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(54) Title: VACCINE CONJUGATES AND USES THEREOF

(57) Abstract: The present invention relates to conjugates, fusion polypeptides and compositions comprising those conjugates or fusion polypeptides for use in the generation of an immune response. In particular, the immune response is generated to treat or prevent cancer or an infectious disease. The present invention relates to a conjugate for inducing an immune response, the conjugate comprising: (a) an antigen presenting cell targeting moiety; (b) a moiety capable of endosomal and/or lysosomal escape; and (c) an antigen; wherein the antigen presenting cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen.



Vaccine conjugates and uses thereof

Field of the invention

The present invention relates to conjugates, fusion polypeptides and compositions comprising those conjugates or fusion polypeptides for use in the generation of an immune response. In particular, the immune response is generated to treat or prevent cancer or an infectious disease.

Associated application

This application claims priority from Australian provisional application AU 2018900032, the entire contents of which are hereby incorporated by reference.

Background of the invention

Most cancer cells elicit an immune response that is evident by the presence of immune cell infiltrates and inflammation. This response, however, is not strong enough to overcome the cancer cell's defence strategies. The lack of understanding of the complex interactions between tumours and the immune system has hindered the development of cancer immunotherapy. Approaches involving using purified tumour antigens and more complex mixtures of tumour antigens have often failed to stimulate adequate immune responses against tumours. The reasons for this are unknown, but may include the genetic instability of tumours and the ability of tumours to evade the immune system by presenting a "normal" appearance or releasing inhibitors. Tumours can respond to an immune response by reducing the amount of targeted antigens, by masking antigens from the immune system or by expressing mutated versions of antigens that are no longer recognised. Such defensive strategies undermine the immune system, making it difficult to maintain an effective immune response at the level required to halt tumour growth and cause regression. Moreover, the responses may be inadequate since they fail to stimulate an adaptive immune response.

T cell-mediated anti-tumor immunity plays a role in regulating tumor growth, placing selective pressure on the antigenically-heterogeneous cancer cell population throughout disease progression. Antigen specific immunotherapy aims to enhance or induce specific immune responses in patients and has been successfully used to control

cancer diseases. T cells play a central role in cell-mediated immunity in humans and animals. The recognition and binding of a particular antigen is mediated by the T cell receptors (TCRs) expressed on the surface of T cells. The T cell receptor (TCR) of a T cell is able to interact with immunogenic peptides (epitopes) bound to major histocompatibility complex (MHC) molecules and presented on the surface of target cells. Specific binding of the TCR in the presence of costimulation triggers a signal cascade inside the T cell leading to proliferation and differentiation into a matured effector T cell.

Vaccines that stimulate antibody production (humoral immunity) have enjoyed success for more than two centuries. Humoral immunity, however, is of limited effectiveness against cancers because many tumor-associated antigens are intracellular and inaccessible to the antibody. Effective cellular immune responses (cell-mediated immunity), especially cytotoxic T lymphocytes (CTLs), are the best weapons amongst the immune system's arsenal against these diseases.

The development of vaccines that generate effective cellular immune responses, in particular, CD8⁺ cytotoxic lymphocytes, however, remains a challenge, partly because exogenous antigens introduced to the body by vaccines, unlike endogenous antigens, have not been efficient in eliciting the production of antigen-class I histocompatibility molecules (MHC class I) complexes required to prime CD8⁺ T cells.

In order for the antigen to be recognized by CD8⁺ T cells, it must be complexed with MHC class I molecules. Endogenous antigens almost always are degraded into peptides by proteasomes. The resultant small peptide fragments are picked up by TAP (transporter associated with antigen processing), and eventually complexed with MHC class I molecules, displayed on the surface of the cell. The antigen-MHC class I complex is recognized by CD8⁺ cytotoxic T cells.

Exogenous antigens, on the other hand, are taken up by antigen-presenting cells (APCs), such as dendritic cells, by multiple mechanisms that include endocytosis or phagocytosis. The endosome or phagosome so formed predominantly mature to the point where they fuse with lysosomes, in which the antigen is degraded into peptide fragments which are then nestled within a class II histocompatibility molecule (MHC II) and displayed at the surface of the cell, and are recognized by CD4⁺ T cells.

There has been a lack of success in the development of vaccines that generate effective cellular immune responses for the treatment or prevention of cancer. A similar lack of success has also applied to infectious diseases. Consequently, there is a need for new or improved compositions that stimulate the immune system for the treatment or
5 prevention of cancer or an infectious disease.

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person
10 in the art.

Summary of the invention

The present invention provides a conjugate for inducing an immune response, the conjugate comprising:

- (a) an antigen presenting cell targeting moiety;
- 15 (b) a moiety capable of endosomal and/or early lysosomal escape; and
- (c) an antigen;

wherein the antigen presenting cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen.

Preferably, the antigen presenting cell is a cell capable of cross presentation.
20 Even more preferably the antigen presenting cell is a professional antigen presenting cell such as a macrophage or dendritic cell.

The antigen presenting cell targeting moiety may localize to and bind a target molecule present on an antigen presenting cell. When an antigen presenting cell targeting moiety localizes to and binds to a target molecule on a dendritic cell, the
25 targeting moiety may be referred to as a dendritic cell targeting moiety. The targeting moieties contemplated for use in any aspect of the invention include antibodies, polypeptides, peptides, aptamers, other ligands, or any combination thereof, that can bind a component of the target cell or molecule.

Exemplary dendritic cell targeting moieties are molecules that bind to any one of XCR1, DEC-205 (CD205), Langerin (CD207), Clec9A receptors, DC-SIGN (CD209) and DNGR1. A preferred dendritic cell targeting moiety binds to XCR1. Typically, a moiety that binds to XCR1 comprises, consists essentially of or consists of an amino acid
5 sequence of XCL1 as described herein.

The antigen presenting cell targeting moiety may be associated with the moiety capable of endosomal escape via a linker which is cleavable in an endosome compartment prior to the endosome maturing into a late endosome. Preferably, the linker is cleavable in an early endosome. In one embodiment, the linker which is
10 cleavable in an early endosome comprises an amino acid sequence of a furin cleavage site. The furin cleavage site may comprise, consist essentially of or consist of the amino acid sequence R-X-K/R-R (SEQ ID NO: 1). Even more preferably, the furin cleavage site may comprise, consist essentially of or consist of the amino acid sequence RKKR (SEQ ID NO: 2).

The linker between the antigen presenting cell targeting moiety and the moiety capable of endosomal escape may also be cleavable in the maturing or late endosome, or in the lysosome. In one embodiment, the linker which is also cleavable in a lysosome comprises an amino acid sequence of a cathepsin B cleavage site. The cathepsin B cleavage site may comprise, consist essentially of or consist of the amino acid
20 sequence V-citrulline (SEQ ID NO: 3; V denotes the amino acid valine) or FKFL (SEQ ID NO: 4).

The linker between the antigen presenting cell targeting moiety and the moiety capable of endosomal escape may comprise, consist essentially of or consist of a furin cleavage site as described herein and a cathepsin B cleavage site as described herein,
25 preferably RKKR (SEQ ID NO: 2) and V-citrulline (SEQ ID NO: 3). In one embodiment, the dual furin/cathepsin B cleavage linker comprises, consists essentially of or consists of the amino acid sequence RKKRXV-citrulline, where X is any small amino acid, preferably a serine. In another embodiment, the dual furin/cathepsin B cleavage linker comprises, consists essentially of or consists of the amino acid sequence
30 GSGTGGSTGRKKRSV-citrulline-GC (SEQ ID NO: 5).

In any aspect of the invention, the moiety capable of endosomal and/or lysosomal escape is capable of escaping from an endosomal and/or lysosomal compartment into the cytosol. Preferably, the moiety is capable of escaping from an endosomal and/or lysosomal compartment into the cytosol prior to the endosome maturing into a late endosome or lysosome. More preferably, the moiety is capable of escaping from an early endosomal compartment into the cytosol.

In one embodiment, the moiety capable of endosomal and/or lysosomal escape mediates significantly greater endosomal escape than the TAT peptide. The degree or level of endosomal escape may be determined by any method known in the art, including any method or assay described herein. For example, the level of endosomal escape may be determined using the beta-lactamase assay described herein. A moiety capable of endosomal and/or lysosomal escape mediate significantly greater endosomal escape than the TAT peptide when measured using the beta-lactamase assay described herein. Alternatively, a moiety capable of endosomal and/or lysosomal escape may result in greater than or equal to 5 %, 10 % or 15 % positive cells at 450 nM, at concentrations of 1 μ M, 2 μ M or 4 μ M respectively, when measured using the beta-lactamase assay described herein.

As used herein, the term endosomal escape may include early and/or late endosomal escape, preferably early endosomal escape. Lysosomal escape may include early and/or late lysosomal escape, preferably early lysosomal escape.

In one embodiment, the moiety capable of endosomal and/or lysosomal escape is a peptide. Preferably, the peptide comprises, consists essentially of, or consists of the amino acid sequence PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKR (SEQ ID NO: 6). Alternatively, the peptide may comprise, consist essentially of or consist of any one or more of the amino acid sequences in WO2014205518 referred to therein as SEQ ID NO: 83-119 and which are herein incorporated in their entirety by reference.

In any aspect of the invention, the moiety capable of endosomal and/or lysosomal escape is associated with the antigen such that when the moiety escapes from an endosomal and/or lysosomal compartment into the cytosol, the antigen also enters the cytosol. Typically, the moiety capable of endosomal and/or lysosomal escape and the antigen are chemically linked, for example, covalently linked. The covalent

linkage may be direct or via a linker. The linker may comprise, consists essentially of or consist of a peptide, for example containing 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids. Preferably the amino acids are selected from alanine, glycine and serine. In one embodiment, the peptide comprises, consists essentially of, or consists of the amino acid sequence GAS (SEQ ID NO: 7).

In any aspect of the invention, the antigen presenting cell targeting moiety mediates internalization upon contact with an antigen presenting cell.

In any aspect of the invention, the moiety capable of endosomal and/or lysosomal escape mediates internalization upon contact with an antigen presenting cell. In this embodiment, the moiety capable of endosomal and/or lysosomal escape may be a cell penetrating peptide as described herein but also have the capacity to escape from endosomal or lysosomal compartments for delivery into the cytosol. Those moieties having the capability of mediating intracellular delivery and escape from endosomal and/or lysosomal compartments for delivery into the cytosol may also be referred to as cytosolic delivery cell penetrating peptide (CPPs).

In any aspect of the invention, the antigen comprises a peptide capable of being presented by an HLA class I molecule. Preferably, the antigen also comprises a peptide capable of being presented by an HLA class II molecule. Typically, the peptide capable of being presented by an HLA class I molecule comprises a CD8+ T cell epitope. Typically, the peptide capable of being presented by an HLA class II molecule comprises a CD4+ T cell epitope. Exemplary antigens include any tumour associated antigens, including those described herein.

The present invention also provides a chimeric or fusion polypeptide for inducing an immune response, the chimeric or fusion polypeptide comprising:

- (a) an antigen presenting cell targeting moiety;
- (b) a moiety capable of endosomal and/or lysosomal escape; and
- (c) an antigen;

wherein the antigen presenting cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen. Preferably, each of the antigen

presenting cell targeting moiety, the moiety capable of endosomal and/or lysosomal escape and the antigen are peptides or polypeptides. Further, the means by which the antigen presenting cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen, is also a peptide or polypeptide.

5 In one embodiment, the chimeric or fusion polypeptide is a contiguous sequence of amino acids. Preferably, the chimeric or fusion polypeptide is expressed from a single expression construct.

 A conjugate, or chimeric or fusion polypeptide of the invention may be produced synthetically or recombinantly. A conjugate, or chimeric or fusion polypeptide of the
10 invention may be substantially purified.

 In another aspect, the present invention also provides a composition such as an antigenic composition, particularly a vaccine composition, comprising a conjugate, or chimeric or fusion polypeptide of the invention described herein, optionally in association with an adjuvant.

15 In another aspect, the present invention also provides a composition such as an antigenic composition, particularly a vaccine composition, wherein the composition comprises a conjugate or a chimeric or fusion polypeptide as described herein, optionally in association with a virus-like particle, liposome or nanoparticle.

 In another aspect, the invention provides a pharmaceutical composition for
20 treating or preventing cancer in a subject comprising a conjugate, or chimeric or fusion polypeptide of the invention described herein and a pharmaceutically acceptable diluent, excipient or carrier.

 In another aspect, the invention provides a pharmaceutical composition for treating or preventing cancer in a subject comprising as an active ingredient a
25 conjugate, or chimeric or fusion polypeptide of the invention described herein, the composition further comprising a pharmaceutically acceptable diluent, excipient or carrier.

 In another aspect, the invention provides a pharmaceutical composition for treating cancer in a subject comprising as a main ingredient a conjugate, or chimeric or

fusion polypeptide of the invention described herein, the composition further comprising a pharmaceutically acceptable diluent, excipient or carrier.

In another aspect, the present invention also provides a conjugate, or chimeric or fusion polypeptide of the invention described herein for use in the treatment or
5 prevention of cancer in a subject.

In another aspect, the invention also provides a nucleic acid molecule comprising, consisting essentially of or consisting of a nucleotide sequence encoding a conjugate, or chimeric or fusion polypeptide of the invention described herein.

In one example, such a nucleic acid is included in an expression construct in
10 which the nucleic acid is operably linked to a promoter. Such an expression construct can be in a vector, e.g., a plasmid.

In examples of the invention the expression construct may comprise a promoter linked to a nucleic acid encoding that polypeptide chain a conjugate, or chimeric or fusion polypeptide of the invention described herein.

15 In another aspect, the invention provides a cell comprising a vector or nucleic acid described herein. Preferably, the cell is isolated, substantially purified or recombinant.

Examples of cells of the present invention include bacterial cells, yeast cells, insect cells or mammalian cells.

20 In another aspect, the present invention also provides a method for eliciting a cell-mediated immune response to an antigen in a subject, the method comprising

providing a conjugate, chimeric or fusion polypeptide, or composition of the invention as described herein, and

administering the conjugate, chimeric or fusion polypeptide, or composition to the
25 subject,

thereby eliciting a cell-mediated immune response to the antigen in a subject.

In another aspect, the present invention also provides a method for treating or preventing cancer or an infectious disease in a subject in need thereof, the method comprising administering a conjugate, chimeric or fusion polypeptide, or composition of the invention as described herein, thereby treating or preventing cancer or an infectious
5 disease in the subject.

In another aspect, the present invention also provides a use of a conjugate, chimeric or fusion polypeptide, or composition of the invention as described herein in the manufacture of a medicament for the treatment or prevention of cancer or an infectious disease in a subject.

10 In any aspect, the cancer may be any one described herein.

In any aspect, the subject may be any one described herein.

The present invention also provides a method for inducing an immune response in a subject suitable for the treatment of cancer or infectious disease, the method including the steps of

15 - contacting antigen presenting cells with a conjugate, chimeric or fusion polypeptide, or composition of the invention as described herein *ex vivo* for a sufficient time and under conditions enabling the antigen to be presented on HLA-I of the antigen presenting cells;

- administering the antigen presenting cells to the subject,

20 thereby forming an immune response in a subject suitable for the treatment of cancer or an infectious disease.

The present invention also provides a method for preparing an isolate or substantially purified composition of antigen presenting cells to treat or prevent cancer or an infectious disease in a subject, the method including the steps of

25 - providing a population of antigen presenting cells, and

- contacting the population of antigen presenting cells with a conjugate, chimeric or fusion polypeptide, or composition of the invention as described herein *ex*

vivo for a sufficient time and under conditions enabling the antigen to be presented on HLA-I of the antigen presenting cells,

thereby preparing an isolate or substantially purified composition of antigen presenting cells to treat or prevent cancer or an infectious disease in a subject.

5 The present invention also provides a method for forming an immune response in a subject suitable for the treatment or prevention of cancer or an infectious disease including the steps of

- obtaining antigen presenting cells from the subject,
- culturing the obtained antigen presenting cells to generate a population of
10 antigen presenting cells,
- contacting the population of antigen presenting cells with a conjugate, chimeric or fusion polypeptide, or composition of the invention as described herein *ex vivo* for a sufficient time and under conditions enabling the antigen to be presented on HLA-I of the antigen presenting cells for forming an immune response,
- 15 - re-introducing the antigen presenting cells to the subject,

thereby producing an immune response in a subject suitable for the treatment of cancer or an infectious disease.

In any aspect of the invention, the population of antigen presenting cells are contacted with a conjugate, chimeric or fusion polypeptide, or composition of the
20 invention as described herein for a sufficient time and under conditions enabling the antigen to be presented on both HLA-I and HLA-II molecules on the cell surface.

The invention also provides a kit or article of manufacture comprising a conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention described herein. Optionally the kit further comprises written instructions for use of the
25 conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention described herein in a method or use of the invention as described herein.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

5 Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

Figure 1 provides a schematic of the proposed pathway for uptake of the
10 conjugates, chimeric or fusion polypeptides of the invention (cytosolic delivery CPP referred to as FPP in this Figure).

Figure 2 provides a schematic of a moiety capable of endosomal escape linked to an antigen (cytosolic delivery CPP – 1746; antigen gD-GTG-gB).

Figure 3 provides a schematic of the assembly of the SpyTag and disulphide
15 linker which may not be cleaved in a cross-presenting DCs endosome due to unique neutral pH in this intracellular compartment. The disulphide bond would typically be cleaved in an acidic environment. MCPA (mercaptopropionic acid) is a synthetic cysteine which forms a disulphide bond with the Cys present.

Figure 4 provides a schematic of the final V5-SpyT-Cys-mcpa-CPP-antigen
20 conjugate.

Figure 5 shows an SDS-PAGE confirming SpyC/SpyT ligation.

Figure 6 provides a schematic of the experimental protocol of peptide vaccine using disulphide linkage technology, to measure specific T cell expansion.

Figure 7 shows the results that disulfide-linked vaccine constructs do not elicit a
25 CD8+ gB-specific T cell expansion whereas disulfide-linked vaccine constructs elicit a CD4+ gD-specific T cell expansion.

Figure 8 provides a schematic of the SpyTag and cleavable linkers.

Figure 9 provides a schematic of the SpyTag-FCB-cytosolic delivery CPP-Antigen delivery module (cytosolic delivery CPP referred to as FPP in this Figure).

Figure 10 provides a schematic of the full peptide vaccine construct.

Figure 11 provides a schematic of the experimental protocol at two different
5 amounts of peptide vaccine.

Figure 12 shows % gD-specific CD4⁺ T cells of total CD4⁺ T cells at 1nmol and 2nmol doses.

Figure 13 shows % gB-specific CD8⁺ T cells of total CD8⁺ T cells at 1nmol and 2nmol doses.

10 **Figure 14** provides a schematic of the experimental prophylactic protocol in the B16 melanoma mouse model and results of percent tumour free survival.

Figure 15 shows B16-gB melanoma tumour growth kinetics 43 days post vaccination.

Figure 16 shows B16-gB melanoma percent survival 43 days post vaccination.

15 **Figure 17** provides a schematic of the experimental therapeutic protocol in the B16 melanoma mouse model.

Figure 18 shows confocal microscopy images of T47D cells at 40X magnification after 1 hour incubation with CPP-SpyC/ SpyC-BLA conjugate. CCF2-AM incubated for 1 hour prior to protein incubation.

20 **Figure 19** is a histogram showing the results of flow cytometry to quantify cytosolic CCF2-AM cleavage by internalized Beta-Lactamase. The ratio of blue to green fluorescence was assessed to determine the cell-penetrating and cytosolic delivery ability of the two CPP sequences 1746 and TAT. The flow cytometry data is further corroborated by confocal microscopy on live cells using the same assay conditions.

25 **Figure 20** shows that CPP peptide vaccines provide efficient protection against HSV skin infection. Cohorts of mice were primed (D0) subcutaneously with 2 nmol of XCL1 constructs linked to either gDgB or 1746-gDgB. All vaccinated groups and an

additional unprimed group received 50 µg poly I:C as an adjuvant (D0). All groups were infected with HSV on the flank (D7) and viral titres in the skin were measured 6 days post infection (D13). Symbols represent individual mice with bars representing the mean.

5 Detailed description of the embodiments

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

10 Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be
15 understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

The present invention is based on the design and production of multicomponent
20 conjugates that are useful in activating both CD8+ and CD4+ T cells for the treatment or prevention of cancer. These conjugates target professional antigen presenting cells and result in presentation of peptides on HLA-I or HLA-II thus activating CD8+ and CD4+ T cells, respectively. The HLA-I pathway is more stringent than the HLA-II pathway in that it generally requires cytosolic processing by the proteasome to generate the antigen to
25 present to CD8+ T cells. CD8+ T cell expansion can only occur if the antigen is presented by HLA-I cytosolic loading. The conjugates and fusion polypeptides of the invention contain moieties capable of endosomal and/or lysosomal escape that traffic an associated antigen into the cytosol for processing and HLA-I cytosolic loading. However, the conjugates and fusion polypeptides also allow the antigen to be located
30 within the maturing late endosome, where it is loaded onto HLA-II via an endosomal processing pathway which is recognised by CD4+ T cells. The activation of key

components of the cellular (CD8+ T cells and CD4+ T cells) immune system, results in the induction of an immune response that, for example, leads to a reduction in tumour growth.

The inventors have surprisingly found that for sufficient endosomal and/or lysosomal escape of the antigen to allow for efficient cross-presentation of antigenic peptides, the moiety capable of escape and the cargo antigen must be separated from the antigen cell targeting moiety. By using linkers that release the targeting moiety from the endosomal and/or lysosomal escape moiety in the early endosome, the endosomal and/or lysosomal escape moiety is then capable of trafficking an associated antigen into the cytosol for processing and HLA-I cytosolic loading. The multicomponent conjugate surprisingly functions even each component is covalently linked in a single construct.

General

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter. Thus, as used herein, the singular forms “a”, “an” and “the” include plural aspects, and vice versa, unless the context clearly dictates otherwise. For example, reference to “a” includes a single as well as two or more; reference to “an” includes a single as well as two or more; reference to “the” includes a single as well as two or more and so forth.

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

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present

invention. The present invention is in no way limited to the methods and materials described.

All of the patents and publications referred to herein are incorporated by reference in their entirety.

5 The present invention is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present invention.

10 Any example or embodiment of the present invention herein shall be taken to apply *mutatis mutandis* to any other example or embodiment of the invention unless specifically stated otherwise.

15 Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

20 Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (editors), Current Protocols in
25 Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein the term "derived from" shall be taken to indicate that a specified
5 integer may be obtained from a particular source albeit not necessarily directly from that source.

Reference herein to a range of, e.g., residues, will be understood to be inclusive. For example, reference to “a region comprising amino acids 56 to 65” will be understood in an inclusive manner, i.e., the region comprises a sequence of amino acids as
10 numbered 56, 57, 58, 59, 60, 61, 62, 63, 64 and 65 in a specified sequence.

Selected definitions

As used herein, the term “chimeric or fusion polypeptide” is used to refer to a polypeptide that is composed of functional units, domains, sequences or regions of amino acids derived from different sources or that are derived from the same source
15 and that have been assembled so as to have an organisation that is distinguished from that observed in a molecule from which the unit, domain, sequence or region is derived or related to.

By “substantially purified” is meant the protein is substantially free of contaminating agents, e.g., at least about 70% or 75% or 80% or 85% or 90% or 95% or
20 96% or 97% or 98% or 99% free of contaminating agents.

