

**(19) World Intellectual Property
Organization**
International Bureau



WIPO | PCT

(10) International Publication Number
WO 2018/067869 A9

(43) International Publication Date
12 April 2018 (12.04.2018)

(51) International Patent Classification:

<i>A61K 38/00</i> (2006.01)	<i>C07K 7/00</i> (2006.01)
<i>A61K 39/00</i> (2006.01)	<i>C07K 14/00</i> (2006.01)

(21) International Application Number:

PCT/US2017/055414

(22) International Filing Date:

05 October 2017 (05.10.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/405,779 07 October 2016 (07.10.2016) US

(71) **Applicant: BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 210 West 7th St., Austin, TX 78701 (US).

(72) **Inventors: LIZEE, Gregory**; 9000 Alameda Rd., Apt. 3301, Houston, TX 77054 (US). **YEE, Cassian**; 8181 Fannin St., Apt. 1332, Houston, TX 77054 (US). **ROSZIK, Janos**; 2475 Underwood St., Apt. 261, Houston, TX 77030 (US).

(74) **Agent: MANN, Monica**; Parker Highlander PLLC, 1120 S. Capital Of Texas Highway, Bldg. One, Suite 200, Austin, TX 78746 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: HLA-RESTRICTED VGLL1 PEPTIDES AND USE THEREOF

(57) Abstract: Provided herein are tumor-antigen VGLL1 specific peptides. Also provided herein are methods of generating VGLL1-specific T cells and their use for the treatment of cancer. In addition, the VGLL1-specific peptides may be used as a vaccine.

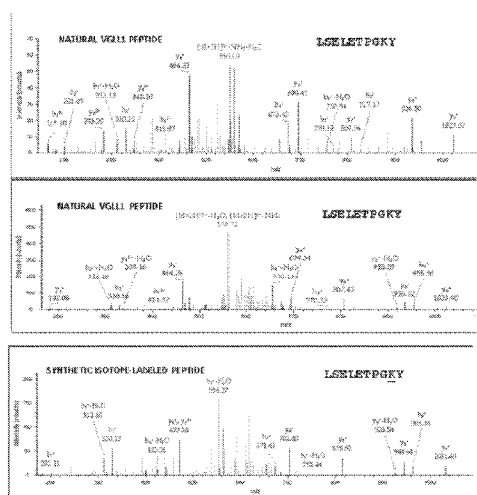


FIG. 1A

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

(48) Date of publication of this corrected version:

31 May 2019 (31.05.2019)

(15) Information about Correction:

see Notice of 31 May 2019 (31.05.2019)

DESCRIPTION

HLA-RESTRICTED VGLL1 PEPTIDES AND USE THEREOF

[0001] This application claims the benefit of United States Provisional Patent Application No. 62/405,779, filed October 7, 2016, the entirety of which is incorporated herein
5 by reference.

[0002] The sequence listing that is contained in the file named “UTFCP1307WO_ST25.txt”, which is 1 KB (as measured in Microsoft Windows) and was created on October 5, 2017, is filed herewith by electronic submission and is incorporated by reference herein.

10 BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] The present invention relates generally to the fields of immunology and medicine. More particularly, it concerns tumor antigen-specific peptides and uses thereof for the treatment of cancer.

15 2. Description of Related Art

[0004] Adoptive T cell therapy (ACT; also referred to as “adoptive cell transfer”) has shown promise as a method for treating melanoma; unfortunately, this approach has also been hindered by limitations including toxicity towards non-cancerous tissues. ACT generally involves infusing a large number of autologous activated tumor-specific T cells into a patient,
20 *e.g.*, to treat a cancer. ACT has resulted in therapeutic clinical responses in melanoma patients (Yee 2002; Dudley 2002; Yee 2014). Generally, to develop effective anti-tumor T cell responses, the following three steps are normally required: priming and activating antigen-specific T cells, migrating activated T cells to the tumor site, and recognizing and killing the tumor by antigen-specific T cells. The choice of the target antigen is important for induction
25 of effective antigen-specific T cells.

[0005] While several tumor-associated antigens have been identified for melanoma and a handful of other solid tumor malignancies, there is a paucity of immunogenic targets for pancreatic, ovarian, gastric, lung, cervical, breast, and head and neck cancer. Thus, identification and validation of novel epitopes and target antigens for these common and
30 difficult to treat malignancies is warranted.

SUMMARY OF THE INVENTION

[0006] In some embodiments, the present disclosure provides VGLL1 peptides and methods for their use, such as in therapies including adoptive T cell therapies. In some embodiments, the peptides may be used to expand VGLL1-specific T cells *in vitro* that are administered to a mammalian subject, such as a human patient, to treat a disease (*e.g.*, a cancer). In further embodiments, the T cells are genetically engineered to express T cell receptors (TCRs) with antigenic specificity for VGLL1. In other embodiments, the peptides may be administered to a mammalian subject to induce an immune response or vaccinate the subject against the peptide, and such an immune response may be useful to treat or reduce the chances of getting or relapsing from a disease, such as a cancer.

