[0043] The individual may be determined as having virus-associated cancer that is not from HPV.

[0044] Methods according to the present disclosure that involve steps of cell stimulation may be performed *in vitro* or *ex vivo*. The term "*in vitro*" is intended to encompass studies with materials, biological substances, cells and/or tissues in laboratory conditions or in culture. "*Ex vivo*" refers to something present or taking place outside an organism, *e.g.* outside the human or animal body, which may be on tissue (*e.g.* whole organs) or cells taken from the organism.

[0045] In one example, there is a method for stimulating peripheral blood cells, the method comprising stimulating peripheral blood T-cells with antigen presenting cells in the presence of interleukin (IL)-7 and IL-15, wherein the antigen presenting cells were previously exposed to one or more peptides, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of one or more viruses that are not HPV.

[0046] In a particular example, there is a method of producing viral antigen-specific T-cells, comprising the step of stimulating a population of peripheral blood T-cells with antigen presenting cells in the presence of IL-7 and IL-15 (for example, ≥100ng/mL), wherein the antigen presenting cells are or were previously exposed to a library of peptides, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of one or more viruses that are not HPV.

[0046a] In one aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of producing viral antigen-specific T-cells, comprising the step of stimulating a population of peripheral blood T-cells with peptide loaded antigen presenting cells in the presence of IL-7 and IL-15, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of one or more viruses that are not HPV, wherein the method: (a) occurs in the absence of IL-4; and (b) comprises depletion of CD45RA-positive cells from the peripheral blood T-cells.

[0047] In particular examples of the method, a population of peripheral blood T-cells have reduced levels, compared to normal levels, of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells, for example when CD45RA+ cells have been depleted from a starting PBMC or apheresis product. In certain cases, PBMCs or peripheral blood T-cells obtained therefrom are subject to a step of reducing the levels of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells. Peripheral blood T-cells may be present in a population of peripheral blood mononuclear cells (PBMCs) or are obtained or isolated therefrom. PBMCs or an apheresis product may be depleted of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells. The PBMCs may be CD45RA-depleted PBMCs. PBMCs in the population may be non-adherent PBMCs. In specific examples, the PBMCs may be depleted of myeloid cells that are suppressive myeloid cells, for example by adherence or by other depletion methods.

activated T-cells, costimulatory cells, IL-7, and/or IL-15. In specific cases, the method occurs in the absence of exposing the T-cells produced by the method to activated B cells that were previously exposed to a library of peptides.

[0053] In some cases, cells produced by the method are modified to express a gene product from an expression vector, such as modified to express a chimeric antigen receptor, αβ T-cell receptor, or combination thereof.

[0054] In certain cases, a therapeutically effective amount of T-cells produced by the method are provided to an individual that has been exposed to EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV, that is seropositive for EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV, or that has disease associated with EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV. In specific examples, the individual is determined as having a medical condition associated with EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV. In certain examples, the cancer is a non-viral cancer.

[0054a] In another example, the present disclosure refers to use of a therapeutically effective amount of T-cells produced by the method as described herein in the manufacture of a medicament for treating a disease associated with EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV in an individual, wherein the individual is one that has been exposed to EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV, that is seropositive for EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV, or that has disease associated with EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV.

[0055] In specific cases, one or more steps of the method lack the presence of exogenously added IL-4, IL-2, or both.

[0056] In an example, there is a method for stimulating T-cells specific for a virus other than HPV, comprising stimulating T-cells specific for the virus with antigen presenting cells in the presence of IL-7 and IL-15 and in the presence of co-stimulatory cells, wherein the antigen presenting cells were previously exposed to one or more peptides, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus other than HPV.

[0056a] In another aspect, the present disclosure refers to an *in vitro* or *ex vivo* method for stimulating T-cells specific for a virus other than HPV, comprising stimulating T-cells specific for the virus with peptide loaded antigen presenting cells in the presence of IL-7 and IL-15 but in the absence of IL-4 and in the presence of co-stimulatory cells, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus other than HPV, and wherein the antigen presenting cells are depleted of CD45RA-positive cells.