The term “recombinant” shall be understood to mean the product of artificial genetic recombination. Accordingly, in the context of a recombinant protein comprising an antibody antigen binding domain, this term does not encompass an antibody naturally-occurring within a subject’s body that is the product of natural recombination
25 that occurs during B cell maturation. However, if such an antibody is isolated, it is to be considered an isolated protein comprising an antibody antigen binding domain. Similarly, if nucleic acid encoding the protein is isolated and expressed using recombinant means, the resulting protein is a recombinant protein comprising an antibody antigen binding domain. A recombinant protein also encompasses a protein

expressed by artificial recombinant means when it is within a cell, tissue or subject, e.g., in which it is expressed.

The term "polypeptide" shall be taken to include a single polypeptide chain, i.e., a series of contiguous amino acids, such as a polymer, linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). For example, the series of polypeptide chains can be covalently linked using a suitable chemical or a disulphide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions. The polypeptide may include amino acid residues and variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally-occurring amino acid, such as a chemical analogue of a corresponding naturally-occurring amino acid, as well as to naturally-occurring amino acid polymers. These terms do not exclude modifications, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid including, for example, unnatural amino acids or polypeptides with substituted linkages.

A polypeptide that contains at least one residue that is not naturally synthesised may be referred to as a "peptidomimetic". A peptidomimetic as used herein is a synthetic chemical compound that has substantially the same structure and/or functional characteristics of a conjugate, or chimeric or fusion polypeptide of the invention as described herein. Non-natural components of peptidomimetic compounds may be according to one or more of: a) residue linkage groups other than the natural amide bond ('peptide bond') linkages; b) non-natural residues in place of naturally occurring amino acid residues; or c) residues which induce secondary structural mimicry, i.e., to induce or stabilize a secondary structure, e.g., a beta turn, gamma turn, beta sheet, alpha helix conformation, and the like. Peptidomimetics can be synthesized using a variety of procedures and methodologies described in the scientific and patent literatures, e.g., Organic Syntheses Collective Volumes, Gilman et al. (Eds) John Wiley & Sons, Inc., NY, al-Obeidi (1998) *Mol. Biotechnol.* 9:205-223; Hruby (1997) *Curr. Opin. Chem. Biol.* 1:114-119; Ostergaard (1997) *Mol. Divers.* 3:17-27; Ostresh (1996) *Methods Enzymol.* 267:220-234.

A "fragment" is a portion of a conjugate, or chimeric or fusion polypeptide of the present invention that retains substantially similar functional activity or substantially the same biological function or activity as the polypeptide, as shown assays disclosed herein.

5 A "derivative" includes all modifications to a conjugate, or chimeric or fusion polypeptide of the present invention that substantially preserve the functions disclosed herein and include additional structure and attendant function.

A "variant" includes polypeptides having an amino acid sequence sufficiently similar to the amino acid sequence of a conjugate, or chimeric or fusion polypeptide of the present invention. The term "sufficiently similar" means a first amino acid sequence that contains a sufficient or minimum number of identical or equivalent amino acid residues relative to a second amino acid sequence such that the first and second amino acid sequences have a common structural domain and/or common functional activity. For example, amino acid sequences that comprise a common structural domain that is
10 at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100%, identical are defined herein as
15 sufficiently similar. Preferably, variants will be sufficiently similar to the amino acid sequence of the preferred conjugates, or chimeric or fusion polypeptides of the present invention. Variants include variants of a conjugate, or chimeric or fusion polypeptide of the present invention encoded by a polynucleotide that hybridizes to a polynucleotide of this invention, or a complement thereof, under stringent conditions. Such variants
20 generally retain the functional activity of a conjugate, or chimeric or fusion polypeptide of the present invention. Variants include polypeptides that differ in amino acid sequence due to mutagenesis.
25

"Substantially similar functional activity" and "substantially the same biological function or activity" each means that the degree of biological activity is within about 50%
30 to 100% or more, within 80% to 100% or more, or within about 90% to 100% or more, of

that biological activity demonstrated by the peptide to which it is being compared when the biological activity of each peptide is determined by the same procedure or assay.

"Similarity" between two peptides is determined by comparing the amino acid sequence of one peptide to the sequence of a second peptide. An amino acid of one peptide is similar to the corresponding amino acid of a second polypeptide if it is identical or a conservative amino acid substitution. Conservative substitutions include those described in Dayhoff, M.O., ed., The Atlas of Protein Sequence and Structure 5, National Biomedical Research Foundation, Washington, D.C. (1978), and in Argos, P. (1989) EMBO J. 8:779-785. For example, amino acids belonging to one of the following groups represent conservative changes or substitutions:

- Ala, Pro, Gly, Gln, Asn, Ser, Thr;
- Cys, Ser, Tyr, Thr;
- Val, Ile, Leu, Met, Ala, Phe;
- Lys, Arg, His;
- Phe, Tyr, Trp, His; and
- Asp, Glu.

Other conservative amino acid substitutions may also be made by another one of the same class, the classes being as follows:

Non-polar: Ala, Val, Leu, Ile, Pro, Met Phe, Trp

Uncharged polar: Gly, Ser, Thr, Cys, Tyr, Asn, Gln

Acidic: Asp, Glu

Basic: Lys, Arg, His

Other conservative amino acid substitutions may also be made as follows:

Aromatic: Phe, Tyr, His

Proton Donor: Asn, Gln, Lys, Arg, His, Trp

Proton Acceptor: Glu, Asp, Thr, Ser, Tyr, Asn, Gln

Within the scope of the invention is any polypeptide of the invention as described herein that contains one or more conservative or non-conservative substitutions provided the conjugate, or chimeric or fusion polypeptide of the present invention retains the ability to activate or induce proliferation of CD8+ and CD4+ T cells. Determining whether a conjugate, or chimeric or fusion polypeptide of the present invention that contains one or more conservative or non-conservative substitutions retains the ability to activate or induce proliferation of CD8+ and CD4+ T cells can be readily determined empirically, for example by any method described herein.

In any aspect of the present invention the conjugate, or chimeric or fusion polypeptide may comprise the following components in order from N-terminus to C-terminus:

an antigen presenting cell targeting moiety – X - a moiety capable of endosomal and/or lysosomal escape - an antigen.

Alternatively, the conjugate, or chimeric or fusion polypeptide may comprise the following components in order from N-terminus to C-terminus:

a moiety capable of endosomal and/or lysosomal escape - an antigen – X - an antigen presenting cell targeting moiety.

Alternatively, the conjugate, or chimeric or fusion polypeptide may comprise the following components in order from N-terminus to C-terminus:

an antigen - a moiety capable of endosomal and/or lysosomal escape – X - an antigen presenting cell targeting moiety.

In all instances above, X denotes that the antigen presenting cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen

In the conjugate, or chimeric or fusion polypeptides of the present invention, the C-terminal residue of the moiety capable of endosomal and/or lysosomal escape may

be covalently linked to the N-terminal residue of an antigen, or the N-terminal residue of the antigen may be covalently linked to the C-terminal residue of moiety capable of endosomal and/or lysosomal escape. In this arrangement, the moiety capable of endosomal and/or lysosomal escape and antigen, are said to be “directly linked” or “adjacent”.

In other embodiments, the conjugate or chimeric or fusion polypeptides include a linker for linking the moiety capable of endosomal and/or lysosomal escape to the antigen. The linker may be any linker, including both amino acid and non-amino acid linkers. Preferably, the linker is non-immunogenic. Suitable linkers may be up to 15 amino acids in length, although less than five amino acids is preferred. The linker may function to bring the moiety capable of endosomal and/or lysosomal escape to the antigen into a close spatial arrangement, alternatively, it may space the moiety capable of endosomal and/or lysosomal escape to the antigen apart. Preferably, the linker is not cleavable in an early endosome. Even more preferably, the linker is not cleavable in an endosomal compartment prior to maturation to a late endosome.

In other embodiments, the conjugate or chimeric or fusion polypeptides are displayed in the context of a virus-like particle, liposome or nanoparticle.

In a further non-limiting embodiment, the antigen and cell-penetrating peptide are associated with a nanoparticle.

In still a further non-limiting embodiment, the conjugate or chimeric or fusion polypeptide includes a sequence enabling enzymatic ligation, including for example, to link the antigen presenting cell targeting moiety and the moiety capable of endosomal and/or lysosomal escape. Examples of such sequences include the peptide “SPYCATCHER”, which has the sequence: SYHHHHHHHDYDIPTTENLYFQGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRD EDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITF TVNEQGQVTVNGKATKGDAHI (SEQ ID NO: 10) and “SPYTAG”: AHIVMVDAYKPTKG (SEQ ID NO: 9). The use of the “SPYCATCHER” and “SPYTAG” ligation system is described in detail in Zakeri et al., (2012), PNAS, 109:E690-E697, the entire contents of which are hereby incorporated by reference. Other systems for enzymatic ligation which

will be known to the skilled person include the use of butelase, asparaginyl peptidase or sortase.

The term "immune response" refers to an integrated bodily response to an antigen and in the present context preferably refers to an adaptive immune response including a cellular immune response. The immune response may be protective/preventive/prophylactic and/or therapeutic.

"Inducing an immune response" may mean that there was no immune response against a particular antigen before induction, but it may also mean that there was a certain level of immune response against a particular antigen before induction and after induction said immune response is enhanced. Thus, "inducing an immune response" also includes "enhancing an immune response". Preferably, after inducing an immune response in a subject, said subject is protected from developing a disease such as a cancer disease or the disease condition is ameliorated by inducing an immune response. For example, an immune response against a tumour expressed antigen may be induced in a patient having cancer or in a subject being at risk of developing cancer. Inducing an immune response in this case may mean that the disease condition of the subject is ameliorated, that the subject does not develop metastases, or that the subject being at risk of developing a cancer disease does not develop a cancer disease.

Antigen presenting cell targeting

The skilled person will appreciate that the conjugate, or chimeric or fusion polypeptide of the invention can be adapted to include any number of antigen presenting cell targeting moieties. Preferred moieties target professional antigen presenting cells such as dendritic cells.

"Antigen presenting cells" (APC) are cells which present peptide fragments of protein antigens in association with HLA molecules on their cell surface. Some APCs may activate antigen specific T cells.

"Antigen processing" or "processing" refers to the degradation of a polypeptide or antigen into procession products, which are fragments of said polypeptide or antigen (e.g., the degradation of a polypeptide into peptides) and the association of one or more

of these fragments (e.g., via binding) with HLA molecules for presentation by cells, preferably antigen presenting cells, to specific T cells.

Professional antigen-presenting cells are very efficient at internalizing antigen, either by phagocytosis or by receptor-mediated endocytosis, and then displaying a
5 fragment of the antigen, bound to a class II HLA molecule, on their membrane. The T cell recognizes and interacts with the antigen-class II HLA molecule complex on the membrane of the antigen-presenting cell. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T cell. The expression of co-stimulatory molecules is a defining feature of professional antigen-
10 presenting cells.

The main types of professional antigen-presenting cells are dendritic cells, which have the broadest range of antigen presentation, and are probably the most important antigen-presenting cells, macrophages, B -cells, and certain activated epithelial cells.

Dendritic cells (DCs) are leukocyte populations that present antigens captured in
15 peripheral tissues to T cells via both HLA class II and I antigen presentation pathways. It is well known that dendritic cells are potent inducers of immune responses and the activation of these cells is a critical step for the induction of antitumoral immunity.

The term "major histocompatibility complex" and the abbreviation "MHC" include MHC class I and MHC class II molecules and relate to a complex of genes which occurs
20 in all vertebrates. MHC proteins or molecules are important for signalling between lymphocytes and antigen presenting cells or diseased cells in immune reactions, wherein the MHC proteins or molecules bind peptides and present them for recognition by T cell receptors. The proteins encoded by the MHC are expressed on the surface of cells, and display both self antigens (peptide fragments from the cell itself) and non-self
25 antigens (e.g., fragments of invading microorganisms) to a T cell.

The MHC region is divided into three subgroups, class I, class II, and class III. MHC class I proteins contain an α (alpha)-chain and β 2 (beta2)-microglobulin (not part of the MHC encoded by chromosome 15). They present antigen fragments to cytotoxic T cells. On most immune system cells, specifically on antigen-presenting cells, MHC class
30 II proteins contain α - and β -chains and they present antigen fragments to T-helper cells.

MHC class III region encodes for other immune components, such as complement components and some that encode cytokines.

In humans, genes in the MHC region that encode antigen-presenting proteins on the cell surface are referred to as human leukocyte antigen (HLA) genes. However the
5 abbreviation MHC is often used to refer to HLA gene products. HLA genes include the nine so-called classical MHC genes: HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB 1.

In one preferred embodiment of all aspects of the invention an MHC molecule is an HLA molecule.

10 As used herein, an “antigen presenting cell targeting moiety” (or moieties) refers to a molecule(s) that has the ability, or capability, to localize to and bind a target molecule present on an antigen presenting cell. Similarly, a “dendritic cell targeting moiety” (or moieties) refers to a molecule(s) that has the ability to localize to and bind a target molecule present on a dendritic cell. In other words, conjugates, fusion proteins
15 or compositions of the invention comprising such an antigen presenting cell targeting moiety, for example a dendritic cell targeting moiety, can bind to a targeted cell or molecule (directly or indirectly). The targeting moieties of the invention contemplated for use with the biologically active agents include antibodies, polypeptides, peptides, aptamers, other ligands, or any combination thereof, that can bind a component of the
20 target cell or molecule. Exemplary dendritic cell targeting moieties are molecules that bind to any one of DEC-205 (CD205), Langerin (CD207), Clec9A receptors, DC-SIGN (CD209) and DNGR1. Preferably the antigen presenting cell is a professional antigen presenting cell. Even more preferably the professional antigen presenting cell is a dendritic cell or a macrophage.

25 The antigen presenting cell targeting moiety may localize to and bind a target molecule present on an antigen presenting cell. When an antigen presenting cell targeting moiety localizes to and binds to a target molecule on a dendritic cell, the targeting moiety may be referred to as a dendritic cell targeting moiety. The targeting moieties contemplated for use in any aspect of the invention include antibodies,
30 polypeptides, peptides, aptamers, other ligands, or any combination thereof, that can bind a component of the target cell or molecule.

In one example, the dendritic cell (DC)-targeting moiety is a ligand for a receptor presented on the surface of a DC. Exemplary dendritic cell targeting moieties are molecules that bind to any one of XCR1, DEC-205 (CD205), Langerin (CD207), Clec9A receptors, DC-SIGN (CD209), DNGR1 and Clec12A. A preferred dendritic cell targeting moiety binds to XCR1. Typically, a moiety that binds to XCR1 comprises, consists essentially of or consists of an amino acid sequence of XCL1 as described herein.

The antigen presenting cell targeting moiety may be associated with the moiety capable of endosomal escape via a linker which is cleavable in an endosome compartment prior to the endosome maturing into a late endosome. Preferably, the linker is cleavable in an early endosome. In one embodiment, the linker which is cleavable in an early endosome comprises, consists essentially of or consists of a cleavage site for an intracellular protease located in a lysosomal/endosomal compartment. The three main classes of intracellular proteases residing in the lysosomal/endosomal compartments and participating in antigen degradation are cysteine (cathepsin B, F, H, L, S, Z, and AEP, for asparaginylendopeptidase), aspartate (cathepsin D, E), and serine (cathepsin A, G) proteases. The protease nomenclature designates the amino acid of the protease active site that catalyzes hydrolysis of the substrate peptide bond.

Preferably, the linker is cleavable in an endosome, even more preferably an early endosome. In one embodiment, the linker which is cleavable in an early endosome comprises an amino acid sequence of a furin (also known as dibasic processing enzyme, Tachyplesin kexin, PACE, PCSK3 peptidase, prohormone convertase, SPC1, prohormone convertase 3, proprotein convertase subtilisin/kexin type 3) cleavage site. The furin cleavage site may comprise, consist essentially of or consist of the amino acid sequence R-X-K/R-R (SEQ ID NO: 1). Even more preferably, the furin cleavage site may comprise, consist essentially of or consist of the amino acid sequence RKKR (SEQ ID NO: 2).

The linker between the antigen presenting cell targeting moiety and the moiety capable of endosomal escape may also be cleavable in late stage or maturing endosome, or a lysosome. In one embodiment, the linker which is also cleavable in late stage or maturing endosome, or a lysosome comprises an amino acid sequence of a

cathepsin B cleavage site. The cathepsin B cleavage site may comprise, consist essentially of or consist of the amino acid sequence V-citrulline (SEQ ID NO: 3; V denotes the amino acid valine) or FKFL (SEQ ID NO: 4).

5 The linker between the antigen presenting cell targeting moiety and the moiety capable of endosomal escape may comprise, consist essentially of or consist of a furin cleavage site as described herein and a cathepsin B cleavage site as described herein, preferably RKKR (SEQ ID NO: 2) and V-citrulline (SEQ ID NO: 3). In one embodiment, the dual furin/cathepsin B cleavage linker comprises, consists essentially of or consists of the amino acid sequence RKKRXV-citrulline, where X is any small amino acid,
10 preferably a serine. In another embodiment, the dual furin/cathepsin B cleavage linker comprises, consists essentially of or consists of the amino acid sequence GSGTGGSTGRKKRSV-citrulline-GC (SEQ ID NO: 5).

In any aspect of the invention, the antigen presenting cell targeting moiety mediates internalization upon contact with an antigen presenting cell.

15 **Moieties capable of endosomal and/or lysosomal escape**

The term “cell penetrating peptide” (CPP) refers to a peptide that is capable of crossing a cellular membrane. In one example, a CPP is capable of translocating across a mammalian cell membrane and entering into a cell. In another example, a CPP may direct a conjugate to a desired subcellular compartment. Thus, a CPP may direct or
20 facilitate penetration of a molecule of interest across a phospholipid, mitochondrial, endosomal, lysosomal, vesicular, or nuclear membrane. CPPs that are able to “escape” the endosomal and lysosomal compartments for cytosolic delivery can be referred to as “cytosolic delivery cell penetrating peptides” as described herein. A CPP may be translocated across the membrane with its amino acid sequence complete and intact, or
25 alternatively partially degraded.

A CPP may direct a molecule of interest from outside a cell through the plasma membrane, and into the cytoplasm or a desired subcellular compartment. Alternatively, or in addition, a CPP may direct a molecule of interest across the blood-brain, trans-mucosal, hematoretinal, skin, gastrointestinal and/or pulmonary barriers.

The capability to translocate across membranes of a CPP may be energy dependent or independent, and/or receptor dependent or independent. In some examples, the CPP is a peptide which is demonstrated to translocate across a plasma membrane as determined by the methods described herein. CPPs encompass peptides that not only become internalized by cells, but also are efficiently able to escape endosomal and/or lysosomal compartments once internalized by cells. Some CPPs, such as TAT, exhibit low to moderate levels of endosomal escape, while others can exhibit superior levels of cytoplasmic delivery. The latter are also referred to as “cytosolic delivery cell penetrating peptides” (cytosolic delivery CPPs) as described herein.

The term “cytosolic delivery CPPs” refers to a subset of CPPs that in addition being able to mediate intracellular delivery, is also able to escape from endosomal and/or lysosomal compartments for delivery into cytosol.

Endosomes comprise three different compartments: early endosomes, late endosomes, and recycling endosomes. They are distinguished by the time it takes for endocytosed material to reach them, and by markers such as Rabs. They also have different morphology. Once endocytic vesicles have uncoated, they fuse with early endosomes. Early endosomes then mature into late endosomes before fusing with lysosomes.

In any aspect of the invention, the moiety capable of endosomal and/or lysosomal escape is capable of escaping from an endosomal and/or lysosomal compartment into the cytosol. Preferably, the moiety is capable of escaping from an endosomal and/or lysosomal compartment into the cytosol prior to the endosome maturing into a late endosome. More preferably, the moiety is capable of escaping from an early endosomal compartment into the cytosol.

In any aspect, the moiety capable of endosomal and/or lysosomal escape is a peptide. In one embodiment, the peptide is the TAT peptide, derived from the HIV virus: GRKKRRQRRRPQ (SEQ ID NO: 8). Preferably, the peptide comprises, consists essentially of, or consists of the amino acid sequence PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKR (SEQ ID NO: 6). Alternatively, the peptide may comprise, consist essentially of or consist of any one or

more of the amino acid sequences in WO2014205518 referred to therein as SEQ ID NO: 83-119 which are herein incorporated by reference.

In any aspect of the invention, the moiety capable of endosomal and/or lysosomal escape is associated with the antigen such that when the moiety escapes
5 from an endosomal and/or lysosomal compartment into the cytosol, the antigen also enters the cytosol. Typically, the moiety capable of endosomal and/or lysosomal escape and the antigen are chemically linked, for example, covalently linked. The covalent linkage may be direct or via a linker. The linker may comprise, consists essentially of or consist of a peptide, for example containing 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids.
10 Preferably the amino acids are selected from alanine, glycine and serine. In one embodiment, the peptide comprises, consists essentially of, or consists of the amino acid sequence GAS (SEQ ID NO: 7).

In any aspect of the invention, the moiety capable of endosomal and/or lysosomal escape mediates internalization upon contact with an antigen presenting cell.
15 In this embodiment, the moiety capable of endosomal and/or lysosomal escape may be a cell penetrating peptide as described herein but also have the capacity to escape from endosomal or lysosomal compartments for delivery into the cytosol. Those moieties having the capability of mediating intracellular delivery and escape from endosomal and/or lysosomal compartments for delivery into the cytosol may also be referred to as
20 cytosolic delivery cell penetrating peptide (cytosolic delivery CPPs).

An exemplary assay to measure peptide cell-penetration and cytosolic delivery, or endosomal or lysosomal escape may be by a fluorescent Beta-Lactamase enzymatic assay as described below in Example 5.

A moiety capable of endosomal and/or lysosomal escape may mediate
25 significantly greater endosomal escape than the TAT peptide. The degree or level of endosomal escape may be determined by any method known in the art, including any method or assay described herein, such as the the beta-lactamase described below in Example 5. For example, the level of endosomal escape may be determined using the beta-lactamase assay described herein. A moiety capable of endosomal and/or
30 lysosomal escape mediate significantly greater endosomal escape than the TAT peptide when measured using the beta-lactamase assay described herein. Alternatively, a

moiety capable of endosomal and/or lysosomal escape may result in greater than or equal to 5%, 10% or 15% positive cells at 450nM, at concentrations of 1µM, 2µM or 4µM respectively, when measured using the beta-lactamase assay described herein.

Antigens

5 An "antigen" according to the invention covers any substance that will elicit an immune response. In particular, an "antigen" relates to any substance, preferably a peptide or protein, that reacts specifically with antibodies or T-lymphocytes (T cells). According to the present invention, the term "antigen" comprises any molecule which comprises at least one epitope. Preferably, an antigen in the context of the present
10 invention is a molecule which, optionally after processing, induces an immune reaction, which is preferably specific for the antigen (including cells expressing the antigen). According to the present invention, any suitable antigen may be used, which is a candidate for an immune reaction, wherein the immune reaction is preferably a cellular immune reaction. In the context of the embodiments of the present invention, the
15 antigen is preferably presented by a cell, preferably by an antigen presenting cell which includes a diseased cell, in particular a cancer cell, in the context of MHC/HLA molecules, which results in an immune reaction against the antigen. An antigen is preferably a product which corresponds to or is derived from a naturally occurring antigen. Such naturally occurring antigens include tumour antigens.

20 Preferably, the antigen peptides according to the invention are MHC class I and/or class II presented peptides or can be processed to produce MHC class I and/or class II presented peptides. Preferably, the antigen peptides comprise an amino acid sequence substantially corresponding to the amino acid sequence of a fragment of an antigen.