[0007] In one embodiment, the present disclosure provides an isolated VGLL1 peptide of 35 amino acids in length or less (*e.g.*, 34, 33, 32, 31, or 30 amino acids) comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:1 (LSELETPGKY), wherein the peptide is capable of inducing cytotoxic T lymphocytes (CTLs). In some aspects, the peptide comprises an amino acid sequence having at least 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent sequence identity to SEQ ID NO:1. In certain aspects, the peptide is 30 amino acids in length or less, such as 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 amino acids in length.

[0008] In some aspects, the peptide binds to a human class I HLA protein. In particular aspects, the human class I HLA protein is HLA-A*0101 or other members of the HLA-A1 family, such as HLA-A*0102 and HLA-A*0103. In other aspects, the class I HLA protein is HLA-A*2902 or other members of the HLA-A29 family, or HLA-A*3002 and other members of the HLA-A30 family. In other aspects, the class I HLA protein is HLA-B*1801 or other members of the HLA-B18 family or HLA-B*4403 or other members of the HLA-B44 family.

[0009] In another embodiment, there is provided a pharmaceutical composition comprising the isolated VGLL1 peptide of the embodiments and a pharmaceutical carrier. In some aspects, the pharmaceutical composition is formulated for parenteral administration, intravenous injection, intramuscular injection, inhalation, or subcutaneous injection. In certain aspects, the peptide is comprised in a liposome, lipid-containing nanoparticle, or in a lipid-based carrier. In some aspects, the pharmaceutical preparation is formulated for injection or inhalation as a nasal spray.

[0010] A further embodiment provides an isolated nucleic acid encoding the VGLL1 peptide of the embodiments. Also provided herein is a vector comprising a contiguous sequence consisting of the nucleic acid encoding the VGLL1 peptide.

5 [0011] In yet another embodiment, there is provided a method of promoting an immune response in a subject, comprising administering an effective amount of the VGLL1 peptide of the embodiments to the subject, wherein the peptide induces antigen-specific T cells in the subject. In some aspects, the subject is diagnosed with cancer. In certain aspects, the cancer is pancreatic, ovarian, gastric, or breast cancer. In particular aspects, the subject is a human.

10 [0012] In additional aspects, the method further comprises administering at least a second anti-cancer therapy. In some aspects, the second anti-cancer therapy is selected from the group consisting of a chemotherapy, a radiotherapy, an immunotherapy, or a surgery. In particular aspects, the immunotherapy is an immune checkpoint inhibitor. In one specific aspects, the immune checkpoint inhibitor is an anti-PD1 monoclonal antibody.

15 [0013] A further embodiment provides a method of producing VGLL1-specific T cells comprising obtaining a starting population of T cells, and contacting the starting population of T cells with the VGLL1 peptide (*e.g.*, a peptide of SEQ ID NO:1) of the embodiments, thereby generating VGLL1-specific T cells. In some aspects, contacting is further defined as co-culturing the starting population of T cells with antigen presenting cells (APCs), wherein the APCs present the VGLL1 peptide of the embodiments on their surface. In particular aspects, 20 the APCs are dendritic cells. In some aspects, the starting population of T cells are CD8⁺ T cells. In certain aspects, the T cells are CTLs. In some aspects, obtaining comprises isolating the starting population of T cells from peripheral blood mononuclear cells (PBMCs). Also provided herein is a pharmaceutical composition comprising the VGLL1-specific T cells produced by the methods herein.

25 [0014] An even further embodiment provides an antigen receptor, such as a T cell receptor (TCR) or chimeric antigen receptor (CAR), with antigenic specificity for VGLL1. Another embodiment provides T cells engineered to express a VGLL1-specific TCR or CAR. In some aspects, the VGLL1-specific chimeric antigen receptor comprises an intracellular signaling domain, a transmembrane domain, and/or an extracellular domain. In certain aspects, 30 DNA encoding the CAR is integrated into the genome of the cell. In some aspects, the extracellular domain of the CAR comprises a VGLL1-binding region. For example, the

VGLL1-binding region may be a F(ab')₂, Fab', Fab, Fv, or scFv. In certain aspects, the intracellular signaling domain of the CAR is a T-lymphocyte activation domain. For example, the intracellular signaling domain of the CAR may comprise CD3 ξ , CD28, OX40/CD134, 4-1BB/CD137, Fc ϵ RI γ , ICOS/CD278, ILRB/CD122, IL-2RG/CD132, DAP molecules, CD70, cytokine receptor, CD40, or a combination thereof. In certain aspects, the transmembrane domain of the CAR comprises CD28 transmembrane domain, IgG4Fc hinge, Fc regions, CD4 transmembrane domain, the CD3 ξ transmembrane domain, cysteine mutated human CD3 ξ domain, CD16 transmembrane domain, CD8 transmembrane domain, or erythropoietin receptor transmembrane domain.

[0015] Another embodiment provides a method of treating cancer in a subject comprising administering an effective amount of the VGLL1-specific T cells of the embodiments to the subject. In some aspects, the cancer is pancreatic, ovarian, gastric, bladder, or breast cancer. In particular aspects, the subject is a human. In some aspects, the antigen-specific T cells are autologous. In some aspects, the subject is determined to have cancer cells which express VGLL1.

[0016] In certain aspects, the method further comprises lymphodepletion of the subject prior to administration of the antigen-specific T cells. In some aspects, lymphodepletion comprises administration of cyclophosphamide and/or fludarabine.

