral dsRNA based adjuvants, imidazochinilines, and/or incomplete Freund's adjuvant (IFA). In another preferred embodiment, the vaccine composition adjuvant is a Montanide ISA adjuvant. In another preferred embodiment the adjuvant is Montanide ISA 51 or Montanide ISA 720. In another preferred embodiment, the adjuvant is Montanide ISA 51. In yet another embodiment, the adjuvant is GM-CSF.

Carriers

The composition and/or vaccine composition of the present invention may comprise any adjuvant substance and/or carrier including any of the above or combinations thereof.

A carrier may be present independently of an adjuvant. The function of a carrier can for example be to increase the molecular weight of in particular peptide fragments in order to increase their activity or immunogenicity, to confer stability, to increase the biological activity, or to increase serum half-life. Furthermore, a carrier may aid in presenting the HIV-1 envelope polypeptide, variant or peptide fragments thereof to T-cells. The carrier may be any suitable carrier known to a person skilled in the art, for example a protein or an antigen presenting cell. A carrier protein could be, but is not limited to, keyhole limpet hemocyanin, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin or ovalbumin, immunoglobulins, or hormones, such as insulin or palmitic acid. For immunization of humans, the carrier must be a physiologically acceptable carrier acceptable to humans and safe. However, tetanus toxoid and/or diptheria toxoid are suitable carriers in one embodiment of the invention. Alternatively, the carrier may be dextrans for example sepharose.

Thus, in one embodiment the compositions of the present invention are provided associated with a carrier such as e.g. a protein of the above or an antigen-presenting cell such as e.g. a dendritic cell (DC).

Dosis and administration

The amount of the plurality of nucleic acid species, and/or components of the composition of the present invention, such as nucleic acid species encoding cytokine or immunogenic polypeptides, vector species retroviral particle species, gene gun particles and/or physical entity species may vary, depending on the particular application and the particular component. However, a single dose of a plurality of nucleic acid species or retroviral particles is preferably anywhere from about 1 ng to about 5000 μg, such as about 1 μg to about 4000 μg more preferably from about 50 μg to about 2500 μg such as about 100 μg to about 1000 μg.

Modes of administration within the scope of the present invention include intradermal, subcutaneous and intravenous administration, implantation in the form of a time release formulation, etc. Any and all forms of administration known to the art are encompassed herein. Also any and all conventional dosage forms that are known in the art to be appropriate for formulating injectable immunogenic peptide composition are encompassed, such as lyophilized forms and solutions, suspensions or emulsion

forms containing, if required, conventional pharmaceutically acceptable carriers, diluents, preservatives, adjuvants, buffer components, etc.

The plurality of nucleic acid species and/or compositions of the present invention may be prepared and administered using any conventional protocol known by a person skilled in the art. The immunoprotective effect of the composition of the invention can be determined using several approaches known to those skilled in the art. A successful immune response may also be determined by the occurrence of DTH reactions after immunization and/or the detection of antibodies specifically recognizing the peptide(s) of the vaccine composition.

The plurality of nucleic acid species and/or compositions of the present invention according to the invention may be administered to an individual in therapeutically effective amounts. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The plurality of nucleic acid species and/or compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular. Administration of pharmaceutical compositions is accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (directly to the tissue), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the methods of prophylaxis and treatment with the vaccine composition.

The plurality of nucleic acid species of the present invention are brought into contact with a population of cells by anu method of administration or method of transfection, which allows for the nucleic acid to be taken op by the cells. Most preferred methods are injection or gene gun. However, the plurality of nucleic acid species and/or vaccine compositions can, for example, be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and

infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the vaccine, comprising any of the herein described compounds can be employed as a prophylactic or therapeutic agent. Also any and all conventional dosage forms that are known in the art to be appropriate for formulating injectable immunogenic peptide composition are encompassed, such as lyophilized forms and solutions, suspensions or emulsion forms containing, if required, conventional pharmaceutically acceptable carriers, diluents, preservatives, adjuvants, buffer components, etc.

Preferred modes of administration of the plurality of nucleic acid species and/or compositions of the invention include, but are not limited to systemic administration, such as intravenous or subcutaneous administration, intradermal administration, intramuscular administration, intranasal administration, oral administration, rectal administration, vaginal administration, pulmonary administration and generally any form of mucosal administration. Furthermore, it is within the scope of the present invention that the means for any of the administration forms mentioned in the herein are included in the present invention.

A plurality of nucleic acid species and/or composition according to the present invention can be administered once, or any number of times such as two, three, four or five times. Administering the vaccine more than once has the effect of boosting the resulting immune response. The immune response can further be boosted by administering the plurality of nucleic acid species and/or composition in a form or body part different from the previous administration. The booster shot is either a homologous or a heterologous booster shot. A homologous booster shot is a where the first and subsequent vaccinations comprise the same constructs and more specifically the same delivery vehicle especially the same viral vector. A heterologous booster shot is where identical constructs are comprised within different viral vectors.

In a broad aspect, the present invention relates to a method of treating, preventing or ameliorating a clinical condition, wherein each individual cell of a population of cells take up at the most 5, such as 4, 3, 2, preferably 1 non-identical nucleic acid species, which are provided a said population of cells, and wherein said nucleic acid species belong to a plurality of nucleic acid species encoding distinct species of cytokine or

hormone, or a functional homologue or part thereof, and/or distinct species of immunogenic polypeptides. It is comprehended that any method of transfection, wherein each individual cell of a population of cells take up at the most 5, such as 4, 3, 2, preferably 1 nucleic acid species, vector species and/or retroviral vector species is within the scope of the present invention. Thus in one embodiment, the present invention relates to a method and/or use, wherein

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined herein above, and/or
b. a plurality of nucleic acid species encoding said immunogenic polypeptide as defined herein above, and/or
c. a vector as defined herein above, and/or
d. a retroviral particle of the present invention, and/or
e. a plurality of physical entity species of the present invention

is brought into contact with said population of cells by any method of transfection, wherein each individual cell of said population of cells take up at the most 5, such as 4, 3, 2, preferably 1 said nucleic acid species, vector species, retroviral particle species and/or physical entity.

To restrict the delivery of only one or a few nucleic acid species per cell, according to the methods of the present invention several means of transfer can be used. In one example, the cells can be transduced by means or retroviral vectors which results in a single integration of the vector into the target cell, thus allowing the transfer of only one nucleic acid species or nucleic acid vector per cell. In another means of transfer, the cells are transduced by means of a gene gun. Thus in one embodiment, the method of transfection is selected from the group consisting of gene gun administration and/or retroviral infection with retroviral particles. In a preferred embodiment, said method of transfection is gene gun administration. Thus in one embodiment of the method of the present invention, the plurality of non-identical nucleic acid species, vector species and/or retroviral vector species are attached to a population of gene gun particles, which are administered by gene gun. The gene gun particles are administered to any population of cells, preferably a population of human cells, such as cancer cells, and/or blood cells.

The gene gun was originally a Crosman air pistol modified to fire dense tungsten particles. The design was first used on onions to deliver particles coated with a marker gene. Genetic transformation can then be proven when the onion tissue expresses the gene. The earliest custom manufactured gene guns (Fabricated by Nelson Allen) used a 22 caliber nailgun cartridge to propel an extruded polyethylene cylinder (bullet) down a 22 cal. Douglas barrel. A droplet of the tungsten powder and genetic material was placed on the bullet and shot down the barrel at a lexan "stopping" disk with a petri dish below. The bullet welded to the disk and the genetic information blasted into the sample in the dish with a doughnut effect (devastation in the middle, a ring of good transformation and little around the edge). The gun was connected to a vacuum pump and was under vacuum while firing. Later the design was refined by removing the "surge tank" and changing to nonexplosive propellants. DuPont added a plastic extrusion to the exterior to visually improve the machine for mass production to the scientific community. Improvements include the use of helium propellant and a multi-disk-collision delivery mechanism. Other heavy metals, such as gold and silver are also used. Gold may be favored because it has better uniformity than tungsten and tungsten can be toxic to cells.

Gene guns have been used for plant cells and bacteria, because these cells have a hard cell wall, where as other methods necessitate the production of protoplasts before gene introduction. However, the gene gun is also useful for transfecting animal cells that are non-dividing, are in primary culture, or are resistant to usual methods of gene transfection. A gene gun is suitable for direct in situ intracellular delivery with a gene. Moreover, it is independent of cell type in various species. The setting for the gene gun can be modulated in various ways allowing for approximately one particle per cell, thereby allowing the transfer of only one or a few attached nucleic acid species, vector species, and/or physical entity species, such as retroviral particles according to the present invention.

In order to obtain expression of one cytokine per cell or one cytokine and one immunogenic peptide per cell, batches of particles, wherein each particle in the batch are coated with only one plasmid encoding one cytokine or one cytokine and one peptide may be produced. In order to obtain a high mucosal protection, immunisation may be performed intrarectal or intranasal. This can also be performed using a gene gun.

In one embodiment of the present invention, at the most 10, such as at the most 9, 8, 7, 6, 5, 4, 3, 2, or 1 gene gun particle on average are provided per cell. The gene gun particles may be produced from any material, however, in a preferred embodiment, the gene gun particles are gold particles. In another embodiment, the gene gun particles are silver or tungsten particles.

When providing the plurality of nucleic acid species to a population of cells according to the present invention by use of gene gun administration, care is to be taken that only one or a few species are provided and taken up by each individual cell. In one embodiment, at the most 5, such as at the most 4, 3, 2, or at the most 1 said

a. nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined herein above, and/or
b. nucleic acid species encoding distinct species of cytokine as defined herein above, and/or
c. vector species as defined herein above, and/or
d. retroviral particle species as defined herein above

are attached to each gene gun particle of said population of gene gun particles.

In one such embodiment, the population of gene gun particles comprises a subpopulation of at least 50%, such as at least 60 %, for example at least 70%, such as at least 80%, such as at least 90%, for example at least 95% of said population of gene gun particles, wherein one and not more than one of said nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof, and/or nucleic acid species encoding distinct species of immunogenic polypeptides, and/or, vector species and/or retroviral particle species is attached to each gene gun particle of said subpopulation of gene gun particles.

In another embodiment, the population of gene gun particles comprises a plurality of non-identical subpopulations, wherein a distinct species of said nucleic acid, vector and/or retroviral particle is attached to the gene gun particles of each subpopulation.

In another embodiment, a plurality of nucleic acid species, vector species and/or retroviral particle species encoding a cytokine or hormone or a functional homologue or part thereof are attached to gene gun particles of a first subpopulation of gene gun

particles, and/or a plurality of nucleic acid species, vector species and/or retroviral particle species encoding an immunogenic polypeptide are attached to gene gun particles of a second subpopulation of gene gun particles. For example, that first subpopulation of gene gun particles comprise at least 10%, such as at least 20%, for example 30%, such as at least 40%, for example at least 50%, such as at least 60 %, for example at least 70%, such as at least 80%, such as at least 90%, for example at least 95%, such as 100% of said population of gene gun particles, and/or the second subpopulation of gene gun particles comprise at least 10%, such as at least 20%, for example 30%, such as at least 40%, for example at least 50%, such as at least 60 %, for example at least 70%, such as at least 80%, such as at least 90%, for example at least 95%, such as 100% of said population of gene gun particles. In one embodiment, said first subpopulations of gene gun particles comprise at least 75%, such as 80%, for example 85%, such as 90%, such as 95%, such as 100% of said population of gene gun particles. In a further embodiment, said first and second subpopulations of gene gun particles together comprise at least 95%, such as 100% of said population of gene gun particles.

In a specific embodiment, the population of gene gun particles comprises:

a. a first subset of subpopulations of gene gun particles, wherein distinct nucleic acid species encoding a cytokine or hormone or a functional homologue or part thereof as defined herein above, or a vector species as defined herein above, a physical entity and/or a retroviral particle species as defined herein are attached to the gene gun particles of each subpopulation of said first subset of subpopulations of gene gun particles, and/or

b. a second subset of subpopulations of gene gun particles, wherein distinct nucleic acid species encoding an immunogenic polypeptide as defined herein above, or a vector species as defined herein above, a physical entity and/or retroviral particle species as defined herein above are attached to the gene gun particles of each subpopulation of said first subset of subpopulations of gene gun particles.

The gene gun particles may be administered by any route of administration known to those of skill within the art. In one embodiment, the plurality of nucleic acid species, vector species, physical entities, retroviral particles and/or population of gene gun

particles are administered by subcutaneous, intraperitonal, intramuscular, intravenous, rectal, and/or nasal administration.

In another specific embodiment, the plurality of nucleic acid species, vector species, and/or retroviral particle species of the present invention are provided to a population of cells by retroviral infection, wherein a retroviral particle is employed, which is capable of infecting specific target cells. Infection of a target cell can occur by receptor mediated endocytosis and/or membrane fusion, as described elsewhere.

Second active ingredient

It is an aspect of the present invention that the composition herein provided is used in combination with a second active ingredient. The administration of the vaccine composition and the second active ingredient may be sequential or combined. The selection of second active ingredient depends on the clinical condition, which is treated by the methods, kits-of-parts, compositions and uses of the present invention. Examples of such additional ingredients, second active ingredients are provided elsewhere herein, and include for example conventional anticancer drugs, anti-inflammatory substances, antibiotic treatment, chemotherapy, and/or treatment with immunostimulating substances. It is a further aspect that the methods and/or compositions may be used in combination with other therapy of relevance for the given clinical condition to be treated. Such therapy may include surgery and/or gene therapy, immunostimulating substances or antibodies; a person skilled in the art is able to determine the appropriate combination treatment for a given scenario.

In some cases it will be appropriate to combine the treatment method of the invention with a further medical treatment such as treatment with immunostimulating substances, gene therapy, treatment with antibodies and/or antibiotics and treatment using dendritic cells.

Kit of Parts

As used herein, the term "kit-of-parts" refers to components packaged or marked for use together. In one aspect, the kit-of-parts contain any component of the present invention, including a composition as defined herein, for example comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined herein above, and/or

b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined herein above, and/or

c. a plurality of non-identical vector species as defined herein above, and/or

d. a plurality of non-identical retroviral particle species as defined herein above, and/or

e. a population of gene gun particles as defined herein above,

f. a plurality of any physical entity species comprising any component of any of a. to e, and

at least one additional active ingredient. The kits-of parts of the present invention are also claimed for use as a medicament.

A kit-of-parts can contain any two components in one container, and a third component and any additional components in one or more separate containers. Optionally, a kit-of-parts further contains instructions for combining the components so as to formulate an immunogenic composition suitable for administration to a mammal. The components of the kit-of-parts are preferably comprised in individual compositions, it is however within the scope of the present invention that the components of the kit-of-parts all are comprised within the same composition. The components of the kit-of-parts may thus be administered simultaneously or sequentially in any order.

One aspect of the present invention, relates to a kit-of-parts comprising the vaccine composition as defined previously herein, and a second active ingredient. The kit-of-parts preferably comprises an adjuvant and/or a carrier. Examples of useful adjuvants are given elsewhere herein. Thus, the vaccine composition may in a kit-of-parts of the present invention be associated with an adjuvant and/or a carrier. As specified previously, adjuvants are any substance whose admixture into the vaccine composition increases or otherwise modifies the immune response to an HIV-1 envelope polypeptide or a peptide fragment thereof, as defined herein. Carriers are scaffold structures, for example a polypeptide or a polysaccharide, to which the HIV-1 envelope or peptide fragment thereof is capable of being associated and which aids in the presentation of especially the peptides of the present invention. Examples of carriers are provided elsewhere herein. Some of the peptide fragments of the invention are relatively small molecules and it may therefore be required in compositions as described herein to combine the peptides with various materials such as adjuvants

and/or carriers, to produce vaccines, immunogenic compositions, etc. Adjuvants, broadly defined, are substances which promote immune responses.

A carrier may be present independently of an adjuvant. The function of a carrier can for example be to increase the molecular weight of in particular peptide fragments in order to increase their activity or immunogenicity, to confer stability, to increase the biological activity, or to increase serum half-life. Furthermore, a carrier may aid in presenting the HIV-1 envelope polypeptide, variant or peptide fragments thereof to T-cells. The carrier may be any suitable carrier known to a person skilled in the art, for example a protein or an antigen presenting cell. A carrier protein could be, but is not limited to, keyhole limpet hemocyanin, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin or ovalbumin, immunoglobulins, or hormones, such as insulin or palmitic acid. For immunization of humans, the carrier must be a physiologically acceptable carrier acceptable to humans and safe. However, tetanus toxoid and/or diptheria toxoid are suitable carriers in one embodiment of the invention. Alternatively, the carrier may be dextrans for example sepharose.