25 In any aspect of the invention, the antigen comprises a peptide capable of being presented by an HLA class I molecule. Preferably, the antigen also comprises a peptide capable of being presented by an HLA class II molecule. Typically, the peptide capable of being presented by an HLA class I molecule comprises a CD8+ T cell epitope. Typically, the peptide capable of being presented by an HLA class II molecule
30 comprises a CD4+ T cell epitope. The antigen typically comprises a HLA-I-restricted T cell epitope and a HLA-II-restricted T cell epitope. Preferably the antigen comprises a

CD4+ T cell epitope and a CD8+ T cell epitope that result in activation and/or proliferation of a CD4+ T cell and a CD8+ T cell respectively. Exemplary antigens are any tumour associated antigens, including those described herein.

5 In any aspect of the invention, the antigen results in an immune response being raised against cells characterized by expression of the antigen and preferably by presentation of the antigen such as diseased cells, in particular cancer cells or infected cells (i.e. viral or bacterial infected).

In any aspect, the antigen may be a pathogen derived antigen. For example, an antigen from a virus, bacterium, parasite or fungi.

10 In a preferred embodiment, the antigen is a tumour antigen, i.e., a part of a tumour cell such as a protein or peptide expressed in a tumour cell which may be derived from the cytoplasm, the cell surface or the cell nucleus, in particular those which primarily occur intracellularly or as surface antigens of tumour cells. According to the present invention, a tumour antigen preferably comprises any antigen which is
15 expressed in and optionally characteristic with respect to type and/or expression level for tumours or cancers as well as for tumour or cancer cells. In one embodiment, the term "tumour antigen" or "tumour-associated antigen" relates to proteins that are under normal conditions specifically expressed in a limited number of tissues and/or organs or in specific developmental stages, for example, the tumour antigen may be under normal
20 conditions specifically expressed in stomach tissue, preferably in the gastric mucosa, in reproductive organs, e.g., in testis, in trophoblastic tissue, e.g., in placenta, or in germ line cells, and are expressed or aberrantly expressed in one or more tumour or cancer tissues. In this context, "a limited number" preferably means not more than 3, more preferably not more than 2. The tumour antigens in the context of the present invention
25 include, for example, differentiation antigens, preferably cell type specific differentiation antigens, i.e., proteins that are under normal conditions specifically expressed in a certain cell type at a certain differentiation stage, cancer/testis antigens, i.e., proteins that are under normal conditions specifically expressed in testis and sometimes in placenta, and germ line specific antigens. Preferably, the tumour antigen or the aberrant
30 expression of the tumour antigen identifies cancer cells. In the context of the present invention, the tumour antigen that is expressed by a cancer cell in a subject, e.g., a

patient suffering from a cancer disease, is preferably a self-protein in said subject. In preferred embodiments, the tumour antigen in the context of the present invention is expressed under normal conditions specifically in a tissue or organ that is non-essential, i.e., tissues or organs which when damaged by the immune system do not lead to death of the subject, or in organs or structures of the body which are not or only hardly accessible by the immune system.

According to the invention, the terms "tumor antigen", "tumor expressed antigen", "cancer antigen" and "cancer expressed antigen" are equivalents and are used interchangeably herein. Those skilled in the art will also appreciate that the terms "tumor antigen", "tumor expressed antigen", "cancer antigen" and "cancer expressed antigen" can include multiple tumor epitopes or antigens encoded within the same contiguous sequence.

Exemplary antigens include those: (a) comprising the minimal HLA-class-I restricted T cell epitopes described in Sachin et al. Nature (2017) 547:222-226, preferably in the Extended Data Table 3; (b) comprising the class I epitopes described in Ott et al. Nature (2017) 547: 217-221; (c) comprising the peptide neoantigens described in Creaney et al. (2015) Oncoimmunology. 4(7):e1011492; (d) comprising the neoantigens described in McGranahan et al. Science (2016) 351(6280):1463-9; comprising the neoantigens described in Rizvi et al. (2015) 351(6280):1463-9.

If a peptide is to be presented directly, i.e., without processing, in particular without cleavage, it has a length which is suitable for binding to an HLA molecule, in particular a class I HLA molecule, and preferably is 7-20 amino acids in length, more preferably 7-12 amino acids in length, more preferably 8-11 amino acids in length, in particular 9 or 10 amino acids in length.

If a peptide is part of a larger entity comprising additional sequences and is to be presented following processing, in particular following cleavage, the peptide produced by processing has a length which is suitable for binding to an HLA molecule, in particular a class I HLA molecule, and preferably is 7-20 amino acids in length, more preferably 7-12 amino acids in length, more preferably 8-11 amino acids in length, in particular 9 or 10 amino acids in length. Preferably, the sequence of the peptide which is to be presented following processing is derived from the amino acid sequence of an

antigen, i.e., its sequence substantially corresponds and is preferably completely identical to a fragment of an antigen.

Peptides having amino acid sequences substantially corresponding to a sequence of a peptide which is presented by the class I HLA may differ at one or more residues that are not essential for T cell receptor (TCR) recognition of the peptide as presented by the class I HLA, or for peptide binding to HLA. Such substantially corresponding peptides are also capable of stimulating an antigen-responsive CTL and may be considered immunologically equivalent.

An antigen peptide when presented by HLA should be recognizable by a T cell receptor. Preferably, the antigen peptide if recognized by a T cell receptor is able to induce in the presence of appropriate co-stimulatory signals, clonal expansion of the T cell carrying the T cell receptor specifically recognizing the antigen peptide. Preferably, antigen peptides, in particular if presented in the context of HLA molecules, are capable of stimulating an immune response, preferably a cellular response against the antigen from which they are derived or cells characterized by expression of the antigen and preferably characterized by presentation of the antigen. Preferably, an antigen peptide is capable of stimulating a cellular response against a cell characterized by presentation of the antigen with class I HLA and preferably is capable of stimulating an antigen-responsive CTL. Activation and/or expansion of CD4+ or CD8+ T cells may be determined by any method known in the art, including any method described herein.

The term "epitope" refers to an antigenic determinant in a molecule such as an antigen, i.e., to a part in or fragment of the molecule that is recognized by the immune system, for example, that is recognized by a T cell, in particular when presented in the context of HLA molecules. An epitope of a protein such as a tumour antigen preferably comprises a continuous or discontinuous portion of said protein and is preferably between 5 and 100, preferably between 5 and 50, more preferably between 8 and 30, most preferably between 10 and 25 amino acids in length, for example, the epitope may be preferably 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length. It is particularly preferred that the epitope in the context of the present invention is a T cell epitope.

Protein Production

The chimeric or fusion proteins of the invention may be produced by recombinant expression systems (such as recombinant DNA technology) or by chemical synthesis (such as solid phase peptide synthesis), or by a combination thereof and joined via chemical or enzymatic ligation chemistry. These techniques are well known in the art.

The present invention provides a nucleic acid molecule including a nucleotide sequence encoding a conjugate, or chimeric or fusion polypeptide of the invention as described herein, optionally operatively linked to at least one regulatory element. In one embodiment the nucleic acid is provided in isolated or substantially purified form.

The nucleic acid molecule may, for example, be inserted into a suitable expression vector for production of the chimeric or fusion polypeptide as a recombinant protein by insertion of the expression vector into a prokaryotic or eukaryotic host cell. Successful expression of the recombinant protein requires that the expression vector contains the necessary regulatory elements for transcription and translation which are compatible with, and recognised by the particular host cell system used for expression. A variety of host cell systems may be utilized to express the recombinant protein, which include, but are not limited to bacteria transformed with a bacteriophage vector, plasmid vector, or cosmid DNA; yeast containing yeast vectors; fungi containing fungal vectors; insect cell lines infected with virus (e.g. baculovirus); and mammalian cell lines transfected with plasmid or viral expression vectors, or infected with recombinant virus (e.g. vaccinia virus, adenovirus, adeno-associated virus, retrovirus, etc).

Using methods known in the art of molecular biology, various promoters and enhancers can be incorporated into the expression vector, to increase the expression of the recombinant protein, provided that the increased expression of the amino acid sequences is compatible with (for example, non-toxic to) the particular host cell system used.

The selection of the promoter will depend on the expression system used. Promoters vary in strength, i.e. ability to facilitate transcription. Generally, it is desirable to use a strong promoter in order to obtain a high level of transcription of the coding nucleotide sequence and expression into recombinant protein. For example, bacterial,

phage, or plasmid promoters known in the art from which a high level of transcription have been observed in a host cell system including *E. coli* include the lac promoter, trp promoter, recA promoter, ribosomal RNA promoter, the P_R and P_L promoters, lacUV5, ompF, bla, lpp, and the like, may be used to provide transcription of the inserted
5 nucleotide sequence encoding amino acid sequences.

Other control elements for efficient transcription or translation include enhancers, and regulatory signals. Enhancer sequences are DNA elements that appear to increase transcriptional efficiency in a manner relatively independent of their position and orientation with respect to a nearby coding nucleotide sequence. Thus, depending on
10 the host cell expression vector system used, an enhancer may be placed either upstream or downstream from the inserted coding sequences to increase transcriptional efficiency. Other regulatory sites, such as transcription or translation initiation signals, can be used to regulate the expression of the coding sequence.

In the case of a recombinant protein, nucleic acid encoding same can be cloned
15 into expression constructs or vectors, which are then transfected into host cells, such as *E. coli* cells, yeast cells, insect cells, or mammalian cells, such as simian COS cells, Chinese Hamster Ovary (CHO) cells, human embryonic kidney (HEK) cells, or myeloma cells that do not otherwise produce the protein. Exemplary cells used for expressing a protein are CHO cells, myeloma cells or HEK cells. Molecular cloning techniques to
20 achieve these ends are known in the art and described, for example in Ausubel *et al.*, (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) or Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989). A wide variety of cloning and *in vitro* amplification methods are suitable for the construction of
25 recombinant nucleic acids. Methods of producing recombinant antibodies are also known in the art, see, e.g., US4816567 or US5530101.

As used herein, the term "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with
30 or without additional regulatory elements (e.g., upstream activating sequences, transcription factor binding sites, enhancers and silencers) that alter expression of a

nucleic acid, e.g., in response to a developmental and/or external stimulus, or in a tissue specific manner. In the present context, the term "promoter" is also used to describe a recombinant, synthetic or fusion nucleic acid, or derivative which confers, activates or enhances the expression of a nucleic acid to which it is operably linked.

- 5 Exemplary promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or temporal expression of said nucleic acid.

As used herein, the term "operably linked to" means positioning a promoter relative to a nucleic acid such that expression of the nucleic acid is controlled by the
10 promoter.

Many vectors for expression in cells are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, a sequence encoding a protein (e.g., derived from the information provided herein), an enhancer element, a promoter, and a transcription termination sequence. The skilled
15 artisan will be aware of suitable sequences for expression of a protein. Exemplary signal sequences include prokaryotic secretion signals (e.g., pelB, alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II), yeast secretion signals (e.g., invertase leader, α factor leader, or acid phosphatase leader) or mammalian secretion signals (e.g., herpes simplex gD signal).

20 Exemplary promoters active in mammalian cells include cytomegalovirus immediate early promoter (CMV-IE), human elongation factor 1- α promoter (EF1), small nuclear RNA promoters (U1a and U1b), α -myosin heavy chain promoter, Simian virus 40 promoter (SV40), Rous sarcoma virus promoter (RSV), Adenovirus major late promoter, β -actin promoter; hybrid regulatory element comprising a CMV enhancer/
25 β -actin promoter or an immunoglobulin promoter or active fragment thereof. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; baby hamster kidney cells (BHK, ATCC CCL 10); or Chinese hamster ovary cells (CHO).

30 Typical promoters suitable for expression in yeast cells such as for example a yeast cell selected from the group comprising *Pichia pastoris*, *Saccharomyces*

cerevisiae and *S. pombe*, include, but are not limited to, the *ADH1* promoter, the *GAL1* promoter, the *GAL4* promoter, the *CUP1* promoter, the *PHO5* promoter, the *nmt* promoter, the *RPR1* promoter, or the *TEF1* promoter.

Means for introducing the isolated nucleic acid or expression construct comprising same into a cell for expression are known to those skilled in the art. The technique used for a given cell depends on the known successful techniques. Means for introducing recombinant DNA into cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

The host cells used to produce the protein may be cultured in a variety of media, depending on the cell type used. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing mammalian cells. Media for culturing other cell types discussed herein are known in the art.

Isolation of Proteins

Methods for isolating a protein are known in the art and/or described herein.

Where a conjugate, or chimeric or fusion polypeptide is secreted into culture medium, supernatants from such expression systems can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants. Alternatively, or additionally, supernatants can be filtered and/or separated from cells expressing the protein, e.g., using continuous centrifugation.

The conjugate, or chimeric or fusion polypeptide prepared from the cells can be purified using, for example, ion exchange, hydroxyapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, affinity chromatography (e.g.,

protein A affinity chromatography or protein G chromatography), or any combination of the foregoing. These methods are known in the art and described, for example in WO99/57134 or Ed Harlow and David Lane (editors) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988).

5 The skilled artisan will also be aware that a protein can be modified to include a tag to facilitate purification or detection, e.g., a poly-histidine tag, e.g., a hexa-histidine tag, or a influenza virus hemagglutinin (HA) tag, or a Simian Virus 5 (V5) tag, or a FLAG tag, or a glutathione S-transferase (GST) tag. The resulting protein is then purified using methods known in the art, such as, affinity purification. For example, a protein
10 comprising a hexa-his tag is purified by contacting a sample comprising the protein with nickel-nitrilotriacetic acid (Ni-NTA) that specifically binds a hexa-his tag immobilized on a solid or semi-solid support, washing the sample to remove unbound protein, and subsequently eluting the bound protein. Alternatively, or in addition a ligand or antibody that binds to a tag is used in an affinity purification method.

15 The skilled person will also be familiar with the use of appropriate tags to allow for enzymatic ligation, including, but not limited to the use of the "SPYCATCHER" and/or the SPYTAG sequence motifs (as shown in SEQ ID NO: 10 and 9, respectively). Other systems for enzymatic ligation which will be known to the skilled person include the use of butelase, asparaginyl peptidase or sortase.

20 **Conditions to be Treated**

 The conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the present invention as described herein are useful in the treatment or prevention of any hyperproliferative condition. An example of a condition is cancer. Exemplary cancers include cystic and solid tumors, bone and soft tissue tumors, including tumors in anal
25 tissue, bile duct, bladder, blood cells, bowel, brain, breast, carcinoid, cervix, eye, esophagus, head and neck, kidney, larynx, leukemia, liver, lung, lymph nodes, lymphoma, melanoma, mesothelioma, myeloma, ovary, pancreas, penis, prostate, skin (e.g. squamous cell carcinoma), sarcomas, stomach, testes, thyroid, vagina, vulva. Soft tissue tumors include Benign schwannoma Monosomy, Desmoid tumor, lipo-blastoma,
30 lipoma, uterine leiomyoma, clear cell sarcoma, dermatofibrosarcoma, Ewing sarcoma, extraskeletal myxoid chondrosarcoma, liposarcoma myxoid, Alveolar

rhabdomyosarcoma and synovial sarcoma. Specific bone tumors include nonossifying fibroma, unicameral bone cyst, enchondroma, aneurismal bone cyst, osteblastoma, chondroblastoma, chondromyxofibroma, ossifying fibroma and adamantinoma, Giant cell tumor, fibrous dysplasia, Ewing's sarcoma eosinophilic granuloma, osteosarcoma, chondroma, chondrosarcoma, malignant fibrous histiocytoma and metastatic carcinoma. Leukemias include acute lymphoblastic, acute myeloblastic, chronic lymphocytic and chronic myeloid.

Other examples include breast tumors, colorectal tumors, adenocarcinomas, mesothelioma, bladder tumors, prostate tumors, germ cell tumor, hepatoma/cholangio, carcinoma, neuroendocrine tumors, pituitary neoplasm, small round cell tumor, squamous cell cancer, melanoma, atypical fibroxanthoma, seminomas, nonseminomas, stromal leydig cell tumors, Sertoli cell tumors, skin tumors, kidney tumors, testicular tumors, brain tumors, ovarian tumors, stomach tumors, oral tumors, bladder tumors, bone tumors, cervical tumors, esophageal tumors, laryngeal tumors, liver tumors, lung tumors, vaginal tumors and Wilm's tumor.

Further, the conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the present invention as described herein are useful in the treatment or prevention of any infectious disease. As used herein an infectious disease is a condition that is associated with or caused by an infectious agent. Typically, an infectious agent is a virus, bacterium, parasite or fungi. Exemplary virus is influenza and Herpes Simplex Virus (HSV).

The words 'treat' or 'treatment' refer to therapeutic treatment wherein the object is to slow down (lessen) an undesired physiological change or disorder. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. 'Treatment' can also mean prolonging survival as compared to expected survival if not receiving treatment. Treatment may not necessarily result in the complete clearance of cancer or infected cells but may reduce or minimise complications and side effects of infection, or the presence or progression of cancer.

The success or otherwise of treatment may be monitored by physical examination of the subject, cytopathological, serological DNA, or mRNA detection techniques.

"Preventing", "prevention", "preventative" or "prophylactic" refers to keeping from occurring, or to hinder, defend from, or protect from the occurrence of a condition, disease, disorder, or phenotype, including an abnormality or symptom. A subject in
5 need of prevention may be prone to develop the condition.

The present invention also includes methods of preventing the development of cancer in a subject. For example the subject for whom prevention of cancer is required may be considered to be at risk of developing cancer, but does not yet have detectable
10 cancer. A subject at risk of the development of cancer may be a subject with a family history of cancer, and/or a subject for whom genetic testing or other testing indicates a high risk or high likelihood of the development of cancer. The subject may have cancer stem cells but does not yet have any detectable tumours. It will be understood that methods of preventing the development of cancer include methods of delaying the
15 onset of cancer in a subject.

The term "ameliorate" or "amelioration" refers to a decrease, reduction or elimination of a condition, disease, disorder, or phenotype, including an abnormality or symptom. A subject in need of treatment may already have the condition, or may be prone to have the condition or may be one in whom the condition is to be prevented.

20 In some examples, a method of the present invention comprises administering a prophylactically or therapeutically effective amount of a conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention as described herein.

The phrase 'therapeutically effective amount' generally refers to an amount of a compound of the present invention that (i) treats the particular disease, condition, or
25 disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease, condition, or disorder, or (iii) delays the onset of one or more symptoms of the particular disease, condition, or disorder described herein. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art and described above,
30 adjustments for systemic versus localized delivery, age, body weight, general health,

sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

As used herein, the term “prophylactically effective amount” shall be taken to mean a sufficient quantity of a protein to prevent or inhibit or delay the onset of one or more detectable symptoms of a clinical condition. The skilled artisan will be aware that such an amount will vary depending on, for example, the specific antigen binding site(s) administered and/or the particular subject and/or the type or severity or level of condition and/or predisposition (genetic or otherwise) to the condition. Accordingly, this term is not to be construed to limit the present invention to a specific quantity, e.g., weight or amount of antigen binding site(s), rather the present invention encompasses any amount of the antigen binding site(s) sufficient to achieve the stated result in a subject.

Suitable dosages of a conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention as described herein will vary depending on the specific the condition to be treated and/or the subject being treated. It is within the ability of a skilled physician to determine a suitable dosage, e.g., by commencing with a sub-optimal dosage and incrementally modifying the dosage to determine an optimal or useful dosage. Alternatively, to determine an appropriate dosage for treatment/prophylaxis, data from the cell culture assays or animal studies are used, wherein a suitable dose is within a range of circulating concentrations that include the ED_{50} of the active compound with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. A therapeutically/prophylactically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration or amount of the compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma maybe measured, for example, by high performance liquid chromatography.

It will be clearly understood that, although this specification refers specifically to applications in humans, the invention is also useful for veterinary purposes. Thus in all aspects the invention is useful for domestic animals such as cattle, sheep, horses and poultry; for companion animals such as cats and dogs; and for zoo animals. Therefore, the general term "subject" or "subject to be / being treated" is understood to include all animals (such as humans, apes, dogs, cats, horses, and cows) that require an enhanced immune response, for example subjects having cancer.

The term "administered" means administration of a therapeutically effective dose of the aforementioned conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the present invention as described herein to the subject.

Subjects requiring treatment include those already having a benign, pre-cancerous, or non-metastatic tumour as well as those in which the occurrence or recurrence of cancer is to be prevented. Subjects may have metastatic cells, including metastatic cells present in the ascites fluid and/or lymph node.

A subject in need of treatment may be one diagnosed with, or at risk of developing, any one of the cancers described herein.

In any method of the invention, one or more of the following effects may be observed: reduction in the reoccurrence of malignant tumours, reduction in metastasis of malignant tumours, reduction in number or size of tumours, differentiation of tumour cells, expression of β -catenin and E-cadherin in malignant tumours to facilitate cell-to-cell adhesion and reduction in metastasis, reduction in tumour cells ability to prevent immunorecognition.

The objective or outcome of treatment may be to reduce the number of cancer cells; reduce the primary tumour size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumour metastasis; inhibit, to some extent, tumour growth; and/or relieve to some extent one or more of the symptoms associated with the disorder.

Efficacy of treatment can be measured by assessing the duration of survival, time to disease progression, the response rates (RR), duration of response, and/or quality of life.

The method is particularly useful for extending time to disease progression.

The method is particularly useful for extending survival of the human, including overall survival as well as progression free survival.

5 The method is particularly useful for providing a complete response to therapy whereby all signs of cancer in response to treatment have disappeared. This does not always mean the cancer has been cured.

The method is particularly useful for providing a partial response to therapy whereby there has been a decrease in the size of one or more tumours or lesions, or in the extent of cancer in the body, in response to treatment.

10 The objective or outcome of treatment may be any one or more of the following:

- to reduce the number of cancer cells;
- reduce the primary tumour size;
- inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs;
- 15 - inhibit (i.e., slow to some extent and preferably stop) tumour metastasis;
- inhibit, to some extent, tumour growth;
- relieve to some extent one or more of the symptoms associated with the disorder.

20 In one embodiment, subjects requiring treatment include those having a benign, pre-cancerous, non-metastatic tumour.

In one embodiment, the cancer is pre-cancerous or pre-neoplastic.

25 In one embodiment, the cancer is a secondary cancer or metastases. The secondary cancer may be located in any organ or tissue, and particularly those organs or tissues having relatively higher hemodynamic pressures, such as lung, liver, kidney, pancreas, bowel and brain. The secondary cancer may be detected in the ascites fluid and/or lymph nodes.

In one embodiment, the cancer may be substantially undetectable.

"Pre-cancerous" or "pre-neoplasia" generally refers to a condition or a growth that typically precedes or develops into a cancer. A "pre -cancerous" growth may have cells that are characterized by abnormal cell cycle regulation, proliferation, or
5 differentiation, which can be determined by markers of cell cycle.

In one embodiment, the cancer is pre-cancerous or pre -neoplastic.

In one embodiment, the cancer is a secondary cancer or metastases. The secondary cancer may be located in any organ or tissue, and particularly those organs or tissues having relatively higher hemodynamic pressures, such as lung, liver, kidney,
10 pancreas, bowel and brain.

In the context of an infectious disease the objective or outcome of treatment may be a reduction in viral, bacterial or fungal titre or symptom associated with the disease.

Compositions

The phrase 'pharmaceutically acceptable' indicates that the substance or
15 composition must be compatible chemically and/or toxicologically, with the other ingredients comprising a formulation, and/or the subject being treated therewith.