[0017] In some aspects, the method further comprises administering at least a second therapeutic agent. In certain aspects, the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy. In particular aspects, the immunotherapy is an immune checkpoint inhibitor. In one specific aspects, the immune checkpoint inhibitor is an anti-PD1 monoclonal antibody.

[0018] In certain aspects, the VGLL1-specific T cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

[0019] Further provided herein is a composition comprising an effective amount of the VGLL1-specific T cells of the embodiments for the treatment of cancer in a subject. In some aspects, the cancer is pancreatic, ovarian, gastric, bladder, or breast cancer. In particular

aspects, the subject is a human. In some aspects, the antigen-specific T cells are autologous. In some aspects, the subject is determined to have cancer cells which express VGLL1.

5 [0020] Further provided herein are methods of determining the prognosis of a subject with cancer comprising measuring the expression level of VGLL1 in a sample obtained from said subject, wherein an elevated level of VGLL1 identifies a subject with a poor prognosis. In some aspects, the sample is a blood sample or tissue sample, such as a tumor sample. In particular aspects, the cancer is pancreatic cancer.

10 [0021] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0023] FIGS. 1A-1D: Mass spectrometry-based identification of a pancreatic tumor-associated antigen derived from VGLL1. (A) Tandem mass spectrometry (MS) analysis of an HLA class I-bound VGLL1 peptide, LSELETPGKY (SEQ ID NO:1), isolated from a pancreatic tumor organoid cell line (hMIA-2D) derived from PDAC patient MP015. The top panel shows the spectra of the original eluted peptide from discovery phase tandem MS. The middle and bottom spectra show a targeted MS experiment in which the isotope-labeled synthetic peptide LSELETPGKY (SEQ ID NO:1) (with heavy lysine residue underlined) was spiked into the tumor sample and co-eluted with the native peptide, demonstrating gold standard confirmation of peptide identity. (B) Same mass spectra as in (A), but with 'y' ions circled to highlight the expected mass shift of 8 atomic mass units for the isotope-labeled peptide, corresponding to the heavy lysine residue. (C) Same mass spectra as in (A), but with 'b' ions circled to highlight the expected mass shift of 8 atomic mass units for only the b₉₊ ion, but no other 'b' ions. (D) Analysis of expected tandem MS fragment ion masses for the native and isotope-labeled VGLL1 peptide LSELETPGKY (SEQ ID NO:1) and LSELETPGKY (SEQ ID NO:1).

[0024] FIGS. 2A-2B: VGLL1 mRNA transcripts are overexpressed in several tumor types compared with normal tissues. (A) RNAseq analysis derived from the GTex Portal normal tissue (gtexportal.org) and TCGA (cancergenome.nih.gov) tumor tissue databases show VGLL1 transcript expression in normal tissues and various cancers, respectively. Each dot represents the results of one analyzed human sample. The left star and arrow indicates the highest mean normal tissue level of VGLL1 transcript, found in bladder. The right star and arrow indicates the level of VGLL1 transcript in the MP015 patient-derived organoid cell line hMIA2D, from which the VGLL1 peptide was detected. Boxes indicate the 5 cancer types that overexpress VGLL1 transcripts: pancreatic, ovarian, bladder, breast, and stomach cancers. TPM, transcripts per million. (B) VGLL1 expression prevalence in five cancer types derived from TCGA data. Tumor-associated expression was considered positive if VGLL1 mRNA expression was >3 TPM, as determined by RNAseq expression.

[0025] **FIG. 3: Elevated VGLL1 expression is a poor prognostic factor in cancer.** Pancreatic patient survival analysis, as divided into quartiles of highest (black line), 2nd highest (dark gray), 3rd highest (medium gray), and lowest (light gray) VGLL1 tumor transcript expression, as determined by TCGA RNAseq data. The lowest VGLL1 expression correlated with highest survival rate.

[0026] **FIGS. 4A-4D:** VGLL1 peptide-specific cytotoxic T cells were isolated and expanded from PDAC patient peripheral blood and infused for treatment. (A) Schematic depicting adoptive T cell treatment process. (B) Peripheral blood from HLA-A*0101-positive PDAC patient MP015 were stimulated twice with autologous LSELETPGKY (SEQ ID NO:1) peptide-pulsed dendritic cells, prior to 2 rounds of tetramer-based OWL sorting and rapid expansion protocol (REP). (C) VGLL1-specific CTLs were expanded to ~20 billion cells and infused into patient MP015 with concurrent anti-PD1 treatment every three weeks. (D) The CTL infusion induced no serious toxicities nor any apparent clinical benefits, despite showing robust autologous antitumor activity *in vitro*. Gene expression analysis performed on multiple longitudinal tumor samples from patient MP015 revealed that VGLL1 antigen loss occurred during the intervening months between harvest of the original tumor (source of the organoid cell line hMIA-2D) and VGLL1-specific CTL infusion.

[0027] **FIGS. 5A-5B:** VGLL1-specific CTLs recognize and kill patient-derived and allogeneic HLA-A*0101+ pancreatic cancer cells. (A) Expanded HLA-A*0101-restricted VGLL1-specific CD8+ T cells were co-cultured with autologous PDAC patient MP015-derived tumor line hMIA-2D, HLA-A*0101-positive PDAC cell line Capan-1, or HLA-A*0101-positive melanoma cell line A375 in a standard 51Cr release assay to measure cytotoxic activity at effector-to-target (E:T) cell ratios of 5:1 and 20:1. (B) Same cytotoxicity assay as in (A), except all three cell lines were pulsed with 1 mM cognate VGLL1 peptide antigen and washed prior to VGLL1-specific CTL exposure.

