Title: T-CELL IMMUNOGENS DERIVED FROM ANTI-VIRAL PROTEINS AND METHODS OF USING SAME

Abstract: Isolated polypeptides related to endogenous anti-viral polypeptides; and compositions, including immunogenic compositions, comprising a subject isolated polypeptide are disclosed herein. A subject isolated polypeptide comprises an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of one or more endogenous anti-viral polypeptides, wherein the endogenous anti-viral polypeptides are polypeptides subject to proteolytic degradation as a result of the activity of one or more viral proteins. Also provided are diagnostic and treatment methods using the subject isolated polypeptides and compositions.
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T-CELL IMMENS DERIVED FROM ANTI-VIRAL PROTEINS AND METHODS OF USING SAME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/101,590, filed September 30, 2008, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] A number of host cell proteins have evolved that inhibit retroviral infection, retroelement mobilization, and/or replication. Examples of such proteins include apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptides, tetherin polypeptides and tripartite motif-containing 5 (TRIM5) polypeptides. Certain retroviruses, e.g., HIV, have evolved proteins which antagonize the anti-viral effects of one or more of these proteins. For example, HIV Vpu has been shown to antagonize the anti-viral activity of the tetherin polypeptide, CD317, and HIV Vif has been shown to antagonize the antiviral activity of APOBEC 3G and 3F polypeptides. It has been shown that Vif triggers proteosomal degradation of APOBEC via a physical interaction with APOBEC 3G.

[0003] Despite recent advances in HIV research, the World Health Organization (WHO) estimates that currently between 30 and 36 million people worldwide are living with HIV/AIDS and that approximately 2.7 million people were newly infected in the last year (UNAIDS 2008 Report on the global AIDS epidemic). There is a need in the art for methods useful in the treatment and/or prophylaxis of HIV infection.

Literature


SUMMARY OF THE INVENTION

[0005] Isolated polypeptides related to endogenous anti-viral polypeptides; and compositions, including immunogenic compositions, comprising a subject isolated polypeptide are disclosed herein. A subject isolated polypeptide comprises an amino acid sequence having substantial
amino acid sequence identity to a contiguous stretch of amino acids of one or more endogenous anti-viral polypeptides, wherein the endogenous anti-viral polypeptides are polypeptides subject to proteolytic degradation as a result of the activity of one or more viral proteins. Also provided are diagnostic and treatment methods using the subject isolated polypeptides and compositions.

[0006] In one embodiment, an immunogenic composition is disclosed, wherein the immunogenic composition includes a) an isolated polypeptide including an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide; and b) a pharmaceutically acceptable carrier.

[0007] In one embodiment of the immunogenic composition, the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID Nos: 1-10 and 23-24. In one embodiment of the immunogenic composition, the isolated polypeptide has a length of about 6 to about 150 amino acids. In one embodiment of the immunogenic composition, the endogenous polypeptide is an apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, or a TRIM5 polypeptide. In one embodiment where the endogenous polypeptide is an apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, or a TRIM5 polypeptide, the endogenous polypeptide is an APOBEC polypeptide. In one embodiment of the immunogenic composition, the isolated polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to any one of SEQ ID Nos: 1-24. In one embodiment of the immunogenic composition, the isolated polypeptide includes an amino acid sequence set forth in any one of SEQ ID Nos: 1-24. The immunogenic composition can be formulated for parenteral administration. The immunogenic composition can also be formulated for administration to a mucosal tissue. In one embodiment of the immunogenic composition, the immunogenic composition also includes an adjuvant. Where the immunogenic composition also includes an adjuvant, the adjuvant can include aluminum hydroxide, MF59, or monophosphoryl lipidA.

[0008] In another embodiment, an immunogenic composition is described, wherein the immunogenic composition includes a nucleic acid including a nucleotide sequence encoding a polypeptide, wherein said polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide.
In one embodiment of the immunogenic composition including a nucleic acid, the polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24. In one embodiment of the immunogenic composition including a nucleic acid, the polypeptide has a length of about 6 to about 150 amino acids. In one embodiment of the immunogenic composition including a nucleic acid, the endogenous polypeptide is an apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, or a TRIM5 polypeptide. In one embodiment of the immunogenic composition including a nucleic acid, the endogenous polypeptide is an APOBEC polypeptide. In one embodiment of the immunogenic composition including a nucleic acid, the encoded polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to any one of SEQ ID NOs: 1-24. In one embodiment of the immunogenic composition including a nucleic acid, the encoded polypeptide includes an amino acid sequence set forth in any one of SEQ ID NOs: 1-24. The immunogenic composition including a nucleic acid can be formulated for parenteral administration. The immunogenic composition including a nucleic acid can also be formulated for administration to a mucosal tissue.

In one embodiment of the immunogenic composition including a nucleic acid, the nucleic acid is a recombinant vector. In one embodiment, the recombinant vector is a recombinant viral vector.

In another embodiment, a method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus is described, wherein the method includes administering to the individual one of the immunogenic compositions described above. In one embodiment of the method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus, the T lymphocyte response includes a CD8+ T cell response or a CD4+ T cell response. In one embodiment of the method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus, the T lymphocyte response includes a mucosal T lymphocyte response. In one embodiment of the method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus, the pathogenic virus is a human immunodeficiency virus. In one embodiment of the method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus, the individual has not been infected with the pathogenic virus. In another embodiment of the method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus, the individual has been infected with the pathogenic virus.
In another embodiment, an isolated polypeptide is described, wherein the isolated polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide. In one embodiment, the isolated polypeptide does not include a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24. In one embodiment, the isolated polypeptide has a length of about 6 to about 150 amino acids. In one embodiment of the isolated polypeptide, the endogenous polypeptide is an apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, a TRIM5 polypeptide. In one embodiment of the isolated polypeptide, the endogenous polypeptide is an APOBEC polypeptide. In one embodiment, the isolated polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to any one of SEQ ID NOs: 1-24. In one embodiment, the isolated polypeptide includes an amino acid sequence set forth in any one of SEQ ID NOs: 1-24. In another embodiment, a composition is described, wherein the composition includes an isolated polypeptide as described above.

In another embodiment, a method of generating a population of CD8+ T cells specific for a polypeptide is described, wherein the method includes contacting a population of unstimulated CD8+ T cells in vitro with an isolated polypeptide in association with an antigen-presenting platform, wherein said isolated polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide, and wherein said contacting provides for production of a population of CD8+ T cells specific for said synthetic polypeptide. In one embodiment of the method of generating a population of CD8+ T cells specific for a polypeptide, the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24. In one embodiment of the method of generating a population of CD8+ T cells specific for a polypeptide, the isolated polypeptide has a length of about 6 to about 150 amino acids.

In another embodiment, a method of generating a population of CD4+ T cells specific for a polypeptide is described, wherein the method includes contacting a population of unstimulated CD4+ T cells in vitro with an isolated polypeptide in association with an antigen-presenting platform, wherein said isolated polypeptide includes an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to
about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide, and wherein said contacting provides for production of a population of CD4+ T cells specific for said synthetic polypeptide. In one embodiment of the method of generating a population of CD4+ T cells specific for a polypeptide, the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID Nos: 1-10 and 23-24. In one embodiment of the method of generating a population of CD4+ T cells specific for a polypeptide, the isolated polypeptide has a length of about 6 to about 150 amino acids.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1 provides a proposed model showing viral protein-mediated proteolytic processing of an endogenous anti-viral polypeptide, subsequent presentation of a fragment of the endogenous anti-viral polypeptide on the surface of a virus-infected cell, and recognition of the displayed fragment by a T cell.

[0016] Figure 2 provides a proposed model of one specific embodiment of the model set forth in Fig. 1.

[0017] Figure 3 provides a table showing patient characteristics and APOBEC polypeptide pool responses based on an enzyme-linked immunospot (ELISPOT) data.

[0018] Figure 4 provides a graph showing T cell responses to an APOBEC polypeptide pool in HIV-I positive children (black triangles) and exposed uninfected children (white circles) measured by interferon-γ ELISPOT. The horizontal lines represent the mean SFU/10^6 PBMC for HIV-I positive children and HIV-I negative children respectively.

[0019] Figure 5 presents ELISPOT responses of peripheral blood mononuclear cells (PBMC) from HIV-infected children to individual APOBEC peptides: RMYRDTFSY (SEQ ID NO:15); RHMDDPPTFTF (SEQ ID NO:19); RP1LSRRNTVWL (SEQ ID NO:22); GPRSRLDA (SEQ ID NO:10); NLKPYYLL (SEQ ID NO:7); AMYPH IFYFHF (SEQ ID NO:11); FLDSKLOEI (SEQ ID NO:12); FYVSEGQPF (SEQ ID NO:13); and VKHHSQSVSW (SEQ ID NO:14).

[0020] Figure 6 provides fluorescence activated cell sorting data for T cell responses in an HIV-I positive child against the APOBEC polypeptide pool.

[0021] Figure 7 provides a table showing T cell responses to an APOBEC polypeptide pool responses based on ELISPOT data for 7 Long Term Non-Progressor (LTNP) patients and 1 chronic progressor. Units are SFU/10^6 PBMC. Bold highlighting = values above background.
Figures 8A and 8B provide a table showing T cell responses for patients including Controllers (individuals who are able to maintain low to undetectable levels of HIV in the absence of any therapy), HAART treated individuals with undetectable plasma HIV RNA levels, and Viremics (individuals with higher levels of viremia). Responses to 12 different APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figures 9A-C provide a table showing T cell responses for chronically infected children. Responses to both pooled and individual APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figure 10 provides a table showing T cell responses for exposed but uninfected children. Responses to both pooled and individual APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figure 11 provides a table showing T cell responses for the Options cohort of patients (a cohort of primary HIV-1 infected subjects). These subjects are HIV-1 infected and enrolled within 6 months of infection, and then followed longitudinally over time. Some members receive antiretroviral treatment, while others remain with drug therapy. Responses to pooled APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figure 12 provides a table showing T cell responses for Controllers (individuals who are able to maintain low to undetectable levels of HIV in the absence of any therapy). Responses to pooled APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figure 13 provides a table showing T cell responses for HAART treated individuals with undetectable plasma HIV RNA levels. Responses to pooled APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figure 14 provides a table showing T cell responses for Viremics (individuals with higher levels of viremia). Responses to pooled APOBEC polypeptides are shown. Results are ELISPOT assay results. Units are SFU/10^6 PBMC. Bold highlighting = values above background.

Figure 15 presents ELISPOT responses of peripheral blood mononuclear cells (PBMC) from HIV-infected adults to individual APOBEC peptides.
DEFINITIONS

[0030] A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents; washed; or enrichment for certain cell populations, such as CD4+ T lymphocytes, CD8+ T lymphocytes, glial cells, macrophages, tumor cells, peripheral blood mononuclear cells (PBMC), and the like. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, tissue samples, organs, bone marrow, blood, plasma, serum, cerebrospinal fluid, and the like.

[0031] The term "retrovirus" is well known in the art, and includes single-stranded, positive sense, enveloped RNA viruses that include, e.g., the genus gammaretrovirus (e.g., murine mammary tumor virus); the genus Epsilonretrovirus; the genus Alpharetrovirus (e.g., avian leukosis virus); the genus Betaretrovirus; the genus Deltaretrovirus (e.g., bovine leukemia virus; human T-lymphotrophic virus (HTLV)); the genus Lentivirus; and the genus Spumavirus. The term "lentivirus," as used herein, refers to a genus of viruses of the Retroviridae family, and includes human immunodeficiency virus-1 (HIV-1); human immunodeficiency virus-2 (HIV-2); simian immunodeficiency virus (SIV); and feline immunodeficiency virus (FIV).

[0032] "Gene delivery vehicle" refers to a construct which is capable of delivering, and, within some embodiments expressing, one or more gene(s) or nucleotide sequence(s) of interest in a host cell. Representative examples of such vehicles include viral vectors, nucleic acid expression vectors, naked DNA, and certain eukaryotic cells (e.g., producer cells).

[0033] "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct-the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

[0034] The terms "polypeptide," "peptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having
modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like. NH2 refer to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxyl group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature, J. Biol. Chem., 243 (1969), 3552-59 is used.

[0035] As used herein the term "isolated" is meant to describe a polynucleotide, a polypeptide, or a cell that is in an environment different from that in which the polynucleotide, the polypeptide, or the cell naturally occurs. An isolated genetically modified host cell may be present in a mixed population of genetically modified host cells. An isolated polypeptide will in some embodiments be synthetic. "Synthetic polypeptides" are assembled from amino acids, and are chemically synthesized in vitro, e.g., cell-free chemical synthesis, using procedures known to those skilled in the art.

[0036] By "purified" is meant a compound of interest (e.g., a polypeptide) has been separated from components that accompany it in nature. "Purified" can also be used to refer to a compound of interest separated from components that can accompany it during manufacture (e.g., in chemical synthesis). In some embodiments, a compound is substantially pure when it is at least 50% to 60%, by weight, free from organic molecules with which it is naturally associated or with which it is associated during manufacture. In some embodiments, the preparation is at least 75%, at least 90%, at least 95%, or at least 99%, by weight, of the compound of interest. A substantially pure compound can be obtained, for example, by extraction from a natural source (e.g., bacteria), by chemically synthesizing a compound, or by a combination of purification and chemical modification. A substantially pure compound can also be obtained by, for example, enriching a sample having a compound that binds an antibody of interest. Purity can be measured by any appropriate method, e.g., chromatography, mass spectroscopy, high performance liquid chromatography analysis, etc.

[0037] The term "endogenous," when used in reference to a polypeptide, means that which is naturally produced (e.g., by an unmodified mammalian or human cell). As used herein, the terms "endogenous" and "native" are interchangeable.

[0038] The term "substantially similar" as used in the context of nucleic acid or amino acid sequence identity refers to two or more sequences which have at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity.
As used herein "% sequence identity" is determined using the EMBOSS Pairwise Alignment Algorithms tool available from The European Bioinformatics Institute (EMBL-EBI), which is part of the European Molecular Biology Laboratory (EMBL). This tool is accessible at the website located by placing "www." in front of "ebi.ac.uk/Tools/emboss/align/". This tool utilizes the Needleman-Wunsch global alignment algorithm (Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453; Kruskal, J. B. (1983) An overview of sequence comparison In D. Sankoff and J. B. Kruskal, (ed.), Time warps, string edits and macromolecules: the theory and practice of sequence comparison, pp. 1-44 Addison Wesley. Default settings are utilized which include Gap Open: 10.0 and Gap Extend 0.5. The default matrix "Blosum62" is utilized for amino acid sequences and the default matrix "DNAfull" is utilized for nucleic acid sequences.

An "antigen" is defined herein to include any substance that may be specifically bound by an antibody molecule or a T cell antigen receptor. An "immunogen" is an antigen that is capable of initiating lymphocyte activation resulting in an antigen-specific immune response.

By "epitope" is meant a site on an antigen to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." B cell epitope sites on proteins, polysaccharides, or other biopolymers may be composed of moieties from different parts of the macromolecule that have been brought together by folding. Epitopes of this kind are referred to as conformational or discontinuous epitopes, since the site is composed of segments of the polymer that are discontinuous in the linear sequence but are continuous in the folded conformation(s). Epitopes that are composed of single segments of biopolymers or other molecules are termed continuous or linear epitopes. T cell epitopes are generally linear peptides. Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

The terms "subject," "individual," "host," and "patient" are used interchangeably herein to refer to a mammal, including, but not limited to, murines (rats, mice), felines, non-human primates (e.g., simians), humans, canines, ungulates, etc.

The terms "treatment," "treating," "treat," and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease or symptom from
occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, i.e., arresting its development; or (c) relieving the disease symptom, i.e., causing regression of the disease or symptom.

