

molecules. If stimulation is too potent, non-specific bystander cells are expanded diluting the antigen-specific T-cells.

**[0008]** Autologous dendritic cells (DCs) are potent antigen-presenting cells, but their numbers are limiting; they do not divide and their monocyte precursors less than 10% of blood mononuclear cells (PBMCs). Large amounts of blood would be required to obtain sufficient DCs for T-cell expansion (300 mLs to 1 liter of blood).

**[0009]** Autologous EBV-transformed B-lymphoblastoid cells lines (LCLs) are also excellent antigen-presenting cells, but it takes at least 6 weeks to establish LCLs from patients and LCLs express highly immunogenic EBV antigens that compete with weaker EBV and non-EBV antigens.

**[0010]** The present disclosure addresses a variety of issues associated with antigen-specific T-cell activation and expansion and provides relief for a long-felt need in the art to treat virus-associated diseases and malignancies with effective immunotherapies.

## SUMMARY

**[0011]** The present disclosure is directed to methods and compositions that concern immune system cells that immunogenically recognize particular targets. In some examples, the present disclosure concerns the development of virus-specific T cells (VSTs) (that may also be referred to as virus antigen-specific T cells or antigen-specific T cell) that target a biological moiety that elicits an immune response in an individual. In specific examples, the present disclosure concerns the development of VSTs that target a viral antigen, including a viral disease-associated antigen. Examples of the disclosure include production of cells that are CD8+ T cells, CD4+ T cells, and cells that do not kill but produce one or more cytokines. In some cases, a mixture of cytotoxic T-cells is produced, and the mixture targets more than one viral antigen, including more than one antigen of more than one virus, in some cases. In particular examples, the virus is not human papilloma virus (HPV). Examples of the disclosure concern the generation and/or expansion of non-HPV-specific T-cells.

**[0012]** Examples of the disclosure concern methods and compositions for providing therapy to individuals infected with a non-HPV virus or that have virus-associated diseases and malignancies that are not associated with HPV, including cancers associated with certain viruses.

In specific examples, the disclosure regards methods and compositions for adoptive cellular immunotherapy that can target virus-associated medical conditions and are therapeutic therefor.

**[0013]** In certain examples, the present disclosure concerns the development of a plurality of T-cells that target antigens from EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV, for example. The present disclosure provides significant and non-obvious improvements on methods for generating T cell lines with specificity against antigens from EBV, CMV, adenovirus, vaccinia virus, and/or VZV, for example, wherein the virus is not HPV.

**[0014]** In some examples of the disclosure, an individual is in need of the methods and/or compositions of the disclosure. In certain examples, an individual has been exposed to EBV, CMV, adenovirus, vaccinia, and/or VZV, for example (the presence of which may or may not be known for the individual), or the individual is suspected of having been exposed to or at risk for being exposed to EBV, CMV, adenovirus, vaccinia virus, and/or VZV, for example. In certain examples, the individual has or is suspected of having or is at risk for having disease associated with EBV, CMV, adenovirus, vaccinia, and/or VZV, or has been vaccinated with EBV, CMV, adenovirus, VZV or vaccinia virus, for example.

**[0015]** In specific examples of at least part of the method, certain antigen(s) associated with EBV, CMV, adenovirus, vaccinia, and/or VZV are presented to APCs in the form of one or more peptides that span some or all of certain antigen(s). The antigenic peptides may be provided to the APCs in a library of peptide mixtures, which may be referred to as pepmixes. In certain examples of the disclosure, there is pooling of a variety of pepmixes for exposure to the APCs. APCs that present the antigens on MHC molecules may be exposed to peripheral blood T-cells under certain conditions to result in stimulation of T-cells specific for the certain viral antigen(s).

**[0016]** In some examples, there are methods for stimulating peripheral blood cells, such as peripheral blood T-cells, wherein the method comprises stimulating peripheral blood T-cells with antigen presenting cells in the presence of interleukin (IL)-7 and IL-15 and, in at least some cases, in the absence of one or more other cytokines, such as IL-6 and/or IL-12, 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 that is not HPV.

**[0017]** Methods for stimulating T-cells specific for a non-HPV virus or for an antigen from a non-HPV virus include stimulating T-cells specific for the virus or for the antigen with antigen presenting cells in the presence of IL-7 and IL-15, and optionally 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 the virus.

**[0018]** In some cases there are methods of producing therapeutic T-cells for non-HPV virus-associated disease(s), comprising the step of stimulating peripheral blood T-cells with antigen presenting cells in the presence of one or more of interleukin IL-7 and IL-15 and, in at least some cases, optionally in the absence of one or more other cytokines, such as IL-6 and/or IL-12, 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 that is not HPV, wherein the stimulating produces T-cells therapeutic for virus-associated diseases and malignancies that are not HPV-associated diseases.

