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(54) **Titre : RECEPTEURS DE LYMPHOCYTES T AYANT UNE SPECIFICITE POUR LE VGLL1 ET LEURS UTILISATIONS**
(54) **Title: T CELL RECEPTORS WITH VGLL1 SPECIFICITY AND USES THEREOF**

(57) **Abrégé/Abstract:**
Provided herein are tumor-antigen VGLL1 specific T cell receptors. The TCR may be utilized in various therapies, such as autologous cell transplantation, to treat a cancer. Methods for expanding a population of T cells that target VGLL1 are also provided.

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Abstract:

Provided herein are tumor-antigen VGLL1 specific T cell receptors. The TCR may be utilized in various therapies, such as autologous cell transplantation, to treat a cancer. Methods for expanding a population of T cells that target VGLL1 are also provided.

DESCRIPTION

T CELL RECEPTORS WITH VGLL1 SPECIFICITY AND USES THEREOF

PRIORITY CLAIM

[0001] This application claims the benefit of United States Provisional Patent Application No. 63/028,262, filed May 21, 2020, the entirety of which is incorporated herein by reference.

INCORPORATION OF SEQUENCE LISTING

[0002] The sequence listing that is contained in the file named "UTFCP1477WO_ST25.txt", which is 58.7 KB (as measured in Microsoft Windows) and was created on May 14, 2021, is filed herewith by electronic submission and is incorporated by reference herein.

BACKGROUND

1. Field

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

2. Description of Related Art

[0004] Pancreatic ductal adenocarcinoma (PDAC), the most aggressive form of pancreatic cancer, remains notorious for its poor prognosis and high mortality rate, with its overall 5-year survival rate of 8% being amongst the lowest of all cancer types. Early detection is unusual, with 85% of patients presenting with locally advanced or metastatic disease. Progress towards effective treatment has been slow and the incidence of PDAC-related deaths has continued to rise. Despite some encouraging recent improvements in survival achieved through optimizing the sequencing of surgery and chemotherapy treatment regimens, developing new and effective therapeutic options remains a dire need for advanced-stage PDAC patients (Strobel *et al.*, 2019).

[0005] Cytotoxic T lymphocyte (CTL)-based immunotherapies have been successful at inducing objective clinical responses in a variety of cancer types. Checkpoint inhibitor (CPI) therapies that act through non- specific activation of T lymphocytes have made a significant

positive impact on long-term patient survival. However, the benefits of CPI have mainly been limited to highly mutated tumor types like melanoma and lung adenocarcinoma that can express a large array of potential neo-antigen peptides in the context of surface HLA molecules (Rizvi *et al.*, 2015). Tumor-infiltrating lymphocyte (TIL) therapy, in which individual cancer patients are re-infused with T cells expanded from their own tumors, has also shown great promise for inducing the regression of bulky tumors. TIL are polyclonal and can recognize both patient-specific neo-antigens as well as shared tumor- associated antigens (TAA) such as melanocyte differentiation antigens (MDA) or cancer-testis antigens (CTA). Targeting of individual validated HLA class I-restricted TAAs through infusion of antigen-specific endogenous T-cells (ETC therapy) or genetically engineered TCR-T cells has also proven successful at inducing clinical responses in patients with melanoma and other solid cancers.

[0006] CPI- and CTL-based immunotherapies have unfortunately not shown the same beneficial impact in treating PDAC patients (Young *et al.*, 2018). This lack of success has been attributed to the highly immune suppressive tumor microenvironment (TME) of PDAC, in addition to the relatively low mutational burden that contributes to a dearth of neo-antigen targets (Yarchoan *et al.*, 2017). A number of potentially targetable HLA class I-restricted peptide antigens have been identified in PDAC, most notably those derived from carcinoembryonic antigen-related cell adhesion molecule (CEACAM), mucin 16 (MUC16), mesothelin (MSLN), and mutated *KRAS*, among others. Although promising, therapies targeting these TAAs have faced inherent limitations, including the induction of toxicities in non-tumor tissues, low prevalence of target antigen expression, or inability to break self-tolerance mechanisms that often hinders the generation of high-affinity CTLs. With limited exceptions, clinical trials targeting these antigens have yielded disappointing results, underscoring the need to identify safe, immunogenic targets that demonstrate higher prevalence in PDAC patients. Thus, there is an unmet medical need for novel T cell-based therapies to additional target antigens for these malignancies.

SUMMARY

[0007] In some embodiments, the present disclosure provides VGLL1 T cell receptors (TCRs) and methods for their use, such as in therapies including adoptive T cell therapies. In one embodiment, there is provided an engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises: (a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID

NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23); (b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46); (c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or (d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92).

[0008] In some aspects, the TCR comprises alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23). In certain aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:15 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:20. For example, the variable region comprises the CDRs and the remaining part of the sequence has at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) sequence identity. In particular aspects, the TCR comprises an alpha chain variable region of SEQ ID NO:15 and a beta chain variable region of amino acid sequence of SEQ ID NO:20. In certain aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:3 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:9. In specific aspects, the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:3 and a beta chain comprising the nucleotide sequence of SEQ ID NO:9. In some aspects, the TCR other than the CDRs has at least 90% identity to SEQ ID NO:13. In particular aspects, the TCR comprises SEQ ID NO:13.