(0044) Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0045] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0046] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0047] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a synthetic polypeptide" includes a plurality of such synthetic polypeptides and reference to "the immunogenic composition" includes reference to one or more immunogenic compositions and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.
The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

**DETAILED DESCRIPTION**

Isolated polypeptides related to endogenous anti-viral polypeptides; and compositions, including immunogenic compositions, comprising a subject isolated polypeptide are disclosed herein. A subject isolated polypeptide comprises an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of one or more endogenous anti-viral polypeptides, wherein the endogenous anti-viral polypeptides are polypeptides that are subject to proteolytic degradation as a result of the activity of one or more viral proteins. For convenience, the disclosed isolated polypeptides are referred to herein as "Polypeptides derived from Endogenous Anti-viral Polypeptides" or PEAPs.

The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a subject PEAP; and compositions, including immunogenic compositions, comprising a subject nucleic acid.

The present disclosure provides immunogenic compositions comprising a nucleic acid comprising a nucleotide sequence encoding a subject PEAP. A subject immunogenic composition is useful for stimulating a specific T cell immune response to a retrovirus infected cell, e.g., a human immunodeficiency virus (HIV)-infected cell. Epitope(s) displayed by a subject isolated polypeptide stimulate or enhance a T cell immune response to the epitope(s). Where the epitopes are also present on the surface of a retrovirus-infected cell, a T cell response to the retrovirus-infected cell also occurs. A "T cell immune response" includes one or more of: 1) an increase in the number and/or activity of CD4+ T cells specific for the epitope; 2) an increase in the number and/or activity (e.g., cytotoxicity) of CD8+ T cells specific for the epitope; and 3) secretion of cytokines or chemokines that induce or are indicative of a T cell immune response. Cytokines or chemokines that induce or are indicative of a T cell immune response include, but are not limited to, interferon-gamma (IFN-γ), IL-2, and tumor necrosis factor-alpha (TNF-α). T cell immune responses that are stimulated with a disclosed immunogenic composition include a mucosal T cell immune response and a systemic T cell immune response.
A subject immunogenic composition can be formulated in any of a variety of ways, including a formulation suitable for intravenous administration, subcutaneous administration, or other parenteral route of administration; a formulation suitable for administration to a mucosal tissue; and the like. The present disclosure provides pharmaceutical formulations comprising a subject immunogenic composition.

The present disclosure further provides polypeptide compositions that are suitable for use in monitoring a patient's response to treatment for a lentivirus infection (e.g., an HIV infection). Thus, the present disclosure further provides methods for monitoring a patient's response to treatment for a lentivirus infection (e.g., an HIV infection).

**POLYPEPTIDES**

The present disclosure provides isolated polypeptides, wherein the isolated polypeptides comprise an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of one or more endogenous anti-viral polypeptides, where the endogenous anti-viral polypeptides are polypeptides that are subject to proteolytic degradation as a result of the activity of one or more viral proteins. A subject isolated polypeptide is referred to herein as a "Polypeptide derived from an Endogenous Anti-viral Polypeptide" or PEAP. In some embodiments, a subject PEAP is synthetic (e.g., chemically synthesized). Thus, the present disclosure provides a synthetic PEAP. In the discussion that follows, the term "subject PEAP," or simply "PEAP" is used; however, it should be understood that the following discussion applies equally to a "subject synthetic PEAP."

A subject PEAP can be from 6 amino acids in length up to the length of a naturally-occurring endogenous anti-viral polypeptide described herein, e.g., a PEAP can be 6 amino acids (aa), 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12-15 aa, 15-20 aa, 20-25 aa, 25-30 aa, 30-40 aa, 40-50 aa, 50-100 aa, or longer than 100 amino acids, e.g., 100 aa to 150 aa, 150 aa to 200 aa.

The present disclosure also provides compositions comprising a subject PEAP. A subject PEAP finds use in, e.g., generating immunogenic compositions (e.g., for enhancing an immune response in an individual to a PEAP and/or an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP; or for enhancing an immune response in an individual to a retrovirus-infected cell); monitoring patient response to therapy, e.g., therapy for a retrovirus infection; staging a disease; detecting a disease; and for generating CD8+ T cells for adoptive transfer methods.

As indicated above, a subject isolated polypeptide comprises an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of one or
more endogenous anti-viral polypeptides, where the endogenous anti-viral polypeptides are polypeptides that are subject to proteolytic degradation as a result of the activity of one or more viral proteins. Endogenous anti-viral polypeptides that are subject to proteolytic degradation as a result of the activity of one or more viral proteins (e.g., one or more human immunodeficiency virus-encoded proteins) include, e.g., APOBEC polypeptides, a tetherin polypeptides and a TRIM5 polypeptides.

APOBEC polypeptides

[0058] In some embodiments, a subject PEAP comprises an amino acid sequence that has substantial amino acid sequence identity to a contiguous stretch of amino acids of one or more apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptides. APOBEC polypeptides are a group of cytidine deaminases, which in humans include AICDA, APOBEC1, APOBEC2, APOBEC4 and a series of seven polypeptides encoded by APOBEC3 genes. APOBEC3 polypeptides include APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3F, APOBEC3G and APOBEC3H (Goila-Gaur and Strebel (2008) Retrovirology 5:51).

[0059] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence of an endogenous APOBEC polypeptide. As used herein, the term "endogenous APOBEC polypeptide" includes known variants of APOBEC polypeptides. For example, known APOBEC3G variants include APOBEC3G polypeptides having an H186R and/or C97A mutation.

[0060] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence of an APOBEC polypeptide; and has a length of 6 amino acids (aa), 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, from 12 aa to 15 aa, from 15 to 20 aa, from 20 to 25 aa, from 25 to 30 aa, from 30 to 40 aa, from 40 to 50 aa, from 50 to 100 aa, from 100 aa to 150 aa, or from 150 aa to 200 aa.

[0061] APOBEC polypeptides include polypeptides having the amino acid sequences set forth in GenBank Accession Nos.:
U72891 (APOBECl): (SEQ ID NO: 1:
MTSEKGPSTGDPLRRRIEPEWDFYDFPRELRKEACLLYEIKWGMSRKI
WHVEDVFKFDSRDFHPSMSCSITWFLSWPSCECSQA] REFLSRHPGVTLVYVARLF
WHMDQGNQRLDDLVMGSETVIQMRASEYYHCWRENFVNYPGEAH WPQYPPlLM
MLYALELHLCCILSPLPCCLIKISRRWQNLTLFFRLHLQNCHYQTIPPHILLATGLIHPVS

[0063] AB040430 (activation-induced cytidine deaminase AICDA): (SEQ ID NO:2:
MDSLLMN RRKFLYQFKNVWRAGKRETELYCYYVKRRDSATSFSFLDFYGLRNNNGCHV
ELLFLRYISDWDLPGRCRYVRTWFTSTSWPSPCYDCARHVADFLRGNPNLSLRIFTARYC
EDRKAAPAFLRLHRAGVQAIATMFDYFYCWNTFVENHTRTKAWEGLHNESVRLS
QRLRILLPYDVLDDLRAFRTLGL;)

U03891 (APOBEC3A): (SEQ ID NO:3:
MEASPASGPRHLPMDPHIFTSN FNGNIGRIKTYLCYEVERLDSGTVKMDQHRGFLHNQ
AKNLLCGFYGRHAELRLFDLVSPLQLDPAQIYRVTWIFSWPSCFSWGCAEGVEVAFQEN
THVRLLIPAFARLIDYDPLYKEALQMLRDAGAQVSMITYDFTKHCWDTFVDHQGCPFPQ
WDGLDEHSQALSGRLRAILQNGN);

NM_004900 (APOBEC3B):(SEQ ID NO:4:
MNPQIRNPMEYMRYTDFYDFNENPEILYGRSYTWCYEVKIKRGRSNNLLWDTGVFRGQ
VYFKPQYHAEMCFLSWFCGNQLPAPYKFQFQTWFVSWTPDCVAKLAEFSLHNPVLTL
TISAARLYYYWERTDRAQCRLQSAGARVTFIMDYEFA YCWENFYNEQGMPMPWY
FDENYAFLHRTLKEILRLMPDPTTFNFFNDPLVLRRRQTYLCYEVERLDSGTVL
MHGMFLCNEAKNNLLCGFYGRHAELRLFDLVSPLQLDPAQIYRVTWIFSWPSCFSWGC
AGEVRAFLQENTHRLLRAIAYDYDPLYKEALQMLRDAGAQVSMITYDFTKHCWDT
FVRQCCFQPWPWDGLEEHSQALSGRLRAILQNGN);

AF165520 (APOBEC3C):(SEQ ID NO:5:
MNPQIRNPMEYMRYTDFYDFNENPEILYGRSYTWCYEVKIKRGRSNLWDTGVFRGQ
QVDSETHCARFCSLFSCDDILSPNTKYQV'TWIFSWPSCPDCAGEVAAELRHSN
NLTITARLYYYFQYPCQYEQEQLRSLEQEGVAVEIMDYEDFKYCEWENFYNDNPFPKWE
GDKNQLSCKETAKGESPVRGLPGHSLPLCSCTGLPSLDPDPCFLCFLVILPSWSWPQGHS
TVLPCLTASSSLFQTLPAEAPFCLHGYSTPTDPVPPACVPLTWLPFSQHQNLNSC);

NMJ 52426 (APOBEC3DE): (SEQ ID NO:6:
MNPQIRNPMEYMRYTDFYDFNENPEILYGRSYTWCYEVKIKRGRSNLWDTGVFRGQ
VLPKRQSNHRQEVYFRFENHAEMCFLSWFCGNRLPANRRFQITWIFSVSNPCPLCVKV
TKFLAEHPNVTiTISAARLYYYYYRDRDWRVLLRLHKARAVKIIMDYEDFAYCWEVNFV
CNEGQPFMPWYKFDDNYASLHRTLKEILRNPMEAMYPHIFFFHFKNLKACGRNESW

[0067]
CFTMEVTKHSAVFRKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTS
WSPCPECAGEVAEFLARHSNVNLTIFFTARLYFWDTYQEGLRLSSEQAGASVKIMGYK
DFVSCWKNFVYSDDEPFKPKWGLQTNFRLKRRRLREILQ);

[0068] BC038808 (APOBEC3F): (SEQ ID NO:7:
MKPHFRNTRVERMYRTDFSYNPYNPIRSRNTTVWLNCYEVTKGSPRPLDAIKIFRGQV
YSQPEHHAEFCFLSWFCGQNLPAAYKQCFQITWFSWT PCPDCVAKLAEFLSEHPN VTLTI
SAARLYYYWERYRRALCRSLAQAGARVKIMDDEEA YCWFENFVYSEQPFPMPFWKF
DDNAYFLHRTLKEILRNPMAMEYPHIFYFHKNLKAYGRNESCWLCTFMEVVKHHSIS
WKRGVFNRQNQVPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGEVAEF
LARHSNVLTIFFTARLYFWDTYQEGLRLSSEQAGASVEIMGKDFKYCWENFVYND
EPFKPKWGLKYNFLFDSKLQIEILE);

[0069] AF182420 (APOBEC3G): (SEQ ID NO:8:
MKPHFRNTRVERMYRTDFSYNPYNPIRSRNTTVWLNCYEVTKGSPRPLDAIKIFRGQVY
SEKYHPERMRFH WFSKWLKLKRDQYEVTWYISWSPCTKCRDMATFLAEDPKVILT
IFVARLYYYWDPDYEQLRSCLQKRDPRATMKMNYDEFQPCHWSKFVYSEQRELFPW
NLNPKYILHILMLGEILRHSMDPPT FTFN FNEPVRGRHETLYCIEEVERMNDTWV
LLNQRGFLCNQAPHKHGFLGRHAECLFLYDPFWKLDLDQDYRTVCFTSWSPCFSCA
QEAMFKISKNKHSLCIFTARIYDDQIRCQEGLRTLAEAGAKISIMTYSEFKHCWDTFTF
DHQGCPFPQDPWGLDEHSQDSLGRRLRAILNQEN);

[0070] BC069023 (APOBEC3H): (SEQ ID NO:9:
MALLTAEFLQFNNKRRLRPYYPRKALLCYQTPNPSTPRTRYFENKKGCAIECF
INEIKSMGLDETOCQYVTCLYTLWSPCASSCAWELDFIKAHDLNLGIFASRLYYWHCKP
QQKGLRLCCGSQVPVEVMGFPEFADCWEN FVDHEKPLSFNPYKMLEEILDNSRAIKR
LERIKS);

[0071] BC02 171 1 (APOBEC4) (SEQ ID NO: 10: MEPIYEEYLHGTIVKPYYWLSFDSNCPHY JRTGEARVSLTEFCQI FGFPYGFITP
QTKHLYEYKTSGLVQKGHASSCTGNYIHPSMLFEMNYGLDAIYNNDSIRHIIILY
NNSPCNEANHCISKMYFLITGILSIFYSQLYHTEMDFPASAWNREALRSALWRL
PVVLSISGGIWHVSLHFSISGVSGHSVFPILCTRALADRHNAYEAINTGVKPYFTDV
LQTKRNPNTKQAEALESPYNAAFPQNYKFPMQPSGQLQPNPLLAPVVFVPLPRLDP
PMHMGQNPXPRNIVRHLNMPQMSFQETKDLGRLPTGRSVEIEITEQFASSKEADEKK
KKKGKK).

[0072] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to
150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in one or more of SEQ ID NOs: 1-10.

In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in one or more of SEQ ID NOs: 1-10; and has a length of 6 amino acids (aa), 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, from 12 aa to 15 aa, from 15 to 20 aa, from 20 to 25 aa, from 25 to 30 aa, from 30 to 40 aa, from 40 to 50 aa, from 50 to 100 aa, from 100 aa to 150 aa, or from 150 aa to 200 aa.

In some embodiments, a subject PEAP does not comprise the full length amino acid sequence disclosed in any one of SEQ ID NOs: 1-10. In one such embodiment, the subject immunogenic composition does not comprise a polypeptide having an amino acid sequence that is at least about 60% identical to the amino acid sequence set forth in SEQ ID NO: 2 or an immunogenic fragment thereof.

As indicated above, in some embodiments, a subject PEAP comprises an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of an APOBEC3F polypeptide. For example, in some embodiments, a subject PEAP comprises about 6, 7, 8, 9, 10 or 11 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 11:

AMYPHFYFHF (SEQ ID NO: 11).

In some embodiments, a subject PEAP comprises about 6, 7, 8, 9, 10 or 11 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 11; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 11 aa, from about 11 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).
In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 12:

FLDSKLQE (SEQ ID NO: 12).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 12; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 13:

FVYSEGPQF (SEQ ID NO: 13).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 13; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 14:

VKKHSPVSW (SEQ ID NO: 14).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at
least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 14; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 15; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 15; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 15; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In some embodiments, a subject PEAP comprises an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of an APOBEC3G polypeptide.
In one example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 17:

NLPKYYYILL (SEQ ID NO: 17).

In one example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 17; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 18:

NTVWLCEYEV (SEQ ID NO: 18).

In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 18; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

In another example, a subject PEAP comprises about 6, 7, 8, 9, 10 or 11 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 19:

RHSMPPPTTFTF (SEQ ID NO: 19).

In another example, a subject PEAP comprises about 6, 7, 8, 9, 10 or 11 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least
about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 19; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 11 aa, from about 11 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

[00103] In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 20:

[00104] FVYSQREL (SEQ ID NO: 20).

[00105] In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 20; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

[00106] In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 21:

[00107] KPHFRNTVE (SEQ ID NO: 21).

[00108] In another example, a subject PEAP comprises about 6, 7, 8 or 9 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 21; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

[00109] In another example, a subject PEAP comprises about 6, 7, 8, 9, 10, 11 or 12 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least
about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:22:


[0011] In another example, a subject PEAP comprises about 6, 7, 8, 9, 10, 11 or 12 contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:22; and has a length of from about 6 amino acids to about 25 amino acids (e.g., from about 6 aa to about 9 aa, from about 9 aa to about 12 aa, from about 12 aa to about 15 aa, from about 15 aa to about 20 aa, or from about 20 aa to about 25 aa).