Thus, it is an aspect of the present invention that the plurality of nucleic acid species encoding distinct cytokine or hormone species and/or immunogenic polypeptide species in the composition of the present invention is associated with a carrier such as e.g. a protein of the above or an antigen-presenting cell such as e.g. a dendritic cell (DC).

One aspect of the present invention relates to a kit-of-parts comprising a therapeutically effective amount of a plurality of nucleic acid species, vector species, provirus species, retroviral particle species, compositions, and/or vaccine composition of the present invention.

In one embodiment, the second active ingredient in the kit-of-parts of the present invention is an immunostimulating composition. Examples of immunostimulatory agents are provided elsewhere herein, however, in one preferred embodiment, the immunostimulating composition comprises one or more interleukins. For example, the interleukins are selected from the group consisting of IL-2 and/or IL-21.

In another embodiment, the second active ingredient in the kit-of-parts of the present invention is an antibiotic, such as an antibiotic is selected from the group consisting of amoxicillin, penicillin, acyclovir and/or vidarabine.

The compositions provided by the kits-of-parts of the present invention are to be administered simultaneously or sequentially.

The invention also relates to a kit-of-parts comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined herein above, for example any polypeptide selected from the group consisting of SEQ ID NO: 47-86 and/or part thereof, and/or

b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined herein above, for example any immunogenic polypeptide selected from the group consisting of SEQ ID NO: 1-46 and/or SEQ ID NO: 95-1159 and/or part thereof, and/or

c. a plurality of non-identical vector species as defined herein above, for example any vector selected from the group consisting of SEQ ID NO: 87-94 and/or part thereof, and/or

d. a plurality of non-identical retroviral particle species as defined herein above, and/or

e. a population of gene gun particles as defined herein above,

f. a plurality of any physical entity species comprising any component of any of a. to e, and

The invention also relates to a kit-of-parts comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined herein above, for example any polypeptide selected from the group consisting of SEQ ID NO: 47-86 and/or part thereof, and/or

b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined herein above, for example any immunogenic polypeptide selected from the group consisting of SEQ ID NO: 1-46 and/or SEQ ID NO: 95-1159 and/or part thereof, and/or

c. a plurality of non-identical vector species as defined herein above, for example any vector selected from the group consisting of SEQ ID NO: 87-94 and/or part thereof, and/or

d. a plurality of non-identical retroviral particle species as defined herein above, and/or

e. a population of gene gun particles as defined herein above,

f. a plurality of any physical entity species comprising any component of any of a. to e, and

and instructions on how to use the kit of parts.

The invention also relates to a kit-of-parts comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined herein above, for example any polypeptide selected from the group consisting of SEQ ID NO: 47-86 and/or part thereof, and/or

b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined herein above, for example any immunogenic polypeptide selected from the group consisting of SEQ ID NO: 1-46 and/or SEQ ID NO: 95-1159 and/or part thereof, and/or

c. a plurality of non-identical vector species as defined herein above, for example any vector selected from the group consisting of SEQ ID NO: 87-94 and/or part thereof, and/or

d. a plurality of non-identical retroviral particle species as defined herein above, and/or

e. a population of gene gun particles as defined herein above,

f. a plurality of any physical entity species comprising any component of any of a. to e, and

and a second active ingredient.

Preferably, the second active ingredient is chosen in correspondence with the clinical condition to be treated so that the second active ingredient is chosen among other clinical agents suitable for treatment of the specific clinical condition, for example HIV, AIDS and/or ARC, which is known the person skilled in the art. For example, if treating a microbial / viral infection, the second active ingredient is preferably an anti-biotic and/or an anti-viral agent.

The components of the kit can be combined ex viva to produce an immunogenic composition, or alternatively, any two components can be combined ex vivo, and administered with a third component, such that an immunogenic composition forms in vivo. For example, an nucleic acid species, vectors, and/or physical entities of the present invention can be emulsified in, dissolved in, mixed with, or adsorbed to an adjuvant and injected into a mammal, preceded or followed by injection of a second component, such as an adjuvant and/or a carrier. Likewise, each component of the kit can be administered separately. Those skilled in the art understand that there are various methods of combining and administering the components of the kit-of-parts, so as to enhance the immune response in a mammal. The kit-of-parts can be administered by the same routes of administration as a vaccine composition of the present invention, for example it can be administered locally or systemically by methods well known in the art, including, but not limited to, intramuscular, intradermal, intravenous, subcutaneous, intraperitoneal, intranasal, oral or other mucosal routes.

Medical applications

The present invention provides a number of therapeutical applications. All components of the present invention including the plurality of nucleic acid species, plurality of vector species, plurality of physical entity species, such as plurality retroviral particles, compositions, and/or kits-of-parts are claimed for use as a medicament. It is understood that said components of the present invention may be used for treating a medical condition. Thus, one aspect of the present invention relates to the use of any component of the present invention for the manufacture of a medicament for the treatment, prevention and/or amelioration of a clinical condition.

Another aspect relates to any component of the present invention including the plurality of nucleic acid species, plurality of vector species, plurality of physical entity species, such as plurality retroviral particles, compositions, and/or kits-of-parts for treating, ameliorating and/or preventing a clinical condition. In a preferred embodiment, said clinical condition is an infectious disorder, such as HIV infection, such as HIV-1 infection and/or AIDS and/or ARC, an autoimmune disorder and/or a cancer as described elsewhere herein. In another preferred embodiment, said treatment is prophylactic treatment of any such disorder, for example by reducing the susceptibility of lentiviral infection, such as HIV infection and/or AIDS and/or ARC.

Thus, one aspect of the present invention relates to the use of any component of the present invention, including the plurality of nucleic acid species, plurality of vector species, plurality of physical entity species, such as plurality retroviral particles, compositions, and/or kits-of-parts for the manufacture of a medicament for the treatment, prevention and/or amelioration of an infectious disorder, an autoimmune disorder, and/or a cancer, said disorders including lentiviral infection, such as HIV infection and/or AIDS and/or ARC. Also, the present invention relates to any said component of the present invention for treating, ameliorating and/or preventing lentiviral infection, including HIV infection and/or AIDS and/or ARC. The present invention also relates to use of any of said component of the present invention, including the plurality of nucleic acid species, plurality of vector species, plurality of physical entity species, such as plurality retroviral particles, compositions, and/or kits-of-parts for the manufacture of a medicament for lentiviral vaccination, such as HIV vaccination, such as HIV-1 vaccination.

In one aspect, the present invention relates to the use of any said component of the present invention for the manufacture of a medicament for gene therapy. Another aspect relates to the use of any component of the present invention for the manufacture of a medicament for immune therapy.

One aspect of the present invention relates to a method of treating, preventing or ameliorating a clinical condition, said method comprising administering to an individual suffering from said clinical condition an effective amount of any said component of the present invention. In one embodiment, the clinical condition is an infection, and/or more specifically HIV infection and/or AIDS and/or ARC. Moreover, the individual suffering from HIV infection and/or AIDS is preferably a human being. In one embodiment, said human being is HIV seronegative. However, in another embodiment, said human being is HIV seropositive. In one embodiment of the methods of treating, preventing or ameliorating a clinical condition according to the present invention, and/or any of said component of the present invention is repeated/administered to said organism two or more times.

Any use of a component of the present invention for the manufacture of a medicament, and/or methods of treating, preventing and/or ameliorating a clinical condition

comprising administering a component of the present invention may be combined with a further treatment. In one example, the further treatment is selected from the group consisting of treatment with immunostimulating substances, gene therapy, treatment with antibodies, treatment using dendritic cells and/or treatments against infections.

In another aspect, the present invention relates to a method of reducing the risk of an individual encountering a clinical condition, said method comprising administration of a composition and/or a kit-of-parts as defined elsewhere herein to said individual in an amount sufficient to generate a protective immune response.

In one embodiment, the clinical condition is an infectious disorder, an autoimmune disorder and/or any cancer form, such as preferably HIV, and/or AIDS.

In another embodiment, the method, composition, kits-of-parts, and uses of the present invention is capable of eliciting INF-γ -producing cells in a PBL population of an individual suffering from a clinical condition at a frequency of at least 10 per 10^4 PBLs.

Monitoring immunization

In preferred embodiments, the composition of the invention is a pharmaceutical composition, such as a vaccine composition. It is therefore of interest, and an aspect of the present invention to monitor the immunization in an individual to whom the vaccine composition of the present invention is administered. The pharmaceutical composition may thus be an immunogenic composition or vaccine capable of eliciting an immune response to a clinical condition as described herein, i.e. an infectious disorder, an autoimmune disorder and/or any cancer form, preferably HIV infection and/or AIDS. The expression "immunogenic composition or vaccine" refers to a composition eliciting at least one type of immune response directed against cells expressing an immunogenic polypeptide species of the present invention, including any peptide selected from the group consisting of SEQ ID NO: 95-1159, including mammalian cells, APCs or DCs and/or retroviral particles. Thus, such an immune response may be any of the following: A CTL response where CTLs are generated that are capable of recognizing the HLA/peptide complex presented on cell surfaces resulting in cell lysis, i.e. the vaccine elicits the production in the vaccinated subject of effector T-cells having a cytotoxic effect against the cancer cells; a B-cell response giving rise to the production of anti-HIV antibodies; and/or a DTH type of immune response. It is on

object of the present invention to monitor the immunization of an individual by monitoring any of the above reactions subsequent to administering the composition of the present invention to said individual.

In one aspect, the invention relates to methods of monitoring immunization, said method comprising the steps of

a. providing a blood sample from an individual,

b. providing a composition as defined in any one of claims 71 to 73 and/or a kit-of-parts as defined in any one of claim 74 to 76, and

c. determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding at least one species of said immunogenic polypeptides or part thereof

thereby determining whether an immune response has been raised in said individual.

In another aspect, the invention relates to methods of monitoring immunization, said method comprising the steps of

a. providing a blood sample from an individual,

b. providing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof to a population of blood cells, wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species

c. determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding at least one species of said immunogenic polypeptides or part thereof

thereby determining whether an immune response has been raised in said individual.

The individual is preferably a human being, for example a human being that has been immunized with an immunogenic polypeptide of the present invention, for example a peptide selected from the group consisting of SEQ ID NO: 95-1159, or a fragment thereof consisting of 7 to 9, or 10 to 11 amino acid residues, a plurality of non-identical nucleic acid species, a plurality of physical entity species, such as retroviral particles, a vaccine composition, and/or a kit-of-parts of the present invention.

Immunogenic response

The components of the present invention including the plurality of nucleic acid species, plurality of vector species, plurality of physical entity species, such as plurality retroviral particles, compositions, and/or kits-of-parts are capable of inducing an immunogenic response in a host animal, for example in a human. The immunogenic response may be divided into to two types of responses, the antibody response and the cytotoxic T lymphocyte (CTL) response.

In the antibody response, specific antibodies are important in and may protect against viral infections. The most effective type of antiviral antibody is "neutralizing" antibody - this is antibody which binds to the virus, usually to the viral envelope of the virus particle or capsid proteins, and which blocks the virus from binding and gaining entry to the host cell. Virus specific antibodies may also act as opsonins in enhancing phagocytosis of virus particles - this effect may be further enhanced by complement activation by antibody-coated virus particles e.g. through production of the viral particles in eukaryotic cells e.g. mouse cells that ads gal-alfa1-3Galbeta1-4GlcNAc-R epitopes on the envelope protein. In addition, in the case of some viral infections, viral proteins are expressed on the surface of the infected cell. These may act as targets for virus-specific antibodies, and may lead to complement-mediated lysis of the infected cell, or may direct a subset of natural killer cells to lyse the infected cell through a process known as antibody-directed cellular cytotoxicity (ADCC). At mucosal surfaces (such as the respiratory and gastrointestinal tracts), virus infection may induce the production of specific antibodies of the IgA isotype, which may be protective against infection at these surfaces. Not all antibodies to viruses are protective, however, and in certain cases an antibody to the virus may facilitate its entry into a cell through Fc receptor-mediated uptake of the antibody coated particle. Such antibodies are called enhancing antibodies.

During the course of a viral infection, antibody is most effective at an early stage, before the virus has gained entry to its target cell. In this respect, antibody is relatively ineffective in primary viral infections, due mainly to the lag phase in antibody production. Preformed antibody, particularly neutralising antibody, however, is an effective form of protective immunity against viral infections, as witnessed by the

success of many viral vaccines, which work by stimulating virus-neutralising antibody responses.

The principal effector cells which are involved in clearing established viral infections are the virus specific cytotoxic T lymphocytes (CTL), for example the CD8+ cytotoxic T lymphocytes. These cells recognise (viral) antigens which have been synthesised within cell's nucleus or cytosol, and which have been degraded. They are presented at the cell's surface as short peptides associated with self class I MHC molecules. The recognition of antigen by CD8+ T cells is, therefore, distinct from that of CD4+ T cells in several respects. It requires synthesis of the target antigen within the cell (and is therefore restricted largely to virally infected or tumour cells); it is "restricted" by class I MHC molecules (as opposed to MHC class II restriction for CD4+ T cells); MHC class I molecules are expressed on almost all somatic cells, so virtually any cell, on infection with virus, can act as a "target" cell for antigen specific CTL (contrasts with the limited tissue distribution of class II MHC); recognition of an antigen presenting cell (APC) by an antigen-specific CTL usually results in the destruction of the APC.

In the context of the present invention the immune response is produced against the immunogenic polypeptide species, preferably derived from HIV envelope polypeptide which is displayed on the host cells or the virus particles carrying the envelope. The virus particles carrying an immunogenic polypeptide species may be given to an animal, including a human, as a vaccine. The vaccine is produced as described herein using a vector of the present invention and/or a retroviral particle and given to an animal for example a human being. The retroviral particle produced according to the present invention may infect a host cell and upon integration of the vector of the present invention into the genome of the host cell, transcription and translation by the host cell, the HIV envelope polypeptide is presented on the surface of the host cell. The host cell targeted in this manner will be subject to a CTL response.

In one embodiment of the present invention a component, such as a vector and/or a biological or physical entity described herein is able to induce an immune response. The response may be an antibody response following vaccination with retroviral particles using the vector of the present invention. However, in yet another embodiment the immune response is a CTL response. In a specific embodiment, the immunogenic

response is a CTL response, wherein said vector, RNA, mRNA of the present invention is integrated into the genome of a host cell.

In one embodiment, the vector or retroviral particle is able to infect, integrate and display the distinct immunogenic polypeptide species on the surface of a host cell, thus providing a means of boosting or enhancing the immune response as compared to viral vaccines that are not able to infect human cells. In a specific embodiment of the present invention, however, is provided a plurality of non-identical non-infectious retroviral particle species, which express distinct immunogenic polypeptide species as described herein and display said immunogenic polypeptide on its surface, thereby allowing the immune system to recognize said immunogenic polypeptide.

The components of the present invention including the plurality of nucleic acid species, plurality of vector species, plurality of physical entity species, such as plurality retroviral particles, compositions, and/or kits-of-parts can thus be used alone or in combination with other vaccines directed against a clinical condition as defined elsewhere herein, for example HIV infection and/or cancer.

During the course of a infection with for example viral infection, such as HIV infection, antibody is most effective at an early stage, before the virus has gained entry to its target cell. In this respect, antibody is relatively ineffective in primary viral infections, due mainly to the lag phase in antibody production. Preformed antibody, particularly neutralising antibody, however, is an effective form of protective immunity against viral infections, as witnessed by the success of many viral vaccines, which work by stimulating virus-neutralising antibody responses. In contrast to existing vaccines against lentiviruses, the present invention thus provides an additional feature which renders the vaccine capable for eliciting a CTL response.

An immunogenic composition of the invention is effective in enhancing an immune response, for example, enhanced beta-chemokine and/or ILl5, IFN, IL2, TNFa production, increased HIV-specific CD4 helper cells, IgG2b antibody production, HIV specific cytotoxic T lymphocyte (CTL) production, IFNy production by CD4+ cells and CD8 T cells, and the like, in a mammal administered the composition. As described in U.S. application serial No. 09/565,906, filed May S. 2000, and WO 00/67787, each of which is incorporated herein by reference, and in Examples I and III, below, production

of the beta -chemokine RANTES can be detected and quantitated using an ELISA assay of supernatants of T cells (such as lymph node cells or peripheral blood cells) from mammals administered the composition. In order to determine antigen-specific beta -chemokine production, T cells from an immunized mammal can be stimulated with HIV antigen in combination with antigen-presenting thymocytes, and the beta -chemokine levels measured in the supernatant. In order to determine non- specific beta -chemokine production, either T cell supernatant or a blood or plasma sample from an immunized mammal can be assayed. Similarly, production of other beta-chemokines, such as MIP-la and MIP-1,B, can be detected and quantitated using commercially available ELISA assays, according to the manufacturer's instructions. Methods of measuring cytokine production, including inteferon, ILLS, IL2, TNFa, IL10 and IL7, by ELISPOT, ELISA, or intracellular cytokine staining are well known to those skilled in the art (see, for example, Robbins et al., AIDS 17:1121-1126 (2003)).

