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(57) Abrégé/Abstract:
The field of the present invention relates to immunotherapeutic peptides, peptide binding agents, and their use, for example, in the immunotherapy of cancer.

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PROTEIN ANTIGENS AND USES THEREOF

CROSS REFERENCE

[0001] This application claims priority to U.S. Provisional Application No. 62/480,593, filed April 3, 2017, U.S. Provisional Application No. 62/480,596, filed April 3, 2017, and U.S. Provisional Application No. 62/480,597, filed April 3, 2017, each of which is incorporated herein by reference in its entirety.

FIELD

[0002] The field of the present invention relates to immunotherapeutic peptides, nucleic acids encoding the peptides, peptide binding agents, and their use, for example, in the immunotherapy of cancer. In one aspect, the invention provides non-mutated protein epitopes expressed in cancer cells, useful alone or in combination with other tumor-associated peptides, anti-cancer, or immunomodulatory agents to treat cancer.

BACKGROUND

[0003] Tumor vaccines are typically composed of tumor antigens and immunostimulatory molecules (e.g., adjuvants, cytokines or TLR ligands) that work together to induce antigen-specific cytotoxic T cells (CTLs) that recognize and lyse tumor cells. Such vaccines contain either shared tissue restricted tumor antigens or a mixture of shared and patient-specific antigens in the form of whole tumor cell preparations. The shared tissue restricted tumor antigens are ideally immunogenic proteins with selective expression in tumors across many individuals and are commonly delivered to patients as synthetic peptides or recombinant proteins. In contrast, whole tumor cell preparations are delivered to patients as autologous irradiated cells, cell lysates, cell fusions, heat-shock protein preparations or total mRNA. Since whole tumor cells are isolated from the autologous patient, the cells may include patient-specific tumor antigens as well as shared tumor antigens. Finally, there is a third class of tumor antigens, neoantigens, which consists of proteins with tumor-specific mutations (which can be patient-specific or shared) that result in altered amino acid sequences. Accordingly, there is still a need for developing additional cancer therapeutics.

SUMMARY

[0004] Provided herein an isolated antigenic peptide comprising an epitope from a sequence in Table 1 or 2. The present disclosure is also directed to an isolated antigenic peptide 100 amino acids or less in length which comprises an epitope from a sequence in Table 1 or 2. The present disclosure is also directed to an isolated antigenic peptide comprising an epitope from a sequence in Table 3 or 4. The present disclosure is also directed to an isolated antigenic peptide 100 amino acids or less in length which comprises an epitope from a sequence in Table 3 or 4. The present disclosure is also directed to an isolated antigenic peptide comprising an epitope from a sequence in Table 5 or 6. The

present disclosure is also directed to an isolated antigenic peptide 100 amino acids or less in length which comprises an epitope from a sequence in Table 5 or 6.

[0005] In one embodiment, the isolated antigenic peptide is a retroviral antigen. In another embodiment, the isolated antigenic peptide is a non-mutated overexpressed antigen. In another embodiment, the isolated antigenic peptide is a viral antigen.

[0006] In one embodiment, the isolated antigenic peptide is between about 5 to about 50 amino acids in length. In another embodiment, the isolated antigenic peptide is between about 15 to about 35 amino acids in length. In another embodiment, the isolated antigenic peptide is about 15 amino acids or less in length. In another embodiment, the isolated antigenic peptide is between about 8 and about 11 amino acids in length. In another embodiment, the isolated antigenic peptide is 9 or 10 amino acids in length. In one embodiment, the isolated antigenic peptide binds major histocompatibility complex (MHC) class I. In another embodiment, the isolated antigenic peptide binds MHC class I with a binding affinity of less than about 500 nM.

[0007] In one embodiment, the isolated antigenic peptide is about 30 amino acids or less in length. In another embodiment, the isolated antigenic peptide is between about 6 and about 25 amino acids in length. In another embodiment, the isolated antigenic peptide is between about 15 and about 24 amino acids in length. In another embodiment, the isolated antigenic peptide is between about 9 and about 15 amino acids in length. In one embodiment, the isolated antigenic peptide binds MHC class II. In another embodiment, the isolated antigenic peptide binds MHC class II with a binding affinity of less than about 1000 nM.

[0008] In one embodiment, the isolated antigenic peptide further comprises flanking amino acids. In another embodiment, the flanking amino acids are not native flanking amino acids. In one embodiment, the isolated antigenic peptide is linked to at least a second antigenic peptide. In another embodiment, the peptides are linked using a poly-glycine or poly-serine linker. In another embodiment, the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 1000 nM. In another embodiment, the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 500 nM. In another embodiment, both of the epitopes bind to human leukocyte antigen (HLA) -A, -B, -C, -DP, -DQ, or -DR. In another embodiment, the isolated antigenic peptide binds a class I HLA and the second antigenic peptide binds a class II HLA. In another embodiment, the isolated antigenic peptide binds a class II HLA and the second antigenic peptide binds a class I HLA.

[0009] In one embodiment, the isolated antigenic peptide further comprises modifications which increase in vivo half-life, cellular targeting, antigen uptake, antigen processing, MHC affinity, MHC

stability, or antigen presentation. In another embodiment, the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, PEGylation, polysialylation HESylation, recombinant PEG mimetics, Fc fusion, albumin fusion, nanoparticle attachment, nanoparticulate encapsulation, cholesterol fusion, iron fusion, acylation, amidation, glycosylation, side chain oxidation, phosphorylation, biotinylation, the addition of a surface active material, the addition of amino acid mimetics, or the addition of unnatural amino acids. In one embodiment, the cells that are targeted are antigen presenting cells. In another embodiment, the antigen presenting cells are dendritic cells. In another embodiment, the dendritic cells are targeted using DEC205, XCR1, CD197, CD80, CD86, CD123, CD209, CD273, CD283, CD289, CD184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, CD141, CD11c, CD83, TSLP receptor, or CD1a marker. In another embodiment, the dendritic cells are targeted using the CD141, DEC205, or XCR1 marker.

[0010] In one embodiment, provided herein is an *in vivo* delivery system comprising an isolated antigenic peptide described herein. In another embodiment, the delivery system includes cell-penetrating peptides, nanoparticulate encapsulation, virus like particles, or liposomes. In another embodiment, the cell-penetrating peptide is TAT peptide, herpes simplex virus VP22, transportan, or Antp.

[0011] In one embodiment, provided herein is a cell comprising an isolated antigenic peptide described herein. In another embodiment, the cell is an antigen presenting cell. In another embodiment, the cell is a dendritic cell.

[0012] In one embodiment, provided herein is a composition comprising an isolated antigenic peptide described herein. In another embodiment, the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated antigenic peptides comprising a tumor-specific epitope defined in Table 1 or 2. In another embodiment, the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated antigenic peptides comprising a tumor-specific epitope defined in Table 3 or 4. In another embodiment, the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26,

at least 27, at least 28, at least 29, or at least 30 of the isolated antigenic peptides comprising a tumor-specific epitope defined in Table 5 or 6. In another embodiment, the composition comprises between 2 and 20 antigenic peptides. In another embodiment, the composition further comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 additional antigenic peptides. In another embodiment, the composition comprises between about 4 and about 20 additional antigenic peptides. In another embodiment, the additional antigenic peptide is specific for an individual patient's tumor. In another embodiment, an antigenic peptide is selected by identifying differences in expression between the transcriptome or proteome of the patient's tumor sample and the transcriptome or proteome of a non-tumor sample. In another embodiment, the samples are fresh or formalin-fixed paraffin embedded tumor tissues, freshly isolated cells, or circulating tumor cells. In some embodiments, the sequences of the antigenic peptides are determined by Next Generation Sequencing.

[0013] In one embodiment, provided herein is an isolated polynucleotide encoding the isolated antigenic peptide described herein. In another embodiment, the isolated polynucleotide is RNA, optionally a self-amplifying RNA. In another embodiment, the RNA is modified to increase stability, increase cellular targeting, increase translation efficiency, adjuvanticity, cytosol accessibility, and/or decrease cytotoxicity. In another embodiment, the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, codon optimization, increased GC-content, incorporation of modified nucleosides, incorporation of 5'-cap or cap analog, and/or incorporation of an unmasked poly-A sequence.

[0014] In one embodiment, provided herein is a cell comprising a polynucleotide described herein.

[0015] In one embodiment, provided herein is a vector comprising a polynucleotide described herein. In another embodiment, the polynucleotide is operably linked to a promoter. In another embodiment, the vector is a self-amplifying RNA replicon, plasmid, phage, transposon, cosmid, virus, or virion. In another embodiment, the vector is an adeno-associated virus, herpesvirus, lentivirus, or pseudotypes thereof.

[0016] In one embodiment, provided herein is an in vivo delivery system comprising an isolated polynucleotide described herein. In another embodiment, the delivery system includes spherical nucleic acids, viruses, virus-like particles, plasmids, bacterial plasmids, or nanoparticles.

[0017] In one embodiment, provided herein is a cell comprising a vector or delivery system described herein. In another embodiment, the cell is an antigen presenting cell. In another

embodiment, the cell is a dendritic cell. In another embodiment, the cell is an immature dendritic cell.

[0018] In one embodiment, provided herein is a composition comprising at least one polynucleotide described herein. In another embodiment, the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated polynucleotides. In another embodiment, the composition comprises between about 2 and about 20 polynucleotides. In another embodiment, the composition further comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 additional antigenic polynucleotides encoding for additional antigenic peptides. In another embodiment, the composition comprises between about 4 and about 20 additional antigenic polynucleotides. In another embodiment, the isolated polynucleotides and the additional antigenic polynucleotides are linked. In another embodiment, the polynucleotides are linked using nucleic acids that encode a poly-glycine or poly-serine linker. In another embodiment, at least one of the additional antigenic peptide is specific for an individual patient's tumor. In another embodiment, an antigenic peptide is selected by identifying differences in expression between the transcriptome or proteome of the patient's tumor sample and the transcriptome or proteome of a non-tumor sample. In another embodiment, the samples are fresh or formalin-fixed paraffin embedded tumor tissues, freshly isolated cells, or circulating tumor cells. In some embodiments, the sequences of the antigenic peptides are determined by Next Generation Sequencing.

[0019] In one embodiment, provided herein is a T cell receptor (TCR) capable of binding at least one antigenic peptide described herein. In another embodiment, the TCR is capable of binding the isolated antigenic peptide in the context of MHC class I or class II.

[0020] In one embodiment, provided herein is a chimeric antigen receptor comprising: (i) a T cell activation molecule; (ii) a transmembrane region; and (iii) an antigen recognition moiety capable of binding an isolated antigenic peptide described herein. In another embodiment, CD3-zeta is the T cell activation molecule. In another embodiment, the chimeric antigen receptor further comprises at least one costimulatory signaling domain. In another embodiment, the signaling domain is CD28, 4-1BB, ICOS, OX40, ITAM, or Fc epsilon RI-gamma. In another embodiment, the antigen recognition

moiety is capable of binding the isolated antigenic peptide in the context of MHC class I or class II. In another embodiment, the chimeric antigen receptor comprises the CD3-zeta, CD28, CTLA-4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, Tim-3, A2aR, or PD-1 transmembrane region. In another embodiment, the tumor-specific epitope is located in the extracellular domain of a tumor associated polypeptide.

[0021] In one embodiment, provided herein is a T cell comprising the T cell receptor or chimeric antigen receptor described herein. In one embodiment, the T cell is a helper or cytotoxic T cell.

[0022] In one embodiment, provided herein is a nucleic acid comprising a promoter operably linked to a polynucleotide encoding a T cell receptor described herein. In another embodiment, the TCR is capable of binding the at least one antigenic peptide in the context of major histocompatibility complex (MHC) class I or class II. In one embodiment, the nucleic acid comprises a promoter operably linked to a polynucleotide encoding a chimeric antigen receptor described herein. In another embodiment, the antigen recognition moiety is capable of binding the at least one antigenic peptide in the context of major histocompatibility complex (MHC) class I or class II. In another embodiment, the tumor-specific epitope is located in the extracellular domain of a tumor associated polypeptide. In another embodiment, the nucleic acid comprises the CD3-zeta, CD28, CTLA-4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, Tim-3, A2aR, or PD-1 transmembrane region.

[0023] In one embodiment, provided herein is an antibody capable of binding at least one antigenic peptide listed in Table 1 or 2. In another embodiment, provided herein is an antibody capable of binding at least one antigenic peptide listed in Table 3 or 4. In another embodiment, provided herein is an antibody capable of binding at least one antigenic peptide listed in Table 5 or 6. In another embodiment, the at least one antigenic peptide listed in Table 1 or 2 is a retroviral antigenic peptide. In another embodiment, the at least one antigenic peptide listed in Table 3 or 4 is a non-mutated overexpressed antigenic peptide. In another embodiment, the at least one antigenic peptide listed in Table 5 or 6 is a viral antigenic peptide.

[0024] In one embodiment, provided herein is a modified cell transfected or transduced with a nucleic acid described herein. In one embodiment, the modified cell is a T cell, tumor infiltrating lymphocyte, NK-T cell, TCR-expressing cell, CD4+ T cell, CD8+ T cell, or NK cell.

[0025] In one embodiment, provided herein is a composition comprising a T cell receptor or chimeric antigen receptor described herein. In another embodiment, a composition comprises autologous patient T cells containing a T cell receptor or chimeric antigen receptor described herein. In another embodiment, the composition further comprises an immune checkpoint inhibitor. In

another embodiment, the composition further comprises at least two immune checkpoint inhibitors. In another embodiment, each of the immune checkpoint inhibitors inhibits a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof. In another embodiment, each of the immune checkpoint inhibitors interacts with a ligand of a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.

[0026] In one embodiment, the composition further comprises an immune modulator or adjuvant. In another embodiment, the immune modulator is a co-stimulatory ligand, a TNF ligand, an Ig superfamily ligand, CD28, CD80, CD86, ICOS, CD40L, OX40, CD27, GITR, CD30, DR3, CD69, or 4-1BB. In another embodiment, the immune modulator is at least one cancer cell or cancer cell extract. In another embodiment, the cancer cell is autologous to the subject in need of the composition. In another embodiment, the cancer cell has undergone lysis or been exposed to UV radiation. In another embodiment, the composition further comprises an adjuvant. In another embodiment, the adjuvant is selected from the group consisting of: Poly(I:C), Poly-ICLC, STING agonist, 1018 ISS, aluminum salts, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312 VG, Montanide ISA 206 VG, Montanide ISA 50 V2, Montanide ISA 51 VG, OK-432, OM-174, OM-197-MP-EC, ISA-TLR2 agonist, ONTAK, PepTel®. vector system, PLG microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Pam3CSK4, acrylic or methacrylic polymers, copolymers of maleic anhydride, and QS21 stimulon. In another embodiment, the adjuvant induces a humoral when administered to a subject. In another embodiment, the adjuvant induces a T helper cell type 1 when administered to a subject.

[0027] In one embodiment, provided herein is a method of inhibiting growth of a tumor cell expressing a tumor-specific epitope defined in Table 1 or 2, comprising contacting a tumor cell with a peptide, polynucleotide, delivery system, vector, composition, antibody, or cells of the invention. In another embodiment, provided herein is a method of inhibiting growth of a tumor cell expressing a tumor-specific epitope defined in Table 3 or 4, comprising contacting the tumor cell with the peptide, polynucleotide, delivery system, vector, composition, antibody, or cells of the invention. In another embodiment, provided herein is a method of inhibiting growth of a tumor cell expressing a

tumor-specific epitope defined in Table 5 or 6, comprising contacting the tumor cell with the peptide, polynucleotide, delivery system, vector, composition, antibody, or cells of the invention.

[0028] In one embodiment, provided herein is a method of treating cancer or initiating, enhancing, or prolonging an anti-tumor response in a subject in need thereof comprising administering to the subject the peptide, polynucleotide, vector, composition, antibody, or cells described herein. In one embodiment, the cancer is selected from the group consisting of CRC, head and neck, stomach, lung squamous, lung adeno., Prostate, Bladder. stomach, renal cell carcinoma, and uterine. In one embodiment, the cancer is selected from the group consisting of melanoma, lung squamous, DLBCL, uterine, head and neck, uterine, liver, and CRC. In one embodiment, the cancer is selected from the group consisting of cervical, head and neck, anal, stomach, Burkitt's lymphoma, and nasopharyngeal carcinoma.

[0029] In one embodiment, the subject is a human. In another embodiment, the subject has cancer. In another embodiment, the cancer is selected from the group consisting of urogenital, gynecological, lung, gastrointestinal , head and neck cancer, malignant glioblastoma, malignant mesothelioma, non-metastatic or metastatic breast cancer, malignant melanoma, triple-negative breast cancer (TNBC), smoldering myeloma (SMM), Merkel Cell Carcinoma or bone and soft tissue sarcomas, hematologic neoplasias, multiple myeloma, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia, non-small cell lung cancer (NSCLC), breast cancer, metastatic colorectal cancers, hormone sensitive or hormone refractory prostate cancer, colorectal cancer, ovarian cancer, hepatocellular cancer, renal cell cancer, pancreatic cancer, gastric cancer, esophageal cancers, hepatocellular cancers, cholangiocellular cancers, head and neck squamous cell cancer soft tissue sarcoma, and small cell lung cancer. In another embodiment, the subject has undergone surgical removal of the tumor. In another embodiment, the peptide, polynucleotide, vector, composition, or cells is administered via intravenous, intraperitoneal, intratumoral, intradermal, or subcutaneous administration. In another embodiment, the peptide, polynucleotide, vector, composition, or cells is administered into an anatomic site that drains into a lymph node basin. In another embodiment, the administration is into multiple lymph node basins. In another embodiment, the administration is by a subcutaneous or intradermal route.

[0030] In one embodiment of the method, a peptide is administered. In another embodiment, the administration is intratumorally. In another embodiment of the method, a polynucleotide, optionally RNA, is administered. In another embodiment, the polynucleotide is administered intravenously. In one embodiment of the method, a cell is administered. In another embodiment, the cell is a T cell or

dendritic cell. In another embodiment, the peptide or polynucleotide comprises an antigen presenting cell targeting moiety.

[0031] One embodiment of the method further comprises administering at least one immune checkpoint inhibitor to a subject. In another embodiment, the checkpoint inhibitor is a biologic therapeutic or a small molecule. In another embodiment, the checkpoint inhibitor is selected from the group consisting of a monoclonal antibody, a humanized antibody, a fully human antibody and a fusion protein or a combination thereof. In another embodiment, the checkpoint inhibitor inhibits a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof. In another embodiment, the checkpoint inhibitor interacts with a ligand of a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof. In another embodiment, two or more checkpoint inhibitors are administered. In another embodiment, the checkpoint inhibitors are: (i) ipilimumab or tremelimumab, and (ii) nivolumab. In another embodiment, the checkpoint inhibitor and the composition are administered simultaneously or sequentially in any order. In another embodiment, the peptide, polynucleotide, vector, composition, or cells is administered prior to the checkpoint inhibitor. In another embodiment, the peptide, polynucleotide, vector, composition, or cells is administered after the checkpoint inhibitor. In another embodiment, administration of the checkpoint inhibitor is continued throughout antigen peptide, polynucleotide, vector, composition, or cell therapy. In another embodiment, the antigen peptide, polynucleotide, vector, composition, or cell therapy is administered to subjects that only partially respond or do not respond to checkpoint inhibitor therapy. In another embodiment, the composition is administered intravenously or subcutaneously. In another embodiment, the checkpoint inhibitor is administered intravenously or subcutaneously. In another embodiment, the checkpoint inhibitor is administered subcutaneously within about 2 cm of the site of administration of the composition. In another embodiment, the composition is administered into the same draining lymph node as the checkpoint inhibitor.

[0032] In one embodiment of the method, an additional agent is administered. In another embodiment, the agent is a chemotherapeutic agent, an immunomodulatory drug, an immune metabolism modifying drug, a targeted therapy, radiation an anti-angiogenesis agent, or an agent that reduces immune-suppression. In another embodiment, the chemotherapeutic agent is an alkylating agent, a topoisomerase inhibitor, an anti-metabolite, or an anti-mitotic agent. In another embodiment,

the additional agent is an anti-glucocorticoid induced tumor necrosis factor family receptor (GITR) agonistic antibody or antibody fragment, ibrutinib, docetaxeol, cisplatin, or cyclophosphamide. In another embodiment, the administration elicits a CD4+ T cell immune response. In another embodiment, the administration elicits a CD4+ T cell immune response and a CD8+ T cell immune response.

[0033] In one embodiment, provided herein is a method for stimulating an immune response in a subject, comprising administering an effective amount of modified cells or composition described herein. In another embodiment, the immune response is cytotoxic and/or humoral immune response. In another embodiment, the method stimulates a T cell-mediated immune response in a subject. In another embodiment, the T cell-mediated immune response is directed against a target cell. In another embodiment, the target cell is a tumor cell. In another embodiment, the modified cells are transfected or transduced in vivo. In another embodiment, the modified cells are transfected or transduced ex vivo. In another embodiment, the modified cells are autologous patient T cells. In another embodiment, the autologous patient T cells are obtained from a patient that has received an antigen peptide or nucleic acid vaccine. In another embodiment, the antigen peptide or nucleic acid vaccine comprises at least one personalized antigen. In another embodiment, the antigen peptide or nucleic acid vaccine comprises at least one additional antigenic peptide listed in Table 1 or 2. In another embodiment, the antigen peptide or nucleic acid vaccine comprises at least one additional antigenic peptide listed in Table 3 or 4. In another embodiment, the antigen peptide or nucleic acid vaccine comprises at least one additional antigenic peptide listed in Table 5 or 6. In another embodiment, the at least one additional antigenic peptide listed in Table 1 or 2 is a retroviral antigenic peptide. In another embodiment, the at least one additional antigenic peptide listed in Table 3 or 4 is a non-mutated overexpressed antigenic peptide. In another embodiment, the at least one additional antigenic peptide listed in Table 5 or 6 is a viral antigenic peptide. In another embodiment, the patient received a chemotherapeutic agent, an immunomodulatory drug, an immune metabolism modifying drug, targeted therapy or radiation prior to and/or during receipt of the antigen peptide or nucleic acid vaccine. In another embodiment, the patient receives treatment with at least one checkpoint inhibitor. In another embodiment, the autologous T cells are obtained from a patient that has already received at least one round of T cell therapy containing an antigen. In another embodiment, the method further comprises adoptive T cell therapy. In another embodiment, the adoptive T cell therapy comprises autologous T-cells. In another embodiment, the autologous T-cells are targeted against tumor antigens. In another embodiment, the adoptive T cell therapy further comprises allogenic T-cells. In another embodiment, the allogenic T-cells are targeted against tumor

antigens. In another embodiment, the adoptive T cell therapy is administered before the checkpoint inhibitor.

[0034] In one embodiment, provided herein is a method for evaluating the efficacy of treatment comprising: (i) measuring the number or concentration of target cells in a first sample obtained from the subject before administering the modified cell, (ii) measuring the number concentration of target cells in a second sample obtained from the subject after administration of the modified cell, and (iii) determining an increase or decrease of the number or concentration of target cells in the second sample compared to the number or concentration of target cells in the first sample. In another embodiment, the treatment efficacy is determined by monitoring a clinical outcome; an increase, enhancement or prolongation of anti-tumor activity by T cells; an increase in the number of anti-tumor T cells or activated T cells as compared with the number prior to treatment; B cell activity; CD4 T cell activity; or a combination thereof. In another embodiment, the treatment efficacy is determined by monitoring a biomarker. In another embodiment, the biomarker is selected from the group consisting of CEA, Her-2/neu, bladder tumor antigen, thyroglobulin, alpha-fetoprotein, PSA, CA 125, CA19.9, CA 15.3, leptin, prolactin, osteopontin, IGF-II, CD98, fascin, sPIgR, 14-3-3 eta, troponin I, and b-type natriuretic peptide. In another embodiment, the clinical outcome is selected from the group consisting of tumor regression; tumor shrinkage; tumor necrosis; anti-tumor response by the immune system; tumor expansion, recurrence or spread; or a combination thereof. In another embodiment, the treatment effect is predicted by presence of T cells or by presence of a gene signature indicating T cell inflammation or a combination thereof.

[0035] In one embodiment, provided herein is a method of treating cancer or initiating, enhancing, or prolonging an anti-tumor response in a subject in need thereof comprising administering to the subject: (a) the peptide, polynucleotide, vector, composition, antibody, or cells described herein; and (b) at least one checkpoint inhibitor. In another embodiment, the method further comprises administration of an immunomodulator or adjuvant. In another embodiment, the immunomodulator or adjuvant is selected from the group consisting of Poly(I:C), Poly-ICLC, STING agonist, 1018 ISS, aluminum salts, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312 VG, Montanide ISA 206 VG, Montanide ISA 50 V2, Montanide ISA 51 VG, OK-432, OM-174, OM-197-MP-EC, ISA-TLR2 agonist, ONTAK, PepTel® vector system, PLG microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Pam3CSK4, acrylic or methacrylic polymers, copolymers of maleic anhydride, and QS21 stimulon. a co-stimulatory ligand, a TNF ligand, an Ig

superfamily ligand, CD28, CD80, CD86, ICOS, CD40L, OX40, CD27, GITR, CD30, DR3, CD69, or 4-1BB. In another embodiment, the immunomodulator or adjuvant is Poly-ICLC. In another embodiment, the checkpoint inhibitor is an anti-PDl antibody or antibody fragment. In another embodiment, the inhibitor of the PD-1 pathway is nivolumab. In another embodiment, the checkpoint inhibitor is an anti-CTLA4 antibody or antibody fragment. In another embodiment, the anti-CTLA4 antibody is ipilimumab or tremelimumab. In another embodiment, the method comprises administering both an anti-PD1 antibody and an anti-CTLA4 antibody. In another embodiment, the administration of the checkpoint inhibitor is initiated before initiation of administration of the peptide, polynucleotide, vector, composition, antibody, or cell. In another embodiment, the administration of the checkpoint inhibitor is initiated after initiation of administration of the peptide, polynucleotide, vector, composition, antibody, or cell. In another embodiment, the administration of the checkpoint inhibitor is initiated simultaneously with the initiation of administration of the peptide, polynucleotide, vector, composition, antibody, or cell. In another embodiment, the peptide, polynucleotide, vector, composition, antibody, or cell is administered intravenously or subcutaneously. In another embodiment, the checkpoint inhibitor is administered intravenously or subcutaneously. In another embodiment, the checkpoint inhibitor is administered subcutaneously within about 2 cm of the site of administration of the peptide, polynucleotide, vector, composition, antibody, or cell. In another embodiment, the peptide, polynucleotide, vector, composition, antibody, or cell is administered into the same draining lymph node as the checkpoint inhibitor.

[0036] In one embodiment of the therapeutic methods, the additional therapeutic agent is for example, a chemotherapeutic or biotherapeutic agent, radiation, or immunotherapy. Any suitable therapeutic treatment for a particular cancer may be administered. Examples of chemotherapeutic and biotherapeutic agents include, but are not limited to, an angiogenesis inhibitor, such as hydroxy angiostatin K 1-3, DL-a-Difluoromethyl-ornithine, endostatin, fumagillin, genistein, minocycline, staurosporine, and thalidomide; a DNA intercalator/cross-linker, such as Bleomycin, Carboplatin, Carmustine, Chlorambucil, Cyclophosphamide, cis-Diammineplatinum (II) dichloride (Cisplatin), Melphalan, Mitoxantrone, and Oxaliplatin; a DNA synthesis inhibitor, such as (±)-Amethopterin (Methotrexate), 3-Amino-1,2,4-benzotriazine 1,4-dioxide, Aminopterin, Cytosine β-D-arabinofuranoside, 5-Fluoro-5'- deoxyuridine, 5-Fluorouracil, Ganciclovir, Hydroxyurea, and Mitomycin C; a DNA-RNA transcription regulator, such as Actinomycin D, Daunorubicin, Doxorubicin, Homoharringtonine, and Idarubicin; an enzyme inhibitor, such as S(-+-)-Camptothecin, Curcumin, (-)-Deguelin, 5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside, Etoposide, Formestane, Fostriecin, Hispidin, 2-Immo-1-imidazoli-dineacetic acid (Cyclocreatine), Mevinolin, Trichostatin A,

Tyrphostin AG 34, and Tyrphostin AG 879; a gene regulator, such as 5-Aza-2'-deoxycytidine, 5-Azacytidine, Cholecalciferol (Vitamin D3), 4-Hydroxytamoxifen, Melatonin, Mifepristone, Raloxifene, all trans-Retinal (Vitamin A aldehyde), Retinoic acid all trans (Vitamin A acid), 9-cis-Retinoic Acid, 13-cis-Retinoic acid, Retinol (Vitamin A), Tamoxifen, and Troglitazone; a microtubule inhibitor, such as Colchicine, docetaxel, Dolastatin15, Nocodazole, Paclitaxel, Podophyllotoxin, Rhizoxin, Vinblastine, Vincristine, Vindesine, and Vinorelbine (Navelbine); and an unclassified therapeutic agent, such as 17-(Allylamino)-l 7-demethoxygeldanamycin, 4-Amino-l,8-naphthalimide, Apigenin, Brefeldin A, Cimetidine, Dichioromethylene-diphosphonic acid, Leuprolide (Leuprorelin), Luteinizing Hormone-Releasing Hormone, Pifithrin-a, Rapamycin, Sex hormone-binding globulin, Thapsigargin, and Urinary trypsin inhibitor fragment (Bikunin). The therapeutic agent may be altretamine, amifostine, asparaginase, capecitabine, cladribine, cisapride, cyiarahirse, dacarbazine (DT1C), dactinomycin, dronabinol, epoetin alpha, "filgrastim, fludarabine, gemcitabine, granisetron, ifosfamide, irinotecan, lansoprazole, levamisole, leucovorin, megestrol, mesna, metoclopramide, mitotane, omeprazole, ondansetron, pilocarpine, prochloroperazine, or topotecan hydrochloride. The therapeutic agent may be a monoclonal antibody such as rituximab (Rituxan®), alemtuzumab (Campath®), Bevacizumab (Avastin®), Cetuximab (Erbitux®), panitumumab (Vectibix®), and trastuzumab (Herceptin®), Vemurafenib (Zelboraf®) imatinib mesylate (Gleevec®), erlotinib (Tarceva®), gefitinib (Iressa®), Vismodegib (Erivedge™), 90Y-ibritumomab tiuxetan, 1311-tosit.umomab, ado-trastuzumab emtansine, lapatinib (Tykerb®), pertuzumab (Perjeta™), ado-trastuzumab emtansine (adcyla™), regorafenib (Stivarga®), sunitinib (Sutent®), Denosumab (Xgeva®), sorafenib (Nexavar®), pazopanib (Votrient®), axitinib (Inita®), dasatinib (Sprycel®), nilotinib (Tasigna®), bosutinib (Bosulif®), ofatumumab (Arzerra®), obinutuzumab (Gazyva™), ibrutinib (Imbruvica™), idelalisib (Zydelig®), crizotinib (Xalkori®), erlotinib (Tarceva®), afatimb dimaleate (Giiotrif®), ceritinib (LDK378/Zykadia), Tositumomab and 1311-tositumomab (Bexxar®), ibritumomab tiuxetan (Zevalin®), brentuximab vedotin (Adcetris®), bortezomib (Velcade®), siltuximab (Sylvant™), trametinib (ekinist®), dabrafenib (Tafmlar®), pembrolizimiab (Keytruda®), carfilzomib (Kyprolis®), Ramucirumab (Cyramza™), Cabozantinib (Cometriq™), vandetanib (Caprelsa®), Optionally, the therapeutic agent is a neoantigen. The therapeutic agent may be a cytokine such as interferons (INFs), interlcukins (ILs), or hematopoietic growth factors. The therapeutic agent may be INF-α, IL-2, Aldesleukin, IL-2, Erythropoietin, Granulocyte-macrophage colony-stimulating factor (GM-CSF) or granulocyte colony-stimulating factor. The therapeutic agent may be a targeted therapy such as toremifene (Fareston®), fulvestrant (Faslodex®), anastrozole (Arimidex®), exemestane (Aromasin®), letrozole (Femara®), ziv-

aflibercept (Zaltrap®), Aiitretinoin (Panretin®), temsirolimus (Torisel®), Tretinoin (Vesanoid®), denileukin diftitox (Ontak®), vorinostat (Zoiinza®), romidepsin (Istodax®), bexarotene (Targretin®), pralatrexate (Foiotyn®), lenaliomide (Revlimid®), belinostat (Beleodaq™), lenaliomide (Revlimid®), pomalidomide (Pomalyst®), Cabazitaxel (Jevtana®), enzaluiamide (Xtandi®), abiraterone acetate (Zytiga®), radium 223 chloride (Xofigo®), or everolimus (Afiniior®). Aditionally, the therapeutic agent may be an epigenetic targeted drug such as HDAC inhibitors, kinase inhibitors, DNA methyltransferase inhibitors, histone demethylase inhibitors, or histone methylation inhibitors. The epigenetic drugs may be Azacitidine (Vidaza), Decitabine (Dacogen), Vorinostat (Zoiinza), Romidepsin (Istodax), or Ruxolitinib (Jakafi). For prostate cancer treatment, a preferred chemotherapeutic agent with which anti-CTLA-4 can be combined is paclitaxel (TAXOL).

[0037] In one embodiment, provided herein is a kit comprising any antigen therapeutic described herein.

[0038] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but also each member of the group individually and all possible subgroups of the main group, and also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

DETAILED DESCRIPTION

[0039] Described herein are novel immunotherapeutic agents and uses thereof based on the discovery of non-mutated protein epitopes expressed in cancer cells. Accordingly, the invention described herein provides peptides, polynucleotides encoding the peptides, and peptide binding agents, that can be used, for example, to stimulate an immune response to a tumor associated antigen, to create an immunogenic composition or cancer vaccine for use in treating disease.

I. Definitions

[0040] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0041] "Non-mutated protein antigens" refer to antigens expressed in cancers either specifically or at a level higher than in non-cancer tissue. They include, but are not limited to, antigens of exogenous viruses, antigens of endogenous retroviruses and overexpressed antigens that do not comprise somatic mutations.

[0042] "Viral antigens" refer to antigens encoded by a exogenous virus.

[0043] "Retroviral antigens" refer to antigens encoded by an endogenous retroviral sequence.

[0044] "Non-mutated overexpressed antigens" refer to non-mutated antigens encoded by a genome of a cancer cell that are expressed at a level higher than in non-cancer tissue.

[0045] A "tumor-specific epitope" refers to an epitope that is either not expressed in non-cancer or germline cells but is found expressed in cancer cells, or that is expressed at a higher level in cancer cells than in non-cancer cells.

[0046] A "reference" can be used to correlate and compare the results obtained in the methods of the invention from a tumor specimen. Typically the "reference" may be obtained on the basis of one or more normal specimens, in particular specimens which are not affected by a cancer disease, either obtained from a patient or one or more different individuals, for example, healthy individuals, in particular individuals of the same species. A "reference" can be determined empirically by testing a sufficiently large number of normal specimens.

[0047] The term "mutation" refers to a change of or difference in the nucleic acid sequence (nucleotide substitution, addition or deletion) compared to a reference. A "somatic mutation" can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. These alterations can (but do not always) cause cancer or other diseases. In some embodiments, a mutation is a non-synonymous mutation. The term "non-synonymous mutation" refers to a mutation, for example, a nucleotide substitution, which does result in an amino acid change such as an amino acid substitution in the translation product.

[0048] Throughout this disclosure, "binding data" results can be expressed in terms of "IC_{50}." IC_{50} is the concentration of the tested peptide in a binding assay at which 50% inhibition of binding of a labeled reference peptide is observed. Given the conditions in which the assays are run (i.e., limiting HLA protein and labeled reference peptide concentrations), these values approximate K_D values. Assays for determining binding are well known in the art and are described in detail, for example, in PCT publications WO 94/20127 and WO 94/03205, and other publications such Sidney et al., Current Protocols in Immunology 18.3.1 (1998); Sidney, et al., J. Immunol. 154:247 (1995); and Sette, et al., Mol. Immunol. 31:813 (1994). Alternatively, binding can be expressed relative to binding by a reference standard peptide. For example, can be based on its IC_{50}, relative to the IC_{50} of a reference standard peptide.

[0049] Binding can also be determined using other assay systems including those using: live cells (e.g., Ceppellini et al., Nature 339:392 (1989); Christnick et al., Nature 352:67 (1991); Busch et al., Int. Immunol. 2:443 (1990); Hill et al., J. Immunol. 147:189 (1991); del Guercio et al., J. Immunol. 154:685 (1995)), cell free systems using detergent lysates (e.g., Cerundolo et al., J. Immunol.

21:2069 (1991)), immobilized purified MHC (e.g., Hill et al., J. Immunol. 152, 2890 (1994); Marshall et al., J. Immunol. 152:4946 (1994)), ELISA systems (e.g., Reay et al., EMBO J. 11:2829 (1992)), surface plasmon resonance (e.g., Khilko et al., J. Biol. Chem. 268:15425 (1993)); high flux soluble phase assays (Hammer et al., J. Exp. Med. 180:2353 (1994)), and measurement of class I MHC stabilization or assembly (e.g., Ljunggren et al., Nature 346:476 (1990); Schumacher et al., Cell 62:563 (1990); Townsend et al., Cell 62:285 (1990); Parker et al., J. Immunol. 149:1896 (1992)).