[0012] In some embodiments, a subject PEAP comprises one or more of the following amino acid sequences:

[0013] AMYPH IFYHFH (SEQ ID NO: 11);
[0014] FLDSKLPQEI (SEQ ID NO: 12);
[0015] FVYSEGQPF (SEQ ID NO: 13);
[0016] VKHHPSPVSW (SEQ ID NO: 14);
[0017] RMYRDTFSY (SEQ ID NO: 15);
[0018] GPSRPRLDA (SEQ ID NO: 16);
[0019] NLPKYYILL (SEQ ID NO: 17);
[0020] NTVWLCYEY (SEQ ID NO: 18);
[0021] RHSMDFPTFTF (SEQ ID NO: 19);
[0022] FVYSEQREL (SEQ ID NO:20);
[0023] KPHFRNTVE (SEQ ID NO:21); and
[0024] RPILSRRNTVWL (SEQ ID NO:22).

Tetherin polypeptides

[0025] In some embodiments, a subject PEAP comprises an amino acid sequence having substantial amino acid sequence identity to a contiguous stretch of amino acids of one or more tetherin polypeptides. Tetherin polypeptides have been shown to inhibit the release of retroviral particles (Neil et al. (2008) Nature 451:425-431).

[0026] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at

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least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%,
at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino
acid sequence identity to the amino acid sequence of an endogenous tetherin polypeptide. As
used herein, the term "endogenous tetherin polypeptide" includes known variants of tetherin polypeptides.

[00127] Tetherin polypeptides include polypeptides having the amino acid sequence set forth in
GenBank Accession No: NM_004335 (BST2, a.k.a., CD317, a.k.a, HM1.24) (SEQ ID NO:23:
MASTSYDCRVMEDGDKCKLILLSLGVILVLLILVPLVIIF
TIKANSEACRDGLRAVMCNRVTLLQQELTEAQKGFQDVEAQAATCNHTVMALMAS
LDAEKAOQGKVEELEGITLHKLQDASAEVERLRRENQVLSSVDIADKKYPPSSQDS
SSAAAPQLLLVLLGLSALLQ).

[00128] As such, in some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11,
12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, from 20 to 25,
from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, from 45 to 50, from 50 to 55, from 55 to 60, or from
100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about
50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least
about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or
100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 23.

[00129] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-
14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, from 20 to 25, from 25 to 30,
from 30 to 35, from 35 to 40, from 40 to 45, from 45 to 50, from 50 to 55, from 55 to 60, or from
100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about
50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least
about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino
acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 23; and has a length
of 6 amino acids (aa), 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, from 12 aa to 15 aa, from 15 to 20 aa, from
20 to 25 aa, from 25 to 30 aa, from 30 to 40 aa, from 40 to 50 aa, from 50 to 100 aa, from 100 aa
to 150 aa, or from 150 aa to 200 aa.

[00130] In some embodiments, a subject PEAP does not comprise the full length amino acid
sequence set forth in SEQ ID NO:23.
TRIM5 polypeptides

[00131] In some embodiments, a subject PEAP comprises an amino acid sequence having
substantial amino acid sequence identity to a contiguous stretch of amino acids of one or more
TRIM5 (tripartite motif-containing 5) polypeptides.

[00132] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-
14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, from 20 to 25, from 25 to 30,
from 30 to 35, from 35 to 40, from 40 to 45, from 45 to 50, from 50 to 55, from 55 to 60, or from 100 to
150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence of an endogenous TRIM5 polypeptide. As used herein, the term "endogenous TRIM5 polypeptide" includes known variants of TRIM5 polypeptides.

[00133] TRIM5 polypeptides include polypeptides having the amino acid sequence set forth in GenBank Accession No: AF220025 (TRIM5, a.k.a., RNF88, a.k.a., TRIM5alpha) (SEQ ID NO:24):

MASGILVMVKEEVTCPLELLTQPLSLDCGHSCFCATLANKSMLDGESCPVCRI
SYQPENIRPNRHVANIVERLKEVSLPEGQKDVHCHARKEGLLLFCQEDGKVICWLCER
SQRHGRHHTFLTEEVALEYQVKLQAAMELRQKQQAEELEADIREEASWKTVIQYD
KTNVLADFEQLRDLDEESNLEQNLEKEDILKSLTNSETMVQTESLRELISDEHL
RLQGSMELLQGVDVGIKTRENTLTKKPETFPKNNQRRVFARPDLMKGMEVRELDTVR
RYWVDVTVAPNNISCAVISEDKRQVSSPKPQIIYARGTRQTFVNFNYCTGILGSQITS
GKHYWEDVSKKTAWILGCAGFQPDAMCNEKNEYQPKYGYWIGLEEGVVKCSAF
QDSSFHTPSVFIVPLSVIICPDRGVFLDYEACTVSSFINTHGFLYKFSHCFSQPVFYPY
LNPRKCGVPMTCSPSS).

[00134] As such, in some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:24.

[00135] In some embodiments, a subject PEAP comprises from about 6, 7, 8, 9, 10, 11, 12, 13-15, 15-17, 17-20, from 20 to 25, from 25 to 50, from 50 to 75, from 75 to 100, or from 100 to 150, or more, contiguous amino acids of an amino acid sequence having at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:24; and has a length of 6 amino acids (aa), 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, from 12 aa to 15 aa, from 15 to 20 aa, from 20 to 25 aa, from 25 to 30 aa, from 30 to 40 aa, from 40 to 50 aa, from 50 to 100 aa, from 100 aa to 150 aa, or from 150 aa to 200 aa.

[00136] In some embodiments, a subject PEAP does not comprise the full length amino acid sequence disclosed in SEQ ID NO:24.
A subject PEAP can be from 6 amino acids in length up to the length of a naturally-occurring endogenous anti-viral polypeptide described herein, e.g., a PEAP can be 6 amino acids (aa), 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12-15 aa, 15-20 aa, 20-25 aa, 25-30 aa, 30-40 aa, 40-50 aa, 50-100 aa, or longer than 100 amino acids, e.g., 100 aa to 150 aa, 150 aa to 200 aa.

A subject PEAP can be in the form of a fusion protein, e.g., a fusion protein comprising one or more of the isolated polypeptides described above covalently linked to a heterologous protein, where the heterologous protein is also referred to as a "fusion partner." In some embodiments, the fusion partner is attached to the N-terminus of an isolated polypeptide disclosed herein, e.g., NH2-fusion partner-isolated polypeptide-COOH. In other embodiments, the fusion partner is attached to the C-terminus of the synthetic polypeptide, e.g., NH2-isolated polypeptide-fusion partner-COOH. In other embodiments, the fusion partner is internal to the synthetic polypeptide, e.g., NH2-PEAP1-PEAP2-COOH, where FP is a fusion partner, and PEAP1 and PEAP2 are N-terminal and C-terminal regions, respectively, of a PEAP.

Suitable fusion partners include, but are not limited to, immunological tags such as epitope tags, including, but not limited to, hemagglutinin, FLAG, myc, and the like; proteins that provide for a detectable signal, including, but not limited to, fluorescent proteins, enzymes (e.g., β-galactosidase, luciferase, horse radish peroxidase, alkaline phosphatase, etc.), and the like; polypeptides that facilitate purification or isolation of the fusion protein, e.g., metal ion binding polypeptides such as 6His tags, glutathione-S-transferase, and the like; polypeptides that provide for subcellular localization; and polypeptides that provide for secretion from a cell. Fusion partners that provide for a detectable signal are also referred to as "reporters." In some embodiments, a fusion partner is an immunomodulatory polypeptide other than a PEAP, e.g., an antigen, a cytokine, etc.

Multimerized PEAPs

In some embodiments, a subject PEAP is multimerized, e.g., two or more PEAPs are linked in tandem. Multimers include dimers, trimers, tetramers, pentamers, etc. Monomeric PEAPs can be linked to one another directly or via a linker. Thus, in some embodiments, a PEAP has the formula $(X_1-Y)_n$, where $X_1$ and $X_2$ are PEAPs. $Y$ is a linker, and $n$ is an integer from 1 to about 10 (e.g., $n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$). Where a linker is used, $Y$ is one or more amino acids, or other linking groups. $X_1$ and $X_2$ can be the same or different, e.g., can have the same amino acid sequence, or can differ from one another in amino acid sequence. Thus, e.g., an PEAP can have the formula $X_1-(Y)_{n=1}$, where the PEAP is a dimer. As another example, a PEAP can have the formula $X_1-(Y)_{n=2}$, e.g., where the PEAP is a trimer.
In some embodiments, the PEAP multimer is a homopolymer, e.g., the individual PEAP peptides in a subject multimer all have the same amino acid sequence. In other embodiments, the PEAP multimer is a heteropolymer, e.g., two or more different PEAPS are multimerized. As a non-limiting example, a PEAP multimer can comprise a first PEAP and at least a second PEAP, where the first and the second PEAPS are two different PEAPS comprising the amino acid sequence of any one of SEQ ID NOs:1 1-22, where the two or more different PEAPS each has a length of from 15 amino acids to about 20 amino acids, from about 20 amino acids to about 25 amino acids, from about 25 amino acids to about 30 amino acids, from about 30 amino acids to about 35 amino acids, from about 35 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

Where Y is a spacer peptide, it is generally of a flexible nature, although other chemical linkages are not excluded. Currently, it is contemplated that the most useful linker sequences will generally be peptides of between about 2 and about 40 amino acids in length, e.g., from about 2 amino acids to about 10 amino acids, from about 10 amino acids to about 20 amino acids, or from about 6 amino acids to about 25 amino acids in length. These linkers are generally produced by using synthetic, linker-encoding oligonucleotides to couple the proteins. Peptide linkers with a degree of flexibility will generally be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that the preferred linkers will have a sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. Exemplary peptide linkers include (Gly), (Ser), and (Ala), where n is 1-40. The creation of such sequences is routine to those of skill in the art. Many different linkers are commercially available and are considered suitable for use according to the disclosed embodiments. However, any flexible linker generally between about 2 amino acids and about 40 amino acids, e.g., from about 6 amino acids to about 10 amino acids in length may be used. Linkers may have virtually any sequence that results in a generally flexible peptide.

Linkages for homo- or hetero-polymers or for coupling to carriers can be provided in a variety of ways. For example, cysteine residues can be added at both the amino- and carboxyl-termini, where the peptides are covalently bonded via controlled oxidation of the cysteine residues. Also useful are a large number of heterobifunctional agents which generate a disulfide link at one functional group end and a peptide link at the other, including N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). This reagent creates a disulfide linkage between itself and a cysteine residue in one protein and an amide linkage through the amino on a lysine or other free amino group in the other. A variety of such disulfide/amide forming agents is known. See, for example, Immun. Rev. 62:185 (1982). Other bifunctional coupling agents form a thioether rather
than a disulfide linkage. Many of these thioether forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimidomethyl) cyclohexane-1-carboxylic acid and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxy-2-nitro-4-sulfonic acid, sodium salt. A particularly preferred coupling agent is succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC). Of course, it will be understood that linkage should not substantially interfere with either of the linked groups to function for its intended use, e.g., as an immunogen.

Carriers

In some embodiments, a subject PEAP is linked to a carrier. The term "linked," as used herein interchangeably with the term "coupled," refers to proximately associated, e.g., the PEAP and the carrier are in close spatial proximity. In some embodiments, the linkage is a covalent linkage. In other embodiments, the linkage is a non-covalent linkage. In some embodiments, the PEAP is linked directly to the carrier. In other embodiments, the PEAP is linked indirectly, e.g., via a linker molecule.

Examples of suitable carriers include large, slowly metabolized macromolecules such as: proteins; polysaccharides, such as sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids such as polyglutamic acid, polylysine, and the like; amino acid copolymers; inactivated virus particles; inactivated bacterial toxins such as toxoid from diphtheria, tetanus, cholera, leukotoxin molecules; liposomes; inactivated bacteria; dendritic cells; and the like. Carriers are described in further detail below.

Suitable carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid; Diphtheria toxoid; polyamino acids such as poly(D-lysine:D-glutamic acid); VP6 polypeptides of rotaviruses; influenza virus hemagglutinin, influenza virus nucleoprotein; hepatitis B virus core protein, hepatitis B virus surface antigen; purified protein derivative (PPD) of tuberculin from Mycobacterium tuberculosis; inactivated Pseudomonas aeruginosa exotoxin A (toxin A); Keyhole Limpet Hemocyanin (KLH); filamentous hemagglutinin (FHA) of Bordetella pertussis; T helper cell (Th) epitopes of tetanus toxoid (TT) and Bacillus Calmette-Guerin (BCG) cell wall; recombinant 10 kDa, 19 kDa and 30-32 kDa proteins from M. leprae or from M. tuberculosis, or any combination of these proteins; and the like. See, e.g., U.S. Patent No. 6,447,778 for a discussion of carriers and methods of conjugating peptides to carriers.

Pseudomonas aeruginosa exotoxin A (toxin A) has been used effectively as a carrier in conjugate vaccines. Pseudomonas aeruginosa exotoxin A may be purified from the supernatant of fermentor-grown cultures of Pseudomonas aeruginosa PA 103. Toxin A has been classified as a superantigen based upon results in animals. Toxin A can be completely and irreversibly
detoxified by covalent coupling to adipic acid dihydrazide (ADH), a 4 carbon spacer molecule. This step destroys the ADPR-transferase activity of the toxin molecule, hence rendering it nontoxic. The non-reacted hydrazide group can be used to covalently couple a polypeptide to toxin A. Toxin A may also be coupled to a polypeptide using a carbodiimide reagent.


[00149] The methods by which a subject polypeptide is conjugated with a carrier include disulfide linkages through a C terminal peptide cysteine linkage, coupling with glutaraldehyde solution for two hours, coupling with tyrosine, or coupling with water soluble carbodiimide.

[00150] In some embodiments, a subject PEAP is lipidated. Lipidation increases a cytotoxic T cell (CTL) response to the peptide that is linked to the lipid. The lipid residue, such as palmitic acid or the like, is attached to the amino terminus of the peptide. The lipid can be attached directly to the peptide, or, indirectly via a linkage, such as a Ser-Ser, Gly, Gly-Gly, Ser linkage or the like. As another example, E. coli lipoprotein, such as tripalmitoyl-S-glycerylcysteinyl-seryl-serine (P₃ CSS), can be used to prime specific CTL when covalently attached to the peptide. See, Deres et al., Nature 342:561-564 (1989). A subject PEAP can be conjugated with uncharged fatty acid residues of different chain lengths and degrees of unsaturation, ranging from acetic to stearic acid as well as to negatively charged succinyl residues via the appropriate carboxylic acid anhydrides. See, e.g., U.S. Patent No. 6,419,931.

[00151] A subject PEAP can be conjugated directly or indirectly, e.g., via a linker molecule, to a carrier. A wide variety of linker molecules are known in the art and can be used in the conjugates. The linkage from the peptide to the carrier may be through a peptide reactive side chain, or the N- or C-terminus of the peptide. A linker may be an organic, inorganic, or semi-organic molecule, and may be a polymer of an organic molecule, an inorganic molecule, or a co-polymer comprising both inorganic and organic molecules.

[00152] If present, the linker molecules are generally of sufficient length to permit the PEAP and a linked carrier to allow some flexible movement between the PEAP and the carrier. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to polypeptides may be used in light of this disclosure.

Compositions

[00153] The present disclosure provides compositions comprising one or more subject PEAPs. Compositions comprising one or more subject PEAPs can include one or more of: a salt, e.g.,
NaCl, MgCl, KCI, MgSO₄, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; and the like. In some embodiments, as described in more detail below, a subject PEAP composition is an immunogenic composition. In other embodiments, as described in more detail below, a subject PEAP composition is a pharmaceutical composition, e.g., a composition comprising a PEAP and a pharmaceutically acceptable excipient.