**[0019]** In specific examples, there are methods of producing therapeutic T-cells for virus-associated diseases and malignancies other than HPV-associated diseases, comprising the step of stimulating T-cells specific for the virus or for a viral antigen with antigen presenting cells in the presence of one or more of interleukin IL-7 and IL-15, and optionally in the presence of co-stimulatory cells, wherein the antigen presenting cells were previously exposed to one or more peptides, wherein the peptides comprise sequences that corresponds to at least part of the sequence of one or more proteins of a virus that is not HPV, wherein the stimulating produces T-cells therapeutic for one or more virus-associated diseases and malignancies.

**[0020]** In some cases, the peripheral blood T-cells being stimulated are obtained from a prior stimulation of peripheral blood cells, such as stimulating peripheral blood cells with antigen presenting cells in the presence of IL-7 and IL-15, and in at least some cases in the presence of one or more other cytokines, such as IL-6 and/or IL-12, 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 that is not HPV. As such, prior to stimulating the peripheral blood T-cells, the methods may further comprise stimulating peripheral blood cells with antigen presenting cells in the presence of IL-7 and IL-15, and in at least some cases in the presence of IL-6 and/or IL-12, 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 that is not HPV, to produce peripheral blood T-cells.

**[0021]** In some examples the one or more peptides comprise sequence that corresponds to at least part of the sequence of one or more proteins of a virus that is not HPV. In some examples the one or more peptides may be a library of peptides, which may also be referred to as a collection of peptides. In specific examples the method may produce T-cells specific for EBV, CMV, adenovirus, vaccinia, and/or VZV or for an antigen from EBV, CMV, adenovirus, Vaccinia, and/or VZV. In some examples the method may expand a population of T-cells present in the peripheral blood T-cells that are specific for EBV, CMV, adenovirus, vaccinia, and/or VZV or for an antigen from EBV, CMV, adenovirus, vaccinia, and/or VZV.

**[0022]** APCs used in one or more methods of the disclosure include monocytes, dendritic cells (DC), B-Blasts (BB), and/or PBMCs, for example. In particular examples the antigen presenting cells are activated T-cells.

**[0023]** In some examples the stimulation of T-cells specific for a virus other than HPV or for an antigen other than one from HPV is not a first stimulation step. The T-cells being stimulated cells may be the product of a prior stimulation. In certain examples stimulation of T-cells specific for a virus other than HPV or for an antigen other than HPV comprises stimulating T-cells specific for the virus or for the viral antigen with antigen presenting cells in the presence of interleukin (IL)-7, IL-15, and in the presence of one or more types of co-stimulatory cells.

**[0024]** In some examples the method may produce T-cells specific for a non-HPV virus or for an antigen that is not an HPV antigen. In some examples the method may expand a population of T-cells specific for a virus or for an antigen other than HPV.

**[0025]** In specific examples, stimulation of peripheral blood T-cells in the presence of IL-7 and IL-15 optionally occurs in the absence of IL-2. In some examples stimulation of peripheral blood T-cells in the presence of IL-7 and IL-15 optionally occurs in the absence of at least IL-4; although in some cases IL-4 is added, for example to increase CD4+ T cells. In some examples stimulation of peripheral blood T-cells in the presence of IL-7 and IL-15 occurs optionally in the absence of IL-6. In some examples stimulation of peripheral blood T-cells

in the presence of IL-7 and IL-15 but alternatively may occur in the absence of IL-7 and/or IL-15. In some examples stimulation of peripheral blood T-cells in the presence of IL-7 and IL-15 optionally occurs in the absence of IL-12. In some examples stimulation of peripheral blood T-cells in the presence of IL-7 and IL-15 optionally occurs in the absence of IL-21.

**[0026]** In some examples, the peripheral blood T-cells utilized in methods of the disclosure may be present in a population of peripheral blood mononuclear cells (PBMCs) or are obtained or isolated therefrom. The PBMCs in the population may be non-adherent PBMCs, or may be CD45RA-depleted PBMCs (for example, to eliminate a combination of Tregs, NK cells and naïve T-cells). The antigen presenting cells may be dendritic cells, B-blasts, or PBMCs, for example.