Various adjuvants are known for use in conjunction with vaccine compositions. The adjuvants aid by modulating the immune response and in attaining a more durable and higher level of immunity using smaller amounts of vaccine antigen or fewer doses
20 than if the vaccine antigen were administered alone. Examples of adjuvants include incomplete Freund's adjuvant (IFA), Adjuvant 65 (containing peanut oil, mannide monooleate and aluminium monostearate), oil emulsions, Ribi adjuvant, the pluronic polyols, polyamines, Avridine, Quil A, saponin, MPL, QS-21, mineral gels such as aluminium salts and calcium salts, nanoparticles such as hydroxyapatite, calcium
25 phosphate, aluminium salts, sugar oligomers and polymers such as mannan, chitosan. Other examples include oil in water emulsions such as SAF-1, SAF-0, MF59, Seppic ISA720, and other particulate adjuvants such ISCOMsTM and ISCOM matrixTM. An extensive but not exhaustive list of other examples of adjuvants are listed in Cox and Coulter 1992 [In: Wong WK (ed.) *Animals parasite control utilising technology*. Bocca

Raton; CRC press, 1992; 49-112]. In addition to the adjuvant, the vaccine composition may include conventional pharmaceutically acceptable carriers, excipients, fillers, buffers or diluents as appropriate.

The skilled person will also be familiar with compounds or compositions that are known to bolster anti-tumor T cell immunity such as including, but not limiting to co-administration with checkpoint blockade drugs. Consequently, also contemplated are co-administration of an inhibitor of immune checkpoint regulators such as PD-1, PDL-1 etc. As such, the present invention also includes the administration of a vaccine composition as herein described, in conjunction with such an inhibitor.

Many methods are known for administration of a vaccine composition to a subject, including but not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, sub-lingual, buccal and oral administration. These routes of administration are particularly useful for vaccination. Other routes include delivery into tumors, subcutaneous delivery, and intranodal,

In another embodiment, a vector comprising a nucleic acid molecule of the invention as described herein may be a viral or bacterial vaccine vector, and used to provide a recombinant viral vaccine, a recombinant bacterial vaccine, a recombinant attenuated bacterial vaccine, or an inactivated recombinant viral vaccine. Vaccinia virus is the best known example, in the art, of an infectious virus that is engineered to express vaccine antigens derived from other organisms. The recombinant live vaccinia virus, which is attenuated or otherwise treated so that it does not cause disease by itself, is used to immunize the host. Subsequent replication of the recombinant virus within the host provides a continual stimulation of the immune system with the vaccine antigens thereby providing long lasting immunity.

The skilled person will also be aware that virus-like particles, liposomes, nanoparticles can be substituted for live viruses, particularly in vaccines with applications such as described herein.

The invention also provides for the use of a nucleic acid molecule including a nucleotide sequence encoding a conjugate, or chimeric or fusion polypeptide of the invention directly as the vaccine formulation. Nucleotide sequences encoding the

chimeric or fusion polypeptides, operatively linked to one or more regulatory elements, can be introduced directly to vaccinate a subject ("direct gene transfer"). Direct gene transfer into a vaccinated subject, resulting in expression of the genetic material by the vaccinated subject's cells such as vascular endothelial cells as well as the tissue of the major organs, has been demonstrated by techniques in the art such as by injecting intravenously an expression plasmid:cationic liposome complex [Zhu *et al.*, 1993, *Science* 261:209-211]. Other effective methods for delivering vector DNA into a target cell are known in the art. In one example, purified recombinant plasmid DNA containing viral genes has been used to inoculate (whether parenterally, mucosally, or via gene-gun immunization) vaccines to induce a protective immune response [Fynan *et al.* 1993, *Proc Natl Acad Sci USA* 90:11478-11482]. In another example, cells removed from a subject can be transfected or electroporated by standard procedures known in the art, resulting in the introduction of the recombinant vector DNA into the target cell. Cells containing the recombinant vector DNA may then be selected for using methods known in the art, such as by use of a selection marker expressed in the vector, and the selected cells may then be re-introduced into the subject to express the recombinant protein.

The pharmaceutical compositions of this invention are particularly useful for parenteral administration, such as intravenous administration. The compositions for administration will commonly comprise a solution of a conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention dissolved in a pharmaceutically acceptable carrier, for example an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of a conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. Exemplary carriers include water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as mixed oils and ethyl oleate may also be used. Liposomes may also be used as carriers. The vehicles

may contain minor amounts of additives that enhance isotonicity and chemical stability, e.g., buffers and preservatives.

Kits

The present invention additionally comprises a kit comprising one or more of the following a conjugate, chimeric or fusion polypeptide, composition or nucleic acid of the invention as described herein.

In the case of a kit for therapeutic/prophylactic use, the kit can additionally comprise a pharmaceutically acceptable carrier.

Optionally a kit of the invention is packaged with instructions for use in a method or use of the invention as described herein.

Examples

Example 1: Methods for construction of peptide vaccines

Protein expression:

The muXCL1-SpyC DNA sequence was synthesized and cloned (ATUM) into NcoI and XhoI sites of pET28a+ vector (Merck Millipore). Recombinant proteins were expressed as 6xHis-N-terminally tagged fusion proteins in *E. coli* strain BL21 (DE3) Gold (Agilent Technologies). The protein was purified using IMAC as previously described (Milech et al 2015, Sci Rep 5, 18329) with an additional Ion Exchange Chromatography (IEX).

The purification step was performed after IMAC. The protein was desalted into 20 mM Tris, pH 8.0 binding buffer and purified through a HiTrap Q HP 5mL column (GE Healthcare) and were eluted using a 0-1M NaCl gradient. The final protein was desalted into PBS pH 7.4, and purity was confirmed by analysis on 4-16 % SDS-PAGE stained with Gel Code Blue Reagent.

Peptide Synthesis:

Peptides were synthesized by Pepscan (Netherlands).

Synthesis of V5-SpyTag-Antigen peptides:*Peptide synthesis:*

Peptide 1: V5-SpyTag-Cys (~15mg at 90%) (32 Amino Acids)

Ac-GKPIPNNLLGLDSTGASAHIVMVDAYKPTKGC-nh2

5 Peptide 2: mcpa-1746-gDgB (~5 mg at 90%) (66 Amino Acids)

(Mercapto propionic acid) -

PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKRGASIPPNNWHIPSIQDAGTGSSIEFARL-nh2

Peptide 3: mcpa-PYC35-gDgB (~5 mg at 90%) (46 Amino Acids)

10 (Mercapto propionic acid) -

AYQSIRSGGIESSSKRER GASIPPNNWHIPSIQDAGTGSSIEFARL-nh2

Peptide 4: mcpa-TAT-gDgB (~5 mg at 90%) (40 Amino Acids)

(Mercapto propionic acid) -

GRKKRRQRRRPQGASIPPNNWHIPSIQDAGTGSSIEFARL-nh2

15 *Peptide Conjugations:*

Conjugation 1: Cysteine Oxidation (disulphide linkage) of V5-SpyTag-Cys + mcpa-1746-gDgB

[Peptide 1 + Peptide 2] (98 Amino Acids)

Ac-GKPIPNNLLGLDSTGASAHIVMVDAYKPTKG-C-C(mcpa)-

20 PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKRGASIPPNNWHIPSIQDAGTGSSIEFARL-nh2

Conjugation 2: Cysteine Oxidation (disulphide linkage) of V5-SpyTag-Cys + mcpa-PYC35-gDgB

[Peptide 1 + Peptide 3] (78 Amino Acids)

Ac-GKPIPPELLGLDSTGASAHIVMVDAYKPTKG-C-C(mcpa)-
AYQSIRSGGIESSSKRERGASIPPNNWHIPSIQDAGTGSSIEFARL-nh2

Conjugation 3: Cysteine Oxidation (disulphide linkage) of V5-SpyTag-Cys +
mcpa-TAT-gDgB

5 [Peptide 1 + Peptide 4] (72 Amino Acids)

Ac-GKPIPPELLGLDSTGASAHIVMVDAYKPTKG-C-C(mcpa)-
GRKKRRQRRRPQGASIPPNNWHIPSIQDAGTGSSIEFARL-nh2

SpyCatcher/ SpyTag Conjugations

10 All conjugations were set up at a SpyCatcher: SpyTag ratio of 1 : 1.1. SpyC
proteins and SpyTag peptides were mixed and incubated for 30 minutes at room
temperature and then left at 4°C overnight. Conjugation efficiencies were analyzed on 4-
16 % SDS-PAGE gels stained with Gel Code Blue Reagent (Thermo Fisher Scientific).

Rationale:

1. Targeting:

15 Cross-presenting DCs are an important cell uniquely capable of presenting
external antigens on both MHC-I and –II, thus activating both CD8+ and CD4+ T cells to
the dangers of a new infection. These cross-presenting DCs exclusively express a cell-
surface marker called XCR1, which allows us to specifically target them using its
endogenous ligand XCL1. Coupling XCL1 in a peptide vaccine construct (eg: XCL1-
20 cytosolic delivery CPP-Antigen) targets the vaccine to the cross-presenting XCR1+
DCs.

2. Internalisation, endosomal escape and MHC loading: cytosolic delivery CPP
and XCR1 ligand facilitate internalisation into cells

25 Upon binding of the peptide vaccine XCL1- cytosolic delivery CPP-Ag, to the
XCR1 cell surface marker, the vaccine is internalised by endocytosis inside the cell
within an organelle called the endosome. Once inside the endosome, the cytosolic
delivery CPP-Ag component is decoupled from the targeting XCL1 component. Two

outcomes can now occur: a proportion of the released cytosolic delivery CPP-Ag component can escape into the cytoplasm of the DC where it is loaded onto MHC-I, which is recognized by CD8⁺ T cells. A proportion of the cytosolic delivery CPP-Antigen is located within the maturing to late stage endosome, where it is loaded onto MHC-II via an endosomal processing pathway which is recognised by CD4⁺ T cells.

Only endosomal escaping cell penetrating peptides will result in CD8⁺ T cell expansion via MHC I loading.

Figure 1 provides a schematic of the proposed pathway for uptake of the conjugates, chimeric or fusion polypeptides of the invention.

10 **Example 2: Disulfide-linked CPP antigen evokes a gDT-specific CD4⁺ T cell expansion but no gBT-specific CD8⁺ expansion**

Peptide vaccines were constructed as shown in Figures 2 to 4. SpyC/SpyT ligation was confirmed by SDS-PAGE (see Figure 5). A disulphide bond may not be cleaved in a cross-presenting DCs endosome due to unique neutral pH in this intracellular compartment. The disulphide bond would typically be cleaved in an acidic environment. As a result, the detectable label will remain bound to the endosomal escape peptide (1746: SEQ ID NO: 6) such that the endosomal escape peptide and associated antigen cannot escape the endosome and therefore cannot enter the cytosol.

20 **gBT.I:** The CD8⁺ T cells in this TCR transgenic animal express a H-2K^b-restricted T cell receptor specific for herpes simplex virus (HSV) glycoprotein B (gB). (Mueller SN, Immunol Cell Biology 2002)

gDT.II: The CD4⁺ T cells in this TCR transgenic animal express a I-A^b-restricted T cell receptor specific for HSV glycoprotein D (gD). (Bedoui S, Nat Immunol 2009)

25 *Peptide vaccine: administration schedule*

On day -2, 5×10^5 gDT.II & 1×10^5 gBT.I transgenic T cells were adoptively transferred via intravenous injection.

On day 0 (priming), 3 x B6 mice were injected with:

- XCL1-SpyC/V5-SpyT-PYC35-gDgB;
- XCL1-SpyC/V5-SpyT-TAT-gDgB;
- XCL1-SpyC/V5-SpyT-1746-gDgB; or
- Poly I:C

5 Peptide concentration was 10 μ M. The peptide amount per injection was 0.5 nmol. The Poly I:C concentration was 1 μ g/ μ l. The Injection volume was 50 μ l.

Poly I:C and peptides were delivered subcutaneously.

10 The injections were repeated on day 14 (boosting). In addition, HSV was inoculated on the flank skin. A schematic of the experimental protocol is provided in Figure 6.

Disulfide-linked vaccine constructs do not elicit gB-specific T cell expansion. Disulfide-linked vaccine constructs elicit gD-specific T cell expansion. (Figure 7). This highlights the importance of a cleavable linker in this conjugate / fusion polypeptide design to allow endosomal escape.

15 **Example 3: Assembly and efficacy of a peptide vaccine comprising a cleavable linker and a moiety capable of endosomal escape**

Figures 8 to 10 show the approach to design and construction of a peptide vaccine comprising a cleavable linker moiety linking the dendritic cell targeting moiety and endosomal escape peptide-antigen moiety.

20 The final peptide vaccine construct comprised a dendritic cell targeting moiety (in the form of XCL-1), a cleavable linker (in the form of a dual furin-cathepsin B (FCB) cleavable linker comprised of a Furin recognition sequence and V-citrulline, linked to cysteine), and a moiety capable of endosomal escape (1746) and associated antigen (gD and gB).

25 The peptide vaccine was injected into mice using a similar protocol to that described in Example 2. Briefly:

On day -2, 1×10^4 gDT.II transgenic T cells were adoptively transferred via intravenous injection.

On day 0 (priming), 3 x B6 mice were injected with:

- XCL1-SpyC/SpyT-FCB-gDgB;
- 5 • XCL1-SpyC/SpyT-FCB-AVI-gDgB;
- XCL1-SpyC/SpyT-FCB-TAT-gDgB;
- XCL1-SpyC/SpyT-FCB-1746-gDgB; or
- Poly I:C

Peptide concentration was 10 μ M. The peptide amount per injection was 0.5
10 nmol. The Poly I:C concentration was 1 μ g/ μ l. The Injection volume was 50 μ l.

Poly I:C and peptides were delivered subcutaneously.

The injections were repeated on day 14 (boosting). In addition, HSV was inoculated on the flank skin. A schematic of the experimental protocol is provided in Figure 11.

15 The results (presented in Figure 12 and 13) demonstrate that the use of a cleavable linker facilitates induction of both CD4⁺ and CD8⁺ T cell proliferation (compare to results in Example 2).

Moreover, the use of a cytosolic delivery cell penetrating peptide (CPP, in the form of “1746”) provided a superior induction of CD8⁺ T cell proliferation as compared
20 with the use of a conventional CPP such as TAT which cannot escape the early endosome.

The efficacy of the peptide vaccine was further assessed using a subcutaneous B16 melanoma model engineered to express glycoprotein B (gB) from Herpes Simplex Virus. The peptide vaccine tested includes a well characterized CD8⁺ T cell gB peptide
25 epitope with and without CPP.

The results (shown in Figure 14) demonstrate that the CPP peptide vaccines result in prolonged tumour-free survival than non-CPP control (gD gB – without CPP capable of endosomal escape).

The results in Figure 15 also show that the peptide vaccine shown as 1746-gDgB with XCL1 targeting, a cleavable furin/cathepsin B linker and the CPP 1746, results in a delay in detectable tumour and a reduction in tumour growth compared to control and a peptide construct without CPP (shown as gDgB). Notably, the 1746-gDgB delayed onset of a detectable tumour equivalent to HSV.

Figure 16 shows a significantly greater percent survival of 1746-gDgB compared to peptide control (gD gB without 1746). Notably, the survival is the same as HSV immunization.

Example 4: Therapeutic vaccination strategy

The same constructs as described above are used in a therapeutic vaccination strategy as outlined in Figure 17.

Example 5: Exemplary assay to measure cell penetration and cytosolic delivery

Beta-Lactamase is a bacterial enzyme that catalyses the opening of Beta-lactam rings. It does not occur naturally in eukaryotic cells. Beta-Lactamase is also not intrinsically cell penetrating and requires the addition of a cell-internalising agent to access the eukaryotic cytoplasm.

CCF2-AM (ThermoFisher Scientific, Australia) is a Fluorescence Resonance Energy Transfer (FRET) substrate that is enzymatically cleaved by Beta-Lactamase. CCF2-AM is an esterified form of 7-hydroxycoumarin linked to fluorescein by a cephalosporin core. Esterification facilitates cell entry of the molecule. Once inside, the molecule is transformed into its anionic form by endogenous cytoplasmic esterases which trap the molecule inside the cell's cytoplasm. When excited at 409 nm, uncleaved CCF2-AM emits a FRET signal at 520 nm (green). In the presence of Beta-Lactamase, the fluorescein moiety of CCF2 is enzymatically cleaved, resulting in the emission wavelength shifting to 447 nm (blue). Beta-Lactamase activity is quantified by measuring the ratio of blue fluorescence to green fluorescence using flow cytometry.

The method described here outlines the measurement of a peptide's cell-penetrating and cytosolic delivery ability by coupling it to Beta-Lactamase and

measuring cytoplasmic Beta-Lactamase enzyme activity by cleavage of cytosolic CCF2, where increased enzyme activity is indicative of increased cell penetration and cytoplasmic delivery of Beta-Lactamase.

Beta-Lactamase was expressed as C-terminal SpyC fusion, making SpyC-BLA
5 (SEQ ID No: 12). The cell-penetrating peptides, 1746 and TAT were synthesized as N-terminal fusions to SpyTag to make CPP-SpyT fusion peptides (SEQ ID Nos: 13 and 14). SpyC-BLA was independently reacted with each CPP-SpyT peptide to be tested to form a CPP conjugate as schematically illustrated in Figure 18 and 19.

The cell-penetrating and cytosolic delivery ability of 1746 and TAT as CPP-
10 SpyT/SpyC-BLA conjugates was assessed by flow cytometry to determine cytosolic CCF2-AM cleavage by internalized Beta-Lactamase. The ratio of blue to green fluorescence was assessed to determine the cell-penetrating and cytosolic delivery ability of the two CPP sequences. The flow cytometry data is further corroborated by confocal microscopy on live cells using the same assay conditions

Example 6: Peptide vaccine and viral challenge

The following groups of mice were treated on D0 & D13 with the following:

- 3 x B6 Poly I:C alone
- 3 x B6 XCL1-gBgD
- 3 x B6 XCL1-Tat gBgD
- 3 x B6 XCL1-1746 gBgD

On D21, all mice were challenged on the skin with Herpes Simplex Virus (HSV). Viral titers are assessed at the primary infectious site 6 days post challenge.

The results (shown in Figure 20) show that CPP peptide vaccines provide efficient protection against HSV skin infection.

Table 1: Vaccine component sequences

Name	Sequence
1746	PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKR (SEQ ID NO: 6)
TAT	GRKKRRQRRRPQ (SEQ ID NO: 8)
PYC35	AYQSIRSGGIESSSKRER(SEQ ID NO: 15)
SpyTag	AHIVMVDAYKPTKG (SEQ ID NO: 9)
SpyCatcher	DSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFY LYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGGAGS WSHPQFEKG (SEQ ID NO: 10)
gD	IPPNWHIPSIQDA (SEQ ID NO: 16)
gB	SSIEFARL (SEQ ID NO: 17)
XCL1	VGTEVLEESSCVNLQTQRLPVQKIKTYIIEWEGAMRAVIFVTKRGLKI CADPEAKWVKAAIKTVDGRASTRKNMAETVPTGAQRSTSTAITLT G (SEQ ID NO: 11)
His	HHHHHH (SEQ ID NO: 18)
V5	GKPIPNNLLGLDST (SEQ ID NO: 19)
FCB	GSGTGGSTGRKKRSV-citrulline-GC (SEQ ID NO: 5).
Furin cleavage site consensus	R-X-K/R-R (SEQ ID NO: 1)
Furin cleavage site	RKKR (SEQ ID NO: 2).
V-citrulline	V-citrulline (SEQ ID NO: 3)
Cathepsin B cleavage site	FKFL (SEQ ID NO: 4).
Linker peptide between moiety capable of endosomal escape and antigen	GAS (SEQ ID NO: 7)

Table 2: Peptide construct sequences

Peptide/ Protein Name	Sequence	Molecular Weight (Da)	Purity
muXCL1 _SpyC	MGHHHHHHGATLEVLFGQPGGSVGTEVLEESSCVNLQTQ RLPVQKIKTYIIWEGAMRAVIFVTKRGLKICADPEAKWWKAAI KTVDGRASTRKNMAETVPTGAQRSTSTAITLTGSGTGATS GDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDG QVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV NGKATKGGAGSWSHPPQFEKG (SEQ ID NO: 20)	24015.9	86%
V5- SpyTag- 1746- gDgB	Ac-GKPIPNPLLGLDSTGASAHIVMVDAYKPTKG-C-(mcpa)- PLKPKPKPTQEKKKKQPPKPKPKPTQEKKKKQPPKPKRGA SIPPNWHIPSIQDAGTGSSIEFARL-NH ₂ (SEQ ID NO: 21)	10721.6	90.60%
V5- SpyTag- PYC35- gDgB	Ac-GKPIPNPLLGLDSTGASAHIVMVDAYKPTKG-C-(mcpa)- AYQSIRSGGIESSSKRER GASIPPNWHIPSIQDAGTGSSIEF ARL-NH ₂ (SEQ ID NO: 22)	8192.6	90%
V5- SpyTag- TAT- gDgB	Ac-GKPIPNPLLGLDSTGASAHIVMVDAYKPTKG-C-(mcpa)- GRKKRRQRRRPQGASIPPNWHIPSIQDAGTGSSIEFARL- NH ₂ (SEQ ID NO: 23)	7803.1	90.20%
mcpa- TAT- gDgB	mcpa- GRKKRRQRRRPQGASIPPNWHIPSIQDAGTGSSIEFARL- NH ₂ (SEQ ID NO: 24)	4513.2	
mcpa- AVI- gDgB	mcpa- GLNDIFEAQKIEFHEGASIPPNWHIPSIQDAGTGSSIEFARL- NH ₂ (SEQ ID NO: 25)	4681.4	
SpyT- FCB-Cys	Ac-GAHIVMVDAYKPTKGSGTGGSTGRKKRSV[Cit]GC-NH ₂ (SEQ ID NO: 26)	3305.0	
1746- gDgB	Ac- PLKPKPKPTQEKKKKQPPKPKPKPTQEKKKKQPPKPKRGA SIPPNWHIPSIQDAGTGSSIEFARL-NH ₂ (SEQ ID NO: 27)	7383.8	
mcpa- 1746- gDgB	mcpa- PLKPKPKPTQEKKKKQPPKPKPKPTQEKKKKQPPKPKRGA SIPPNWHIPSIQDAGTGSSIEFARL-NH ₂ (SEQ ID NO: 28)	7429.1	
mcpa- gDgB	mcpa-IPPNWHIPSIQDAGTGSSIEFARL-NH ₂ (SEQ ID NO: 29)	2693.2	
mcpa- MEM- gDgB	mcpa- YGTIAVIVFALIGFTIGYLGYPNWHIPSIQDAGTGSSIEFARL -NH ₂ (SEQ ID NO: 30)	4926.9	
gDgB	Ac-IPPNWHIPSIQDAGTGSSIEFARL-NH ₂ (SEQ ID NO 31)	2647.9	
SpyT- FCB- TAT- gDgB	Ac- GAHIVMVDAYKPTKGSGTGGSTGRKKRSV[Cit]GC(mcpa- GRKKRRQRRRPQGASIPPNWHIPSIQDAGTGSSIEFARL- NH ₂)-NH ₂ (SEQ ID NO: 32)	7816.2	
SpyT- FCB-Avi- gDgB	Ac- GAHIVMVDAYKPTKGSGTGGSTGRKKRSV[Cit]GC(mcpa- GLNDIFEAQKIEFHEGASIPPNWHIPSIQDAGTGSSIEFARL-	7984.4	