[0028] **FIGS. 6A-6C:** VGLL1-specific CTLs kill HLA-A*0101+ tumor cell lines derived from pancreatic, bladder, ovarian, and triple-negative breast cancers. (A) Expanded HLA-A*0101-restricted VGLL1-specific CD8+ T cells were co-cultured with HLA-A*0101-positive Mel888 melanoma cells pulsed with titrated amounts of cognate VGLL1 peptide in a standard 51Cr release cytotoxicity assay at a 5:1 effector-to-target (E:T) cell ratio. (B) Cytotoxicity assay results of VGLL1-specific CTLs tested against six HLA-A*0101-positive cell lines, including four PDAC lines (hMIA-2D, Capan-1, BXPC3, and MP081), a triple-

negative breast cancer line (BT20), and a bladder cancer line (UBLC1) at a 5:1 E:T cell ratio. (C) Cytotoxicity assay results of VGLL1-specific CTLs tested against two additional HLA-A*0101-positive cell lines, a triple-negative breast cancer line (BT549) and an ovarian cancer line (OAW28), with or without prior interferon-gamma treatment at a 5:1 E:T cell ratio.

5 [0029] **FIGS. 7A-7B:** Gastric cancer line MKN74 is killed by VGLL1-specific CTLs following HLA-A*0101 transduction. (A) Flow cytometric analysis of parental MKN74 gastric cancer cells and MKN74 cells transduced with a lentiviral vector to express HLA-A*0101. Cells were stained with a HLA-A*0101-specific mAb and analyzed by flow cytometry. The MKN74 transduced cells are shifted to the right. (B) Cytotoxicity assay results of VGLL1-
10 specific CTLs tested against untransduced HLA-A*0101-positive melanocytes or the same melanocytes lentivirally transduced to express VGLL1 at a 5:1 E:T cell ratio.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0030] For patients with many different cancer types, T cell based immunotherapies represent a promising approach with proven efficacy. However, antigen-specific T cell therapy for most cancer types is not feasible due to the lack of tumor-associated antigens currently known, which has stalled their clinical development. Studies in the present disclosure profiled the immunopeptidome of pancreatic cell lines and tumor specimens through mass spectrophotometry and identified peptide epitopes derived from a novel tumor-associated antigen VGLL1 that is expressed at substantially higher levels in pancreatic, ovarian, gastric, bladder, and breast cancer compared with normal tissues. Using these peptide epitopes, antigen-specific cytotoxic T lymphocytes (CTLs) were generated from pancreatic patient peripheral blood mononuclear cells (PBMCs) that recognized the endogenously-presented antigen on HLA-matched allogeneic tumor cell lines, leading to tumor cell killing. Thus, these antigen-specific CTLs provided herein may be used to target solid cancers (*e.g.*, pancreatic, ovarian, gastric, and breast cancer).

[0031] Accordingly, the present disclosure provides tumor antigen-specific peptides, such as to tumor antigen VGLL1, for use as immunotherapy for the treatment of a cancer. The present studies identified VGLL1 as a tumor-associated antigen from a pancreatic elution. The VGLL1 peptide had a predicted HLA-A*0101 affinity of 51 nM which has a world prevalence of 10-20%. Thus, in some embodiments, VGLL1 peptides (*e.g.* comprising SEQ ID NO:1) are provided herein. For example, a VGLL1-specific peptide may be contacted with or used to stimulate a population of T cells to induce proliferation of the T cells that recognize or bind the VGLL1-specific peptide. In other embodiments, a VGLL1-specific peptide of the present disclosure may be administered to a subject, such as a human patient, to enhance the immune response of the subject against a cancer.

[0032] A VGLL1-specific peptide may be included in an active immunotherapy (*e.g.*, a cancer vaccine) or a passive immunotherapy (*e.g.*, an adoptive immunotherapy). Active immunotherapies include immunizing a subject with a purified tumor antigen or an immunodominant VGLL1-specific peptide (native or modified). Alternately, antigen presenting cells (APCs) pulsed with a VGLL1-specific peptide (or transfected with genes encoding the tumor antigen) may be administered to a subject. The VGLL1-specific peptide may be modified or contain one or more mutations, such as a substitution mutation. Passive immunotherapies include adoptive immunotherapies. Adoptive immunotherapies generally

involve administering cells to a subject, wherein the cells (*e.g.*, CTLs) have been sensitized *in vitro* to the VGLL1-specific peptide (see, *e.g.*, US 7,910,109), such as by using APCs to present the VGLL1 epitope to the CTLs *in vitro*.

[0033] It was observed in the present studies that survival rate corresponds with VGLL1 expression as the lowest VGLL1 expression correlated with the highest survival rate. Thus, VGLL1 is a tumor marker that may be used as a prognostic marker in cancer patients, specifically pancreatic cancer patients.

I. Definitions

[0034] As used herein, “essentially free,” in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%, preferably below 0.01%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0035] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one.