[0050] "Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

[0051] The term "derived" when used to discuss an epitope is a synonym for "prepared." A derived epitope can be isolated from a natural source, or it can be synthesized according to standard protocols in the art. Synthetic epitopes can comprise artificial amino acid residues "amino acid mimetics," such as D isomers of natural occurring L amino acid residues or non-natural amino acid residues such as cyclohexylalanine. A derived or prepared epitope can be an analog of a native epitope.

[0052] A "diluent" includes sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is also a diluent for pharmaceutical compositions. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as diluents, for example, in injectable solutions.

[0053] An "epitope" is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by, for example, an immunoglobulin, T cell receptor, HLA molecule, or chimeric antigen receptor. Alternatively, an epitope can be defined as a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins, chimeric antigen receptors, and/or Major Histocompatibility Complex (MHC) receptors. Epitopes can be prepared by isolation from a natural source, or they can be synthesized according to standard protocols in the art. Synthetic epitopes can comprise artificial amino acid residues, "amino acid mimetics," such as D isomers of naturally-occurring L amino acid residues or non-naturally-occurring amino acid residues such as cyclohexylalanine. Throughout this disclosure, epitopes may be referred to in some cases as peptides or peptide epitopes.

[0054] It is to be appreciated that proteins or peptides that comprise an epitope or an analog described herein as well as additional amino acid(s) are still within the bounds of the invention. In certain embodiments, the peptide comprises a fragment of an antigen.

[0055] In certain embodiments, there is a limitation on the length of a peptide of the invention. The embodiment that is length-limited occurs when the protein or peptide comprising an epitope described herein comprises a region (i.e., a contiguous series of amino acid residues) having 100% identity with a native sequence. In order to avoid the definition of epitope from reading, e.g., on whole natural molecules, there is a limitation on the length of any region that has 100% identity with a native peptide sequence. Thus, for a peptide comprising an epitope described herein and a region with 100% identity with a native peptide sequence, the region with 100% identity to a native sequence generally has a length of: less than or equal to 600 amino acid residues, less than or equal to 500 amino acid residues, less than or equal to 400 amino acid residues, less than or equal to 250 amino acid residues, less than or equal to 100 amino acid residues, less than or equal to 85 amino acid residues, less than or equal to 75 amino acid residues, less than or equal to 65 amino acid residues, and less than or equal to 50 amino acid residues. In certain embodiments, an "epitope" described herein is comprised by a peptide having a region with less than 51 amino acid residues that has 100% identity to a native peptide sequence, in any increment down to 5 amino acid residues; for example 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues.

[0056] "Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (see, e.g., Stites, et al., IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, Calif. (1994).

[0057] An "HLA supertype or HLA family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into such HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where "xx" denotes a particular HLA type), are synonyms.

[0058] The terms "identical" or percent "identity," in the context of two or more peptide sequences or antigen fragments, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

[0059] An "immunogenic" peptide or an "immunogenic" epitope or "peptide epitope" is a peptide that comprises an allele-specific motif such that the peptide will bind an HLA molecule and induce a cell-mediated or humoral response, for example, cytotoxic T lymphocyte (CTL), helper T

lymphocyte (HTL) and/or B lymphocyte response. Thus, immunogenic peptides described herein are capable of binding to an appropriate HLA molecule and thereafter inducing a CTL (cytotoxic) response, or a HTL (and humoral) response, to the peptide.

[0060] As used herein, a "chimeric antigen receptor" or "CAR" refers to an antigen binding protein in that includes an immunoglobulin antigen binding domain (e.g., an immunoglobulin variable domain) and a T cell receptor (TCR) constant domain. As used herein, a "constant domain" of a TCR polypeptide includes a membrane-proximal TCR constant domain, and may also include a TCR transmembrane domain and/or a TCR cytoplasmic tail. For example, in some embodiments, the CAR is a dimer that includes a first polypeptide comprising a immunoglobulin heavy chain variable domain linked to a TCR-beta constant domain and a second polypeptide comprising an immunoglobulin light chain variable domain (e.g., a κ or λ variable domain) linked to a TCRα constant domain. In some embodiments, the CAR is a dimer that includes a first polypeptide comprising a immunoglobulin heavy chain variable domain linked to a TCRα constant domain and a second polypeptide comprising an immunoglobulin light chain variable domain (e.g., a κ or λ variable domain) linked to a TCRβ constant domain.

[0061] The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides described herein do not contain some or all of the materials normally associated with the peptides in their in situ environment. An "isolated" epitope refers to an epitope that does not include the whole sequence of the antigen from which the epitope was derived. Typically the "isolated" epitope does not have attached thereto additional amino acid residues that result in a sequence that has 100% identity over the entire length of a native sequence. The native sequence can be a sequence such as a tumor-associated antigen from which the epitope is derived. Thus, the term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or peptide present in a living animal is not isolated, but the same polynucleotide or peptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such a polynucleotide could be part of a vector, and/or such a polynucleotide or peptide could be part of a composition, and still be "isolated" in that such vector or composition is not part of its natural environment. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules described herein, and further include such molecules produced synthetically.

[0062] "Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the

MHC complex is also known as the human leukocyte antigen (HLA) complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3.sup.RD ED., Raven Press, New York (1993).

[0063] A "native" or a "wild type" sequence refers to a sequence found in nature. Such a sequence can comprise a longer sequence in nature.

[0064] A "T-cell epitope" is to be understood as meaning a peptide sequence which can be bound by the MHC molecules of class I or II in the form of a peptide-presenting MHC molecule or MHC complex and then, in this form, be recognized and bound by cytotoxic T-lymphocytes or T-helper cells, respectively.

[0065] A "receptor" is to be understood as meaning a biological molecule or a molecule grouping capable of binding a ligand. A receptor may serve, to transmit information in a cell, a cell formation or an organism. The receptor comprises at least one receptor unit, for example, where each receptor unit may consist of a protein molecule. The receptor has a structure which complements that of a ligand and may complex the ligand as a binding partner. The information is transmitted in particular by conformational changes of the receptor following complexation of the ligand on the surface of a cell. In some embodiments, a receptor is to be understood as meaning in particular proteins of MHC classes I and II capable of forming a receptor/ligand complex with a ligand, in particular a peptide or peptide fragment of suitable length.

[0066] A "ligand" is to be understood as meaning a molecule which has a structure complementary to that of a receptor and is capable of forming a complex with this receptor. In some embodiments, a ligand is to be understood as meaning a peptide or peptide fragment which has a suitable length and suitable binding motifs in its amino acid sequence, so that the peptide or peptide fragment is capable of forming a complex with proteins of MHC class I or MHC class II.

[0067] In some embodiments, a "receptor/ligand complex" is also to be understood as meaning a "receptor/peptide complex" or "receptor/peptide fragment complex", including a peptide- or peptide fragment-presenting MHC molecule of class I or of class II.

[0068] "Proteins or molecules of the major histocompatibility complex (MHC)", "MHC molecules", "MHC proteins" or "HLA proteins" are to be understood as meaning proteins capable of binding peptides resulting from the proteolytic cleavage of protein antigens and representing potential lymphocyte epitopes, (e.g., T cell epitope and B cell epitope) transporting them to the cell surface and presenting them there to specific cells, in particular cytotoxic T-lymphocytes, T-helper cells, or B cells. The major histocompatibility complex in the genome comprises the genetic region whose gene products expressed on the cell surface are important for binding and presenting

endogenous and/or foreign antigens and thus for regulating immunological processes. The major histocompatibility complex is classified into two gene groups coding for different proteins, namely molecules of MHC class I and molecules of MHC class II. The cellular biology and the expression patterns of the two MHC classes are adapted to these different roles.

[0069] The terms "peptide" and "peptide epitope" are used interchangeably with "oligopeptide" in the present specification to designate a series of residues connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acid residues.

[0070] "Synthetic peptide" refers to a peptide that is obtained from a non-natural source, e.g., is man-made. Such peptides can be produced using such methods as chemical synthesis or recombinant DNA technology. "Synthetic peptides" include "fusion proteins."

[0071] A "PanDR binding" peptide, a "PanDR binding epitope" is a member of a family of molecules that binds more than one HLA class II DR molecule.

[0072] "Pharmaceutically acceptable" refers to a generally non-toxic, inert, and/or physiologically compatible composition or component of a composition.

[0073] A "pharmaceutical excipient" or "excipient" comprises a material such as an adjuvant, a carrier, pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like. A "pharmaceutical excipient" is an excipient which is pharmaceutically acceptable.

[0074] The term "motif" refers to a pattern of residues in an amino acid sequence of defined length, for example, a peptide of less than about 15 amino acid residues in length, or less than about 13 amino acid residues in length, for example, from about 8 to about 13 amino acid residues (e.g., 8, 9, 10, 11, 12, or 13) for a class I HLA motif and from about 6 to about 25 amino acid residues (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) for a class II HLA motif, which is recognized by a particular HLA molecule. Motifs are typically different for each HLA protein encoded by a given human HLA allele. These motifs differ in their pattern of the primary and secondary anchor residues. In some embodiments, an MHC class I motif identifies a peptide of 9, 10, or 11 amino acid residues in length.

[0075] A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. In some embodiments, a supermotif-bearing peptide described herein is recognized with high or intermediate affinity (as defined herein) by two or more HLA antigens.

[0076] The term "naturally occurring" as used herein refers to the fact that an object can be found in nature. For example, a peptide or nucleic acid that is present in an organism (including viruses) and can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

[0077] According to the invention, the term "vaccine" relates to a pharmaceutical preparation (pharmaceutical composition) or product that upon administration induces an immune response, for example, a cellular or humoral immune response, which recognizes and attacks a pathogen or a diseased cell such as a cancer cell. A vaccine may be used for the prevention or treatment of a disease. The term "individualized cancer vaccine" or "personalized cancer vaccine" concerns a particular cancer patient and means that a cancer vaccine is adapted to the needs or special circumstances of an individual cancer patient.

[0078] A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an pathogenic antigen (e.g., a tumor antigen), which in some way prevents or at least partially arrests disease symptoms, side effects or progression. The immune response can also include an antibody response which has been facilitated by the stimulation of helper T cells.

[0079] "Antigen processing" or "processing" refers to the degradation of a polypeptide or antigen into procession products, which are fragments of said polypeptide or antigen (e.g., the degradation of a polypeptide into peptides) and the association of one or more of these fragments (e.g., via binding) with MHC molecules for presentation by cells, for example, antigen presenting cells, to specific T cells.

[0080] "Antigen presenting cells" (APC) are cells which present peptide fragments of protein antigens in association with MHC molecules on their cell surface. Some APCs may activate antigen specific T cells. Professional antigen-presenting cells are very efficient at internalizing antigen, either by phagocytosis or by receptor-mediated endocytosis, and then displaying a fragment of the antigen, bound to a class II MHC molecule, on their membrane. The T cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the antigen presenting cell. An additional co-stimulatory signal is then produced by the antigen presenting cell, leading to activation of the T cell. The expression of co-stimulatory molecules is a defining feature of professional antigen-presenting cells.

[0081] The main types of professional antigen-presenting cells are dendritic cells, which have the broadest range of antigen presentation, and are probably the most important antigen presenting cells, macrophages, B-cells, and certain activated epithelial cells.

[0082] Dendritic cells (DCs) are leukocyte populations that present antigens captured in peripheral tissues to T cells via both MHC class II and I antigen presentation pathways. It is well known that dendritic cells are potent inducers of immune responses and the activation of these cells is a critical step for the induction of antitumoral immunity.

[0083] Dendritic cells are conveniently categorized as “immature” and “mature” cells, which can be used as a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation.

[0084] Immature dendritic cells are characterized as antigen presenting cells with a high capacity for antigen uptake and processing, which correlates with the high expression of Fey receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e. g. CD54 and CD1 1) and costimulatory molecules (e. g., CD40, CD80, CD86 and 4-1 BB).

[0085] The term “residue” refers to an amino acid residue or amino acid mimetic residue incorporated into a peptide or protein by an amide bond or amide bond mimetic, or nucleic acid (DNA or RNA) that encodes the amino acid or amino acid mimetic.

[0086] The nomenclature used to describe peptides or proteins follows the conventional practice wherein the amino group is presented to the left (the amino- or N-terminus) and the carboxyl group to the right (the carboxy- or C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the residue located at the amino terminal end of the epitope, or the peptide or protein of which it can be a part.

[0087] In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acid residues having D-forms is represented by a lower case single letter or a lower case three letter symbol. However, when three letter symbols or full names are used without capitals, they can refer to L amino acid residues. Glycine has no asymmetric carbon atom and is simply referred to as “Gly” or “G”. The amino acid sequences of peptides set forth herein are generally designated using the standard single letter symbol. (A, Alanine; C, Cysteine; D, Aspartic Acid; E, Glutamic Acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; W, Tryptophan; and Y, Tyrosine.)

[0088] The terms “polynucleotide” and “nucleic acid” are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA, for example, mRNA. The

nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. In some embodiments, the polynucleotide and nucleic acid can be in vitro transcribed mRNA. In some embodiments, the polynucleotide that is administered is mRNA.

[0089] The terms "identical" or percent "identity" in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that can be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variations thereof. In some embodiments, two nucleic acids or polypeptides described herein are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 40-60 residues, at least about 60-80 residues in length or any integral value 2between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a nucleotide sequence.

[0090] A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate peptide function are well-known in the art.

[0091] The term "vector" as used herein means a construct, which is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

[0092] A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure. In one embodiment, an "isolated polynucleotide" encompasses a PCR or quantitative PCR reaction comprising the polynucleotide amplified in the PCR or quantitative PCR reaction.

[0093] The term "substantially pure" as used herein refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0094] The term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0095] The terms "effective amount" or "therapeutically effective amount" or "therapeutic effect" refer to an amount of a therapeutic effective to "treat" a disease or disorder in a subject or mammal. The therapeutically effective amount of a drug has a therapeutic effect and as such can prevent the development of a disease or disorder; slow down the development of a disease or disorder; slow down the progression of a disease or disorder; relieve to some extent one or more of the symptoms associated with a disease or disorder; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

[0096] The terms "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0097] As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[0098] It is understood that terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention. Nothing herein is intended as a promise.

[0099] The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

II. Non-mutated protein antigens expressed in cancer cells

[0100] Applicants have discovered antigens expressed by cancer cells encoded by the following genes: ERVH-2 matrix protein, ERVH-2 gag, ERVH48-1 coat protein, ERVH48-1 syncytin, ERVE-4 reverse transcriptase, ERVK-5 gag, env, pol protein, and ERVI-1 envelope protein.

[0101] Applicants have discovered antigens expressed by cancer encoded by the following genes: TYR, MAGEC1, MAGEA10, MAGEB17, MAGEA4, MABEB16, MAGEA1, MAGEA8, MAGEB4, CT45A5, ALPPL2, MMP13, CTAG1B, DCT, CLDN6, MLANA, AFP, DKK4, ASCL2, GAGE1, GAGE10, SLC45A2, PAGE5, PAGE2, and PMEL.

[0102] Applicants have discovered antigens expressed by cancer encoded by the following genes: HPV-16, E6, HPV-16 E7, EBV LF2, EBV BALF5, EBV RPMS1, EBV A73, EBV BALF4, EBV BALF3, and EBV BARF0.

Non-mutated protein epitope polypeptides

[0103] In aspects, the invention provides isolated peptides that comprise a non-mutated protein epitope expressed in a cancer cell. In some embodiments, the non-mutated protein epitope is a retroviral antigen. In some embodiments, the non-mutated protein epitope is a non-mutated overexpressed antigen. In some embodiments, the non-mutated protein epitope is a viral antigen.

[0104] In aspects, the invention provides an isolated peptide that comprises a peptide from Tables 1-6. The term "peptide" is used in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. Similarly, the term "polypeptide" is used in the present

specification to designate a series of residues, e.g., L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. The polypeptides or peptides can be a variety of lengths, either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the biological activity of the polypeptides as herein described.

[0105] In some embodiments, sequencing methods are used to identify tumor specific epitopes. Any suitable sequencing method can be used according to the invention, for example, Next Generation Sequencing (NGS) technologies. Third Generation Sequencing methods might substitute for the NGS technology in the future to speed up the sequencing step of the method. For clarification purposes: the terms "Next Generation Sequencing" or "NGS" in the context of the present invention mean all novel high throughput sequencing technologies which, in contrast to the "conventional" sequencing methodology known as Sanger chemistry, read nucleic acid templates randomly in parallel along the entire genome by breaking the entire genome into small pieces. Such NGS technologies (also known as massively parallel sequencing technologies) are able to deliver nucleic acid sequence information of a whole genome, exome, transcriptome (all transcribed sequences of a genome) or methylome (all methylated sequences of a genome) in very short time periods, e.g. within 1-2 weeks, for example, within 1-7 days or within less than 24 hours and allow, in principle, single cell sequencing approaches. Multiple NGS platforms which are commercially available or which are mentioned in the literature can be used in the context of the invention e.g. those described in detail in WO 2012/159643.

[0106] In certain embodiments a non-mutated protein epitope peptide described herein molecule can comprise, but is not limited to, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120 or greater amino acid residues, and any range derivable therein. In specific embodiments, a non-mutated protein epitope peptide molecule is equal to or less than 100 amino acids.

[0107] In some embodiments, non-mutated protein epitope peptides and polypeptides described herein for MHC Class I are 13 residues or less in length and usually consist of between about 8 and

about 11 residues, particularly 9 or 10 residues. In some embodiments, non-mutated protein epitope peptides and polypeptides described herein for MHC Class II are 9-24 residues in length.

[0108] A longer non-mutated protein epitope peptide can be designed in several ways. In some embodiments, when HLA-binding peptides are predicted or known, a longer non-mutated protein epitope peptide could consist of (1) individual binding peptides with extensions of 2-5 amino acids toward the N- and C-terminus of each corresponding peptide; or (2) a concatenation of some or all of the binding peptides with extended sequences for each. In some embodiments, use of a longer peptide is presumed to allow for endogenous processing by patient cells and can lead to more effective antigen presentation and induction of T cell responses. In some embodiments, two or more peptides can be used, where the peptides overlap and are tiled over the long non-mutated protein epitope peptide.

[0109] In some embodiments, the non-mutated protein epitope peptides and polypeptides bind an HLA protein (e.g., HLA class I or HLA class II). In specific embodiments the non-mutated protein epitope peptide or polypeptide has an IC_{50} of at least less than 5000 nM, at least less than 500 nM, at least less than 100 nM, at least less than 50 nM or less.

[0110] In some embodiments, a non-mutated protein epitope peptide described herein can comprise carriers such as those well known in the art, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acid residues such as poly L-lysine, poly L-glutamic acid, influenza virus proteins, hepatitis B virus core protein, and the like.

[0111] In some embodiments, a non-mutated protein epitope peptide described herein can be modified by terminal-NH_2 acylation, e.g., by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some embodiments these modifications can provide sites for linking to a support or other molecule.

[0112] In some embodiments, a non-mutated protein epitope peptide described herein can contain modifications such as but not limited to glycosylation, side chain oxidation, biotinylation, phosphorylation, addition of a surface active material, e.g. a lipid, or can be chemically modified, e.g., acetylation, etc. Moreover, bonds in the peptide can be other than peptide bonds, e.g., covalent bonds, ester or ether bonds, disulfide bonds, hydrogen bonds, ionic bonds, etc.

[0113] In some embodiments, a non-mutated protein epitope peptide described herein can contain substitutions to modify a physical property (e.g., stability or solubility) of the resulting peptide. For example, non-mutated protein epitope peptides can be modified by the substitution of a cysteine (C) with α-amino butyric acid ("B"). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity.

Substituting α-amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances. Substitution of cysteine with α-amino butyric acid can occur at any residue of a non-mutated protein epitope peptide, e.g., at either anchor or non-anchor positions of an epitope or analog within a peptide, or at other positions of a peptide.

[0114] In some embodiments, a non-mutated protein epitope peptide described herein can comprise amino acid mimetics or unnatural amino acid residues, e.g. D- or L-naphylalanine; D- or L-phenylglycine; D- or L-2-thieneylalanine; D- or L-1, -2, 3-, or 4-pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoro-methyl)-phenylalanine; D-.rho.-fluorophenylalanine; D- or L-.rho.-biphenyl-phenylalanine; D- or L-ρ-methoxybiphenylphenylalanine; D- or L-2-indole(allyl)alanines; and, D- or L-alkylalanines, where the alkyl group can be a substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acid residues. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings. Modified peptides that have various amino acid mimetics or unnatural amino acid residues are particularly useful, as they tend to manifest increased stability in vivo. Such peptides can also possess improved shelf-life or manufacturing properties.

[0115] Peptide stability can be assayed in a number of ways. For instance, peptidases and various biological media, such as human plasma and serum, have been used to test stability. See, e.g., Verhoef, et al., Eur. J. Drug Metab. Pharmacokinetics 11:291 (1986). Half-life of the peptides described herein is conveniently determined using a 25% human serum (v/v) assay. The protocol is as follows: pooled human serum (Type AB, non-heat inactivated) is dilapidated by centrifugation before use. The serum is then diluted to 25% with RPMI-1640 or another suitable tissue culture medium. At predetermined time intervals, a small amount of reaction solution is removed and added to either 6% aqueous trichloroacetic acid (TCA) or ethanol. The cloudy reaction sample is cooled ($4^{o}C$) for 15 minutes and then spun to pellet the precipitated serum proteins. The presence of the peptides is then determined by reversed-phase HPLC using stability-specific chromatography conditions.

[0116] In some embodiments, a non-mutated protein epitope peptide described herein can be in solution, lyophilized, or can be in crystal form.

[0117] In some embodiments, a non-mutated protein epitope peptide described herein can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or can be isolated from natural sources such as native tumors or pathogenic organisms. Epitopes can be synthesized

individually or joined directly or indirectly in a peptide. Although a non-mutated protein epitope peptide described herein will be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptide can be synthetically conjugated to be joined to native fragments or particles.

[0118] In some embodiments, a non-mutated protein epitope peptide described herein can be prepared in a wide variety of ways. In some embodiments, the peptides can be synthesized in solution or on a solid support according to conventional techniques. Various automatic synthesizers are commercially available and can be used according to known protocols. (See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984). Further, individual peptides can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

[0119] Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes a peptide inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989). Thus, recombinant peptides, which comprise or consist of one or more epitopes described herein, can be used to present the appropriate T cell epitope.

[0120] In one aspect, the invention described herein also provides compositions comprising one, at least two, or more than two non-mutated protein epitope peptides. In some embodiments a composition described herein contains at least two distinct peptides. In some embodiments, the at least two distinct peptides are derived from the same polypeptide. By distinct polypeptides is meant that the peptide vary by length, amino acid sequence or both. The peptides are derived from any polypeptide known to or have been found to contain a tumor specific epitope.

Non-mutated protein epitope polynucleotides

[0121] Polynucleotides encoding each of the peptides described herein are also part of the invention. As appreciated by one of ordinary skill in the art, various nucleic acids will encode the same peptide due to the redundancy of the genetic code. Each of these nucleic acids falls within the scope of the present invention. This embodiment of the invention comprises DNA and RNA, for example, mRNA, and in certain embodiments a combination of DNA and RNA. In one embodiment, the mRNA is a self-amplifying mRNA. (Brito et al., Adv. Genet. 2015; 89:179-233). It is to be appreciated that any polynucleotide that encodes a peptide described herein falls within the scope of this invention.

[0122] The term "RNA" includes and in some embodiments relates to "mRNA". The term "mRNA" means "messenger-RNA" and relates to a "transcript" which is generated by using a DNA template and encodes a peptide or polypeptide. Typically, an mRNA comprises a 5'-UTR, a protein coding region, and a 3'-UTR. mRNA only possesses limited half-life in cells and in vitro. In one embodiment, the mRNA is self-amplifying mRNA. In the context of the present invention, mRNA may be generated by in vitro transcription from a DNA template. The in vitro transcription methodology is known to the skilled person. For example, there is a variety of in vitro transcription kits commercially available.

[0123] The stability and translation efficiency of RNA may be modified as required. For example, RNA may be stabilized and its translation increased by one or more modifications having a stabilizing effects and/or increasing translation efficiency of RNA. Such modifications are described, for example, in PCT/EP2006/009448 incorporated herein by reference. In order to increase expression of the RNA used according to the present invention, it may be modified within the coding region, i.e. the sequence encoding the expressed peptide or protein, without altering the sequence of the expressed peptide or protein, so as to increase the GC-content to increase mRNA stability and to perform a codon optimization and, thus, enhance translation in cells.

[0124] The term "modification" in the context of the RNA used in the present invention includes any modification of an RNA which is not naturally present in said RNA. In one embodiment of the invention, the RNA used according to the invention does not have uncapped 5'-triphosphates. Removal of such uncapped 5'-triphosphates can be achieved by treating RNA with a phosphatase. The RNA according to the invention may have modified ribonucleotides in order to increase its stability and/or decrease cytotoxicity. For example, in one embodiment, in the RNA used according to the invention 5-methylcytidine is substituted partially or completely, for example, completely, for cytidine. Alternatively or additionally, in one embodiment, in the RNA used according to the invention pseudouridine is substituted partially or completely, for example, completely, for uridine.

[0125] In one embodiment the term "modification" relates to providing an RNA with a 5'-cap or 5'- cap analog. The term "5'-cap" refers to a cap structure found on the 5'-end of an mRNA molecule and generally consists of a guanosine nucleotide connected to the mRNA via an unusual 5' to 5' triphosphate linkage. In one embodiment, this guanosine is methylated at the 7-position. The term "conventional 5'-cap" refers to a naturally occurring RNA 5'-cap, to the 7-methylguanosine cap (m G). In the context of the present invention, the term "5'-cap" includes a 5'-cap analog that resembles the RNA cap structure and is modified to possess the ability to stabilize RNA and/or enhance translation of RNA if attached thereto, in vivo and/or in a cell.

[0126] In certain embodiments, an mRNA encoding a non-mutated protein epitope is administered to a subject in need thereof. In one embodiment, the invention provides RNA, oligoribonucleotide, and polyribonucleotide molecules comprising a modified nucleoside, gene therapy vectors comprising same, gene therapy methods and gene transcription silencing methods comprising same. In one embodiment, the mRNA to be administered comprises at least one modified nucleoside.

[0127] The polynucleotides encoding peptides described herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, et al., J. Am. Chem. Soc. 103:3185 (1981). Polynucleotides encoding peptides comprising or consisting of an analog can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native epitope.

[0128] A large number of vectors and host systems suitable for producing and administering a non-mutated protein epitope peptide described herein are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pBluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); pCR (Invitrogen). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia); p75.6 (Valentis); pCEP (Invitrogen); pCEI (Epimmune). However, any other plasmid or vector can be used as long as it is replicable and viable in the host.

[0129] As representative examples of appropriate hosts, there can be mentioned: bacterial cells, such as *E. coli*, *Bacillus subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

[0130] Thus, the present disclosure is also directed to vectors, and expression vectors useful for the production and administration of the non-mutated protein epitope peptides described herein, and to host cells comprising such vectors.

[0131] Host cells are genetically engineered (transduced or transformed or transfected) with the vectors which can be, for example, a cloning vector or an expression vector. The vector can be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting

transformants or amplifying the polynucleotides. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[0132] For expression of the non-mutated protein epitope peptides described herein, the coding sequence will be provided operably linked start and stop codons, promoter and terminator regions, and in some embodiments, and a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts.

[0133] Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and in some embodiments, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

[0134] Yeast, insect or mammalian cell hosts can also be used, employing suitable vectors and control sequences. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. Such promoters can also be derived from viral sources, such as, e.g., human cytomegalovirus (CMV-IE promoter) or herpes simplex virus type-1 (HSV TK promoter). Nucleic acid sequences derived from the SV40 splice, and polyadenylation sites can be used to provide the nontranscribed genetic elements.

[0135] Polynucleotides encoding non-mutated protein epitope peptides described herein can also comprise a ubiquitination signal sequence, and/or a targeting sequence such as an endoplasmic

reticulum (ER) signal sequence to facilitate movement of the resulting peptide into the endoplasmic reticulum.

[0136] Polynucleotides described herein can be administered and expressed in human cells (e.g., immune cells, including dendritic cells). A human codon usage table can be used to guide the codon choice for each amino acid. Such polynucleotides comprise spacer amino acid residues between epitopes and/or analogs, such as those described above, or can comprise naturally-occurring flanking sequences adjacent to the epitopes and/or analogs (and/or CTL, HTL, and B cell epitopes).

[0137] In some embodiments, a non-mutated protein epitope peptide described herein can also be administered/expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. As an example of this approach, vaccinia virus is used as a vector to express nucleotide sequences that encode the non-mutated protein epitope peptides described herein. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described by Stover et al., Nature 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the non-mutated protein epitope polypeptides described herein, e.g. adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, Sendai virus vectors, poxvirus vectors, canarypox vectors, and fowlpox vectors, and the like, will be apparent to those skilled in the art from the description herein. In some embodiments, the vector is Modified Vaccinia Ankara (VA) (e.g. Bavarian Noridic (MVA-BN)).

[0138] Standard regulatory sequences well known to those of skill in the art can be included in the vector to ensure expression in the human target cells. Several vector elements are desirable: a promoter with a downstream cloning site for polynucleotide, e.g., minigene insertion; a polyadenylation signal for efficient transcription termination; an E. coli origin of replication; and an E. coli selectable marker (e.g. ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, e.g., the human cytomegalovirus (hCMV) promoter. See, e.g., U.S. Pat. Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences. In some embodiments, the promoter is the CMV-IE promoter.

[0139] Polynucleotides described herein can comprise one or more synthetic or naturally-occurring introns in the transcribed region. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells can also be considered for increasing polynucleotide expression.

[0140] In addition, a polynucleotide described herein can comprise immunostimulatory sequences (ISSs or CpGs). These sequences can be included in the vector, outside the polynucleotide coding sequence to enhance immunogenicity.

Non-mutated protein epitope binding peptides

[0141] In certain embodiments, the present invention provides a binding protein (e.g., an antibody or antigen-binding fragment thereof), or a T cell receptor (TCR), or a chimeric antigen receptor (CAR) capable of binding with a high affinity to a non-mutated protein epitope peptide:human leukocyte antigen (HLA) complex. In some embodiments, the present invention provides a CAR that is capable of binding with a high affinity to a non-mutated protein epitope peptide derived from the extracellular domain of a protein. In certain embodiments, an antigen-specific binding protein or TCR or CAR as described herein includes variant polypeptide species that have one or more amino acid substitutions, insertions, or deletions in the native amino acid sequence, provided that the binding protein retains or substantially retains its specific binding function. Conservative substitutions of amino acids are well known and may occur naturally or may be introduced when the binding protein or TCR is recombinantly produced. Amino acid substitutions, deletions, and additions may be introduced into a protein using mutagenesis methods known in the art (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, N Y, 2001). Oligonucleotide-directed site-specific (or segment specific) mutagenesis procedures may be employed to provide an altered polynucleotide that has particular codons altered according to the substitution, deletion, or insertion desired. Alternatively, random or saturation mutagenesis techniques, such as alanine scanning mutagenesis, error prone polymerase chain reaction mutagenesis, and oligonucleotide-directed mutagenesis may be used to prepare immunogen polypeptide variants (see, e.g., Sambrook et al., supra).

[0142] A variety of criteria known to persons skilled in the art indicate whether an amino acid that is substituted at a particular position in a peptide or polypeptide is conservative (or similar). For example, a similar amino acid or a conservative amino acid substitution is one in which an amino acid residue is replaced with an amino acid residue having a similar side chain. Similar amino acids may be included in the following categories: amino acids with basic side chains (e.g., lysine, arginine, histidine); amino acids with acidic side chains (e.g., aspartic acid, glutamic acid); amino acids with uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, histidine); amino acids with nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); amino acids with beta-branched side chains (e.g., threonine, valine, isoleucine), and amino acids with aromatic side chains (e.g., tyrosine,

phenylalanine, tryptophan). Proline, which is considered more difficult to classify, shares properties with amino acids that have aliphatic side chains (e.g., leucine, valine, isoleucine, and alanine) In certain circumstances, substitution of glutamine for glutamic acid or asparagine for aspartic acid may be considered a similar substitution in that glutamine and asparagine are amide derivatives of glutamic acid and aspartic acid, respectively. As understood in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide (e.g., using GENEWORKS, Align, the BLAST algorithm, or other algorithms described herein and practiced in the art).

[0143] In certain embodiments, a non-mutated protein epitope specific binding protein, TCR or CAR is capable of (a) specifically binding to an antigen:HLA complex on a cell surface independent or in the absence of CD8. In certain embodiments, a non-mutated protein epitope specific binding protein is a T cell receptor (TCR), a chimeric antigen receptor or an antigen-binding fragment of a TCR, any of which can be chimeric, humanized or human. In further embodiments, an antigen-binding fragment of the TCR comprises a single chain TCR (scTCR).

[0144] In certain embodiments, there is provided a composition comprising a non-mutated protein epitope-specific binding protein or high affinity recombinant TCR according to any one of the above embodiments and a pharmaceutically acceptable carrier, diluent, or excipient.

[0145] Methods useful for isolating and purifying recombinantly produced soluble TCR, by way of example, can include obtaining supernatants from suitable host cell/vector systems that secrete the recombinant soluble TCR into culture media and then concentrating the media using a commercially available filter. Following concentration, the concentrate can be applied to a single suitable purification matrix or to a series of suitable matrices, such as an affinity matrix or an ion exchange resin. One or more reverse phase HPLC steps may be employed to further purify a recombinant polypeptide. These purification methods can also be employed when isolating an immunogen from its natural environment. Methods for large scale production of one or more of the isolated/recombinant soluble TCR described herein include batch cell culture, which is monitored and controlled to maintain appropriate culture conditions. Purification of the soluble TCR may be performed according to methods described herein and known in the art.

III. Immunogenic and vaccine compositions

[0146] In one embodiment, provided herein is an immunogenic composition, e.g., a vaccine composition capable of raising a non-mutated protein epitope-specific response (e.g., a humoral or

cell-mediated immune response). In some embodiments, the immunogenic composition comprises non-mutated protein epitope therapeutics (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) described herein corresponding to tumor specific non-mutated protein epitope identified herein.

[0147] A person skilled in the art will be able to select non-mutated protein epitope therapeutics by testing, for example, the generation of T-cells in vitro as well as their efficiency and overall presence, the proliferation, affinity and expansion of certain T-cells for certain peptides, and the functionality of the T-cells, e.g. by analyzing the IFN-γ production or tumor killing by T-cells. The most efficient peptides can then combined as an immunogenic composition.

[0148] In one embodiment of the present invention the different non-mutated protein epitope peptides and/or polypeptides are selected so that one immunogenic composition comprises non-mutated protein epitope peptides and/or polypeptides capable of associating with different MHC molecules, such as different MHC class I molecule. In some embodiments, an immunogenic composition comprises non-mutated protein epitope peptides and/or polypeptides capable of associating with the most frequently occurring MHC class I molecules. Hence immunogenic compositions described herein comprise different peptides capable of associating with at least 2, at least 3, or at least 4 MHC class I or class II molecules.

[0149] In one embodiment, an immunogenic composition described herein is capable of raising a specific cytotoxic T-cells response, specific helper T-cell response, or a B cell response.

[0150] In some embodiments, an immunogenic composition described herein can further comprise an adjuvant and/or a carrier. Examples of useful adjuvants and carriers are given herein below. Polypeptides and/or polynucleotides in the composition can be associated with a carrier such as e.g. a protein or an antigen-presenting cell such as e.g. a dendritic cell (DC) capable of presenting the peptide to a T-cell or a B cell. In further embodiments, DC-binding peptides are used as carriers to target the non-mutated protein epitope peptides and polynucleotides encoding the non-mutated protein epitope peptides to dendritic cells (Sioud et al. FASEB J 27: 3272-3283 (2013)).

[0151] In embodiments, the non-mutated protein epitope polypeptides or polynucleotides can be provided as antigen presenting cells (e.g., dendritic cells) containing such polypeptides or polynucleotides. In other embodiments, such antigen presenting cells are used to stimulate T cells for use in patients.

[0152] In some embodiments, the antigen presenting cells are dendritic cells. In related embodiments, the dendritic cells are autologous dendritic cells that are pulsed with the non-mutated

protein epitope peptide or nucleic acid. The non-mutated protein epitope peptide can be any suitable peptide that gives rise to an appropriate T-cell response. T-cell therapy using autologous dendritic cells pulsed with peptides from a tumor associated antigen is disclosed in Murphy et al. (1996) The Prostate 29, 371-380 and Tjua et al. (1997) The Prostate 32, 272-278. In some embodiments, the T cell is a CTL. In some embodiments, the T cell is a HTL.