In some embodiments, a composition comprises a single type (or "species") of PEAP, e.g., in some embodiments, the PEAPs in a subject composition all comprise substantially the same amino acid sequence. In other embodiments, a subject immunogenic composition comprises two or more different PEAPs, e.g., the composition comprises a population of PEAPs, the members of which population can differ in amino acid sequence. A composition can comprise from two to about 20 different PEAPs, e.g., a subject composition can comprise two, three, four, five, six, seven, eight, nine, ten, 11-15, or 15-20 different PEAPs, each having an amino acid that differs from the amino acid sequences of the other PEAPs. For example, in some embodiments, a composition comprises a first PEAP having a first amino acid sequence; and at least a second PEAP having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence. As another example, in some embodiments, a composition comprises a first PEAP having a first amino acid sequence; a second PEAP having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence; and at least a third PEAP having a third amino acid sequence, where the third amino acid sequence differs from both the first and the second amino acid sequences. In other embodiments, a subject composition comprises a multimerized PEAP, as described above.

Production of PEAPs

A subject PEAP can be produced in a number of ways, including, e.g., by chemical synthesis, where the PEAP is a "synthetic" polypeptide; by isolation and purification from a naturally-occurring source; and by recombinant means, where the PEAP is a "recombinant" polypeptide. Recombinant means for producing a subject PEAP are well known in the art, and involve genetically modifying a host cell with a polynucleotide comprising a nucleotide sequence encoding a subject PEAP, culturing the host cell in vitro under conditions and for a suitable time such that the PEAP is produced by the genetically modified cell, and isolating the PEAP produced by the genetically modified cell. Methods of chemically synthesizing a
polypeptide are known in the art and can be used to synthesize a subject PEAP. For example, Standard 9H-fluoren-9-yl-methoxycarbonyl (Fmoc) chemistry can be used. See, e.g., "Fmoc Solid Phase Peptide Synthesis: A Practical Approach" W.C. Chan and P.D. White, eds. (2000) Oxford Univ. Press.

Pharmaceutical compositions

The present disclosure provides a pharmaceutical composition comprising a subject PEAP, the composition comprising a subject PEAP and a pharmaceutically acceptable excipient.

A wide variety of pharmaceutically acceptable excipients is known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents.

A subject PEAP pharmaceutical composition can be prepared by dissolving, suspending or emulsifying a subject PEAP in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

IMMUNOGENIC COMPOSITIONS COMPRISING A PEAP

The present disclosure contemplates immunogenic compositions comprising a subject PEAP. A subject immunogenic composition can comprise a subject PEAP that comprises one or more T cell epitopes that, when presented on the surface of a retrovirus-infected cell, induce a T cell immune response specific for a retrovirus-infected cell, e.g., a human immunodeficiency virus (HIV)-infected cell. A "T cell immune response" includes one or more of: 1) an increase in the number and/or activity of CD4+ T cells specific for the PEAP epitope; 2) an increase in the
number and/or activity of CD8+ T cells specific for the PEAP epitope; and 3) secretion of cytokines or chemokines that induce or are indicative of a T cell immune response. Cytokines that induce or are indicative of a T cell immune response include, but are not limited to, interferon-gamma (IFN-γ), IL-2, IL-7, and tumor necrosis factor-alpha (TNF-α).

[00162] In certain embodiments, administration of a subject immunogenic composition results in T cell mediated killing of a retrovirus-infected cell, e.g. an HIV infected cell, via specific T cell recognition of a PEAP or fragment thereof on the surface of a retrovirus-infected cell. In other embodiments, administration of a disclosed immunogenic composition results in T cell mediated killing of a retrovirus-infected cell, e.g. an HIV infected cell, via cross-reactivity of a T cell specific for a PEAP or fragment thereof with a fragment of an endogenous anti-viral polypeptide presented on the surface of a retrovirus-infected cell.

[00163] In one embodiment, a subject immunogenic composition does not comprise a polypeptide having an amino acid sequence that is at least about 60% identical to the amino acid sequence set forth in SEQ ID NO: 2 or an immunogenic fragment thereof.

[00164] In certain embodiments, a subject immunogenic composition comprises a peptide comprising the amino acid sequence of any one of SEQ ID NOs: 11-22, where the peptide has a length of from 15 amino acids to about 20 amino acids, from about 20 amino acids to about 25 amino acids, from about 25 amino acids to about 30 amino acids, from about 30 amino acids to about 35 amino acids, from about 35 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[00165] In certain embodiments, a subject immunogenic composition comprises two or more different PEAPS. For example, in some embodiments, a subject immunogenic composition comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 different PEAPS, where each PEAP comprises a peptide comprising the amino acid sequence of any one of SEQ ID NOs: 1 1-22, where the peptide has a length of from 15 amino acids to about 20 amino acids, from about 20 amino acids to about 25 amino acids, from about 25 amino acids to about 30 amino acids, from about 30 amino acids to about 35 amino acids, from about 35 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[00166] In some embodiments, a subject immunogenic composition comprises a multimerized PEAP, as described above.

[00167] A subject immunogenic composition can be formulated in a number of ways, as described in more detail below. In one example, a subject immunogenic composition comprises single species of PEAP, e.g., the immunogenic composition comprises a population of PEAPs, substantially all of which have the same amino acid sequence. In other examples, a subject
immunogenic composition comprises two or more different PEAPs, i.e., the immunogenic composition comprises a population of PEAPs, wherein two or more of the members differ in amino acid sequence. A subject immunogenic composition can comprise from two to about 20 different PEAPs, e.g., a subject immunogenic composition can comprise two, three, four, five, six, seven, eight, nine, ten, 11-15, or 15-20 different PEAPs, each having an amino acid that differs from the amino acid sequences of the other PEAPs. For example, in some embodiments, a subject immunogenic composition comprises a first PEAP having a first amino acid sequence; and at least a second PEAP having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence. As another example, in some embodiments, a subject immunogenic composition comprises a first PEAP having a first amino acid sequence; a second PEAP having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence; and at least a third PEAP having a third amino acid sequence, where the third amino acid sequence differs from both the first and the second amino acid sequences. In other embodiments, a subject immunogenic composition comprises a multimerized PEAP, as described above.

Adjuvants

[00168] In some embodiments, a subject immunogenic composition comprises a subject PEAP, and an adjuvant. Examples of suitable adjuvants that can be used in humans include, but are not necessarily limited to, alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v Tween 80, 0.5% w/v Span 85), CpG-containing nucleic acid (where the cytosine is unmethylated), QS21, MPL, 3DMPL, extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, and the like. For veterinary applications including but not limited to animal experimentation, one can use Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(r-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic antigen.

[00169] Further exemplary adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO90/14837; Chapter 10 in Vaccine design: the submit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80
(polyoxyethylene sorbitan mono-oleate), and 0.5% Span 85 (sorbitan trioleate) (optionally containing muramyl tri-peptide covalently linked to dipalmitoyl phosphatidylethanolamine (MTP-PE)) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBIT™ adjuvant system (RAS), (Ribi Immunoclem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), e.g., MPL + CWS (DETOX™); (2) saponin adjuvants, such as QS21 or STIMULON™ (Cambridge Bioscience, Worcester, MA) can be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent e.g. WOOO/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) e.g. GB-222022 1, EP-A-0689454, optionally in the substantial absence of alum e.g. WOOO/56358; (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions e.g. EP-A-08353 18, EP-A-0735898, EP-A-0761 23; (7) oligonucleotides comprising CpG motifs [Krieg Vaccine 2000, 19, 6 38-622; Krieg Curr Opin Mol Ther 2001 3:1 5-24; Roman et al., Nat. Med., 1997, 3, 849-854; Weinere/al., PNAS USA, 1997, 94, 10833-10837; Davis et al., J. Immunol., 1998, 160, 870-876; Chu et al., J. Exp.Med., 1997, 186, 1623-1631; Lipford et al., Eur. J. Immunol., 1997, 27, 2340-2344; Moldoveanu et al., Vaccine, 1988, 10, 1216-1224, Krieg et al., Nature, 1995, 374, 546-549; Klinman et al., PNAS USA, 1996, 93, 2879-2883; Ballas et al., J. Immunol., 1996, 157, 1840-1845; Cowdery et al., J. Immunol., 1996, 156, 4570-4575; Halpern et al., Cell Immunol., 1996, 167, 72-78; Yamamoto et al., Jpn. J. Cancer Res., 1988, 79, 866-873; Stacey et al., J. Immunol., 1996, 157, 2 116-2122; Messina et al. J. Immunol., 1991, 147, 1759-1764; Yi et al., J. Immunol., 1996, 157, 4918-4925; Yi et al. J. Immunol., 1996, 157, 5394-5402; Yi et al., J. Immunol., 1998, 160, 4755-4761; and Yi et al., J. Immunol., 1998, 160, 5898-5906; International patent publications WO96/02555, WO98/16247, WO98/18810, WO98/40100, WO98/55495, WO98/37919 and WO98/52581] i.e. containing at least one CG dinucleotide, where the cytosine is unmethylated; (8) a polyoxyethylene ether or a polyoxyethylene ester e.g. WO99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (WO91/2 1207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO91/21 152); (10) a saponin and an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) (WO90/62800); (11) an immunostimulant and a particle of metal salt e.g. WOOO/23 105; (12) a saponin and an oil-in-water emulsion e.g. WOOO/1 124 1; (13)
a saponin (e.g. QS21) + 3dMPL + IM2 (optionally + a sterol) e.g. WO98/57659; (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sα-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE, etc.

A subject immunogenic composition can include a conventional pharmaceutically acceptable excipient, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antigen in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient’s needs. The resulting compositions may be in the form of a solution, suspension, tablet, pill, capsule, powder, gel, cream, lotion, ointment, aerosol or the like.

The protein concentration of a subject PEAP in the pharmaceutical formulations can vary widely, i.e. from less than about 0.1%, from about 2% to about 20% to 50%, or more, by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

In some embodiments, a subject PEAP is formulated with one or more lipids. For example, liposomes of various sizes can be made. Small liposomes or vesicles formed are unilamellar and have a size in the range of about 20 to 400 nanometers and can be produced by subjecting multi-lamellar vesicles to ultrasound, by extrusion under pressure through membranes having pores of defined size, or by high pressure homogenization. Larger unilamellar liposomes having a size in the range of about 0.1 to 1 μm in diameter can be obtained when the lipid is solubilized in an organic solvent or a detergent and the solubilized agent is removed by evaporation or dialysis, respectively. The fusion of smaller unilamellar liposomes by methods requiring particular lipids or stringent dehydration-hydration conditions can yield unilamellar vessels as large or larger than cells.

Liposomes may comprise one or more cationic lipids, e.g., DDAB, dimethyldioctadecyl ammonium bromide; N-{[1-(2,3-Dioloyloxy)propyl]-N,N,N-trimethylammonium methylsulfate; 1,2-diacyl-3-trimethylammonium-propanes, (including but not limited to, dioleoyl (DOTAP), dimyristoyl, dipalmitoyl, disearoyl); 1,2-diacyl-3-dimethylammonium-propanes, (including but not limited to, dioleoyl, dimyristoyl, dipalmitoyl, disearoyl) DOTMA,
N-[1-[2,3-bis(oleoyloxy)]propyl]-N,N,N-trimethylammonium chloride; DOGS, diocadecylamidoglycylspermine; DC-cholesterol, 3β-[N-(N',N' -dimethylaminoethane)carbamoyl]cholesterol; DOSPA, 2,3-dioleoyloxy-N-(2(sperminecarboxamido)-ethyl)-N,N-dimethyl-1-propanaminium trifluoroacetate; \(^1\) \(^\omega\)-diacetyl-sn-glycero-\(^\omega\)-ethylphosphocholines (including but not limited to dioleoyl (DOEPC), dilauroyl, dimyristoyl, dipalmitoyl, distearoyl, palmitoyl-oleoyl); \(\beta\)-alanyl cholesterol; CTAB, cetyl trimethyl ammonium bromide; diC14-amidine, N-t-butyl-N'-tetradecyl-3-tetradecylaminopropionamidine; 14Dea2, O,O'-ditetradecanolyl-N-(trimethylammonioacetyl) diethanolamine chloride; DOSPER, 1,3-dioleoyloxy-2-(6-carboxy-spermyl)-propylamide; N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxylethyl)-2,3-dioleoyloxy-1,4-butanediammonium iodide; l-[2-acyloxy]ethyl]2-alkyl (alkenyl)-3-(2-hydroxyethyl)imidazolinium chloride derivatives such as l-[2-(9(Z)-octadecenoyloxy)ethyl]-2-(8(Z)-heptadecenyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), l-[2-(hexadecanoyloxy)ethyl]-2-pentadecyl-3-(2-hydroxyethyl)imidazolinium chloride (DPTIM); l-[2-tetradecanoyloxy]ethyl]-2-tridecyl-3-(2-hydroxyethyl)imidazolinium chloride (DMTIM) - as described in Solodin et al. (1995) Biochem. 43:13537-13544; 2,3-dialkyloxypropyl quaternary ammonium compound derivates, containing a hydroxyalkyl moiety on the quaternary amine, such as 1,2-dioleoyl-3-dimethyl- hydroxyethyl ammonium bromide (DORI); l,2-dioleoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE); 1,2-dioleoyloxypropyl-3-dimethyl- hydroxypropyl ammonium bromide (DORIE-HP); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB); 1,2-dioleoyloxypropyl-3-dimethyl- hydroxypentyl ammonium bromide (DORIE-HPe); 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE); 1,2-dipalmitoyloxypropyl-3- dimethyl-hydroxyethyl ammonium bromide (DPRIE); 1,2-disteroyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE) - as described, e.g., in Feigner et al. (1994) J. Biol. Chem. 269:2550-2561. Many of the above-mentioned lipids are available commercially from, e.g., Avanti Polar Lipids, Inc.; Sigma Chemical Co.; Molecular Probes, Inc.; Northern Lipids, Inc.; Roche Molecular Biochemicals; and Promega Corp.

(Liposomes may comprise cationic lipids alone, or in admixture with other lipids, particularly neutral lipids such as: cholesterol; 1,2-diacyl-sn-glycero-3-phosphoethanolamines, (including but not limited to dioleoyl (DOPE), 1,2-diacyl-sn-glycero-3-phosphocholines; natural egg yolk phosphatidyl choline (PC), and the like; synthetic mono- and diacyl phosphocholines (e.g., monoacyl phosphatidyl choline (MOPC)) and phosphoethanolamines. Asymmetric fatty
acids, both synthetic and natural, and mixed formulations, for the above diacyl derivatives may also be included.

Other suitable liposome compositions include dimyristoylphosphatidylcholine (DMPC) and cholesterol. Such liposomes are described in, e.g., U.S. Patent No. 5,916,588. Additional suitable liposomal compositions, and methods of preparing same, are known in the art, and are described in various publications, including, e.g., U.S. Patent Nos. 4,241,046 and 6,355,267.

**PEAP POLYNUCLEOTIDES**

The present disclosure provides a recombinant (e.g., synthetic) nucleic acid comprising a nucleotide sequence encoding a subject PEAP. A recombinant (e.g., synthetic) nucleic acid comprising a nucleotide sequence encoding a subject PEAP is referred to herein as a "subject PEAP-encoding nucleic acid," a "subject PEAP-encoding polynucleotide," or simply a "PEAP nucleic acid" or "PEAP polynucleotide." The present disclosure further provides compositions, including pharmaceutical compositions and immunogenic compositions, comprising a subject PEAP polynucleotide.

In certain embodiments, a subject PEAP polynucleotide comprises a nucleotide sequence encoding subject PEAP, where the PEAP comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to the amino acid sequence as set forth in any one of SEQ ID NOs: 11-22.