[0057] In another example, there is a method of producing therapeutic T-cells for a virus-associated disease or a non-virus-associated disease, the method comprising the step of stimulating T-cells specific for a virus other than HPV with antigen presenting cells in the presence of one or more of IL-7 and IL-15 and in the presence of co-stimulatory cells, wherein the antigen presenting cells were previously exposed to one or more peptides, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus other than HPV, wherein the stimulating produces T-cells therapeutic for the virus-associated disease or malignancy.

[0057a] In another aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of producing therapeutic T-cells for a virus-associated disease or a non-virus-associated disease, the method comprising the step of stimulating T-cells specific for a virus other than HPV with peptide loaded antigen presenting cells in the presence of one or more of IL-7 and IL-15 but in the absence of IL-4 and in the presence of co-stimulatory cells, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus other than HPV, wherein the stimulating produces T-cells therapeutic for the virus-associated disease, wherein the antigen presenting cells are depleted of CD45RA-positive cells.

The disclosure encompasses at least the following:

1. A method for stimulating peripheral blood cells, the method comprising stimulating peripheral blood T-cells with antigen presenting cells in the presence of interleukin (IL)-7 and IL-15, wherein the antigen presenting cells were previously exposed to one or more peptides, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of one or more viruses that are not human papillomavirus (HPV).

2. A method of producing viral antigen-specific T-cells, comprising the step of stimulating a population of peripheral blood T-cells with antigen presenting cells in the presence of IL-7 and IL-15, wherein the antigen presenting cells are or were previously exposed to a library of peptides, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of one or more viruses that are not HPV.

3. The method of paragraph 1 or 2, wherein the concentration of IL-15 is ≥ 100 ng per mL.

4. The method of paragraph 1, 2, or 3, wherein the stimulating occurs in the absence of IL-6, IL-12, IL-2, IL-4, IL-7, IL-21, or a combination thereof.

5. The method of any one of paragraphs 1-4, wherein the population of peripheral blood T-cells have reduced levels, compared to normal levels, of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells.

6. The method of paragraph 5, wherein the PBMCs or peripheral blood T-cells obtained therefrom are subject to a step of reducing the levels of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells.

7. The method of any one of paragraphs 1-6, wherein the virus is from the family *Herpesviridae* or is a poxvirus, adenovirus, polyomavirus, lentivirus, rhabdovirus or other oncolytic virus.

8. The method of any one of paragraphs 1-7, wherein the virus is selected from the group consisting of Epstein-Barr virus (EBV), Cytomegalovirus (CMV), adenovirus, Vaccinia, and/or Varicella zoster virus (VZV), HIV, influenza, marabavirus vesicular stomatitis virus and an oncolytic virus.

CLAIMS

What is claimed is:

1. An *in vitro* or *ex vivo* method of producing viral antigen-specific T-cells, comprising the step of stimulating a population of peripheral blood T-cells with peptide loaded antigen presenting cells in the presence of IL-7 and IL-15, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of one or more viruses that are not HPV, wherein the method:

(a) occurs in the absence of IL-4; and

(b) comprises depletion of CD45RA-positive cells from the peripheral blood T-cells.

2. The method of claim 1, wherein the stimulating occurs in the absence of IL-6, IL-12, IL-2, IL-21, or a combination thereof.

3. The method of claim 1 or 2, wherein the population of peripheral blood T-cells have reduced levels, compared to normal levels, of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells.

4. The method of claim 3, wherein peripheral blood mononuclear cells (PBMCs) or peripheral blood T-cells obtained therefrom are subject to a step of reducing the levels of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; 3) T-regulatory cells and/or 4) inhibitory myeloid cells.

5. The method of any one of claims 1-4, wherein the virus is from the family *Herpesviridae* or is a poxvirus, adenovirus, polyomavirus, lentivirus, rhabdovirus or other oncolytic virus.