	NH ₂)-NH ₂ (SEQ ID NO: 33)		
SpyT-FCB-1746-gDgB	Ac-GAHIVMVDAYKPTKGSGTGGSTGRKKRSV[Cit]GC(mcpa-PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKRGA SIPPNWHIPSIQDAGTGSSIEFARL-NH ₂)-NH ₂ (SEQ ID NO: 34)	10732.1	
SpyT-FCB-gDgB	Ac-GAHIVMVDAYKPTKGSGTGGSTGRKKRSV[Cit]GC(mcpa-IPPNWHIPSIQDAGTGSSIEFARL-NH ₂)-NH ₂ (SEQ ID NO: 35)	5996.2	
SpyT-FCB-MEM-gDgB	Ac-GAHIVMVDAYKPTKGSGTGGSTGRKKRSV[Cit]GC(mcpa-YGTIAVIVFALIGFTIGYLYIPPNWHIPSIQDAGTGSSIEFARL-NH ₂)-NH ₂ (SEQ ID NO: 36)	8213.3	
SpyC-BLA:	MGHHHHHHGATLEVLFGPGGSGSDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVET AAPDGYEVATAITFTVNEQGQVTVNGKATKGSHPETLVKVK DAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLL CGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGM TVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHV TRLDREWEPELNEAIPNDERDTTMAAMATTLRKLLTGELLT LASRQQLIDWMEADKVAGPLLR SALPAGWFIADKSGAGER GSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGAS LIKHWQLGSASGTTGATS GEF (SEQ ID NO: 12)		
1746-SpyT	Ac-PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKRGASAHIVMVDAYKPTKG-NH ₂ (SEQ ID NO: 13)		
TAT-SpyT	Ac-RQIKIWFQNR RMKWKKGASAHIVMVDAYKPTKG-NH ₂ (SEQ ID NO: 14)		
muXCL1_SpyC+V5-SpyTag-1746-gDgB	MGHHHHHHGATLEVLFGPGGSGVTEVLEESSCVNLQTQRLPVQKIKTYIWE G AMRAVIFVTKRGLKICADPEAKWKA AIKTVDGRASTRKNMAETVPTGAQRSTSTAITLTGGSGTGATSGDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGAGSWSH PQFEKG+Ac-GKPIPNPLLGLDSTGASAHIVMVDAYKPTKG-C-(mcpa)-PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKRGA	34737.5	~80%

	SIPPNWHIPSIQDAGTGSSIEFARL-NH ₂ (SEQ ID NO: 37)		
muXCL1	MGHHHHHHGATLEVLFGPGGSGVGTEVLEESSCVNLQTQ	32208.5	~80%
_SpyC+V	RLPVQKIKTYIIEWEGAMRAVIFVTKRGLKICADPEAKWWKAAI		
5-	KTVDGRASTRKNMAETVPTGAQRSTSTAITLTGGSGTGATS		
SpyTag-	GDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDG		
PYC35-	QVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV		
gDgB	NGKATKGGAGSWSHPPQFEKG+Ac-		
	GKPIPNPLLGLDSTGASAHIVMVDAYKPTKG-C-(mcpa)-		
	AYQSIRSGGIESSSKRERGASIPPNWHIPSIQDAGTGSSIEF		
	ARL-NH ₂ (SEQ ID NO: 38)		
muXCL1	MGHHHHHHGATLEVLFGPGGSGVGTEVLEESSCVNLQTQ	31819	~80%
_SpyC+V	RLPVQKIKTYIIEWEGAMRAVIFVTKRGLKICADPEAKWWKAAI		
5-	KTVDGRASTRKNMAETVPTGAQRSTSTAITLTGGSGTGATS		
SpyTag-	GDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDG		
TAT-	QVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV		
gDgB	NGKATKGGAGSWSHPPQFEKG+Ac-		
	GKPIPNPLLGLDSTGASAHIVMVDAYKPTKG-C-(mcpa)-		
	GRKKRRQRRRPQGASIPPNWHIPSIQDAGTGSSIEFARL-		
	NH ₂ (SEQ ID NO: 39)		

Table 3: Protein expression construct

DNA insert for pET28a+ muXCL1-SpyC	CCATGGGTCATCATCATCATCACGGCGCAACTCTGGAAGTTTT GTTTCAAGGTCCTGGCGGCAGCGTTGGTACCGAAGTGCTGGAAGA GAGCTCCTGCGTCAATCTGCAAACCCAACGCCTGCCGGTGACAGAA AATCAAAACCTATATCATCTGGGAGGGTGCGATGCGTGCCGTCATT TTCGTAACCAAGCGTGGTCTGAAGATTTGTGCGGACCCAGAGGCG AAATGGGTAAAGCTGCCATCAAACCGTTGACGGCCGTGCGAGC ACGCGTAAGAACATGGCAGAGACTGTGCCGACCGGTGCCAGCG CTCTACGAGCACCGCGATCACCTGACCGGTGGCAGCGGCACGG GTGCGACCTCGGGCGATAGCGCAACGCACATCAAATTCTCTAAGC GCGACGAAGATGGCAAAGAACTGGCGGGTGCGACGATGGAAGT CGTGATAGCAGCGGCAAGACGATTTCCACGTGGATTAGCGATGGC CAGGTCAAAGACTTCTATTTGTACCCGGGTAAGTACACGTTTGTGG AGACTGCGGCTCCGGACGGTTACGAAGTTGCAACCGCCATTACCT TCACCGTGAACGAGCAAGGTCAGGTCACCGTTAATGGTAAGGCAA CCAAGGGTGCGCTGGTAGCTGGAGCCACCCGCAGTTTGAGAAA GGTTAAATGATTAACCTCGAG (SEQ ID NO: 40)
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CLAIMS

1. A conjugate for inducing an immune response, the conjugate comprising:

(a) an antigen presenting cell targeting moiety;

(b) a moiety capable of endosomal and/or lysosomal escape; and

5 (c) an antigen;

wherein the antigen presenting cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen.

2. A conjugate according to claim 1, wherein the antigen presenting cell is a cell capable of cross presentation.

10 3. A conjugate according to claim 1 or 2, wherein the antigen presenting cell is a professional antigen presenting cell.

4. A conjugate according to any one of claims 1 to 3, wherein the antigen presenting cell is a macrophage or dendritic cell.

15 5. A conjugate according to any one of claims 1 to 3, wherein the antigen presenting cell is a dendritic cell.

6. A conjugate according to any one of claims 1 to 5, wherein the antigen presenting cell targeting moiety is capable of binding to a target molecule present on an antigen presenting cell.

20 7. A conjugate according to claim 6, wherein the antigen presenting cell targeting moiety is selected from the group consisting of antibodies, polypeptides, peptides, aptamers, other ligands, or any combination thereof.

8. A conjugate according to claim 5, wherein the antigen presenting cell moiety is a molecule that is capable of binding to any one of XCR1, DEC-205 (CD205), Langerin (CD207), Clec9A receptors, DC-SIGN (CD209) and DNGR1.

25 9. A conjugate according to claim 8, wherein the targeting moiety is capable of binding to XCR1.

10 A conjugate according to claim 9, wherein the moiety that is capable of binding to XCR1 comprises, consists essentially of or consists of an amino acid sequence of XCL1 (SEQ ID NO: 11) as described herein.

11. A conjugate according to any one of claims 1 to 10, wherein the antigen
5 presenting cell targeting moiety is associated with the moiety capable of endosomal escape via a linker which is cleavable in an endosome compartment prior to the endosome maturing into a late endosome.

12. A conjugate according to claim 11, wherein the linker is cleavable in an early endosome.

10 13. A conjugate according to claim 12, wherein the linker which is cleavable in an early endosome comprises an amino acid sequence of a furin cleavage site.

14. A conjugate according to claim 12, wherein the furin cleavage site comprises, consists essentially of or consists of the amino acid sequence R-X-K/R-R (SEQ ID NO: 1), preferably RKKR (SEQ ID NO: 2).

15 15. A conjugate according to any one of claims 11 to 14, wherein the linker between the antigen presenting cell targeting moiety and the moiety capable of endosomal escape, is also cleavable in the late endosome or in a lysosome.

16. A conjugate according to claim 15, wherein the linker which is also
20 cleavable in a lysosome comprises an amino acid sequence of a cathepsin B cleavage site.

17. A conjugate according to claim 16, wherein the cathepsin B cleavage site may comprise, consist essentially of or consist of the amino acid sequence V-citrulline (SEQ ID NO: 3; V denotes the amino acid valine) or FKFL (SEQ ID NO: 4).

18. A conjugate according to claim 17, wherein the linker between the antigen
25 presenting cell targeting moiety and the moiety capable of endosomal escape may comprise, consist essentially of or consist of a dual furin/cathepsin B cleavage linker in the form of a furin cleavage site and a cathepsin B cleavage site.

19. A conjugate according to claim 18, wherein the furin cleavage site comprises the sequence. RKKR (SEQ ID NO: 2).

20. A conjugate according to claim 18 or 19, wherein the cathepsin B cleavage site comprises V-citrulline (SEQ ID NO: 3).

5 21. A conjugate according to any one of claims 18 to 20, wherein the dual furin/cathepsin B cleavage linker comprises, consists essentially of or consists of the amino acid sequence GSGTGGSTGRKKRSV-citrulline-GC (SEQ ID NO: 5).

22. A conjugate according to any one of claims 1 to 21, wherein the antigen presenting cell targeting moiety and the moiety capable of endosomal and/or lysosomal escape are linked using enzymatic ligation, optionally via the peptide sequences as set forth in SEQ ID NOs: 9 and 10.

23. A conjugate according to any one of claims 1 to 22, wherein the moiety capable of endosomal and/or lysosomal escape is capable of escaping from an endosomal and/or lysosomal compartment into the cytosol.

15 24. A conjugate according to claim 23, wherein the moiety is capable of escaping from an endosomal and/or lysosomal compartment into the cytosol prior to the endosome maturing into a late endosome.

25. A conjugate according to claim 24, wherein the moiety is capable of escaping from an early endosomal compartment into the cytosol.

20 26. A conjugate according to any one of claims 1 to 25, wherein the moiety capable of endosomal and/or lysosomal escape mediates significantly greater endosomal escape than the TAT peptide from HIV having the amino acid sequence GRKKRRQRRRPQ (SEQ ID NO: 8).

25 27. A conjugate according to any one of claims 1 to 26, wherein the moiety capable of endosomal and/or lysosomal escape is a peptide.

28. A conjugate according to claim 27, wherein the peptide comprises, consists essentially of, or consists of the amino acid sequence PLKPKKPKTQEKKKKQPPKPKKPKTQEKKKKQPPKPKR (SEQ ID NO: 6).

29. A conjugate according to any one of claims 1 to 28, wherein the moiety capable of endosomal and/or lysosomal escape is associated with the antigen such that when the moiety escapes from an endosomal and/or lysosomal compartment into the cytosol, the antigen also enters the cytosol.

5 30. A conjugate according to claim 29, wherein the moiety capable of endosomal and/or lysosomal escape and the antigen are chemically linked.

31. A conjugate according to claim 30, wherein the chemical linkage is covalent.

10 32. A conjugate according to claim 31, wherein the covalent linkage may be direct or via a linker.

33. A conjugate according to claim 32, wherein the linker is formed by enzymatic ligation, optionally via the peptide sequences as set forth in SEQ ID NOs: 9 and 10.

15 34. A conjugate according to claim 33, wherein the linker comprises, consists essentially of or consists of a peptide, preferably having 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids.

35. A conjugate according to claim 34, wherein the amino acids are selected from alanine, glycine and serine.

20 36. A conjugate according to claim 35, wherein the peptide comprises, consists essentially of, or consists of the amino acid sequence GAS (SEQ ID NO: 7).

37. A conjugate according to any one of claims 1 to 36, wherein the antigen presenting cell targeting moiety mediates internalization upon contact with an antigen presenting cell.

25 38. A conjugate according to any one of claims 1 to 37, wherein the moiety capable of endosomal and/or lysosomal escape mediates internalization upon contact with an antigen presenting cell.

39. A conjugate according to claim 38, wherein the moiety capable of endosomal and/or lysosomal escape is a cell penetrating peptide that also has the capacity to escape from endosomal or lysosomal compartments for delivery into the cytosol.

5 40. A conjugate according to any one of claims 1 to 39, wherein the antigen comprises a peptide capable of being presented by an HLA class I molecule.

41. A conjugate according to any one of claims 1 to 40, wherein the antigen comprises a peptide capable of being presented by an HLA class II molecule.

10 42. A conjugate according to claim 41, wherein the peptide capable of being presented by an HLA class I molecule comprises a CD8+ T cell epitope.

43. A conjugate according to claim 41, wherein the peptide capable of being presented by an HLA class II molecule comprises a CD4+ T cell epitope.

44. A chimeric or fusion polypeptide, the chimeric or fusion polypeptide comprising:

- 15 (a) an antigen presenting cell targeting moiety;
- (b) a moiety capable of endosomal and/or lysosomal escape; and
- (c) an antigen;

wherein the dendritic cell targeting moiety is releasable from the moiety capable of endosomal escape and the antigen.

20 45. A composition such as an antigenic composition, particularly a vaccine composition, comprising a conjugate, or chimeric or fusion polypeptide according to any one of claims 1 to 44, optionally in association with an adjuvant.

25 46. A pharmaceutical composition for treating or preventing cancer in a subject comprising a conjugate, or chimeric or fusion polypeptide according to any one of claims 1 to 44 and a pharmaceutically acceptable diluent, excipient or carrier.

47. A pharmaceutical composition for treating or preventing cancer in a subject comprising as an active ingredient a conjugate, or chimeric or fusion polypeptide according to any one of claims 1 to 44, the composition further comprising a pharmaceutically acceptable diluent, excipient or carrier.

5 48. A pharmaceutical composition for treating cancer in a subject comprising as a main ingredient a conjugate, or chimeric or fusion polypeptide according to any one of claims 1 to 44, the composition further comprising a pharmaceutically acceptable diluent, excipient or carrier.

49. A pharmaceutical composition according to any one of claims 46 to 48,
10 wherein the conjugate, or chimeric or fusion polypeptide is in association with a virus-like particle, liposome or nanoparticle.

50. A conjugate, or chimeric or fusion polypeptide according to any one of claims 1 to 44 for use in the treatment or prevention of cancer in a subject.

41. A nucleic acid molecule comprising, consisting essentially of or consisting
15 of a nucleotide sequence encoding a conjugate, or chimeric or fusion polypeptide according to any one of claims 1 to 44.

52. A vector comprising a nucleic acid molecule according to claim 51.

53. A vector according to claim 52, wherein the vector comprises an expression construct in which the nucleic acid is operably linked to a promoter.

20 54. A cell comprising a vector or nucleic acid according to any one of claims 51 to 53.

55. A method for eliciting a cell-mediated immune response to an antigen in a subject, the method comprising

providing a conjugate, chimeric or fusion polypeptide, or composition according
25 to any one of claims 1 to 49, and

administering the conjugate, chimeric or fusion polypeptide, or composition to the subject,

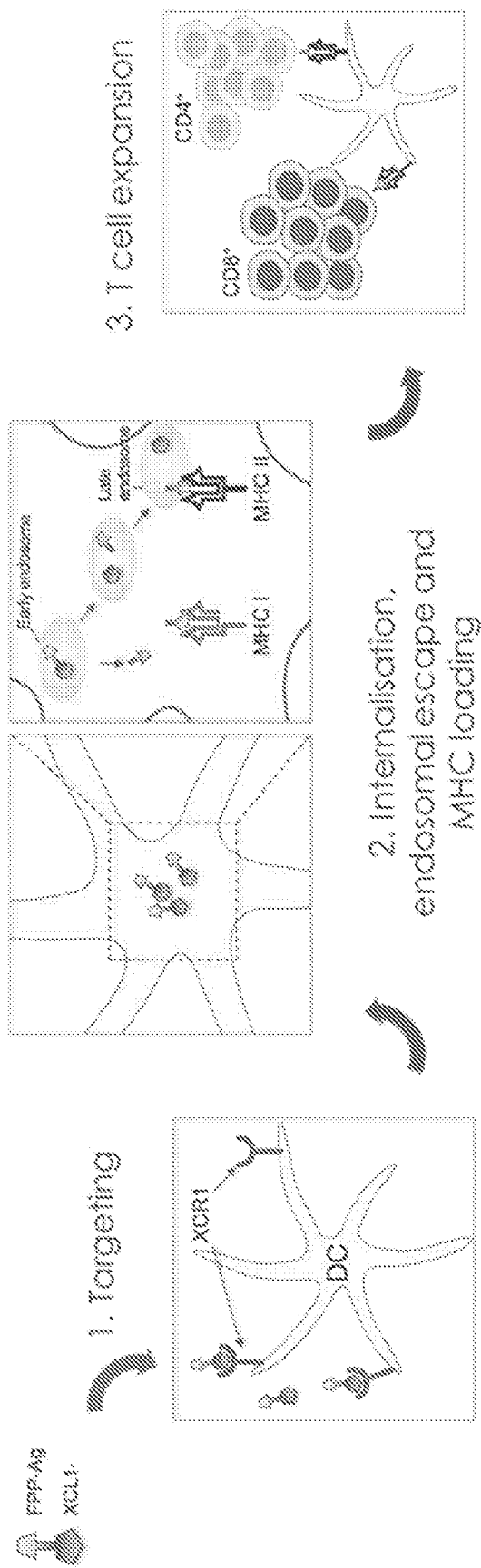
thereby eliciting a cell-mediated immune response to the antigen in a subject.

56. A method for treating or preventing cancer or an infectious disease in a subject in need thereof, the method comprising administering a conjugate, chimeric or fusion polypeptide, or composition according to any one of claims 1 to 49, thereby
5 treating or preventing cancer or infectious disease in the subject.

57. A use of a conjugate, chimeric or fusion polypeptide, or composition according to any one of claims 1 to 49 in the manufacture of a medicament for the treatment or prevention of cancer in a subject.

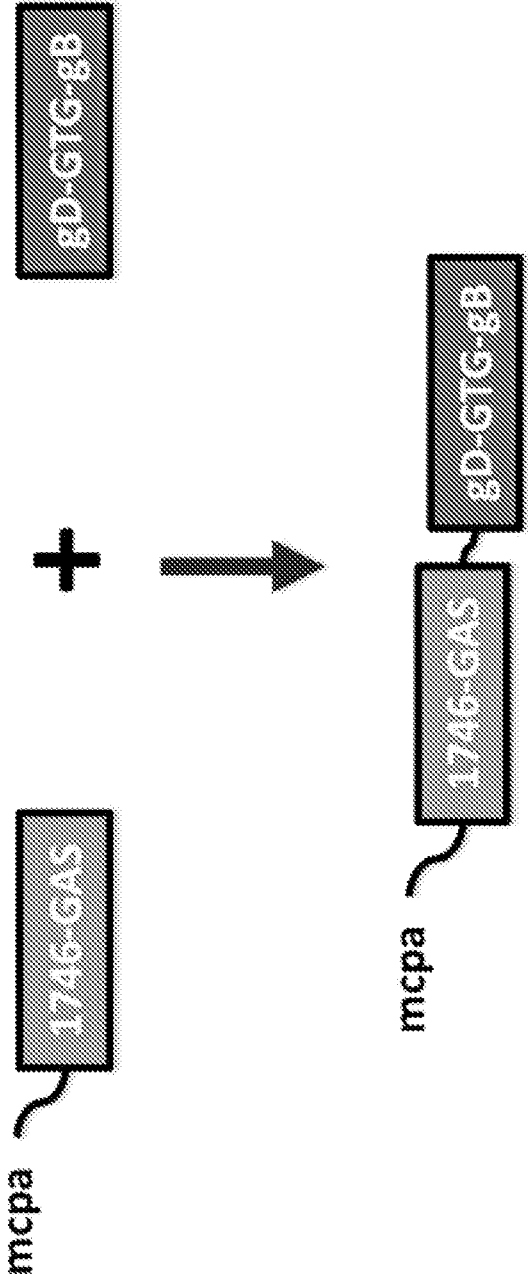
58. A kit or article of manufacture comprising a conjugate, chimeric or fusion
10 polypeptide, composition, nucleic acid, vector or cell according to any one of claims 1 to 54, optionally the kit further comprises written instructions for use in a method or use of the invention according to any one of claims 55 to 57.