[0153] Thus, one embodiment of the present invention an immunogenic composition containing at least one antigen presenting cell (e.g., a dendritic cell) that is pulsed or loaded with one or more non-mutated protein epitope polypeptides or polynucleotides described herein. In embodiments, such APCs are autologous (e.g., autologous dendritic cells). Alternatively, peripheral blood mononuclear cells (PBMCs) isolated from a patient can be loaded with non-mutated protein epitope peptides or polynucleotides ex vivo. In related embodiments, such APCs or PBMCs are injected back into the patient.

[0154] The polynucleotide can be any suitable polynucleotide that is capable of transducing the dendritic cell, thus resulting in the presentation of a non-mutated protein epitope peptide and induction of immunity. In one embodiment, the polynucleotide can be naked DNA that is taken up by the cells by passive loading. In another embodiment, the polynucleotide is part of a delivery vehicle, for example, a liposome, virus like particle, plasmid, or expression vector. In another embodiment, the polynucleotide is delivered by a vector-free delivery system, for example, high performance electroporation and high-speed cell deformation). In embodiments, such antigen presenting cells (APCs) (e.g., dendritic cells) or peripheral blood mononuclear cells (PBMCs) are used to stimulate a T cell (e.g., an autologous T cell). In related embodiments, the T cell is a CTL. In other related embodiments, the T cell is an HTL. Such T cells are then injected into the patient. In some embodiments, CTL is injected into the patient. In some embodiments, HTL is injected into the patient. In some embodiments, both CTL and HTL are injected into the patient. Administration of either therapeutic can be performed simultaneously or sequentially and in any order.

[0155] The pharmaceutical compositions (e.g., immunogenic compositions) described herein for therapeutic treatment are intended for parenteral, topical, nasal, oral or local administration. In some embodiments, the pharmaceutical compositions described herein are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. In embodiments, the composition can be administered intratumorally. The compositions can be administered at the site of surgical excision to induce a local immune response to the tumor. In some embodiments, described herein are compositions for parenteral administration which comprise a solution of the non-mutated protein epitope peptides and immunogenic compositions are dissolved or suspended in an acceptable carrier,

for example, an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions can be sterilized by conventional, well known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

[0156] The concentration of non-mutated protein epitope peptides and polynucleotides described herein in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected by fluid volumes, viscosities, etc., according to the particular mode of administration selected.

[0157] The non-mutated protein epitope peptides and polynucleotides described herein can also be administered via liposomes, which target the peptides to a particular cells tissue, such as lymphoid tissue. Liposomes are also useful in increasing the half-life of the peptides. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the DEC205 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide or polynucleotide described herein can be directed to the site of lymphoid cells, where the liposomes then deliver the selected therapeutic/immunogenic polypeptide/polynucleotide compositions. Liposomes can be formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, for example, cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9; 467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728, 4,501,728, 4,837,028, and 5,019,369.

[0158] For targeting to the immune cells, a non-mutated protein epitope polypeptides or polynucleotides to be incorporated into the liposome for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide can be administered intravenously, locally, topically, etc. in a dose which varies according to, inter alia, the manner of

administration, the polypeptide or polynucleotide being delivered, and the stage of the disease being treated.

[0159] In some embodiments, non-mutated protein epitope polypeptides and polynucleotides are targeted to dendritic cells. In one embodiment, the non-mutated protein epitope polypeptides and polynucleotides are target to dendritic cells using the markers DEC205, XCR1, CD197, CD80, CD86, CD123, CD209, CD273, CD283, CD289, CD184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, TSLP receptor, or CD1a.

[0160] For solid compositions, conventional or nanoparticle nontoxic solid carriers can be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more non-mutated protein epitope polypeptides or polynucleotides described herein at a concentration of 25%-75%.

[0161] For aerosol administration, the non-mutated protein epitope polypeptides or polynucleotides can be supplied in finely divided form along with a surfactant and propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides can be employed. The surfactant can constitute 0.1%-20% by weight of the composition, or 0.25-5%. The balance of the composition can be propellant. A carrier can also be included as desired, as with, e.g., lecithin for intranasal delivery.

[0162] Additional methods for delivering the non-mutated protein epitope polynucleotides described herein are also known in the art. For instance, the nucleic acid can be delivered directly, as "naked DNA". This approach is described, for instance, in Wolff et al., Science 247: 1465-1468 (1990) as well as U.S. Pat. Nos. 5,580,859 and 5,589,466. The nucleic acids can also be administered using ballistic delivery as described, for instance, in U.S. Pat. No. 5,204,253. Particles comprised solely of DNA can be administered. Alternatively, DNA can be adhered to particles, such as gold particles.

[0163] For therapeutic or immunization purposes, mRNA encoding the non-mutated protein epitope peptides, or peptide binding agents can also be administered to the patient. In one embodiment, the mRNA is self-amplifying RNA. In a further embodiment, the self-amplifying RNA

is a part of a synthetic lipid nanoparticle formulation (Geall et al., Proc Natl Acad Sci U S A. 109: 14604–14609 (2012)).

[0164] The nucleic acids can also be delivered complexed to cationic compounds, such as cationic lipids. Lipid-mediated gene delivery methods are described, for instance, in WO 96/18372, WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682-691 (1988); U.S. Pat. No. 5,279,833; WO 91/06309; and Felgner et al., Proc. Natl. Acad. Sci. USA 84: 7413-7414 (1987).

[0165] The non-mutated protein epitope peptides and polypeptides described herein can also be expressed by attenuated viruses, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptide described herein. Upon introduction into an acutely or chronically infected host or into a noninfected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460 (1991)). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides described herein will be apparent to those skilled in the art from the description herein.

[0166] Adjuvants are any substance whose admixture into the immunogenic composition increases or otherwise modifies the immune response to the therapeutic agent. Carriers are scaffold structures, for example a polypeptide or a polysaccharide, to which a non-mutated protein epitope polypeptide or polynucleotide, is capable of being associated. Optionally, adjuvants are conjugated covalently or non-covalently to the polypeptides or polynucleotides described herein.

[0167] The ability of an adjuvant to increase the immune response to an antigen is typically manifested by a significant increase in immune-mediated reaction, or reduction in disease symptoms. For example, an increase in humoral immunity can be manifested by a significant increase in the titer of antibodies raised to the antigen, and an increase in T-cell activity can be manifested in increased cell proliferation, or cellular cytotoxicity, or cytokine secretion. An adjuvant can also alter an immune response, for example, by changing a primarily humoral or T helper 2 response into a primarily cellular, or T helper 1 response.

[0168] Suitable adjuvants are known in the art (*see*, WO 2015/095811) and include, but are not limited to poly(I:C), poly-ICLC, STING agonist, 1018 ISS, aluminum salts, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-

MP-EC, ONTAK, PepTel®. vector system, PLG microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Pam3CSK4, Aquila's QS21 stimulon (Aquila Biotech, Worcester, Mass., USA) which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and other proprietary adjuvants such as Ribi's Detox. Quil or Superfos. Adjuvants also include incomplete Freund's or GM-CSF. Several immunological adjuvants (e.g., MF59) specific for dendritic cells and their preparation have been described previously (Dupuis M, et al., Cell Immunol. 1998; 186(1):18-27; Allison A C; Dev Biol Stand. 1998; 92:3-11) (Mosca et al. Frontiers in Bioscience, 2007; 12:4050-4060) (Gamvrellis et al. Immunol & Cell Biol. 2004; 82: 506-516). Also cytokines can be used. Several cytokines have been directly linked to influencing dendritic cell migration to lymphoid tissues (e.g., TNF-alpha), accelerating the maturation of dendritic cells into efficient antigen-presenting cells for T-lymphocytes (e.g., GM-CSF, PGE1, PGE2, IL-1, IL-1b, IL-4, IL-6 and CD40L) (U.S. Pat. No. 5,849,589 incorporated herein by reference in its entirety) and acting as immunoadjuvants (e.g., IL-12) (Gabrilovich D I, et al., J Immunother Emphasis Tumor Immunol. 1996 (6):414-418).

[0169] CpG immunostimulatory oligonucleotides have also been reported to enhance the effects of adjuvants in a vaccine setting. Without being bound by theory, CpG oligonucleotides act by activating the innate (non-adaptive) immune system via Toll-like receptors (TLR), mainly TLR9. CpG triggered TLR9 activation enhances antigen-specific humoral and cellular responses to a wide variety of antigens, including peptide or protein antigens, live or killed viruses, dendritic cell vaccines, autologous cellular vaccines and polysaccharide conjugates in both prophylactic and therapeutic vaccines. Importantly, it enhances dendritic cell maturation and differentiation, resulting in enhanced activation of TH1 cells and strong cytotoxic T-lymphocyte (CTL) generation, even in the absence of CD4 T-cell help. The TH1 bias induced by TLR9 stimulation is maintained even in the presence of vaccine adjuvants such as alum or incomplete Freund's adjuvant (IFA) that normally promote a TH2 bias. CpG oligonucleotides show even greater adjuvant activity when formulated or co-administered with other adjuvants or in formulations such as microparticles, nanoparticles, lipid emulsions or similar formulations, which are especially necessary for inducing a strong response when the antigen is relatively weak. They also accelerate the immune response and enabled the antigen doses to be reduced with comparable antibody responses to the full-dose vaccine without CpG in some experiments (Arthur M. Krieg, Nature Reviews, Drug Discovery, 5, June 2006, 471-484). U.S. Pat. No. 6,406,705 B1 describes the combined use of CpG oligonucleotides, non-nucleic acid adjuvants and an antigen to induce an antigen-specific immune response. A commercially available CpG TLR9 antagonist is dSLIM (double Stem Loop Immunomodulator) by Mologen

(Berlin, GERMANY), which is a component of the pharmaceutical composition described herein. Other TLR binding molecules such as RNA binding TLR 7, TLR 8 and/or TLR 9 can also be used.

[0170] Other examples of useful adjuvants include, but are not limited to, chemically modified CpGs (e.g. CpR, Idera), Poly(I:C)(e.g. polyi:CI2U), non-CpG bacterial DNA or RNA, ssRNA40 for TLR8, as well as immunoactive small molecules and antibodies such as cyclophosphamide, sunitinib, bevacizumab, celebrex, NCX-4016, sildenafil, tadalafil, vardenafil, sorafinib, XL-999, CP-547632, pazopanib, ZD2171, AZD2171, ipilimumab, tremelimumab, and SC58175, which can act therapeutically and/or as an adjuvant. The amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan without undue experimentation. Additional adjuvants include colony-stimulating factors, such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim).

[0171] In some embodiments, an immunogenic composition according to the present invention can comprise more than one different adjuvants. Furthermore, the invention encompasses a therapeutic composition comprising any adjuvant substance including any of the above or combinations thereof. It is also contemplated that the non-mutated protein epitope therapeutic (e.g., a humoral or cell-mediated immune response). In some embodiments, the immunogenic composition comprises non-mutated protein epitope therapeutics (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) and the adjuvant can be administered separately in any appropriate sequence.

[0172] A carrier can be present independently of an adjuvant. The function of a carrier can for example be to increase the molecular weight of in particular mutant in order to increase their activity or immunogenicity, to confer stability, to increase the biological activity, or to increase serum half-life. Furthermore, a carrier can aid presenting peptides to T-cells. The carrier can be any suitable carrier known to the person skilled in the art, for example a protein or an antigen presenting cell. A carrier protein could be but is not limited to keyhole limpet hemocyanin, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin or ovalbumin, immunoglobulins, or hormones, such as insulin or palmitic acid. In one embodiment, the carrier comprises a human fibronectin type III domain (Koide et al. Methods Enzymol. 2012;503:135-56). For immunization of humans, the carrier must be a physiologically acceptable carrier acceptable to humans and safe. However, tetanus toxoid and/or diptheria toxoid are suitable carriers in one embodiment of the invention. Alternatively, the carrier can be dextrans for example sepharose.

[0173] In some embodiments, the polypeptides can be synthesized as multiply linked peptides as an alternative to coupling a polypeptide to a carrier to increase immunogenicity. Such molecules are also known as multiple antigenic peptides (MAPS).

IV. Combinations of CTL peptides and HTL peptides

[0174] Immunogenic or vaccine compositions comprising the non-mutated protein epitope polypeptides and polynucleotides described herein, or analogs thereof, which have immunostimulatory activity can be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

[0175] For instance, the ability of the non-mutated protein epitope peptides to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. In one embodiment, CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus can be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide can be linked to the T helper peptide without a spacer.

[0176] Although the CTL peptide epitope can be linked directly to the T helper peptide epitope, CTL epitope/HTL epitope conjugates can be linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus can be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. The CTL peptide epitope can be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide can be acylated.

[0177] HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their resistance to

proteases and thus extend their serum half-life. Also, the epitope peptides can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. For example, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

[0178] In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

[0179] Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (see, e.g., PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (e.g., PADRE, Epimmune, Inc., San Diego, CA) are designed to bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and a is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

[0180] In some embodiments it can be desirable to include in a non-mutated protein epitope therapeutic (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) in pharmaceutical compositions (e.g., immunogenic compositions) at least one component of which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL in vivo against viral antigens. For example, palmitic acid residues can be attached to the ε-and α- amino groups of a lysine residue and then linked, e.g., via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic non-mutated protein epitope peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. In one embodiment, a particularly effective immunogenic construct

comprises palmitic acid attached to ε- and α- amino groups of Lys, which is attached via linkage, e.g., Ser-Ser, to the amino terminus of the immunogenic peptide.

[0181] As another example of lipid priming of CTL responses, E. coli lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. (See, e.g., Deres, et al., Nature 342:561, 1989). Non-mutated protein epitope peptides described herein can be coupled to P3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P3CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

[0182] As noted herein, additional amino acids can be added to the termini of a non-mutated protein epitope peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide. However, it is to be noted that modification at the carboxyl terminus of a T cell epitope can, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH2 acylation, e.g., by alkanoyl (C1-C20) or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some instances these modifications can provide sites for linking to a support or other molecule.

[0183] An embodiment of an immunogenic composition described herein comprises *ex vivo* administration of a cocktail of epitope-bearing non-mutated protein epitope polypeptide or polynucleotides to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of dendritic cells (DCs) can be used, including GM-CSF, IL-4, IL-6, IL-1β, and TNFα. After pulsing the DCs with peptides or polynucleotides encoding the peptides, and prior to reinfusion into patients, the DC are washed to remove unbound peptides. In this embodiment, a vaccine or immunogenic composition comprises peptide-pulsed DCs which present the pulsed peptide epitopes complexed with HLA molecules on their surfaces. The composition is then administered to the patient. In other embodiments, such pulsed DCs are used to stimulate T cells suitable for use in T cell therapy.

V. Multi-epitope immunogenic compositions

[0184] A number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the non-mutated protein epitope peptides described herein are a particularly useful embodiment of the invention. In one embodiment, the nucleic acid is RNA. In some embodiments, minigene constructs encoding a non-mutated protein epitope peptide comprising one or multiple epitopes described herein are used to administer nucleic acids encoding the non-mutated protein epitope peptides described herein uses.

[0185] The use of multi-epitope minigenes is described An, L. and Whitton, J. L., J. Virol. 71:2292, 1997; Thomson, S. A. et al., J. Immunol. 157:822, 1996; Whitton, J. L. et al., J. Virol. 67:348, 1993; Hanke, R. et al., Vaccine 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing antigen peptides, a universal helper T cell epitope (or multiple tumor associated antigen HTL epitopes), and an endoplasmic reticulum-translocating signal sequence can be engineered.

[0186] The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of immune response induced against the epitopes tested. Further, the immunogenicity of DNA-encoded epitopes in vivo can be correlated with the in vitro responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a cell mediated and/or humoral response and 2.) that the induced immune cells recognized cells expressing the encoded epitopes.

[0187] For example, to create a DNA sequence encoding the selected non-mutated protein epitope (minigene) for expression in human cells, the amino acid sequences of the epitopes can be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These non-mutated protein epitope-encoding DNA sequences can be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes can be improved by including synthetic (e.g. poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

[0188] The minigene sequence can be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) can be synthesized, phosphorylated, purified and annealed under appropriate conditions using

well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

[0189] Standard regulatory sequences well known to those of skill in the art can be included in the vector to ensure expression in the target cells. For example, a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an E. coli origin of replication; and an E. coli selectable marker (e.g. ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, e.g., the human cytomegalovirus (hCMV) promoter. See, e.g., U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

[0190] Additional vector modifications can be used to optimize minigene expression and immunogenicity. In some cases, introns are utilized for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells can also be considered for increasing minigene expression.

[0191] Once an expression vector is selected, the minigene can be cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate E. coli strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, can be confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

[0192] In addition, immunomodulatory sequences appear to play a role in the immunogenicity of DNA vaccines. These sequences can be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity. In one embodiment, the sequences are immunostimulatory. In another embodiment, the sequences are ISSs or CpGs.

[0193] In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL-2, IL-12, GM-CSF), cytokine-inducing molecules (e.g., LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins. Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL

induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF-β) can be beneficial in certain diseases.

[0194] Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in E. coli, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well-known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

[0195] Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA can be used. A variety of methods have been described, and new techniques can become available. Cationic lipids can also be used in the formulation (see, e.g., as described by WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, et al., Proc. Nat'l Acad. Sci. USA 84:7413 (1987). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

[0196] In another embodiment, the nucleic acid is introduced into cells by use of high-speed cell deformation. During high-speed deformation, cells are squeezed such that temporary disruptions occur in the cell membrane, thus allowing the nucleic acid to enter the cell. Alternatively, protein can be produced from expression vectors – in a bacterial expression vector, for example, and the proteins can then be delivered to the cell.

[0197] Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct in vitro transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and

used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes can be evaluated in an analogous manner using assays to assess HTL activity.

[0198] In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (e.g., IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA). An exemplary protocol is twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ^{51}Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for in vivo induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

[0199] Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

VI. Cells

[0200] In one aspect, the present invention also provides cells expressing a non-mutated protein epitope-recognizing receptor that activates an immunoresponsive cell (e.g., T cell receptor (TCR) or chimeric antigen receptor (CAR)), and methods of using such cells for the treatment of a disease that requires an enhanced immune response.

[0201] Such cells include genetically modified immunoresponsive cells (e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTL) cells, helper T lymphocyte (HTL) cells) expressing an antigen-recognizing receptor (e.g., TCR or CAR) that binds one of the non-mutated protein epitope peptides described herein, and methods of use therefore for the treatment of neoplasia and other pathologies where an increase in an antigen-specific immune response is desired. T cell activation is mediated by a TCR or a CAR targeted to an antigen.

[0202] The present invention provides cells expressing a combination of an antigen-recognizing receptor that activates an immunoresponsive cell (e.g., TCR, CAR) and a chimeric co-stimulating receptor (CCR), and methods of using such cells for the treatment of a disease that requires an enhanced immune response. In one embodiment, tumor antigen-specific T cells, NK cells, CTL cells

or other immunoresponsive cells are used as shuttles for the selective enrichment of one or more co-stimulatory ligands for the treatment or prevention of neoplasia. Such cells are administered to a human subject in need thereof for the treatment or prevention of a particular cancer.

[0203] In one embodiment, the tumor antigen-specific human lymphocytes that can be used in the methods of the invention include, without limitation, peripheral donor lymphocytes genetically modified to express chimeric antigen receptors (CARs) (Sadelain, M., et al. 2003 Nat Rev Cancer 3:35-45), peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the a and p heterodimer (Morgan, R. A., et al. 2006 Science 314:126-129), lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies (Panelli, M. C., et al. 2000 J Immunol 164:495-504; Panelli, M. C., et al. 2000 J Immunol 164:4382-4392), and selectively in vitro-expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells (Dupont, J., et al. 2005 Cancer Res 65:5417-5427; Papanicolaou, G. A., et al. 2003 Blood 102:2498-2505). The T cells may be autologous, allogeneic, or derived in vitro from engineered progenitor or stem cells.

Co-Stimulatory Ligands

[0204] In one embodiment, the cells of the invention are provided with at least one co-stimulatory ligand which is a non-antigen specific signal important for full activation of an immune cell. Co-stimulatory ligands include, without limitation, tumor necrosis factor (TNF) ligands, cytokines (such as IL-2, IL-12, IL-15 or IL21), and immunoglobulin (Ig) superfamily ligands.

[0205] Tumor necrosis factor (TNF) is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Tumor necrosis factor (TNF) ligands share a number of common features. The majority of the ligands are synthesized as type II transmembrane proteins containing a short cytoplasmic segment and a relatively long extracellular region. TNF ligands include, without limitation, nerve growth factor (NGF), CD4OL (CD4OL)/CD154, CD137L/4-1BBL, tumor necrosis factor alpha (TNFα), CD134L/OX4OL/CD252, CD27L/CD70, Fas ligand (FasL), CD3OL/CD153, tumor necrosis factor β (TNF(3)/lymphotoxin-alpha (LTa), lymphotoxin-beta (ur(3), CD257/B cell-activating factor (BAFF)/Blys/THANK/Ta11-1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TNF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF14). The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with

immunoglobulins--they possess an immunoglobulin domain (fold). Immunoglobulin superfamily ligands include, without limitation, CD80 and CD86, both ligands for CD28.

[0206] Compositions comprising genetically modified immunoresponsive cells of the invention can be provided systemically or directly to a subject for the treatment of a neoplasia. In one embodiment, cells of the invention are directly injected into an organ of interest (e.g., an organ affected by a tumor). Alternatively, compositions comprising genetically modified immunoresponsive cells are provided indirectly to the organ of interest, for example, by administration into the circulatory system (e.g., the tumor vasculature). Expansion and differentiation agents can be provided prior to, during or after administration of the cells to increase production of T cells, NK cells, or CTL cells in vitro or in vivo.

[0207] The modified cells can be administered in any physiologically acceptable vehicle, normally intravascularly, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (e.g., thymus). Genetically modified immunoresponsive cells of the invention can comprise a purified population of cells. Those skilled in the art can readily determine the percentage of genetically modified immunoresponsive cells in a population using various well-known methods, such as fluorescence activated cell sorting (FACS). Dosages can be readily adjusted by those skilled in the art (e.g., a decrease in purity may require an increase in dosage). The cells can be introduced by injection, catheter, or the like. If desired, factors can also be included, including, but not limited to, interleukins, e.g. IL-2, IL-3, IL-6, and IL-11, as well as the other interleukins, the colony stimulating factors, such as G-, M- and GM-CSF, interferons, e.g. γ-interferon and erythropoietin.

[0208] Compositions of the invention include pharmaceutical compositions comprising genetically modified immunoresponsive cells or their progenitors and a pharmaceutically acceptable carrier. Administration can be autologous or heterologous. For example, immunoresponsive cells, or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells of the invention or their progeny (e.g., in vivo, ex vivo or in vitro derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition of the present invention (e.g., a pharmaceutical composition containing a genetically modified immunoresponsive cell), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

VII. Methods of use and pharmaceutical compositions

[0209] The non-mutated protein epitope therapeutics (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) described herein are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment of cancer. In some embodiments, the therapeutic treatment methods comprise immunotherapy. In certain embodiments, a non-mutated protein epitope peptide is useful for activating, promoting, increasing, and/or enhancing an immune response, redirecting an existing immune response to a new target, increasing the immunogenicity of a tumor, inhibiting tumor growth, reducing tumor volume, increasing tumor cell apoptosis, and/or reducing the tumorigenicity of a tumor. The methods of use can be *in vitro, ex vivo*, or *in vivo* methods.

[0210] In some aspects, the present invention provides methods for activating an immune response in a subject using a non-mutated protein epitope therapeutic described herein. In some embodiments, the invention provides methods for promoting an immune response in a subject using a non-mutated protein epitope therapeutic described herein. In some embodiments, the invention provides methods for increasing an immune response in a subject using a non-mutated protein epitope peptide described herein. In some embodiments, the invention provides methods for enhancing an immune response using a non-mutated protein epitope peptide. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing cell-mediated immunity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing T-cell activity or humoral immunity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CTL or HTL activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing T-cell activity and increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CTL activity and increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises inhibiting or decreasing the suppressive activity of Tregs. In some embodiments, the immune response is a result of antigenic stimulation. In some embodiments, the antigenic stimulation is a tumor cell. In some embodiments, the antigenic stimulation is cancer.

[0211] In some embodiments, the invention provides methods of activating, promoting, increasing, and/or enhancing of an immune response using a non-mutated protein epitope therapeutic described herein. In some embodiments, a method comprises administering to a subject in need thereof a

therapeutically effective amount of a non-mutated protein epitope therapeutic that delivers a non-mutated protein epitope polypeptide or polynucleotide to a tumor cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope therapeutic that binds the tumor associated antigen and is internalized by the tumor cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope polypeptide that is internalized by a tumor cell, and the non-mutated protein epitope peptide is processed by the cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope polypeptide that is internalized by a tumor cell, and an antigenic peptide is presented on the surface of the tumor cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope polypeptide that is internalized by the tumor cell, is processed by the cell, and an antigenic peptide is presented on the surface of the tumor cell.

[0212] In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class I molecule. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class II molecule.

[0213] In some embodiments, a method comprises contacting a tumor cell with a non-mutated protein epitope polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to the tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class I molecule. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class II molecule.

[0214] In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell, and an immune response against the tumor cell is induced. In some embodiments, the immune response against the tumor cell is increased. In some embodiments, the non-mutated protein epitope

polypeptide or polynucleotide delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell, and tumor growth is inhibited.

[0215] In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a non-mutated protein epitope polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell, and T-cell killing directed against the tumor cell is induced. In some embodiments, T-cell killing directed against the tumor cell is enhanced. In some embodiments, T-cell killing directed against the tumor cell is increased.

[0216] In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of a non-mutated protein epitope therapeutic described herein, wherein the agent is an antibody that specifically binds the non-mutated protein epitope described herein. In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of the antibody.

[0217] The present invention provides methods of redirecting an existing immune response to a tumor. In some embodiments, a method of redirecting an existing immune response to a tumor comprises administering to a subject a therapeutically effective amount of a non-mutated protein epitope therapeutic described herein. In some embodiments, the existing immune response is against a virus. In some embodiments, the virus is selected from the group consisting of: measles virus, varicella-zoster virus (VZV; chickenpox virus), influenza virus, mumps virus, poliovirus, rubella virus, rotavirus, hepatitis A virus (HAV), hepatitis B virus (HBV), Epstein Barr virus (EBV), and cytomegalovirus (CMV). In some embodiments, the virus is varicella-zoster virus. In some embodiments, the virus is cytomegalovirus. In some embodiments, the virus is measles virus. In some embodiments, the existing immune response has been acquired after a natural viral infection. In some embodiments, the existing immune response has been acquired after vaccination against a virus. In some embodiments, the existing immune response is a cell-mediated response. In some embodiments, the existing immune response comprises cytotoxic T-cells (CTLs) or HTLs.

[0218] In some embodiments, a method of redirecting an existing immune response to a tumor in a subject comprises administering a fusion protein comprising (i) an antibody that specifically binds a non-mutated protein epitope and (ii) at least one non-mutated protein epitope peptide described herein, wherein (a) the fusion protein is internalized by a tumor cell after binding to the tumor-

associated antigen; (b) the non-mutated protein epitope peptide is processed and presented on the surface of the tumor cell associated with a MHC class I molecule; and (c) the non-mutated protein epitope peptide/MHC Class I complex is recognized by cytotoxic T-cells. In some embodiments, the cytotoxic T-cells are memory T-cells. In some embodiments, the memory T-cells are the result of a vaccination with the non-mutated protein epitope peptide.

[0219] The present invention provides methods of increasing the immunogenicity of a tumor. In some embodiments, a method of increasing the immunogenicity of a tumor comprises contacting the tumor or tumor cells with an effective amount of a non-mutated protein epitope therapeutic described herein. In some embodiments, a method of increasing the immunogenicity of a tumor comprises administering to a subject a therapeutically effective amount of a non-mutated protein epitope therapeutic described herein.

[0220] The present invention also provides methods for inhibiting growth of a tumor using a non-mutated protein epitope therapeutic described herein. In certain embodiments, a method of inhibiting growth of a tumor comprises contacting a cell mixture with a non-mutated protein epitope therapeutic in vitro. For example, an immortalized cell line or a cancer cell line mixed with immune cells (e.g., T-cells) is cultured in medium to which a non-mutated protein epitope peptide is added. In some embodiments, tumor cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample, mixed with immune cells (e.g., T-cells), and cultured in medium to which an antigen therapeutic is added. In some embodiments, a non-mutated protein epitope therapeutic increases, promotes, and/or enhances the activity of the immune cells. In some embodiments, a non-mutated protein epitope therapeutic inhibits tumor cell growth. In some embodiments, a non-mutated protein epitope therapeutic activates killing of the tumor cells.

[0221] In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or the subject had a tumor which was at least partially removed.

[0222] In some embodiments, a method of inhibiting growth of a tumor comprises redirecting an existing immune response to a new target, comprising administering to a subject a therapeutically effective amount of a non-mutated protein epitope therapeutic, wherein the existing immune response is against an antigenic peptide delivered to the tumor cell by the non-mutated protein epitope peptide.

[0223] In certain embodiments, the tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the non-mutated protein epitope therapeutic. In some embodiments, a method of reducing the frequency of cancer stem cells

in a tumor in a subject, comprising administering to the subject a therapeutically effective amount of a non-mutated protein epitope therapeutic is provided.

[0224] In addition, in some aspects the invention provides a method of reducing the tumorigenicity of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of a non-mutated protein epitope therapeutic described herein. In certain embodiments, the tumor comprises cancer stem cells. In some embodiments, the tumorigenicity of a tumor is reduced by reducing the frequency of cancer stem cells in the tumor. In some embodiments, the methods comprise using the non-mutated protein epitope therapeutic described herein. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of a non-mutated protein epitope therapeutic described herein.

[0225] In some embodiments, the tumor is a solid tumor. In certain embodiments, the tumor is a tumor selected from the group consisting of: colorectal tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, neuroendocrine tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor. In certain embodiments, the tumor is a colorectal tumor. In certain embodiments, the tumor is an ovarian tumor. In some embodiments, the tumor is a breast tumor. In some embodiments, the tumor is a lung tumor. In certain embodiments, the tumor is a pancreatic tumor. In certain embodiments, the tumor is a melanoma tumor. In some embodiments, the tumor is a solid tumor.

[0226] The present invention further provides methods for treating cancer in a subject comprising administering to the subject a therapeutically effective amount of a non-mutated protein epitope therapeutic described herein.

[0227] In some embodiments, a method of treating cancer comprises redirecting an existing immune response to a new target, the method comprising administering to a subject a therapeutically effective amount of non-mutated protein epitope therapeutic, wherein the existing immune response is against an antigenic peptide delivered to the cancer cell by the non-mutated protein epitope peptide.

[0228] The present invention provides for methods of treating cancer comprising administering to a subject a therapeutically effective amount of a non-mutated protein epitope therapeutic described herein (e.g., a subject in need of treatment). In certain embodiments, the subject is a human. In certain embodiments, the subject has a cancerous tumor. In certain embodiments, the subject has had a tumor at least partially removed.

[0229] In certain embodiments, the cancer is a cancer selected from the group consisting of colorectal cancer, renal cancer, pancreatic cancer, lung cancer, ovarian cancer, liver cancer, breast

cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, neuroendocrine cancer, bladder cancer, glioblastoma, triple-negative breast cancer (TNBC), smoldering myeloma (SMM), and head and neck cancer. In certain embodiments, the cancer is pancreatic cancer. In certain embodiments, the cancer is ovarian cancer. In certain embodiments, the cancer is colorectal cancer. In certain embodiments, the cancer is breast cancer. In certain embodiments, the cancer is prostate cancer. In certain embodiments, the cancer is lung cancer. In certain embodiments, the cancer is melanoma. In some embodiments, the cancer is a solid cancer. In some embodiments, the cancer comprises a solid tumor.

[0230] In some embodiments, the cancer is a hematologic cancer. In some embodiment, the cancer is selected from the group consisting of: acute myelogenous leukemia (AML), Hodgkin lymphoma, multiple myeloma, T-cell acute lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML), non-Hodgkin lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), and cutaneous T-cell lymphoma (CTCL).

[0231] In some embodiments, the non-mutated protein epitope therapeutic is administered as a combination therapy. Combination therapy with two or more therapeutic agents uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action can result in additive or synergetic effects. Combination therapy can allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent(s). Combination therapy can decrease the likelihood that resistant cancer cells will develop. In some embodiments, combination therapy comprises a therapeutic agent that affects the immune response (e.g., enhances or activates the response) and a therapeutic agent that affects (e.g., inhibits or kills) the tumor/cancer cells.

[0232] In some embodiments, the combination of an agent described herein and at least one additional therapeutic agent results in additive or synergistic results. In some embodiments, the combination therapy results in an increase in the therapeutic index of the agent. In some embodiments, the combination therapy results in an increase in the therapeutic index of the additional therapeutic agent(s). In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the agent. In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the additional therapeutic agent(s).

[0233] In certain embodiments, in addition to administering a non-mutated protein epitope therapeutic described herein, the method or treatment further comprises administering at least one additional therapeutic agent. An additional therapeutic agent can be administered prior to,

concurrently with, and/or subsequently to, administration of the agent. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

[0234] Therapeutic agents that can be administered in combination with the non-mutated protein epitope therapeutic described herein include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the administration of an agent described herein in combination with a chemotherapeutic agent or in combination with a cocktail of chemotherapeutic agents. Treatment with an agent can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in The Chemotherapy Source Book, 4th Edition, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

[0235] Useful classes of chemotherapeutic agents include, for example, anti-tubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, anti-folates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[0236] Chemotherapeutic agents useful in the instant invention include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin,

carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel (TAXOL) and docetaxel (TAXOTERE); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine (XELODA); and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4 hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain embodiments, the additional therapeutic agent is cisplatin. In certain embodiments, the additional therapeutic agent is carboplatin.

[0237] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HCl, daunorubicin citrate, mitoxantrone HCl, actinomycin D, etoposide, topotecan HCl,

teniposide (VM-26), and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In some embodiments, the additional therapeutic agent is irinotecan.

[0238] In certain embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite utilized for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6 mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain embodiments, the additional therapeutic agent is gemcitabine.

[0239] In certain embodiments, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some embodiments, the agent is a taxane. In certain embodiments, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain embodiments, the agent is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative embodiments, the antimitotic agent comprises a vinca alkaloid, such as vincristine, vinblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some embodiments, the antimitotic agent is an inhibitor of kinesin Eg5 or an inhibitor of a mitotic kinase such as Aurora A or Plk1. In certain embodiments, the additional therapeutic agent is paclitaxel. In some embodiments, the additional therapeutic agent is albumin-bound paclitaxel.

[0240] In some embodiments, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of an agent of the present invention with a small molecule that acts as an inhibitor against tumor-associated antigens including, but not limited to, EGFR, HER2 (ErbB2), and/or VEGF. In some embodiments, an agent is administered in combination with a protein kinase inhibitor selected from the group consisting of: gefitinib (IRESSA), erlotinib (TARCEVA), sunitinib (SUTENT), lapatanib, vandetanib (ZACTIMA), AEE788, CI-1033, cediranib (RECENTIN), sorafenib (NEXAVAR), and pazopanib (GW786034B). In some embodiments, an additional therapeutic agent comprises an mTOR inhibitor. In another embodiment, the additional therapeutic agent is chemotherapy or other inhibitors that reduce the number of Treg cells. In certain embodiments, the therapeutic agent is cyclophosphamide or an anti-CTLA4 antibody. In another embodiment, the additional therapeutic reduces the presence of myeloid-derived suppressor cells. In a further embodiment, the additional therapeutic is

carbotaxol. In another embodiment, the additional therapeutic agent shifts cells to a T helper 1 response. In a further embodiment, the additional therapeutic agent is ibrutinib.

[0241] In some embodiments, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of an agent of the present invention with antibodies against tumor-associated antigens including, but not limited to, antibodies that bind EGFR, HER2/ErbB2, and/or VEGF. In certain embodiments, the additional therapeutic agent is an antibody specific for a cancer stem cell marker. In certain embodiments, the additional therapeutic agent is an antibody that is an angiogenesis inhibitor (e.g., an anti-VEGF or VEGF receptor antibody). In certain embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), ramucirumab, trastuzumab (HERCEPTIN), pertuzumab (OMNITARG), panitumumab (VECTIBIX), nimotuzumab, zalutumumab, or cetuximab (ERBITUX).

[0242] In certain embodiments, an additional therapeutic agent comprises a second immunotherapeutic agent. In some embodiments, the additional immunotherapeutic agent includes, but is not limited to, a colony stimulating factor, an interleukin, an antibody that blocks immunosuppressive functions (e.g., an anti-CTLA-4 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-TIGIT antibody), an antibody that enhances immune cell functions (e.g., an anti-GITR antibody, an anti-OX-40 antibody, an anti-CD40 antibody, or an anti-4-1BB antibody), a toll-like receptor (e.g., TLR4, TLR7, TLR9), a soluble ligand (e.g., GITRL, GITRL-Fc, OX-40L, OX-40L-Fc, CD40L, CD40L-Fc, 4-1BB ligand, or 4-1BB ligand-Fc), or a member of the B7 family (e.g., CD80, CD86). In some embodiments, the additional immunotherapeutic agent targets CTLA-4, CD28, CD3, PD-1, PD-L1, TIGIT, GITR, OX-40, CD-40, or 4-1BB.