**[0027]** Methods of the present disclosure include methods of producing therapeutic T-cells for virus-associated diseases and malignancies that are not HPV. The stimulation of cells may produce T-cells that are therapeutic for virus-associated diseases and malignancies that are not HPV-associated diseases. In some examples a method of producing therapeutic T-cells for HPV-associated diseases is provided, the method comprising:

**[0028]** (i) stimulating peripheral blood cells, wherein the method comprises stimulating peripheral blood T-cells with antigen presenting cells in the presence of interleukin (IL)-7 and IL-15 and optionally in the absence of IL-6 and/or IL-12, 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 non-HPV virus; or

**[0029]** (ii) stimulating T-cells obtained from (i) with antigen presenting cells in the presence of interleukin (IL)-7 and IL-15 and optionally in the absence of IL-6 and/or IL-12, 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 the virus, wherein (ii) is optionally repeated one or more times;

**[0030]** (iii) stimulating T-cells obtained from (ii) with antigen presenting cells in the presence of IL-7 and IL-15, and optionally 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 the virus, wherein (iii) is optionally repeated one or more times.

**[0031]** In some examples the antigen presenting cells used in (i) and (ii) are monocytes, dendritic cells (DC) B-Blasts (BB) or PBMCs. In some examples the antigen presenting cells used in (iii) are activated T-cells, dendritic cells (DC), B-Blasts (BB) or PBMCs. In some examples the antigen presenting cells used in (iii) are different than the antigen presenting cells used in (i) and/or (ii). In preferred examples the antigen presenting cells used in (iii) are activated T-cells.

**[0032]** In particular examples, stimulation occurs in the presence of co-stimulatory cells. In some examples the co-stimulatory cells are one or more cell types selected from the group consisting of CD80+ cells, CD86+ cells, CD83+ cells, 4-1BBL+ cells, and a combination thereof. The co-stimulatory cells may be CD80+/CD86+/CD83+/4-1BBL+ cells. The co-stimulatory cells may be HLA-negative lymphoblastoid cells.

**[0033]** In some particular examples, methods of the present disclosure are for producing T-cells specific for EBV, CMV, adenovirus, Vaccinia, and/or VZV. In some particular examples methods of the present disclosure are for producing T-cells specific for diseases and malignancies associated with EBV, CMV, adenovirus, Vaccinia, and/or VZV.

**[0034]** In some examples, peripheral blood T-cells may be obtained from an individual that is known to be infected or suspected of being infected or vaccinated with EBV, CMV, adenovirus, vaccinia, and/or VZV. Antigen presenting cells may be obtained from an individual that is known to be infected or suspected of being infected or vaccinated with EBV, CMV, adenovirus, vaccinia virus, and/or VZV.

**[0035]** In some examples, the method may occur 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.

**[0036]** In some examples, antigen presenting cells may be autologous or allogeneic to an individual intended to be treated with the therapeutic T-cells obtained.

**[0037]** In some examples, the one or more peptides comprise sequence that corresponds at least part to the sequence of one or more proteins of EBV, CMV, adenovirus, vaccinia virus, and/or VZV. The peptides may correspond to a contiguous amino acid sequence present within the viral protein. A peptide may have a length of at least or no more than 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length, or of 15 amino acids in length. The

collection of peptides may form a library and peptides in the library may overlap in sequence with other peptides by any suitable amount, including 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids, for example. The peptides may comprise sequence that corresponds to the viral protein.

**[0038]** T-cells produced by the methods of the present disclosure may be isolated and/or purified, *e.g.*, isolated/purified from other cells.

**[0039]** In some examples, a therapeutically effective amount of T-cells produced by the methods of the present disclosure are provided to an individual that has been exposed to a virus that is not HPV, or that has a virus-associated disease that is not from HPV. In a related example T-cells produced by the methods of the present disclosure are provided for use in the treatment of virus-associated disease that is not from HPV. In another related example the use of T-cells produced by the method of the present disclosure are provided for use in the manufacture of a medicament for use in the treatment of virus-associated disease that is not HPV.

**[0040]** In particular examples, the methods of the disclosure encompass the use of viruses to promote the expansion of VSTs modified with one or more engineered receptors, for example chimeric antigen receptors (CARs). For example, if the virus was VZV and the VSTs were VZV-specific (VZVSTs), then a VZV vaccine (*e.g.*, ZOSTAVAX or VARIVAX) could be used to stimulate proliferation of CAR-modified VZVSTs after infusion. If the VSTs were specific for an oncolytic virus such as adenovirus, maraba virus or vaccinia or VSV, then the oncolytic virus (OV) could not only kill tumor cells, but also stimulate CAR-modified T-cells specific for that oncolytic virus. The CAR-modified OV-specific T cells (CAR-OVSTs) could then kill uninfected or metastatic tumors cells *via* the CAR.

**[0041]** The individual to be treated may be a human. The individual may be a patient. The individual may have been exposed to EBV, CMV, adenovirus, vaccinia virus, and/or VZV, or has a disease associated with EBV, CMV, adenovirus, vaccinia virus, and/or VZV. The disease may be a neoplasm, such as a cancer of any kind.