Figure 1



Assembly of the Delivery System: FPP with gD and gB antigens

1746 sincludes C-terminal GAS linker:	gD separated from gB by GTG linker:
PLKPKKPKTQEKKKKKQPPKPKKPKTQEKK	IPPNWHIPSIQDA-GTG-SSIEFARL
KKQPPKPKR-GAS	



FPP-Antigen module for endosomal/ cytosolic delivery

Figure 2

Figure 3

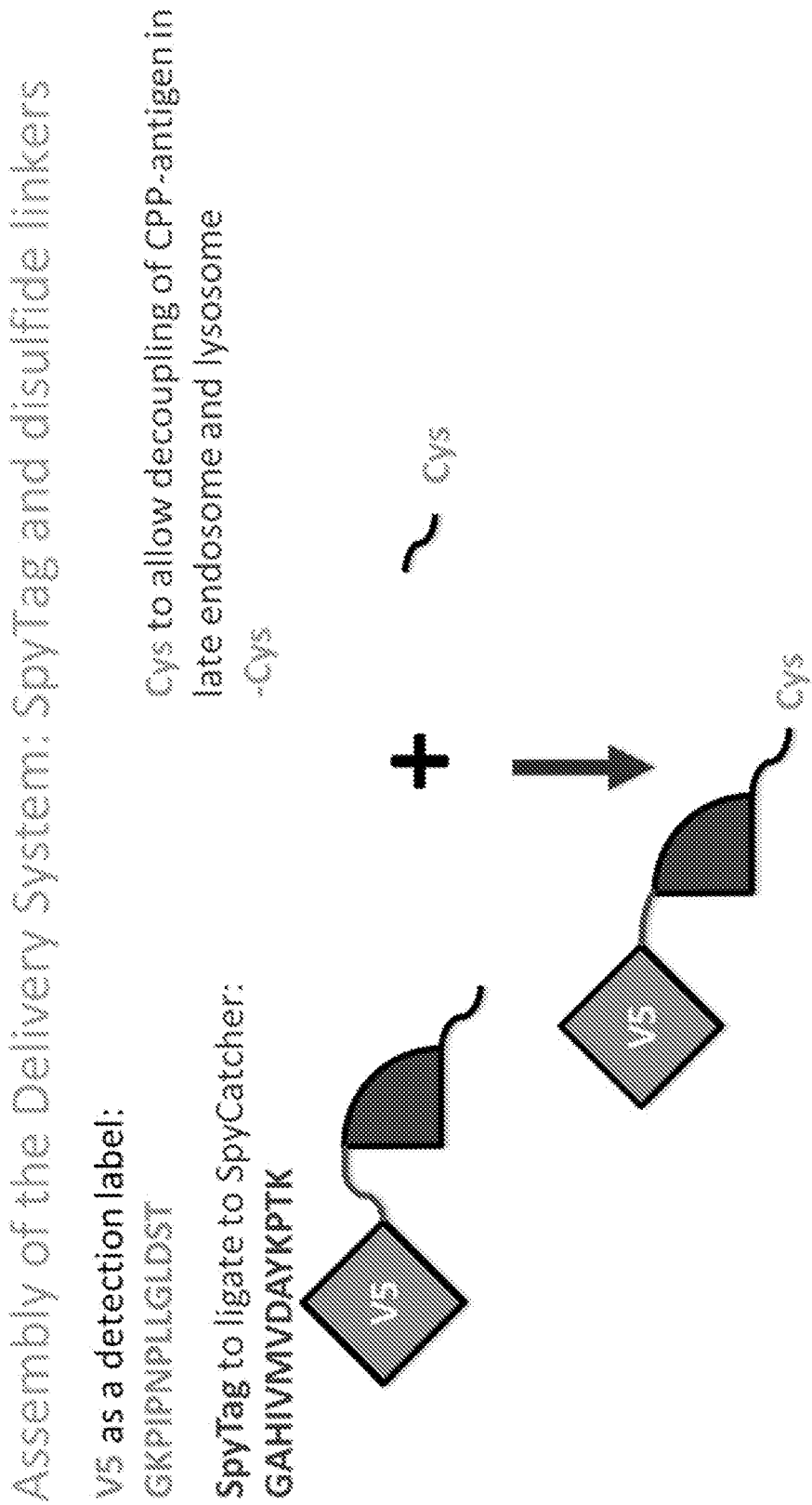


Figure 4

Assembly of final V5-SpyT-Cys-mcpa-CPP-antigen units

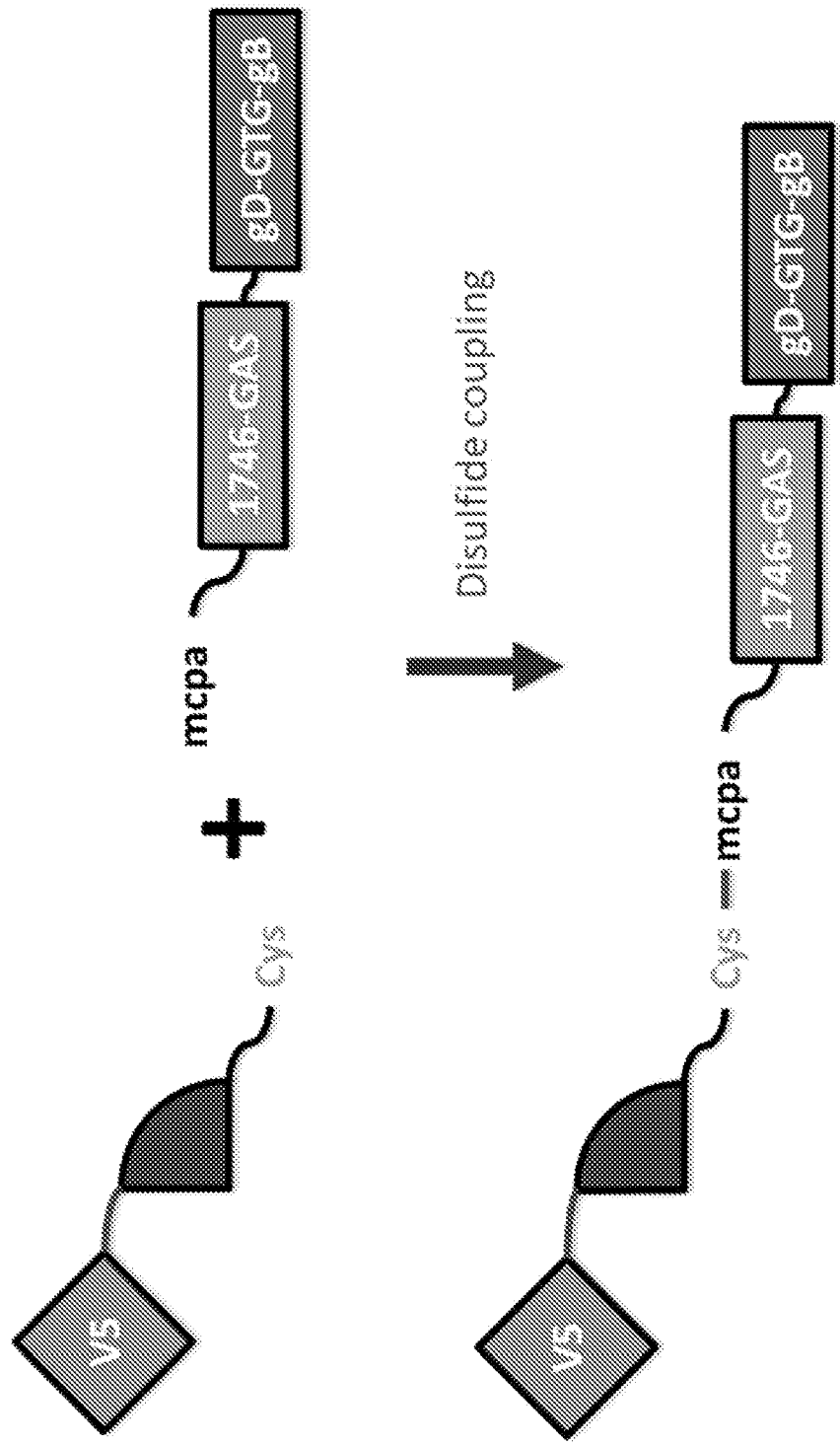


Figure 5

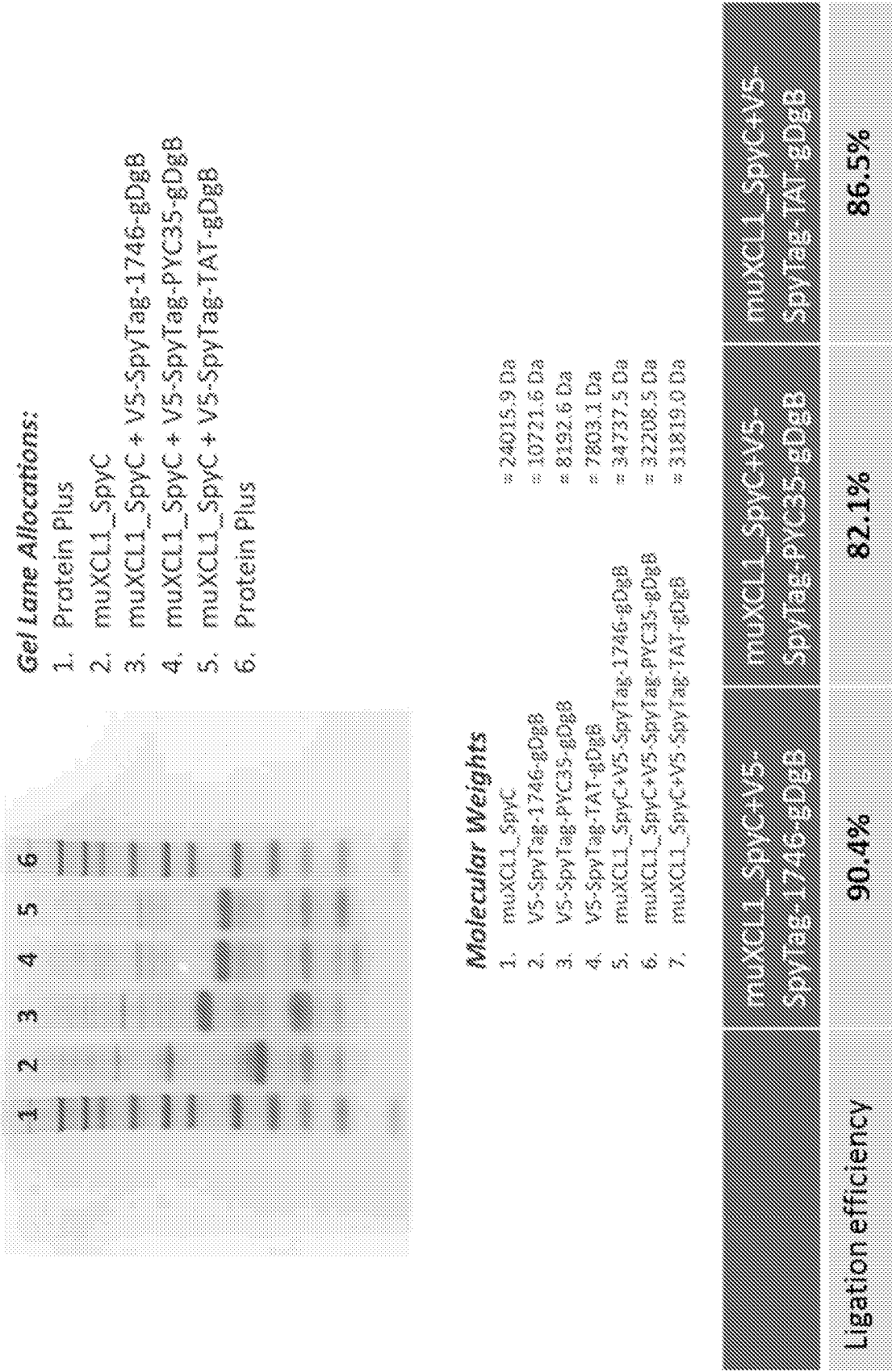
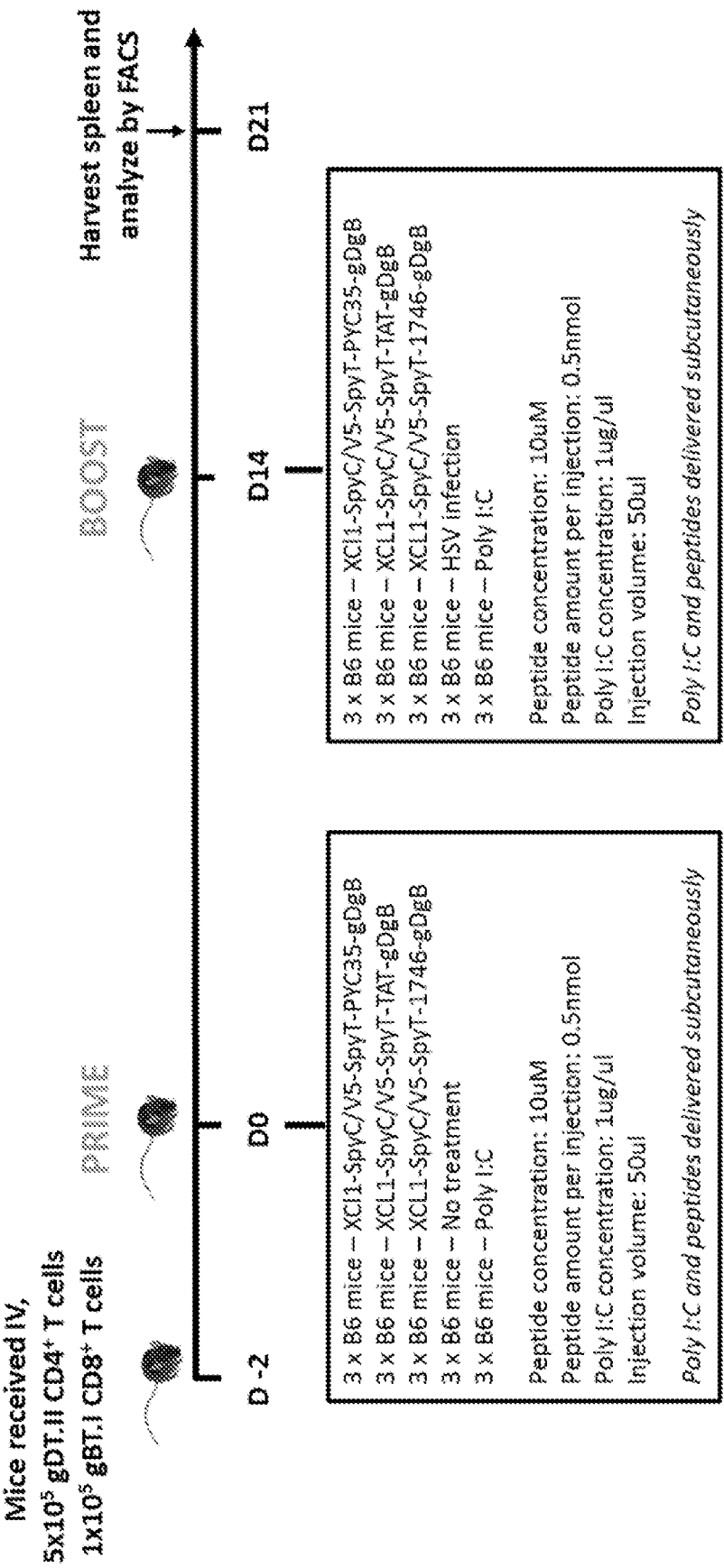
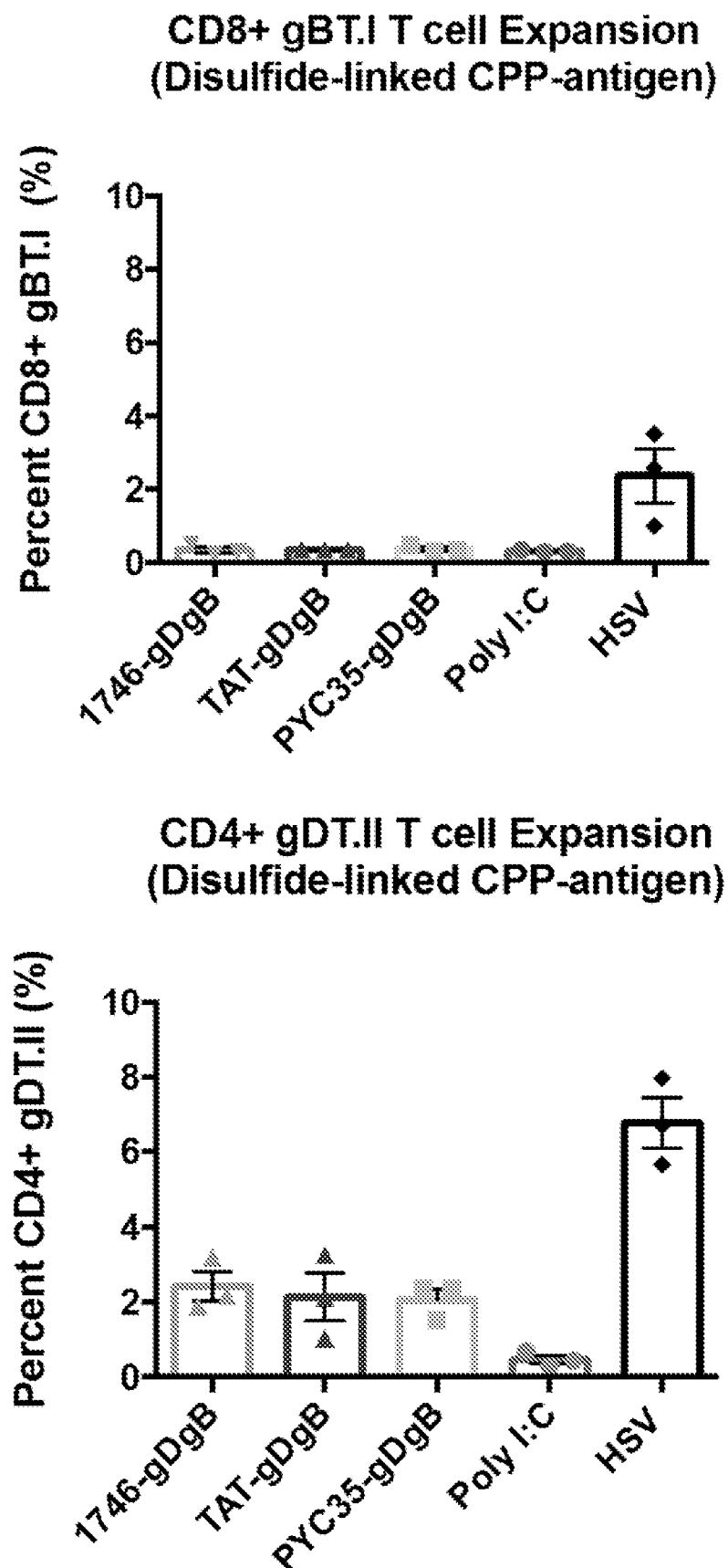


Figure 6



• Prime boost vaccination strategy (0.5nmol per injection)

Figure 7



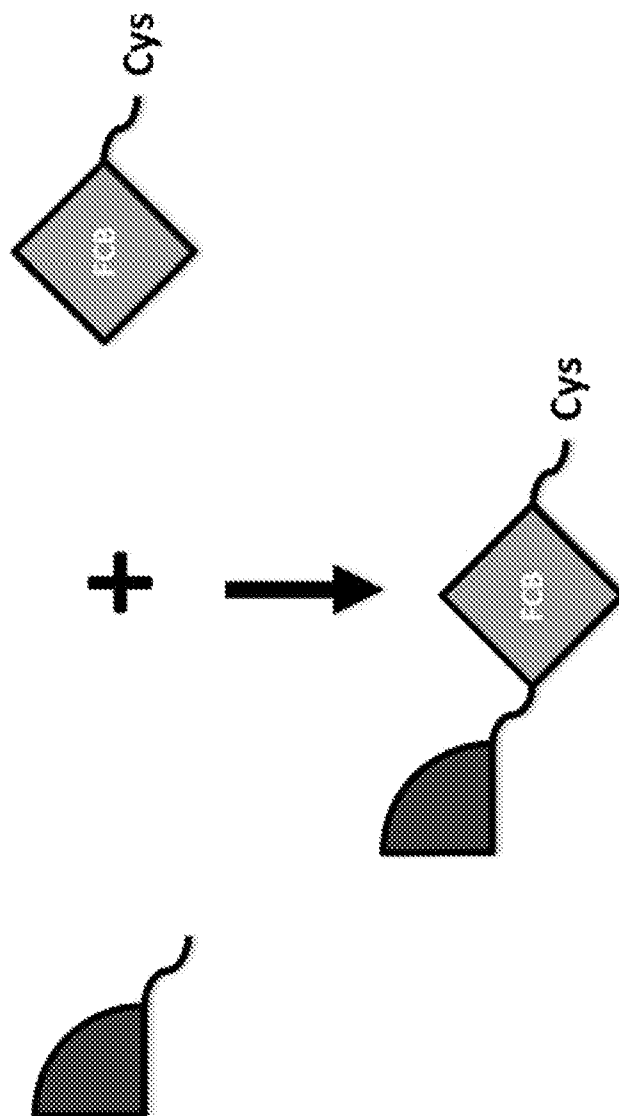
Assembly of the Delivery System: SpyTag and cleavable linkers

SpyTag to ligate to SpyCatcher:

GAHIVMVDAYKPTK

FCB to allow cleavage in both endosome
(Furin) and lysosome (Cathepsin-B)

GSGTGGSTGRKKRSV-Citrulline-G-Cys



SpyTag-FCB module for linkage to Targeting system

Figure 8

Figure 8 continued...