[0243] In some embodiments, the additional therapeutic agent is an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-CD28 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, an anti-TIM3 antibody, an anti-GITR antibody, an anti-4-1BB antibody, or an anti-OX-40 antibody. In some embodiments, the additional therapeutic agent is an anti-TIGIT antibody. In some embodiments, the additional therapeutic agent is an anti-PD-1 antibody selected from the group consisting of: nivolumab (OPDIVO), pembrolizumab (KEYTRUDA), pidilzumab, MEDI0680, REGN2810, BGB-A317, and PDR001. In some embodiments, the additional therapeutic agent is an anti-PD-L1 antibody selected from the group consisting of: BMS935559 (MDX-1105), atexolizumab (MPDL3280A), durvalumab (MEDI4736), and avelumab (MSB0010718C). In some embodiments, the additional therapeutic agent is an anti-CTLA-4 antibody selected from the group consisting of:

ipilimumab (YERVOY) and tremelimumab. In some embodiments, the additional therapeutic agent is an anti-LAG-3 antibody selected from the group consisting of: BMS-986016 and LAG525. In some embodiments, the additional therapeutic agent is an anti-OX-40 antibody selected from the group consisting of: MEDI6469, MEDI0562, and MOXR0916. In some embodiments, the additional therapeutic agent is an anti-4-1BB antibody selected from the group consisting of: PF-05082566.

[0244] In some embodiments, the non-mutated protein epitope therapeutic can be administered in combination with a biologic molecule selected from the group consisting of: adrenomedullin (AM), angiopoietin (Ang), BMPs, BDNF, EGF, erythropoietin (EPO), FGF, GDNF, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor (SCF), GDF9, HGF, HDGF, IGF, migration-stimulating factor, myostatin (GDF-8), NGF, neurotrophins, PDGF, thrombopoietin, TGF-α, TGF-β, TNF-α, VEGF, PlGF, gamma-IFN, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, and IL-18.

[0245] In some embodiments, treatment with a non-mutated protein epitope therapeutic described herein can be accompanied by surgical removal of tumors, removal of cancer cells, or any other surgical therapy deemed necessary by a treating physician.

[0246] In certain embodiments, treatment involves the administration of a non-mutated protein epitope therapeutic described herein in combination with radiation therapy. Treatment with an agent can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Dosing schedules for such radiation therapy can be determined by the skilled medical practitioner.

[0247] Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously.

[0248] It will be appreciated that the combination of a non-mutated protein epitope therapeutic described herein and at least one additional therapeutic agent can be administered in any order or concurrently. In some embodiments, the agent will be administered to patients that have previously undergone treatment with a second therapeutic agent. In certain other embodiments, the non-mutated protein epitope therapeutic and a second therapeutic agent will be administered substantially simultaneously or concurrently. For example, a subject can be given an agent while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In certain embodiments, a non-mutated protein epitope therapeutic will be administered within 1 year of the treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments

can be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[0249] For the treatment of a disease, the appropriate dosage of a non-mutated protein epitope therapeutic described herein depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the agent is administered for therapeutic or preventative purposes, previous therapy, the patient's clinical history, and so on, all at the discretion of the treating physician. The non-mutated protein epitope therapeutic can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual agent. The administering physician can determine optimum dosages, dosing methodologies, and repetition rates.

[0250] In some embodiments, a non-mutated protein epitope therapeutic can be administered at an initial higher "loading" dose, followed by one or more lower doses. In some embodiments, the frequency of administration can also change. In some embodiments, a dosing regimen can comprise administering an initial dose, followed by additional doses (or "maintenance" doses) once a week, once every two weeks, once every three weeks, or once every month. For example, a dosing regimen can comprise administering an initial loading dose, followed by a weekly maintenance dose of, for example, one-half of the initial dose. Or a dosing regimen can comprise administering an initial loading dose, followed by maintenance doses of, for example one-half of the initial dose every other week. Or a dosing regimen can comprise administering three initial doses for 3 weeks, followed by maintenance doses of, for example, the same amount every other week.

[0251] As is known to those of skill in the art, administration of any therapeutic agent can lead to side effects and/or toxicities. In some cases, the side effects and/or toxicities are so severe as to preclude administration of the particular agent at a therapeutically effective dose. In some cases, therapy must be discontinued, and other agents can be tried. However, many agents in the same therapeutic class display similar side effects and/or toxicities, meaning that the patient either has to stop therapy, or if possible, suffer from the unpleasant side effects associated with the therapeutic agent.

[0252] In some embodiments, the dosing schedule can be limited to a specific number of administrations or "cycles". In some embodiments, the agent is administered for 3, 4, 5, 6, 7, 8, or more cycles. For example, the agent is administered every 2 weeks for 6 cycles, the agent is administered every 3 weeks for 6 cycles, the agent is administered every 2 weeks for 4 cycles, the

agent is administered every 3 weeks for 4 cycles, etc. Dosing schedules can be decided upon and subsequently modified by those skilled in the art.

[0253] The present invention provides methods of administering to a subject a non-mutated protein epitope therapeutic described herein comprising using an intermittent dosing strategy for administering one or more agents, which can reduce side effects and/or toxicities associated with administration of an agent, chemotherapeutic agent, etc. In some embodiments, a method for treating cancer in a human subject comprises administering to the subject a therapeutically effective dose of a non-mutated protein epitope therapeutic in combination with a therapeutically effective dose of a chemotherapeutic agent, wherein one or both of the agents are administered according to an intermittent dosing strategy. In some embodiments, a method for treating cancer in a human subject comprises administering to the subject a therapeutically effective dose of a non-mutated protein epitope therapeutic in combination with a therapeutically effective dose of a second immunotherapeutic agent, wherein one or both of the agents are administered according to an intermittent dosing strategy. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a non-mutated protein epitope therapeutic to the subject, and administering subsequent doses of the agent about once every 2 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a non-mutated protein epitope therapeutic to the subject, and administering subsequent doses of the agent about once every 3 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a non-mutated protein epitope therapeutic to the subject, and administering subsequent doses of the agent about once every 4 weeks. In some embodiments, the agent is administered using an intermittent dosing strategy and the additional therapeutic agent is administered weekly.

[0254] The present invention provides compositions comprising the non-mutated protein epitope therapeutic described herein. The present invention also provides pharmaceutical compositions comprising a non-mutated protein epitope therapeutic described herein and a pharmaceutically acceptable vehicle. In some embodiments, the pharmaceutical compositions find use in immunotherapy. In some embodiments, the compositions find use in inhibiting tumor growth. In some embodiments, the pharmaceutical compositions find use in inhibiting tumor growth in a subject (e.g., a human patient). In some embodiments, the compositions find use in treating cancer. In some embodiments, the pharmaceutical compositions find use in treating cancer in a subject (e.g., a human patient).

[0255] Formulations are prepared for storage and use by combining an antigen therapeutic of the present invention with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Those of

skill in the art generally consider pharmaceutically acceptable carriers, excipients, and/or stabilizers to be inactive ingredients of a formulation or pharmaceutical composition. Exemplary formulations are listed in WO 2015/095811.

[0256] Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (Remington: The Science and Practice of Pharmacy, 22st Edition, 2012, Pharmaceutical Press, London.). In one embodiment, the vehicle is 5% dextrose in water.

[0257] The pharmaceutical compositions described herein can be administered in any number of ways for either local or systemic treatment. Administration can be topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal; oral; or parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular).

[0258] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories.

[0259] The non-mutated protein epitope peptides described herein can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in

macroemulsions as described in Remington: The Science and Practice of Pharmacy, 22st Edition, 2012, Pharmaceutical Press, London.

[0260] In certain embodiments, pharmaceutical formulations include a non-mutated protein epitope therapeutic described herein complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes can be extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0261] In certain embodiments, sustained-release preparations comprising the non-mutated protein epitope peptides described herein can be produced. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing an agent, where the matrices are in the form of shaped articles (e.g., films or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

VIII. Kits

[0262] The non-mutated protein epitope therapeutic described herein can be provided in kit form together with instructions for administration. Typically the kit would include the desired antigen therapeutic in a container, in unit dosage form and instructions for administration. Additional therapeutics, for example, cytokines, lymphokines, checkpoint inhibitors, antibodies, can also be included in the kit. Other kit components that can also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

[0263] The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments according to the invention. All patents, patent applications, and printed publications listed herein are incorporated herein by reference in their entirety.

EXAMPLES

Example 1: Identification of mutant sequences with immunogenic potential

[0264] Applicants have discovered that the following epitopes are recurrent in cancer patients.

Table 1

ERV element (nomenclature see: ncbi.nlm.nih.gov/pmc/articles/PMC3113919/)	Translated Sequence
ERVH-2: retroviral matrix	MGNLPPSIPPSPLACVLKNLKPLQLTPDLKPKCLIFFCNTAWPQYKLDN GSKWPENGTFDFSILQDLNNSCRKMGKWSEVPDVQAFFYTSVPS
ERVH48-1: coat protein	MPNRAIRLQAVLEIITNQTASALEMLAQQQNQMRAAIYQNRLALDYLL AEEGAGCGKFNISNCCLNIGNNGEEVLEIASNIRKVARVPVQTWEGWD PANLLGGWFSNLGGFKMLVGTVIFITGVLLFLPCGIPLKLLLKLQLTS
ERVH48-1: syncytin	MACIYPTTFYTSLPTKSLNMGISLTTILILSVAVLLSTAAPPSCRECYQSL HYRGEMQQYFTYHTHIERSCYGNLIEECVESGKSYYKVKNLGVCGSRN GAICPRGKQWLCFTKIGQWGVNTQVLEDIKREQIIAKAKASKPTTPPEN RPRHFHSFIQKL
ERVH-2: gag	MARSAATLRRFTALDPKRSKGRLILNIHFITQSAPDIK
ERVE-4: reverse transcriptase	RLFLTKPGKEIGPALAQWWPKVCAEDNPPGLAVNQAPVLREVKPEAQ PVRQNQYPVPREALEGIQVHLKHLRTFGIIVPCQSPWNTPLLPVPKPGT KDYRPVQDLRLVNQATVTFHPTVPNPYTLLGLLPAKDSWFTCLDLKD AFFSIRLAPESQKLFAFQWEDPGSGVTTHYTWTRLPQGFKNFPHHLWG GTGSRPPKVSCQRPRLRVVPVHRQPPAGTPHGSRVRQRNRRPASAPGG LWV
ERVE-4: reverse transcriptase	MAVGCVKGTDALLQHLEDYGYKVSKKKAQICRQQVRYLGFTIRQREC SLGSERKQVICNLLEPKTRRQLRELLGAVGFCRLWIPNFAVLAKPLVPS YKGG
ERVE-4: protease	NADLLAAAIRGVPLKGQGNGGSRKNTQSDRPRLQRNQCAYCKETGH WKDKCPQLKEKQGGSEQKTPDKDEGALFNLAEGLLDRRGPGSRAPKE PMVRMTVGGKDIKFLVNTGAEHSVVTTPVAPLSKKAIDIIGATGVLTK QAFCLPRTCSVGGHEVIHQFLYIPDCPLPLLGRDLLSKLRAIFLYQARLF TTEVAWNRSYHGPDSSPRGRVATLPNQTRQRDRASSGPVVAKSMRRR QPSWIGSQSSSCTQGS
ERVE-4: unknown	SLFLHKTSVREVLSATIPATFLGSLTWKRGD
ERVK-5: gag, env, pol	MQNEAIEQVRAICLRAWGKIQDPGTAFPINSIRQGSKEPYPDFVARLQD AAQKSITDDNARKVIVELMAYENANPECQSAIKPLKGKVPAGVDVITE YVKACDGIGGAMHKAMLMAQAMRGLTLGGQVRTFGKKCYNCGQIG HLKRSCPVLNKQNIINQAITAKNKKPSGLCPKCGKGKHWANQCHSKFD KDGQPLSGNRKRGQPQAPQQTGAFPVQLFVPQGFQGQQPLQKIPPLQG VSQLQQSNSCPAPQQAAPQ
ERVI-1: envelope	MEWIKYSICTLNKSNCYACAHGRPEAQIVPFPLRWSSSRPSMGCMVAL FQDSTAWGNISCQALSLLYPEVQHPAGQPPRAIQLPSPNVSFISCLS

For each epitope, the full-length amino acid sequence of the non-mutated protein epitope was derived. Any constituent 9mer or 10mer not found in the germline protein sequence was flagged and scored for binding potential on six common HLA alleles (HLA-A01:01, HLA-A02:01. HLA-A03:01, HLA-A24:02, HLA-B07:02, and HLA-B08:01) using available algorithms. Any peptide scoring better than 1000 nM was nominated.

Table 2

ERV element (nomenclature see: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3113919/)	Translated Sequence
ERVH-2: retroviral matrix	MGNLPPSIPPSPLACVLKNLKPLQLTPDLKPKCLIFFCNTAWPQYKLD NGSKWPENGTFDFSILQDLNNSCRKMGKWSEVPDVQAFFYTSVPS
ERVH48-1: coat protein	MPNRAIRLQAVLEIITNQTASALEMLAQQQNQMRAAIYQNRLALDY LLAEEGAGCGKFNISNCCLNIGNNGEEVLEIASNIRKVARVPVQTWE GWDPANLLGGWFSNLGGFKMLVGTVIFITGVLLFLPCGIPLKLLLKL QLTS
ERVH48-1: syncytin	MACIYPTTFYTSLPTKSLNMGISLTTILILSVAVLLSTAAPPSCRECYQS LHYRGEMQQYFTYHTHIERSCYGNLIEECVESGKSYYKVKNLGVCG SRNGAICPRGKQWLCFTKIGQWGVNTQVLEDIKREQIIAKAKASKPT TPPENRPRHFHSFIQKL
ERVH-2: gag	MARSAATLRRFTALDPKRSKGRLILNIHFITQSAPDIK
ERVE-4: reverse transcriptase	RLFLTKPGKEIGPALAQWWPKVCAEDNPPGLAVNQAPVLREVKPEA QPVRQNQYPVPREALEGIQVHLKHLRTFGIIVPCQSPWNTPLLPVPKP GTKDYRPVQDLRLVNQATVTFHPTVPNPYTLLGLLPAKDSWFTCLD LKDAFFSIRLAPESQKLFAFQWEDPGSGVTTHYTWTRLPQGFKNFPH HLWGGTGSRPPKVSCQRPRLRVVPVHRQPPAGTPHGSRVRQRNRRP ASAPGGLWV
ERVE-4: reverse transcriptase	MAVGCVKGTDALLQHLEDYGYKVSKKKAQICRQQVRYLGFTIRQR ECSLGSERKQVICNLLEPKTRRQLRELLGAVGFCRLWIPNFAVLAKPL VPSYKGG
ERVE-4: protease	NADLLAAAIRGVPLKGQGNGGSRKNTQSDRPRLQRNQCAYCKETGH WKDKCPQLKEKQGGSEQKTPDKDEGALFNLAEGLLDRRGPGSRAPK EPMVRMTVGGKDIKFLVNTGAEHSVVTTPVAPLSKKAIDIIGATGVL TKQAFCLPRTCSVGGHEVIHQFLYIPDCPLPLLGRDLLSKLRAIFLYQ ARLFTTEVAWNRSYHGPDSSPRGRVATLPNQTRQRDRASSGPVVAK SMRRRQPSWIGSQSSSCTQGS
ERVE-4: unknown	SLFLHKTSVREVLSATIPATFLGSLTWKRGD
ERVK-5: gag, env, pol	MQNEAIEQVRAICLRAWGKIQDPGTAFPINSIRQGSKEPYPDFVARLQ DAAQKSITDDNARKVIVELMAYENANPECQSAIKPLKGKVPAGVDVI TEYVKACDGIGGAMHKAMLMAQAMRGLTLGGQVRTFGKKCYNCG QIGHLKRSCPVLNKQNIINQAITAKNKKPSGLCPKCGKGKHWANQC HSKFDKDGQPLSGNRKRGQPQAPQQTGAFPVQLFVPQGFQGQQPLQ KIPPLQGVSQLQQSNSCPAPQQAAPQ

ERVI-1: envelope	MEWIKYSICTLNKSNCYACAHGRPEAQIVPFPLRWSSSRPSMGCMVA LFQDSTAWGNISCQALSLLYPEVQHPAGQPPRAIQLPSPNVSFISCLS

Table 3

Over-expressed gene	UCSC ID	Full Sequence
TYR	uc001pcs.3	MLLAVLYCLLWSFQTSAGHFPRACVSSKNLMEKECCPPWSGDRSP CGQLSGRGSCQNILLSNAPLGPQFPFTGVDDRESWPSVFYNRTCQC SGNFMGFNCGNCKFGFWGPNCTERRLLVRRNIFDLSAPEKDKFFAY LTLAKHTISSDYVIPIGTYGQMKNGSTPMFNDINIYDLFVWMHYYV SMDALLGGSEIWRDIDFAHEAPAFLPWHRLFLLRWEQEIQKLTGDE NFTIPYWDWRDAEKCDICTDEYMGGQHPTNPNLLSPASFFSSWQIV CSRLEEYNSHQSLCNGTPEGPLRRNPGNHDKSRTPRLPSSADVEFCL SLTQYESGSMDKAANFSFRNTLEGFASPLTGIADASQSSMHNALHI YMNGTMSQVQGSANDPIFLLHHAFVDSIFEQWLRRHRPLQEVYPE ANAPIGHNRESYMVPFIPLYRNGDFFISSKDLGYDYSYLQDSDPDSF QDYIKSYLEQASRIWSWLLGAAMVGAVLTALLAGLVSLLCRHKRK QLPEEKQPLLMEKEDYHSLYQSHL
MAGEC1	uc004fbt.3	MGDKDMPTAGMPSLLQSSSESPQSCPEGEDSQSPLQIPQSSPESDDT LYPLQSPQSRSEGEDSSDPLQRPPEGKDSQSPLQIPQSSPEGDDTQSP LQNSQSSPEGKDSLSPLEISQSPPEGEDVQSPLQNPASSFFSSALLSIF QSSPESTQSPFEGFPQSVLQIPVSAASSSTLVSIFQSSPESTQSPFEGFP QSPLQIPVSRSFSSTLLSIFQSSPERTQSTFEGFAQSPLQIPVSPSSSSTL LSLFQSFSERTQSTFEGFAQSSLQIPVSPSFSSTLVSLFQSSPERTQSTF EGFPQSPLQIPVSSSSSSTLLSLFQSSPERTHSTFEGFPQSLLQIPMTSS FSSTLLSIFQSSPESAQSTFEGFPQSPLQIPGSPSFSSTLLSLFQSSPERT HSTFEGFPQSPLQIPMTSSFSSTLLSILQSSPESAQSAFEGFPQSPLQIP VSSSFSYTLLSLFQSSPERTHSTFEGFPQSPLQIPVSSSSSSSTLLSLFQ SSPECTQSTFEGFPQSPLQIPQSPPEGENTHSPLQIVPSLPEWEDSLSP HYFPQSPPQGEDSLSPHYFPQSPPQGEDSLSPHYFPQSPQGEDSLSPH YFPQSPPQGEDSMSPLYFPQSPLQGEEFQSSLQSPVSICSSSTPSSLPQ SFPESSQSPPEGPVQSPLHSPQSPPEGMHSQSPLQSPESAPEGEDSLSP LQIPQSPLEGEDSLSSLHFPQSPPEWEDSLSPLHFPQFPPQGEDFQSSL QSPVSICSSSTSLSLPQSFPESPQSPPEGPAQSPLQRPVSSFFSYTLASL LQSSHESPQSPPEGPAQSPLQSPVSSFPSSTSSSLSQSSPVSSFPSSTSS SLSKSSPESPLQSPVISFSSSTSLSPFSEESSSPVDEYTSSSDTLLESDSL TDSESLIESEPLFTYTLDEKVDELARFLLLKYQVKQPITKAEMLTNVI SRYTGYFPVIFRKAREFIEILFGISLREVDPDDSYVFVNTLDLTSEGCL SDEQGMSQNRLLILILSIIFIKGTYASEEVIWDVLSGIGVRAGREHFA FGEPRELLTKVWVQEHYLEYREVPNSSPPRYEFLWGPRAHSEVIKR KVVEFLAMLKNTVPITFPSSYKDALKDVEERAQAIIDTTDDSTATES ASSSVMSPSFSSE

Over-expressed gene	UCSC ID	Full Sequence
MAGEA10	uc022cgz.1	MPRAPKRQRCMPEEDLQSQSETQGLEGAQAPLAVEEDASSSTSTSS SFPSSFPSSSSSSSSSCYPLIPSTPEEVSADDETPNPPQSAQIACSSPSV VASLPLDQSDEGSSSQKEESPSTLQVLPDSESLPRSEIDEKVTDLVQF LLFKYQMKEPITKAEILESVIRNYEDHFPLLFSEASECMLLVFGIDVK EVDPTGHSFVLVTSLGLTYDGMLSDVQSMPKTGILILILSIVFIEGYC TPEEVIWEALNMMGLYDGMEHLIYGEPRKLLTQDWVQENYLEYR QVPGSDPARYEFLWGPRAHAEIRKMSLLKFLAKVNGSDPRSFPLW YEEALKDEEERAQDRIATTDDTTAMASASSSATGSFSYPE
MAGEB17	uc031tgu.1	MPRGQASKRRAREKRRQARGEDQCLGGAQATAAEKEKLPSSSSPA CQSPPQSFPNAGIPQESQRASYPSSPASAVSLTSSDEGAKGQKGESP NSFHGPSSSESTGRDLLNTKTGELVQFLLNKYIRKEPITREAMLKVI NRKYKQHFPEILRRSTENVEVVFGLYLKEMDPSRQSYVLVGKLDFP NQGSLSDGGGFPLSGLLMVLLSTIFMHGNRATEEEMWECLNALGM YKGRKHFIYGEPQELVTKDLVREGYLEYQQVPSSDPPRYEFLWGPR ARAETSKMKVLEFVAKLNDTVASTYKSRYEEALREEEEQARARARAV ARDSARARASRSFQP
MAGEA4	uc022cgu.1	MLPLSVGLWVPIAQLLPALLPAALTRVIMSSEQKSQHCKPEEGVEA QEEALGLVGAQAPTTEEQEAAVSSSSPLVPGTLEEVPAAESAGPPQS PQGASALPTTISFTCWRQPNEGSSSQEEEGPSTSPDAESLFREALSNK VDELAHFLLRKYRAKELVTKAEMLERVIKNYKRCFPVIFGKASESL KMIFGIDVKEVDPASNTYTLVTCLGLSYDGLLGNNQIFPKTGLLIIVL GTIAMEGDSASEEEIWEELGVMGVYDGREHTVYGEPRKLLTQDWV QENYLEYRQVPGSNPARYEFLWGPRALAETSYVKVLEHVVRVNAR VRIAYPSLREAALLEEEEGV
MABEB16	uc022bus.1	MSQDQESPRCTHDQHLQTFSETQSLEVAQVSKALEKTLLSSSHPLV PGKLKEAPAAKAESPLEVPQSFCSSSIAVTTTSSSESDEASSNQEEED SPSSSEDTSDPRNVPADALDQKVAFLVNFMLHKCQMKKPITKADM LKIIIKDDESHFSEILLRASEHLEMIFGLDVVEVDPTTHCYGLFIKLGL TYDGMLSGEKGVPKTGLLIIVLGVIFMKGNRATEEEVWEVLNLTGV YSGKKHFIFGEPRMLITKDFVKEKYLEYQQVANSDPARYEFLWGPR AKAETSKMKVLEFVAKVHGSYPHSFPSQYAEALKEEEERARARI
MAGEA1	uc022chs.1	MSLEQRSLHCKPEEALEAQQEALGLVCVQAATSSSSPLVLGTLEEV PTAGSTDPPQSPQGASAFPTTINFTRQRQPSEGSSSREEEGPSTSCILE SLFRAVITKKVADLVGFLLLKYRAREPVTKAEMLESVIKNYKHCFP EIFGKASESLQLVFGIDVKEADPTGHSYVLVTCLGLSYDGLLGDNQI MPKTGFLIIVLVMIAMEGGHAPEEEIWEELSVMEVYDGREHSAYGE PRKLLTQDLVQEKYLEYRQVPDSDPARYEFLWGPRALAETSYVKV LEYVIKVSARVRFFFPSLREAALREEEEGV
MAGEA8	uc022cgo.1	MLLGQKSQRYKAEEGLQAQGEAPGLMDVQIPTAEEQKAASSSSTLI MGTLEEVTDSGSPSPPQSPEGASSSLTVTDSTLWSQSDEGSSSNEEE GPSTSPDPAHLESLFREALDEKVAELVRFLLRKYQIKEPVTKAEMLE SVIKNYKNHFPDIFSKASECMQVIFGIDVKEVDPAGHSYILVTCLGL SYDGLLGDDQSTPKTGLLIIVLGMILMEGSRAPEEAIWEALSVMGL YDGREHSVYWKLRKLLTQEWVQENYLEYRQAPGSDPVRYEFLWG PRALAETSYVKVLEHVVRVNARVRISYPSLHEEALGEEKGV

Over-expressed gene	UCSC ID	Full Sequence
MAGEB4	uc004dcb.3	MPRGQKSKLRAREKRQRTRGQTQDLKVGQPTAAEKEESPSSSSSVLRDTASSSLAFGIPQEPQREPPTTSAAAAMSCTGSDKGDESQDEENASSSQASTSTERSLKDSLTRKTKMLVQFLLYKYKMKEPTTKAEMLKIISKKYKEHFPEIFRKVSQRTELVFGLALKEVNPTTHSYILVSMLGPNDGNQSSAWTLPRNGLLMPLLSVIFLNGNCAREEEIWEFLNMLGIYDGKRHLIFGEPRKLITQDLVQEKYLEYQQVPNSDPPRYQFLWGPRAHAETSKMKVLEFLAKVNDTTPNNFPLLYEEALRDEEERAGARPRVAARRGTTAMTSAYSRATSSSSQPM
CT45A5	uc011mvu.2	MTDKTEKVAVDPETVFKRPRECDSPSYQKRQRMALLARKQGAGDSLIAGSAMSKEKKLMTGHAIPPSQLDSQIDDFTGFSKDGMMQKPGSNAPVGGNVTSNFSGDDLECRGIASSPKSQQEINADIKCQVVKEIRCLGRKYEKIFEMLEGVQGPTAVRKRFFESIIKEAARCMRRDFVKHLKKKLKRMI
ALPPL2	uc002vss.4	MQGPWVLLLLGLRLQLSLGIIPVEEENPDFWNRQAAEALGAAKKLQPAQTAAKNLIIFLGDGMGVSTVTAARILKGQKKDKLGPETFLAMDRFPYVALSKTYSVDKHVPDSGATATAYLCGVKGNFQTIGLSAAARFNQCNTTRGNEVISVMNRAKKAGKSVGVVTTTRVQHASPAGAYAHTVNRNWYSDADVPASARQEGCQDIATQLISNMDIDVILGGGRKYMFPMGTPDPEYPDDYSQGGTRLDGKNLVQEWLAKHQGARYVWNRTELLQASLDPSVTHLMGLFEPGDMKYEIHRDSTLDPSLMEMTEAALLLLSRNPRGFFLFVEGGRIDHGHHESRAYRALTETIMFDDAIERAGQLTSEEDTLSLVTADHSHVFSFGGYPLRGSSIFGLAPGKARDRKAYTVLLYGNGPGYVLKDGARPDVTESESGSPEYRQQSAVPLDGETHAGEDVAVFARGPQAHLVHGVQEQTFIAHVMAFAACLEPYTACDLAPRAGTTDAAHPGPSVVPALLPLLAGTLLLLGTATAP
MMP13	uc001phl.3	MHPGVLAAFLFLSWTHCRALPLPSGGDEDDLSEEDLQFAERYLRSYYHPTNLAGILKENAASSMTERLREMQSFFGLEVTGKLDDNTLDVMKKPRCGVPDVGEYNVFPRTLKWSKMNLTYRIVNYTPDMTHSEVEKAFKKAFKVWSDVTPLNFTRLHDGIADIMISFGIKEHGDFYPFDGPSGLLAHAFPPGPNYGGDAHFDDDETWTSSSKGYNLFLVAAHEFGHSLGLDHSKDPGALMFPIYTYTGKSHFMLPDDDVQGIQSLYGPGDEDPNPKHPKTPDKCDPSLSLDAITSLRGETMIFKDRFFWRLHPQQVDAELFLTKSFWPELPNRIDAAYEHPSHDLIFIFRGRKFWALNGYDILEGYPKKISELGLPKEVKKISAAVHFEDTGKTLLFSGNQVWRYDDTNHIMDKDYPRLIEEDFPGIGDKVDAVYEKNGYIYFFNGPIQFEYSIWSNRIVRVMPANSILWC
CTAG1B	uc004fmf.1	MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGAPRGPHGGAASGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR

Over-expressed gene	UCSC ID	Full Sequence
DCT	uc010afh.3	MSPLWWGFLLSCLGCKILPGAQGQFPRVCMTVDSLVNKECCPRLG AESANVCGSQQGRGQCTEVRADTRPWSGPYILRNQDDRELWPRKF FHRTCKCTGNFAGYNCGDCKFGWTGPNCERKKPPVIRQNIHSLSPQ EREQFLGALDLAKKRVHPDYVITTQHWLGLLGPNGTQPQFANCSV YDFFVWLHYYSVRDTLLGPGRPYRAIDFSHQGPAFVTWHRYHLLC LERDLQRLIGNESFALPYWNFATGRNECDVCTDQLFGAARPDDPTL ISRNSRFSSWETVCDSLDDYNHLVTLCNGTYEGLLRRNQMGRNSM KLPTLKDIRDCLSLQKFDNPPFFQNSTFSFRNALEGFDKADGTLDSQ VMSLHNLVHSFLNGTNALPHSAANDPIFVVISNRLLYNATTNILEHV RKEKATKELPSLHVLVLHSFTDAIFDEWMKRFNPPADAWPQELAPI GHNRMYNMVPFFPPVTNEELFLTSDQLGYSYAIDLPVSVEETPGWP TTLLVVMGTLVALVGLFVLLAFLQYRRLRKGYTPLMETHLSSKRY TEEA
CLDN6	uc021tbb.1	MASAGMQILGVVLTLLGWVNGLVSCALPMWKVTAFIGNSIVVAQ VVWEGLWMSCVVQSTGQMQCKVYDSLLALPQDLQAARALCVIAL LVALFGLLVYLAGAKCTTCVEEKDSKARLVLTSGIVFVISGVLTLIP VCWTAHAIIRDFYNPLVAEAQKRELGASLYLGWAASGLLLLGGGL LCCTCPSGGSQGPSHYMARYSTSAPAISRGPSEYPTKNYV
MLANA	uc003zjo.1	MPREDAHFIYGYPKKGHGHSYTTAEEAAGIGILTVILGVLLLIGCWY CRRRNGYRALMDKSLHVGTQCALTRRCPQEGFDHRDSKVSLQEK NCEPVVPNAPPAYEKLSAEQSPPPYSP
AFP	uc003hgz.1	MKWVESIFLIFLLNFTESRTLHRNEYGIASILDSYQCTAEISLADLATI FFAQFVQEATYKEVSKMVKDALTAIEKPTGDEQSSGCLENQLPAFL EELCHEKEILEKYGHSDCCSQSEEGRHNCFLAHKKPTPASIPLFQVP EPVTSCEAYEEDRETFMNKFIYEIARRHPFLYAPTILLWAARYDKIIP SCCKAENAVECFQTKAATVTKELRESSLLNQHACAVMKNFGTRTF QAITVTKLSQKFTKVNFTEIQKLVLDVAHVHEHCCRGDVLDCLQD GEKIMSYICSQQDTLSNKITECCKLTTLERGQCIIHAENDEKPEGLSP NLNRFLGDRDFNQFSSGEKNIFLASFVHEYSRRHPQLAVSVILRVAK GYQELLEKCFQTENPLECQDKGEEELQKYIQESQALAKRSCGLFQK LGEYYLQNAFLVAYTKKAPQLTSSELMAITRKMAATAATCCQLSE DKLLACGEGAADIIIGHLCIRHEMTPVNPGVGQCCTSSYANRRPCFS SLVVDETYVPPAFSDDKFIFHKDLCQAQGVALQTMKQEFLINLVKQ KPQITEEQLEAVIADFSGLLEKCCQGQEQEVCFAEEGQKLISKTRAA LGV
DKK4	uc003xpb.3	MVAAVLLGLSWLCSPLGALVLDFNNIRSSADLHGARKGSQCLSDT DCNTRKFCLQPRDEKPFCATCRGLRRRCQRDAMCCPGTLCVNDVC TTMEDATPILERQLDEQDGTHAEGTTGHPVQENQPKRKPSIKKSQG RKGQEGESCLRTFDCGPGLCCARHFWTKICKPVLLEGQVCSRRGH KDTAQAPEIFQRCDCGPGLLCRSQLTSNRQHARLRVCQKIEKL
ASCL2	uc021qcf.1	MDGGTLPRSAPPAPPVPVGCAARRRPASPELLRCSRRRRPATAETG GGAAAVARRNERERNRVKLVNLGFQALRQHVPHGGASKKLSKVE TLRSAVEYIRALQRLLAEHDAVRNALAGGLRPQAVRPSAPRGPPGT TPVAASPSRASSSPGRGGSSEPGSPRSAYSSDDSGCEGALSPAERELL DFSSWLGGY

Over-expressed gene	UCSC ID	Full Sequence
GAGE1	uc004dok.2	MSWRGRSTYYWPRPRRYVQPPEMIGPMRPEQFSDEVEPATPEEGEPATQRQDPAAAQEGEDEGASAGQGPKPEADSQEQGHPQTGCECEDGPDGQEMDPPNPEEVKTPEEEMRSHYVAQTGILWLLMNNCFLNLSPRKP
GAGE10	uc010nir.1	MSWRGRSTYRSRPRLYVEPPEMIGPMLPEQFSDEVEPATPEEGEPATQRQDPAAAQEGEDEGASAGQGPKPEADSQEQVHPKTGCECGDGPDGQEMGLPNPEEVKRPEEGEKQSQC
SLC45A2	uc003jid.3	MGSNSGQAGRHIYKSLADDGPFDSVEPPKRPTSRLIMHSMAMFGREFCYAVEAAYVTPVLLSVGLPSSLYSIVWFLSPILGFLLQPVVGSASDHCRSRWGRRRPYILTLGVMMLVGMALYLNGATVVAALIANPRRKLVWAISVTMIGVVLFDFAADFIDGPIKAYLFDVCSHQDKEKGLHYHALFTGFGGALGYLLGAIDWAHLELGRLLGTEFQVMFFFSALVLTLCFTVHLCSISEAPLTEVAKGIPPQQTPQDPPLSSDGMYEYGSIEKVKNGYVNPELAMQGAKNKNHAEQTRRAMTLKSLLRALVNMPPHYRYLCISHLIGWTAFLSNMLFFTDFMGQIVYRGDPYSAHNSTEFLIYERGVEVGCWGFCINSVFSSLYSYFQKVLVSYIGLKGLYFTGYLLFGLGTGFIGLFPNVYSTLVLCSLFGVMSSTLYTVPFNLITEYHREEEKERQQAPGGDPDNSVRGKGMDCATLTCMVQLAQILVGGGLGFLVNTAGTVVVVVITASAVALIGCCFVALFVRYVD
PAGE5	uc004duj.3	MQAPWAGNRGWAGTREEVRDMSEHVTRSQSSERGNDQESSQPVGPVIVQQPTEEKRQEEEPPTDNQGIAPSGEIKNEGAPAVQGTDVEAFQQELALLKIEDAPGDGPDVREGTLPTFDPTKVLEAGEGQL
PAGE2	uc004duf.1	MSELLRARSQSSERGNDQESSQPVGSVIVQEPTEEKRQEEEPPTDNQGIAPSGEIENQAVPAFQGPDMEAFQQELALLKIEDEPGDGPDVREGIMPTFDLTKVLEAGDAQP
PMEL	uc001siq.3	MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVSRQLRTKAWNRQLYPEWTEAQRLDCWRGGQVSLKVSNDGPTLIGANASFSIALNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQPVYPQETDDACIFPDGGPCPSGSWSQKRSFVYVWKTWGQYWQVLGGPVSGLSIGTGRAMLGTHTMEVTVYHRRGSRSYVPLAHSSSAFTITDQVPFSVSVSQLRALDGGNKHFLRNQPLTFALQLHDPSGYLAEADLSYTWDFGDSSGTLISRALVVTHTYLEPGPVTAQVVLQAAIPLTSCGSSPVPGTTDGHRPTAEAPNTTAGQVPTTEVVGTTPGQAPTAEPSGTTSVQVPTTEVISTAPVQMPTAESTGMTPEKVPVSEVMGTTLAEMSTPEATGMTPAEVSIVVLSGTTAAQVTTTEWVETTARELPIPEPEGPDASSIMSTESITGSLGPLLDGTATLRLVKRQVPLDCVLYRYGSFSVTLDIVQGIESAEILQAVPSGEGDAFELTVSCQGGLPKEACMEISSPGCQPPAQRLCQPVLPSPACQLVLHQILKGGSGTYCLNVSLADTNSLAVVSTQLIMPVPGILLTGQEAGLGQVPLIVGILLVLMAVVLASLIYRRRLMKQDFSVPQLPHSSSHWLRLPRIFCSCPIGENSPLLSGQQV

For each epitope, the full-length amino acid sequence of the non-mutated protein epitope was derived. Any constituent 9mer or 10mer not found in the germline protein sequence was flagged and scored for binding potential on six common HLA alleles (HLA-A01:01, HLA-A02:01. HLA-A03:01,

HLA-A24:02, HLA-B07:02, and HLA-B08:01) using available algorithms. Any peptide scoring better than 1000 nM was nominated.