An immunogenic composition of the invention can further be capable of enhancing HIV-specific IgG2b antibody production in a mammal administered the composition. High levels of IgG2b antibodies, which are associated with a Th1 type response, are correlated with protection against HIV infection and progression to AIDS. Thus, the invention provides compositions that can increase a TH1 response. An immunogenic composition of the invention can further be capable of enhancing HIV-specific cytotoxic T lymphocyte (CTL) responses in a mammal administered the composition. An immunogenic composition of the invention can increase IFN-y production by both CD4+ T cells and CD8+ T cells. IFN-y production by CD4+ T cells is characterized as a classic CD4 helper 2 5 response important to cell-mediated immunity. CD4+ T cells producing both IFN and IL2 may be most effective. IFN- production by CD8+ T cells is representative of a cytotoxic -= T lymphocyte (CTL) response, and is highly correlated with cytolytic activity. Cells producing both IFN and TNFa may be most effective. CTL activity is an important component of an effective prophylactic or therapeutic anti-HIV immune response. Methods of determining whether a CTL response is enhanced following administration of an immunogenic composition of the invention are well known in the art, and include cytolytic assays and LPA assays (described, for example, in Deml et al. supra (1999); see Example III), and ELISA and ELISPOT assays for CD8-specific IFN-y production (see U.S. application serial No. 09/565,906 and WO 00/67787 and Examples I and II below), intracellular staining and FACS analysis using a myriad of antibodies against cell surface markers.

Examples

Example 1

Detection of cytokine

The Enzyme-Linked ImmunoSpot (ELISpot) assay is a very sensitive immunoassay, allowing the detection of a secreted cytokine at the single cell level. With detection levels that can be as low as one cell in 100 000, the ELISpot is one of the most sensitive cellular assays available. Depending on the substance analyzed, it is between 20 and 200 times more sensitive than a conventional ELISA. The assay is schematically depicted in figure1.

In fact, the ELISpot displays a similar sensitivity as a RT-PCR analysis but detects the secreted protein instead of the mRNA. For cytokine analyses this is advantageous since many cytokines are translationally regulated.

ELISpot analyses are also less impaired by binding proteins and protease activity since the analyte is bound to the capture antibody immediately after secretion. Due to its high sensitivity, the ELISpot has proven particularly useful when studying small populations of active cells such as those regularly found in specific immune responses.

Assay Procedure

As a first step, cytokine-specific monoclonal antibodies are immobilized on a solid phase. In the second step, the cells to be investigated are added to the wells in presence or absence of stimuli and incubated for a relevant time period to allow cytokine production.

The secreted cytokine will bind to the capture antibodies in the vicinity of the producing cells and, after removal of the cells by washing, detection anti-cytokine antibodies are added. These antibodies are either directly conjugated with enzyme or biotinylated, in which case a third step with enzyme conjugated Streptavidin is required. Finally a substrate is added which will form a colored, insoluble precipitate when catalyzed by the enzyme. This way a visible spot corresponding to the location of the producing cell is formed. The spots can be counted under a dissection microscope or with an automated ELISpot reader and the frequency of positive cells registered.

The immune response in immunized mice may for example be investigated by the three different ELISpot Assays described below, each of which is giving valuable information about the nature of the elicited immune response

The secretion of IFN-γ.
IFN- γ is the hallmark cytokine of the Th1 cells that regulate the cell mediated immune response. IFN-γ has antiviral, immunoregulatory, and anti-tumour properties.

The secretion of IL-2.
Antigen binding to the T cell receptor (TCR) stimulates the secretion of IL-2, and the expression of IL-2 receptors IL-2R. The IL-2/IL-2R interaction then stimulates the growth, differentiation and survival of antigen-selected cytotoxic T cells via the activation of the expression of specific genes. As such, IL-2 is necessary for the development of T cell immunologic memory, one of the unique characteristics of the immune system, which depends upon the expansion of the number and function of antigen-selected T cell clones.

The secretion of Granzyme b
Cytolytic T lymphocytes (CTL) have the remarkable ability to recognize, bind, and lyse specific target cells. They are thought to protect their host by lysing cells bearing on their surface 'MHC-1 bound antigens, usually peptides or proteins resulting from infection by intracellular pathogens. Granzyme b is crucial for the rapid induction of target cell apoptosis by CTL in cell-mediated immune response.

Example 2
Asssessment of peptides pressented by MHC molecules.doc

Assessment of presentation of antigenic peptides on MHC class I molecules in a quantitative manner can be done by various well described methods. Methods include the use of HPLC fractionation of extracted peptides (Anton et al., 1997), monoclonal Ab (mAb) or multivalent TCR specific for a given MHC–peptide complex (Andersen et al., 1996; O'Herrin et al., 1997; Porgador et al., 1997), T cell hybridomas (Sanderson and Shastri, 1994; Usherwood et al., 1999; Canaday et al., 2003), TCR transgenic cells (Robson et al., 2003), T cell lines (Chen et al., 2000; Schnurr et al., 2005) or Dynamic

quantification of MHC class I–peptide presentation to CD8+ T cells via intracellular cytokine staining (Pang et al 2006).

Anton, L.C., Yewdell, J.W., Bennink, J.R., 1997. MHC class I associated peptides produced from endogenous gene products with vastly different efficiencies. J. Immunol. 158, 2535.

Andersen, P.S., Stryhn, A., Hansen, B.E., Fugger, L., Engberg, J., Buus, S., 1996. A recombinant antibody with the antigen-specific, major histocompatibility complex-restricted specificity of T cells. Proc. Natl. Acad. Sci. U. S. A. 93, 1820.

O'Herrin, S.M., Lebowitz, M.S., Bieler, J.G., al-Ramadi, B.K., Utz, U., Bothwell, A.L., Schneck, J.P., 1997. Analysis of the expression of peptide–major histocompatibility complexes using high affinity soluble divalent T cell receptors. J. Exp. Med. 186, 1333.

Porgador, A., Yewdell, J.W., Deng, Y., Bennink, J.R., Germain, R.N., 1997. Localization, quantitation, and in situ detection of specific peptide–MHC class I complexes using a monoclonal antibody. Immunity 6, 715.

Sanderson, S., Shastri, N., 1994. LacZ inducible, antigen/MHCspecific T cell hybrids. Int. Immunol. 6, 369.

Usherwood, E.J., Hogg, T.L., Woodland, D.L., 1999. Enumeration of antigen-presenting cells in mice infected with Sendai virus. J. Immunol. 162, 3350.

Canaday, D.H., Gehring, A., Leonard, E.G., Eilertson, B., Schreiber, J.R., Harding, C.V., Boom, W.H., 2003. T-cell hybridomas from HLA-transgenic mice as tools for analysis of human antigen processing. J. Immunol. Methods 281, 129.

Robson, N.C., Beacock-Sharp, H., Donachie, A.M., Mowat, A.M., 2003. Dendritic cell maturation enhances CD8+ T-cell responses to exogenous antigen via a proteasome-independent mechanism of major histocompatibility complex class I loading. Immunology 109, 374.

Chen,W., Anton, L.C., Bennink, J.R., Yewdell, J.W., 2000. Dissecting the multifactorial causes of immunodominance in class I-restricted T cell responses to viruses. Immunity 12, 83.

Schnurr, M., Chen, Q., Shin, A., Chen, W., Toy, T., Jenderek, C., Green, S., Miloradovic, L., Drane, D., Davis, I.D., Villadangos, J., Shortman, K., Maraskovsky, E., Cebon, J., 2005. Tumor antigen processing and presentation depends critically on dendritic cell type and the mode of antigen delivery. Blood 105, 2465.

Pang KC, Wei JQ, Chen W. Dynamic quantification of MHC class I-peptide presentation to CD8+ T cells via intracellular cytokine staining. J Immunol Methods. 2006 Apr 20;311(1-2):12-8. Epub 2006 Feb 20.

Example 3

Human Immunodeficiency Virus (HIV)

Introduction

Infection with HIV is one of the major global health problems with an estimated number of 33.2 million people living with HIV worldwide in the year 2007. In the same year 2.5 million people are estimated to have been newly infected with HIV and approximately 2.1 million people died from AIDS (1).

HIV is an enveloped retrovirus that infects cells of the immune system expressing the CD4 receptor and one of the coreceptors CCR5 / CXCR4. HIV is mainly transmitted sexually and the first cells to be infected are likely to be CD4+ T lymphocytes (CD4+ T cells) of the vaginal or gut mucosa. Monocytes, machrophages and dendritic cells are also susceptible to HIV (2,3). The virus enters the host cells by binding of the viral surface protein Env gp 120 subunit to the CD4 receptor and one of the coreceptors upon which the viral RNA genome is released into the cytoplasm. The RNA genome is translated into a double-stranded DNA copy which is transported into the cell nucleus and integrated into the host cell genome (4). From that point a permanent infection is established, and both cellular mechanisms and viral enzymes participate in the continuous production of new HIV particles that contribute to a gradual breakdown of the immune system.

Despite the promise of effective anti-HIV drugs, there is no medical cure for HIV, and the ultimate solution to the pandemic would be an effective preventive HIV vaccine. More than 20 years of intensive studies of HIV pathology has not resulted in a major scientific breakthrough in the vaccine area. What complicates matters the most is the genetics of HIV. HIV has a lot of different clades and subtypes and they all have different antigen composition. Furthermore, HIV is a moving target that readily mutates in the individual patient. Previous viral vaccines have all been against viruses that do not change over time. How to address the mutations and massive genetic variety of HIV in a vaccine remains a monumental scientific challenge.

<u>HIV Vaccine development</u>

Correlates of immunity against HIV?

Despite 20 years of intensive studies and search for an HIV vaccine, the correlates of protective immunity have yet to be defined in terms of an HIV vaccine. Yet, the

existence of people who have been exposed to HIV on numerous occasions and still remain uninfected indicates that protection against HIV is possible.

A recent prospective study among of HIV-exposed seronegative individuals (HEPS) - Kenyan female sex workers - shows that the presence of genital HIV-specific neutralizing IgA and systemic proliferative responses in circulating peripheral blood mononuclear cells (PBMC) at enrolment were markers that correlated well with the absence of HIV-infection (5). The study showed no relation between the systemic CTL-response as measured by IFN-γ ELISPOT and protection from HIV-infection. The study is unique with regards to the prospective, blinded design which adds to the value of the data presented. A previous study among female sex workers in Kenya equally demonstrated the frequent presence of genital anti-HIV IgA capable of neutralizing a primary HIV isolate (6). A study from 2000 among 26 Gambian HEPS fail to show any vaginal anti-HIV antibodies and the authors thus conclude that resistance against HIV infection can occur in the absence of specific antibodies against HIV at the genital mucosa (7).

Another HEPS-study compares the HIV-specific cellular immune response between HIV-infected and uninfected female sex workers in Kenya (8). The study interestingly shows an HIV-specific CTL-response among infected as well as uninfected HEPS, suggesting that in the HEPS population, a small population of cells is likely to have been HIV-infected at some point. The main finding of the study is that the CTL-response among HEPS was predominantly mucosal whereas it was mainly systemic among the HIV-infected women.

Though unable to conclusively demonstrate the nature of protective immunity against HIV, the different findings among HEPS add to the idea that a future HIV vaccine will have to elicit a mucosal immune response and that both antibodies and CTL-responses are likely to contribute to the protection from HIV-infection.

Animal Studies

A lot of the current knowledge in the HIV vaccine field derives from studies in different animal models. In rhesus macaques it has been shown that intravenous (IV) administration of monoclonal neutralizing anti-HIV IgG antibodies prior to vaginal challenge with SHIV were sufficient to protect the animals from infection or pathogenic manifestations of SHIV-infection (9,10). Though administered IV, the antibodies could be identified in the vaginal mucosa of the macaques. The above mentioned studies both support the important role of mucosal anti-HIV antibodies in a future vaccine.

Superior vaccine protection in macaques is primarily observed from strategies based on a live□attenuated vaccine strain. A recent review (11) focused on this issue from the Indian macaque model and the findings have been compiled in figure 3.

Almost all of the macaques immunized with live attenuated virus were protected when they were challenged with SIV, whereas few macaques immunized by alternative approaches were protected. However the macaque studies revealed that with time, attenuated SIV regains its virulence and a significant number of macaques immunized with live attenuated SIV developed AIDS from the vaccine. Thus the use of live attenuated HIV for vaccine in humans is not an option and an obvious strategy would be to pursue the development of a vaccine modality that imitates live attenuated HIV the most. So far, though, there seems to have been a general reluctance using retroviral vectors in HIV vaccine development because of fear of insertional mutagenesis.

Clinical Trials in Humans

Some HIV-vaccine approaches have been tested in humans as proof-of-concept, but only a few have reached phase IIb/III – and the ones tested so far have all failed. In 2003 a vaccine candidate using recombinant gp 120 from HIV envelope as vaccine-antigen to elicit neutralizing antibodies failed in a phase III trial. More than 5.000 volunteers – primarily men who have sex with men - enrolled in the clinical trial were randomized to receive 7 intramuscular (IM) injections of either recombinant gp 120 or placebo. There was no overall protection of the vaccine candidate. Another approach aiming at eliciting CTL responses (ALVAC-HIV) was tested in phase I and II trials but it never reached phase III because the vector used for antigen delivery lacked significant immogenicity (12). In late 2003, a 16.000-person phase III trial was initiated combining AIDSVax with ALVAC-HIV, two vaccine candidates that had already failed individually as described above. The AIDSVax- ALVAC-HIV study is to be completed in 2009. A recent phase IIb HIV-vaccine trial conducted by Merck used an adenovirus vector, Ad5, for delivery of three HIV genes. The study was designed to elicit a cell mediated immune response, and the purpose was rather to reduce viral load in those infected by HIV than to prevent infection. The trial was stopped prematurely when preliminary results indicated that the vaccine might increase the susceptibility to HIV among persons with preexisting antibodies to the Ad5-vector (13). Furthermore, the results showed no difference in viral load between those infected by HIV in the vaccine arm

and those infected by HIV in the placebo arm (14). The disappointing results from clinical trials in humans urge the search for novel HIV□1 vaccine strategies, and the above mentioned results from immunization studies in rhesus macaques suggest that imitating the use of live attenuated HIV might be a reasonable way to pursue.

γ-retroviral VaccineTechnology

Different viral vectors have been tested for efficient delivery of antigens in the ongoing search for an HIV-vaccine, eg adenoviral vectors, lentiviral vectors and γ-retroviral vectors. γ-retroviral vectors derived from murine leukemia virus (MLV) can be pseudotyped with HIV envelope protein, (see figure 4).

HIV env-pseudotyped γ-retroviral particles can to a large extent imitate a real HIV infection, their route of infection being the same. The use of MLV-vectors expressing HIV-envelope on the surface has previously been tested for vaccine purposes in rhesus macaques (15). CD4+ cells were harvested from a rhesus macaque, the cells were ex vivo transduced with HIV-Env expressing MLV vectors and subsequently re-inoculated into the macaque. The MLV vectors induced both a potent systemic cell mediated and humoral immune response. No viral challenge was performed and hence the efficiency of the measured immune response cannot be judged.

CMVbipep is a bicistronic γ-retroviral vector derived from MLV. The bicistronic design enables the placement of two gene fragments of choise in the vector in two cloning positions of the vector (see figure 5). The first cloning position is designed for small fragments, eg 8-10mer peptides, that will be processed and presented in MHC I complexes by the cells infected by the virus (16). On the second cloning position, another gene of choice can be placed – eg the gene of fluorescent egfp-marker that enables ex vivo monitoring of cells transduced with the virus. Another obvious strategy would be to place the gene of an adjuvant of choice which would be delivered specifically to the exact cells that present the immunogenic epitope in MHC I complex.