[0036] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0037] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0038] “Treatment” and “treating” refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For example, a treatment may include administration of a T cell therapy.

[0039] "Subject" and "patient" refer to either a human or non-human, such as primates, mammals, and vertebrates. In particular embodiments, the subject is a human.

[0040] The term "therapeutic benefit" or "therapeutically effective" as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the size of a tumor, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

[0041] An "anti-cancer" agent is capable of negatively affecting a cancer cell/tumor in a subject, for example, by promoting killing of cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer.

[0042] The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0043] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to

humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multi-specific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against
5 different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0044] The phrases “pharmaceutical or pharmacologically acceptable” refers to
10 molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, such as a human, as appropriate. The preparation of a pharmaceutical composition comprising an antibody or additional active ingredient will be known to those of skill in the art in light of the present disclosure. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility,
15 pyrogenicity, general safety, and purity standards as required by FDA Office of Biological Standards.

[0045] As used herein, “pharmaceutically acceptable carrier” includes any and all aqueous solvents (*e.g.*, water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles, such as sodium chloride, Ringer's dextrose, *etc.*), non-aqueous solvents (*e.g.*, propylene glycol,
20 polyethylene glycol, vegetable oil, and injectable organic esters, such as ethyloleate), dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial or antifungal agents, anti-oxidants, chelating agents, and inert gases), isotonic agents, absorption delaying agents, salts, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, fluid and nutrient replenishers, such like
25 materials and combinations thereof, as would be known to one of ordinary skill in the art. The pH and exact concentration of the various components in a pharmaceutical composition are adjusted according to well-known parameters.

[0046] The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the therapeutic composition
30 calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the effect

desired. The actual dosage amount of a composition of the present embodiments administered to a patient or subject can be determined by physical and physiological factors, such as body weight, the age, health, and sex of the subject, the type of disease being treated, the extent of disease penetration, previous or concurrent therapeutic interventions, idiopathy of the patient, the route of administration, and the potency, stability, and toxicity of the particular therapeutic substance. For example, a dose may also comprise from about 1 µg/kg/body weight to about 1000 mg/kg/body weight (this such range includes intervening doses) or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 µg/kg/body weight to about 100 mg/kg/body weight, about 5 µg/kg/body weight to about 500 mg/kg/body weight, *etc.*, can be administered. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. In some embodiments, the dosage of antigen-specific T cell infusion may comprise about 100 million to about 30 billion cells, such as 10, 15, or 20 billion cells.

[0047] The term "immune checkpoint" refers to a molecule such as a protein in the immune system which provides inhibitory signals to its components in order to balance immune reactions. Known immune checkpoint proteins comprise CTLA-4, PD-1 and its ligands PD-L1 and PD-L2 and in addition LAG-3, BTLA, B7H3, B7H4, TIM3, KIR. The pathways involving LAG3, BTLA, B7H3, B7H4, TIM3, and KIR are recognized in the art to constitute immune checkpoint pathways similar to the CTLA-4 and PD-1 dependent pathways (see *e.g.* Pardoll, 2012. *Nature Rev Cancer* 12:252-264; Mellman *et al.*, 2011. *Nature* 480:480- 489).

[0048] An "immune checkpoint inhibitor" refers to any compound inhibiting the function of an immune checkpoint protein. Inhibition includes reduction of function and full blockade. In particular the immune checkpoint protein is a human immune checkpoint protein. Thus the immune checkpoint protein inhibitor in particular is an inhibitor of a human immune checkpoint protein.

[0049] As used herein, a "protective immune response" refers to a response by the immune system of a mammalian host to a cancer. A protective immune response may provide a therapeutic effect for the treatment of a cancer, *e.g.*, decreasing tumor size or increasing survival.

[0050] As used herein, the term “antigen” is a molecule capable of being bound by an antibody or T-cell receptor. An antigen may generally be used to induce a humoral immune response and/or a cellular immune response leading to the production of B and/or T lymphocytes.

5 [0051] The terms “tumor-associated antigen,” “tumor antigen” and “cancer cell antigen” are used interchangeably herein. In each case, the terms refer to proteins, glycoproteins or carbohydrates that are specifically or preferentially expressed by cancer cells.

[0052] The term “chimeric antigen receptors (CARs),” as used herein, may refer to artificial T-cell receptors, chimeric T-cell receptors, or chimeric immunoreceptors, for example, and encompass engineered receptors that graft an artificial specificity onto a particular immune effector cell. CARs may be employed to impart the specificity of a monoclonal antibody onto a T cell, thereby allowing a large number of specific T cells to be generated, for example, for use in adoptive cell therapy. In specific embodiments, CARs direct specificity of the cell to a tumor associated antigen, for example. In some embodiments, CARs
10 comprise an intracellular activation domain, a transmembrane domain, and an extracellular domain comprising a tumor associated antigen binding region. In particular aspects, CARs comprise fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies, fused to CD3-zeta a transmembrane domain and endodomain. The specificity of other CAR designs may be derived from ligands of receptors (*e.g.*, peptides) or from pattern-recognition receptors, such as Dectins. In certain cases, the spacing of the antigen-recognition domain can be modified to reduce activation-induced cell death. In certain cases, CARs
15 comprise domains for additional co-stimulatory signaling, such as CD3 ζ , FcR, CD27, CD28, CD137, DAP10, and/or OX40. In some cases, molecules can be co-expressed with the CAR, including co-stimulatory molecules, reporter genes for imaging (*e.g.*, for positron emission tomography), gene products that conditionally ablate the T cells upon addition of a pro-drug,
20 homing receptors, chemokines, chemokine receptors, cytokines, and cytokine receptors.