Table 4

Over-expressed gene	UCSC ID	Full Sequence
TYR	uc001pcs.3	MLLAVLYCLLWSFQTSAGHFPRACVSSKNLMEKECCPPWSGDRS PCGQLSGRGSCQNILLSNAPLGPQFPFTGVDDRESWPSVFYNRTC QCSGNFMGFNCGNCKFGFWGPNCTERRLLVRRNIFDLSAPEKDK FFAYLTLAKHTISSDYVIPIGTYGQMKNGSTPMFNDINIYDLFVW MHYYVSMDALLGGSEIWRDIDFAHEAPAFLPWHRLFLLRWEQEI QKLTGDENFTIPYWDWRDAEKCDICTDEYMGGQHPTNPNLLSPA SFFSSWQIVCSRLEEYNSHQSLCNGTPEGPLRRNPGNHDKSRTPRL PSSADVEFCLSLTQYESGSMDKAANFSFRNTLEGFASPLTGIADAS QSSMHNALHIYMNGTMSQVQGSANDPIFLLHHAFVDSIFEQWLR RHRPLQEVYPEANAPIGHNRESYMVPFIPLYRNGDFFISSKDLGYD YSYLQDSDPDSFQDYIKSYLEQASRIWSWLLGAAMVGAVLTALL AGLVSLLCRHKRKQLPEEKQPLLMEKEDYHSLYQSHL
MAGEC1	uc004fbt.3	MGDKDMPTAGMPSLLQSSSESPQSCPEGEDSQSPLQIPQSSPESDD TLYPLQSPQSRSEGEDSSDPLQRPPEGKDSQSPLQIPQSSPEGDDTQ SPLQNSQSSPEGKDSLSPLEISQSPPEGEDVQSPLQNPASSFFSSALL SIFQSSPESTQSPFEGFPQSVLQIPVSAASSSTLVSIFQSSPESTQSPFE GFPQSPLQIPVSRSFSSTLLSIFQSSPERTQSTFEGFAQSPLQIPVSPS SSSTLLSLFQSFSERTQSTFEGFAQSSLQIPVSPSFSSTLVSLFQSSPE RTQSTFEGFPQSPLQIPVSSSSSSTLLSLFQSSPERTHSTFEGFPQSLL QIPMTSSFSSTLLSIFQSSPESAQSTFEGFPQSPLQIPGSPSFSSTLLSL FQSSPERTHSTFEGFPQSPLQIPMTSSFSSTLLSILQSSPESAQSAFEG FPQSPLQIPVSSSFSYTLLSLFQSSPERTHSTFEGFPQSPLQIPVSSSS SSSTLLSLFQSSPECTQSTFEGFPQSPLQIPQSPPEGENTHSPLQIVPS LPEWEDSLSPHYFPQSPPQGEDSLSPHYFPQSPPQGEDSLSPHYFPQ SPQGEDSLSPHYFPQSPPQGEDSMSPLYFPQSPLQGEEFQSSLQSPV SICSSSTPSSLPQSFPESSQSPPEGPVQSPLHSPQSPPEGMHSQSPLQ SPESAPEGEDSLSPLQIPQSPLEGEDSLSSLHFPQSPPEWEDSLSPLH FPQFPPQGEDFQSSLQSPVSICSSSTSLSLPQSFPESPQSPPEGPAQSP LQRPVSSFFSYTLASLLQSSHESPQSPPEGPAQSPLQSPVSSFPSSTS SSLSQSSPVSSFPSSTSSSLSKSSPESPLQSPVISFSSSTSLSPFSEESSS PVDEYTSSSDTLLESDSLTDSESLIESEPLFTYTLDEKVDELARFLL LKYQVKQPITKAEMLTNVISRYTGYFPVIFRKAREFIEILFGISLRE VDPDDSYVFVNTLDLTSEGCLSDEQGMSQNRLLILILSIIFIKGTYA SEEVIWDVLSGIGVRAGREHFAFGEPRELLTKVWVQEHYLEYRE VPNSSPPRYEFLWGPRAHSEVIKRKVVEFLAMLKNTVPITFPSSYK DALKDVEERAQAIIDTTDDSTATESASSSVMSPSFSSE

Over-expressed gene	UCSC ID	Full Sequence
MAGEA10	uc022cgz.1	MPRAPKRQRCMPEEDLQSQSETQGLEGAQAPLAVEEDASSSTSTS SSFPSSFPSSSSSSSSSCYPLIPSTPEEVSADDETPNPPQSAQIACSSPS VVASLPLDQSDEGSSSQKEESPSTLQVLPDSESLPRSEIDEKVTDLV QFLLFKYQMKEPITKAEILESVIRNYEDHFPLLFSEASECMLLVFGI DVKEVDPTGHSFVLVTSLGLTYDGMLSDVQSMPKTGILILILSIVFI EGYCTPEEVIWEALNMMGLYDGMEHLIYGEPRKLLTQDWVQEN YLEYRQVPGSDPARYEFLWGPRAHAEIRKMSLLKFLAKVNGSDP RSFPLWYEEALKDEEERAQDRIATTDDTTAMASASSSATGSFSYP E	
MAGEB17	uc031tgu.1	MPRGQASKRRAREKRRQARGEDQCLGGAQATAAEKEKLPSSSSP ACQSPPQSFPNAGIPQESQRASYPSSPASAVSLTSSDEGAKGQKGE SPNSFHGPSSSESTGRDLLNTKTGELVQFLLNKYIRKEPITREAML KVINRKYKQHFPEILRRSTENVEVVFGLYLKEMDPSRQSYVLVGK LDFPNQGSLSDGGGFPLSGLLMVLLSTIFMHGNRATEEEMWECL NALGMYKGRKHFIYGEPQELVTKDLVREGYLEYQQVPSSDPPRY EFLWGPRARAETSKMKVLEFVAKLNDTVASTYKSRYEEALREEE EQARARARAVARDSARARASRSFQP
MAGEA4	uc022cgu.1	MLPLSVGLWVPIAQLLPALLPAALTRVIMSSEQKSQHCKPEEGVE AQEEALGLVGAQAPTTEEQEAAVSSSSPLVPGTLEEVPAAESAGP PQSPQGASALPTTISFTCWRQPNEGSSSQEEEGPSTSPDAESLFREA LSNKVDELAHFLLRKYRAKELVTKAEMLERVIKNYKRCFPVIFGK ASESLKMIFGIDVKEVDPASNTYTLVTCLGLSYDGLLGNNQIFPKT GLLIIVLGTIAMEGDSASEEEIWEELGVMGVYDGREHTVYGEPRK LLTQDWVQENYLEYRQVPGSNPARYEFLWGPRALAETSYVKVLE HVVRVNARVRIAYPSLREAALLEEEEGV
MAGEA1	uc022chs.1	MSQDQESPRCTHDQHLQTFSETQSLEVAQVSKALEKTLLSSSHPL VPGKLKEAPAAKAESPLEVPQSFCSSSIAVTTTSSSESDEASSNQEE EDSPSSSEDTSDPRNVPADALDQKVAFLVNFMLHKCQMKKPITK ADMLKIIIKDDESHFSEILLRASEHLEMIFGLDVVEVDPTTHCYGLF IKLGLTYDGMLSGEKGVPKTGLLIIVLGVIFMKGNRATEEEVWEV LNLTGVYSGKKHFIFGEPRMLITKDFVKEKYLEYQQVANSDPARY EFLWGPRAKAETSKMKVLEFVAKVHGSYPHSFPSQYAEALKEEE ERARARI
MAGEA1	uc022chs.1	MSLEQRSLHCKPEEALEAQQEALGLVCVQAATSSSSPLVLGTLEE VPTAGSTDPPQSPQGASAFPTTINFTRQRQPSEGSSSREEEGPSTSCI LESLFRAVITKKVADLVGFLLLKYRAREPVTKAEMLESVIKNYKH CFPEIFGKASESLQLVFGIDVKEADPTGHSYVLVTCLGLSYDGLLG DNQIMPKTGFLIIVLVMIAMEGGHAPEEEIWEELSVMEVYDGREH SAYGEPRKLLTQDLVQEKYLEYRQVPDSDPARYEFLWGPRALAE TSYVKVLEYVIKVSARVRFFFPSLREAALREEEEGV

Over-expressed gene	UCSC ID	Full Sequence
MAGEA8	uc022cgo.1	MLLGQKSQRYKAEEGLQAQGEAPGLMDVQIPTAEEQKAASSSST LIMGTLEEVTDSGSPSPPQSPEGASSSLTVTDSTLWSQSDEGSSSNE EEGPSTSPDPAHLESLFREALDEKVAELVRFLLRKYQIKEPVTKAE MLESVIKNYKNHFPDIFSKASECMQVIFGIDVKEVDPAGHSYILVT CLGLSYDGLLGDDQSTPKTGLLIIVLGMILMEGSRAPEEAIWEALS VMGLYDGREHSVYWKLRKLLTQEWVQENYLEYRQAPGSDPVR YEFLWGPRALAETSYVKVLEHVVRVNARVRISYPSLHEEALGEE KGV
MAGEB4	uc004dcb.3	MPRGQKSKLRAREKRQRTRGQTQDLKVGQPTAAEKEESPSSSSS VLRDTASSSLAFGIPQEPQREPPTTSAAAAMSCTGSDKGDESQDEE NASSSQASTSTERSLKDSLTRKTKMLVQFLLYKYKMKEPTTKAE MLKIISKKYKEHFPEIFRKVSQRTELVFGLALKEVNPTTHSYILVS MLGPNDGNQSSAWTLPRNGLLMPLLSVIFLNGNCAREEEIWEFLN MLGIYDGKRHLIFGEPRKLITQDLVQEKYLEYQQVPNSDPPRYQF LWGPRAHAETSKMKVLEFLAKVNDTTPNNFPLLYEEALRDEEER AGARPRVAARRGTTAMTSAYSRATSSSSSQPM
CT45A5	uc011mvu.2	MTDKTEKVAVDPETVFKRPRECDSPSYQKRQRMALLARKQGAG DSLIAGSAMSKEKKLMTGHAIPPSQLDSQIDDFTGFSKDGMMQKP GSNAPVGGNVTSNFSGDDLECRGIASSPKSQQEINADIKCQVVKEI RCLGRKYEKIFEMLEGVQGPTAVRKRFFESIIKEAARCMRRDFVK HLKKKLKRMI
ALPPL2	uc002vss.4	MQGPWVLLLLGLRLQLSLGIIPVEEENPDFWNRQAAEALGAAKK LQPAQTAAKNLIIFLGDGMGVSTVTAARILKGQKKDKLGPETFLA MDRFPYVALSKTYSVDKHVPDSGATATAYLCGVKGNFQTIGLSA AARFNQCNTTRGNEVISVMNRAKKAGKSVGVVTTTRVQHASPA GAYAHTVNRNWYSDADVPASARQEGCQDIATQLISNMDIDVILG GGRKYMFPMGTPDPEYPDDYSQGGTRLDGKNLVQEWLAKHQG ARYVWNRTELLQASLDPSVTHLMGLFEPGDMKYEIHRDSTLDPS LMEMTEAALLLLSRNPRGFFLFVEGGRIDHGHHESRAYRALTETI MFDDAIERAGQLTSEEDTLSLVTADHSHVFSFGGYPLRGSSIFGLA PGKARDRKAYTVLLYGNGPGYVLKDGARPDVTESESGSPEYRQQ SAVPLDGETHAGEDVAVFARGPQAHLVHGVQEQTFIAHVMAFA ACLEPYTACDLAPRAGTTDAAHPGPSVVPALLPLLAGTLLLLGTA TAP
MMP13	uc001phl.3	MHPGVLAAFLFLSWTHCRALPLPSGGDEDDLSEEDLQFAERYLRS YYHPTNLAGILKENAASSMTERLREMQSFFGLEVTGKLDDNTLD VMKKPRCGVPDVGEYNVFPRTLKWSKMNLTYRIVNYTPDMTHS EVEKAFKKAFKVWSDVTPLNFTRLHDGIADIMISFGIKEHGDFYPF DGPSGLLAHAFPPGPNYGGDAHFDDDETWTSSSKGYNLFLVAAH EFGHSLGLDHSKDPGALMFPIYTYTGKSHFMLPDDDVQGIQSLYG PGDEDPNPKHPKTPDKCDPSLSLDAITSLRGETMIFKDRFFWRLHP QQVDAELFLTKSFWPELPNRIDAAYEHPSHDLIFIFRGRKFWALN GYDILEGYPKKISELGLPKEVKKISAAVHFEDTGKTLLFSGNQVW RYDDTNHIMDKDYPRLIEEDFPGIGDKVDAVYEKNGYIYFFNGPI QFEYSIWSNRIVRVMPANSILWC

Over-expressed gene	UCSC ID	Full Sequence
CTAG1B	uc004fmf.1	MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGR GPRGAGAARASGPGGGAPRGPHGGAASGLNGCCRCGARGPESRL LEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNI LTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQ RR
DCT	uc010afh.3	MSPLWWGFLLSCLGCKILPGAQGQFPRVCMTVDSLVNKECCPRL GAESANVCGSQQGRGQCTEVRADTRPWSGPYILRNQDDRELWPR KFFHRTCKCTGNFAGYNCGDCKFGWTGPNCERKKPPVIRQNIHSL SPQEREQFLGALDLAKKRVHPDYVITTQHWLGLLGPNGTQPQFA NCSVYDFFVWLHYYSVRDTLLGPGRPYRAIDFSHQGPAFVTWHR YHLLCLERDLQRLIGNESFALPYWNFATGRNECDVCTDQLFGAA RPDDPTLISRNSRFSSWETVCDSLDDYNHLVTLCNGTYEGLLRRN QMGRNSMKLPTLKDIRDCLSLQKFDNPPFFQNSTFSFRNALEGFD KADGTLDSQVMSLHNLVHSFLNGTNALPHSAANDPIFVVISNRLL YNATTNILEHVRKEKATKELPSLHVLVLHSFTDAIFDEWMKRFNP PADAWPQELAPIGHNRMYNMVPFFPPVTNEELFLTSDQLGYSYAI DLPVSVEETPGWPTTLLVVMGTLVALVGLFVLLAFLQYRRLRKG YTPLMETHLSSKRYTEEA
CLDN6	uc021tbb.1	MASAGMQILGVVLTLLGWVNGLVSCALPMWKVTAFIGNSIVVA QVVWEGLWMSCVVQSTGQMQCKVYDSLLALPQDLQAARALCV IALLVALFGLLVYLAGAKCTTCVEEKDSKARLVLTSGIVFVISGVL TLIPVCWTAHAIIRDFYNPLVAEAQKRELGASLYLGWAASGLLLL GGGLLCCTCPSGGSQGPSHYMARYSTSAPAISRGPSEYPTKNYV
MLANA	uc003zjo.1	MPREDAHFIYGYPKKGHGHSYTTAEEAAGIGILTVILGVLLLIGC WYCRRRNGYRALMDKSLHVGTQCALTRRCPQEGFDHRDSKVSL QEKNCEPVVPNAPPAYEKLSAEQSPPPYSP
AFP	uc003hgz.1	MKWVESIFLIFLLNFTESRTLHRNEYGIASILDSYQCTAEISLADLA TIFFAQFVQEATYKEVSKMVKDALTAIEKPTGDEQSSGCLENQLP AFLEELCHEKEILEKYGHSDCCSQSEEGRHNCFLAHKKPTPASIPL FQVPEPVTSCEAYEEDRETFMNKFIYEIARRHPFLYAPTILLWAAR YDKIIPSCCKAENAVECFQTKAATVTKELRESSLLNQHACAVMK NFGTRTFQAITVTKLSQKFTKVNFTEIQKLVLDVAHVHEHCCRGD VLDCLQDGEKIMSYICSQQDTLSNKITECCKLTTLERGQCIIHAEN DEKPEGLSPNLNRFLGDRDFNQFSSGEKNIFLASFVHEYSRRHPQL AVSVILRVAKGYQELLEKCFQTENPLECQDKGEEELQKYIQESQA LAKRSCGLFQKLGEYYLQNAFLVAYTKKAPQLTSSELMAITRKM AATAATCCQLSEDKLLACGEGAADIIIGHLCIRHEMTPVNPGVGQ CCTSSYANRRPCFSSLVVDETYVPPAFSDDKFIFHKDLCQAQGVA LQTMKQEFLINLVKQKPQITEEQLEAVIADFSGLLEKCCQGQEQE VCFAEEGQKLISKTRAALGV
DKK4	uc003xpb.3	MVAAVLLGLSWLCSPLGALVLDFNNIRSSADLHGARKGSQCLSD TDCNTRKFCLQPRDEKPFCATCRGLRRRCQRDAMCCPGTLCVND VCTTMEDATPILERQLDEQDGTHAEGTTGHPVQENQPKRKPSIKK SQGRKGQEGESCLRTFDCGPGLCCARHFWTKICKPVLLEGQVCSR RGHKDTAQAPEIFQRCDCGPGLLCRSQLTSNRQHARLRVCQKIEK L

Over-expressed gene	UCSC ID	Full Sequence
ASCL2	uc021qcf.1	MDGGTLPRSAPPAPPVPVGCAARRRPASPELLRCSRRRRPATAET GGGAAAVARRNERERNRVKLVNLGFQALRQHVPHGGASKKLSK VETLRSAVEYIRALQRLLAEHDAVRNALAGGLRPQAVRPSAPRGP PGTTPVAASPSRASSSPGRGGSSEPGSPRSAYSSDDSGCEGALSPA ERELLDFSSWLGGY
GAGE1	uc004dok.2	MSWRGRSTYYWPRPRRYVQPPEMIGPMRPEQFSDEVEPATPEEG EPATQRQDPAAAQEGEDEGASAGQGPKPEADSQEQGHPQTGCEC EDGPDGQEMDPPNPEEVKTPEEEMRSHYVAQTGILWLLMNNCFL NLSPRKP
GAGE10	uc010nir.1	MSWRGRSTYRSRPRLYVEPPEMIGPMLPEQFSDEVEPATPEEGEP ATQRQDPAAAQEGEDEGASAGQGPKPEADSQEQVHPKTGCECG DGPDGQEMGLPNPEEVKRPEEGEKQSQC
SLC45A2	uc003jid.3	MGSNSGQAGRHIYKSLADDGPFDSVEPPKRPTSRLIMHSMAMFG REFCYAVEAAYVTPVLLSVGLPSSLYSIVWFLSPILGFLLQPVVGS ASDHCRSRWGRRRPYILTLGVMMLVGMALYLNGATVVAALIAN PRRKLVWAISVTMIGVVLFDFAADFIDGPIKAYLFDVCSHQDKEK GLHYHALFTGFGGALGYLLGAIDWAHLELGRLLGTEFQVMFFFS ALVLTLCFTVHLCSISEAPLTEVAKGIPPQQTPQDPPLSSDGMYEY GSIEKVKNGYVNPELAMQGAKNKNHAEQTRRAMTLKSLLRALV NMPPHYRYLCISHLIGWTAFLSNMLFFTDFMGQIVYRGDPYSAHN STEFLIYERGVEVGCWGFCINSVFSSLYSYFQKVLVSYIGLKGLYF TGYLLFGLGTGFIGLFPNVYSTLVLCSLFGVMSSTLYTVPFNLITE YHREEEKERQQAPGGDPDNSVRGKGMDCATLTCMVQLAQILVG GGLGFLVNTAGTVVVVVITASAVALIGCCFVALFVRYVD
PAGE5	uc004duj.3	MQAPWAGNRGWAGTREEVRDMSEHVTRSQSSERGNDQESSQPV GPVIVQQPTEEKRQEEEPPTDNQGIAPSGEIKNEGAPAVQGTDVEA FQQELALLKIEDAPGDGPDVREGTLPTFDPTKVLEAGEGQL
PAGE2	uc004duf.1	MSELLRARSQSSERGNDQESSQPVGSVIVQEPTEEKRQEEEPPTDN QGIAPSGEIENQAVPAFQGPDMEAFQQELALLKIEDEPGDGPDVR EGIMPTFDLTKVLEAGDAQP
PMEL	uc001siq.3	MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVSRQLRTKA WNRQLYPEWTEAQRLDCWRGGQVSLKVSNDGPTLIGANASFSIA LNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQPVYPQETDDACIF PDGGPCPSGSWSQKRSFVYVWKTWGQYWQVLGGPVSGLSIGTG RAMLGTHTMEVTVYHRRGSRSYVPLAHSSSAFTITDQVPFSVSVS QLRALDGGNKHFLRNQPLTFALQLHDPSGYLAEADLSYTWDFGD SSGTLISRALVVTHTYLEPGPVTAQVVLQAAIPLTSCGSSPVPGTT DGHRPTAEAPNTTAGQVPTTEVVGTTPGQAPTAEPSGTTSVQVPT TEVISTAPVQMPTAESTGMTPEKVPVSEVMGTTLAEMSTPEATG MTPAEVSIVVLSGTTAAQVTTTEWVETTARELPIPEPEGPDASSIM STESITGSLGPLLDGTATLRLVKRQVPLDCVLYRYGSFSVTLDIVQ GIESAEILQAVPSGEGDAFELTVSCQGGLPKEACMEISSPGCQPPA QRLCQPVLPSPACQLVLHQILKGGSGTYCLNVSLADTNSLAVVST QLIMPVPGILLTGQEAGLGQVPLIVGILLVLMAVVLASLIYRRRLM KQDFSVPQLPHSSSHWLRLPRIFCSCPIGENSPLLSGQQV

Table 5

Virus, Gene	Genbank Accession	Sequence
HPV-16, E6	NC_001526	MHQKRTAMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRREVYDFAFRDLCIVYRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQL
HPV-16, E7	NC_001526	MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP
EBV, LF2	KC207813	MAEAYPGGAHAALASRRSSFRNSLRRLRPTEKPDTSFMRGVWKYEIFPSYVRVTNKQVLQLDAQCQELPPCPSVGQILSFKLPSFSFNTTTYGSRYFTVAFLFFGAEDNEVFLKPFFVMHSDQDIVLSVLNPRSLFIEKGKFTWYIVPIRLVKNPYLYLQILPGQSDIQLTRSCTQSGDKLNTSEPQIFLSGSPVTSQDECLPYLLAQHTPPFLKSYARIHTFPGKVCPVNAIRRGKGYVRVSVDTPDLKREGPLNVKVGMTLLDDVIIAFRYNPYPKSHWRWDGESTDIRYFGSPVIIPPNFITELEYNNTYEAPLSSKITAIVVSHSSNPVFYVYPQEWKPGQTLKLTVRNISNNPITIVTGQSMAQAFFIYAGDPSISTIMRRYIQRQGCALTLPGNIVVESSSLPTFERINKTFNGNIVASEGTL
EBV, BALF5	KC207813	MSGGLFYNPFLRPNKGLLKKPDKEYLRLIPKCFQTPGAAGVVDVRGPQPPLCFYQDSLTVVGGDEDGKGMWWRQRAQEGTARPEADTHGSPLDFHVYDILETVYTHEKCAVIPSDKQGYVVPCGIVIKLLGRRKADGASVCVNVFGQQAYFYASAPQGLDVEFAVLSALKASTFDRRTPCRVSVEKVTRRSIMGYGNHAGDYHKITLSHPNSVCHVATWLQDKHGCRIFEANVDATRRFVLDNDFVTFGWYSCRRAIPRLQHRDSYAELEYDCEVGDLSVRREDSSWPSYQALAFDIECLGEEGFPTATNEADLILQISCVLWSTGEEAGRYRRILLTLGTCEDIEGVEVYEFPSELDMLYAFFQLIRDLSVEIVTGYNVANFDWPYILDRARHIYSINPASLGKIRAGGVCEVRRPHDAGKGFLRANTKVRITGLIPIDMYAVCRDKLSLSDYKLDTVARHLLGAKKEDVHYKEIPRLFAAGPEGRRRLGMYCVQDSALVMDLLNHFVIHVEVAEIAKIAHIPCRRVLDDGQQIRVFSCLLAAAQKENFILPMPSASDRDGYQGATVIQPLSGFYNSPVLVVDFASLYPSIIQAHNLCYSTMITPGEEHRLAGLRPGEDYESFRLTGGVYHFVKKHVHESFLASLLTSWLAKRKAIKKLLAACEDPRQRTILDKQQLAIKCTCNAVYGFTGVANGLFPCLSIAETVTLQGRTMLERAKAFVEALSPANLQALAPSPDAWAPLNPEGQLRVIYGDTDSLFIECRGFSESETLRFAEALAAHTTRSLFVAPISLEAEKTFSCLMLITKKRYVGVLTDGKTLMKGVELVRKTACKFVQTRCRRVLDLVLADARVKEAASLLSHRPFQESFTQGLPVGFLPVIDILNQAYTDLREGRVPMGELCFSTELSRKLSAYKSTQMPHLAVYQKFVERNEELPQIHDRIQYVFVEPKGGVKGARKTEMAEDPAYAERHGVPVAVDHYFDKLLQGAANILQCLFDNNSGAALSVLQNFTARPPF
EBV, RPMS1	KC207813	MAGARRRARCPASAGCAYSARPPPLSTRGRRISAGSGQPRWWPWGSPPPLDTRYRRPGPGRRARSCLHAGPRGRPPHSRTRARRTSPGAGGGGWRGGSCTSQR
EBV, A73	KC207813	MSMPPKGFLKKEMKPETRLLNKPPTVLTRPAMFCAWKLYSRKMPSRSKTLEARCSSRPPCDSPACQTRDTGCPRRSGTGRRGWRARRLGKESWFADAWRMARYWGCAVKAAAQSAFSASTASPEEL

Virus, Gene	Genbank Accession	Sequence
EBV, BALF4	KC207813	MTRRRVLSVVVLLAALACRLGAQTPEQPAPPATTVQPTATRQQT SFPFRVCELSSHGDLFRFSSDIQCPSFGTRENHTEGLLMVFKDNIIP YSFKVRSYTKIVTNILIYNGWYADSVTNRHEEKFSVDSYETDQM DTIYQCYNAVKMTKDGLTRVYVDRDGVNITVNLKPTGGLANGV RRYASQTELYDAPGWLIWTYRTRTTVNCLITDMMAKSNSPFDFF VTTTGQTVEMSPFYDGKNKETFHERADSFHVRTNYKIVDYDNR GTNPQGERRAFLDKGTYTLSWKLENRTAYCPLQHWQTFDSTIAT ETGKSIHFVTDEGTSSFVTNTTVGIELPDAFKCIEEQVNKTMHEK YEAVQDRYTKGQEAITYFITSGGLLLAWLPLTPRSLATVKNLTEL TTPTSSPPSSPSPPAPPAARGSTSAAVLRRRRRDAGNATTPVPPAA PGKSLGTLNNPATVQIQFAYDSLRRQINRMLGDLARAWCLEQKR QNMVLRELTKINPTTVMSSIYGKAVAAKRLGDVISVSQCVPVNQ ATVTLRKSMRVPGSETMCYSRPLVSFSFINDTKTYEGQLGTDNEI FLTKKMTEVCQATSQYYFQSGNEIHVYNDYHHFKTIELDGIATL QTFISLNTSLIENIDFASLELYSRDEQRASNVFDLEGIFREYNFQAQ NIAGLRKDLDNAVSNGRNQFVDGLGELMDSLGSVGQSITNLVST VGGLFSSLVSGFISFFKNPFGGMLILVLVAGVVILVISLTRRTRQM SQQPVQMLYPGIDELAQQHASGEGPGINPISKTELQAIMLALHEQ NQEQKRAAQRAAGPSVASRALQAARDRFPGLRRRRYHDPETAA ALLGEAETEF
EBV, BALF3	KC207813	MSGLLAAAYSQVYALAVELSVCARLDPRSLDVAAVVRNAGLLA ELEAILLPRLRRQNDRACSALSLELVHLLENSREASAALLAPGRK GTRVPPLRTPSVAYSVEFYGGHKVDVSLCLINDIEILMKRINSVFY CMSHTMGLESLERALDLLGRFRGVSPIPDPRLYITSVPCWRCVGE LMVLPNHGNPSTAEGTHVSCNHLAVPVNPEPVSGLFENEVRQAG LGHLLEAEEKARPGGPEEGAVPGPGRPEAEGATRALDTYNVFST VPPEVAELSELLYWNSGGHAIGATGQGEGGGHSRLSALFARERR LALVRRACEEALAGARLTHLFDAVAPGATERLFCGGVYSSSGDA VEALKADCAAAFTAHPQYRAILQKRNELYTRLNRAMQRLGRGE EEASRESPEVPRPAGAREPGPSGALSDALKRKEQYLRQVATEGL AKLQSCLAQQSETLTETLCLRVWGDVVYWELARMRNHFLYRR AFVSGPWEDRRAGEGAAFENSKYIKTHLFTQTLSSEHLHALTHSL YTFITGPLAEESGLFPPPSNVALARCCDAAGTLPHQKAFLTSLIWP GIEPSDWIETSFNSFYSVPGGSLASSQQILCRALREAVLTVSLYNK TWGRSLILRRADAVSPGQALPPDGLYLTYDSDRPLILLYKGRGW VFKDLYALLYLHLQMRDDSA
EBV, BARF0	KC207813	APGYAVEAVEGGLYPVARLDAWPYQGSQERLLVRQRTCGVTA ASQGHVAGWGKEPALLRQGPRDEGVQAVRQRVQVLRAQGLGK QVCFDVLGILKGGTLAGAPVLPGTRDEGPSVEEVVAHAGQLPVD HVPPDAQAQGLGQGLALLRQAGLQLGQTLGGHLAQVLLLALER VREGAGRAGLSCPSRPGHLRALPGRLLLASAQPLHGSVEPRVEL VPLLQDGPVLGVRREGGGAVRLQRLHRVARGAVDPAAEEPLCG PGSHGIKQVSQPCPRQRLLAGPPHQGQATLPGKQGREAGMSATL PLPRCTDSMAARVPIEELREFRHLRGHCREDVVGVQRSGRPLCL RPPRARDRALLWAARPRLLLSLQQVPEPSLPDFILKQSRDRLRIH RHRQVVTGDVGPLCRGRVAVVGQNHQLAHTAPAGHRGDVEAR VWDGTYAPKAAQQIQGPFQALQPHGVRHAIKHAIDSLH

For each epitope, the full-length amino acid sequence of the non-mutated protein epitope was derived. Any constituent 9mer or 10mer not found in the germline protein sequence was flagged and scored for binding potential on six common HLA alleles (HLA-A01:01, HLA-A02:01. HLA-A03:01, HLA-A24:02, HLA-B07:02, and HLA-B08:01) using available algorithms. Any peptide scoring better than 1000 nM was nominated.