Aim of study

The aim of the study of this example was to evaluate the possibility of inducing an antigen-specific, cell mediated immune response in Balb/c mice by in vivo injection of CMVbipep-retroviruses encoding an immunogenic HIV CTL epitope from the HIV envelope gene. The following routes of administration of virus were tested:

intramuscular (IM), subcutaneous (SC), intraperatoneal (IP) and rectal. γ-retroviruses only infect dividing cells and we also wanted to test if we could enhance any given cell mediated immune response by co-administration of CpG, a stimulatory TLR-9 agonist known to induce cell proliferation and enhance immune responses to various vaccines (17).

Materiale and methods

Synthesis and cloning of antigen

RGPGRAFVT, an immunodominant CTL epitope from the HIV env V3 loop known to induce a cell mediated immune response in Balb/c mice was synthesized using PCR amplification. Primers used for synthesis of antigens and DNA sequence analysis were purchased from DNA Technology A/S. The primers were designed with BamHI and XbaI restrictionssites matching those of CMVBipep, as described in (16).The PCR product was visualized using an agarose gel band and purified using illistra GFX PCR DNA and Gel Band Purification Kit from GE Healthcare. PCR product and CMVBipep were digested with restriction endonucleases BamH I and XbaI, purchased from New England Biolabs. Both PCR product and CMVBipep were subsequently ran on an agarose gel band and purified with the GFX Kit. Ligation of RGPGRAFVT into CMVBipep was performed in 20 µL reactions at 16oC for one hour, using T4 DNA Ligase from Invitrogen.

Cloning of adjuvants

Immunization studies in Balb/c mice have demonstrated that the cytokines GM-csf and IL-2 can enhance the cell mediated immune response to vaccine antigens. Genes encoding murine GM-csf and IL-2 were purchased from Eurofins MWG GmbH, Germany. The genes had MluI and AFIII restrictionssites matching the second cloning position of CMVbipep. The genes and CMVbipep were digested with restriction endonucleases MluI and AFIII, purchased from New England Biolabs. Purifucation and cloning were performed as described above.

Verification of cloning

Chemically competent DHα5 cells were purchased from Invitrogen. DHα5 cells were transformed with CMVBipep encoding RGPGRAFVT and cytokines IL-2/GM-csf by addition of 2 µL of the ligation mix to the cells. These were heat-shocked for 45 seconds at 42oC. 250 µL of S.O.C. medium from invitrogen was added and the tube

was shaken horizontally (225 rpm) at 37oC for one hour. 10 µL was spread on agar plates with 100 µg/ml ampicillin. Only DHα5 cells containing a circular plasmid can grow on ampicillin agar plates. Plates were incubated over night at 37 oC. Single bacterial colonies were picked from the agarplate and subsequently grown in ampicillin-containing LB-medium overnight. Plasmid DNA from transduced cells was purified using NucleoBond Xtra Midi from Macherey-Nagel as described by the manufacturer. The successful cloning was verified by performing DNA sequence analysis of the DNA purified from single bacterial colonies. Sequence primers were purchased from DNA Technology A/S and DNA sequence analysis was performed using a Hitachi 3130 Genetic Analyser from Applied Biosystems.

Production of γ - retroviral particles

Plat-e cells were maintained in DMEM with 10 % FCS supplemented with 1 % PenStrep. DMEM and FCS were purchased from Invitrogen A/S. The cells were kept at 37 degrees C in a 5 % CO^2, 95 % air humidified incubator. Plat-e cells are modified human 293 cells that continuously produce γ-retroviral particles pseudotyped with an ecotrophic envelope. γ-retroviral particles pseudotyped with an ecotrop envelope were produced by transfecting Plat-e cells with CmvBipep-RGPGRAFVT using Lipofectamine 2000 Transfection Reagent as described by the manufacturer, Invitrogen A/S. On day 2 and 3 after the transfection, cell supernatant was collected, filtered through a 0,45 µm filter and centrifuged 90 minutes at 27.000 RPM in a Beckman L80 ultracentrifuge. Supernatant was discharged, and a small visible viruspellet was dissolved in sterile PBS containing 10 % DMSO. The virus was frozen at – 80 degrees C.

In Vitro testing of γ - retroviral particles

A Balb/c fibroblast cell line, generously donated by Mogens Duch, Institute of Molecular Biology, University of Aarhus, was used for in vitro analysis of the γ - retroviral particles. Cells were maintained in DMEM with 10 % Newborn Calf Serum supplemented with 1 % PenStrep and kept at 37 degrees C in a 5 % CO2, 95 % air humidified incubator. 10^5 cells were seeded in each well in a 6 well dish. CMVbipep-RGPGRAFVT-Neo was thawed and the Balb/c cells were transduced with a dilution series of the virus. Cells infected by virus were positively selected by adding G418 to the cell culture media. Cell colonies were counted after two weeks, and virus titer was approximately 2 * 10^7/mL.

Previous studies have shown that cells transduced by CMVbipep-virus precents the viral encoded peptide bound in MHC I molecules on the cell surface (16). DNA from transduced cells was purified and analyzed as described above. DNA-sequence and encoded peptide are shown in figure 6.

Cells were subsequently transduced with CMVbipep-virus encoding RGPGRAFVT and GM-csf or IL-2. Cell supernatant collected on day 2 after transfection was used for performed Elisa measurement of GM-csf and IL-2, repectively. GM-csf Elisa kit was purchased from R&D Systems, IL-2 Elisa kit from BIOSOURCE was purchased from Invitrogen. Elisa results are shown in figure 7 and 8:

Immunization of mice

Female Balb/c mice 6-8 weeks old were supplied by Taconic Europe and housed in the animal facility of Pipeline Biotech A/S. Groups of 3 mice were primed on day 0 with 10^6 mixed particles (8 x 105 CMVBipep-RGPGRAFVT, 105 CMVBipep-RGPGRAFVT+IL-2 and 10^5 CMVBipep-RGPGRAFVT+GM-csf) and boosted on day 7 with 4 x 10^5 CMVBipep-RGPGRAFVT+GM-csf. The immunization schedule is shown in figure 9.

The TLR-9 agonist, CpG, (ODN1826) was purchased from Invivogen. In mice receiving CpG, 10 µg of CpG dissolved in sterile PBS was co-administered with particles.
The mice were terminated 14 days after the first dosing and spleens were harvested from all mice. Spleens were dissected out and placed in a RPMI solution in a 10 ml test tube.

IFN-γ and IL-2 Elispot Assays

IFN-γ and IL-2 Elispot assays were purchased from Mabtech. Microplates precoated with capture antibody were blocked with RPMI containing 10 % fetal calf serum. Spleens were mashed in a homogenisator and splenocytes were isolated by centrifugation 15 min at 2000 RPM (4 oC) in a Hereus Christ Cryofuge 8000 over a Ficoll-Paque PLUS gradient. Splenocytes were counted and IFN-γ and IL-2 Elispot Assays were performed as follows. Splenocytes from immunized mice were added to the plates in triplicates at 2,5 x 10^5 cells/well and stimulated with 10 µg/ml of a stimulatory peptide, INCTRPNNNTRKRIRIQRGPGRAFVTIGKIGN, purchased from CASLO Laboratory. Plates were put in a 370C humidified incubator with 5% CO2. After

36 hours, spots were revealed with the biotin-conjugated antibody, followed by streptavidin ALP and BCIP/NBT substrate solution. Spots were counted using an ImmunoScan reader from Cellular Technology LTD, and results were expressed as spot-forming cells per million splenocytes.

Results

CD8+ cells that are activated by recognition of the specific antigens they are primed to recognize secrete IFN-γ among other molecules and ELISPOT assays are a widely used method for the detection of IFN-γ secreting cells at single cell level. Results from IFN-γ Elispot assay are summarized in figure 10.

As figure 10 illustrates, the injection of CMVBipep-RGPGRAFVT particles by different routes in Balb/c mice elicited an antigen-specific CTL-immune response. IP-, SC- and IM-immunization gave quite strong immune reactions in the mice whereas the rectal administration of particles gave a minor yet significant positive result. Our data furthermore show that the co-administration of CpG resulted in an enhanced immune response when mice were immunized IP and SC. CpG seemingly diminished the immune response in mice that received intrarectal and IM-immunizations which we did not expect. In various studies it has been shown that CpG increases the immune response when used as an adjuvant in IM-immunizations. Bearing that in mind and watching the results in the SC- and IP-immunizations, we speculate that the particles with and without CpG used for the IM- and intrarectal immunizations might have been exchanged by mistake.

Activated lymphocytes proliferate in response to autocrine IL-2-secretion. We performed IL-2 Elispot assay to get a rough idea, whether splenocytes will be able to proliferate in the presence of antigen. Results from IL-2 Elispot assay are summarized in figure 11.

Figure 11 shows that splenocytes from mice immunized IP, SC, IM and intrarectally all secreted IL-2 in the presence of specific antigen. As for the role of CpG, the picture from the IFN-γ Elispot repeats itself: In the IP and SC immunization, CpG increased the immune response as measured by IL-2 secretion quite strongly, whereas CpG diminished the immune response in mice given an IM-injection. Once again, we believe that the particles with and without CpG used for the IM- and intrarectal immunizations have been exchanged by mistake.

Discussion

In this study we demonstrated that in vivo injection of γ-retroviral particles in Balb/c mice elicited an antigen specific cell mediated immune response. 4 different routes of administration were tested and all 4 routes resulted in a systemic immune response as measured by IFN-γ and IL-s ELISPOT. These results are very encouraging and they call for further studies. As regards the role of co-administration of the TLR-9 agonist CpG with the particles, the results are somehow inconclusive. CpG seemingly increases the immune response when administered IP and SC whereas it seems to diminish the immune response when administered IM and intrarectally. We would have expected CpG to have the same - positive, if any - effect by all the routes and we speculate that the particles with and without CpG for IM- and rectal administration might have been mixed up. To clarify this issue, a repetition of the study has been planned. The mice used for negative control in this study were injected with PBS. A different negative control will be used in the upcoming studies as we shall inject CpG and integration-defective CMV-Bipep particles encoding the same epitope. Injection this formulation will clearly demonstrate whether the immune response adheres from integration of the viral RNA into the genome of the host cells. At this point we cannot know with certainty if the packaging cells used for production of particles make a reverse transcription of the RNA of the vector, process the DNA and secrete the epitope, although it does not seem likely. If that should be the case, the injection of the epitope secreted from the packaging cells could cause the immune reaction in the mice. A lot of questions are still to be answered and we envisage clarifying the following matters in upcoming studies: Can we induce a mucosal immune response by injection of our particles? Can we influence the immune response by injecting particles that encode for the epitope and specific cytokines known to function as adjuvants? Longitudinal studies will demonstrate if a pool of memory cells are created by the immunizations and we intend to include a Granzyme B ELISPOT assay to asses if the CD8+ cells are capable of mediating lysis when they are stimulated with the epitope. Finally we shall also establish a flow based cell proliferation assay to analyze how many, and which subpopulations of cells that divide when stimulated with a peptide covering the epitope they are encoded to recognize.

References

(1) UNAIDS. 2008 Report on the global AIDS epidemic.

(2) Picker LJ. Immunopathogenesis of acute AIDS virus infection. Curr.Opin.Immunol. 2006 Aug;18(4):399-405.

(3) Levy JA. HIV pathogenesis: knowledge gained after two decades of research. Adv.Dent.Res. 2006 Apr 1;19(1):10-16.

(4) Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. Nat.Rev.Immunol. 2008 Jun;8(6):447-457.

(5) Hirbod T, Kaul R, Reichard C, Kimani J, Ngugi E, Bwayo JJ, et al. HIV-neutralizing immunoglobulin A and HIV-specific proliferation are independently associated with reduced HIV acquisition in Kenyan sex workers. AIDS 2008 Mar 30;22(6):727-735.

(6) Devito C, Hinkula J, Kaul R, Lopalco L, Bwayo JJ, Plummer F, et al. Mucosal and plasma IgA from HIV-exposed seronegative individuals neutralize a primary HIV-1 isolate. AIDS 2000 Sep 8;14(13):1917-1920.

(7) Dorrell L, Hessell AJ, Wang M, Whittle H, Sabally S, Rowland-Jones S, et al. Absence of specific mucosal antibody responses in HIV-exposed uninfected sex workers from the Gambia. AIDS 2000 Jun 16;14(9):1117-1122.

(8) Kaul R, Plummer FA, Kimani J, Dong T, Kiama P, Rostron T, et al. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J.Immunol. 2000 Feb 1;164(3):1602-1611.

(9) Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson CE, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat.Med. 2000;6(2):207-210.

(10) Baba TW, Liska V, Hofmann-Lehmann R, Vlasak J, Xu W, Ayehunie S, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian−human immunodeficiency virus infection. Nat.Med. 2000;6(2):200-205.

(11) Koff WC, Johnson PR, Watkins DI, Burton DR, Lifson JD, Hasenkrug KJ, et al. HIV vaccine design: insights from live attenuated SIV vaccines. Nat.Immunol. 2006 Jan;7(1):19-23.

(12) Editorial. Cold shower for AIDS vaccines. Nat.Med. 2007;13(12):1389-1390.

(13) Sekaly RP. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? J.Exp.Med. 2008 Jan 21;205(1):7-12.

(14) Cohen J. AIDS research. Promising AIDS vaccine's failure leaves field reeling. Science 2007 Oct 5;318(5847):28-29.

(15) Neumann J, Stitz J, Konig R, Seibold E, Norley S, Flory E, et al. Retroviral vectors for vaccine development: induction of HIV-1-specific humoral and cellular immune

responses in rhesus macaques using a novel MLV(HIV-1) pseudotype vector. J.Biotechnol. 2006 Jul 25;124(3):615-625.

(16) Tolstrup AB, Duch M, Dalum I, Pedersen FS, Mouritsen S. Functional screening of a retroviral peptide library for MHC class I presentation. Gene 2001 Jan 24;263(1-2):77-84.

(17) Klinman DM. Immunotherapeutic uses of CpG oligodeoxynucleotides. Nat.Rev.Immunol. 2004;4(4):249-258.

Example 4

Treatment of cancer by the methods, compositions, kits-of-parts or uses of the present invention.

Many types of cancers can be treated by the methods, compositions, kits-of-parts and/or uses of the present invention. Specifically, malignant melanomas can be treated. This example displays two routes for the treatment of malignant melanoma according to the present invention.

Route 1: Direct transduction/transfer of cytokines directly into melanoma cells by either retroviral vectors or gene gun. Multiple batches of retroviral vector particles or gold particles will be produced where each batch will transfer one expression vector expressing one of the cytokines as defined in the present invention, for example any of SEQ ID NO: 47-86. The whole population of batches will contain vectors expressing at least five of said cytokines. After production of the individual batches of expression vectors these will be mixed to constitute a mixed population of particles each expressing only a single (or a few) nuceic acid species encoding a distinct cytokine. This mixed batch will be used to transfer the expression vectors directly into the tumor cells using settings allowing for on average one particle per cell. After transfer the tumor cells will constitute a heterogenous population of cells where the targeted population of tumor cells will express all of the cytokine species provided to the population in total but where the individual targeted tumor cells on the average only will express a single cytokine.

Route 2 Direct transduction/transfer of vectors expressing a cytokine and an additional polypeptide for immune recognition by peptides presented on MHC molecules. A population of different batches of vectors or gene gun particles will be produced,

wherein each batch contains a vector expressing one immunogenic polypeptide of the present invention, for example any peptide selected from the group consisting of SEQ ID NO: 95-1159 and one cytokine as defined in the present invention, for example any of SEQ ID NO: 47-86. The population of batches will contain at least five distinct combinations of particles expressing an immunogenic polypeptide of the present invention, for example any peptide selected from the group consisting of SEQ ID NO: 95-1159 and a cytokine of the present invention. In the extreme case batches of particles will be produced, which comprises expression vectors for all combinations of immunogenic polypeptides as listed herein and cytokines as listed herein. After production of the individual batches of expression vectors these will be mixed to form a mixed population of particles each expressing only a single cytokine and a single immunogenic peptide, i.e. I distinct combination of immunogenic polypeptide and cytokine or hormone. This mixed batch will be used to transfer the expression vectors directly into the patient as described elsewhere (IV, IM, SC etc) using settings allowing for on average one particle per transfected cell of the targeted population of cells. After transfer the target cells will constitute a heterogeneous population of cells which will express a plurality of distinct combinations of immunogenic peptides and cytokines transferred but where the individual target cell on the average only will express a single cytokine and a single immunogenic polypeptide combination.