In some embodiments, a subject PEAP nucleic acid comprises a nucleotide sequence encoding a single type (or "species") of PEAP, e.g., in some embodiments, the PEAP nucleic acids all comprise nucleotide sequences substantially the same amino acid sequence. In other embodiments, a subject PEAP nucleic acid composition comprises two or more different PEAP nucleic acids, e.g., the composition comprises a population of PEAP nucleic acids encoding a population of PEAP, the members of which population can differ in amino acid sequence. A population of encoded PEAPs can comprise from two to about 20 different PEAPs, e.g., a subject composition can comprise two, three, four, five, six, seven, eight, nine, ten, 11-15, or 15-20 different PEAPs, each having an amino acid that differs from the amino acid sequences of the other PEAPs. For example, in some embodiments, a population of encoded PEAPs comprises a first PEAP having a first amino acid sequence; and at least a second PEAP having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence. As another example, in some embodiments, a population of encoded PEAPs a first PEAP having a first amino acid sequence; second PEAP having a second amino acid sequence, where the second amino acid sequence differs from the first amino acid sequence; and at least a
third PEAP having a third amino acid sequence, where the third amino acid sequence differs from both the first and the second amino acid sequences. In other embodiments, the encoded PEAP is a multimerized PEAP, as described above.

Expression vectors and delivery vehicles

In some embodiments, a subject PEAP polynucleotide is an expression vector. The expression vector will provide a transcriptional and translational initiation region, which may be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. Thus, e.g., a subject PEAP polynucleotide can comprise a nucleotide sequence encoding a subject PEAP, where the PEAP-encoding nucleotide sequence is operably linked to a transcriptional control element (e.g., a promoter), where the transcriptional control element can be inducible or constitutive.

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins (e.g., to provide for insertion of a nucleotide sequence encoding a subject PEAP). A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Ophthmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191 ; WO 94/28938; WO 95/1 1984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Ophthmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:103 19 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK.3, pBPV, pMSG, and pSVLSV40.
(Pharmacia). However, any other vector may be used so long as it is compatible with the host cell.

[00182] Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g., Bitter et al. (1987) Methods in Enzymology, 153:516-544).

[00183] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression.

[00184] A subject recombinant vector will in some embodiments include one or more selectable markers. In addition, the expression vectors can include one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture.


[00186] Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/1 1092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Nos. WO 95/13796, WO 94/23697, and WO 91/14445, and EP No. 524 968.
Liposome or lipid nucleic acid delivery vehicles can also be used. Liposome complexes for gene delivery are described in, e.g., U.S. Patent No. 7,001,614. For example, liposomes comprising DOTAP and at least one cholesterol and/or cholesterol-derivative, present in a molar ratio range of 2.0 mM to 10 mM provide an effective delivery system, e.g., where the molar ratio of DOTAP to cholesterol is 1:1 to 3:1. The cationic lipid N-[(2,3-dioleoyloxy)propyl]-L-lysinamide (LADOP) can be used in a composition for delivering a PEAP polynucleotide, where LADOP-containing liposomes are described in, e.g., U.S. Patent No. 7,067,697. Liposome formulations comprising amphipathic lipids having a polar headgroup and aliphatic components capable of promoting transfection are suitable for use and are described in, e.g., U.S. Patent No. 6,433,017.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al, (1994) Proc. Natl. Acad. Sci. USA 91:1 1581-1 1585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Patent No. 5,206,152 and PCT No. WO 92/1 1033. Compositions

The present disclosure provides compositions comprising a subject PEAP nucleic acid. Compositions comprising a subject PEAP nucleic acid can include one or more of: a salt, e.g., NaCl, MgCl, KCl, MgSO₄, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-amino propanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a nuclease inhibitor; and the like. In some embodiments, as described in more detail below, a subject PEAP nucleic acid composition is an immunogenic composition.

Pharmaceutical compositions

The present disclosure provides a pharmaceutical composition comprising a subject PEAP nucleic acid and a pharmaceutically acceptable excipient. A wide variety of pharmaceutically acceptable excipients is known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and

(00191) The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

(00192) Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents.

Immunogenic compositions

(00193) The present disclosure provides an immunogenic composition comprising a subject PEAP polynucleotide. When administered to an individual in need thereof, a polynucleotide comprising a nucleotide sequence encoding a subject PEAP is taken up by a cell, e.g., an antigen-presenting cell, the encoded PEAP is produced in the cell, and the PEAP is processed into polypeptide fragments ("epitope fragments") that are then displayed on the surface of the cell in association with an MHC molecule. The encoded PEAP stimulates or enhances a T cell response to the epitope(s) displayed on the cell surface. Where epitopes having the amino acid sequence of the PEAP epitopes are also present on a retrovirus-infected cell, a T cell response to the retrovirus-infected cell also occurs.

(00194) A subject immunogenic composition comprising a subject PEAP nucleic acid includes, in addition to a subject PEAP nucleic acid, one or more additional components, as described above for immunogenic compositions comprising a subject PEAP polypeptide.

Adjuvants

(00195) In some embodiments, a subject immunogenic composition comprises a subject PEAP polynucleotide and an adjuvant. Suitable adjuvants include those suitable for use in humans. Examples of known suitable adjuvants that can be used in humans include, but are not necessarily limited to, alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v polysorbate 80 (Tween 80), 0.5% w/v sorbitan trioleate (Span 85)), a CpG-containing nucleic acid (where the cytosine is unmethylated), QS21 (saponin adjuvant), MPL (Monophosphoryl Lipid A), 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS (see, e.g., Sölander et al. (1998) J. Leukocyte Biol. 64:713), LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, and the like. For veterinary applications including but not limited to animal experimentation, one can use Freund's, N-
acetyl-muramyl-L-threonyl-D-isoglutamine (Cthr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-
isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylMuramyl-L-alanyl-D-
isoglutaminyl-L-alanine-2-(l’-2’-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine
(CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted
from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton
(MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

Further exemplary adjuvants to enhance effectiveness of the composition include, but
are not limited to: (1) oil-in-water emulsion formulations (with or without other specific
immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall
components), such as for example (a) MF59™ (WO90/14837; Chapter 10 in Vaccine design: the
subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5%
Squalene, 0.5% Tween 80 (polyoxyethylene sorbitan mono-oleate), and 0.5% Span 85 (sorbitan
triol) (optionally containing muramyl tri-peptide covalently linked to dipalmitoyl
phosphatidylethanolamine (MTP-PE)) formulated into submicron particles using a
microfluidizer, (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked
polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to
generate a larger particle size emulsion, and (c) RIBI™ adjuvant system (RAS), (Ribi
Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more
bacterial cell wall components such as monophosphoryl lipid A (MPL), trehalose dimycolate
(TDM), and cell wall skeleton (CWS), e.g., MPL + CWS (DETOX™); (2) saponin adjuvants,
such as QS21 or STIMULON™ (Cambridge Bioscience, Worcester, MA) may be used or
particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS
may be devoid of additional detergent e.g. WO00/07621; (3) Complete Freund's Adjuvant (CFA)
and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-
4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), etc.), interferons (e.g. gamma interferon), macrophage
colony stimulating factor (M-CSF), tumor necrosis factor (TNF), other TNF superfamily
molecules (e.g., CH40L,OX40L, and the like), etc.; (5) monophosphoryl lipid A (MPL) or 3-0-
deacetylated MPL (3dMPL) e.g. GB-2220221, EP-A-0689454, optionally in the substantial
absence of alum when used with pneumococcal saccharides e.g. WO00/56358; (6) combinations
of 3dMPL with, for example, QS21 and/or oil-in-water emulsions e.g. EP-A-0835318, EP-A-
0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs [Krieg Vaccine 2000, 19,
2344; Moldoveanu et al., Vaccine, 1988, 16, 1216-1224, Krieg et al., Nature, 1995, 374, 546-
A subject immunogenic composition can include a conventional pharmaceutically acceptable excipient, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. A subject immunogenic composition can include one or more pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of a subject PEAP nucleic acid in these formulations can vary widely, and can be selected based on various factors such as fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. The resulting compositions may be in the form of a solution, suspension, tablet, pill, capsule, powder, gel, cream, lotion, ointment, aerosol or the like.

The concentration of a subject PEAP polynucleotide in the pharmaceutical formulations can vary widely, e.g., less than about 0.1%, from about 0.1% to about 2%, from about 2% to 20%, or from about 20% to about 50%, or more, by weight, and will be selected on the basis of
various factors such as fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

In some embodiments, a subject PEAP polynucleotide is formulated with one or more lipids. For example, liposomes of various sizes can be made. Small liposomes or vesicles formed are unilamellar and have a size in the range of about 20 to 400 nanometers and can be produced by subjecting multi-lamellar vesicles to ultrasound, by extrusion under pressure through membranes having pores of defined size, or by high pressure homogenization. Larger unilamellar liposomes having a size in the range of about 0.1 to 1 μm in diameter can be obtained when the lipid is solubilized in an organic solvent or a detergent and the solubilized agent is removed by evaporation or dialysis, respectively. The fusion of smaller unilamellar liposomes by methods requiring particular lipids or stringent dehydration-hydration conditions can yield unilamellar vessels as large as or larger than cells.

Liposomes can comprise one or more cationic lipids, e.g., DDAB, dimethyldioctadecyl ammonium bromide; N-[l(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate; 1,2-diacyl-3-trimethylammonium-propanes, (including but not limited to, dioleoyl (DOTAP), dimyristoyl, dipalmitoyl, distearoyl); 1,2-diacyl-3-dimethylammonium- propanes, (including but not limited to, dioleoyl, dimyristoyl, dipalmitoyl, distearoyl) DOTMA, N-[l-2,3-bis(oleoyloxy)propyl]-N,N,N-trimethylammonium chloride; DOGS, dioctadecylamidoglycylspermine; DC-cholesterol, 3β-[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol; DOSPA, 2,3-dioleoyloxy-N-(2(sperminecarboxamido)- ethyl)-N,N-dimethyl-1-propanaminium trifluoroacetate; 1,2-diacyl-sn-glycero-3-ethylphosphocholines (including but not limited to dioleoyl (DOEPC), dilauroyl, dimyristoyl, dipalmitoyl, distearoyl, palmitoyl-oleoyl); β-alanyl cholesterol; CTAB, cetyl trimethyl ammonium bromide; diC14-amidine, N-t-butyl-N'-tetradecyl-3- tetradecylaminopropionamidine; 14Dea2, 0,0'-ditetradecanoyl-N-(trimethylammonioacetyl) diethanolamine chloride; DOSPER, 1,3dioleoyloxy-2-(6-carboxy-spermyl)-propylamide; N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-2,3-dioleoyloxy-1,4-butanedi ammonium iodide; 1-[2-acyloxy]ethyl]2-alkyl (alkenyl)-3-(2-hydroxyethyl)imidazolinium chloride derivatives such as 1-[2-(9(Z)-octadecenoyloxy)ethyl]-2-(8(Z)-heptadecenyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 1-[2-(hexadecanoyloxy)ethyl]-2-pentadecyl-3-(2-hydroxyethyl)imidazolinium chloride (DPTIM); 1-[2-tetradecanoyloxyethyl]-2-tridecyl-3-(2-hydroxyethyl)imidazolium chloride (DMTIM) - as described in Solodin et al. (1995) Biochem. 43: 13537-13544;
2,3-dialkyloxypropyl quaternary ammonium compound derivates, containing a hydroxyalkyl moiety on the quaternary amine, such as 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxypropyl ammonium bromide (DORIE-HP); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxypentyl ammonium bromide (DORIE-HPe); 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE); 1,2-dipalmityloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DPRIE); 1,2-disteryloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE) - as described, e.g., in Feigner et al. (1994) J. Biol. Chem. 269:2550-2561. Many of the above-mentioned lipids are available commercially from, e.g., Avanti Polar Lipids, Inc.; Sigma Chemical Co.; Molecular Probes, Inc.; Northern Lipids, Inc.; Roche Molecular Biochemicals; and Promega Corp.

Liposomes may comprise cationic lipids alone, or in admixture with other lipids, particularly neutral lipids such as: cholesterol; 1,2-diacyl-sn-glycero-3-phosphoethanolamines, including but not limited to dioleoyl (DOPE), 1,2-diacyl-sn-glycero-3-phosphocholines; natural egg yolk phosphatidyl choline (PC), and the like; synthetic mono- and diacyl phosphocholines (e.g., monoacyl phosphatidyl choline (MOPC)) and phosphoethanolamines. Asymmetric fatty acids, both synthetic and natural, and mixed formulations, for the above diacyl derivatives may also be included.

Other suitable liposome compositions include dimyristoylphosphatidylcholine (DMPC) and cholesterol. Such liposomes are described in, e.g., U.S. Patent No. 5,916,588. Additional suitable liposomal compositions, and methods of preparing same, are known in the art, and are described in various publications, including, e.g., U.S. Patent Nos. 4,241,046 and 6,355,267.

TREATMENT AND/OR PROPHYLAXIS METHODS

A variety of treatment and/or prophylaxis methods are contemplated by the present disclosure, which methods utilize a subject PEAP, a subject PEAP nucleic acid, or a subject PEAP composition (e.g., a subject PEAP immunogenic composition, e.g., a subject PEAP immunogenic composition comprising a subject PEAP polypeptide, or a subject PEAP immunogenic composition comprising a subject PEAP polynucleotide). The treatment and/or prophylaxis methods include methods of inducing an immune response in an individual to a PEAP or an endogenous polypeptide having substantial amino acid sequence identity to a PEAP, and methods of enhancing a subject's immune response to a PEAP or an endogenous polypeptide having substantial amino acid sequence identity to a PEAP, e.g., for the treatment of a retrovirus infection (e.g., a lentivirus infection). Thus, e.g., the present disclosure provides
methods of inducing an immune response in an individual to a retrovirus-infected cell (e.g., an HTLV-I-infected cell or an HIV-infected cell), methods of enhancing an immune response to a retrovirus-infected cell (e.g., an HTLV-I-infected cell or an HIV-infected cell), for the treatment of a retrovirus infection (e.g., a retroviral infection, such as an HTLV-I infection or an HIV infection).

**Methods of inducing or enhancing an immune response to a retrovirus-infected cell**

[00204] The present disclosure provides methods for inducing, eliciting, or enhancing a T cell immune response to a retrovirus-infected cell, e.g., an HTLV-I-infected cell or an HIV-infected cell, in an individual in need thereof. The methods generally involve administering an effective amount of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) to the individual.

[00205] In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, reduces retroviral load in the individual by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 75%, at least about 85%, or at least about 90%, compared to the viral load in the individual before treatment with the subject PEAP, the subject PEAP polynucleotide, or the subject PEAP composition.

[00206] For example, in some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces retroviral load in the individual by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 75%, at least about 85%, or at least about 90%, compared to the viral load in the individual before treatment with the immunogenic composition.

[00207] In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell. In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP
polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell, compared with the number of T cells specific for the epitope of the endogenous anti-viral polypeptide in the individual before treatment with the subject PEAP, the subject PEAP polynucleotide, or the subject PEAP composition.

[00208] For example, in some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell, compared with the number of T cells specific for the epitope of the endogenous anti-viral polypeptide in the individual before treatment with the immunogenic composition.

[00209] In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, results in an increase in the number of CD8+ T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell. In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of CD8+ T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell, compared with the
number of CD8\(^+\) T cells specific for the epitope of the endogenous anti-viral polypeptide in the individual before treatment with the subject PEAP, the subject PEAP polynucleotide, or the subject PEAP composition.

For example, in some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of CD8\(^+\) T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25\%, at least about 50\%, at least about 100\% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of CD8\(^+\) T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell, compared with the number of CD8\(^+\) T cells specific for the epitope of the endogenous anti-viral polypeptide in the individual before treatment with the immunogenic composition.

Prophylactic Methods

In some embodiments, a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is administered to a naïve individual (e.g., an individual not infected with a retrovirus such as HTLV-I or HIV) or an individual seronegative for a retrovirus such as HTLV-I or HIV. In such embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, reduces the likelihood that the individual, if later infected with a retrovirus such as HTLV-I or HIV, would develop disease symptoms from the retrovirus infection. In some embodiments where a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is administered to a naïve individual (e.g., an individual not infected with a retrovirus) or an individual seronegative for the retrovirus, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject immunogenic composition, such as a subject immunogenic composition comprising a subject PEAP, or a subject immunogenic composition comprising a subject PEAP nucleic acid) is an amount that, when administered to an individual in one or more doses, results in an increase in the number of CD8\(^+\) T cells specific for an epitope of an endogenous anti-viral polypeptide, which epitope is present on a retrovirus-infected cell, compared with the number of CD8\(^+\) T cells specific for the epitope of the endogenous anti-viral polypeptide in the individual before treatment with the immunogenic composition.
doses, increases the likelihood that the individual, if later infected with a retrovirus such as HTLV-I or HIV, would limit and/or clear the retrovirus infection.