[0053] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 80%, 85%, 90%, or 95%) of “sequence identity” or “homology” to another sequence means that, when aligned, that
30 percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in CURRENT PROTOCOLS IN

MOLECULAR BIOLOGY (F. M. Ausubel *et al.*, eds., 1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR.

II. VGLL1 Peptides

[0054] Certain embodiments of the present disclosure concern tumor antigen-specific peptides, such as to the VGLL1 tumor antigen. In particular embodiments, the tumor antigen-specific peptides have the amino acid sequence of a VGLL1 peptide (LSELETPGKY: SEQ ID NO:1). The tumor antigen-specific peptide may have an amino acid sequence with at least 80, 85, 90, 95, 96, 97, 98, 99, or 100 percent sequence identity with the peptide sequences of SEQ ID NO:1.

[0055] As used herein, the term “peptide” encompasses amino acid chains comprising 7-35 amino acids, preferably 8-35 amino acid residues, and even more preferably 8-25 amino acids, or 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 amino acids in length, or any range derivable therein. For example, a VGLL1 peptide of the present disclosure may, in some embodiments, comprise or consist of the VGLL1 peptide of SEQ ID NO:1. As used herein, an “antigenic peptide” is a peptide which, when introduced into a vertebrate, can stimulate the production of antibodies in the vertebrate, *i.e.*, is antigenic, and wherein the antibody can selectively recognize and/or bind the antigenic peptide. An antigenic peptide may comprise an immunoreactive VGLL1 peptide, and may comprise additional sequences. The additional sequences may be derived from a native antigen and may be heterologous, and such sequences may, but need not, be immunogenic. In some embodiments, a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) can selectively bind with a HLA-A*0101. In certain embodiments, the VGLL1 peptide is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 amino acids in length, or any range derivable therein. Preferably, the tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) is from 8 to 35 amino acids in length. In some embodiments, the tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) is from 8 to 10 amino acids in length.

[0056] As would be appreciated by one of skill in the art, MHC molecules can bind peptides of varying sizes, but typically not full length proteins. While MHC class I molecules have been traditionally described to bind to peptides of 8-11 amino acids long, it has been shown that peptides 15 amino acids in length can bind to MHC class I molecules by bulging in the middle of the binding site or extending out of the MHC class I binding groove (Guo *et al.*, 1992; Burrows *et al.*, 2006; Samino *et al.*, 2006; Stryhn *et al.*, 2000; Collins *et al.*, 1994; Blanchard and Shastri, 2008). Further, recent studies also demonstrated that longer peptides may be more efficiently endocytosed, processed, and presented by antigen-presenting cells (Zwaveling *et al.*, 2002; Bijker *et al.*, 2007; Melief and van der Burg, 2008; Quintarelli *et al.*, 2011). As demonstrated in Zwaveling *et al.* (2002) peptides up to 35 amino acids in length may be used to selectively bind a class II MHC and are effective. As would be immediately appreciated by one of skill, a naturally occurring full-length tumor antigen, such as VGLL1, would not be useful to selectively bind a class II MHC such that it would be endocytosed and generate proliferation of T cells. Generally, the naturally occurring full-length tumor antigen proteins do not display these properties and would thus not be useful for these immunotherapy purposes.

[0057] In certain embodiments, a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) is immunogenic or antigenic. As shown in the below examples, tumor antigen-specific peptides (*e.g.*, a VGLL1 peptide) of the present disclosure can promote the proliferation of T cells. It is anticipated that such peptides may be used to induce some degree of protective immunity.

[0058] A tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) may be a recombinant peptide, synthetic peptide, purified peptide, immobilized peptide, detectably labeled peptide, encapsulated peptide, or a vector-expressed peptide (*e.g.*, a peptide encoded by a nucleic acid in a vector comprising a heterologous promoter operably linked to the nucleic acid). In some embodiments, a synthetic tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) may be administered to a subject, such as a human patient, to induce an immune response in the subject. Synthetic peptides may display certain advantages, such as a decreased risk of bacterial contamination, as compared to recombinantly expressed peptides. A tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) may also be comprised in a pharmaceutical composition such as, *e.g.*, a vaccine composition, which is formulated for administration to a mammalian or human subject.

A. Cell Penetrating Peptides

[0059] In some embodiments, an immunotherapy may utilize a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) of the present disclosure that is associated with a cell penetrator, such as a liposome or a cell penetrating peptide (CPP). Antigen presenting cells (such as dendritic cells) pulsed with peptides may be used to enhance antitumour immunity (Celluzzi *et al.*, 1996; Young *et al.*, 1996). Liposomes and CPPs are described in further detail below. In some embodiments, an immunotherapy may utilize a nucleic acid encoding a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) of the present disclosure, wherein the nucleic acid is delivered, *e.g.*, in a viral vector or non-viral vector.