The list of peptides and cytokines of the present invention are only to be considered suggestive as additional immunogenic peptides and cytokines may be used according to the present invention if such cytokines or immunogenic peptides are shown to have any impact on the desired immune response

Example 5

Test of HIV immunogenic polypeptide

The HIV envelope (RGPGRAFVT) and gag (AMQMLKETI) epitopes were cloned. These epitopes have been chosen for mouse studies because studies have shown them to be immunogenic in mice. The gag epitope AMQMLKETI is recognized by both mouse and human MHC genotypes (A2, H-2^{d}, H-2^{kd}, H-$2D^{d}$, H-$2K^{d}$, H-2d).

Example 6

Hepatitis C Virus - vaccine development

Hepatitis C (HCV) is a single stranded RNA virus belonging to the flavivirus family. Six genotypes and a number of subtypes are identified. The virus poses a global problem with more than 170 million people infected worldwide (2). The infection is characterized by a huge productivity – 1012 viral particles being produced daily and an error prone polymerase causing the virus to exist as quasispecies with a high variability (3). These characteristics are probably the underlying reason why the infection establishes itself as a chronic disease, which is often the case, since on the average 75% of all persons exposed to HCV develop chronic disease (4). The infection causes a serious health problem since a significant proportion of patients develop cirrhosis and is at risk to develop incompensated liver disease and hepatocellular carcinoma within 15-20 years. Both are conditions with a very poor prognosis. Treatment with interferon and ribavirin though improved over the years is still succesfull in only about 50% of those infected with genotype 1, the most prevalent genotype in Europe and the U.S.A. The treatment is associated with severe side-effects and is given for 6-12 months. A lot of contraindications exist and only a minority of patients are offered treatment. A significant number of infected patients have a history of or present I.V. abuse.

Immunity

The correlates of limited disease without progression to chronicity have been studied on a number of occasions. The T-cell response seems to be of greatest importance since a strong and multispecific and sustained CD4 and CD8 response is seen in patients who recovers after the acute stage (5,6), whereas those progressing to chronicity have a weak, monospecific or even absent CD4 response (7). As for the CD8 response the picture is less clear, both weak and narrowly focused (8), and lack of correlation between the CD8 response in self-limited and chronic disease have been reported (4). In the latter study T-cell dysfunction and maturation defects were described, however. In general the CD8 response is weak in patients with chronic HCV and seems to target only a few epitopes (9), exhibit defects such as reduced secretion of IFN-gamma and reduced proliferative capacity (10). The reason for these defects is probably multifactor and several causes have been suggested: Impairment of antigen presenting cells (11), impairment of CD8 activation by HCV core protein (12), lack of CD4 assistance (13), or suppression by regulatory T-cells (14).

Viral escape mutants are frequently the result of the T cell defects mentioned combined with the high viral productivity and variability. This phenomenon is described in the chimpanzee model (15) as well as in human (16). The HLA background is probably

important for this phenomenon (17). The importance of the humeral response is less clear. Sterilizing immunity intuitively would depend on neutralizing immunity; however reinfection is seen with the same genotype, both in man and chimpanzees. Neutralizing antibodies are often not seen in patients recovering from acute hepatitis and also such antibodies can be found in patients with chronic hepatitis (18). In one study, where the primary viral isolate was available neutralizing antibodies were associated with HCV clearance. Interesting epitopes for neutralizing antibodies have been described on the E1 E2 glucoproteins with a “hot spot” adjacent to the hypervaraible region (HVR1) (19).

Viral regions of interest in vaccine development T-cell response.

An immundominant region in E1 (aa 121-135) was used in a polypeptide vaccine and stimulated a potent CD4 response in a mouse model when used with a TLR-3 agonist (Poly i:C) and anti CD 40 antibody. No CD8 response was seen (20). In the same study NS3 peptides (aa 1073-1081 and aa 1038-1047) elicited a strong CD8 and CD4 response when used together with a full length rNS3 peptide. A consensus sequence was constructed from 15 genotype 1a NS3 isolates, and from 19 1b NS3 isolates. The peptides from these constructs elicited a cellular immunresponse in mice targeting an immunogenic epitope near the c terminal part of NS3. In rhesus monkeys the response was directed against different parts of NS3 and NS4a (21). peptides

E1 from 20 patients (genotype 1-5) were cloned and used as a plasmid DNA vaccine in mice (IM injection x 4). A moderate and cross reactive T cell response and a low to moderate humoral response was seen (22)

A polypeptide constructed from
1) E1 and E2 envelope antigen (aa192-746) and
2) NS3, NS4 and NS5 (aa1027-3012)
The vaccine was used with a TLR agonist (CpG) and MF59 as an adjuvant and injected twice in mice and boosted with a defective alfa virus (Sindbis-VEE) expressing E1, E2 and NS3, 4 and 5. When using 1) a good CD8 reactivity against E2 epitope and a good CD4 reactivity against an E1 and less well against anE2 epitope. TLR agonist important for a Th1 response. With 2) a strong CD4 and CD8 response was seen. With regimen 1) CD81 blocking antibodies and cross neutralization against heterolog virus (same genotype?) were found (23). A vaccine using a defective adenovirus transfected

with a vector encoding the NS3-NS5b region from a 1b strain (BK-strain) was given week 0 and 4 and boosted at week 25 to 10 chimpanzees. Five microgram DNA plasmid was further injected weeks 35, 37 and 39 (+ electrical treatment)
All chimpanzees were then challenged with 100 cid of HCV strain H77 (genotype 1a).

Nine of 10 viruses had a short viraemic infection and only became chronic. Peripheral and intrahepatic HCV CD8 and CD4 lymphocytes reacting with vaccine and virus epitopes. The Chimpanzee developing a chronic infection had an immune escape variant (I308 epitope) (26).

Humoral response
Humanized SCID mice treated with reconvalescens serum and challenged. No correlation between protection and titer of neutralizing antibodies was seen (24).
Monoclonal antibodies from mice, chimpanzees and man treated for HCV with interferon-Alpha were made. 2 MoAbs could neutralize pseudotyped particles carrying 1a, 1b, 4a, 5 b and 6a but not 2a or 3a. Epitopes were mapped to E1 epitopes (aa313-327). E1 epitopes more conserved than E2 8 (25).

Pseudoparticles carrying envelope from 1a strain were used to detect neutralizing antibodies from 8 chimpanzees and 4 humans with resolving infection. Neutralizing antibodies were detected only in 1 of 4 chimps and no humans. In 1 chimpanzee and 2 humans with chronic infection neutralizing antibodies were detected. These antibodies could neutralize 4a and 6a strains. The hypervariable region was targeted. (27).

Conclusion
T cell immunresponse seems to be critical to avoid chronicity, but cannot confer sterilizing immunity. Since most acute cases are subclinical and with relatively few symptoms a T cell vaccine could be interesting. To be effective a broad CD4 and CD8 response most be obtained. The NS3 (+NS4 and 5 perhaps) seem to be good candidates and most involve Consenus Sequences? from all major genotypes and probably some of the subtypes. TLR agonists are valuable adjuvants.
Envelope (E1 and E2) can elicit some T-cell response but are of more value to stimulate antibody production. Protection is difficult since failure and chronicity can be seen even with repeated challenge with the same virus (28).

References

1 Rice CM: In Fields BN, Knippe DM, Hawley PM editors Fields Virology. Lipnicot and Raven: Philadephia 1996: 931-60.
2 J Viral Hepat 1999;1:564-9
3 Semin Liver Dis 1995;15:41-63.
4 Hepatology 2002;36:s35-46.
5 J Clin Virol 2006:36: 24-31.
6 Hepatol 2005;42: 104-12
7 Antvir Res 2006;69:129-41
8 J Exp Med 2001;194:1395-1406.
9 Gastroenterol 2004;127:924-36
10 Hepatol 2005;42:828-37
11 Immunol 2007;121:281-292
12 J Clin Invest 2000;106:1239-49.
13 Journal of Infectious Diseases 2000;181:1528-36.
14 J Virol 2005;79:7860-67.
15 Immunity 2001;15:885-95.
16 J Exp Med 2005;201:1741-52.
17 Hepatol 2006;43:563-72.
18 Clinical Infect Dis 2005;41:667-75.
19 Biol Chem 2008;389:457-67 (Review)
20 Antivir Res 2007;74:25-35.
21 Vaccine 2008 in press
22 Eur J Clin Invest 2007;37:396-406.
23 J Virol 2008;82:7492-7503.
24 Hepatol 2008;47:1846-55.
25 J Virol 2008;82:966-73.
26 Nature Medicine 2006;12:190-7.
27 PNAS 2005;102:4560-65.
28 J Virol 82:8183-95.

Sequences

SEQ ID NOs: 1-46: HIV envelope polypeptide prototypes for different HIV-1 subtypes. SEQ ID NO: 47 – 86: Immunomodulating polypeptides, such as cytokines; SEQ ID NO: 47-48: IL-1β; SEQ ID NO: 49-50: IL-2; SEQ ID NO: 51-52: IL-2 receptor subunit alpha; SEQ ID NO: 53-54: IL-4; SEQ ID NO: 55-56: IL-5; SEQ ID NO: 57-58: IL-6; SEQ ID NO: 59-60: IL-7; SEQ ID NO: 1160 amino acid sequence: human il-8; SEQ ID NO: 61-62: IL-10; SEQ ID NO: 63-64: IL-12; SEQ ID NO: 65-66: IL-15; SEQ ID NO: 67-68: IL-18; SEQ ID NO: 69-70: IL-21; SEQ ID NO: 71-72: GM-csf; SEQ ID NO: 73-75: TNF-α; SEQ ID NO: 76-77: IFN-γ; SEQ ID NO: 78-79: CCL2; SEQ ID NO: 80-81: CCL3; SEQ ID NO: 82-83: CCL5; SEQ ID NO: 84: Cholera toxin; SEQ ID NO: 85: C3d; SEQ ID NO: 86: CpG 1826-ODN; SEQ ID NO: 87: CMVbipep vector sequence; SEQ ID NO: 88: CMVbipep vector sequence, CMVbipep with immunogenic RGPGRAFVT-epitop from HIV envelope (underscored); SEQ ID NO: 89: CMVbipep vector sequence; SEQ ID NO: 90: CMVbipep vector sequence, CMVbipep with immunogenic RGPGRAFVT-

epitop from HIV envelope (underscored) and mouse IL-2 (in bold); SEQ ID NO: 91: CMVbipep vector sequence, CMVbipep with immunogenic RGPGRAFVT-epitop from HIV envelope (underscored) and mouse GM-csf (in bold); SEQ ID NO: 92: CMVbipep vector sequence, CMVbipep with immunogenic AMQMLKETI-epitop from HIV gag (underlined); SEQ ID NO: 93: CMVbipep vector sequence, CMVbipep with immunogenic AMQMLKETI-epitop from HIV gag (underlined) and mouse IL-2 (in bold); SEQ ID NO: 94: CMVbipep vector sequence, CMVbipep with immunogenic AMQMLKETI-epitop from HIV gag (underlined) and mouse GM-csf (in bold); SEQ ID NO: 95-1159: Immunogenic polypeptides for HIV treatment, see table 1b.; SEQ ID NO: 1160: Human IL-8; SEQ ID NO: 1161: CpG 2006/7909-ODN

Claims

1. A method of treating, preventing or ameliorating a clinical condition, said method comprising

    a. providing a population of cells,

    b. bringing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof,

    c. obtaining an immunogenic response of said population of cells,

    wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species.

2. The method according to claim 2, wherein said plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone or a functional homologue or part thereof further comprises a plurality of additional non-identical nucleic acid species, wherein each said additional non-identical nucleic acid species encodes a distinct species of immunogenic polypeptide comprising at least 3, such as at least 7 consecutive amino acids, wherein each individual cell of said population of cells takes up at the most 5 of said additional non-identical nucleic acid species.

3. The method according to any of the preceding claims, wherein said distinct species of cytokine or hormone, or a functional homologue or part thereof and/or said distinct immunogenic polypeptide is expressed within each said individual cell.

4. The method according to any of the preceding claims, wherein

    i) each individual cell of a first subpopulation of said population of cells takes up one and not more than one nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or one and not more than one additional nucleic acid species encoding said immunogenic polypeptide, and/or

ii) each individual cell of a second subpopulation of said population of cells takes up two and not more than two nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or two and not more than two additional nucleic acid species encoding said immunogenic polypeptide, and/or

iii) each individual cell of a third subpopulation of said population of cells takes up three and not more than three nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or three and not more than three additional nucleic acid species encoding said immunogenic polypeptide, and/or

iv) each individual cell of a fourth subpopulation of said population of cells takes up four and not more than four nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or four and not more than four additional nucleic acid species encoding said immunogenic polypeptide, and/or

v) each individual cell of a fifth subpopulation of said population of cells takes up five and not more than five nucleic acid species encoding said cytokine or hormone or functional homologue or part thereof and/or five and not more than five additional nucleic acid species encoding said immunogenic polypeptide.

5. The method according to claim 4, wherein the cells of said first, second, third and fourth subpopulations together comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

6. The method according to claim 4, wherein the cells of said first, second, and third subpopulations together comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

7. The method according to claim 4, wherein the cells of said first and second subpopulations together comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

8. The method according to any of the preceding claims, wherein said first subpopulation comprise at least 1 percent, such as at least 5 percent, for example at least 10 percent, such as at least 15 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as 100 percent of said population of cells.

9. The method according to any of the preceding claims, wherein said second subpopulation comprise at least 10 percent, such as at least 20 percent, such as at least 30, for example at least 40, such as at least 50, for example at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 95, such as at least 98 percent of said population of cells.

10. The method according to any of the preceding claims, wherein said clinical condition is an infectious disorder, an autoimmune disorder, and/or a cancer form.

11. The method according to claim 10, wherein said infectious disorder is selected from the group consisting of AIDS, Anal Warts, Anthrax, Bronchitis, Bug-borne Diseases, Campylobacter, Cellulitis, Chickenpox, Chlamydia Infections, Common Cold, Crab Lice, Cryptosporidiosis, Cytomegalovirus Infections, Dengue, Diphtheria, E. Coli Infections, Ehrlichiosis, Fever, Flea Bites, Foodborne Diseases, Fungal Infections, Gonorrhea, Hepatitis, Hepatitis C, Herpes Simplex, Herpes Zoster, HPV, Impetigo, Infectious Diseases (General), Influenza, Legionnaire's Disease, Leprosy, Lyme Disease, Malaria, Measles, Meningitis, MRSA, Mumps, Norwalk Virus Infections, Parasitic Diseases, Pelvic Inflammatory Disease, Pfiesteria piscicida, PID, Pneumonia, Polio and Post-Polio Syndrome, Rubella, Salmonella Infections, Scabies, Severe Acute

Respiratory Syndrome, Sexually Transmitted Diseases, Shigella Infections, Shingles (Herpes Zoster), Sinusitis, Smallpox, Staphylococcal Infections, STD, Streptococcal Infections, Syphilis, Tetanus, Tick Bites, Trichomoniasis, Tuberculosis, Viral Infections, and/or West Nile Virus.

12. The method according to claim 10, wherein said infectious disorder is selected from the group consisting of AIDS, Anthrax, Campylobacter, Chlamydia Infections, Common Cold, Cytomegalovirus Infections, Dengue, Diphtheria, E. Coli Infections, Gonorrhea, Hepatitis C, Herpes Simplex, Herpes Zoster, HPV, Influenza, Legionnaire's Disease, Leprosy, Lyme Disease, Malaria, Measles, Norwalk Virus Infections, Salmonella Infections, Scabies, Shigella Infections, Syphilis, Tuberculosis, and/or West Nile Virus.

13. The method according to claim 10, wherein said infectious disorder is selected from the group consisting of HIV, such as HIV-1 or HIV-2, AIDS and/or AIDS related complex (ARC).

14. The method according to claim 10, wherein said infectious disorder is hepatitis C.

15. The method according to claim 10, wherein said clinical condition is an autoimmune disorder selected from the group consisting of Rheumatoid Arthritis (RA), Type 1 diabetes, Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE), Inflammatory Bowel Disease (IBD), and/or Autoimmune Thyroid Disease (ATD).

16. The method according to claim 10, wherein said autoimmune disorder is a food allergy selected from the group consisting of Egg Allergy, Milk Allergy, Peanut Allergy, and/or Soy Allergy.

17. The method according to claim 10, wherein said autoimmune disorder is an airborne allergy selected from the group consisting of Pollen Allergy, Mold Allergy, Dust Mite Allergy, and/or Animal Allergy.