For example, in some embodiments where a subject immunogenic composition is administered to a naïve individual (e.g., an individual not infected with a retrovirus such as HTLV-I or HIV) or an individual seronegative for a retrovirus such as HTLV-I or HIV, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces the likelihood that the individual, if later infected with a retrovirus such as HTLV-I or HIV, would develop disease symptoms from the retrovirus infection. In some embodiments where a subject immunogenic composition is administered to a naïve individual (e.g., an individual not infected with a retrovirus) or an individual seronegative for a retrovirus, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, increases the likelihood that the individual, if later infected with a retrovirus such as HTLV-I or HIV, would limit and/or clear the retrovirus infection.

Combination therapies

A subject immunogenic composition can be administered in conjunction with one or more therapeutic agents for the treatment of a retroviral, e.g., a lentiviral infection, or for the treatment of a disorder that may accompany a retroviral, e.g., a lentiviral infection (e.g., a bacterial infection, a fungal infection, and the like). Therapeutic agents include, e.g., beta-lactam antibiotics, tetracyclines, chloramphenicol, neomycin, gramicidin, bacitracin, sulfonamides, nitrofurazone, nalidixic acid, cortisone, hydrocortisone, prednisolone, triamcinolone, dexamethasone, fluocortolone, acyclovir, amantadine, rimantadine, recombinant soluble CD4 (rsCD4), anti-receptor antibodies (e.g., for rhinoviruses), nevirapine, didanosine (ddI, Videx™), stavudine (d4T, Zerit™), zalcitabine (ddC, Hivid™), lamivudine (Epivir™), nevirapine (Viramune™), protease inhibitors, saquinavir (Invirase™, Fortovase™), ritonavir (Norvir™), lopinavir (Viracept™), efavirenz (Sustiva™), abacavir (Ziagen™), amprenavir (Agenerase™) indinavir (Crixivan™), ganciclovir, AzDU, delavirdine (Rescriptor™), kaletra, trizivir, rifampin, clathiromycin, erythropoietin, colony stimulating factors (G-CSF and GM-CSF), non-nucleoside reverse transcriptase inhibitors, nucleoside inhibitors, adriamycin, fluorouracil, methotrexate, asparaginase and combinations thereof.
Methods of treating cancer

[00214] The present disclosure further provides methods of treating cancer in an individual, where the cancerous state is associated with aberrant expression of an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP or increased expression of an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP, e.g., where the cancer comprises a cancer cell or a pre-cancerous cell that exhibits aberrant expression of an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP (e.g., expresses an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP at a level that is at least about 15%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 5-fold, or at least about 10-fold, or more than 10-fold, higher than the level of the endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP expressed by a non-cancerous (normal) cell of the same cell type). Such cancers include, but are not limited to, myeloma, melanoma, ovarian cancer, breast cancer, and testicular cancer (including teratoma, seminoma, and embryonal carcinoma or mixed tumors composed of one or more of these types). The methods generally involve administering to an individual in need thereof an effective amount of a subject PEAP (e.g., a subject synthetic PEAP), a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject PEAP pharmaceutical composition or a subject PEAP immunogenic composition). In some embodiments, the methods generally involve administering to an individual in need thereof an effective amount of a subject PEAP immunogenic composition (e.g., a subject PEAP immunogenic composition comprising one or more subject PEAPs or one or more subject PEAP polynucleotides).

[00215] The present disclosure provides methods for treating a cancer (e.g., myeloma, melanoma, ovarian cancer, breast cancer, and testicular cancer (including teratoma, seminoma, and embryonal carcinoma or mixed tumors composed of one or more of these types) in an individual, the methods generally involving administering to an individual in need thereof an effective amount of a subject PEAP (e.g. a subject synthetic PEAP), a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject PEAP pharmaceutical composition or a subject PEAP immunogenic composition). In some embodiments, the present disclosure provides methods for treating cancer in an individual, the methods generally involving administering to an individual in need thereof an effective amount of a subject PEAP immunogenic composition, e.g., a subject immunogenic composition comprising a subject PEAP or a subject PEAP polynucleotide. The present disclosure provides use of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition in the preparation of a medicament.
for the treatment of a cancer in an individual. The present disclosure provides use of a subject PEAP immunogenic composition (e.g., a subject immunogenic composition comprising a subject PEAP or a subject PEAP polynucleotide) in the preparation of a medicament for the treatment of a cancer in an individual. The present disclosure provides a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition for treating a cancer in an individual. The present disclosure provides a subject PEAP immunogenic composition (e.g., a subject immunogenic composition comprising a subject PEAP or a subject PEAP polynucleotide) for treating a cancer in an individual.

For example, an effective amount of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is administered to an individual having a tumor (e.g., a solid tumor), wherein the cells of the tumor express an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP as a marker of the cancerous state.

For example, an effective amount of a subject immunogenic composition comprising one or more PEAPs is administered to an individual having a tumor (e.g., a solid tumor), wherein the cells of the tumor express an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP as a marker of the cancerous state.

As another example, an effective amount of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is administered to a subject having a tumor, wherein the tissue from which the tumor expresses an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP in the non-cancerous state and such tissue exhibits an increase (e.g., an at least about 15%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 5-fold, or at least about 10-fold, or more than 10-fold, increase) in expression of the endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP as a marker of the cancerous state.

As another example, an effective amount of a subject immunogenic composition is administered to a subject having a tumor, wherein the tissue from which the tumor expresses an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP in the non-cancerous state exhibits an increase (e.g., an at least about 15%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 5-fold, or at least about 10-fold, or more than 10-fold, increase) in expression of the endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP as a marker of the cancerous state.
Cancers amenable to treatment with subject immunogenic compositions include ovarian cancer, breast cancer, myeloma, melanoma, prostate cancer, and testicular cancer (including seminoma, teratoma, and embryonal carcinoma).

In some embodiments, in the context of cancer treatment, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is an amount that, when administered to an individual in one or more doses, reduces one or more of tumor size, cancer cell number, and cancer cell metastasis by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, up to total eradication of the cancer.

In some embodiments, in the context of cancer treatment, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, reduces one or more of tumor size, cancer cell number, and cancer cell metastasis by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, up to total eradication of the cancer.

In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for an epitope present on a cancer cell. In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for an epitope present on a cancer cell, compared with the number of T cells specific for a cancer cell epitope in the individual before treatment with the subject PEAP, subject PEAP polynucleotide, or the subject PEAP composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of T cells specific for an epitope present on a cancer cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of T cells specific for an epitope present on a
cancer cell, compared with the number of T cells specific for a cancer cell epitope in the individual before treatment with the immunogenic composition.

In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of CD8+ T cells specific for an epitope present on a cancer cell. In some embodiments, an "effective amount" of a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of CD8+ T cells specific for an epitope present on a cancer cell, compared with the number of CD8+ T cells specific for a cancer cell epitope in the individual before treatment with the subject PEAP, the subject PEAP polynucleotide, or the subject PEAP composition.

In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase in the number of CD8+ T cells specific for an epitope present on a cancer cell. In some embodiments, an "effective amount" of a subject immunogenic composition is an amount that, when administered to an individual in one or more doses, results in an increase of at least about 25%, at least about 50%, at least about 100% or 2-fold, at least about 5-fold, at least about 10-fold, or at least about 100-fold, or more, in the number of CD8+ T cells specific for an epitope present on a cancer cell, compared with the number of CD8+ T cells specific for a cancer cell epitope in the individual before treatment with the immunogenic composition.

In some embodiments, a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition (e.g., a subject PEAP immunogenic composition) is administered to an individual in need thereof as an adjuvant therapy to a standard cancer therapy. Standard cancer therapies include surgery (e.g., surgical removal of cancerous tissue), radiation therapy, bone marrow transplantation, chemotherapeutic treatment, biological response modifier treatment, and certain combinations of the foregoing.

Radiation therapy includes, but is not limited to, x-rays or gamma rays that are delivered from either an externally applied source such as a beam, or by implantation of small radioactive sources.

Chemotherapeutic agents are non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-
limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.

Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan®), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlorothymine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), flouxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, IO-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, e.g. vincristine, vinblastine, vinorelbine, vindesine, etc.; podophyllotoxins, e.g. etoposide, teniposide, etc.; antibiotics, e.g. anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, etc.; phenoxizone biscyclopeptides, e.g. dactinomycin; basic glycopeptides, e.g. bleomycin; anthraquinone glycosides, e.g. plicamycin (mithramycin); anthracenediones, e.g. mitoxantrone; azirinopyrrolo indolenediones, e.g. mitomycin; macrocyclic immunosuppressants, e.g. cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, etc.; and the like.

Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate,
vincristine sulfate, natural and synthetic epothilones including but not limited to, epothilone A, epothilone B, discodermolide; estramustine, nociodazole, and the like.

Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, e.g. prednisone, dexamethasone, etc.; estrogens and pregnestins, e.g. hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; etc.; and adrenocortical suppressants, e.g. aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation; therefore, compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

Other chemotherapeutic agents include metal complexes, e.g. cisplatin (cis-DDP), carboplatin, etc.; ureas, e.g. hydroxyurea; and hydrazines, e.g. N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc. Other antiproliferative agents of interest include immunosuppressants, e.g. mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinoxy)propoxy)quinazoline); etc.

"Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3H-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from Taxus brevifolia; or T-19 12 from Taxus yannanensis).

Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., TaxotereU docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).
Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/181 13; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

Biological response modifiers suitable for use in connection with the methods of the invention include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN-α; (7) IFN-γ colony-stimulating factors; and (9) inhibitors of angiogenesis.

In some embodiments, in the context of cancer treatment, a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition as described above does not comprise an amino acid sequence (or a nucleotide sequence encoding the amino acid sequence, in the case of a polynucleotide) of an endogenous tetherin (a.k.a., BST2, a.k.a., CD3 17, a.k.a, HM 1.24) polypeptide. In some embodiments, in the context of cancer treatment, a subject PEAP, a subject PEAP polynucleotide, or a subject PEAP composition does not comprise more than 5 contiguous amino acids (or a nucleotide sequence encoding more than 5 contiguous amino acids, in the case of a polynucleotide) of SEQ ID NO:23.

**Formulations**

A subject PEAP, as described above, can be formulated in any of a variety of ways for administration to an individual in need thereof. The present disclosure provides pharmaceutical formulations comprising a PEAP. Immunogenic compositions comprising a PEAP or a nucleic acid encoding a PEAP are described above. Additional formulations are described below.

A formulation comprising a PEAP can include one or more excipients (e.g., sucrose, starch, mannitol, sorbitol, lactose, glucose, cellulose, tcalc, calcium phosphate or calcium carbonate), a binder (e.g., cellulose, methylcellulose, hydroxyethylcellulose, polypropylpyrrolidone, polyvinylpyrrolidone, gelatin, gum arabic, polyethylene glycol, sucrose or starch), a disintegrator (e.g., starch, carboxymethylcellulose, hydroxypropylstarch, low substituted hydroxypropylcellulose, sodium bicarbonate, calcium phosphate or calcium citrate), a
lubricant (e.g., magnesium stearate, light anhydrous silicic acid, talc or sodium lauryl sulfate), a
flavoring agent (e.g., citric acid, menthol, glycine or orange powder), a preservative (e.g.,
sodium benzoate, sodium bisulfite, methylparaben or propylparaben), a stabilizer (e.g., citric
acid, sodium citrate or acetic acid), a suspending agent (e.g., methylcellulose,
polyvinylpyrrolidone or aluminum stearate), a dispersing agent (e.g.,
hydroxypropylmethylcellulose), a diluent (e.g., water), and base wax (e.g., cocoa butter, white
petrolatum or polyethylene glycol).

Tablets comprising an active agent may be coated with a suitable film-forming agent,
e.g., hydroxypropylmethyl cellulose, hydroxypropyl cellulose or ethyl cellulose, to which a
suitable excipient may optionally be added, e.g., a softener such as glycerol, propylene glycol,
diethylphthalate, or glycerol triacetate; a filler such as sucrose, sorbitol, xylitol, glucose, or
lactose; a colorant such as titanium hydroxide; and the like.

Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or
the like, and combinations thereof. In addition, if desired, the vehicle may contain minor
amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents.
Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled
in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton,
Pa., 17th edition, 1985. The composition or formulation to be administered will, in any event,
contain a quantity of the agent adequate to achieve the desired state in the subject being treated.
The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are
readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such
as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and
the like, are readily available to the public.

In some embodiments, e.g., for use in inducing or enhancing an immune response to a
lentivirus, a PEAP is formulated for vaginal delivery. A subject formulation for intravaginal
administration is formulated as an intravaginal bioadhesive tablet, intravaginal bioadhesive
microparticle, intravaginal cream, intravaginal lotion, intravaginal foam, intravaginal ointment,
intravaginal paste, intravaginal solution, or intravaginal gel.

Dosages

The appropriate dosage of a subject PEAP that, when administered in one or multiple
doses, has the desired effect (e.g., increases a T cell immune response to a lentivirus-infected
cell), will vary, depending on various factors, but will generally be in the range of from about 1
µg to about 100 mg, e.g., from about 1 µg to about 5 µg, from about 5 µg to about 10 µg, from
about 10 µg to about 25 µg, from about 25 µg to about 50 µg, from about 50 µg to about 100 µg,
from about 100 µg to about 500 µg, from about 500 µg to about 1 mg, from about 1 mg to about
10 mg, from about 10 mg to about 50 mg, or from about 50 mg to about 100 mg, administered in one dose or divided into multiple doses.

In some embodiments, the amount of PEAP per dose is determined on a per body weight basis. For example, in some embodiments, a PEAP is administered in an amount of from about 0.5 mg/kg to about 100 mg/kg, e.g., from about 0.5 mg/kg to about 1 mg/kg, from about 1 mg/kg to about 2 mg/kg, from about 2 mg/kg to about 3 mg/kg, from about 3 mg/kg to about 5 mg/kg, from about 5 mg/kg to about 7 mg/kg, from about 7 mg/kg to about 10 mg/kg, from about 10 mg/kg to about 15 mg/kg, from about 15 mg/kg to about 20 mg/kg, from about 20 mg/kg to about 25 mg/kg, from about 25 mg/kg to about 30 mg/kg, from about 30 mg/kg to about 40 mg/kg, from about 40 mg/kg to about 50 mg/kg per dose, from about 50 mg/kg to about 60 mg/kg, from about 60 mg/kg to about 70 mg/kg, from about 70 mg/kg to about 80 mg/kg, from about 80 mg/kg to about 90 mg/kg, or from about 90 mg/kg to about 100 mg/kg, or more than about 100 mg/kg.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

In some embodiments, multiple doses of a subject PEAP are administered. The frequency of administration of a PEAP can vary depending on any of a variety of factors, e.g., severity of the symptoms, etc. For example, in some embodiments, a PEAP is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

The duration of administration of a PEAP, e.g., the period of time over which a PEAP is administered, can vary, depending on any of a variety of factors, e.g., patient response, etc. For example, a PEAP can be administered over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

**Routes of Administration**

Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, intratumoral, transdermal, subcutaneous, intradermal, topical application, intravenous, vaginal, nasal, and other parenteral routes of administration.
Suitable routes of administration also include oral and rectal routes. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. The composition can be administered in a single dose or in multiple doses.

A subject PEAP composition can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the disclosure include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, vaginal, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrastemal, intratumoral, peritumoral, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of the agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

A subject PEAP composition can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (e.g., using a suppository) delivery.

A subject immunogenic composition can be delivered to mucosal tissue, e.g., to vaginal tissue, to rectal tissue, etc.