Table 6

Peptide	Virus, Gene	Affinity
CMSCCRSSR	HPV-16, E6	A03.01=610
CPEEKQRHL	HPV-16, E6	B07.02=720
CVYCKQQLL	HPV-16, E6	B08.01=880
CVYCKQQLLR	HPV-16, E6	A03.01=250
CYSLYGTTL	HPV-16, E6	A24.02=290
DKKQRFHNI	HPV-16, E6	B08.01=200
EYRHYCYSL	HPV-16, E6	A24.02=350;B08.01=360
FAFRDLCIV	HPV-16, E6	A02.01=150
IILECVYCK	HPV-16, E6	A03.01=150
ISEYRHYCY	HPV-16, E6	A01.01=81
IVYRDGNPY	HPV-16, E6	A03.01=700
IVYRDGNPYA	HPV-16, E6	A02.01=760
KFYSKISEY	HPV-16, E6	A03.01=670
KISEYRHYCY	HPV-16, E6	A03.01=570
KLPQLCTEL	HPV-16, E6	A02.01=130
LIRCINCQK	HPV-16, E6	A03.01=230
LLIRCINCQK	HPV-16, E6	A03.01=130
MHQKRTAMF	HPV-16, E6	A24.02=980;B08.01=580
NPYAVCDKCL	HPV-16, E6	B07.02=550
QYNKPLCDLL	HPV-16, E6	A24.02=520
RFHNIRGRW	HPV-16, E6	A24.02=620
RGRWTGRCM	HPV-16, E6	B07.02=720
RPRKLPQLC	HPV-16, E6	B07.02=310
RPRKLPQLCT	HPV-16, E6	B07.02=79
SEYRHYCYSL	HPV-16, E6	B08.01=390
SSRTRRETQL	HPV-16, E6	B08.01=230
TIHDIILECV	HPV-16, E6	A02.01=140
TTLEQQYNK	HPV-16, E6	A03.01=520
VYDFAFRDL	HPV-16, E6	A24.02=600
GIVCPICSQK	HPV-16, E7	A03.01=200
GTLGIVCPI	HPV-16, E7	A02.01=120
HGDTPTLHEY	HPV-16, E7	A01.01=270
IVCPICSQK	HPV-16, E7	A03.01=200
LLMGTLGIV	HPV-16, E7	A02.01=20
RAHYNIVTF	HPV-16, E7	A24.02=330
RLCVQSTHV	HPV-16, E7	A02.01=770

Peptide	Virus, Gene	Affinity
TLEDLLMGTL	HPV-16, E7	A02.01=480
TLHEYMLDL	HPV-16, E7	A02.01=95
TPTLHEYML	HPV-16, E7	B07.02=490
YMLDLQPET	HPV-16, E7	A02.01=7
YMLDLQPETT	HPV-16, E7	A02.01=25
AALASRRSSF	EBV, LF2	B07.02=160
ALASRRSSF	EBV, LF2	B07.02=290;B08.01=41
ALASRRSSFR	EBV, LF2	A03.01=160
ALTLPGNIVV	EBV, LF2	A02.01=470
APLSSKITA	EBV, LF2	B07.02=490
APLSSKITAI	EBV, LF2	B07.02=31
AQHTPPFLK	EBV, LF2	A03.01=140
AYPGGAHAAL	EBV, LF2	A24.02=470
CPSVGQILSF	EBV, LF2	B07.02=190
EVFLKPFFV	EBV, LF2	A02.01=210
FFGAEDNEVF	EBV, LF2	A24.02=910
FIEKGKFTWY	EBV, LF2	A01.01=450
FIYAGDPSI	EBV, LF2	A02.01=13
FLSGSPVTS	EBV, LF2	A02.01=630
FMRGVWKYEI	EBV, LF2	A02.01=140;B08.01=340
FTVAFLFFGA	EBV, LF2	A02.01=17
FTWYIVPIRL	EBV, LF2	A02.01=63
FVMHSDQDIV	EBV, LF2	A02.01=130
GPLNVKVGM	EBV, LF2	B07.02=610
GQSMAQAFFI	EBV, LF2	A02.01=510
HSDQDIVLSV	EBV, LF2	A01.01=860
HSSNPVFYV	EBV, LF2	A02.01=800
HSSNPVFYVY	EBV, LF2	A01.01=170
HTFPGKVCPV	EBV, LF2	A02.01=200
IAFRYNPYPK	EBV, LF2	A03.01=58
IIPPNFITEL	EBV, LF2	A02.01=350
ILPGQSDIQL	EBV, LF2	A02.01=350
IPPNFITEL	EBV, LF2	B07.02=200
ITELEYNNTY	EBV, LF2	A01.01=61
IVVSHSSNPV	EBV, LF2	A02.01=160
KFTWYIVPI	EBV, LF2	A24.02=200
KLNTSEPQI	EBV, LF2	A02.01=270
KLPSFSFNT	EBV, LF2	A02.01=63
KLPSFSFNTT	EBV, LF2	A02.01=520
KNPYLYLQI	EBV, LF2	A24.02=900
KPDTSFMRGV	EBV, LF2	B07.02=920
KPGQTLKLTV	EBV, LF2	B07.02=320
KSYARIHTF	EBV, LF2	A24.02=210
KVCPVNAIRR	EBV, LF2	A03.01=850
KYEIFPSYV	EBV, LF2	A24.02=740
LAQHTPPFLK	EBV, LF2	A03.01=440
LDDVIIAFRY	EBV, LF2	A01.01=860

Peptide	Virus, Gene	Affinity
LFIEKGKFTW	EBV, LF2	A24.02=230
LLAQHTPPF	EBV, LF2	A02.01=540;B07.02=750;B08.01=190
LLAQHTPPFL	EBV, LF2	A02.01=9.6;B08.01=480
LLDDVIIAF	EBV, LF2	A02.01=120
LPGQSDIQL	EBV, LF2	B07.02=470
LPPCPSVGQI	EBV, LF2	B07.02=930
LPSFSFNTT	EBV, LF2	B07.02=490
LPSFSFNTTT	EBV, LF2	B07.02=560
LQLDAQCQEL	EBV, LF2	A02.01=220
LTLPGNIVV	EBV, LF2	A02.01=880
MAQAFFIYA	EBV, LF2	A02.01=450
NPYLYLQIL	EBV, LF2	B07.02=170;B08.01=150
NSLRRLRPT	EBV, LF2	B08.01=370
NTTTYGSRY	EBV, LF2	A01.01=210
NTYEAPLSSK	EBV, LF2	A03.01=120
PFLKSYARI	EBV, LF2	A24.02=390
PSYVRVTNK	EBV, LF2	A03.01=620
PYPKSHWRW	EBV, LF2	A24.02=100
QIFLSGSPV	EBV, LF2	A02.01=380
QLDAQCQEL	EBV, LF2	A02.01=370
QSMAQAFFI	EBV, LF2	A02.01=890
QSMAQAFFIY	EBV, LF2	A01.01=190
RLVKNPYLY	EBV, LF2	A03.01=410
RLVKNPYLYL	EBV, LF2	A02.01=120
RPTEKPDTSF	EBV, LF2	B07.02=39
RSLFIEKGK	EBV, LF2	A03.01=430
RSSFRNSLR	EBV, LF2	A03.01=230
RSSFRNSLRR	EBV, LF2	A03.01=490
RVSVDTPDLK	EBV, LF2	A03.01=600
RYFGSPVII	EBV, LF2	A24.02=25
RYFTVAFLF	EBV, LF2	A24.02=3.7
RYFTVAFLFF	EBV, LF2	A24.02=5.2
RYIQRQGCAL	EBV, LF2	A24.02=450
RYNPYPKSHW	EBV, LF2	A24.02=530
SFKLPSFSF	EBV, LF2	A24.02=98
SFMRGVWKY	EBV, LF2	A24.02=460
SLPTFERINK	EBV, LF2	A03.01=420
SLRRLRPTEK	EBV, LF2	A03.01=120
SMAQAFFIY	EBV, LF2	A01.01=870;A03.01=340
SMAQAFFIYA	EBV, LF2	A02.01=18
SPVTSQDECL	EBV, LF2	B07.02=540
SQDECLPYL	EBV, LF2	A02.01=18
SQDECLPYLL	EBV, LF2	A02.01=45
SRRSSFRNSL	EBV, LF2	B07.02=620;B08.01=150
SSFRNSLRR	EBV, LF2	A03.01=350
SSNPVFYVY	EBV, LF2	A01.01=110
SVGQILSFK	EBV, LF2	A03.01=73

Peptide	Virus, Gene	Affinity
TFERINKTF	EBV, LF2	A24.02=540
TIMRRYIQR	EBV, LF2	A03.01=280
TLLDDVIIA	EBV, LF2	A02.01=51
TLLDDVIIAF	EBV, LF2	A02.01=650
TPDLKREGPL	EBV, LF2	B07.02=100
TSFMRGVWK	EBV, LF2	A03.01=83
TSFMRGVWKY	EBV, LF2	A01.01=730
TSQDECLPY	EBV, LF2	A01.01=74
TTYGSRYFTV	EBV, LF2	A02.01=130
TVAFLFFGA	EBV, LF2	A02.01=290
TVRNISNNPI	EBV, LF2	B07.02=450
TWYIVPIRL	EBV, LF2	A24.02=680
TYEAPLSSKI	EBV, LF2	A24.02=420
TYGSRYFTV	EBV, LF2	A24.02=19
VFLKPFFVM	EBV, LF2	A24.02=600
VFYVYPQEW	EBV, LF2	A24.02=110
VFYVYPQEWK	EBV, LF2	A03.01=650
VLNPRSLFI	EBV, LF2	A02.01=110
VLSVLNPRSL	EBV, LF2	A02.01=680
VMHSDQDIV	EBV, LF2	A02.01=440
VMHSDQDIVL	EBV, LF2	A02.01=910
VSHSSNPVFY	EBV, LF2	A01.01=330
VTSQDECLPY	EBV, LF2	A01.01=26
VVSHSSNPV	EBV, LF2	A02.01=690
WYIVPIRLV	EBV, LF2	A24.02=700
YARIHTFPG	EBV, LF2	B08.01=430
YARIHTFPGK	EBV, LF2	A03.01=660
YFTVAFLFF	EBV, LF2	A24.02=62
YIQRQGCAL	EBV, LF2	B07.02=500;B08.01=890
YIVPIRLVK	EBV, LF2	A03.01=300
YLLAQHTPPF	EBV, LF2	A02.01=69;A24.02=550;B08.01=310
YLYLQILPG	EBV, LF2	A02.01=320
YLYLQILPGQ	EBV, LF2	A02.01=800
YPGGAHAAL	EBV, LF2	B07.02=7;B08.01=700
YPGGAHAALA	EBV, LF2	B07.02=410
YVRVTNKQVL	EBV, LF2	B07.02=70;B08.01=470
AGRYRRILL	EBV, BALF5	B08.01=810
AIKCTCNAV	EBV, BALF5	B08.01=760
ALAAHTTRSL	EBV, BALF5	A02.01=93;B07.02=200;B08.01=340
ALAFDIECL	EBV, BALF5	A02.01=70
ALAPSPDAWA	EBV, BALF5	A02.01=280
ALKASTFDR	EBV, BALF5	A03.01=1000
ALSPANLQA	EBV, BALF5	A02.01=530
ALSPANLQAL	EBV, BALF5	A02.01=65
ALSVLQNFTA	EBV, BALF5	A02.01=260
APLNPEGQL	EBV, BALF5	B07.02=85
APQGLDVEF	EBV, BALF5	B07.02=280

Peptide	Virus, Gene	Affinity
APSPDAWAPL	EBV, BALF5	B07.02=18
ASLLTSWLAK	EBV, BALF5	A03.01=140
AVYGFTGVA	EBV, BALF5	A02.01=830
AVYQKFVER	EBV, BALF5	A03.01=340
AYKSTQMPHL	EBV, BALF5	A24.02=770
CLFDNNSGA	EBV, BALF5	A02.01=130
CLFDNNSGAA	EBV, BALF5	A02.01=390
CLGEEGFPT	EBV, BALF5	A02.01=680
CLGEEGFPTA	EBV, BALF5	A02.01=120
CLSIAETVTL	EBV, BALF5	A02.01=580
CVNVFGQQAY	EBV, BALF5	A01.01=750
DARVKEAASL	EBV, BALF5	B08.01=330
DLLNHFVIHV	EBV, BALF5	A02.01=290
DLREGRVPM	EBV, BALF5	B07.02=690;B08.01=600
DMLYAFFQL	EBV, BALF5	A02.01=340
DNDFVTFGWY	EBV, BALF5	A01.01=820
DRARHIYSI	EBV, BALF5	B08.01=350
DVRGPQPPL	EBV, BALF5	B07.02=870
EAGRYRRIL	EBV, BALF5	B08.01=770
ELSRKLSAYK	EBV, BALF5	A03.01=450
EMAEDPAYA	EBV, BALF5	A02.01=480
EYLRLIPKCF	EBV, BALF5	A24.02=310
FLASLLTSW	EBV, BALF5	A02.01=150
FLASLLTSWL	EBV, BALF5	A02.01=4.1
FLRANTKVRI	EBV, BALF5	B08.01=260
FLRPNKGLL	EBV, BALF5	B08.01=250
FLRPNKGLLK	EBV, BALF5	A03.01=96
FPTATNEADL	EBV, BALF5	B07.02=180
FQESFTQGL	EBV, BALF5	A02.01=920
FQLIRDLSV	EBV, BALF5	A02.01=210;B08.01=390
FQTPGAAGV	EBV, BALF5	A02.01=48
FQTPGAAGVV	EBV, BALF5	A02.01=640
FSESETLRF	EBV, BALF5	A01.01=180
FTQGLPVGFL	EBV, BALF5	A02.01=960
FVAPISLEA	EBV, BALF5	A02.01=26
FVEALSPANL	EBV, BALF5	A02.01=860
FVIHVEVAEI	EBV, BALF5	A02.01=64
FVKKHVHESF	EBV, BALF5	B08.01=130
FVLDNDFVT	EBV, BALF5	A02.01=650
FVLDNDFVTF	EBV, BALF5	A24.02=750
FVQTRCRRV	EBV, BALF5	B08.01=340
FVQTRCRRVL	EBV, BALF5	B07.02=330;B08.01=100
FYASAPQGL	EBV, BALF5	A24.02=100
GLDVEFAVL	EBV, BALF5	A02.01=640
GLFPCLSIA	EBV, BALF5	A02.01=33
GLFYNPFLR	EBV, BALF5	A03.01=100
GLIPIDMYA	EBV, BALF5	A02.01=28

Peptide	Virus, Gene	Affinity
GLIPIDMYAV	EBV, BALF5	A02.01=5.9
GLPVGFLPV	EBV, BALF5	A02.01=68
GLPVGFLPVI	EBV, BALF5	A02.01=330
GMYCVQDSA	EBV, BALF5	A02.01=100
GMYCVQDSAL	EBV, BALF5	A02.01=64
GPEGRRRLGM	EBV, BALF5	B07.02=47
GQQAYFYASA	EBV, BALF5	A02.01=800
GVANGLFPCL	EBV, BALF5	A02.01=340
GVYHFVKKH	EBV, BALF5	A03.01=990
GYNVANFDW	EBV, BALF5	A24.02=270
HIYSINPASL	EBV, BALF5	A02.01=360
HLAVYQKFV	EBV, BALF5	A02.01=140
HPNSVCHVA	EBV, BALF5	B07.02=530
HPNSVCHVAT	EBV, BALF5	B07.02=380
HVATWLQDK	EBV, BALF5	A03.01=380
HVHESFLASL	EBV, BALF5	A02.01=670;B07.02=260;B08.01=990
HVYDILETV	EBV, BALF5	A02.01=12
HVYDILETVY	EBV, BALF5	A03.01=990
HYKEIPRLF	EBV, BALF5	A24.02=71
IAHIPCRRVL	EBV, BALF5	B07.02=620
IIQAHNLCY	EBV, BALF5	A01.01=600;A03.01=790
ILDKQQLAI	EBV, BALF5	A02.01=180
ILDKQQLAIK	EBV, BALF5	A03.01=310
ILDRARHIY	EBV, BALF5	A01.01=160
ILETVYTHEK	EBV, BALF5	A03.01=290
ILNQAYTDL	EBV, BALF5	A02.01=750
ILQISCVLW	EBV, BALF5	A24.02=990
IPRLFAAGPE	EBV, BALF5	B07.02=640
IPRLQHRDSY	EBV, BALF5	B07.02=360
IPSDKQGYV	EBV, BALF5	B07.02=610
IPSDKQGYVV	EBV, BALF5	B07.02=150
IQYVFVEPK	EBV, BALF5	A03.01=78
ITGLIPIDMY	EBV, BALF5	A01.01=340
ITKKRYVGV	EBV, BALF5	B08.01=520
ITKKRYVGVL	EBV, BALF5	B08.01=240
ITLSHPNSV	EBV, BALF5	A02.01=610
IVIKLLGRRK	EBV, BALF5	A03.01=350
IYGDTDSLF	EBV, BALF5	A24.02=37
IYGDTDSLFI	EBV, BALF5	A24.02=95
IYSINPASL	EBV, BALF5	A24.02=110
KAIKKLLAA	EBV, BALF5	B08.01=790
KEYLRLIPK	EBV, BALF5	A03.01=810
KGFLRANTK	EBV, BALF5	A03.01=170
KIAHIPCRR	EBV, BALF5	A03.01=340
KITLSHPNSV	EBV, BALF5	A02.01=920
KLDTVARHL	EBV, BALF5	A02.01=900
KLDTVARHLL	EBV, BALF5	A02.01=170

Peptide	Virus, Gene	Affinity
KLLQGAANI	EBV, BALF5	A02.01=25
KLLQGAANIL	EBV, BALF5	A02.01=63
KLSAYKSTQM	EBV, BALF5	A02.01=620
KLSLSDYKL	EBV, BALF5	A02.01=160
KPDKEYLRL	EBV, BALF5	B07.02=810
KTACKFVQTR	EBV, BALF5	A03.01=740
KTEMAEDPAY	EBV, BALF5	A01.01=200
KTFSCLMLI	EBV, BALF5	A02.01=46;A24.02=400
KTLMKGVELV	EBV, BALF5	A02.01=730
KVRITGLIPI	EBV, BALF5	B07.02=260
KVTRRSIMGY	EBV, BALF5	A03.01=140
LAAHTTRSL	EBV, BALF5	B07.02=76
LAKRKAIKKL	EBV, BALF5	B08.01=180
LDRARHIYSI	EBV, BALF5	B08.01=410
LILQISCVL	EBV, BALF5	A02.01=810
LIPIDMYAV	EBV, BALF5	A02.01=150
LITKKRYVGV	EBV, BALF5	B08.01=860
LLNHFVIHV	EBV, BALF5	A02.01=7.3
LLQGAANIL	EBV, BALF5	A02.01=360
LLTSWLAKRK	EBV, BALF5	A03.01=240
LMKGVELVRK	EBV, BALF5	A03.01=130
LMLITKKRYV	EBV, BALF5	A02.01=910
LPVGFLPVI	EBV, BALF5	B07.02=530
LQISCVLWST	EBV, BALF5	A02.01=220
LSRKLSAYK	EBV, BALF5	A03.01=94
LTDGKTLMK	EBV, BALF5	A01.01=410;A03.01=610
LTGGVYHFV	EBV, BALF5	A02.01=200
LTGGVYHFVK	EBV, BALF5	A03.01=330
LTSWLAKRK	EBV, BALF5	A03.01=560
LVMDLLNHFV	EBV, BALF5	A02.01=5.3
LVVDFASLY	EBV, BALF5	A01.01=460
MLERAKAFV	EBV, BALF5	A02.01=400
MLITKKRYV	EBV, BALF5	A02.01=190;B08.01=130
MLITKKRYVG	EBV, BALF5	B08.01=560
MLYAFFQLI	EBV, BALF5	A02.01=6.6;A24.02=800;B08.01=640
MLYAFFQLIR	EBV, BALF5	A03.01=40
MPHLAVYQKF	EBV, BALF5	B07.02=150
MSGGLFYNPF	EBV, BALF5	A24.02=490
MYAVCRDKL	EBV, BALF5	A24.02=210
NPEGQLRVI	EBV, BALF5	B07.02=650
NPFLRPNKGL	EBV, BALF5	B07.02=140;B08.01=630
NTKVRITGL	EBV, BALF5	B08.01=120
NTKVRITGLI	EBV, BALF5	B08.01=940
NVANFDWPY	EBV, BALF5	A01.01=300
NVANFDWPYI	EBV, BALF5	A02.01=320
PLSGFYNSPV	EBV, BALF5	A02.01=520
QIHDRIQYV	EBV, BALF5	A02.01=180

Peptide	Virus, Gene	Affinity
QIRVFSCLL	EBV, BALF5	B08.01=960
QLIRDLSVEI	EBV, BALF5	A02.01=94
QMPHLAVYQK	EBV, BALF5	A03.01=900
QQIRVFSCLL	EBV, BALF5	A02.01=980
QTRCRRVLDL	EBV, BALF5	B08.01=200
RAKAFVEAL	EBV, BALF5	B07.02=270;B08.01=990
RIFEANVDA	EBV, BALF5	A02.01=870
RIQYVFVEPK	EBV, BALF5	A03.01=85
RLFAAGPEGR	EBV, BALF5	A03.01=260
RLIPKCFQT	EBV, BALF5	A02.01=130
RLTGGVYHF	EBV, BALF5	A24.02=340
RLTGGVYHFV	EBV, BALF5	A02.01=8.1
RPGEDYESF	EBV, BALF5	B07.02=630
RPHDAGKGF	EBV, BALF5	B07.02=21
RPHDAGKGFL	EBV, BALF5	B07.02=9.1
RTMLERAKAF	EBV, BALF5	B07.02=920
RTPCRVSVEK	EBV, BALF5	A03.01=430
RVFSCLLAA	EBV, BALF5	A02.01=69;A03.01=810
RVFSCLLAAA	EBV, BALF5	A02.01=58
RVIYGDTDSL	EBV, BALF5	B07.02=560
RVKEAASLL	EBV, BALF5	B07.02=380
RVLDLVLADA	EBV, BALF5	A02.01=670
RYRRILLTL	EBV, BALF5	A24.02=23
SFLASLLTSW	EBV, BALF5	A24.02=56
SFTQGLPVGF	EBV, BALF5	A24.02=350
SIMGYGNHA	EBV, BALF5	A02.01=760
SINPASLGK	EBV, BALF5	A03.01=63
SLFVAPISL	EBV, BALF5	A02.01=30
SLLTSWLAK	EBV, BALF5	A03.01=23
SLLTSWLAKR	EBV, BALF5	A03.01=490
SLSDYKLDTV	EBV, BALF5	A02.01=22
SLYPSIIQA	EBV, BALF5	A02.01=23
SLYPSIIQAH	EBV, BALF5	A03.01=240
SPANLQALA	EBV, BALF5	B07.02=500
SPLDFHVYDI	EBV, BALF5	B07.02=570
STFDRRTPCR	EBV, BALF5	A03.01=600
STGEEAGRY	EBV, BALF5	A01.01=130
STQMPHLAVY	EBV, BALF5	A01.01=130
SWLAKRKAI	EBV, BALF5	B08.01=340
SWPSYQALAF	EBV, BALF5	A24.02=49
SYQALAFDI	EBV, BALF5	A24.02=13
TKKRYVGVL	EBV, BALF5	B08.01=460
TLMKGVELV	EBV, BALF5	A02.01=7.9
TMLERAKAF	EBV, BALF5	B08.01=100
TMLERAKAFV	EBV, BALF5	A02.01=33;B08.01=840
TVARHLLGAK	EBV, BALF5	A03.01=90
VARHLLGAK	EBV, BALF5	A03.01=600

Peptide	Virus, Gene	Affinity
VARHLLGAKK	EBV, BALF5	A03.01=820
VIDILNQAY	EBV, BALF5	A01.01=34
VIKLLGRRK	EBV, BALF5	A03.01=720
VIQPLSGFY	EBV, BALF5	A01.01=990;A03.01=920
VIYGDTDSL	EBV, BALF5	A02.01=680
VLADARVKEA	EBV, BALF5	A02.01=450
VLDDGQQIRV	EBV, BALF5	A02.01=17
VLDLVLADA	EBV, BALF5	A02.01=540
VLTDGKTLMK	EBV, BALF5	A03.01=200
VLVVDFASL	EBV, BALF5	A02.01=190
VLWSTGEEA	EBV, BALF5	A02.01=210
VMDLLNHFV	EBV, BALF5	A01.01=780;A02.01=9.7
VMDLLNHFVI	EBV, BALF5	A02.01=79
VTFGWYSCR	EBV, BALF5	A03.01=290
VTFGWYSCRR	EBV, BALF5	A03.01=230
VTRRSIMGY	EBV, BALF5	A03.01=250
VYTHEKCAVI	EBV, BALF5	A24.02=490
WLAKRKAIK	EBV, BALF5	A03.01=470
WLAKRKAIKK	EBV, BALF5	A03.01=190
WLQDKHGCRI	EBV, BALF5	A02.01=790;B08.01=810
WPSYQALAF	EBV, BALF5	B07.02=31
WSTGEEAGRY	EBV, BALF5	A01.01=370
YAERHGVPV	EBV, BALF5	B08.01=700
YAVCRDKLSL	EBV, BALF5	B08.01=330
YFYASAPQGL	EBV, BALF5	A24.02=650
YILDRARHI	EBV, BALF5	A02.01=740;B08.01=770
YLRLIPKCF	EBV, BALF5	B08.01=330
YPSIIQAHNL	EBV, BALF5	B07.02=76
YQGATVIQPL	EBV, BALF5	A02.01=62
YSINPASLGK	EBV, BALF5	A03.01=230
YTDLREGRV	EBV, BALF5	A01.01=510
YVFVEPKGGV	EBV, BALF5	A02.01=650
AYSARPPPL	EBV, RPMS1	A24.02=550
CAYSARPPPL	EBV, RPMS1	B07.02=420;B08.01=770
GARRRARCPA	EBV, RPMS1	B08.01=240
GPGRRARSCL	EBV, RPMS1	B07.02=52;B08.01=650
MAGARRRARC	EBV, RPMS1	B08.01=460
RPGPGRRARS	EBV, RPMS1	B07.02=520
RPPHSRTRA	EBV, RPMS1	B07.02=93
RRRARCPASA	EBV, RPMS1	B08.01=610
SGQPRWWPW	EBV, RPMS1	A24.02=350
STRGRRISA	EBV, RPMS1	B07.02=990;B08.01=240
WPWGSPPPL	EBV, RPMS1	B07.02=7.8
WWPWGSPPPL	EBV, RPMS1	A24.02=650
AMFCAWKLY	EBV, A73	A03.01=120
AMFCAWKLYS	EBV, A73	A02.01=700
AVKAAAQSAF	EBV, A73	B07.02=750

Peptide	Virus, Gene	Affinity
CAWKLYSRK	EBV, A73	A03.01=350
FADAWRMARY	EBV, A73	A01.01=11
KLYSRKMPS	EBV, A73	A03.01=400
KLYSRKMPSR	EBV, A73	A03.01=19
KPPTVLTRPA	EBV, A73	B07.02=540
KTLEARCSSR	EBV, A73	A03.01=770
MARYWGCAV	EBV, A73	B07.02=37;B08.01=51
MARYWGCAVK	EBV, A73	A03.01=420
MPSRSKTLEA	EBV, A73	B07.02=92;B08.01=910
MSMPPKGFLK	EBV, A73	A03.01=29
PPTVLTRPAM	EBV, A73	B07.02=350
RGWRARRLGK	EBV, A73	A03.01=290
RKMPSRSKTL	EBV, A73	B07.02=580
RLGKESWFA	EBV, A73	A02.01=56
RLLNKPPTV	EBV, A73	A02.01=21
RLLNKPPTVL	EBV, A73	A02.01=290
RMARYWGCAV	EBV, A73	A02.01=41;B08.01=740
RPAMFCAWKL	EBV, A73	B07.02=40
SMPPKGFLK	EBV, A73	A03.01=200
SMPPKGFLKK	EBV, A73	A03.01=230
SPACQTRDT	EBV, A73	B07.02=440
SWFADAWRM	EBV, A73	A24.02=530
VLTRPAMFCA	EBV, A73	A02.01=650
WRMARYWGC	EBV, A73	B08.01=880
WRMARYWGCA	EBV, A73	B08.01=190
YSRKMPSRSK	EBV, A73	A03.01=680
AARDRFPGL	EBV, BALF4	B07.02=720;B08.01=270
AARGSTSAA	EBV, BALF4	B07.02=110
AARGSTSAAV	EBV, BALF4	B07.02=61
AFLDKGTYTL	EBV, BALF4	A24.02=960
ALHEQNQEQK	EBV, BALF4	A03.01=730
APGKSLGTL	EBV, BALF4	B07.02=16
APPAARGST	EBV, BALF4	B07.02=170
AQNIAGLRK	EBV, BALF4	A03.01=570
ATLQTFISL	EBV, BALF4	A02.01=980
ATRQQTSFPF	EBV, BALF4	B07.02=720
ATVQIQFAY	EBV, BALF4	A01.01=170
CLEQKRQNM	EBV, BALF4	B08.01=650
CLITDMMAK	EBV, BALF4	A03.01=74
CPLQHWQTF	EBV, BALF4	B07.02=88;B08.01=630
CQATSQYYF	EBV, BALF4	A24.02=620
CYSRPLVSF	EBV, BALF4	A24.02=12
DMMAKSNSPF	EBV, BALF4	B08.01=190
DSFHVRTNYK	EBV, BALF4	A03.01=850
ELMDSLGSV	EBV, BALF4	A02.01=21
ELYDAPGWLI	EBV, BALF4	A02.01=400
ENRTAYCPL	EBV, BALF4	B08.01=680

Peptide	Virus, Gene	Affinity
EQKRQNMVL	EBV, BALF4	B08.01=470
ETDQMDTIY	EBV, BALF4	A01.01=13
ETMCYSRPL	EBV, BALF4	B08.01=160
FFKNPFGGML	EBV, BALF4	B08.01=980
FISLNTSLI	EBV, BALF4	A02.01=140
FITSGGLLL	EBV, BALF4	A02.01=350
FITSGGLLLA	EBV, BALF4	A02.01=330
FLDKGTYTL	EBV, BALF4	A02.01=3
FLDKGTYTLS	EBV, BALF4	A02.01=200
FLTKKMTEV	EBV, BALF4	A02.01=10;B08.01=980
FPGLRRRRY	EBV, BALF4	B07.02=850
FQAQNIAGL	EBV, BALF4	A02.01=25
FQSGNEIHV	EBV, BALF4	A02.01=79
FSFINDTKTY	EBV, BALF4	A01.01=740
FVDGLGELM	EBV, BALF4	A01.01=760
FVTNTTVGI	EBV, BALF4	A02.01=340
FYDGKNKETF	EBV, BALF4	A24.02=190
GFISFFKNPF	EBV, BALF4	A24.02=160
GIATLQTFI	EBV, BALF4	A02.01=490
GIFREYNFQA	EBV, BALF4	A02.01=310
GLFSSLVSG	EBV, BALF4	A02.01=870
GLFSSLVSGF	EBV, BALF4	A02.01=850
GLGELMDSL	EBV, BALF4	A02.01=73
GLLLAWLPL	EBV, BALF4	A02.01=85
GLLLAWLPLT	EBV, BALF4	A02.01=280
GLLMVFKDNI	EBV, BALF4	A02.01=610
GLRKDLDNAV	EBV, BALF4	A02.01=680
GMLILVLVA	EBV, BALF4	A02.01=570
GPSVASRAL	EBV, BALF4	B07.02=7.9
GQEAITYFI	EBV, BALF4	A02.01=430
GTDNEIFLTK	EBV, BALF4	A03.01=690
GTLNNPATV	EBV, BALF4	A02.01=940
GTYTLSWKL	EBV, BALF4	A02.01=410
GVNITVNLK	EBV, BALF4	A03.01=120
HTEGLLMVFK	EBV, BALF4	A03.01=450
HVYNDYHHFK	EBV, BALF4	A03.01=22
HWQTFDSTI	EBV, BALF4	A24.02=490
ILIYNGWYA	EBV, BALF4	A02.01=15
IQFAYDSLR	EBV, BALF4	A03.01=920
IQFAYDSLRR	EBV, BALF4	A03.01=610
IYNGWYADSV	EBV, BALF4	A24.02=290
IYQCYNAVKM	EBV, BALF4	A24.02=140
KGTYTLSWK	EBV, BALF4	A03.01=570
KIVTNILIY	EBV, BALF4	A03.01=460
KMTEVCQAT	EBV, BALF4	A02.01=400
KMTKDGLTRV	EBV, BALF4	A02.01=96
KPTGGLANGV	EBV, BALF4	B07.02=580

Peptide	Virus, Gene	Affinity
KSNSPFDFFV	EBV, BALF4	A02.01=570
KTMHEKYEAV	EBV, BALF4	A02.01=250
LARAWCLEQK	EBV, BALF4	A03.01=920
LFSSLVSGF	EBV, BALF4	A24.02=490
LILVLVAGV	EBV, BALF4	A02.01=58
LILVLVAGVV	EBV, BALF4	A02.01=940
LLAALACRL	EBV, BALF4	A02.01=37
LLAWLPLTPR	EBV, BALF4	A03.01=440
LLLAWLPLT	EBV, BALF4	A02.01=21
LLMVFKDNI	EBV, BALF4	A02.01=130
LLMVFKDNII	EBV, BALF4	A02.01=320;B08.01=700
LPLTPRSLA	EBV, BALF4	B07.02=250
LPLTPRSLAT	EBV, BALF4	B07.02=110
LRRQINRML	EBV, BALF4	B08.01=770
LTPRSLATV	EBV, BALF4	A02.01=900
LVAGVVILV	EBV, BALF4	A02.01=46
LVSGFISFFK	EBV, BALF4	A03.01=27
LYDAPGWLI	EBV, BALF4	A24.02=110
LYDAPGWLIW	EBV, BALF4	A24.02=170
MCYSRPLVSF	EBV, BALF4	B08.01=760
MLILVLVAGV	EBV, BALF4	A02.01=7.6
MLYPGIDEL	EBV, BALF4	A02.01=4.2
MLYPGIDELA	EBV, BALF4	A02.01=16
MMAKSNSPF	EBV, BALF4	A02.01=920;A24.02=240;B07.02=290;B08.01=630
MSQQPVQMLY	EBV, BALF4	A01.01=67
MTRRRVLSV	EBV, BALF4	B07.02=200;B08.01=7.4
MTRRRVLSVV	EBV, BALF4	B07.02=490;B08.01=39
MVFKDNIIPY	EBV, BALF4	A03.01=360
MVLRELTKI	EBV, BALF4	A02.01=580
NIDFASLELY	EBV, BALF4	A01.01=24
NIIPYSFKV	EBV, BALF4	A02.01=11
NILIYNGWYA	EBV, BALF4	A02.01=470
NLTELTTPT	EBV, BALF4	A02.01=1000
NMVLRELTK	EBV, BALF4	A03.01=840
NPATVQIQF	EBV, BALF4	B07.02=810
NPFGGMLIL	EBV, BALF4	B07.02=110
NPQGERRAF	EBV, BALF4	B07.02=48
NPQGERRAFL	EBV, BALF4	B07.02=73
NPTTVMSSI	EBV, BALF4	B07.02=240
PPAAPGKSL	EBV, BALF4	B07.02=84
PPAARGSTSA	EBV, BALF4	B07.02=800
QMDTIYQCY	EBV, BALF4	A01.01=75
QMLYPGIDEL	EBV, BALF4	A02.01=210
QPAPPATTV	EBV, BALF4	B07.02=95
QQTSFPFRV	EBV, BALF4	A02.01=400
QTVEMSPFY	EBV, BALF4	A01.01=320
QVNKTMHEK	EBV, BALF4	A03.01=520

Peptide	Virus, Gene	Affinity
QYYFQSGNEI	EBV, BALF4	A24.02=320
RMLGDLARA	EBV, BALF4	A02.01=47
RMLGDLARAW	EBV, BALF4	A24.02=1000
RPLVSFSFI	EBV, BALF4	B07.02=87
RQQTSFPFR	EBV, BALF4	A03.01=790
RQQTSFPFRV	EBV, BALF4	A02.01=250
RTAYCPLQH	EBV, BALF4	A03.01=420
RTNYKIVDY	EBV, BALF4	A03.01=890
RTRQMSQQPV	EBV, BALF4	B07.02=160
RTRTTVNCL	EBV, BALF4	B07.02=220
RVLSVVVLL	EBV, BALF4	A02.01=150
RVLSVVVLLA	EBV, BALF4	A02.01=500
RYASQTELY	EBV, BALF4	A24.02=870
RYTKGQEAI	EBV, BALF4	A24.02=350
SFHVRTNYK	EBV, BALF4	A03.01=1000
SFHVRTNYKI	EBV, BALF4	A24.02=580
SFKVRSYTKI	EBV, BALF4	B08.01=410
SFPFRVCEL	EBV, BALF4	A24.02=670;B08.01=970
SITNLVSTV	EBV, BALF4	A02.01=370
SIYGKAVAA	EBV, BALF4	A02.01=490
SIYGKAVAAK	EBV, BALF4	A03.01=17
SLGSVGQSI	EBV, BALF4	A02.01=790
SLIENIDFA	EBV, BALF4	A02.01=7.7
SLIENIDFAS	EBV, BALF4	A02.01=320
SLNTSLIENI	EBV, BALF4	A02.01=76
SLRRQINRM	EBV, BALF4	B08.01=77
SLRRQINRML	EBV, BALF4	B07.02=560;B08.01=80
SLTRRTRQM	EBV, BALF4	B08.01=55
SLVSGFISF	EBV, BALF4	A02.01=990
SLVSGFISFF	EBV, BALF4	A02.01=770
SMRVPGSETM	EBV, BALF4	B07.02=270;B08.01=780
SPPSSPSPPA	EBV, BALF4	B07.02=260
SPSPPAPPA	EBV, BALF4	B07.02=55
SPSPPAPPAA	EBV, BALF4	B07.02=58
STIATETGK	EBV, BALF4	A03.01=320
STVGGLFSSL	EBV, BALF4	A02.01=490
SVGQSITNLV	EBV, BALF4	A02.01=810
SVVVLLAAL	EBV, BALF4	A02.01=980
SYTKIVTNI	EBV, BALF4	A24.02=62
SYTKIVTNIL	EBV, BALF4	A24.02=120
TFHERADSF	EBV, BALF4	A24.02=860
TFISLNTSLI	EBV, BALF4	A24.02=200
TIYQCYNAV	EBV, BALF4	A02.01=160
TIYQCYNAVK	EBV, BALF4	A03.01=43
TMCYSRPLV	EBV, BALF4	A02.01=630
TMHEKYEAV	EBV, BALF4	A02.01=61;B08.01=300
TTVMSSIYGK	EBV, BALF4	A03.01=220

Peptide	Virus, Gene	Affinity
TVGGLFSSLV	EBV, BALF4	A02.01=690
TVMSSIYGK	EBV, BALF4	A03.01=44
TYFITSGGLL	EBV, BALF4	A24.02=480
VILVISLTR	EBV, BALF4	A03.01=750
VILVISLTRR	EBV, BALF4	A03.01=850
VISVSQCVPV	EBV, BALF4	A02.01=510
VLLAALACR	EBV, BALF4	A03.01=980
VLLAALACRL	EBV, BALF4	A02.01=31
VLRRRRRDA	EBV, BALF4	B08.01=190
VLRRRRRDAG	EBV, BALF4	B08.01=490
VLSVVVLLA	EBV, BALF4	A02.01=340
VLSVVVLLAA	EBV, BALF4	A02.01=250
VLVAGVVIL	EBV, BALF4	A02.01=150
VLVAGVVILV	EBV, BALF4	A02.01=21
VMSSIYGKA	EBV, BALF4	A02.01=910
VMSSIYGKAV	EBV, BALF4	A02.01=140
VPPAAPGKSL	EBV, BALF4	B07.02=82
VPVNQATVTL	EBV, BALF4	B07.02=42
VSFSFINDTK	EBV, BALF4	A03.01=220
VSGFISFFK	EBV, BALF4	A03.01=65
VTDEGTSSF	EBV, BALF4	A01.01=110
VTDEGTSSFV	EBV, BALF4	A01.01=220;A02.01=150
VYVDRDGVNI	EBV, BALF4	A24.02=690
YADSVTNRH	EBV, BALF4	A01.01=950
YCPLQHWQTF	EBV, BALF4	A24.02=690
YFITSGGLLL	EBV, BALF4	A24.02=630
YSFKVRSYTK	EBV, BALF4	A03.01=52
YSRPLVSFSF	EBV, BALF4	A24.02=900
YTKGQEAITY	EBV, BALF4	A01.01=940
YYFQSGNEI	EBV, BALF4	A24.02=38
AAAYSQVYAL	EBV, BALF3	B07.02=440
AAFENSKYIK	EBV, BALF3	A03.01=430
AAFTAHPQYR	EBV, BALF3	A03.01=790
AAYSQVYAL	EBV, BALF3	A02.01=610;B07.02=490;B08.01=980
AGARLTHLF	EBV, BALF3	A24.02=640
AILLPRLRR	EBV, BALF3	A03.01=490
AILQKRNEL	EBV, BALF3	B08.01=250
ALAGARLTH	EBV, BALF3	A03.01=800
ALAGARLTHL	EBV, BALF3	A02.01=120
ALARCCDAA	EBV, BALF3	A02.01=570
ALAVELSVCA	EBV, BALF3	A02.01=170
ALDTYNVFST	EBV, BALF3	A02.01=150
ALFARERRL	EBV, BALF3	B08.01=800
ALKRKEQYL	EBV, BALF3	B08.01=100
ALLYLHLQM	EBV, BALF3	A02.01=540
ALPPDGLYL	EBV, BALF3	A02.01=330
ALPPDGLYLT	EBV, BALF3	A02.01=940