18. The method according to claim 10, wherein said clinical condition is cancer.

19. The method according to claim 18, wherein said cancer is selected from the group consisting of Acute Lymphoblastic Leukemia - Adult, Acute

Lymphoblastic Leukemia - Childhood, Acute Myeloid Leukemia - Adult, Acute Myeloid Leukemia - Childhood, Adrenocortical Carcinoma, Adrenocortical Carcinoma - Childhood, AIDS-Related Cancers, AIDS-Related Lymphoma, Anal Cancer, Appendix Cancer, Astrocytoma - Childhood Cerebellar, Astrocytoma - Childhood Cerebral, Basal Cell Carcinoma - Skin Cancer (Nonmelanoma), Bile Duct Cancer - Extrahepatic, Bladder Cancer, Bladder Cancer - Childhood, Bone Cancer - Osteosarcoma and Malignant Fibrous Histiocytoma, Brain Stem Glioma - Childhood, Brain Tumor - Adult, Brain Tumor - Brain Stem Glioma - Childhood, Brain Tumor - Central Nervous System Embryonal Tumors - Childhood, Brain Tumor - Cerebellar Astrocytoma - Childhood, Brain Tumor - Cerebral Astrocytoma/Malignant Glioma - Childhood, Brain Tumor - Ependymoblastoma - Childhood, Brain Tumor - Ependymoma - Childhood, Brain Tumor - Medulloblastoma - Childhood, Brain Tumor - Medulloepithelioma - Childhood, Brain Tumor - Pineal Parenchymal Tumors of Intermediate Differentiation - Childhood , Brain Tumor - Supratentorial Primitive Neuroectodermal Tumors and Pineoblastoma - Childhood, Brain Tumor - Visual Pathway and Hypothalamic Glioma - Childhood, Brain and Spinal Cord Tumors - Childhood (Other), Breast Cancer, Breast Cancer and Pregnancy, Breast Cancer - Childhood, Breast Cancer - Male, Bronchial Tumors - Childhood, Burkitt Lymphoma, Carcinoid Tumor - Childhood, Carcinoid Tumor,Gastrointestinal, Carcinoma of Unknown Primary, Central Nervous System Embryonal Tumors - Childhood , Central Nervous System Lymphoma - Primary, Cerebellar Astrocytoma - Childhood, Cerebral Astrocytoma/Malignant Glioma - Childhood, Cervical Cancer, Cervical Cancer - Childhood, Childhood Cancers, Chordoma - Childhood, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer - Childhood, Cutaneous T-Cell Lymphoma - see Mycosis Fungoides and Sézary Syndrome, Embryonal Tumors - Central Nervous System - Childhood , Endometrial Cancer, Ependymoblastoma - Childhood , Ependymoma - Childhood, Esophageal Cancer, Esophageal Cancer - Childhood, Ewing Family of Tumors, Extracranial Germ Cell Tumor - Childhood, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer - Intraocular Melanoma, Eye Cancer - Retinoblastoma, Gallbladder Cancer, Gastric (Stomach) Cancer, Gastric (Stomach) Cancer - Childhood, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST),

Gastrointestinal Stromal Cell Tumor - Childhood, Germ Cell Tumor - Extracranial - Childhood, Germ Cell Tumor - Extragonadal, Germ Cell Tumor - Ovarian, Gestational Trophoblastic Tumor, Glioma - Adult, Glioma - Childhood Brain Stem, Glioma - Childhood Cerebral Astrocytoma, Glioma - Childhood Visual Pathway and Hypothalamic, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular (Liver) Cancer - Adult (Primary), Hepatocellular (Liver) Cancer - Childhood (Primary), Hodgkin Lymphoma - Adult, Hodgkin Lymphoma - Childhood, Hypopharyngeal Cancer, Hypothalamic and Visual Pathway Glioma - Childhood, Intraocular Melanoma, Islet Cell Tumors (Endocrine Pancreas), Kaposi Sarcoma, Kidney (Renal Cell) Cancer, Kidney Cancer - Childhood, Laryngeal Cancer, Laryngeal Cancer - Childhood, Leukemia - Acute Lymphoblastic - Adult, Leukemia - Acute Lymphoblastic - Childhood, Leukemia - Acute Myeloid - Adult, Leukemia - Acute Myeloid - Childhood, Leukemia - Chronic Lymphocytic, Leukemia - Chronic Myelogenous, Leukemia - Hairy Cell, Lip and Oral Cavity Cancer, Liver Cancer - Adult (Primary), Liver Cancer - Childhood (Primary), Lung Cancer - Non-Small Cell, Lung Cancer - Small Cell, Lymphoma - AIDS-Related, Lymphoma - Burkitt, Lymphoma - Cutaneous T-Cell - see Mycosis Fungoides and Sézary Syndrome, Lymphoma - Hodgkin - Adult, Lymphoma - Hodgkin - Childhood, Lymphoma - Non-Hodgkin - Adult, Lymphoma - Non-Hodgkin - Childhood, Lymphoma - Primary Central Nervous System, Macroglobulinemia - Waldenström, Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Medulloblastoma - Childhood, Medulloepithelioma - Childhood , Melanoma, Melanoma - Intraocular (Eye), Merkel Cell Carcinoma, Mesothelioma - Adult Malignant, Mesothelioma - Childhood, Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Multiple Endocrine Neoplasia Syndrome - Childhood, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Diseases, Myelogenous Leukemia - Chronic, Myeloid Leukemia - Adult Acute, Myeloid Leukemia - Childhood Acute, Myeloma - Multiple, Myeloproliferative Disorders - Chronic, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Nasopharyngeal Cancer - Childhood, Neuroblastoma, Non-Hodgkin Lymphoma - Adult, Non-Hodgkin Lymphoma - Childhood, Non-Small Cell Lung Cancer, Oral Cancer - Childhood, Oral Cavity Cancer - Lip and, Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma of Bone, Ovarian Cancer - Childhood, Ovarian

Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Pancreatic Cancer - Childhood, Pancreatic Cancer - Islet Cell Tumors, Papillomatosis - Childhood, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pheochromocytoma, Pineal Parenchymal Tumors of Intermediate Differentiation - Childhood , Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors - Childhood, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Pleuropulmonary Blastoma, Pregnancy and Breast Cancer, Primary Central Nervous System Lymphoma, Prostate Cancer, Rectal Cancer, Renal Cell (Kidney) Cancer, Renal Cell (Kidney) Cancer - Childhood, Renal Pelvis and Ureter - Transitional Cell Cancer, Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Retinoblastoma, Rhabdomyosarcoma - Childhood, Salivary Gland Cancer, Salivary Gland Cancer - Childhood, Sarcoma - Ewing Family of Tumors, Sarcoma - Kaposi, Sarcoma - Soft Tissue - Adult, Sarcoma - Soft Tissue - Childhood, Sarcoma - Uterine, Sézary Syndrome, Skin Cancer (Nonmelanoma), Skin Cancer - Childhood, Skin Cancer (Melanoma), malignant melanoma, Skin Carcinoma - Merkel Cell, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma - Adult, Soft Tissue Sarcoma - Childhood, Squamous Cell Carcinoma - see Skin Cancer (Nonmelanoma), Squamous Neck Cancer with Occult Primary - Metastatic, Stomach (Gastric) Cancer, Stomach (Gastric) Cancer - Childhood, Supratentorial Primitive Neuroectodermal Tumors - Childhood, T-Cell Lymphoma - Cutaneous - see Mycosis Fungoides and Sézary Syndrome, Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thymoma and Thymic Carcinoma - Childhood, Thyroid Cancer, Thyroid Cancer - Childhood, Transitional Cell Cancer of the Renal Pelvis and Ureter, Trophoblastic Tumor - Gestational, Unknown Primary Site - Carcinoma of - Adult, Unknown Primary Site - Cancer of - Childhood, Unusual Cancers of Childhood, Ureter and Renal Pelvis - Transitional Cell Cancer, Urethral Cancer, Uterine Cancer - Endometrial, Uterine Sarcoma, Vaginal Cancer, Vaginal Cancer - Childhood, Visual Pathway and Hypothalamic Glioma - Childhood, Vulvar Cancer, Waldenström Macroglobulinemia, Wilms Tumor and/or Women's Cancers.

20. The method according to any of the preceding claims, wherein said plurality of non-identical nucleic acid species encoding distinct species of cytokine or

hormone or a functional homologue or part thereof comprises at least 2, such as at least 3, for example at least 4, such as at least 5 nucleic acid species, such as at least 10, such as at least 15 species, for example at least 20, such as at least 25 species, such as at least 30, such as at least 40 species, for example at least 50, such as at least 60 species such as at least 70, such as at least 80 species, for example at least 90, such as at least 100 species, for example at least 150 nucleic acid species.

21. The method according to claim 20, wherein said cytokine or hormone is selected from the group consisting of IL-1β, IL-2, IL-2 receptor subunit alpha, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, IL-21, GM-csf, TNF-α, IFN-α, IFN-γ, CCL2, CCL3, and/or CCL5 and/or fragments or functional homologes thereof.

22. The method according to claim 20, wherein said cytokine or hormone is selected from the group consisting of SEQ ID NO: 47-86, and/or functional homologs or parts thereof.

23. The method according to claim 20, wherein said cytokine or hormone are selected from the group consisting of IL-2, IL-4, IL-5, IL-7, IL-12, GM-csf, and/or TNF-α.

24. The method according to any of the preceding claims, wherein said plurality of non-identical nucleic acid species encoding a distinct species of immunogenic polypeptide comprises at least 2, such as at least 3, for example at least 4, such as at least 5 nucleic acid species, such as at least 10, such as at least 15 species, for example at least 20, such as at least 25 species, such as at least 30, such as at least 40 species, for example at least 50, such as at least 60 species such as at least 70, such as at least 80 species, for example at least 90, such as at least 100 species, for example at least 150 nucleic acid species.

25. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species are selected from any region of a gene involved in a clinical condition according to any of claims 10 to 19.

26. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species are selected from any region of a gene involved in HIV infection.

27. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species are selected from any region of a gene encoded by HIV.

28. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species are selected from any region of an HIV envelope polypeptide, such as defined in any of SEQ ID NO: 1-46, or a functional homolog thereof, or a polypeptide having at least 70% identity to any of said polypeptides.

29. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species are selected from the group consisting of SEQ ID NO: 95 to 1159.

30. The method according to claim 29, wherein the plurality of nucleic acid species encoding immunogenic polypeptide species comprise at least one nucleic acid species encoding an immunogenic polypeptide selected from the group consisting of polypeptide sequences RGPGRAFVT, AMQMLKETI, MRGPGRAFVT, MAMQMLKETI, and/or a part comprising at least 7 amino acids thereof.

31. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species consist of 7-10 consecutive amino acids.

32. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide species are pressented by an MHC class I molecule.

33. The method according to claim 32, wherein said MHC Class I-presented polypeptide species have at least one of the following characteristics:

a) capable of eliciting INF-γ -producing cells in a PBL population of a cancer patient at a frequency of at least 1 per 10^4 PBLs as determined by an ELISPOT assay, and/or

b) capable of in situ detection in an individual of cytotoxic T-lymphocytes (CTLs) that are reactive with the immunogenic peptide epitope, and/or

c) capable of inducing the growth in vitro of T-cells specific for any polypeptide selected from the group consisting of SEQ ID NO: 1-46 and/or 95-1159, and/or

d) capable of inducing the growth in vitro of T-cells specific for an infectious disorder, an autoimmune disorder and/or any cancer form as defined in any one of claims 10 to 19.

34. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide consist of 10-13 consecutive amino acids.

35. The method according to any of the preceding claims, wherein said distinct immunogenic polypeptide is restricted by an MHC class II molecule.

36. The method according to any of the preceding claims, wherein each said non-identical nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof is comprised in a nucleic acid expression vector species, and/or each said additional non-identical nucleic acid species encoding a distinct species of said immunogenic polypeptide is comprised in a nucleic acid expression vector species.

37. The method according to claim 36, wherein each said expression vector species comprises one and not more than one nucleic acid encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof and/or one and not more than one nucleic acid encoding a distinct species of said immunogenic polypeptide.

38. The method according to claim 36, wherein said nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and said additional nucleic acid species encoding a distinct species of said immunogenic polypeptide are comprised in separate nucleic acid expression vectors species.

39. The method according to claim 36, wherein said nucleic acid species encoding a distinct species of cytokine or hormone, or a functional homologue or part thereof, and said additional nucleic acid species encoding a distinct species of said immunogenic polypeptide are comprised in the same nucleic acid expression vector species.

40. The method according to claim 39, wherein said cytokine or hormone, or a functional homologue or part thereof, and/or said immunogenic polypeptide is translated from the vector transcript by means of an internal ribosomal entry site (IRES).

41. The method according to any of the preceding claims, wherein said nucleic acid species encoding said cytokine or hormone, or a functional homologue or part thereof, and said nucleic acid species encoding said immunogenic polypeptide are expressed by different promoters.

42. The method according to any of the preceding claims, wherein said nucleic acid species encoding said cytokine or hormone, or a functional homologue or part thereof, and said nucleic acid species encoding said immunogenic polypeptide comprise the identical 3'-untranslated region, including polyadenylation signal and/or 3'-processing elements.

43. The method according to any of the preceding claims, wherein said nucleic acid species encoding said cytokine or hormone, or a functional homologue or part thereof, and said nucleic acid species encoding said immunogenic polypeptide comprise different 3'-untranslated region.

44. The method according to any of the preceding claims, wherein said nucleic acid species encoding said cytokine or hormone, or a functional homologue or part thereof, and said nucleic acid species encoding said immunogenic polypeptide are produced by different splicing of the same vector transcript species.

45. The method according to any of the preceding claims, wherein said nucleic acid species encoding said cytokine or hormone, or a functional homologue or part thereof, and/or said nucleic acid species encoding said immunogenic polypeptide are comprised in a retroviral vector species, or a retroviral vector transcript species.

46. The method according to claim 45, wherein said vector is a retroviral expression vector.

47. The method according to any of claims 45 to 46, wherein said vector is a non-integrating retroviral vector.

48. The method according to claim 36, wherein said vector is selected from the group consisting of SEQ ID NO: 87-94.

49. The method according to any of claims 45 to 48, wherein said nucleic acid species and/or retroviral vector is comprised in a retroviral particle.

50. The method according to any of the preceding claims, wherein

a. said plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined in any of claims 20 to 23, and/or

b. said plurality of nucleic acid species encoding said immunogenic polypeptide as defined in any of claims 24 to 35, and/or

c. said vector as defined in any of claims 36 to 49, and/or

d. said retroviral particle of claim 49

is brought into contact with said population of cells by any method of transfection, wherein each individual cell of said population of cells take up at the most 5, preferably 1 nucleic acid species, vector species and/or retroviral vector species.

51. The method according to claim 50, wherein said method of transfection is selected from the group consisting of gene gun administration and/or retroviral infection with retroviral particles.

52. The method according to claim 50, wherein said method of transfection is gene gun administration.

53. The method according to claim 52, wherein said plurality of non-identical nucleic acid species, vector species and/or retroviral vector species are attached to a population of gene gun particles, which are administered by gene gun.

54. The method according to any of claims 52 to 53, wherein at the most 10, such as at the most 9, 8, 7, 6, 5, 4, 3, 2, or 1 gene gun particle on average are provided per cell.

55. The method according to any of claims 52 to 54, wherein said gene gun particles are gold particles.

56. The method according to any of claims 52 to 55, wherein at the most 5, such as at the most 4, 3, 2, or at the most 1 said

a. nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof, and/or

b. nucleic acid species encoding distinct species of cytokine, and/or

c. vector species, and/or

d. retroviral particle species

is attached to each gene gun particle of said population of gene gun particles.

57. The method according to claim 56, wherein said population of gene gun particles comprises a subpopulation of at least 50%, such as at least 60 %, for example at least 70%, such as at least 80%, such as at least 90%, for example at least 95% of said population of gene gun particles, wherein one and not more than one of said nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof, and/or nucleic acid species encoding distinct species of immunogenic polypeptides, and/or, vector species and/or retroviral particle species is attached to each gene gun particle of said subpopulation of gene gun particles.

58. The method according to any one of claims 56 and 57, wherein said population of gene gun particles comprises a plurality of non-identical subpopulations, wherein a distinct species of said nucleic acid, vector and/or retroviral particle is attached to the gene gun particles of each subpopulation.

59. The method according to any one of claims 56 to 58, wherein

a. a plurality of nucleic acid species, vector species and/or retroviral particle species encoding a cytokine or hormone or a functional homologue or part thereof are attached to gene gun particles of a first subpopulation of gene gun particles, and/or

b. a plurality of nucleic acid species, vector species and/or retroviral particle species encoding an immunogenic polypeptide are attached to gene gun particles of a second subpopulation of gene gun particles.