METHODS OF GENERATING PEAP-SPECIFIC CD8+ T CELLS

The present disclosure provides methods of generating a population of PEAP-specific CD8+ T cells in vitro. The methods generally involve contacting a CD8+ T cell, or a precursor thereof, with a subject PEAP in association with an antigen-presenting platform, where the contacting is performed in vitro. The methods are useful for generating a population of PEAP-specific CD8+ T cells, which are in turn useful in methods of treating disorders such as retrovirus infection, e.g., lentivirus infection (e.g., HIV infection).

In some embodiments, CD8+ T cells are obtained from an individual, and are contacted in vitro with a PEAP in association with an antigen-presenting platform. In some embodiments, a mixed population of cells that comprises CD8+ T cells is obtained from an individual; and CD8+ T cells are isolated from the mixed population, generating an unstimulated CD8+ T cell population. The unstimulated CD8+ T cell population is then contacted in vitro to a PEAP in association with an antigen-presenting platform. The contacting step activates at least a portion
of the unstimulated CD8+ T cell population having T cell receptors capable of binding a PEAP to become specific for a PEAP.

The source of the mixed cell population that comprises a CD8+ T cell can be, e.g., whole blood. The mixed cell population can be manipulated in one or more ways or steps, e.g., to remove red blood cells; to select for CD8+ T cells; and/or to select against CD4+ T cells or other non-CD8+ cell populations. The number of unstimulated CD8+ cells can range from about 10^2 to about 10^9 cells, e.g., from about 10^2 cells to about 10^3 cells, from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells.

The antigen-presenting platform can be an antigen-presenting cell (APC), e.g., an APC pulsed with a PEAP, where the APC can be live or can be inactivated. In some embodiments, the antigen-presenting platform is a bead (e.g., a plastic bead, a magnetic bead, etc.), or other particle, to which a PEAP is bound. Antigen-presenting platforms other than naturally-occurring APCs are known in the art and include, but are not limited to, beads; inactivated surface-engineered viruses (see, e.g., Mosca et al. (2007) Retroviral 4:32); artificial APCs, e.g., liposomes (see, e.g., U.S. Patent Publication No. 2006/0034865); and the like.

The antigen-presenting platform will include, in addition to a PEAP, one or more surface molecules sufficient for stimulating expansion of a PEAP-specific CD8+ T cell population, e.g., MHC class I molecules (e.g., HLA Class I molecules), etc. The antigen-presenting platform can also include one or more co-stimulatory molecules, where suitable co-stimulatory molecules include, but are not limited to, an anti-CD28 antibody, an anti-CD49d antibody, and the like.

The unstimulated CD8+ T cells are contacted in vitro with a PEAP in association with an antigen-presenting platform; and the number of PEAP-specific CD8+ T cells is increased. The method results in a 10-fold to a 10^6-fold increase in the number of PEAP-specific CD8+ T cells. The number of PEAP-specific CD8+ cells obtained by the disclosed method can range from about 10^3 to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells.

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The present disclosure provides treatment methods using the PEAP-specific CD8+ T cells. In some embodiments, the methods are methods of treating an HIV infection. The methods generally involve administering to an individual in need thereof an effective amount of PEAP-specific CD8+ T cells. In some embodiments, the PEAP-specific CD8+ T cells are autologous, e.g., the PEAP-specific CD8+ T cells are administered to the same individual from which the mixed cell population was obtained (i.e., the donor individual and the recipient individual are the same). In other embodiments, the PEAP-specific CD8+ T cells are allogeneic, e.g., the PEAP-specific CD8+ T cells are administered to an individual (a recipient individual) not genetically identical to the individual from which the mixed cell population was obtained (the donor individual).

In some embodiments, the PEAP-specific CD8+ T cells are administered to a recipient individual in an amount of from about 10^3 to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 10^6 cells, from about 10^6 cells to about 10^7 cells, from about 10^7 cells to about 10^8 cells, from about 10^8 cells to about 10^9 cells, in one or more doses.

METHODS OF GENERATING PEAP-SPECIFIC CD4+ T CELLS

The present disclosure also provides methods of generating a population of PEAP-specific CD4+ T cells in vitro. The methods generally involve contacting a CD4+ T cell, or a precursor thereof, with a subject PEAP in association with an antigen-presenting platform, where the contacting is performed in vitro. The methods are useful for generating a population of PEAP-specific CD4+ T cells, which are in turn useful in methods of treating disorders such as retrovirus infection, e.g., lentivirus infection (e.g., HIV infection).

In some embodiments, CD4+ T cells are obtained from an individual, and are contacted in vitro with a PEAP in association with an antigen-presenting platform. In some embodiments, a mixed population of cells that comprises CD4+ T cells is obtained from an individual; and CD4+ T cells are isolated from the mixed population, generating an unstimulated CD4+ T cell population. The unstimulated CD4+ T cell population is then contacted in vitro to a PEAP in association with an antigen-presenting platform. The contacting step activates at least a portion of the unstimulated CD4+ T cell population having T cell receptors capable of binding a PEAP to become specific for a PEAP.

The source of the mixed cell population that comprises a CD4+ T cell can be, e.g., whole blood. The mixed cell population can be manipulated in one or more ways or steps, e.g., to
remove red blood cells; to select for CD4+ T cells; and/or to select against CD8+ T cells or other non-CD4+ cell populations. The number of unstimulated CD4+ cells can range from about 10^2 to about 10^9 cells, e.g., from about 10^2 cells to about 10^3 cells, from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells.

[00268] The antigen-presenting platform can be an antigen-presenting cell (APC), e.g., an APC pulsed with a PEAP, where the APC can be live or can be inactivated. In some embodiments, the antigen-presenting platform is a bead (e.g., a plastic bead, a magnetic bead, etc.), or other particle, to which a PEAP is bound. Antigen-presenting platforms other than naturally-occurring APCs are known in the art and include, but are not limited to, beads; inactivated surface-engineered viruses (see, e.g., Mosca et al. (2007) Retrovirol. 4:32); artificial APCs, e.g., liposomes (see, e.g., U.S. Patent Publication No. 2006/0034865); and the like.

[00269] The antigen-presenting platform will include, in addition to a PEAP, one or more surface molecules sufficient for stimulating expansion of a PEAP-specific CD4+ T cell population, e.g., MHC class II molecules (e.g., HLA Class II molecules), etc. The antigen-presenting platform can also include one or more co-stimulatory molecules, where suitable co-stimulatory molecules include, but are not limited to, an anti-CD28 antibody, an anti-CD49d antibody, and the like.

[00270] The unstimulated CD4+ T cells are contacted in vitro with a PEAP in association with an antigen-presenting platform; and the number of PEAP-specific CD4+ T cells is increased. The method results in a 10-fold to a 10^6-fold increase in the number of PEAP-specific CD4+ T cells. The number of PEAP-specific CD4+ cells obtained by the disclosed method can range from about 10^3 to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells.

[00271] The present disclosure provides treatment methods using the PEAP-specific CD4+ T cells. In some embodiments, the methods are methods of treating an HIV infection. The methods generally involve administering to an individual in need thereof an effective amount of PEAP-specific CD4+ T cells. In some embodiments, the PEAP-specific CD4+ T cells are autologous, e.g., the PEAP-specific CD4+ T cells are administered to the same individual from which the mixed cell population was obtained (i.e., the donor individual and the recipient individual are the
same). In other embodiments, the PEAP-specific CD4+ T cells are allogeneic, e.g., the PEAP-specific CD4+ T cells are administered to an individual (a recipient individual) not genetically identical to the individual from which the mixed cell population was obtained (the donor individual).

[00272] In some embodiments, the PEAP-specific CD4+ T cells are administered to a recipient individual in an amount of from about 10^1 to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 5 x 10^5 cells, from about 5 x 10^5 cells to about 10^6 cells, from about 10^6 cells to about 5 x 10^6 cells, from about 5 x 10^6 cells to about 10^7 cells, from about 10^7 cells to about 5 x 10^7 cells, from about 5 x 10^7 cells to about 10^8 cells, from about 10^8 cells to about 5 x 10^8 cells, or from about 5 x 10^8 cells to about 10^9 cells, in one or more doses.

DIAGNOSTIC METHODS

[00273] The present disclosure provides various diagnostic methods, which methods utilize a subject PEAP polypeptide or a subject PEAP composition. Subject diagnostic methods include methods for monitoring a patient's response to treatment; methods for staging a disease; and methods for detecting a disease.

[00274] Diagnostic methods can involves detecting the number of PEAP-specific CD8+ T cells in a biological sample obtained from an individual. The number of PEAP-specific CD8+ T cells can be determined using, e.g., a ^5^Cr release assay, where target cells pulsed with a PEAP and labeled with ^5^Cr are contacted with a test sample that may contain PEAP-specific CD8+ T cells. The number of PEAP-specific CD8+ T cells is determined by measuring release of ^5^Cr from the target cells.

[00275] In other embodiments, a disclosed diagnostic method involves detecting a PEAP or an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP in the serum or plasma (or other biological fluid) of an individual. Detection of a PEAP or an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP in a biological fluid obtained from an individual can be carried out using, e.g., immunological assays employing antibody specific for a PEAP. Suitable immunological assays include, but are not limited to, enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), protein blot ("Western blot") assays, immunoprecipitation assays, and the like.
PEAP-specific antibodies

As noted above, in some embodiments, a subject diagnostic assay will employ an antibody specific for a PEAP (an "anti-PEAP antibody"). Suitable anti-PEAP antibodies include whole antibody of any isotype; epitope-binding fragments of an anti-PEAP antibody; polyclonal antibodies; monoclonal antibodies; artificial antibodies; single-chain antibodies; and the like.

Monoclonal antibodies are produced by conventional techniques. Generally, the spleen and/or lymph nodes of an immunized host animal provide a source of plasma cells. The plasma cells are immortalized by fusion with myeloma cells to produce hybridoma cells. Culture supernatant from individual hybridomas is screened using standard techniques to identify those producing antibodies with the desired specificity. Suitable animals for production of monoclonal antibodies include mouse, rat, hamster, guinea pig, rabbit, etc. The antibody may be purified from the hybridoma cell supernatants or ascites fluid by conventional techniques, e.g. affinity chromatography using protein bound to an insoluble support, protein A sepharose, etc.

The antibody may be produced as a single chain, instead of the normal multimeric structure. Single chain antibodies are described in Jost et al. (1994) J.B.C. 269:26267-73, and others. DNA sequences encoding the variable region of the heavy chain and the variable region of the light chain are ligated to a spacer encoding at least about 4 amino acids of small neutral amino acids, including glycine and/or serine. The protein encoded by this fusion allows assembly of a functional variable region that retains the specificity and affinity of the original antibody.

Suitable anti-PEAP antibodies also include "artificial" antibodies, e.g., antibodies and antibody fragments produced and selected in vitro. In some embodiments, such antibodies are displayed on the surface of a bacteriophage or other viral particle. In many embodiments, such artificial antibodies are present as fusion proteins with a viral or bacteriophage structural protein, including, but not limited to, M13 gene 111 protein. Methods of producing such artificial antibodies are well known in the art. See, e.g., U.S. Patent Nos. 5,516,637; 5,223,409; 5,658,727; 5,667,988; 5,498,538; 5,403,484; 5,571,698; and 5,625,033.

Antibody fragments, such as Fv, F(\(\text{ab}'\))\(_2\) and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(\(\text{ab}'\))\(_2\) fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

An anti-PEAP antibody will in some embodiments be detectably labeled, e.g., with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, a
chromogenic protein, and the like. An anti-PEAP antibody may be further conjugated to other moieties, such as members of specific binding pairs, e.g., biotin (member of biotin-avidin specific binding pair), and the like. An anti-PEAP antibody may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, magnetic beads, test strips, membranes, and the like.

[00282]  An antibody specific for a PEAP can be labeled, directly or indirectly. Direct labels include radioisotopes (e.g., $^{125}$I; $^{35}$S, and the like); enzymes whose products are detectable (e.g., luciferase, $\beta$-galactosidase, horse radish peroxidase, alkaline phosphatase, and the like); fluorescent labels (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, and the like); fluorescence emitting metals, e.g., $^{152}$Eu, or others of the lanthanide series, attached to the antibody through metal chelating groups such as EDTA; chemiluminescent compounds, e.g., luminol, isoluminol, acridinium salts, and the like; bioluminescent compounds, e.g., luciferin; fluorescent proteins (e.g., a green fluorescent protein, a yellow fluorescent protein, etc.); and the like. Indirect labels include second antibodies specific for PEAP-specific antibodies, wherein the second antibody is labeled as described above; and members of specific binding pairs, e.g., biotin-avidin, and the like.

[00283]  In some embodiments, an anti-PEAP antibody comprises, covalently linked to the antibody, a protein that provides for a detectable signal. Suitable proteins include, but are not limited to, fluorescent proteins and enzymes (e.g., $\beta$-galactosidase, luciferase, horse radish peroxidase, alkaline phosphatase, etc.). Suitable fluorescent proteins include, but are not limited to, a green fluorescent protein (GFP), including, but not limited to, a GFP derived from Aequorina victoria or a derivative thereof, a number of which are commercially available; a GFP from a species such as Renilla reniformis, Renilla mulleri, or Ptilosarcus guernyi, as described in, e.g., WO 99/49019 and Peelle et al. (2001) J. Protein Chem. 20:507-519; any of a variety of fluorescent and colored proteins from Anthozoa species, as described in, e.g., Matz et al. (1999) Nature Biotechnol. 17:969-973, U.S. Patent Publication No. 2002/0197676, or U.S. Patent Publication No. 2005/0032085; and the like.

[00284]  In certain embodiments, a subject diagnostic assay employs an antibody specific for a PEAP, wherein the antibody specific for the PEAP specifically excludes antibodies, or binding fragments thereof, having binding affinity for a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.

Monitoring patient response to treatment for a retrovirus infection

[00285]  In some embodiments, a subject PEAP composition is useful for monitoring a patient's response to treatment for a retrovirus infection, e.g., an HIV infection. Thus, the present disclosure further provides methods for monitoring a patient's response to treatment for a
lentivirus infection, e.g., an HIV infection. The methods generally involve contacting a white blood cell (WBC) from a patient in vitro with a disclosed PEAP; and detecting a cytokine secreted by the WBC in response to contact with the PEAP. A reduction in cytokine production by the WBC in response to contact with a PEAP is an indication that the treatment is effective in treating a lentivirus infection (e.g., in achieving a reduction in viral load, in achieving an increase in CD4+ T lymphocyte levels (in the case of an HIV infection), and the like). Suitable WBCs include, but are not limited to, peripheral blood mononuclear cells (PBMC), isolated T lymphocytes, isolated CD4+ T lymphocytes, isolated CD8+ T lymphocytes, natural killer (NK) cells, natural killer T lymphocytes (NKT, e.g., NKLI+ T lymphocytes), and the like.

PEAPs suitable for use in the disclosed monitoring method can be 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, 11 amino acids, 12 amino acids, 12-15 amino acids, 15-18 amino acids, 18-20 amino acids, or 20-25 amino acids long, or longer. Suitable PEAPs include any of the PEAPs discussed above. In some embodiments, the PEAP comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 1-10.

Cytokines that are secreted from PBMCs and that are detected in a disclosed patient monitoring method include, but are not limited to, IFN-γ, TNF-α, and IL-2.

Methods for detecting secreted cytokines that are suitable for use in a disclosed patient monitoring method include, but are not limited to, immunological assays, e.g., enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), an enzyme-linked immunospot (ELISPOT) assay; cellular assays; and the like.

In some embodiments, a reduction of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% or more, in cytokine production by WBCs in response to contact with a PEAP indicates that the treatment for the lentivirus infection is efficacious.

Patient samples comprising white blood cells (WBCs) can be obtained before and after treatment; or at various times during the course of treatment, and the level of cytokine production compared between a sample taken at a first time point and a sample taken at a second (later) time point.

