

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.~~

45. The method of any one of claims 1-~~34~~, wherein the virus is from the family *Herpesviridae* or is a poxvirus, adenovirus, polyomavirus, lentivirus, rhabdovirus or other oncolytic virus.

56. The method of any one of claims 1-~~45~~, 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.

67. The method of any one of claims 1-~~57~~, wherein the peripheral blood T-cells are present in a population of PBMCs or are obtained or isolated therefrom.

78. The method of claim ~~64~~or 7, wherein the PBMCs or peripheral blood T-cells obtained therefrom, 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; and/or 4) inhibitory myeloid cells.

89. The method of ~~any one of claims 4 or 7-8~~ 6 or claim 7, wherein the PBMCs in the population are non-adherent PBMCs.

940. The method of any one of claims 1-89, wherein the antigen presenting cells are dendritic cells or PBMCs.

1044. The method of any one of claims 1-940, wherein the step of stimulating a population of peripheral blood T-cells occurs in the presence of costimulatory cells.

1142. The method of claim 1044, wherein the costimulatory cells are CD80+, CD86+, CD83+, 4-1BBL+, or a combination thereof, or wherein the costimulatory cells are HLA-negative lymphoblastoid cells.

1243. The method of any one of claims 1-1142, wherein the stimulating occurs in the presence of activated T-cells, dendritic cells, or PBMCs; and optionally in the presence of ~~or~~ HLA-negative costimulatory cells.

1344. The method of claim 1243, wherein when the stimulating occurs in the presence of activated T-cells, dendritic cells, or PBMCs, ~~or~~; and optionally in the presence of HLA-negative costimulatory cells, the stimulating step is not a first stimulation step.

1445. The method of any one of claims 1-1344, 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.

1546. The method of claim 1445, 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.

16. The method of any one of claims 1 to 15, wherein the peptide loaded antigen presenting cells are loaded, or have been previously loaded, by a method comprising exposing the antigen presenting cells to a library of peptides, the library of peptides comprising a mixture of peptides

from one or more viral antigens, wherein the antigens are EBV, CMV, adenovirus, vaccinia virus and/or VZV antigens.

17. The method of ~~any one of claim s 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 1-16~~ or claim 17, wherein the peptide library comprises peptides of 15 amino acids in length.

19. The method of any one of claims ~~16~~-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, $\alpha\beta$ 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.