60. The method according to claim 59, wherein said first subpopulation of gene gun particles comprise at least 10%, such as at least 20%, for example 30%, such as at least 40%, for example at least 50%, such as at least 60 %, for example at least 70%, such as at least 80%, such as at least 90%, for example at least 95%, such as 100% of said population of gene gun particles, and/or wherein said second subpopulation of gene gun particles comprise at least 10%, such as at least 20%, for example 30%, such as at least 40%, for example at least 50%, such as at least 60 %, for example at least 70%, such as at least 80%, such as at least 90%, for example at least 95%, such as 100% of said population of gene gun particles.

61. The method according to claim 60, wherein said first subpopulations of gene gun particles comprise at least 95%, such as 100% of said population of gene gun particles.

62. The method according to claim 60, wherein said first and second subpopulations of gene gun particles together comprise at least 95%, such as 100% of said population of gene gun particles.

63. The method according to any of claims 52 to 62, wherein said population of gene gun particles comprises:

a. a first subset of subpopulations of gene gun particles, wherein distinct nucleic acid species encoding a cytokine or hormone or a functional homologue or part thereof according to any of claims 20 to 23, or a vector species according to any of claims 36 to 48, and/or a retroviral particle species according to claim 49 are attached to the gene gun particles of each subpopulation of said first subset of subpopulations of gene gun particles, and/or

b. a second subset of subpopulations of gene gun particles, wherein distinct nucleic acid species encoding an immunogenic polypeptide according to any of claims 24 to 35, or a vector species according to any

of claims 36 to 48, and/or retroviral particle species according to claim 49 are attached to the gene gun particles of each subpopulation of said first subset of subpopulations of gene gun particles.

64. The method according to any of the preceding claims, wherein said plurality of nucleic acid species, vector species, retroviral particles and/or population of gene gun particles are administered by subcutaneous, intraperitonal, intramuscular, intravenous, rectal, and/or nasal administration.

65. The method according to any of the preceding claims, which is capable of eliciting INF-γ -producing cells in a PBL population of an individual suffering from a clinical condition at a frequency of at least 10 per 10^4 PBLs.

66. The method according to any of the preceding claims, wherein the method is capable of eliciting a clinical response in a subject, wherein the clinical response is characterised by a stable disease, a partial response or complete remission.

67. A composition comprising

a. a plurality of non-identical nucleic acid species encoding distinct species of cytokine or hormone, or a functional homologue or part thereof as defined in any of claims 20 to 23, and/or

b. a plurality of non-identical nucleic acid species encoding distinct species of immunogenic polypeptides as defined in any of claims 24 to 35, and/or

c. a plurality of non-identical vector species as defined in any of claims 36 to 48, and/or

d. a plurality of non-identical retroviral particle species as defined in claim 49, and/or

e. a population of gene gun particles as defined in any of claims 54 to 63,

f. a plurality of any physical entity species comprising any component of any of a. to e.

for use as a medicament.

68. The composition according to claim 67, further comprising at least one adjuvant and/or carrier.

69. The composition according to claim 68, wherein said adjuvant is selected from the group consisting of $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, silica, alum, $Al(OH)_3$, $Ca_3(PO_4)_2$, kaolin, carbon, aluminum hydroxide, muramyl dipeptides, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-DMP), N-acetyl-nornuramyl-L-alanyl-D-isoglutamine (CGP 11687, also referred to as nor-MDP), N-acetylmuramyul-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'2'-dipalmitoyl-sn - glycero-3-hydroxphosphoryloxy)-ethylamine (CGP 19835A, also referred to as MTP-PE), RIBI (MPL+TDM+CWS) in a 2% squalene/Tween-80.RTM. emulsion, lipopolysaccharides and its various derivatives, including lipid A, Freund's Complete Adjuvant (FCA), Freund´s Incomplete Adjuvants, Merck Adjuvant 65, polynucleotides (for example, poly IC and poly AU acids), wax D from Mycobacterium, tuberculosis, substances found in Corynebacterium parvum, Bordetella pertussis, and members of the genus Brucella, Titermax, ISCOMS, Quil A, ALUN (see US 58767 and 5,554,372), Lipid A derivatives, choleratoxin derivatives, HSP derivatives, LPS derivatives, synthetic peptide matrixes or GMDP, Interleukin 1, Interleukin 2, Montanide ISA-51 and QS-21.

70. The composition according to any of claims 67 to 69 for the treatment, amelioration and/or prevention of a clinical condition as defined in any of claims 10 to 19.

71. A kit-of-parts comprising a composition as defined in any of claims 67 to 70, and at least one additional active ingredient for use as a medicament.

72. The kit-of-parts according to claim 71, wherein said at least one active ingredient is an adjuvant and/or a carrier.

73. The kit-of-parts according to claim 72, wherein said carrier is selected from the group consisting of carrier is selected from the group consisting of keyhole limpet hemocyanin, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin or ovalbumin, immunoglobulins, or hormones, such as insulin or palmitic acid.

74. The kit-of-parts according to claim 71, wherein said at least one active ingredient is an immunostimulating agent, such as an interleukin selected from the group consisting of IL-2 and/or IL-21.

75. The kit-of-parts according to claim 71, wherein at least one additional active ingredient is an antibiotic, such as an antibiotic is selected from the group consisting of amoxicillin, penicillin, acyclovir and/or vidarabine.

76. The kit-of-parts according to any of claims 71 and/or 75, wherein the components of the kit-of-parts may thus be administered simultaneously or sequentially in any order

77. The kit-of-parts according to any of claims 71 to 76, further contains instructions for combining the components so as to formulate an immunogenic composition suitable for administration to a mammal.

78. A method of reducing the risk of an individual encountering a clinical condition, said method comprising administration of a composition as defined in any one of claims 67 to 70 and/or a kit-of-parts as defined in any one of claim 71 to 77 to said individual in an amount sufficient to generate a protective immune response.

79. The method according to claim 78, wherein said clinical condition is a clinical condition as defined in any of any one of claims 10 to 19.

80. A method of immunizing an animal, said method comprising administration of a composition as defined in any one of claims 67 to 70 and/or a kit-of-parts as defined in any one of claim 71 to 77 to said individual in an amount sufficient to generate a protective immune response.

81. A method of monitoring immunization, said method comprising the steps of

a. providing a blood sample from an individual,

b. providing a composition as defined in any one of claims 67 to 70 and/or a kit-of-parts as defined in any one of claim 71 to 77, and

c. determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding at least one species of said immunogenic polypeptides or part thereof

thereby determining whether an immune response has been raised in said individual.

82. A method of monitoring immunization, said method comprising the steps of

a. providing a blood sample from an individual,

b. providing a plurality of non-identical nucleic acid species into contact with said population of cells, wherein said contact results in uptake of said nucleic acid species in said cells, and wherein each of said non-identical nucleic acid species encodes a distinct species of cytokine or hormone, or a functional homologue or part thereof to a population of blood cells, wherein each individual cell of said population of cells take up at the most 5 non-identical nucleic acid species

c. determining whether said blood sample comprises antibodies or T-cells comprising T-cell receptors specifically binding at least one species of said immunogenic polypeptides or part thereof

thereby determining whether an immune response has been raised in said individual.

83. Use of a composition as defined in any one of claims 67 to 70 and/or a kit-of-parts as defined in any one of claim 71 to 77 for the manufacture of a medicament for the treatment, amelioration and/or prevention of a disorder as defined in any of claim 10 to 19.

84. A composition as defined in any one of claims 67 to 70 and/or a kit-of-parts as defined in any one of claim 71 to 77 for the treatment, amelioration and/or prevention of a disorder as defined in any of claim 10 to 19.

85. A pharmaceutical composition for the treatment, amelioration and/or prevention of a disorder as defined in any of claim 10 to 19 comprising a composition as defined in any one of claims 67 to 70 and/or a kit-of-parts as defined in any one of claim 71 to 77.

Figure 1

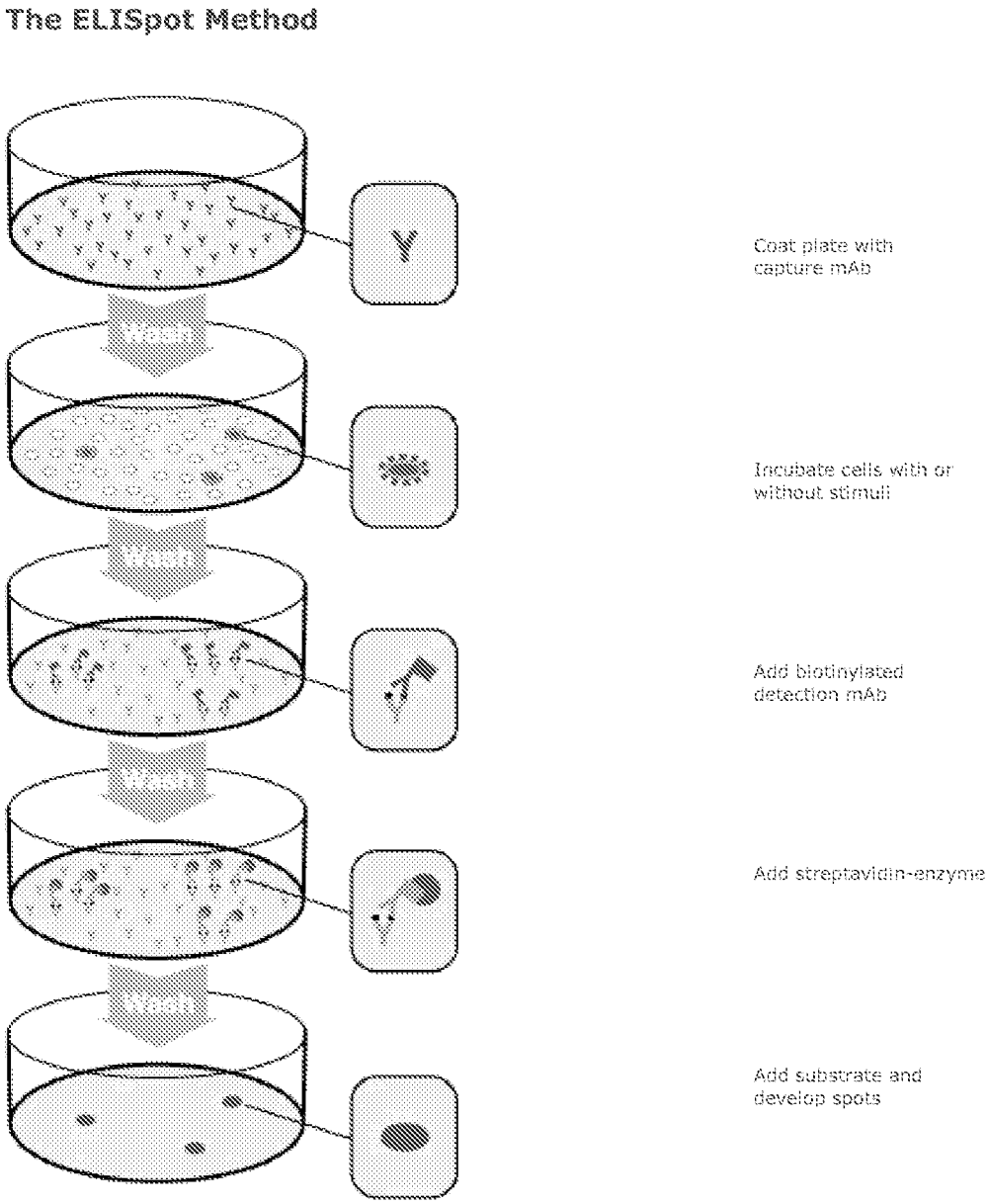

The ELISpot Method
Wash
Wash
Wash
Wash
Coat plate with capture mAb
Incubate cells with or without stimuli
Add biotinylated detection mAb
Add streptavidin-enzyme
Add substrate and develop spots

Figure 2

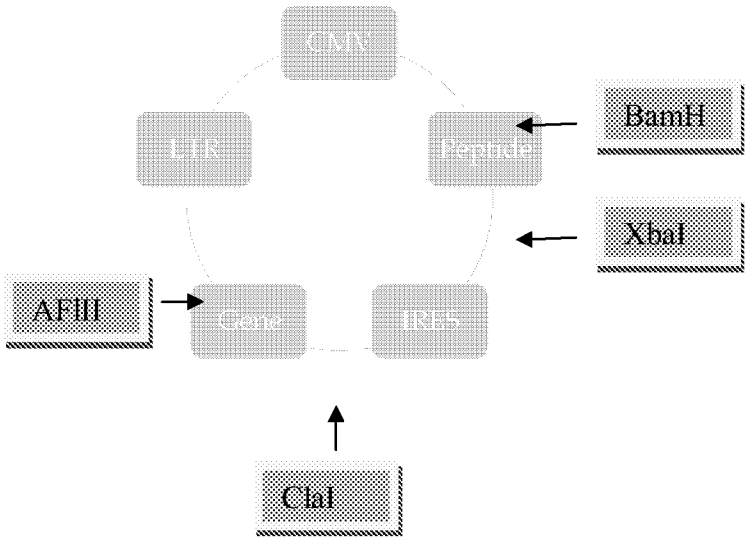


Figure 3

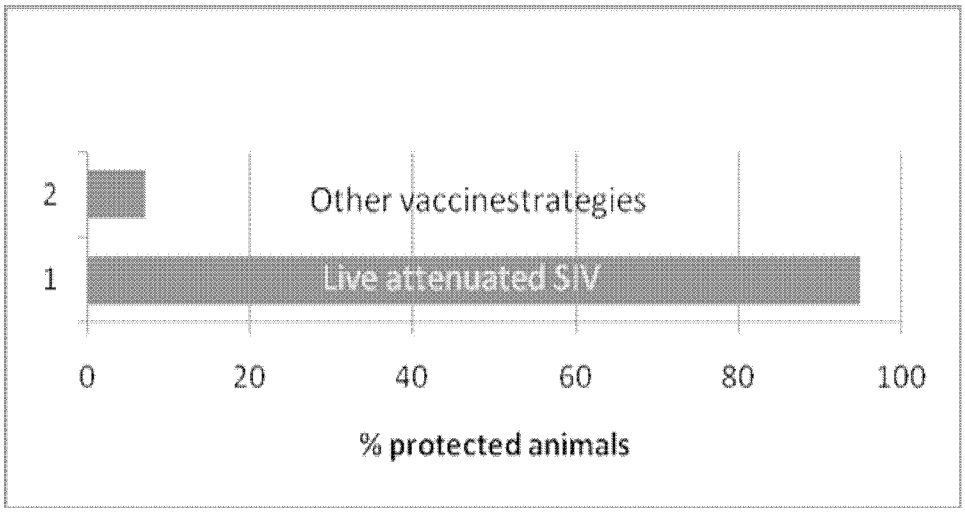

2
Other vaccinestrategies
1
Live attenuated SIV
0
20
40
60
80
100
% protected animals

Figure 4

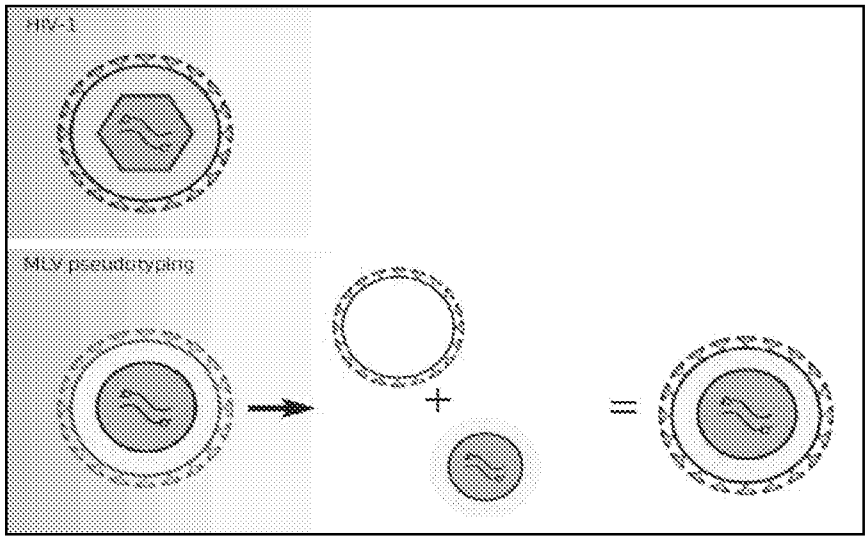


Figure 5

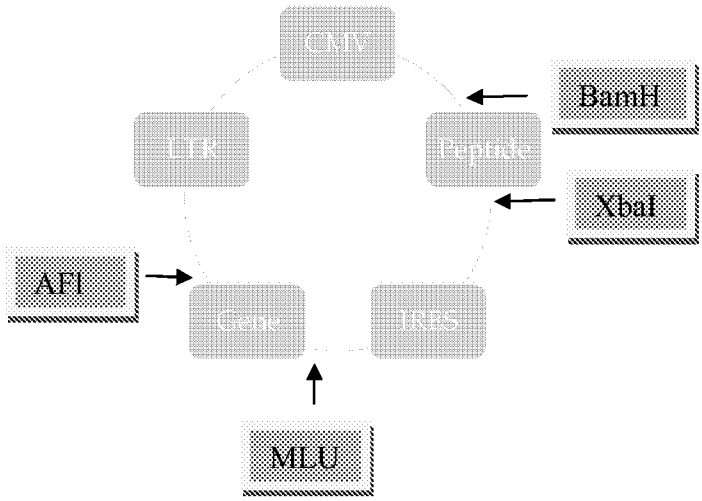


Figure 6


ccagagctcgcgagctttgaaaaacacgcggccgccatg-
P E L A S F E K H A A A M-
agaggaccagggagagcatttgttacatagtctagaacg
R G P G R A F V T - S R T