Peptide	Virus, Gene	Affinity
ALREAVLTV	EBV, BALF3	A02.01=40
ALSDALKRK	EBV, BALF3	A03.01=320
ALSLELVHL	EBV, BALF3	A02.01=130
ALSLELVHLL	EBV, BALF3	A02.01=50
ALTHSLYTF	EBV, BALF3	A24.02=540
ALTHSLYTFI	EBV, BALF3	A02.01=35
APGATERLF	EBV, BALF3	B07.02=990
APGRKGTRV	EBV, BALF3	B07.02=210
AVLTVSLYNK	EBV, BALF3	A03.01=60
AYSQVYALA	EBV, BALF3	A24.02=540
AYSQVYALAV	EBV, BALF3	A24.02=330
CARLDPRSL	EBV, BALF3	B07.02=530;B08.01=940
CLAQQSETL	EBV, BALF3	A02.01=570
CLINDIEIL	EBV, BALF3	A02.01=220
CLINDIEILM	EBV, BALF3	A02.01=540
DLLGRFRGV	EBV, BALF3	B08.01=620
DLYALLYLHL	EBV, BALF3	A02.01=620
DPRLYITSV	EBV, BALF3	B07.02=430;B08.01=220
DPRSLDVAAV	EBV, BALF3	B07.02=580
DSDRPLILLY	EBV, BALF3	A01.01=10
DWIETSFNSF	EBV, BALF3	A24.02=810
EILMKRINSV	EBV, BALF3	B08.01=120
ELARMRNHFL	EBV, BALF3	B08.01=350
ELYTRLNRA	EBV, BALF3	B08.01=550
ELYTRLNRAM	EBV, BALF3	B08.01=130
EVAELSELLY	EBV, BALF3	A01.01=220
FARERRLAL	EBV, BALF3	B07.02=8.5;B08.01=3.6
FARERRLALV	EBV, BALF3	B07.02=910;B08.01=20
FKDLYALLY	EBV, BALF3	A01.01=480
FLTSLIWPG	EBV, BALF3	A02.01=30
FLTSLIWPGI	EBV, BALF3	A02.01=6.6
FLYRRAFVS	EBV, BALF3	A02.01=230;B08.01=600
FLYRRAFVSG	EBV, BALF3	A02.01=610;B08.01=380
FPPPSNVAL	EBV, BALF3	B07.02=44
FYCMSHTMGL	EBV, BALF3	A24.02=660
GGHSRLSAL	EBV, BALF3	B08.01=800
GLAKLQSCL	EBV, BALF3	A02.01=350
GLAKLQSCLA	EBV, BALF3	A02.01=390
GLFENEVRQA	EBV, BALF3	A02.01=140
GLFPPPSNV	EBV, BALF3	A02.01=25
GLFPPPSNVA	EBV, BALF3	A02.01=430
GLLAAAYSQV	EBV, BALF3	A02.01=10
GLLAELEAI	EBV, BALF3	A02.01=25
GLLAELEAIL	EBV, BALF3	A02.01=100
GPSGALSDAL	EBV, BALF3	B07.02=47
HALTHSLYTF	EBV, BALF3	A24.02=630
HFLYRRAFV	EBV, BALF3	B08.01=350

Peptide	Virus, Gene	Affinity
HLFDAVAPG	EBV, BALF3	A02.01=420
HLFDAVAPGA	EBV, BALF3	A02.01=12
HLHALTHSL	EBV, BALF3	A02.01=78;B07.02=450;B08.01=160
HLHALTHSLY	EBV, BALF3	A03.01=120
HLLEAEEKA	EBV, BALF3	A02.01=810
HLLENSREA	EBV, BALF3	A02.01=350
HLQMRDDSA	EBV, BALF3	B08.01=870
HQKAFLTSL	EBV, BALF3	B08.01=450
HVSCNHLAV	EBV, BALF3	A02.01=960;B07.02=950
ILCRALREAV	EBV, BALF3	A02.01=390
ILLYKGRGWV	EBV, BALF3	A02.01=930
ILMKRINSV	EBV, BALF3	A02.01=6.7;B08.01=9.4
ILMKRINSVF	EBV, BALF3	A24.02=660;B08.01=75
IWPGIEPSDW	EBV, BALF3	A24.02=350
KTWGRSLIL	EBV, BALF3	A02.01=650
KTWGRSLILR	EBV, BALF3	A03.01=120
KVDVSLCLI	EBV, BALF3	A02.01=900
LAAAYSQVY	EBV, BALF3	A01.01=800
LAGARLTHL	EBV, BALF3	B08.01=320
LARMRNHFL	EBV, BALF3	B07.02=350;B08.01=22
LFARERRLAL	EBV, BALF3	B08.01=280
LGRFRGVSPI	EBV, BALF3	B08.01=300
LILRRADAV	EBV, BALF3	B08.01=350
LINDIEILM	EBV, BALF3	A02.01=880
LINDIEILMK	EBV, BALF3	A03.01=190
LLAAAYSQV	EBV, BALF3	A02.01=12
LLAAAYSQVY	EBV, BALF3	A03.01=850
LLAELEAIL	EBV, BALF3	A02.01=28
LLAELEAILL	EBV, BALF3	A02.01=17
LLYKGRGWV	EBV, BALF3	A02.01=460
LLYKGRGWVF	EBV, BALF3	B08.01=720
LLYLHLQMR	EBV, BALF3	A03.01=410
LLYWNSGGH	EBV, BALF3	A03.01=610
LLYWNSGGHA	EBV, BALF3	A02.01=750
LMKRINSVF	EBV, BALF3	B08.01=130
LMKRINSVFY	EBV, BALF3	A03.01=450
LPNHGNPST	EBV, BALF3	B07.02=400
LPNHGNPSTA	EBV, BALF3	B07.02=240
LSLELVHLL	EBV, BALF3	A02.01=630
LVRRACEEAL	EBV, BALF3	B07.02=370
LYALLYLHL	EBV, BALF3	A24.02=64
LYITSVPCW	EBV, BALF3	A24.02=60
LYKGRGWVF	EBV, BALF3	A24.02=68
LYNKTWGRSL	EBV, BALF3	A24.02=630
LYTFITGPL	EBV, BALF3	A24.02=350
LYWNSGGHAI	EBV, BALF3	A24.02=150
MSGLLAAAY	EBV, BALF3	A01.01=91

Peptide	Virus, Gene	Affinity
NSREASAAL	EBV, BALF3	B07.02=230
NVFSTVPPEV	EBV, BALF3	A02.01=46
PQYRAILQK	EBV, BALF3	A03.01=850
QVYALAVEL	EBV, BALF3	A02.01=520
RAILQKRNEL	EBV, BALF3	B07.02=260
RLDPRSLDV	EBV, BALF3	A02.01=500
RLFCGGVYS	EBV, BALF3	A02.01=390
RLFCGGVYSS	EBV, BALF3	A02.01=150
RLNRAMQRL	EBV, BALF3	A02.01=810
RLSALFARER	EBV, BALF3	A03.01=400
RLTHLFDAV	EBV, BALF3	A02.01=90
RMRNHFLYR	EBV, BALF3	A03.01=17
RMRNHFLYRR	EBV, BALF3	A03.01=61
RPAGAREPG	EBV, BALF3	B07.02=200
RPGGPEEGAV	EBV, BALF3	B07.02=180
RQAGLGHLL	EBV, BALF3	A02.01=460
RQVATEGLAK	EBV, BALF3	A03.01=780
RTPSVAYSV	EBV, BALF3	A02.01=430
SAALLAPGRK	EBV, BALF3	A03.01=1000
SLASSQQIL	EBV, BALF3	A02.01=600
SLCLINDIEI	EBV, BALF3	A02.01=690
SLILRRADA	EBV, BALF3	B08.01=330
SLILRRADAV	EBV, BALF3	A02.01=490;B08.01=190
SLYNKTWGR	EBV, BALF3	A03.01=170
SLYTFITGPL	EBV, BALF3	A02.01=31;B08.01=790
SPEVPRPAGA	EBV, BALF3	B07.02=630
SPIPDPRLYI	EBV, BALF3	B07.02=150
SQVYALAVEL	EBV, BALF3	A02.01=340
SVAYSVEFY	EBV, BALF3	A01.01=690
SVEFYGGHK	EBV, BALF3	A03.01=900
SVFYCMSHTM	EBV, BALF3	A02.01=900
TLSSEHLHAL	EBV, BALF3	A02.01=99
TLTETLCLRV	EBV, BALF3	A02.01=45
TPSVAYSVEF	EBV, BALF3	B07.02=180
TSFNSFYSV	EBV, BALF3	A02.01=210
TYDSDRPLI	EBV, BALF3	A24.02=990
VAELSELLY	EBV, BALF3	A01.01=74
VFKDLYALL	EBV, BALF3	A24.02=390
VFYCMSHTM	EBV, BALF3	A24.02=350
VLTVSLYNK	EBV, BALF3	A03.01=88
VPCWRCVGEL	EBV, BALF3	B07.02=170
VPGPGRPEA	EBV, BALF3	B07.02=260
VPPLRTPSV	EBV, BALF3	B07.02=250;B08.01=470
VPPLRTPSVA	EBV, BALF3	B07.02=850
VPRPAGARE	EBV, BALF3	B07.02=720
VPRPAGAREP	EBV, BALF3	B07.02=620
VVYWELARMR	EBV, BALF3	A03.01=660

Peptide	Virus, Gene	Affinity
VWGDVVYWEL	EBV, BALF3	A24.02=97
VYALAVELSV	EBV, BALF3	A24.02=340
WIETSFNSFY	EBV, BALF3	A01.01=120
WVFKDLYAL	EBV, BALF3	A02.01=46
WVFKDLYALL	EBV, BALF3	A02.01=130
YALAVELSV	EBV, BALF3	A02.01=110
YALLYLHLQM	EBV, BALF3	B08.01=690
YCMSHTMGL	EBV, BALF3	A02.01=940;B08.01=420
YLRQVATEGL	EBV, BALF3	A02.01=180;B07.02=860;B08.01=570
YLTYDSDRPL	EBV, BALF3	A02.01=98
YNKTWGRSL	EBV, BALF3	B08.01=240
YTFITGPLA	EBV, BALF3	A02.01=960
YWNSGGHAI	EBV, BALF3	A24.02=770
AARPRLLLSL	EBV, BARF0	B07.02=58;B08.01=70
AARVPIEEL	EBV, BARF0	B07.02=950
AGMSATLPL	EBV, BARF0	B07.02=550
ALLRQAGLQL	EBV, BARF0	A02.01=450
ALLWAARPR	EBV, BARF0	A03.01=590
ALLWAARPRL	EBV, BARF0	A02.01=130
ALPGRLLLA	EBV, BARF0	A02.01=620
APAGHRGDV	EBV, BARF0	B07.02=23
APAGHRGDVE	EBV, BARF0	B07.02=840
APGYAVEAV	EBV, BARF0	B07.02=130
AVEAVEGGLY	EBV, BARF0	A01.01=540
AVEGGLYPV	EBV, BARF0	A02.01=63
AVRLQRLHRV	EBV, BARF0	B08.01=730
AVRQRVQVL	EBV, BARF0	B07.02=37;B08.01=47
CPRQRLLAG	EBV, BARF0	B07.02=190;B08.01=180
CPSRPGHLRA	EBV, BARF0	B07.02=680
CTDSMAARV	EBV, BARF0	A01.01=220
EPRVELVPL	EBV, BARF0	B07.02=64;B08.01=410
EPRVELVPLL	EBV, BARF0	B07.02=430
FQALQPHGV	EBV, BARF0	A02.01=44
GLALLRQAGL	EBV, BARF0	A02.01=910
GLGKQVCFDV	EBV, BARF0	A02.01=99
GLGQGLALL	EBV, BARF0	A02.01=96
GMSATLPLPR	EBV, BARF0	A03.01=410
GPLCRGRVA	EBV, BARF0	B07.02=150
GPLCRGRVAV	EBV, BARF0	B07.02=23;B08.01=360
GPPHQGQATL	EBV, BARF0	B07.02=180
GPRDEGVQA	EBV, BARF0	B07.02=670
GPRDEGVQAV	EBV, BARF0	B07.02=35
GVQRSGRPL	EBV, BARF0	B07.02=330
GVRREGGGAV	EBV, BARF0	B07.02=860
HAIKHAIDSL	EBV, BARF0	B07.02=700
HLAQVLLLA	EBV, BARF0	A02.01=99
HLAQVLLLAL	EBV, BARF0	A02.01=42

Peptide	Virus, Gene	Affinity
HLRALPGRL	EBV, BARF0	B07.02=970;B08.01=700
HLRALPGRLL	EBV, BARF0	B07.02=370;B08.01=700
HLRGHCREDV	EBV, BARF0	B08.01=680
HQLAHTAPA	EBV, BARF0	A02.01=360;B08.01=880
ILKGGTLAGA	EBV, BARF0	A02.01=860
KQVCFDVLGI	EBV, BARF0	A02.01=140
LALLRQAGL	EBV, BARF0	B08.01=170
LCRGRVAVV	EBV, BARF0	B08.01=400
LLASAQPLH	EBV, BARF0	A03.01=950
LLLASAQPL	EBV, BARF0	A02.01=20;B08.01=790
LLQDGPVLGV	EBV, BARF0	A02.01=18
LLRQAGLQL	EBV, BARF0	B07.02=110;B08.01=280
LLVRQRTCGV	EBV, BARF0	A02.01=300
LLWAARPRL	EBV, BARF0	A02.01=39
LLWAARPRLL	EBV, BARF0	A02.01=73;B08.01=860
LPGKQGREA	EBV, BARF0	B07.02=280
LPGRLLLASA	EBV, BARF0	B07.02=560
LPLPRCTDSM	EBV, BARF0	B07.02=40;B08.01=600
LPRCTDSMA	EBV, BARF0	B07.02=140
LPRCTDSMAA	EBV, BARF0	B07.02=44;B08.01=860
LQDGPVLGV	EBV, BARF0	A02.01=200
LRIHRHRQV	EBV, BARF0	B08.01=840
LRIHRHRQVV	EBV, BARF0	B08.01=490
LVRQRTCGV	EBV, BARF0	B07.02=690;B08.01=72
LWAARPRLLL	EBV, BARF0	A24.02=770
LYPVARLDAW	EBV, BARF0	A24.02=85
MSATLPLPR	EBV, BARF0	A03.01=760
PPHQGQATL	EBV, BARF0	B07.02=320
PPRARDRAL	EBV, BARF0	B07.02=44;B08.01=760
PPRARDRALL	EBV, BARF0	B07.02=230
QPCPRQRLL	EBV, BARF0	B07.02=540
QPHGVRHAI	EBV, BARF0	B07.02=22
QTLGGHLAQV	EBV, BARF0	A02.01=600
QVLRAQGLGK	EBV, BARF0	A03.01=430
RALLWAARPR	EBV, BARF0	A03.01=960
RALPGRLLL	EBV, BARF0	B07.02=230
RARDRALLWA	EBV, BARF0	B07.02=1000
RIHRHRQVV	EBV, BARF0	B07.02=340;B08.01=130
RLLLASAQPL	EBV, BARF0	A02.01=54
RLLLSLQQV	EBV, BARF0	A02.01=22
RLRIHRHRQV	EBV, BARF0	B07.02=530;B08.01=58
RPGHLRALPG	EBV, BARF0	B07.02=410
RPLCLRPPRA	EBV, BARF0	B07.02=320
RPPRARDRA	EBV, BARF0	B07.02=640
RPPRARDRAL	EBV, BARF0	B07.02=9.5
RPRLLLSLQQ	EBV, BARF0	B07.02=630
RVQVLRAQGL	EBV, BARF0	B07.02=700

Peptide	Virus, Gene	Affinity
RVREGAGRA	EBV, BARF0	B07.02=990
RVREGAGRAG	EBV, BARF0	B07.02=970
RVWDGTYAPK	EBV, BARF0	A03.01=52
SLQQVPEPSL	EBV, BARF0	A02.01=230
SQGHVAGWGK	EBV, BARF0	A03.01=950
TLGGHLAQV	EBV, BARF0	A02.01=18
TLGGHLAQVL	EBV, BARF0	A02.01=760
TYAPKAAQQI	EBV, BARF0	A24.02=61
VLLLALERV	EBV, BARF0	A02.01=31
VLRAQGLGK	EBV, BARF0	A03.01=33
VPIEELREF	EBV, BARF0	B07.02=790
VPLLQDGPV	EBV, BARF0	B07.02=170
VPLLQDGPVL	EBV, BARF0	B07.02=68
VVAHAGQLPV	EBV, BARF0	A02.01=530
WAARPRLLL	EBV, BARF0	B07.02=260
WPYQGSQERL	EBV, BARF0	B07.02=35
YPVARLDAW	EBV, BARF0	B07.02=250

Example 2: HLA Class I and Class II Binding Assays

[0265] The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

[0266] Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.22 transfectants are used as sources of HLA class I molecules. Cell lysates are prepared and HLA molecules purified in accordance with disclosed protocols (Sidney et al., Current Protocols in Immunology 18.3.1 (1998); Sidney, et al., J. Immunol. 154:247 (1995); Sette, et al., Mol. Immunol. 31:813 (1994)). HLA molecules are purified from lysates by affinity chromatography. The lysates are passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The anti-HLA column is then washed with 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, and PBS containing 0.4% n-octylglucoside and HLA molecules are eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, is added to the eluate to reduce the pH to ~8.0. Eluates are then concentrated by centrifugation in Centriprep 30 concentrators (Amicon, Beverly, MA). Protein content is evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, IL) and confirmed by SDS-PAGE.

[0267] A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette et al., Mol. Immunol. 31:813, 1994; Sidney et al., in Current Protocols in Immunology, Margulies, Ed., John Wiley & Sons, New York, Section 18.3,

1998). Briefly, purified MHC molecules (5 to 500nM) are incubated with various unlabeled peptide inhibitors and 1-10nM 125I-radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. All assays are at pH 7.0 with the exception of DRB1*0301, which was performed at pH 4.5, and DRB1*1601 (DR2w21β1) and DRB4*0101 (DRw53), which were performed at pH 5.0.

[0268] Following incubation, MHC-peptide complexes are separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215, Montgomeryville, PA). Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2β1) assay makes separation of bound from unbound peaks more difficult under these conditions, all DRB1*1501 (DR2w2β1) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mLs/min. The eluate from the TSK columns is passed through a Beckman 170 radioisotope detector, and radioactivity is plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound is determined.

[0269] Radiolabeled peptides are iodinated using the chloramine-T method. Typically, in preliminary experiments, each MHC preparation is titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays are performed using these HLA concentrations.

[0270] Since under these conditions [label]<[HLA] and IC_{50}≥[HLA], the measured IC_{50} values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC_{50} of a positive control for inhibition by the IC_{50} for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC_{50} nM values by dividing the IC_{50} nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

[0271] Because the antibody used for HLA-DR purification (LB3.1) is α-chain specific, β1 molecules are not separated from β3 (and/or β4 and β5) molecules. The β1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and

DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404 (DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2β1), DRB5*0101 (DR2w2β2), DRB1*1601 (DR2w21β1), DRB5*0201 (DR51Dw21), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DRβ molecule specificity have been described previously (see, e.g., Southwood et al., J. Immunol. 160:3363-3373, 1998).

[0272] The live cell/flow cytometry-based assays can also be used. This is a well-established assay utilizing the TAP-deficient hybridoma cell line T2 (American Type Culture Collection (ATCC Accession No. CRL-1992), Manassas, Va.). The TAP deficiency in this cell line leads to inefficient loading of MHCI in the ER and an excess of empty MHCIs. Salter and Cresswell, EMBO J. 5:943-49 (1986); Salter, Immunogenetics 21:235-46 (1985). Empty MHCIs are highly unstable, and are therefore short-lived. When T2 cells are cultured at reduced temperatures, empty MHCIs appear transiently on the cell surface, where they can be stabilized by the exogenous addition of MHCI-binding peptides. To perform this binding assay, peptide-receptive MHCIs were induced by culturing aliquots of 10^7 T2 cells overnight at 26°C in serum free AIM-V medium alone, or in medium containing escalating concentrations (0.1 to 100 μM) of peptide. Cells were then washed twice with PBS, and subsequently incubated with a fluorescent tagged HLA-A0201-specific monoclonal antibody, BB7.2, to quantify cell surface expression. Samples were acquired on a FACS Calibur instrument (Becton Dickinson) and the mean fluorescence intensity (MFI) determined using the accompanying Cellquest software.

Example 3: Confirmation of Immunogenicity

[0273] In vitro education (IVE) assays are used to test the ability of each test peptide to expand CD8+ T-cells. Mature professional APCs are prepared for these assays in the following way. 80-90x10^6 PBMCs isolated from a healthy human donor are plated in 20 ml of RPMI media containing 2% human AB serum, and incubated at 37°C for 2 hours to allow for plastic adherence by monocytes. Non-adherent cells are removed and the adherent cells are cultured in RPMI, 2% human AB serum, 800 IU/ml of GM-CSF and 500 IU/ml of IL-4. After 6 days, TNF-alpha is added to a final concentration of 10 ng/ml. On day 7, the dendritic cells (DC) are matured either by the addition of 12.5 μg/ml poly I:C or 0.3 μg/ml of CD40L. The mature dendritic cells (mDC) are harvested on day 8, washed, and either used directly or cryopreserved for future use.

[0274] For the IVE of CD8+ T-cells, aliquots of 2x10^5 mDCs are pulsed with each peptide at a final concentration of 100 μM, incubated for 4 hours at 37°C, and then irradiated (2500 rads). The peptide-pulsed mDCs are washed twice in RPMI containing 2% human AB serum. 2x10^5 mDCs and

$2x10^6$ autologous CD8+ cells are plated per well of a 24-well plate in 2 ml of RPMI containing 2% human AB, 20 ng/ml IL-7 and 100 pg/ml of IL-12, and incubated for 12 days. The CD8+ T-cells are then re-stimulated with peptide-pulsed, irradiated mDCs. Two to three days later, 20 IU/ml IL-2 and 20 ng/IL7 are added. Expanding CD8+ T-cells are re-stimulated every 8-10 days, and are maintained in media containing IL-2 and IL-7. Cultures are monitored for peptide-specific T-cells using a combination of functional assays and/or tetramer staining. Parallel IVEs with the modified and parent peptides allowed for comparisons of the relative efficiency with which the peptides expanded peptide-specific T-cells.

Quantitative and Functional Assessment of CD8+ T-Cells

Tetramer Staining

[0275] MHC tetramers are purchased or manufactured on-site, and are used to measure peptide-specific T-cell expansion in the IVE assays. For the assessment, tetramer is added to $1x10^5$ cells in PBS containing 1% FCS and 0.1% sodium azide (FACS buffer) according to manufacturer's instructions. Cells are incubated in the dark for 20 minutes at room temperature. Antibodies specific for T-cell markers, such as CD8, are then added to a final concentration suggested by the manufacturer, and the cells are incubated in the dark at 4^oC for 20 minutes. Cells are washed with cold FACS buffer and resuspended in buffer containing 1% formaldehyde. Cells are acquired on a FACS Calibur (Becton Dickinson) instrument, and are analyzed by use of Cellquest software (Becton Dickinson). For analysis of tetramer positive cells, the lymphocyte gate is taken from the forward and side-scatter plots. Data are reported as the percentage of cells that were CD8+/Tetramer+.

ELISPOT

[0276] Peptide-specific T-cells are functionally enumerated using the ELISPOT assay (BD Biosciences), which measures the release of IFNgamma from T-cells on a single cell basis. Target cells (T2 or HLA-A0201 transfected C1Rs) were pulsed with 10 μM peptide for 1 hour at 37^oC, and washed three times. $1x10^5$ peptide-pulsed targets are co-cultured in the ELISPOT plate wells with varying concentrations of T-cells ($5x10^2$ to 2x103) taken from the IVE culture. Plates are developed according to the manufacturer's protocol, and analyzed on an ELISPOT reader (Cellular Technology Ltd.) with accompanying software. Spots corresponding to the number of IFNgamma-producing T-cells are reported as the absolute number of spots per number of T-cells plated. T-cells expanded on modified peptides are tested not only for their ability to recognize targets pulsed with the modified peptide, but also for their ability to recognize targets pulsed with the parent peptide.

CD107 Staining

[0277] CD107a and b are expressed on the cell surface of CD8+ T-cells following activation with cognate peptide. The lytic granules of T-cells have a lipid bilayer that contains lysosomal-associated membrane glycoproteins ("LAMPs"), which include the molecules CD107a and b. When cytotoxic T-cells are activated through the T-cell receptor, the membranes of these lytic granules mobilize and fuse with the plasma membrane of the T-cell. The granule contents are released, and this leads to the death of the target cell. As the granule membrane fuses with the plasma membrane, C107a and b are exposed on the cell surface, and therefore are markers of degranulation. Because degranulation as measured by CD107 a and b staining is reported on a single cell basis, the assay is used to functionally enumerate peptide-specific T-cells. To perform the assay, peptide is added to HLA-A0201-transfected cells C1R to a final concentration of 20 μM, the cells were incubated for 1 hour at 37°C, and washed three times. $1x10^5$ of the peptide-pulsed C1R cells were aliquoted into tubes, and antibodies specific for CD107 a and b are added to a final concentration suggested by the manufacturer (Becton Dickinson). Antibodies are added prior to the addition of T-cells in order to "capture" the CD107 molecules as they transiently appear on the surface during the course of the assay. $1x10^5$ T-cells from the IVE culture are added next, and the samples were incubated for 4 hours at 37°C. The T-cells are further stained for additional cell surface molecules such as CD8 and acquired on a FACS Calibur instrument (Becton Dickinson). Data is analyzed using the accompanying Cellquest software, and results were reported as the percentage of CD8+ CD107 a and b+ cells.

CTL Lysis

[0278] Cytotoxic activity is measured using a chromium release assay. Target T2 cells are labeled for 1 hour at 37°C with $Na^{51}Cr$ and washed $5x10^3$ target T2 cells were then added to varying numbers of T-cells from the IVE culture. Chromium release is measured in supernatant harvested after 4 hours of incubation at 37°C. The percentage of specific lysis is calculated as:
Experimental release-spontaneous release/Total release-spontaneous releasex100

Example 4: Selection of CTL and HTL epitopes for inclusion in an tumor-specific vaccine.

[0279] This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition can be in the form of a nucleic acid sequence, either single or one or more sequences (i.e., minigene) that encodes peptide(s), or may be single and/or polyepitopic peptides.

[0280] Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For example, vaccine can include 1-2 epitopes that

come from at least one tumor antigen region. Epitopes from one region can be used in combination with epitopes from one or more additional tumor antigen regions.

[0281] Epitopes can be selected that have a binding affinity of an IC_{50} of 500 nM or less for an HLA class I molecule, or for class II, an IC_{50} of 1000 nM or less.

[0282] When creating a polyepitopic compositions, e.g. a minigene, it is typically desirable to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same, as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, e.g., by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope, which is not present in a native protein sequence.

[0283] Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in the Tables. A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response similar in magnitude of an immune response that inhibits tumor growth.

Example 5: Peptide Composition for Prophylactic or Therapeutic Uses

[0284] Immunogenic or vaccine compositions of the invention are used to inhibit tumor growth. For example, a polyepitopic composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes is administered to individuals having tumors. The composition is provided as a single lipidated polypeptide that encompasses multiple epitopes. The composition is administered in an aqueous carrier comprised of alum. The dose of peptide for the initial immunization is from about 1 to about 50,000 μg, generally 100-5,000 μg, for a 70 kg patient. The initial administration is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious to inhibit tumor growth.

[0285] Alternatively, the polyepitopic composition can be administered as a nucleic acid, for example as RNA, in accordance with methodologies known in the art and disclosed herein.

[0286] Non-mutated protein epitope binding agents, such as TCR or CARs can be can be administered in accordance with methodologies known in the art and disclosed herein. The binding

agents can be administered as polypeptides or polynucleotides, for example RNA, encoding the binding agents, or as a cellular therapy, by administering cells expressing the binding agents.

[0287] Non-mutated protein epitope peptides, polynucleotides, binding agents, or cells expressing these molecules can be delivered to the same patient via multiple methodologies known in the art, and can further be combined with other cancer therapies (e.g., chemotherapy, surgery, radiation, checkpoint inhibitors, etc.).

Example 6. Administration of Compositions Using Dendritic Cells

[0288] Vaccines comprising epitopes of the invention may be administered using dendritic cells. In this example, the peptide-pulsed dendritic cells can be administered to a patient to stimulate a CTL response *in vivo*. In this method dendritic cells are isolated, expanded, and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced CTL and HTL then destroy (CTL) or facilitate destruction (HTL) of the specific target tumor cells that bear the proteins from which the epitopes in the vaccine are derived.

[0289] Alternatively, *ex vivo* CTL or HTL responses to a particular tumor-associated antigen can be induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells, such as dendritic cells, and the appropriate immunogenic peptides.

[0290] After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, i.e., tumor cells.

Paragraphs of the Embodiments

[0291] An isolated antigenic peptide comprising an epitope from a sequence in Table 1 or 2.

[0292] An isolated antigenic peptide 100 amino acids or less in length which comprises an epitope from a sequence in Table 1 or 2.

[0293] An isolated antigenic peptide comprising an epitope from a sequence in Table 3 or 4.

[0294] An isolated antigenic peptide 100 amino acids or less in length which comprises an epitope from a sequence in Table 3 or 4.

[0295] An isolated antigenic peptide comprising an epitope from a sequence in Table 5 or 6.

[0296] An isolated antigenic peptide 100 amino acids or less in length which comprises an epitope from a sequence in Table 5 or 6.

[0297] The isolated antigenic peptide of paragraph [00291] or [00292], wherein the isolated antigenic peptide is a retroviral antigen.

[0298] The isolated antigenic peptide of paragraph [00293] or [00294], wherein the isolated antigenic peptide is a non-mutated overexpressed antigen.

[0299] The isolated antigenic peptide of paragraph [00295] or [00296], wherein the isolated antigenic peptide is a viral antigen.

[0300] The isolated antigenic peptide of any of paragraphs [00291]-[00299], which is between about 5 to about 50 amino acids in length.

[0301] The isolated antigenic peptide of any of paragraphs [00291]-[00300], which is between about 15 to about 35 amino acids in length.

[0302] The isolated antigenic peptide of paragraph [00301], which is about 15 amino acids or less in length.

[0303] The isolated antigenic peptide of paragraph [00302], which is between about 8 and about 11 amino acids in length.

[0304] The isolated antigenic peptide of paragraph [00303], which is 9 or 10 amino acids in length.

[0305] The isolated antigenic peptide of any of paragraphs [00291]-[00304], which binds major histocompatibility complex (MHC) class I.

[0306] The isolated antigenic peptide of paragraph [00305], which binds MHC class I with a binding affinity of less than about 500 nM.

[0307] The isolated antigenic peptide of any of paragraphs [00291]-[00296], which is about 30 amino acids or less in length.

[0308] The isolated antigenic peptide of paragraph [00307], which is between about 6 and about 25 amino acids in length.

[0309] The isolated antigenic peptide of paragraph [00308], which is between about 15 and about 24 amino acids in length.

[0310] The isolated antigenic peptide of paragraph [00308], which is between about 9 and about 15 amino acids in length.

[0311] The isolated antigenic peptide of any of paragraphs [00291]-[00296] and [00307]-[00310], which binds MHC class II.

[0312] The isolated antigenic peptide of paragraph [00311], which binds MHC class II with a binding affinity of less than about 1000 nM.

[0313] The isolated antigenic peptide of any of paragraphs [00291]-[00312], further comprising flanking amino acids.

[0314] The isolated antigenic peptide of paragraph [00313], wherein the flanking amino acids are not native flanking amino acids.

[0315] The isolated antigenic peptide of any of paragraphs [00291]-[00314], which is linked to at least a second antigenic peptide.

[0316] The isolated antigenic peptide of paragraph [00315], wherein peptides are linked using a poly-glycine or poly-serine linker.

[0317] The isolated antigenic peptide of paragraph [00315] or [00316], wherein the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 1000 nM.

[0318] The isolated antigenic peptide of paragraph [00317], wherein the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 500 nM.

[0319] The isolated antigenic peptide of paragraph [00317] or [00318], wherein both of the epitopes bind to human leukocyte antigen (HLA) -A, -B, -C, -DP, -DQ, or -DR.

[0320] The isolated antigenic peptide of any of paragraphs [00317]-[00319], wherein the isolated antigenic peptide binds a class I HLA and the second antigenic peptide binds a class II HLA.

[0321] The isolated antigenic peptide of any of paragraphs [00317]-[00319], wherein the isolated antigenic peptide binds a class II HLA and the second antigenic peptide binds a class I HLA.

[0322] The isolated antigenic peptide of any of paragraphs [00291]-[00321], further comprising modifications which increase in vivo half-life, cellular targeting, antigen uptake, antigen processing, MHC affinity, MHC stability, or antigen presentation.

[0323] The isolated antigenic peptide of paragraph [00322], wherein the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, PEGylation, polysialylation HESylation, recombinant PEG mimetics, Fc fusion, albumin fusion, nanoparticle attachment, nanoparticulate encapsulation, cholesterol fusion, iron fusion, acylation, amidation, glycosylation, side chain oxidation, phosphorylation, biotinylation, the addition of a surface active material, the addition of amino acid mimetics, or the addition of unnatural amino acids.

[0324] The isolated antigenic peptide of paragraph [00322], wherein the cells that are targeted are antigen presenting cells.

[0325] The isolated antigenic peptide of paragraph [00324], wherein the antigen presenting cells are dendritic cells.

[0326] The isolated antigenic peptide of paragraph [00325], wherein the dendritic cells are targeted using DEC205, XCR1, CD197, CD80, CD86, CD123, CD209, CD273, CD283, CD289, CD184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, CD141, CD11c, CD83, TSLP receptor, or CD1a marker.

[0327] The isolated antigenic peptide of paragraph [00326], wherein the dendritic cells are targeted using the CD141, DEC205, or XCR1 marker.

[0328] An in vivo delivery system comprising the isolated antigenic peptide of any of paragraphs [00291]-[00327].

[0329] The delivery system of paragraph [00328], wherein the delivery system includes cell-penetrating peptides, nanoparticulate encapsulation, virus like particles, or liposomes.

[0330] The delivery system of paragraph [00328], wherein the cell-penetrating peptide is TAT peptide, herpes simplex virus VP22, transportan, or Antp.

[0331] A cell comprising the isolated antigenic peptide of any of paragraphs [00291]-[00327].

[0332] The cell of paragraph [00331], which is an antigen presenting cell.

[0333] The cell of paragraph [00332], which is a dendritic cell.

[0334] A composition comprising the isolated antigenic peptide of any of paragraphs [00291]-[00327].

[0335] The composition of paragraph [00334], wherein the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated antigenic peptides comprising a tumor-specific epitope defined in Table 1 or 2.

[0336] The composition of paragraph [00334], wherein the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated antigenic peptides comprising a tumor-specific epitope defined in Table 3 or 4.

[0337] The composition of paragraph [00334], wherein the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated antigenic peptides comprising a tumor-specific epitope defined in Table 5 or 6.

[0338] The composition of any of paragraphs [00335]-[00337], wherein the composition comprises between 2 and 20 antigenic peptides.

[0339] The composition of any one of paragraphs [00334]-[00338], wherein the composition further comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at

least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 additional antigenic peptides.

[0340] The composition of paragraph [00339], wherein the composition comprises between about 4 and about 20 additional antigenic peptides.

[0341] The composition of any of paragraphs [00334]-[00340], wherein the additional antigenic peptide is specific for an individual patient's tumor.

[0342] The composition of paragraph [00341], wherein the patient specific antigenic peptide is selected by identifying sequence differences between the genome, exome, and/or transcriptome of the patient's tumor sample and the genome, exome, and/or transcriptome of a non-tumor sample.

[0343] The composition of paragraph [00337], wherein the samples are fresh or formalin-fixed paraffin embedded tumor tissues, freshly isolated cells, or circulating tumor cells.

[0344] The composition of paragraph [00342] or [00343], wherein the sequence differences are determined by Next Generation Sequencing.

[0345] An isolated polynucleotide encoding the isolated antigenic peptide of any of paragraphs [00291]-[00300].

[0346] The isolated polynucleotide paragraph [00345], which is RNA, optionally a self-amplifying RNA.

[0347] The isolated polynucleotide of paragraph [00346], wherein the RNA is modified to increase stability, increase cellular targeting, increase translation efficiency, adjuvanticity, cytosol accessibility, and/or decrease cytotoxicity.

[0348] The isolated polynucleotide of paragraph [00347], wherein the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, codon optimization, increased GC-content, incorporation of modified nucleosides, incorporation of 5'-cap or cap analog, and/or incorporation of an unmasked poly-A sequence.

[0349] A cell comprising the polynucleotide of any of paragraphs [00345]-[00348].

[0350] A vector comprising the polynucleotide of any one of paragraphs [00345]-[00348].

[0351] The vector of paragraph [00350], in which the polynucleotide is operably linked to a promoter.

[0352] The vector of paragraphs [00350] or [00351], which is a self-amplifying RNA replicon, plasmid, phage, transposon, cosmid, virus, or virion.

[0353] The vector of paragraph [00352], which is an adeno-associated virus, herpesvirus, lentivirus, or pseudotypes thereof.

[0354] An in vivo delivery system comprising the isolated polynucleotide of any of paragraphs [00345]-[00348].