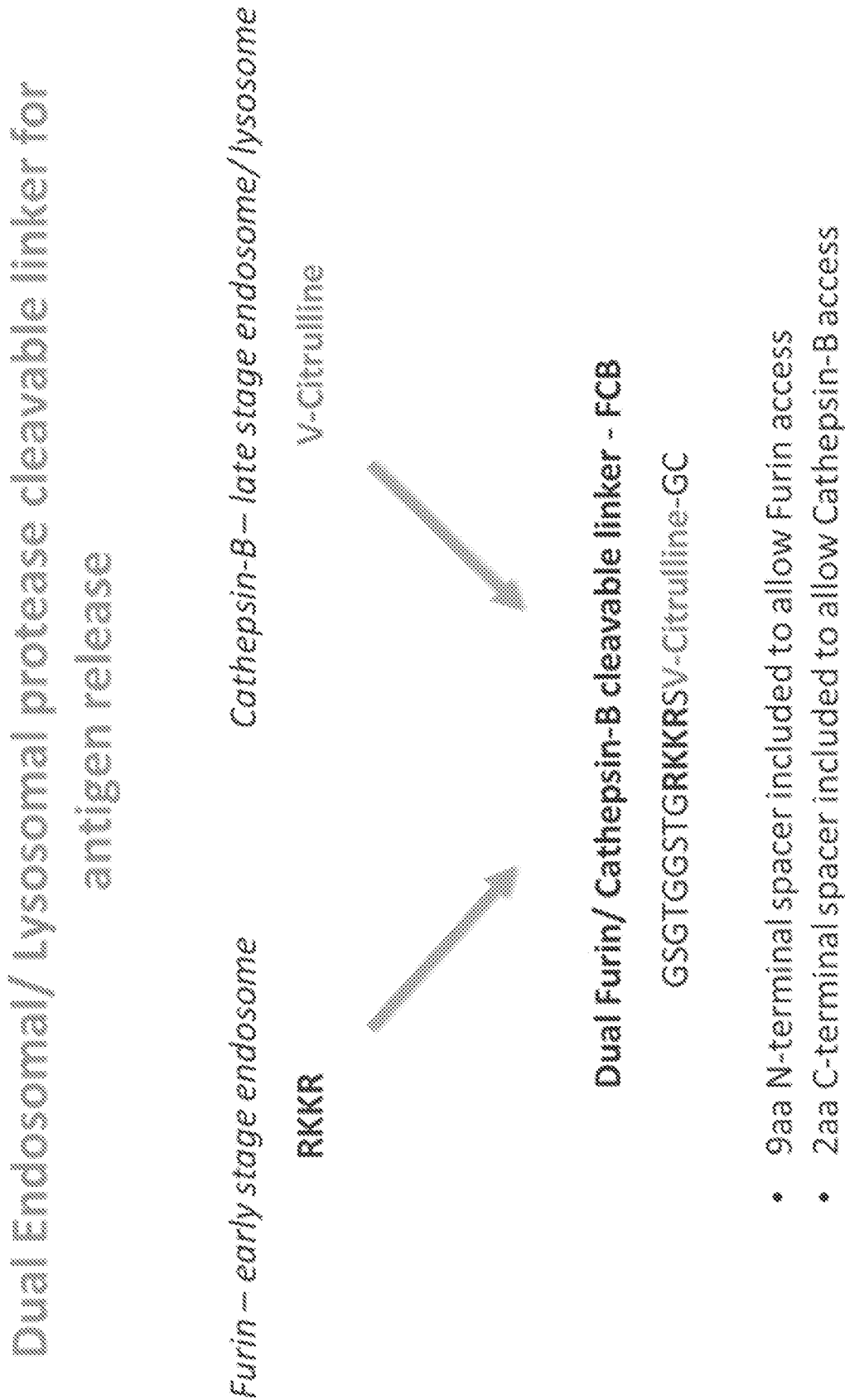


Figure 9

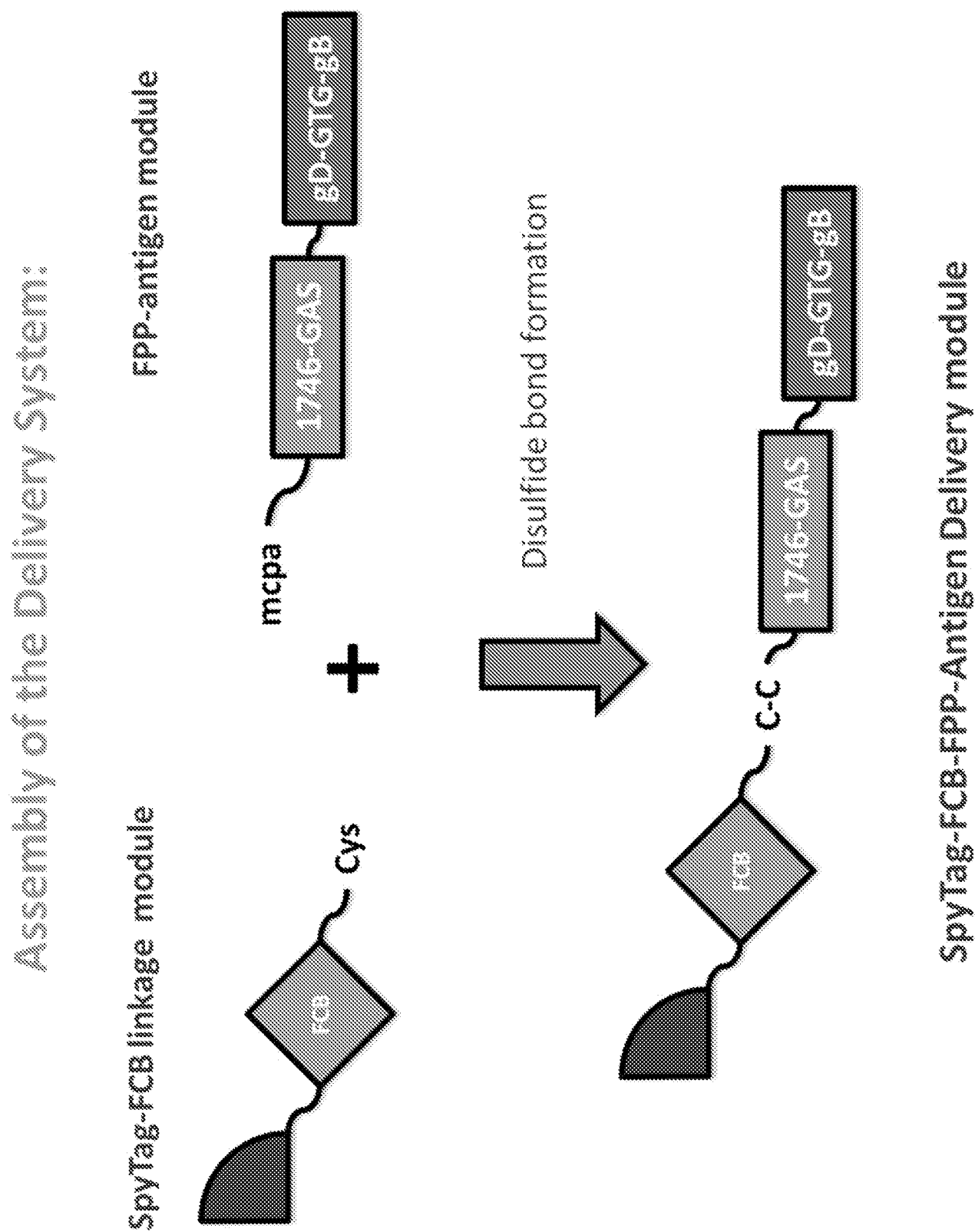


Figure 10

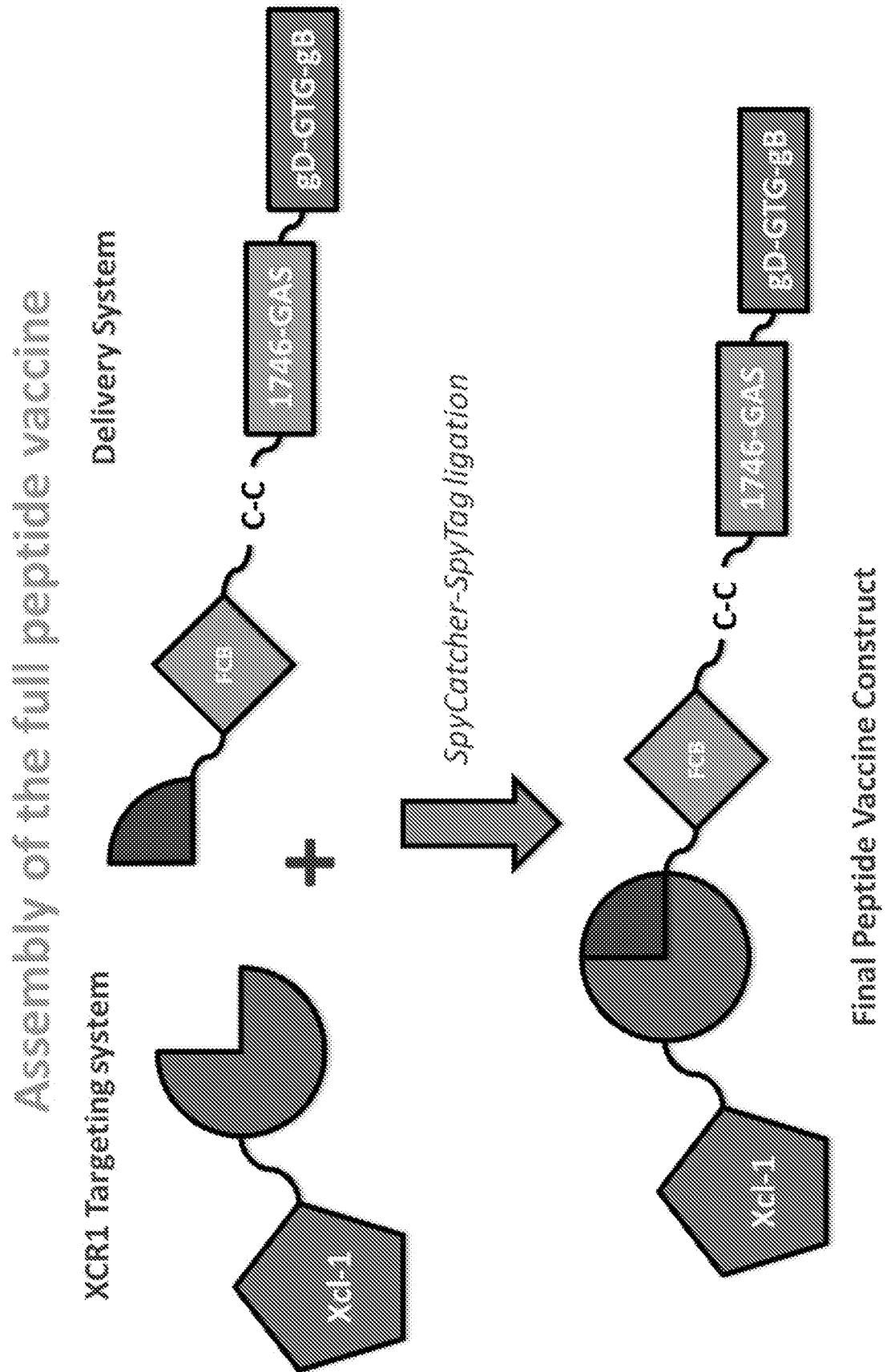


Figure 11

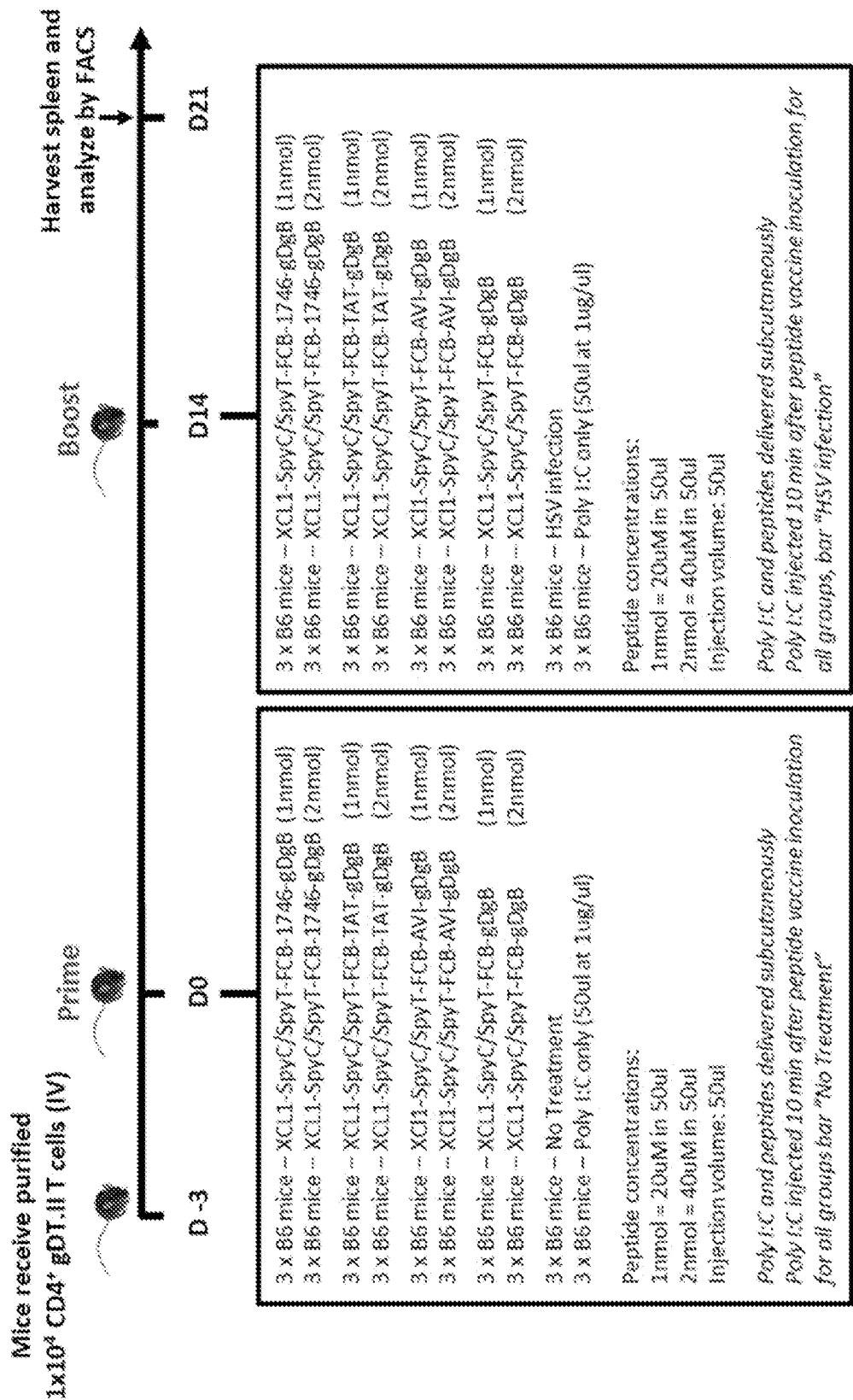


Figure 12

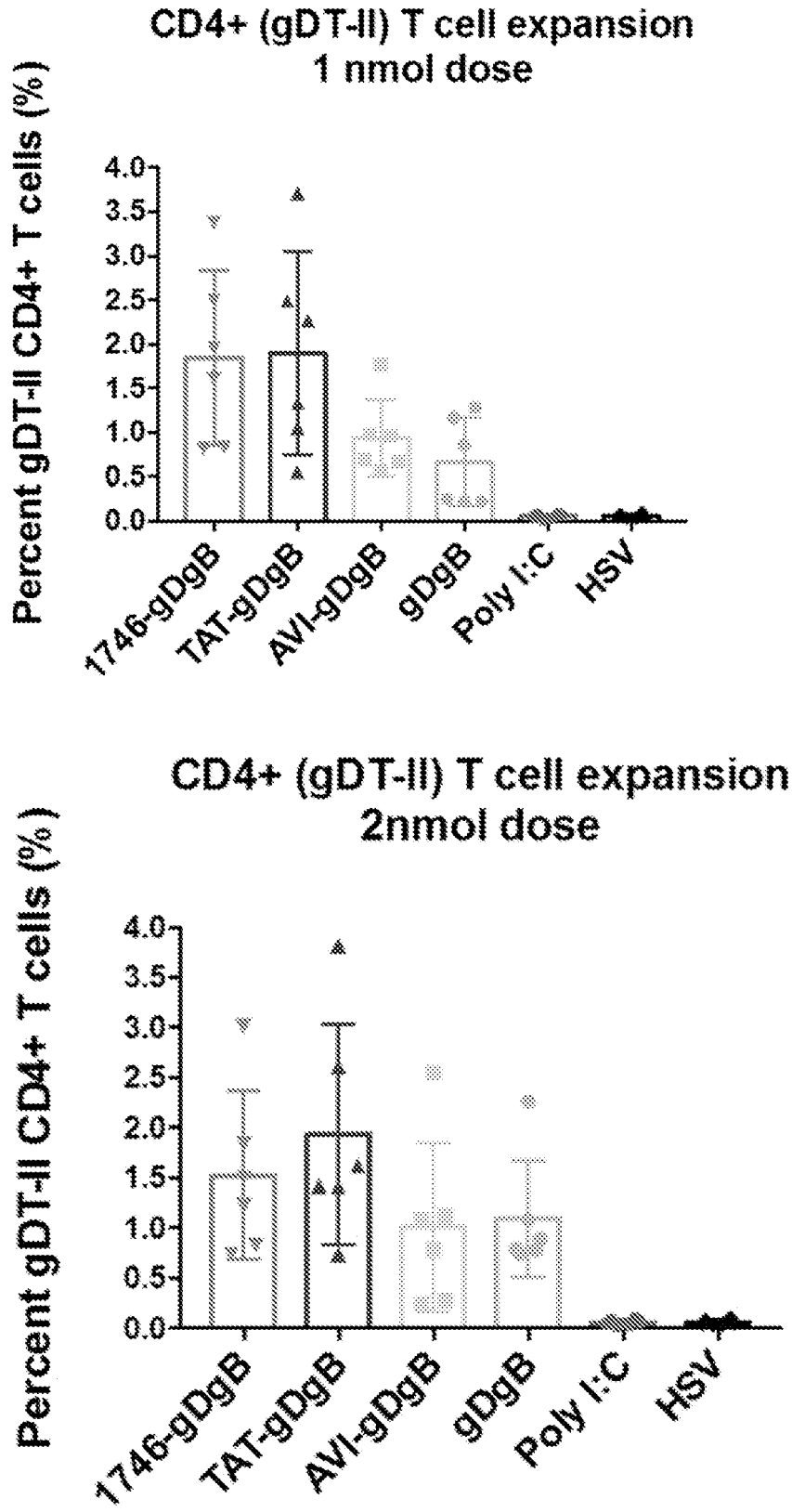


Figure 13

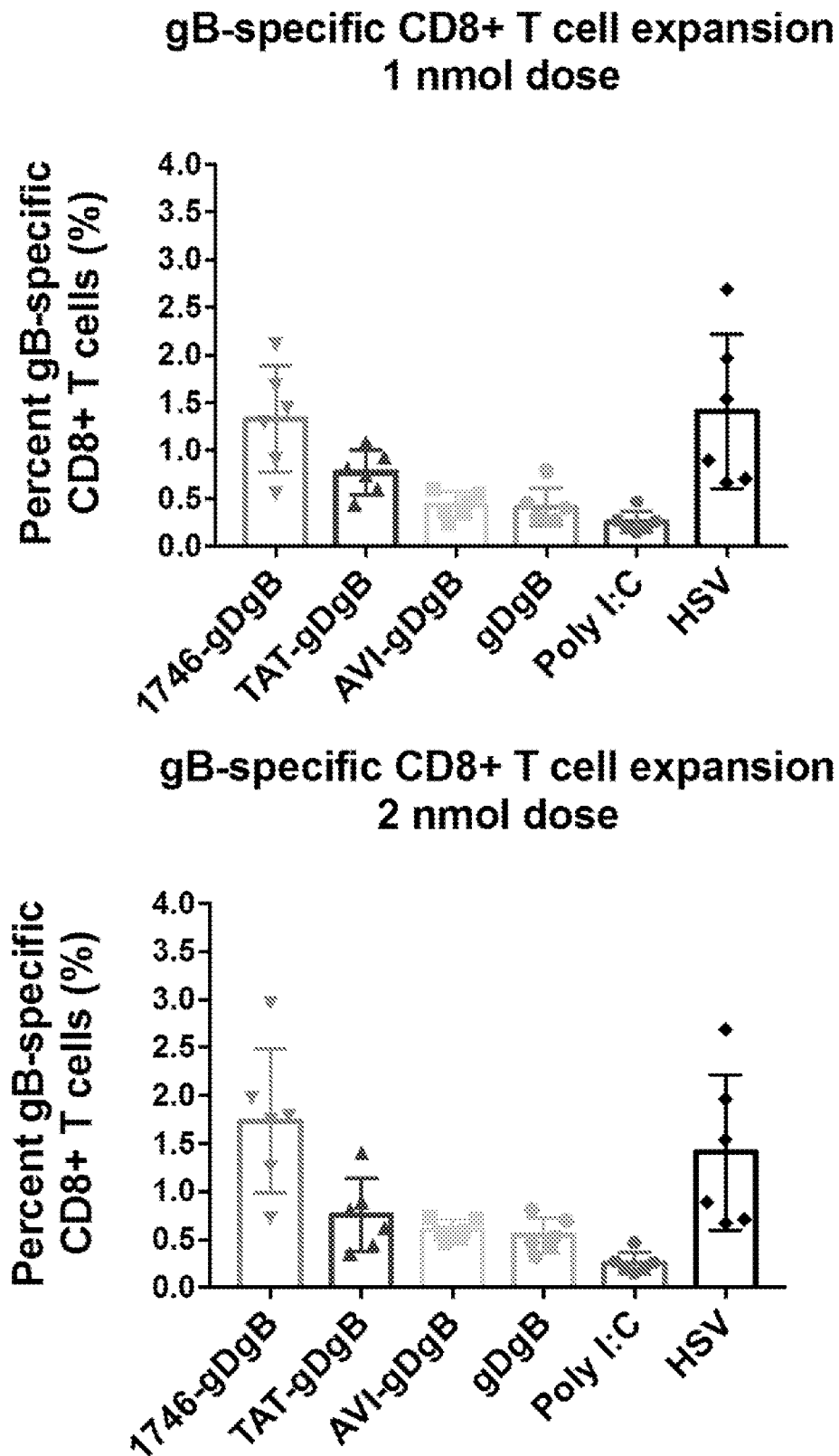
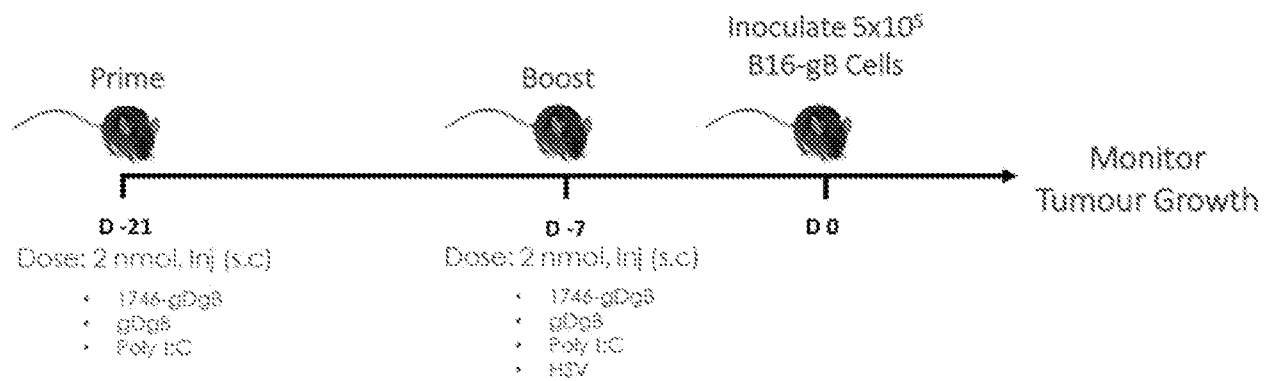
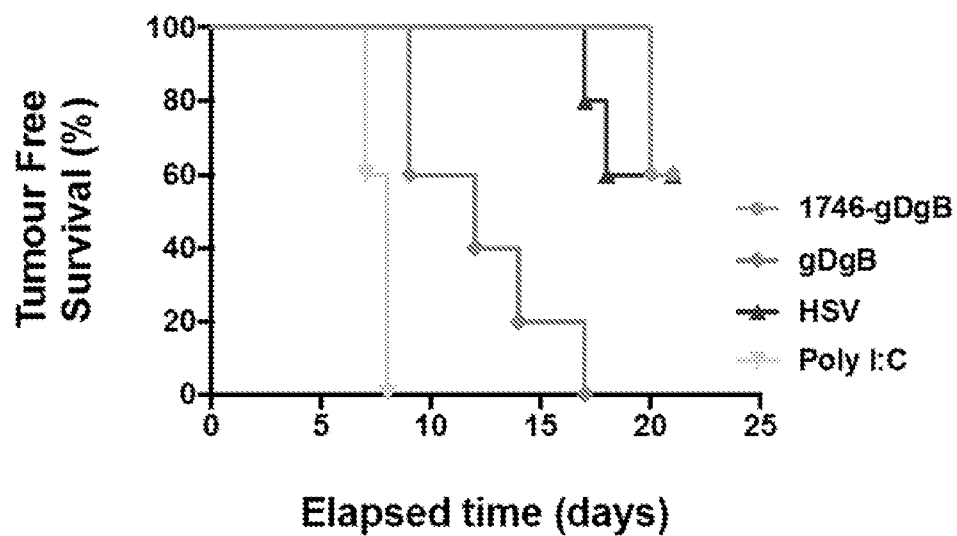