Figure 7

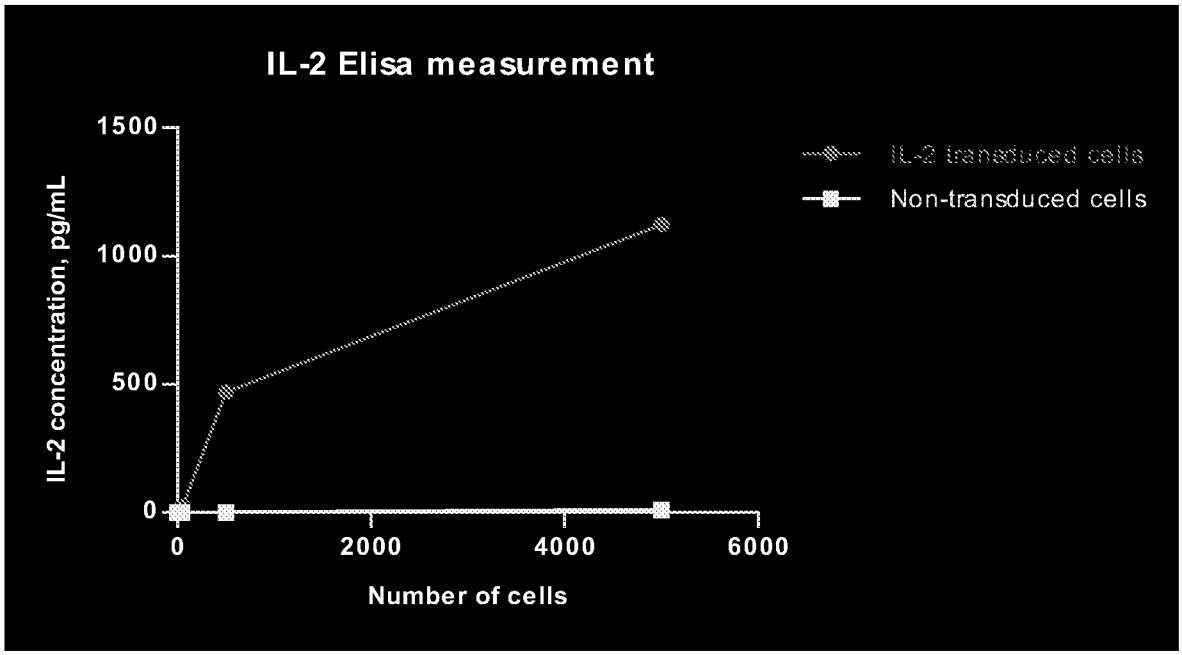

IL-2 Elisa measurement
IL-2 concentration, pg/mL
1500
1000
500
0
0
2000
4000
6000
Number of cells
IL-2 transduced cells
Non-transduced cells

Figure 8

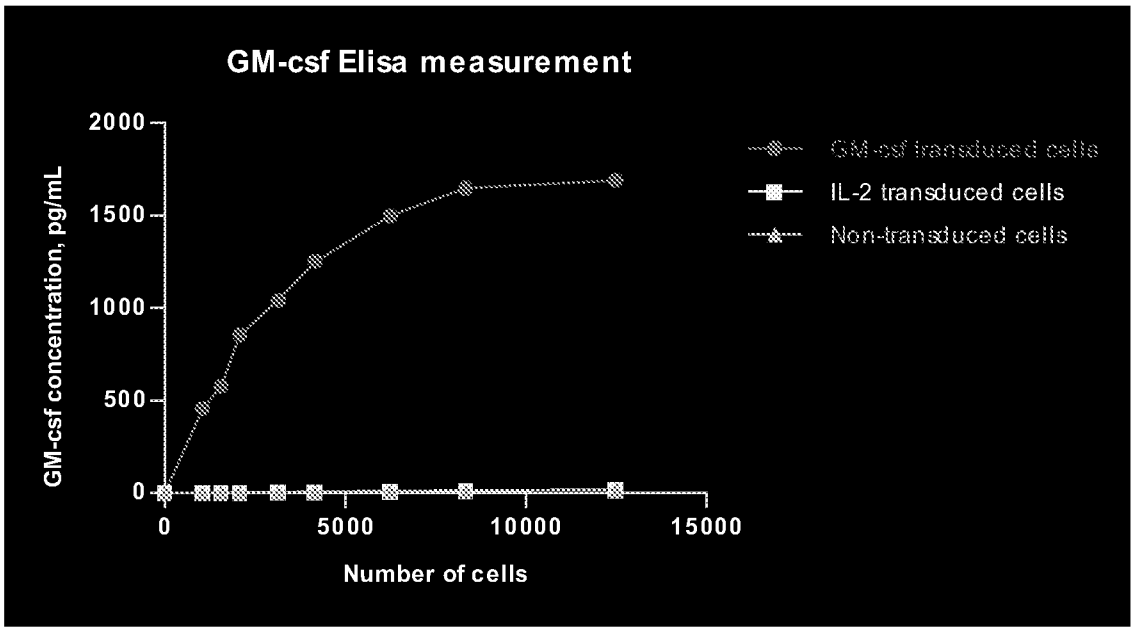

GM-csf Elisa measurement
GM-csf concentration, pg/mL
2000
1500
1000
500
0
0
5000
10000
15000
Number of cells
GM-csf transduced cells
IL-2 transduced cells
Non-transduced cells

Figure 9

Number of mice	Administration	Prime	Boost
3	IP	particles	particles
3	IP	particles + CpG	particles + CpG
3	SC	particles	particles
3	SC	particles + CpG	particles + CpG
3	IM	particles	particles
3	IM	particles + CpG	particles + CpG
3	Rectal	particles	particles
3	Rectal	particles + CpG	particles + CpG
2	IP	PBS	PBS

Figure 10

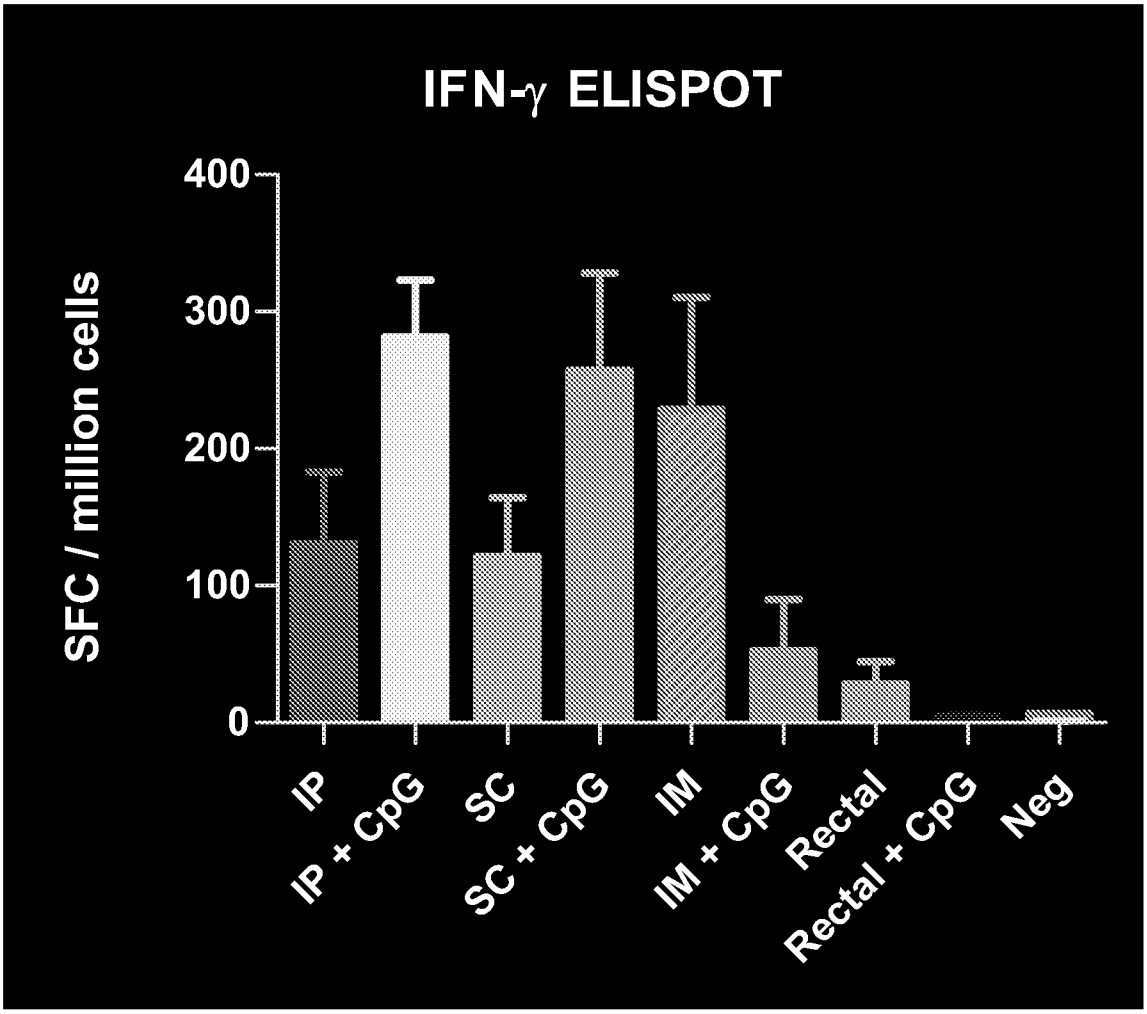
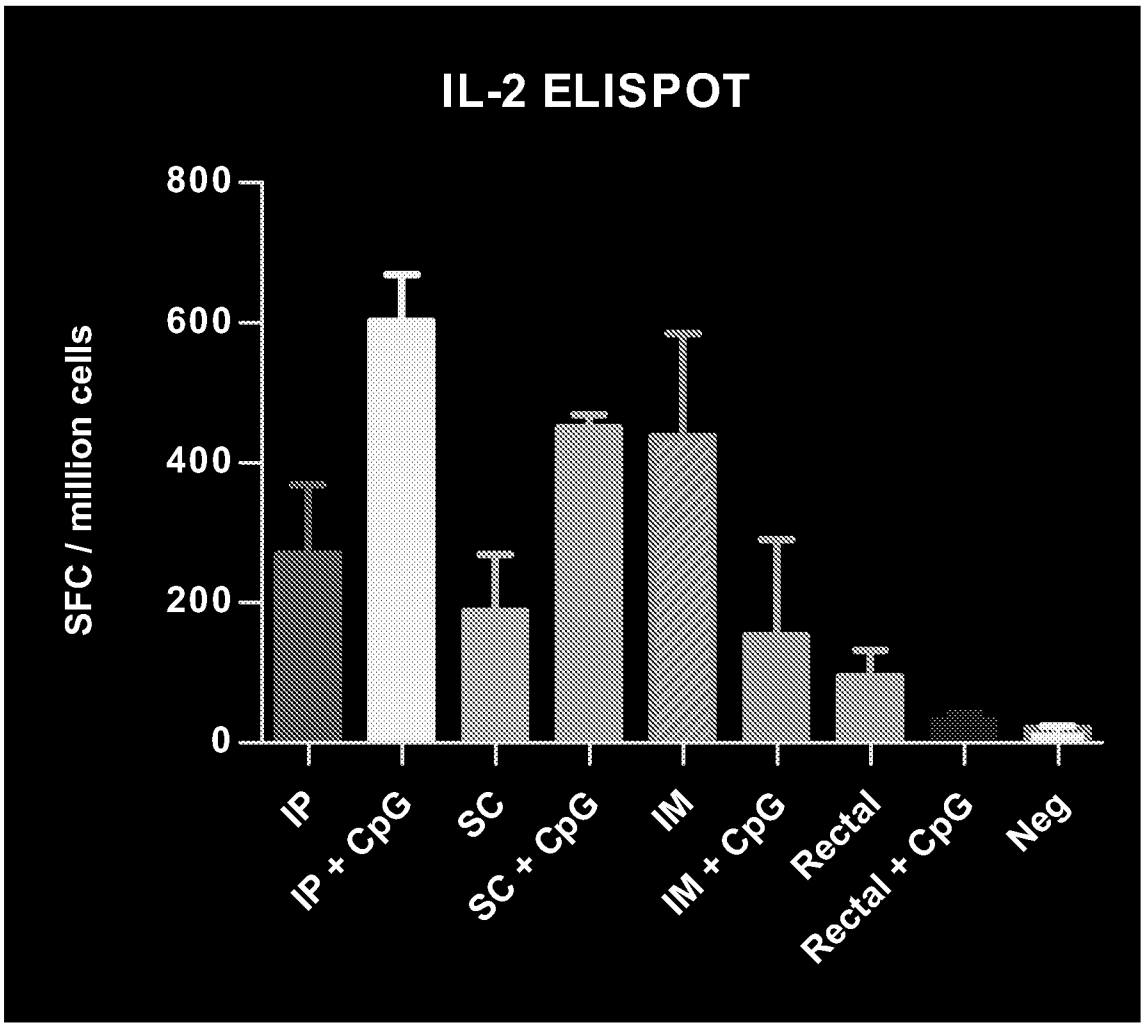

IFN-γ ELISPOT
SFC / million cells
400
300
200
100
0
IP
IP + CpG
SC
SC + CpG
IM
IM + CpG
Rectal
Rectal + CpG
Neg

Figure 11


IL-2 ELISPOT
SFC / million cells
800
600
400
200
0
IP
IP + CpG
SC
SC + CpG
IM
IM + CpG
Rectal
Rectal + CpG
Neg

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2009/050294

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61K48/00

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2004/266713 A1 (LU SHAN [US] ET AL) 30 December 2004 (2004-12-30) the whole document	67-85 1-66
Y	WO 98/17799 A1 (UNIV PENNSYLVANIA [US]; APOLLON INC [US]; KIM JONG J [US]; BAGARAZZI M) 30 April 1998 (1998-04-30) the whole document	1-85
Y	WO 00/67787 A2 (IMMUNE RESPONSE CORP INC [US]; MOSS RONALD B [US]) 16 November 2000 (2000-11-16) the whole document	1-85
A	US 2004/219169 A1 (BERMUDES DAVID G [US] ET AL) 4 November 2004 (2004-11-04)	1-85

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2010	01/03/2010

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2009/050294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004266713 A1	30-12-2004	NONE	
WO 9817799 A1	30-04-1998	AU 729579 B2	01-02-2001
		AU 5002297 A	15-05-1998
		BR 9712852 A	16-11-1999
		CA 2269074 A1	30-04-1998
		CN 1242045 A	19-01-2000
		CN 1868545 A	29-11-2006
		EP 0958364 A1	24-11-1999
		JP 2001507216 T	05-06-2001
		JP 2008194046 A	28-08-2008
		JP 2009232860 A	15-10-2009
		KR 20000052710 A	25-08-2000
		KR 20050053796 A	08-06-2005
		KR 20070108418 A	09-11-2007
WO 0067787 A2	16-11-2000	NONE	
US 2004219169 A1	04-11-2004	NONE	