In some embodiments, PBMC obtained from a patient are contacted with one or more PEAPs in vitro; and an ELISPOST assay is used to detect cytokine production. The ELISPOST assay has been described in the art. See, e.g., Lalvani et al. (1997) J. Exp. Med. 186:859; and U.S. Patent No. 5,853,697. In these embodiments, the level of cytokines produced by the PBMC is expressed as the number of spot-forming units (SFU) per 10⁶ PBMC. A reduction in the number of SFU indicates that a treatment for a lentivirus infection is effective.
Staging a disease

The present disclosure provides methods of staging a disease in an individual, where the level of a PEAP or an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP is associated with the stage or severity of the disease. The methods generally involve detecting the level of a PEAP or an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP in a biological sample obtained from the individual. The level of the PEAP or the endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP in the biological sample is correlated with the severity of the disease or disorder, and used to stage the disease.

A disclosed method of staging a disease involves detecting the number of CD8+ T cells, in a biological sample obtained from an individual, that are specific for a subject PEAP. In some embodiments, the number of PEAP-specific CD8+ T cells is an indication of the stage of the disease.

SUBJECTS SUITABLE FOR TREATMENT AND/OR PROPHYLAXIS
Treatment and/or prophylaxis of retroviral infection

The present disclosure contemplates methods which are suitable for treating individuals who have a retroviral infection, e.g., a lentiviral infection; uninfected individuals who are at risk of contracting a retroviral infection; individuals who were treated for a retroviral infection, but failed to respond to the treatment; and individuals who were treated for a retroviral infection, but who relapsed.

Individuals suitable for treatment with a subject method of inducing an immune response to a retrovirus-infected cell, e.g., an HIV-infected cell, include naïve individuals, e.g., individuals who are not infected with HIV.

For example, the methods of the present disclosure are suitable for treating individuals who have a human immunodeficiency virus (HIV) infection (e.g., individuals who have been diagnosed as having an HIV infection); individuals who are naïve with respect to HIV infection, but who at risk of contracting an HIV infection; and individuals who were treated for an HIV infection, but who either failed to respond to the treatment, or who initially responded to treatment but subsequently relapsed. For example, a suitable subject includes an individual who has been treated with highly active antiretroviral therapy (HAART).

Subjects suitable for treatment with a subject method include, but are not limited to, uninfected individuals with healthy, intact immune systems, but who are at greater risk for becoming HIV infected ("at-risk" individuals). At-risk individuals include, but are not limited to,
individuals who have a greater likelihood than the general population of becoming HIV infected. Individuals at risk for becoming HIV infected include, but are not limited to, individuals at risk for HIV infection due to sexual activity with HIV-infected individuals; intravenous drug users; individuals who may have been exposed to HIV-infected blood, blood products, or other HIV-contaminated body fluids; and babies who are being nursed by HIV-infected mothers. Individuals suitable for treatment include individuals infected with, or at risk of becoming infected with, HIV-1 and/or HIV-2 and/or H1V-3, or any variant thereof.

[00298] The above-described methods can be used to treat a human T cell leukemia virus I (HTLV-I) infection in an individual. Thus, a disclosed method is also suitable for treating individuals who have been infected with HTLV-I; individuals who have not yet been infected with HTLV-I, but who are at risk of becoming infected with HTLV-I; and individuals who have not yet been infected with HTLV, but who may in the future become infected with HTLV-I.

Treatment of Cancer

[00299] As discussed above, the present disclosure contemplates methods which are suitable for treating individuals who have cancer. Individuals suitable for treatment with a subject method of treating cancer include individuals having cancer wherein the cancerous state is associated with aberrant expression of an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP or increased expression of an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP, e.g., where the cancer comprises a cancer cell or a pre-cancerous cell that exhibits aberrant expression of an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP (e.g., expresses an endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP at a level that is at least about 15%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 5-fold, or at least about 10-fold, or more than 10-fold, higher than the level of the endogenous polypeptide having an amino acid sequence substantially similar to that of a subject PEAP expressed by a non-cancerous (normal) cell of the same cell type). Such cancers include, but are not limited to, myeloma, melanoma, ovarian cancer, breast cancer, and testicular cancer (including teratoma, seminoma, and embryonal carcinoma or mixed tumors composed of one or more of these types). As such, individuals suitable for treatment with the subject methods include, but are not limited to, individuals with myeloma, melanoma, ovarian cancer, breast cancer, and testicular cancer (including teratoma, seminoma, and embryonal carcinoma or mixed tumors composed of one or more of these types).
EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pi, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

EXAMPLE 1: IDENTIFICATION OF APOBEC PEPTIDE SEQUENCES WHICH ELICIT A T CELL RESPONSE IN HIV INFECTED SUBJECTS

In order to determine whether HIV infected subjects exhibit a T cell response to APOBEC self-peptides presented on the surface of cells of HIV infected subjects, peptide epitopes from the APOBEC 3F and 3G proteins were identified and tested via ELISPOT assay as described below.

**Materials/Methods**

**Immunogenicity prediction software (NetCTL1.2 (Larsen et al. (2005) European Journal of Immunology 35(8): 2295-303) was used to identify peptide epitopes from the APOBEC 3F and 3G proteins presented by HLA-A2, -B7 and -B58 superfamilies.**

Top-scoring peptides (shown in Table 1 below) were tested, in a "pool" or individually, in an interferon-gamma (IFN-γ) ELISPOT analysis of T cell responses performed on cryopreserved PBMC.

T cell reactivity to APOBEC peptides was tested in:

1) Low risk healthy volunteers N=33 ("Healthy HIV adults" in Figure 3);

2) Exposed through maternal route, but uninfected children N=7 ("children exposed uninfected" in Figure 3);

3) Long term non progressors (LTNP) N=7 ("LTNP" in Figure 3);

4) HIV-I infected adults in primary HIV-I infection N=13;
5) Chronically HIV-I infected adults (low to undetectable levels of HIV-I in the absence of any therapy ("controllers", with less than 5000 copies/ml HIV-I plasma vial load without HAART therapy) N=19 ("chronic infection - natural controllers" in Figure 3);

6) individuals who had higher levels of viremia ("non-controllers") N=21 ("chronic infection - viremics" in Figure 3);

7) HAART-treated individuals with undetectable plasma HIV-I RNA levels ("HAART suppressed") N=20 ("chronic infection - Haart suppressed" in Figure 3); and

8) HIV-I vertically infected children N=73 ("children: chronic infection" in Figure 3).

A total of 193 HIV-I negative and positive subjects were tested in this cross sectional study.

Table 1

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* = shared epitope in different HLA supertypes
# = shared epitope between APOBEC 3G and 3F  
° = shared epitope within same protein, same HLA supertype

Peptides were tested in an IFN-γ ELISPOT using cryopreserved PBMC; a positive response was considered as >50 SFU over background. Peptides were tested at a concentration of 10 µg/ml (either individually or in pools) with 100,000 PBMC per well. Spot totals for duplicate wells were averaged, and all spot numbers were normalized to numbers of IFN-γ spot-forming units (SFU) per 1x10^6 PBMC. Spot values from medium control wells were subtracted to determine responses to each polypeptide, with a minimum response value of 50 SFU/1x10^6 PBMC.
Results

A table showing patient characteristics and APOBEC polypeptide pool responses is set forth in Figure 3. 2/33 HIV-I negative low risk volunteers and 0/7 exposed uninfected children had responses to the pool of APOBEC peptides (Figure 3). 5/7 of the LTNP had responses to the APOBEC peptide pool with a mean of 486 SFU/10^6 PBMC (Figure 3). In primary HIV-I infected subjects, 5/13 had responses, with a lower mean of 84 SFU/10^6 PBMC. The cohort of chronically infected subjects had the lowest responses of all HIV-I infected people. The non controllers had the lowest mean T cell response to the APOBEC pool (34 SFU/10^6 PBMC), although there was no statistically difference compared to the HAART suppressed group (54 SFU/10^6 PBMC), or the controllers (45 SFU/10^6 PBMC). There were 13/77 responders in the group of HIV-I infected children (88 SFU/10^6 PBMC).

Specific ELISPOT results for HIV-I positive children (black triangles) and exposed uninfected children (white circles) are provided in Figure 4. The horizontal lines represent the mean SFU/10^6 PBMC for HIV-I positive children and HIV-I negative children respectively. The results of these experiments indicate that peptides derived from APOBEC 3F and 3G are immunogenic in the context of HIV-I infection.

ELISPOT results for HIV-infected children to individual APOBEC peptides from among the 13 APOBEC pool responders are shown in Figure 5.

Example 2: T cell responses against APOBEC proteins are CD8 mediated

Materials/Methods

APOBEC polypeptides were identified as indicated in Example 1 above. PBMCs from HIV-I-infected individuals were stimulated with or without the pool of twelve APOBEC peptides for six hours with anti-CD28, anti-CD49d, and brefeldin A. The cells were stained with fluorophore-conjugated antibodies to CD3, CD4, CD8, and interferon-γ to determine phenotype and function and an amine dye to discriminate between live and dead cells. Data were acquired with a LSR-II system. At least 100,000 events were collected and analyzed with FlowJo software.

Results

The results demonstrate that T cell responses against APOBEC polypeptides are CD8 mediated. In one specific example, Figure 6 shows T cell responses for an HIV-I positive child against the APOBEC peptides pool.

Additional results for both pooled and individual APOBC polypeptides are provided in Figures 7-14. Some of the data are represented graphically in Figure 15.
Figure 15 presents ELISPOT responses of peripheral PMBC from HIV-infected adults to individual APOBEC peptides. Seven HIV-infected adults were tested individually against individual APOBEC peptides. Each graph represents responses of an individual adult. Peptide sequences are shown below the bars. Each bar corresponds to the interferon gamma ELISPOT T cell response of the individual to an individual peptide. The absence of a bar indicates absence of a significant response above the "medium" control.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.
CLAIMS

What is claimed is:

1. An immunogenic composition comprising:
   a) an isolated polypeptide comprising an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide; and
   b) a pharmaceutically acceptable carrier.

2. The immunogenic composition of claim 1, wherein the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24.

3. The immunogenic composition of claim 1, wherein the isolated polypeptide has a length of about 6 to about 150 amino acids.

4. The immunogenic composition of claim 1, wherein the endogenous polypeptide is an apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, or a TRIM5 polypeptide.

5. The immunogenic composition of claim 4, wherein the endogenous polypeptide is an APOBEC polypeptide.

6. The immunogenic composition of claim 1, wherein the isolated polypeptide comprises an amino acid sequence having at least about 85% amino acid sequence identity to any one of SEQ ID NOs: 1-24.

7. The immunogenic composition of claim 1, wherein the isolated polypeptide comprises an amino acid sequence set forth in any one of SEQ ID NOs: 1-24.

8. The immunogenic composition of claim 1, wherein the composition is formulated for parenteral administration.
9. The immunogenic composition of claim 1, wherein the composition is formulated for
administration to a mucosal tissue.

10. The immunogenic composition of claim 1, further comprising an adjuvant.

11. The immunogenic composition of claim 10, wherein the adjuvant comprises aluminum
hydroxide, MF59, or monophosphoryl lipidA.

12. An immunogenic composition comprising a nucleic acid comprising a nucleotide
sequence encoding a polypeptide, wherein said polypeptide comprises an amino acid sequence having at
least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to
about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein
interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic
degradation of the endogenous polypeptide.

13. The immunogenic composition of claim 12, wherein the polypeptide does not comprise
a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24.

14. The immunogenic composition of claim 12, wherein the polypeptide has a length of
about 6 to about 150 amino acids.

15. The immunogenic composition of claim 12, wherein the endogenous polypeptide is an
apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, or a TRIM5
polypeptide.

16. The immunogenic composition of claim 15, wherein the endogenous polypeptide is an
APOBEC polypeptide.

17. The immunogenic composition of claim 12, wherein the encoded polypeptide comprises
an amino acid sequence having at least about 85% amino acid sequence identity to any one of SEQ ID

18. The immunogenic composition of claim 12, wherein the encoded polypeptide comprises
an amino acid sequence set forth in any one of SEQ ID NOs: 1-24.
19. The immunogenic composition of claim 12, wherein the composition is formulated for parenteral administration.

20. The immunogenic composition of claim 12, wherein the composition is formulated for administration to a mucosal tissue.

21. The immunogenic composition of claim 12, wherein the nucleic acid is a recombinant vector.

22. The immunogenic composition of claim 21, wherein the recombinant vector is a recombinant viral vector.

23. A method of inducing a T lymphocyte response in an individual to a host cell infected with or at risk of infection with a pathogenic virus, the method comprising administering to the individual the immunogenic composition of claim 1 or claim 12.

24. The method of claim 23, wherein the T lymphocyte response comprises a CD8+ T cell response or a CD4+ T cell response.

25. The method of claim 23, wherein the T lymphocyte response comprises a mucosal T lymphocyte response.

26. The method of claim 23, wherein the pathogenic virus is a human immunodeficiency virus.

27. The method of claim 23, wherein the individual has not been infected with the pathogenic virus.

28. The method of claim 23, wherein the individual has been infected with the pathogenic virus.

29. An isolated polypeptide comprising an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the
endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide.

30. The isolated polypeptide of claim 29, wherein the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24.

31. The isolated polypeptide of claim 29, wherein the isolated polypeptide has a length of about 6 to about 150 amino acids.

32. The isolated polypeptide of claim 29, wherein the endogenous polypeptide is an apolipoprotein B mRNA-editing catalytic (APOBEC) polypeptide, a tetherin polypeptide, a TRIM5 polypeptide.

33. The isolated polypeptide of claim 29, wherein the endogenous polypeptide is an APOBEC polypeptide.

34. The isolated polypeptide of claim 29, wherein the isolated polypeptide comprises an amino acid sequence having at least about 85% amino acid sequence identity to any one of SEQ ID NOs: 1-24.

35. The isolated polypeptide of claim 29, wherein the isolated polypeptide comprises an amino acid sequence set forth in any one of SEQ ID NOs: 1-24.

36. A composition comprising the isolated polypeptide of claim 29.

37. A method of generating a population of CD8+ T cells specific for a polypeptide, the method comprising contacting a population of unstimulated CD8+ T cells in vitro with an isolated polypeptide in association with an antigen-presenting platform, wherein said isolated polypeptide comprises an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide, and wherein said contacting provides for production of a population of CD8+ T cells specific for said synthetic polypeptide.
38. The method of claim 37, wherein the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24.

39. The method of claim 37, wherein the isolated polypeptide has a length of about 6 to about 150 amino acids.

40. A method of generating a population of CD4+ T cells specific for a polypeptide, the method comprising contacting a population of unstimulated CD4+ T cells \textit{in vitro} with an isolated polypeptide in association with an antigen-presenting platform, wherein said isolated polypeptide comprises an amino acid sequence having at least about 85% amino acid sequence identity to a contiguous stretch of from about 6 amino acids to about 60 amino acids of an endogenous polypeptide that interacts with a retroviral polypeptide, wherein interaction of the endogenous polypeptide with the retroviral polypeptide results in proteolytic degradation of the endogenous polypeptide, and wherein said contacting provides for production of a population of CD4+ T cells specific for said synthetic polypeptide.

41. The method of claim 40, wherein the isolated polypeptide does not comprise a full-length amino acid sequence as set forth in any one of SEQ ID NOs: 1-10 and 23-24.

42. The method of claim 40, wherein the isolated polypeptide has a length of about 6 to about 150 amino acids.
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<th>group</th>
<th>number of subjects</th>
<th>CD4 mean (mm$^3$)</th>
<th>Viral load mean (copies/ml)</th>
<th>number of responders against APOBEC pool</th>
<th>APOBEC pool responses mean (SFU / 10^6 PBMC$^*$)</th>
<th>% of responders</th>
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*background SFU have been subtracted

**FIG. 3**
FIG. 4
ELISPOT Responses to individual APOBEC peptides among 13 APOBEC pool responders from HIV infected children

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*Bold highlighting = values above background.

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FIG. 10 (Table 5)
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FIG. 11 (Table 6)
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**FIG. 12 (Table 7)**
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mean: 54  
median: 45

FIG. 13 (Table 8)
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**FIG. 14 (Table 9)**

mean: 33.57142857
median: 10
FIG. 15 (Cont.)