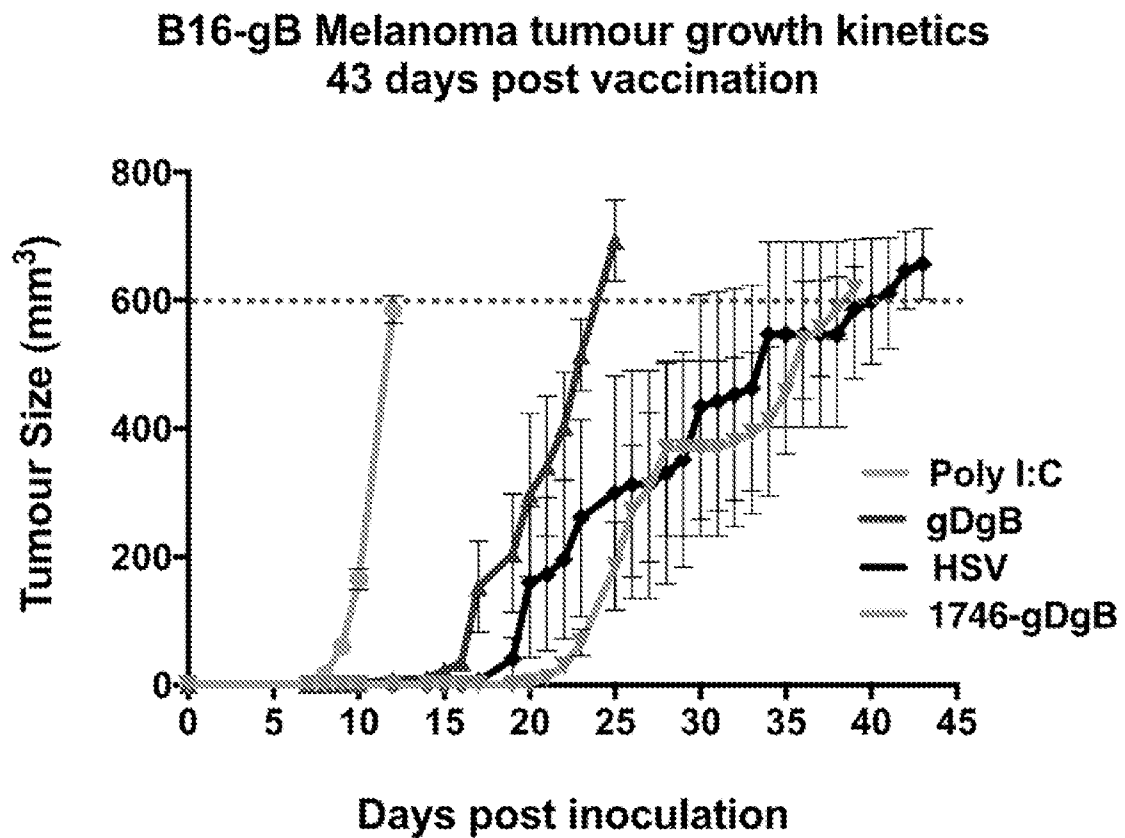
Figure 14

Tumour free survival at day 23 post-inoculation



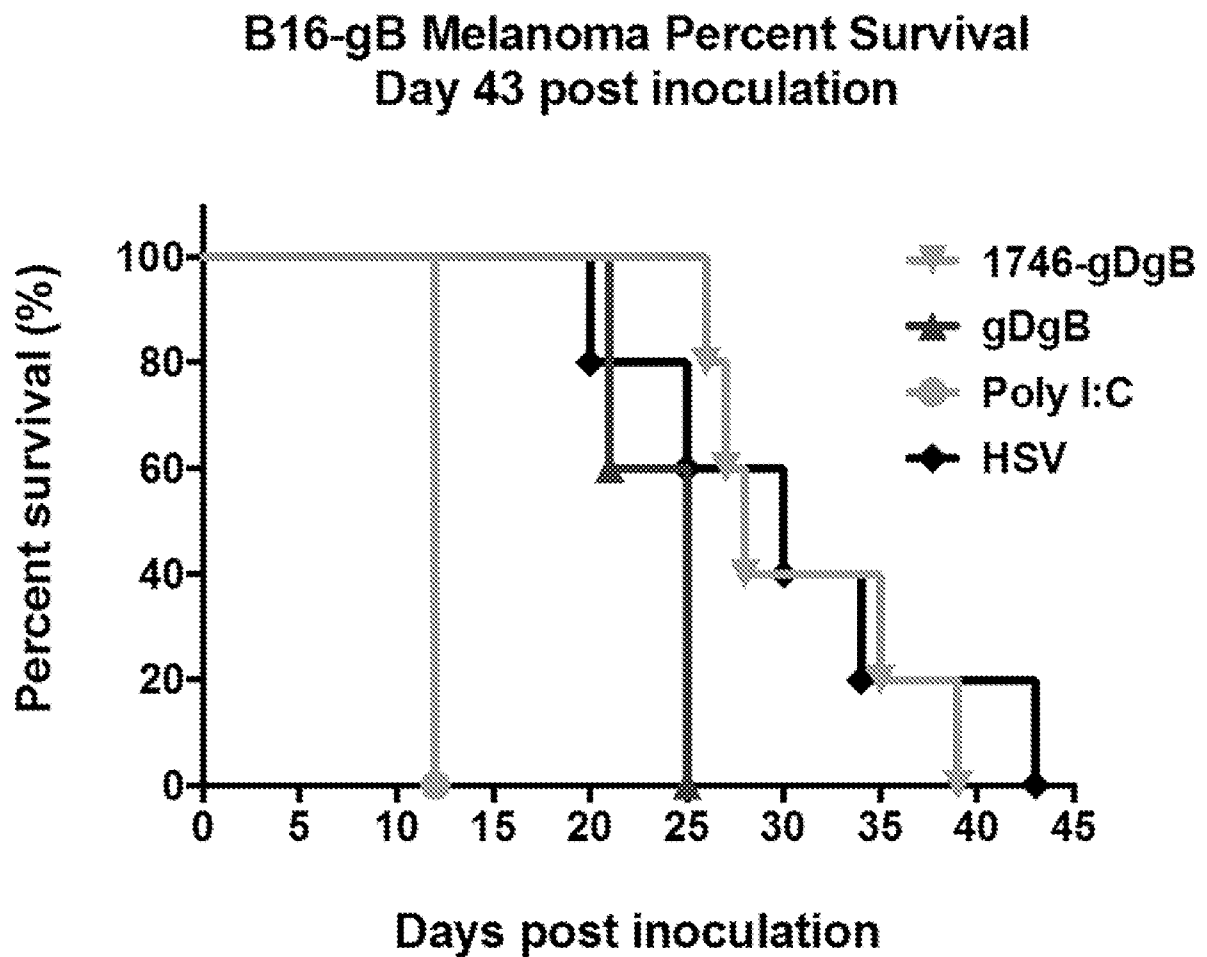
Data is from one experiment with 5 mice per group
 HSV = Herpes Simplex Virus

Figure 15



Data is from one experiment with 5 mice per group
HSV = Herpes Simplex Virus

Figure 16



Data is from one experiment with 5 mice per group
HSV = Herpes Simplex Virus

Figure 17

Therapeutic vaccination strategy

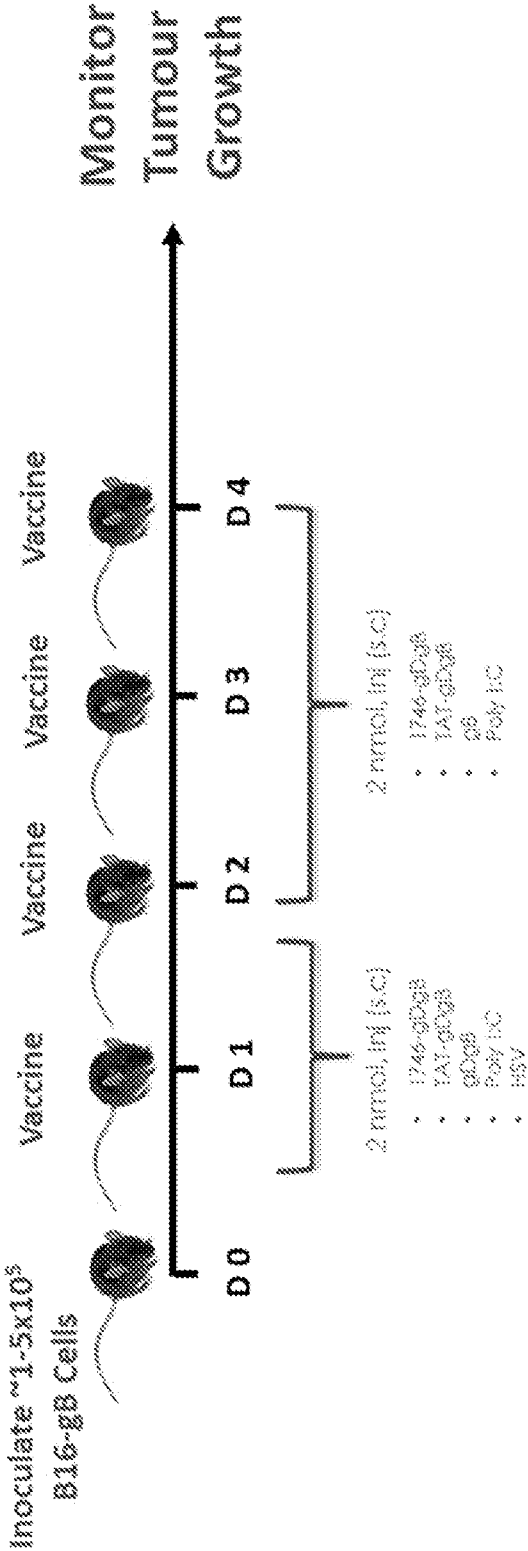


Figure 18

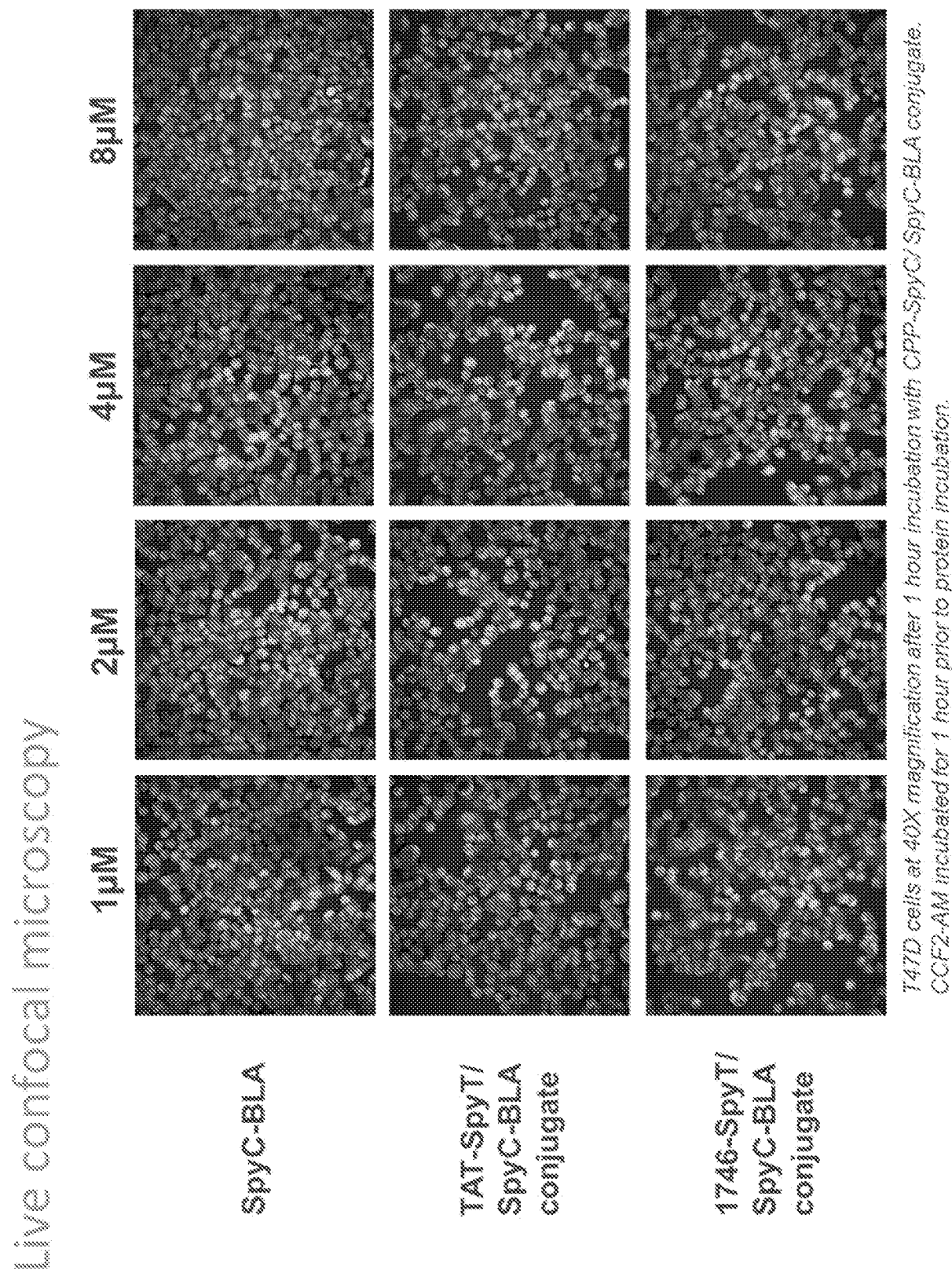


Figure 19

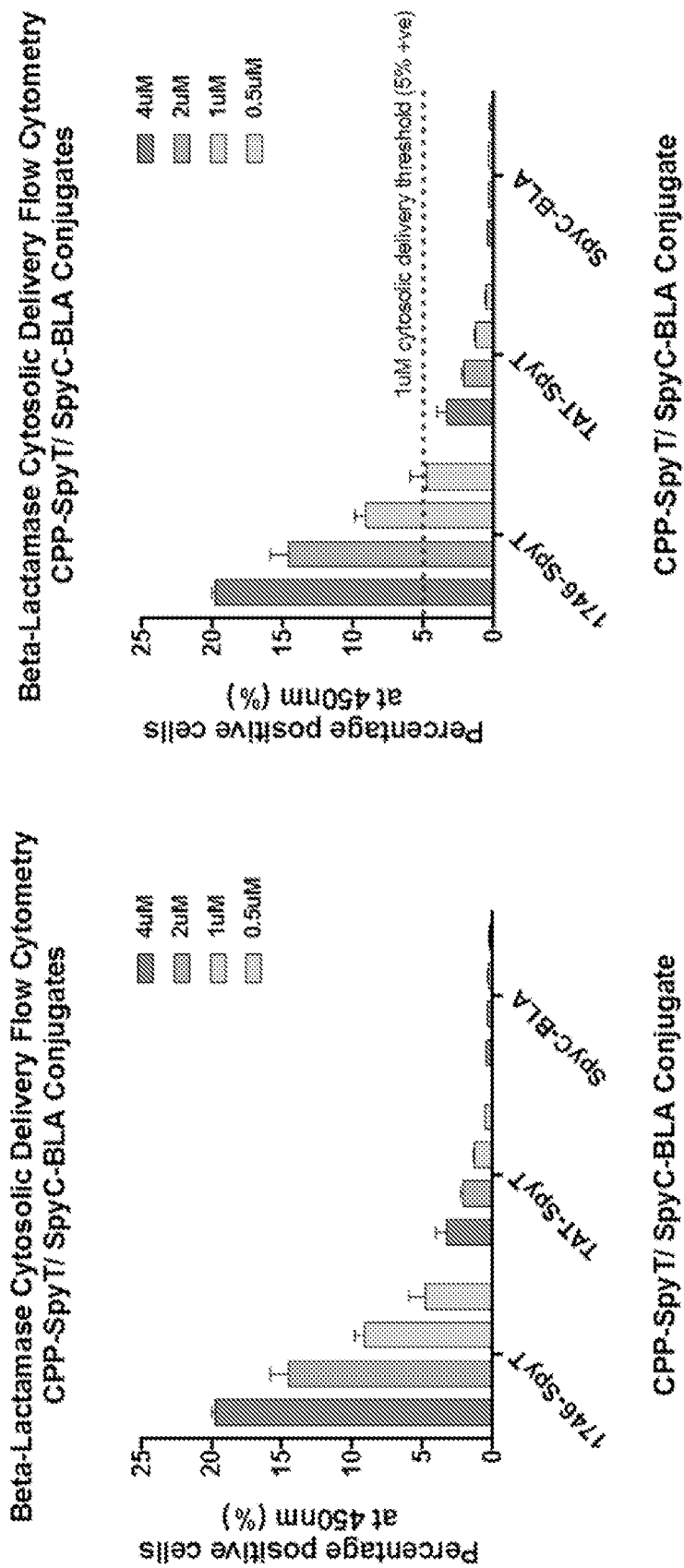
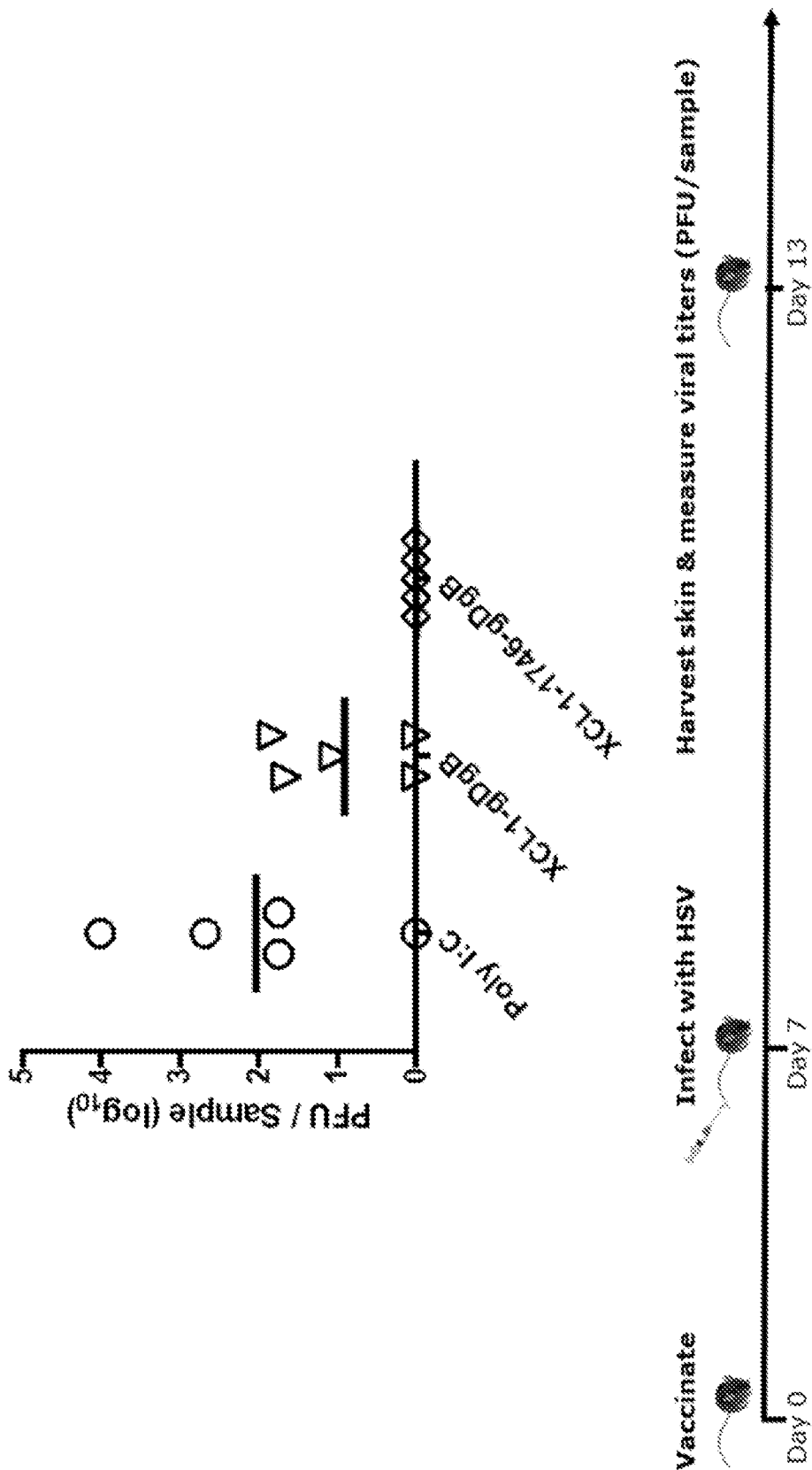


Figure 20



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/051408

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/385 (2006.01) A61K 38/57 (2006.01) A61P 35/00 (2006.01) A61P 31/00 (2006.01) A61P 37/00 (2006.01)
A61K 47/64 (2017.01) A61K 47/65 (2017.01)

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)

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

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

PATENW (English language full text databases), REGISTRY, HCAPLUS, MEDLINE, BIOSIS, EMBASE: SEQ ID NO:5, C07K2319/00, XCR1, DEC205, CD205, LANGERIN, CD207, CLE9A RECEPTOR, DCSIGN, CD209, DNGR1, Antigen Presenting Cell peptide, Dendritic Cell peptide, Macrophage peptide, Cell Penetrating Peptide, Tat peptide, Glycoprotein B or D, Furin, Val-Cit linker, SpyTag, SpyCatcher, Endosomal escape, Lysosomal escape and related terms.

GenomeQuest: SEQ ID NO:6 and 15

Google Scholar: "furin cathepsin linker"

PubMed and Espacenet: SpyCatcher, Phylogica, Telethon Kids Institute, Waithman, Stone S with CPP, Watt P with cell or CPP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 4 February 2019		Date of mailing of the international search report 04 February 2019	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Vivian Turner AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262108484	

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

SEQ ID NO:5, 6 and 15 searched.

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2018/051408
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/146260 A1 (AMAL THERAPEUTICS SA) 22 September 2016 Abstract, p14 lines 1-5, p15 lines 12-16, p19 line 1-9, p28 line 11-14, p84 line 9-10, p134 lines 26-28, p137, SEQ ID NO:21-25	1-25, 27, 29-58
X	WO 2017/025057 A1 (COHERENT BIOPHARMA) 16 February 2017 Abstract, [013], [019], [023], [041], [058], [086], Table 2	1-7, 11-12, 15-17, 23-25, 27, 29-32, 37-50, 55-58
X	WO 2007/015704 A2 (UNIVERSITY OF ROCHESTER) 08 February 2007 p20 lines 16 to p21 line 3, p25 line 28 to p26 line 9, p41 line 14-26, p45 lines 22-29, p48 line 20 to p49 line 16, p54 lines 18-22, p61 lines 8-15	1-8, 22-25, 27, 29-35, 37-58
A	CHEN, X. et al., "Fusion protein linkers: Property, design and functionality", Advanced Drug Delivery Reviews, (2013), vol. 65, pages 1357-1369 Whole Document	1-58
A	REDDINGTON, S. et al., "Secrets of a covalent interaction for biomaterials and biotechnology: SpyTag and SpyCatcher", Current Opinion in Chemical Biology, (2015), vol. 29, pages 94-99 Whole Document	1-58

Form PCT/ISA/210 (fifth sheet) (January 2015)

INTERNATIONAL SEARCH REPORT		International application No.			
Information on patent family members		PCT/AU2018/051408			
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.					
Patent Document/s Cited in Search Report		Patent Family Member/s			
Publication Number	Publication Date	Publication Number	Publication Date		
WO 2016/146260 A1	22 September 2016	WO 2016146260 A1	22 Sep 2016		
		AU 2016232656 A1	10 Aug 2017		
		AU 2016232657 A1	10 Aug 2017		
		AU 2016232659 A1	10 Aug 2017		
		BR 112017015906 A2	10 Apr 2018		
		CA 2973747 A1	22 Sep 2016		
		CA 2973757 A1	22 Sep 2016		
		CA 2973770 A1	22 Sep 2016		
		CL 2017002345 A1	18 May 2018		
		CN 107428845 A	01 Dec 2017		
		EA 201792045 A1	30 Mar 2018		
		EP 3270954 A1	24 Jan 2018		
		EP 3270955 A1	24 Jan 2018		
		EP 3270957 A1	24 Jan 2018		
		JP 2018509935 A	12 Apr 2018		
		JP 2018509936 A	12 Apr 2018		
		JP 2018510215 A	12 Apr 2018		
		KR 20170128516 A	22 Nov 2017		
		MX 2017011796 A	11 Apr 2018		
		PH 12017501287 A1	05 Feb 2018		
		US 2018133295 A1	17 May 2018		
		US 2018133327 A1	17 May 2018		
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		US 2018133339 A1	17 May 2018		
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		WO 2016146259 A1	22 Sep 2016		
		WO 2016146261 A1	22 Sep 2016		
		WO 2016146262 A1	22 Sep 2016		
		WO 2017/025057 A1	16 February 2017	WO 2017025057 A1	16 Feb 2017
				AU 2016305703 A1	23 Nov 2017
				CA 2987322 A1	16 Feb 2017
				CN 106466484 A	01 Mar 2017
CN 106466485 A	01 Mar 2017				
CN 108135881 A	08 Jun 2018				
		EP 3334500 A1	20 Jun 2018		
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
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INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2018/051408	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2007/015704 A2	08 February 2007	JP 2018529632 A	11 Oct 2018
		KR 20180033513 A	03 Apr 2018
		MX 2018001723 A	17 May 2018
		US 2018200377 A1	19 Jul 2018
		WO 2007015704 A2	08 Feb 2007
		CA 2585267 A1	08 Feb 2007
		EP 1846041 A2	24 Oct 2007
		US 2008305119 A1	11 Dec 2008
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(January 2015)			