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 embodiments, 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 embodiments, the present disclosure concerns the development of VSTs that target a viral antigen, including a viral disease-associated antigen. Embodiments of the disclosure include production of cells that are CD8+ T cells, CD4+ T cells, and cells that do no 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 embodiments, the virus is not human papilloma virus (HPV). Embodiments of the disclosure concern the generation and/or expansion of non-HPV-specific T-cells.

[0012] Embodiments 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 embodiments, the disclosure regards methods and compositions for adoptive cellular immunotherapy that can target virus-associated medical conditions and are therapeutic therefor.

[0013] In certain embodiments, 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 embodiments of the disclosure, an individual is in need of the methods and/or compositions of the disclosure. In certain embodiments, 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 embodiments, 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 embodiments 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 embodiments 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 embodiments, 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.

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 embodiments, 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 embodiment 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 embodiment 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 embodiments, 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 embodiment, 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 embodiment, 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, there is provided 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, wherein the method comprises one or more of the following: (a) the method occurs in the absence of IL-4; (b) the concentration of IL-15 is ≥ 100 ng per mL; and (c) the method comprises depletion of CD45RA-positive cells from the peripheral blood T-cells.

[0047] In particular embodiments 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 embodiments, 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 embodiments, the individual is determined as having a medical condition associated with EBV, CMV, adenovirus, vaccinia virus, HIV, and/or VZV. In certain embodiments, the cancer is a non-viral cancer.

[0054a] In another aspect, there is provided 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 embodiment, 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, there is provided 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 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 embodiment, 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, there is provided 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 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 embodiment 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

accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawings.

[0060] FIG. 1 illustrates general embodiments of virus-specific T-cell (VST) generation methods of the disclosure.

[0061] FIG. 2 demonstrates improved specificity of methods of the disclosure that employ IL-7 and IL-15 as compared to known methods that employ IL-4 and IL-7.

[0062] FIG. 3 shows improved specificity of lymphoma patient EBVSTs grown in the presence of IL-7 and IL-15.

[0063] FIG. 4 demonstrates that high doses of IL-15 increase specificity of VSTs.

[0064] FIG. 5 shows that high dose of IL-15 increases central memory EBVSTs.

[0065] FIG. 6 shows excessive NK-cell outgrowth in EBVSTs from some patients.

[0066] FIG. 7 illustrates generation of pepmix-activated EBVSTs from CD45RA-depleted PBMCs.

[0067] FIG. 8 demonstrates that CD45RA depletion decreases the frequency of CD3-CD56+ NK cells in EBVSTs expanded from healthy donors.

[0068] FIG. 9 shows that removal of CD45RA+ cells increases proliferation of EBVSTs.

[0069] FIG. 10 demonstrates that CD45RA depletion enhanced the fold expansion of EBVSTs.

[0070] FIG. 11 shows CD45RA depletion enhances antigen specificity of EBVSTs at the end of a second stimulation (at day 16).

CLAIMS

What is claimed is:

1. 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, wherein the method comprises one or more of the following:

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

(b) the concentration of IL-15 is $\geq$ 100 ng per mL; and

(c) the method 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 or apheresis product 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 a stimulating step 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 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, αβ 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. 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 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. 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 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.