[0060] A tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) may also be associated with or covalently bound to a cell penetrating peptide (CPP). Cell penetrating peptides that may be covalently bound to a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) include, *e.g.*, HIV Tat, herpes virus VP22, the *Drosophila* Antennapedia homeobox gene product, signal sequences, fusion sequences, or protegrin I. Covalently binding a peptide to a CPP can prolong the presentation of a peptide by dendritic cells, thus enhancing antitumour immunity (Wang and Wang, 2002). In some embodiments, a tumor antigen-specific peptide (*e.g.*, the VGLL1 peptide) of the present disclosure (*e.g.*, comprised within a peptide or polypeptide string) may be covalently bound (*e.g.*, via a peptide bond) to a CPP to generate a fusion protein. In other embodiments, a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) or nucleic acid encoding a tumor antigen-specific peptide may be encapsulated within or associated with a liposome, such as a multilamellar, vesicular, or multivesicular liposome.

[0061] As used herein, “association” means a physical association, a chemical association or both. For example, an association can involve a covalent bond, a hydrophobic interaction, encapsulation, surface adsorption, or the like.

[0062] As used herein, “cell penetrator” refers to a composition or compound which enhances the intracellular delivery of the peptide/polypeptide string to the antigen presenting cell. For example, the cell penetrator may be a lipid which, when associated with the peptide, enhances its capacity to cross the plasma membrane. Alternatively, the cell penetrator may be a peptide. Cell penetrating peptides (CPPs) are known in the art, and include, *e.g.*, the Tat protein of HIV (Frankel and Pabo, 1988), the VP22 protein of HSV (Elliott and O'Hare, 1997) and fibroblast growth factor (Lin *et al.*, 1995).

[0063] Cell-penetrating peptides (or “protein transduction domains”) have been identified from the third helix of the *Drosophila* Antennapedia homeobox gene (Antp), the HIV Tat, and the herpes virus VP22, all of which contain positively charged domains enriched for arginine and lysine residues (Schwarze *et al.*, 2000; Schwarze *et al.*, 1999). Also, hydrophobic peptides derived from signal sequences have been identified as cell-penetrating peptides. (Rojas *et al.*, 1996; Rojas *et al.*, 1998; Du *et al.*, 1998). Coupling these peptides to marker proteins such as β -galactosidase has been shown to confer efficient internalization of the marker protein into cells, and chimeric, in-frame fusion proteins containing these peptides have been used to deliver proteins to a wide spectrum of cell types both *in vitro* and *in vivo* (Drin *et al.*, 2002). Fusion of these cell penetrating peptides to a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) in accordance with the present disclosure may enhance cellular uptake of the polypeptides.

[0064] In some embodiments, cellular uptake is facilitated by the attachment of a lipid, such as stearate or myristate, to the polypeptide. Lipidation has been shown to enhance the passage of peptides into cells. The attachment of a lipid moiety is another way that the present disclosure increases polypeptide uptake by the cell. Cellular uptake is further discussed below.

[0065] A tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) of the present disclosure may be included in a liposomal vaccine composition. For example, the liposomal composition may be or comprise a proteoliposomal composition. Methods for producing proteoliposomal compositions that may be used with the present disclosure are described, *e.g.*, in Neelapu *et al.* (2007) and Popescu *et al.* (2007). In some embodiments, proteoliposomal compositions may be used to treat a melanoma.

[0066] By enhancing the uptake of a tumor antigen-specific polypeptide, it may be possible to reduce the amount of protein or peptide required for treatment. This in turn can significantly reduce the cost of treatment and increase the supply of therapeutic agent. Lower dosages can also minimize the potential immunogenicity of peptides and limit toxic side effects.

[0067] In some embodiments, a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) may be associated with a nanoparticle to form nanoparticle-polypeptide complex. In some embodiments, the nanoparticle is a liposomes or other lipid-based nanoparticle such as a lipid-based vesicle (*e.g.*, a DOTAP:cholesterol vesicle). In other embodiments, the nanoparticle is an iron-oxide based superparamagnetic nanoparticles. Superparamagnetic nanoparticles

ranging in diameter from about 10 to 100 nm are small enough to avoid sequestering by the spleen, but large enough to avoid clearance by the liver. Particles this size can penetrate very small capillaries and can be effectively distributed in body tissues. Superparamagnetic nanoparticles-polypeptide complexes can be used as MRI contrast agents to identify and follow
5 those cells that take up the tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide). In some embodiments, the nanoparticle is a semiconductor nanocrystal or a semiconductor quantum dot, both of which can be used in optical imaging. In further embodiments, the nanoparticle can be a nanoshell, which comprises a gold layer over a core of silica. One advantage of nanoshells is that polypeptides can be conjugated to the gold layer using standard chemistry.
10 In other embodiments, the nanoparticle can be a fullerene or a nanotube (Gupta *et al.*, 2005).

[0068] Peptides are rapidly removed from the circulation by the kidney and are sensitive to degradation by proteases in serum. By associating a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) with a nanoparticle, the nanoparticle-polypeptide complexes of the present disclosure may protect against degradation and/or reduce clearance by the kidney. This
15 may increase the serum half-life of polypeptides, thereby reducing the polypeptide dose need for effective therapy. Further, this may decrease the costs of treatment, and minimizes immunological problems and toxic reactions of therapy.