[0355] The delivery system of paragraph [00350], wherein the delivery system includes spherical nucleic acids, viruses, virus-like particles, plasmids, bacterial plasmids, or nanoparticles.

[0356] A cell comprising the vector or delivery system of any of paragraphs [00350]-[00355].

[0357] The cell of paragraph [00356], which is an antigen presenting cell.

[0358] The cell of paragraph [00357], which is a dendritic cell.

[0359] The cell of paragraph [00358], which is an immature dendritic cell.

[0360] A composition comprising at least one polynucleotide of any of paragraphs [00345]-[00348].

[0361] The composition of paragraph [00360], wherein the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of the isolated polynucleotides.

[0362] The composition of paragraph [00361], wherein the composition comprises between about 2 and about 20 polynucleotides.

[0363] The composition of any one of paragraphs [00360]-[00362], wherein the composition further comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 additional antigenic polynucleotides encoding for additional antigenic peptides.

[0364] The composition of paragraph [00363], wherein the composition comprises between about 4 and about 20 additional antigenic polynucleotides.

[0365] The composition of paragraph [00363], wherein the isolated polynucleotides and the additional antigenic polynucleotides are linked.

[0366] The composition of paragraph [00365], wherein the polynucleotides are linked using nucleic acids that encode a poly-glycine or poly-serine linker.

[0367] The composition of any of paragraphs [00360]-[00366], wherein at least one of the additional antigenic peptide is specific for an individual patient's tumor.

[0368] The composition of paragraph [00367], wherein the patient specific antigenic peptide is selected by identifying sequence differences between the genome, exome, and/or transcriptome of the patient's tumor sample and the genome, exome, and/or transcriptome of a non-tumor sample.

[0369] The composition of paragraph [00368], wherein the samples are fresh or formalin-fixed paraffin embedded tumor tissues, freshly isolated cells, or circulating tumor cells.

[0370] The composition of paragraphs [00368] or [00369], wherein the sequence differences are determined by Next Generation Sequencing.

[0371] A T cell receptor (TCR) capable of binding at least one antigenic peptide listed in any of paragraphs [00291]-[00324].

[0372] The TCR of paragraph [00371], which is capable of binding the isolated antigenic peptide in the context of MHC class I or class II.

[0373] A chimeric antigen receptor comprising: (i) a T cell activation molecule; (ii) a transmembrane region; and (iii) an antigen recognition moiety capable of binding an isolated antigenic peptide of any one of paragraphs [00291]-[00324].

[0374] The chimeric antigen receptor of paragraph [00373], wherein CD3-zeta is the T cell activation molecule.

[0375] The chimeric antigen receptor of paragraph [00373] or [00374], further comprising at least one costimulatory signaling domain.

[0376] The chimeric antigen receptor of any of paragraphs [00373]-[00375], wherein the signaling domain is CD28, 4-1BB, ICOS, OX40, ITAM, or Fc epsilon RI-gamma.

[0377] The chimeric antigen receptor of any of paragraphs [00373]-[00376], wherein the antigen recognition moiety is capable of binding the isolated antigenic peptide in the context of MHC class I or class II.

[0378] The chimeric antigen receptor of any of paragraphs [00373]-[00377], comprising the CD3-zeta, CD28, CTLA-4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, Tim-3, A2aR, or PD-1 transmembrane region.

[0379] The chimeric antigen receptor of any of paragraphs [00373]-[00378], wherein the tumor-specific epitope is located in the extracellular domain of a tumor associated polypeptide.

[0380] A T cell comprising the T cell receptor or chimeric antigen receptor of any of paragraphs [00371]-[00379].

[0381] The T cell of paragraph [00380], which is a helper or cytotoxic T cell.

[0382] A nucleic acid comprising a promoter operably linked to a polynucleotide encoding the T cell receptor of paragraph [00371] or [00372].

[0383] The nucleic acid of paragraph [00382], wherein the TCR is capable of binding the at least one antigenic peptide in the context of major histocompatibility complex (MHC) class I or class II.

[0384] A nucleic acid comprising a promoter operably linked to a polynucleotide encoding the chimeric antigen receptor of any of paragraphs [00373]-[00379].

[0385] The nucleic acid of paragraph [00384], wherein the antigen recognition moiety is capable of binding the at least one antigenic peptide in the context of major histocompatibility complex (MHC) class I or class II.

[0386] The nucleic acid of paragraph [00384] or [00385], wherein the tumor-specific epitope is located in the extracellular domain of a tumor associated polypeptide.

[0387] The nucleic acid of any of paragraphs [00384]-[00386], comprising the CD3-zeta, CD28, CTLA-4, ICOS, BTLA, KIR, LAG3, CD137, OX40, CD27, CD40L, Tim-3, A2aR, or PD-1 transmembrane region.

[0388] An antibody capable of binding at least one antigenic peptide listed in Table 1 or 2.

[0389] An antibody capable of binding at least one antigenic peptide listed in Table 3 or 4.

[0390] An antibody capable of binding at least one antigenic peptide listed in Table 5 or 6.

[0391] An antibody of paragraph [00388], wherein the at least one antigenic peptide listed in Table 1 or 2 is a retroviral antigenic peptide.

[0392] An antibody of paragraph [00389], wherein the at least one antigenic peptide listed in Table 3 or 4 is a non-mutated overexpressed antigenic peptide.

[0393] An antibody of paragraph [00390], wherein the at least one antigenic peptide listed in Table 5 or 6 is a viral antigenic peptide.

[0394] A modified cell transfected or transduced with the nucleic acid of any one of paragraphs [00382]-[00387].

[0395] The modified cell of paragraph [00394], wherein the modified cell is a T cell, tumor infiltrating lymphocyte, NK-T cell, TCR-expressing cell, CD4+ T cell, CD8+ T cell, or NK cell.

[0396] A composition comprising the T cell receptor or chimeric antigen receptor of any of paragraphs [00371]-[00379].

[0397] A composition comprising autologous patient T cells containing the T cell receptor or chimeric antigen receptor of any of paragraphs [00371]-[00379].

[0398] The composition of paragraph [00395] or [00396], further comprising an immune checkpoint inhibitor.

[0399] The composition of paragraph [00396] or [00397], further comprising at least two immune checkpoint inhibitors.

[0400] The composition of paragraph [00398] or [00399], wherein each of the immune checkpoint inhibitors inhibits a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.

[0401] The composition of paragraph [00398] or [00399], wherein each of the immune checkpoint inhibitors interacts with a ligand of a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.

[0402] The composition of any of paragraphs [00334]-[00344], [00360]-[00369], and [00396]-[00401], further comprising an immune modulator or adjuvant.

[0403] The composition of paragraph [00402], wherein the immune modulator is a co-stimulatory ligand, a TNF ligand, an Ig superfamily ligand, CD28, CD80, CD86, ICOS, CD40L, OX40, CD27, GITR, CD30, DR3, CD69, or 4-1BB.

[0404] The composition of paragraph [00402], wherein the immune modulator is at least one cancer cell or cancer cell extract.

[0405] The composition of paragraph [00404], wherein the cancer cell is autologous to the subject in need of the composition.

[0406] The composition of paragraph [00405], wherein the cancer cell has undergone lysis or been exposed to UV radiation.

[0407] The composition of paragraph [00402], wherein the composition further comprises an adjuvant.

[0408] The composition of paragraph [00407], wherein the adjuvant is selected from the group consisting of: Poly(I:C), Poly-ICLC, STING agonist, 1018 ISS, aluminum salts, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312 VG, Montanide ISA 206 VG, Montanide ISA 50 V2, Montanide ISA 51 VG, OK-432, OM-174, OM-197-MP-EC, ISA-TLR2 agonist, ONTAK, PepTel®. vector system, PLG microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Pam3CSK4, acrylic or methacrylic polymers, copolymers of maleic anhydride, and QS21 stimulon.

[0409] The composition of paragraph [00407] or [00408], wherein the adjuvant induces a humoral when administered to a subject.

[0410] The composition of paragraph [00409], wherein the adjuvant induces a T helper cell type 1 when administered to a subject.

[0411] A method of inhibiting growth of a tumor cell expressing a tumor-specific epitope defined in Table 1 or 2, comprising contacting the tumor cell with the peptide, polynucleotide, delivery system, vector, composition, antibody, or cells of any of paragraphs [00291]-[00410].

[0412] A method of inhibiting growth of a tumor cell expressing a tumor-specific epitope defined in Table 3 or 4, comprising contacting the tumor cell with the peptide, polynucleotide, delivery system, vector, composition, antibody, or cells of any of paragraphs [00291]-[00410].

[0413] A method of inhibiting growth of a tumor cell expressing a tumor-specific epitope defined in Table 5 or 6, comprising contacting the tumor cell with the peptide, polynucleotide, delivery system, vector, composition, antibody, or cells of any of paragraphs [00291]-[00410].

[0414] A method of treating cancer or initiating, enhancing, or prolonging an anti-tumor response in a subject in need thereof comprising administering to the subject the peptide, polynucleotide, vector, composition, antibody, or cells of any of paragraphs [00291]-[00410].

[0415] The method of any of paragraphs [00411]-[00414], wherein the subject is a human.

[0416] The method of paragraph [00415], wherein the subject has cancer.

[0417] The method of paragraph [00416], wherein the cancer is selected from the group consisting of urogenital, renal, gynecological, lung, gastrointestinal , head and neck cancer, malignant glioblastoma, malignant mesothelioma, non-metastatic or metastatic breast cancer, malignant melanoma, Merkel Cell Carcinoma or bone and soft tissue sarcomas, hematologic neoplasias, multiple myeloma, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia, non-small cell lung cancer (NSCLC), triple-negative breast cancer (TNBC), smoldering myeloma (SMM), breast cancer, metastatic colorectal cancers, hormone sensitive or hormone refractory prostate cancer, colorectal cancer, ovarian cancer, hepatocellular cancer, renal cell cancer, pancreatic cancer, gastric cancer, esophageal cancers, hepatocellular cancers, cholangiocellular cancers, head and neck squamous cell cancer soft tissue sarcoma, and small cell lung cancer.

[0418] The method of any of paragraphs [00411]-[00417], wherein the subject has undergone surgical removal of the tumor.

[0419] The method of any of paragraphs [00411]-[00418], wherein the peptide, polynucleotide, vector, composition, or cells is administered via intravenous, intraperitoneal, intratumoral, intradermal, or subcutaneous administration.

[0420] The method of paragraph [00419], wherein the peptide, polynucleotide, vector, composition, or cells is administered into an anatomic site that drains into a lymph node basin.

[0421] The method of paragraph [00420], wherein administration is into multiple lymph node basins.

[0422] The method of any one of paragraphs [00411]-[00421], wherein administration is by a subcutaneous or intradermal route.

[0423] The method of paragraph [00419], wherein peptide is administered.

[0424] The method of paragraph [00423], wherein administration is intratumorally.

[0425] The method of paragraph [00419], wherein polynucleotide, optionally RNA, is administered.

[0426] The method of paragraph [00419] or [00425], wherein the polynucleotide is administered intravenously.

[0427] The method of paragraph [00419], wherein the cell is a T cell or dendritic cell.

[0428] The method of paragraph [00419] or [00427], wherein the peptide or polynucleotide comprises an antigen presenting cell targeting moiety.

[0429] The method of any of paragraphs [00411]-[00428], further comprising administering at least one immune checkpoint inhibitor to the subject.

[0430] The method of paragraph [00429], wherein the checkpoint inhibitor is a biologic therapeutic or a small molecule.

[0431] The method of paragraph [00429] or [00430], wherein the checkpoint inhibitor is selected from the group consisting of a monoclonal antibody, a humanized antibody, a fully human antibody and a fusion protein or a combination thereof.

[0432] The method of any of paragraphs [00429]-[00431], wherein the checkpoint inhibitor inhibits a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.

[0433] The method of any of paragraphs [00429]-[00432], wherein the checkpoint inhibitor interacts with a ligand of a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.

[0434] The method of any of paragraphs [00429]-[00433], wherein two or more checkpoint inhibitors are administered.

[0435] The method of paragraph [00434], wherein the checkpoint inhibitors are: (i) ipilimumab or tremelimumab, and (ii) nivolumab.

[0436] The method of any of paragraphs [00429]-[00435], wherein the checkpoint inhibitor and the composition are administered simultaneously or sequentially in any order.

[0437] The method of paragraph [00436], wherein the peptide, polynucleotide, vector, composition, or cells is administered prior to the checkpoint inhibitor.

[0438] The method of paragraph [00436], wherein the peptide, polynucleotide, vector, composition, or cells is administered after the checkpoint inhibitor.

[0439] The method of paragraph [00436], wherein administration of the checkpoint inhibitor is continued throughout antigen peptide, polynucleotide, vector, composition, or cell therapy.

[0440] The method of any of paragraphs [00429]-[00439], wherein the antigen peptide, polynucleotide, vector, composition, or cell therapy is administered to subjects that only partially respond or do not respond to checkpoint inhibitor therapy.

[0441] The method of any one of paragraphs [00411]-[00428], wherein the composition is administered intravenously or subcutaneously.

[0442] The method of any one of paragraphs [00429]-[00440], wherein the checkpoint inhibitor is administered intravenously or subcutaneously.

[0443] The method of any one of paragraphs [00429]-[00441], wherein the checkpoint inhibitor is administered subcutaneously within about 2 cm of the site of administration of the composition.

[0444] The method of paragraph [00443], wherein the composition is administered into the same draining lymph node as the checkpoint inhibitor.

[0445] The method of any of paragraphs [00411]-[00444], further comprising administering an additional therapeutic agent to the subject either prior to, simultaneously with, or after treatment with the peptide, polynucleotide, vector, composition, or cells.

[0446] The method of paragraph [00445], wherein the additional agent is a chemotherapeutic agent, an immunomodulatory drug, an immune metabolism modifying drug, a targeted therapy, radiation an anti-angiogenesis agent, or an agent that reduces immune-suppression.

[0447] The method of paragraph [00446], wherein the chemotherapeutic agent is an alkylating agent, a topoisomerase inhibitor, an anti-metabolite, or an anti-mitotic agent.

[0448] The method of paragraph [00445], wherein the additional agent is an anti-glucocorticoid induced tumor necrosis factor family receptor (GITR) agonistic antibody or antibody fragment, ibrutinib, docetaxeol, cisplatin, or cyclophosphamide.

[0449] The method of any of paragraphs [00411]-[00448], which elicits a CD4+ T cell immune response.

[0450] The method of any of paragraphs [00411]-[00449], which elicits a CD4+ T cell immune response and a CD8+ T cell immune response.

[0451] A method for stimulating an immune response in a subject, comprising administering an effective amount of modified cells or composition of any of paragraphs [00394]-[00410].

[0452] The method of paragraph [00451], wherein the immune response is cytotoxic and/or humoral immune response.

[0453] The method of paragraph [00451], wherein the method stimulates a T cell-mediated immune response in a subject.

[0454] The method of paragraph [00453], wherein the T cell-mediated immune response is directed against a target cell.

[0455] The method of paragraph [00454], wherein the target cell is a tumor cell.

[0456] The method of any of paragraphs [00451]-[00455], wherein the modified cells are transfected or transduced in vivo.

[0457] The method of any of paragraphs [00451]-[00456], wherein the modified cells are transfected or transduced ex vivo.

[0458] The method of any of paragraphs [00451]-[00457], wherein the modified cells are autologous patient T cells.

[0459] The method of paragraph [00458], wherein the autologous patient T cells are obtained from a patient that has received an antigen peptide or nucleic acid vaccine.

[0460] The method of paragraph [00459], wherein the antigen peptide or nucleic acid vaccine comprises at least one personalized antigen.

[0461] The method of paragraph [00460], wherein the antigen peptide or nucleic acid vaccine comprises at least one additional antigenic peptide listed in Table 1 or 2.

[0462] The method of paragraph [00460], wherein the antigen peptide or nucleic acid vaccine comprises at least one additional antigenic peptide listed in Table 3 or 4.

[0463] The method of paragraph [00460], wherein the antigen peptide or nucleic acid vaccine comprises at least one additional antigenic peptide listed in Table 5 or 6.

[0464] The method of paragraph [00461], wherein the at least one additional antigenic peptide listed in Table 1 or 2 is a retroviral antigenic peptide.

[0465] The method of paragraph [00462], wherein the at least one additional antigenic peptide listed in Table 3 or 4 is a non-mutated overexpressed antigenic peptide.

[0466] The method of paragraph [00463], wherein the at least one additional antigenic peptide listed in Table 5 or 6 is a viral antigenic peptide.

[0467] The method of any of paragraphs [00461]-[00466], wherein the patient received a chemotherapeutic agent, an immunomodulatory drug, an immune metabolism modifying drug, targeted therapy or radiation prior to and/or during receipt of the antigen peptide or nucleic acid vaccine.

[0468] The method of any of paragraphs [00459]-[00467], wherein the patient receives treatment with at least one checkpoint inhibitor.

[0469] The method of any of paragraphs [00459]-[00468], wherein the autologous T cells are obtained from a patient that has already received at least one round of T cell therapy containing an antigen.

[0470] The method of any of paragraphs [00459]-[00469], wherein the method further comprises adoptive T cell therapy.

[0471] The method of paragraph [00470], wherein the adoptive T cell therapy comprises autologous T-cells.

[0472] The method of paragraph [00471], wherein the autologous T-cells are targeted against tumor antigens.

[0473] The method of paragraph [00470] or [00471], wherein the adoptive T cell therapy further comprises allogenic T-cells.

[0474] The method of paragraph [00473], wherein the allogenic T-cells are targeted against tumor antigens.

[0475] The method of any of paragraphs [00470]-[00474], wherein the adoptive T cell therapy is administered before the checkpoint inhibitor.

[0476] A method for evaluating the efficacy of any of paragraphs [00411]-[00475], comprising: (i) measuring the number or concentration of target cells in a first sample obtained from the subject before administering the modified cell, (ii) measuring the number concentration of target cells in a second sample obtained from the subject after administration of the modified cell, and (iii) determining an increase or decrease of the number or concentration of target cells in the second sample compared to the number or concentration of target cells in the first sample.

[0477] The method of paragraph [00476], wherein treatment efficacy is determined by monitoring a clinical outcome; an increase, enhancement or prolongation of anti-tumor activity by T cells; an increase in the number of anti-tumor T cells or activated T cells as compared with the number prior to treatment; B cell activity; CD4 T cell activity; or a combination thereof.

[0478] The method of paragraph [00477], wherein treatment efficacy is determined by monitoring a biomarker.

[0479] The method of paragraph [00478], wherein the biomarker is selected from the group consisting of CEA, Her-2/neu, bladder tumor antigen, thyroglobulin, alpha-fetoprotein, PSA, CA 125, CA19.9, CA 15.3, leptin, prolactin, osteopontin, IGF-II, CD98, fascin, sPIgR, 14-3-3 eta, troponin I, and b-type natriuretic peptide.

[0480] The method of paragraph [00477], wherein clinical outcome is selected from the group consisting of tumor regression; tumor shrinkage; tumor necrosis; anti-tumor response by the immune system; tumor expansion, recurrence or spread; or a combination thereof.

[0481] The method of paragraph [00477], wherein the treatment effect is predicted by presence of T cells or by presence of a gene signature indicating T cell inflammation or a combination thereof.

[0482] A method of treating cancer or initiating, enhancing, or prolonging an anti-tumor response in a subject in need thereof comprising administering to the subject:

[0483] the peptide, polynucleotide, vector, composition, antibody, or cells of any of paragraphs [00291]-[00410]; and

[0484] at least one checkpoint inhibitor.

[0485] The method of paragraph [00482], further comprising administration of an immunomodulator or adjuvant.

[0486] The method of paragraph [00483], wherein the immunomodulator or adjuvant is selected from the group consisting of Poly(I:C), Poly-ICLC, STING agonist, 1018 ISS, aluminum salts, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, JuvImmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312 VG, Montanide ISA 206 VG, Montanide ISA 50 V2, Montanide ISA 51 VG, OK-432, OM-174, OM-197-MP-EC, ISA-TLR2 agonist, ONTAK, PepTel® vector system, PLG microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Pam3CSK4, acrylic or methacrylic polymers, copolymers of maleic anhydride, and QS21 stimulon. a co-stimulatory ligand, a TNF ligand, an Ig superfamily ligand, CD28, CD80, CD86, ICOS, CD40L, OX40, CD27, GITR, CD30, DR3, CD69, or 4-1BB.

[0487] The method of paragraph [00484], wherein the immunomodulator or adjuvant is Poly-ICLC.

[0488] The method of any one of paragraphs [00482]-[00485], wherein the checkpoint inhibitor is an anti-PDl antibody or antibody fragment.

[0489] The method of paragraph [00486], wherein the inhibitor of the PD-1 pathway is nivolumab.

[0490] The method of any one of paragraphs [00482]-[00485], wherein the checkpoint inhibitor is an anti-CTLA4 antibody or antibody fragment.

[0491] The method of paragraph [00488], wherein the anti-CTLA4 antibody is ipilimumab or tremelimumab.

[0492] The method of any one of paragraphs [00482]-[00489], wherein the method comprises administering both an anti-PD1 antibody and an anti-CTLA4 antibody.

[0493] The method of any one of paragraphs [00482]-[00489], wherein administration of the checkpoint inhibitor is initiated before initiation of administration of the peptide, polynucleotide, vector, composition, antibody, or cell.

[0494] The method of any one of paragraphs [00482]-[00489], wherein administration of the checkpoint inhibitor is initiated after initiation of administration of the peptide, polynucleotide, vector, composition, antibody, or cell.

[0495] The method of any one of paragraphs [00482]-[00489], wherein administration of the checkpoint inhibitor is initiated simultaneously with the initiation of administration of the peptide, polynucleotide, vector, composition, antibody, or cell.

[0496] The method of any one of paragraphs [00482]-[00493], wherein the peptide, polynucleotide, vector, composition, antibody, or cell is administered intravenously or subcutaneously.

[0497] The method of any one of paragraphs [00482]-[00493], wherein the checkpoint inhibitor is administered intravenously or subcutaneously.

[0498] The method of any one of paragraphs [00482]-[00495], wherein the checkpoint inhibitor is administered subcutaneously within about 2 cm of the site of administration of the peptide, polynucleotide, vector, composition, antibody, or cell.

[0499] The method of paragraph [00496], wherein the peptide, polynucleotide, vector, composition, antibody, or cell is administered into the same draining lymph node as the checkpoint inhibitor.

[0500] A kit comprising an antigen therapeutic of any of paragraphs [00291]-[00410].

[0501] The method of paragraph [00414], wherein the cancer is selected from the group consisting of: CRC, head and neck, stomach, lung squamous, lung adeno., prostate, bladder, stomach, renal cell carcinoma, and uterine.

[0502] The method of paragraph [00414], wherein the cancer is selected from the group consisting of: melanoma, lung squamous, DLBCL, uterine, head and neck, uterine, liver, and CRC.

[0503] The method of paragraph [00414], wherein the cancer is selected from the group consisting of: cervical, head and neck, anal, stomach, Burkitt's lymphoma, and nasopharyngeal carcinoma.

[0504] Provided herein is an immunogenic vaccine composition comprising a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the peptide is a synthetic peptide. In some embodiments, the peptide is a recombinant peptide. In some embodiments, the peptide comprises a sequence from an endogenous retroviral protein. In some embodiments, the peptide comprises a sequence from an exogenous viral protein. In some embodiments, the peptide comprises a sequence of a protein expressed by a cancer cell of a subject with cancer, wherein the protein is expressed by the cancer cell at a level that is higher than a level expressed by a non-cancer cell of the subject. In some embodiments, the peptide is 100 amino acids or less in length. In some embodiments, the peptide is from about 5 to about 50 amino acids in length or from about 15 to about 35 amino acids in length. In some embodiments, the peptide is about 30 amino acids or less in length or about 15 amino acids or less in length. In some embodiments, the peptide comprises a sequence which binds a major histocompatibility complex (MHC) class I with a binding affinity of less than about 500 nM. In some embodiments, the peptide comprises a sequence which binds a major histocompatibility complex (MHC) class II with a binding affinity of less than about 1000 nM. In some embodiments, the peptide further comprises non-native amino acids flanking the at least 8 contiguous amino acids. In some embodiments, the composition further comprises a second peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6, wherein the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 1000 nM. In some embodiments, the peptides are linked using a poly-glycine or poly-serine linker. In some embodiments, the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 1000 nM or less than about 500 nM. In some embodiments, the peptide further comprises a modification which increases in vivo half-life, cellular targeting, antigen uptake, antigen processing, MHC affinity, MHC stability, or antigen presentation. In some embodiments, the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, PEGylation, polysialylation HESylation, recombinant PEG mimetics, Fc fusion, albumin fusion, nanoparticle attachment, nanoparticulate encapsulation, cholesterol fusion, iron fusion, acylation, amidation, glycosylation, side chain oxidation, phosphorylation, biotinylation, the addition of a surface active material, the addition of amino acid mimetics, or the addition of unnatural amino acids. In some embodiments, the peptide comprises a modification which increases targeting by antigen presenting cells. In some embodiments, the antigen presenting cells are dendritic cells. In some embodiments, the modification which increases targeting by the dendritic cells is a

DEC205, XCR1, CD197, CD80, CD86, CD123, CD209, CD273, CD283, CD289, CD184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, CD141, CD11c, CD83, TSLP receptor, or CD1a marker. In some embodiments, the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of peptides each comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the composition comprises from 2 to 20 peptides each comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the composition further comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 additional antigenic peptides. In some embodiments, the additional antigenic peptides are specific for an individual patient's tumor. In some embodiments, the additional antigenic peptides are selected by identifying sequence differences between the genome, exome, and/or transcriptome of the patient's tumor sample and the genome, exome, and/or transcriptome of a non-tumor sample. In some embodiments, identifying sequence differences comprises performing Next Generation Sequencing. Provided herein is a composition comprising an antigen presenting cell comprising a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the antigen presenting cell is a dendritic cell.

[0505] Provided herein is an in vivo delivery system comprising a composition described herein. In some embodiments, the delivery system includes a cell-penetrating peptide, nanoparticulate encapsulation, a virus like particle, or a liposome. In some embodiments, the cell-penetrating peptide is a TAT peptide, herpes simplex virus VP22, transportan, or Antp.

[0506] Provided herein is an immunogenic vaccine composition comprising a recombinant polynucleotide encoding a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the recombinant polynucleotide is RNA, optionally a self-amplifying RNA. In some embodiments, the RNA is modified to increase stability, increase cellular targeting, increase translation efficiency, adjuvanticity, cytosol accessibility, and/or decrease cytotoxicity. In some embodiments, the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, codon optimization, increased GC-content, incorporation of

modified nucleosides, incorporation of 5'-cap or cap analog, and/or incorporation of an unmasked poly-A sequence.

[0507] Provided herein is a composition comprising a cell comprising a recombinant polynucleotide encoding a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.

[0508] Provided herein is a composition comprising a vector comprising a polynucleotide comprising a sequence encoding a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the polynucleotide is operably linked to a promoter. In some embodiments, the polynucleotide is a self-amplifying RNA replicon, plasmid, phage, transposon, cosmid, virus, or virion. In some embodiments, the virus is an adeno-associated virus, herpesvirus, lentivirus, or pseudotypes thereof.

[0509] Provided herein is an in vivo delivery system comprising a composition described herein. In some embodiments, the delivery system includes spherical nucleic acids, viruses, virus-like particles, plasmids, bacterial plasmids, or nanoparticle.

[0510] Provided herein is a T cell receptor (TCR) that specifically binds to a peptide:MHC complex, wherein the peptide of the peptide of the peptide:MHC complex is a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.

[0511] Provided herein is a T cell comprising a T cell receptor (TCR) that specifically binds to a peptide:MHC complex, wherein the peptide of the peptide of the peptide:MHC complex is a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the T cell is a helper or cytotoxic T cell. In some embodiments, the T cell is an autologous patient T cell.

[0512] Provided herein is a method of treating cancer in a subject in need thereof comprising administering to the subject a composition described herein; wherein the subject comprises cancer cells expressing a protein comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6. In some embodiments, the subject is a human. In some embodiments, the cancer is selected from the group consisting of urogenital, gynecological, lung, gastrointestinal , head and neck cancer, malignant glioblastoma, malignant mesothelioma, non-metastatic or metastatic breast cancer, triple-negative breast cancer (TNBC), malignant melanoma, Merkel Cell Carcinoma or bone and soft tissue sarcomas, hematologic neoplasias, multiple myeloma, smoldering myeloma (SMM), acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia, non-small cell lung cancer (NSCLC), breast cancer, metastatic colorectal cancers, hormone sensitive or hormone refractory prostate cancer, colorectal cancer, ovarian cancer,

hepatocellular cancer, renal cell cancer, pancreatic cancer, gastric cancer, esophageal cancers, hepatocellular cancers, cholangiocellular cancers, head and neck squamous cell cancer soft tissue sarcoma, and small cell lung cancer. In some embodiments, the method further comprises administering at least one immune checkpoint inhibitor to the subject. In some embodiments, the checkpoint inhibitor inhibits a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.

CLAIMS

WHAT IS CLAIMED IS:

1. An immunogenic vaccine composition comprising a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
2. The immunogenic vaccine composition of claim 1, wherein the peptide is a synthetic peptide.
3. The immunogenic vaccine composition of claim 1, wherein the peptide is a recombinant peptide.
4. The immunogenic vaccine composition of claim 1, wherein the peptide comprises a sequence from an endogenous retroviral protein.
5. The immunogenic vaccine composition of claim 1, wherein the peptide comprises a sequence from an exogenous viral protein.
6. The immunogenic vaccine composition of claim 1, wherein the peptide comprises a sequence of a protein expressed by a cancer cell of a subject with cancer, wherein the protein is expressed by the cancer cell at a level that is higher than a level expressed by a non-cancer cell of the subject.
7. The immunogenic vaccine composition of any of claims 1-6, wherein the peptide is 100 amino acids or less in length.
8. The immunogenic vaccine composition of any of claims 1-7, wherein the peptide is from about 5 to about 50 amino acids in length or from about 15 to about 35 amino acids in length.
9. The immunogenic vaccine composition of any of claims 1-8, wherein the peptide is about 30 amino acids or less in length or about 15 amino acids or less in length.
10. The immunogenic vaccine composition of any of claims 1-9, wherein the peptide comprises a sequence which binds a major histocompatibility complex (MHC) class I with a binding affinity of less than about 500 nM.
11. The immunogenic vaccine composition of any of claims 1-9, wherein the peptide comprises a sequence which binds a major histocompatibility complex (MHC) class II with a binding affinity of less than about 1000 nM.
12. The immunogenic vaccine composition of any of claims 1-11, wherein the peptide further comprises non-native amino acids flanking the at least 8 contiguous amino acids.
13. The immunogenic vaccine composition of any of claims 1-12, wherein the composition further comprises a second peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6, wherein the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 1000 nM.

14. The immunogenic vaccine composition of claim 13, wherein peptides are linked using a poly-glycine or poly-serine linker.
15. The immunogenic vaccine composition of claim 13 or 14, wherein the second antigenic peptide binds MHC class I or class II with a binding affinity of less than about 1000 nM or less than about 500 nM.
16. The immunogenic vaccine composition of any of claims 1-15, wherein the peptide further comprises a modification which increases in vivo half-life, cellular targeting, antigen uptake, antigen processing, MHC affinity, MHC stability, or antigen presentation.
17. The immunogenic vaccine composition of claim 16, wherein the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, PEGylation, polysialylation HESylation, recombinant PEG mimetics, Fc fusion, albumin fusion, nanoparticle attachment, nanoparticulate encapsulation, cholesterol fusion, iron fusion, acylation, amidation, glycosylation, side chain oxidation, phosphorylation, biotinylation, the addition of a surface active material, the addition of amino acid mimetics, or the addition of unnatural amino acids.
18. The immunogenic vaccine composition of claim 16, wherein the peptide comprises a modification which increases targeting by antigen presenting cells.
19. The immunogenic vaccine composition of claim 18, wherein the antigen presenting cells are dendritic cells.
20. The immunogenic vaccine composition of claim 19, wherein the modification which increases targeting by the dendritic cells is a DEC205, XCR1, CD197, CD80, CD86, CD123, CD209, CD273, CD283, CD289, CD184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, CD141, CD11c, CD83, TSLP receptor, or CD1a marker.
21. The immunogenic vaccine composition of any of claims 1-20, wherein the composition comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 of peptides each comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
22. The immunogenic vaccine composition of any of claims 1-20, wherein the composition comprises from 2 to 20 peptides each comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
23. The immunogenic vaccine composition of any of claims 1-22, wherein the composition further comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at

least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 additional antigenic peptides.

24. The immunogenic vaccine composition of claim 23, wherein the additional antigenic peptides are specific for an individual patient's tumor.
25. The immunogenic vaccine composition of claim 24, wherein the additional antigenic peptides are selected by identifying sequence differences between the genome, exome, and/or transcriptome of the patient's tumor sample and the genome, exome, and/or transcriptome of a non-tumor sample.
26. The immunogenic vaccine composition of claim 25, wherein identifying sequence differences comprises performing Next Generation Sequencing.
27. A composition comprising an antigen presenting cell comprising a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
28. The composition of claim 27, wherein the antigen presenting cell is a dendritic cell.
29. An in vivo delivery system comprising the composition of any of claims 1-28.
30. The delivery system of claim 29, wherein the delivery system includes a cell-penetrating peptide, nanoparticulate encapsulation, a virus like particle, or a liposome.
31. The delivery system of claim 29, wherein the cell-penetrating peptide is a TAT peptide, herpes simplex virus VP22, transportan, or Antp.
32. An immunogenic vaccine composition comprising a recombinant polynucleotide encoding a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
33. The immunogenic vaccine composition of claim 32, wherein the recombinant polynucleotide is RNA, optionally a self-amplifying RNA.
34. The immunogenic vaccine composition of claim 33, wherein the RNA is modified to increase stability, increase cellular targeting, increase translation efficiency, adjuvanticity, cytosol accessibility, and/or decrease cytotoxicity.
35. The immunogenic vaccine composition of claim 34, wherein the modification is conjugation to a carrier protein, conjugation to a ligand, conjugation to an antibody, codon optimization, increased GC-content, incorporation of modified nucleosides, incorporation of 5'-cap or cap analog, and/or incorporation of an unmasked poly-A sequence.
36. A composition comprising a cell comprising a recombinant polynucleotide encoding a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.

37. A composition comprising a vector comprising a polynucleotide comprising a sequence encoding a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
38. The composition of claim 37, wherein the polynucleotide is operably linked to a promoter.
39. The composition of claims 37 or 38, wherein the polynucleotide is a self-amplifying RNA replicon, plasmid, phage, transposon, cosmid, virus, or virion.
40. The composition of claim 39, wherein the virus is an adeno-associated virus, herpesvirus, lentivirus, or pseudotypes thereof.
41. An in vivo delivery system comprising the composition of any of claims 32-40.
42. The delivery system of claim 41, wherein the delivery system includes spherical nucleic acids, viruses, virus-like particles, plasmids, bacterial plasmids, or nanoparticle.
43. A T cell receptor (TCR) that specifically binds to a peptide:MHC complex, wherein the peptide of the peptide of the peptide:MHC complex is a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
44. A T cell comprising a T cell receptor (TCR) that specifically binds to a peptide:MHC complex, wherein the peptide of the peptide of the peptide:MHC complex is a peptide comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
45. The T cell of claim 44, which is a helper or cytotoxic T cell.
46. The T cell of claim 44 or 45, which is an autologous patient T cell.
47. A method of treating cancer in a subject in need thereof comprising administering to the subject the composition of any of claims 1-28 and 32-40; the delivery system of any of claims 29-31, 41 and 42; the TCR of claim 43; or the T cell of any of claims 44-46; wherein the subject comprises cancer cells expressing a protein comprising at least 8 contiguous amino acids of a sequence in any one of Tables 1-6.
48. The method of claim 47, wherein the subject is a human.
49. The method of claim 47 or 48, wherein the cancer is selected from the group consisting of urogenital, gynecological, lung, gastrointestinal , head and neck cancer, malignant glioblastoma, malignant mesothelioma, non-metastatic or metastatic breast cancer, triple-negative breast cancer (TNBC), malignant melanoma, Merkel Cell Carcinoma or bone and soft tissue sarcomas, hematologic neoplasias, multiple myeloma, smoldering myeloma (SMM), acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia, non-small cell lung cancer (NSCLC), breast cancer, metastatic colorectal cancers, hormone sensitive or hormone refractory prostate cancer, colorectal cancer, ovarian cancer,

hepatocellular cancer, renal cell cancer, pancreatic cancer, gastric cancer, esophageal cancers, hepatocellular cancers, cholangiocellular cancers, head and neck squamous cell cancer soft tissue sarcoma, and small cell lung cancer.

50. The method of any of claims 47-49, further comprising administering at least one immune checkpoint inhibitor to the subject.

51. The method of claim 50, wherein the checkpoint inhibitor inhibits a checkpoint protein selected from the group consisting of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, and B-7 family ligands or a combination thereof.