**[0042]** The individual may have received, be receiving, or will receive an additional therapy for the disease including, where appropriate, an additional cancer therapy, such as surgery, radiation, hormone therapy, chemotherapy, immunotherapy, or a combination thereof.

**[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,  $\geq 100\text{ng/mL}$ ), 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 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, 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.

**[0048]** Viruses encompassed by the disclosure include those from the family *Herpesviridae* or is a poxvirus, adenovirus, polyomavirus, lentivirus, rhabdovirus or other oncolytic virus. In specific examples, 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 or any other oncolytic virus.

**[0049]** Antigen presenting cells utilized in methods of the disclosure may be dendritic cells or PBMCs, in specific examples.

**[0050]** In methods encompassed by the disclosure a stimulating step occurs in the absence of IL-6, IL-12, IL-2, IL-4, IL-21, or a combination thereof. In certain examples, a stimulating step occurs in the presence of costimulatory cells, such as costimulatory cells that are CD80+, CD86+, CD83+, 4-1BBL+, or a combination thereof, or wherein the costimulatory cells are HLV-negative lymphoblastoid cells. The stimulating may occur in the presence of activated T-cells, dendritic cells, PBMCs, or HLA-negative costimulatory cells. The stimulating may occur in the presence of activated T-cells, dendritic cells, PBMCs, or HLA-negative costimulatory cells, the stimulating step is not a first stimulation step. Activated T-cells may be autologous to the individual. Stimulating may occur in the presence of pepmixes, pepmix-pulsed autologous activated T-cells, in the presence of HLA-negative costimulatory cells, or both. In specific cases, 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. In such cases, two stimulations are performed and the second stimulation occurs in the presence of costimulatory cells and pepmix-pulsed (autologous activated T cells (AATCs).

**[0051]** Peptide libraries utilized in methods of the disclosure may comprise 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. In specific cases, the peptide library comprises peptides of 15 amino acids in length and/or peptides in the library overlap in sequence with other peptides by 11 amino acids.

**[0052]** In certain examples, T-cells produced by a first stimulating step are subject to one or more subsequent stimulating steps, such as a subsequent stimulating step that occurs in the presence of IL-7 and IL-15. A subsequent stimulating step may occur in the presence of

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,  $\alpha\beta$  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 aspect, 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 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 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, 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 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 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, 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  $\geq 100$  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.

Vaccinia, HIV, and/or VZV, or that has disease associated with EBV, CMV, adenovirus, Vaccinia, HIV, and/or VZV.

31. The method of any one of paragraphs 1-30, wherein the individual is determined as having a medical condition associated with EBV, CMV, adenovirus, Vaccinia, HIV, and/or VZV.

32. The method of any one of paragraphs 1-31, wherein one or more steps of the method lack the presence of exogenously added IL-4, IL-2, or both.

33. 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.

34. 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 viral-associated disease.

**[0058]** The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description of the disclosure that follows may be better understood. Additional features and advantages of the disclosure will be described hereinafter which form the subject of the claims of the disclosure. It should be appreciated by those skilled in the art that the conception and specific example disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present disclosure. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the disclosure as set forth in the appended claims. The novel features which are believed to be characteristic of the disclosure, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the

## 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 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, 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 claim 13 or 14, wherein the activated T-cells are autologous to the individual.
16. The method of any one of claims 1-15, 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.
17. The method of claim 16, 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.

18. The method of any one of claims 1-17, 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.
19. The method of any one of claims 1-18, wherein the peptide library comprises peptides of 15 amino acids in length.
20. The method of any one of claims 1-19, wherein peptides in the library overlap in sequence with other peptides by 11 amino acids.
21. The method of any one of claims 1-20, wherein T-cells produced by a first stimulating step are subject to one or more subsequent stimulating steps.
22. The method of claim 21, wherein a subsequent stimulating step occurs in the presence of IL-7 and IL-15.
23. The method of claim 21 or 22, wherein a subsequent stimulating step occurs in the presence of activated T-cells, costimulatory cells, IL-7, and IL-15.
24. The method of any one of claims 1-23, 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.
25. The method of any one of claims 1-24, wherein the viral antigen-specific T-cells are modified to express a gene product from an expression vector.
26. The method of claim 25, wherein the cells are modified to express a chimeric antigen receptor,  $\alpha\beta$  T-cell receptor, or combination thereof.
27. The method of any one of claims 1-26, wherein one or more steps of the method lack the presence of exogenously added IL-4, IL-2, or both.
28. Use of a therapeutically effective amount of T-cells produced by the method of any one of claims 1-27 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.

29. The use of claim 28, wherein the individual is determined as having a medical condition associated with EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV.

30. 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 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 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, and wherein the antigen presenting cells are depleted of CD45RA-positive cells.

31. 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 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 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, wherein the antigen presenting cells are depleted of CD45RA-positive cells.