B. Polyepitope Strings

[0069] In some embodiments, a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide)
20 is included or comprised in a polyepitope string. A polyepitope string is a peptide or polypeptide containing a plurality of antigenic epitopes from one or more antigens linked together. A polyepitope string may be used to induce an immune response in a subject, such as a human subject. Polyepitope strings have been previously used to target malaria and other pathogens (Baraldo *et al.*, 2005; Moorthy *et al.*, 2004; Baird *et al.*, 2004). A polyepitope string
25 may refer to a nucleic acid (*e.g.*, a nucleic acid encoding a plurality of antigens including a VGLL1 peptide) or a peptide or polypeptide (*e.g.*, containing a plurality of antigens including a VGLL1 peptide). A polyepitope string may be included in a cancer vaccine composition.

C. Biological Functional Equivalents

[0070] A tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) of the present
30 disclosure may be modified to contain amino acid substitutions, insertions and/or deletions that do not alter their respective interactions with an HLA class protein, such as HLA-A*0101,

binding regions. Such a biologically functional equivalent of a tumor antigen-specific peptide (e.g., a VGLL1 peptide) could be a molecule having like or otherwise desirable characteristics, e.g., binding of HLA-A*0101. As a nonlimiting example, certain amino acids may be substituted for other amino acids in a tumor antigen-specific peptide (e.g., a VGLL1 peptide) disclosed herein without appreciable loss of interactive capacity, as demonstrated by detectably unchanged peptide binding to HLA-A*0101. In some embodiments, the tumor antigen-specific peptide has a substitution mutation at an anchor residue, such as a substitution mutation at one, two, or all of positions: 1 (P1), 2 (P2), and/or 9 (P9). It is thus contemplated that a tumor antigen-specific peptide (e.g., a VGLL1 peptide) disclosed herein (or a nucleic acid encoding such a peptide) which is modified in sequence and/or structure, but which is unchanged in biological utility or activity remains within the scope of the compositions and methods disclosed herein.

[0071] It is also well understood by the skilled artisan that, inherent in the definition of a biologically functional equivalent peptide, is the concept that there is a limit to the number of changes that may be made within a defined portion of the molecule while still maintaining an acceptable level of equivalent biological activity. Biologically functional equivalent peptides are thus defined herein as those peptides in which certain, not most or all, of the amino acids may be substituted. Of course, a plurality of distinct peptides with different substitutions may easily be made and used in accordance with the present disclosure.

[0072] The skilled artisan is also aware that where certain residues are shown to be particularly important to the biological or structural properties of a peptide, e.g., residues in specific epitopes, such residues may not generally be exchanged. This may be the case in the present disclosure, as a mutation in an tumor antigen-specific peptide (e.g., the VGLL1 peptide) disclosed herein could result in a loss of species-specificity and in turn, reduce the utility of the resulting peptide for use in methods of the present disclosure. Thus, peptides which are antigenic (e.g., bind HLA-A*0101 specifically) and comprise conservative amino acid substitutions are understood to be included in the present disclosure. Conservative substitutions are least likely to drastically alter the activity of a protein. A "conservative amino acid substitution" refers to replacement of amino acid with a chemically similar amino acid, i.e., replacing nonpolar amino acids with other nonpolar amino acids; substitution of polar amino acids with other polar amino acids, acidic residues with other acidic amino acids, etc.

[0073] Amino acid substitutions, such as those which might be employed in modifying a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) disclosed herein are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. An analysis of the size, shape and type of the amino acid side-chain substituents reveals that arginine, lysine and histidine are all positively charged residues; that alanine, glycine and serine are all a similar size; and that phenylalanine, tryptophan and tyrosine all have a generally similar shape. Therefore, based upon these considerations, arginine, lysine and histidine; alanine, glycine and serine; and phenylalanine, tryptophan and tyrosine; are defined herein as biologically functional equivalents. In some embodiments, the mutation may enhance TCR-pMHC interaction and/or peptide-MHC binding.

[0074] The present disclosure also contemplates isoforms of the tumor antigen-specific peptides (*e.g.*, a VGLL1 peptide) disclosed herein. An isoform contains the same number and kinds of amino acids as a peptide of the present disclosure, but the isoform has a different molecular structure. The isoforms contemplated by the present disclosure are those having the same properties as a peptide of the present disclosure as described herein.

[0075] Nonstandard amino acids may be incorporated into proteins by chemical modification of existing amino acids or by *de novo* synthesis of a peptide disclosed herein. A nonstandard amino acid refers to an amino acid that differs in chemical structure from the twenty standard amino acids encoded by the genetic code.

[0076] In select embodiments, the present disclosure contemplates a chemical derivative of a tumor antigen-specific peptide (*e.g.*, a VGLL1 peptide) disclosed herein. "Chemical derivative" refers to a peptide having one or more residues chemically derivatized by reaction of a functional side group, and retaining biological activity and utility. Such derivatized peptides include, for example, those in which free amino groups have been derivatized to form specific salts or derivatized by alkylation and/or acylation, *p*-toluene sulfonyl groups, carbobenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups, formyl or acetyl groups among others. Free carboxyl groups may be derivatized to form organic or inorganic salts, methyl and ethyl esters or other types of esters or hydrazides and preferably amides (primary or secondary). Chemical derivatives may include those peptides which comprise one or more naturally occurring amino acids derivatives of the twenty standard amino