6. The method of any one of claims 1-5, wherein the virus is selected from the group consisting of Epstein-Barr virus (EBV), Cytomegalovirus (CMV), adenovirus, vaccinia virus, and/or Varicella zoster virus (VZV), HIV, influenza, marabavirus vesicular stomatitis virus and an oncolytic virus.

7. The method of any one of claims 1-7, wherein the peripheral blood T-cells are present in a population of PBMCs or are obtained or isolated therefrom.

8. The method of claim 4 or 7, wherein the PBMCs are depleted of one or more of the following: 1) NK cells; 2) naïve cells that can grow as bystander cells; and/or 3) T-regulatory cells.

9. The method of any one of claims 4 or 7-8, wherein the PBMCs in the population are non-adherent PBMCs.

10. The method of any one of claims 1-9, wherein the antigen presenting cells are dendritic cells or PBMCs.

11. The method of any one of claims 1-10, wherein the step of stimulating a population of peripheral blood T-cells occurs in the presence of costimulatory cells.

12. The method of claim 11, wherein the costimulatory cells are CD80+, CD86+, CD83+, 4-1BBL+, or a combination thereof, or wherein the costimulatory cells are HLA-negative lymphoblastoid cells.

13. The method of any one of claims 1-12, wherein the stimulating occurs in the presence of activated T-cells, dendritic cells, PBMCs, or HLA-negative costimulatory cells.

14. The method of claim 13, wherein when the stimulating occurs in the presence of activated T-cells, dendritic cells, PBMCs, or HLA-negative costimulatory cells, the stimulating step is not a first stimulation step.

15. The method of any one of claims 1-14, wherein the stimulating occurs in the presence of pepmixes, pepmix-pulsed autologous activated T-cells, in the presence of HLA-negative costimulatory cells, or both.

16. The method of claim 15, wherein when the stimulating occurs in the presence of pepmix-pulsed autologous activated T-cells, in the presence of HLA-negative costimulatory cells, or both, the stimulating step is not a first stimulation step.

17. The method of any one of claims 1-16, wherein the peptide library comprises peptides of at least or no more than 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acids in length.

18. The method of any one of claims 1-17, wherein the peptide library comprises peptides of 15 amino acids in length.

19. The method of any one of claims 1-18, wherein peptides in the library overlap in sequence with other peptides by 11 amino acids.

20. The method of any one of claims 1-19, wherein T-cells produced by a first stimulating step are subject to one or more subsequent stimulating steps.

21. The method of claim 20, wherein a subsequent stimulating step occurs in the presence of IL-7 and IL-15.

22. The method of claim 20 or 21, wherein a subsequent stimulating step occurs in the presence of activated T-cells, costimulatory cells, IL-7, and IL-15.

23. The method of any one of claims 1-22, wherein the method occurs in the absence of exposing the T-cells produced by the method to activated B cells that were previously exposed to a library of peptides.

24. The method of any one of claims 1-23, wherein the viral antigen-specific T-cells are modified to express a gene product from an expression vector.

25. The method of claim 24, wherein the cells are modified to express a chimeric antigen receptor, αβ T-cell receptor, or combination thereof.

26. The method of any one of claims 1-25, wherein one or more steps of the method lack the presence of exogenously added IL-4, IL-2, or both.

27. An *in vitro* or *ex vivo* method for stimulating T-cells specific for a virus other than HPV, comprising stimulating T-cells specific for the virus with peptide loaded antigen presenting cells in the presence of IL-7 and IL-15 but in the absence of IL-4 and in the presence of co-stimulatory cells, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus other than HPV, and wherein the antigen presenting cells are depleted of CD45RA-positive cells.

28. An *in vitro* or *ex vivo* method of producing therapeutic T-cells for a virus-associated disease or a non-virus-associated disease, the method comprising the step of stimulating T-cells

specific for a virus other than HPV with peptide loaded antigen presenting cells in the presence of one or more of IL-7 and IL-15 but in the absence of IL-4 and in the presence of co-stimulatory cells, wherein the peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus other than HPV, wherein the stimulating produces T-cells therapeutic for the virus-associated disease, wherein the antigen presenting cells are depleted of CD45RA-positive cells.