[0009] In certain aspects, the TCR comprises alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46). In certain aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:38 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%,

94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:43. In certain aspects, the TCR comprises an alpha chain variable region of SEQ ID NO:38 and a beta chain variable region of amino acid sequence of SEQ ID NO:43. In some aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:26 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:32. In some aspects, the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:26 and a beta chain variable region comprising the nucleotide sequence of SEQ ID NO:32. In some aspects, the TCR other than the CDRs has at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to SEQ ID NO:36. In certain aspects, the TCR comprises SEQ ID NO:36.

[0010] In certain aspects, the TCR comprises alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69). In some aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:61 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:66. In some aspects, the TCR comprises an alpha chain variable region of SEQ ID NO:61 and a beta chain variable region of amino acid sequence of SEQ ID NO:66. In certain aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:49 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:55. In some aspects, the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:49 and a beta chain comprising the nucleotide sequence of SEQ ID NO:55. In particular aspects, the TCR other than the CDRs has at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to SEQ ID NO:59. In specific aspects, the TCR comprises SEQ ID NO:59.

[0011] In some aspects, the TCR comprises alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2

(SEQ ID NO:91), and CDR3 (SEQ ID NO:92). In certain aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:84 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the amino acid sequence of SEQ ID NO:89. In some aspects, the TCR comprises an alpha chain variable region of SEQ ID NO:84 and a beta chain variable region of amino acid sequence of SEQ ID NO:89. In certain aspects, the TCR comprises an alpha chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:72 and a beta chain variable region other than the CDRs having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to the nucleotide sequence of SEQ ID NO:78. In some aspects, the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:72 and a beta chain comprising the nucleotide sequence of SEQ ID NO:78. In particular aspects, the TCR other than the CDRs has at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97, 98%, or 99%) identity to SEQ ID NO:82. In specific aspects, the TCR comprises SEQ ID NO:82.

[0012] In some aspects, the engineered TCR binds HLA-A*0101. In certain aspects, the TCR is further defined as a soluble TCR, wherein the soluble TCR does not comprise a transmembrane domain. In additional aspects, the TCR further comprises a detectable label. In some aspects, the TCR is covalently bound to a therapeutic agent, such as an immunotoxin or a chemotherapeutic agent.

[0013] A further embodiment provides a multivalent TCR complex comprising a plurality of TCRs of the present embodiments or aspects, thereof (e.g., an engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises: (a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23); (b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46); (c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or (d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID

NO:92)). In some aspects, the multivalent TCR comprises 2, 3, 4 or more TCRs associated with one another. In certain aspects, the multivalent TCR is present in a lipid bilayer, in a liposome, or is attached to a nanoparticle. In some aspects, the TCRs are associated with one another via a linker molecule.

[0014] Another embodiment provides a polypeptide encoding the TCR of the present embodiments or aspects thereof (e.g., an engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises: (a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23); (b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46); (c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or (d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92). A further embodiment provides a polynucleotide encoding the polypeptide encoding the TCR of the present embodiments or aspects thereof.

[0015] In yet another embodiment, there is provided an expression vector encoding the TCR of the present embodiments or aspects thereof (e.g., an engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises: (a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23); (b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46); (c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or (d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92)). In some aspects, the sequence encoding the TCR is under the control of a promoter. In certain aspects, the expression vector is a viral vector. In some aspects, the viral vector is a retroviral vector. In additional aspects, the vector further encodes a linker domain. For example, the linker domain is positioned between the alpha chain and beta chain.

[0016] A further embodiment provides a host cell engineered to express the TCR of the present embodiments or aspects thereof (e.g., an engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises: (a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23); (b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46); (c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or (d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92)). In some aspects, the cell is a T cell, NK cell, invariant NK cell, NKT cell, mesenchymal stem cell (MSC), or induced pluripotent stem (iPS) cell. In certain aspects, the host cell is an immune cell. In particular aspects, the host cell is isolated from an umbilical cord. In some aspects, the T cell is a $CD8^+$ T cell, CD4+ T cell, or γδ T cell. In particular aspects, the T cell is a regulatory T cell (Treg). In some aspects, the cell is autologous. In certain aspects, the cell is allogeneic.

[0017] Another embodiment provides a method for engineering the host cell of the present embodiments or aspects thereof comprising contacting said immune cell with the TCR of the present embodiments or aspects thereof (e.g., an engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises: (a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23); (b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46); (c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or (d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92)) or the expression vector of any one of the present embodiments or aspects thereof. In some aspects, the immune cell is a T cell, or a peripheral blood lymphocyte. In certain aspects, contacting is further defined as transfecting or transducing. In some aspects, transfecting comprises electroporating RNA encoding the TCR of the present embodiments or aspects thereof into the immune cell. In some aspects, the method further comprises generating viral supernatant from

the expression vector of claim 41 prior to transducing the immune cell. In some aspects, the immune cell is a stimulated lymphocyte, such as a human lymphocyte. In particular aspects, stimulating comprises contacting the immune cell with or incubating the immune cell in OKT3 and/or IL-2. In additional aspects, the method further comprises sorting the immune cells to isolate TCR engineered T cells. In some aspects, the method further comprises performing T cell cloning by serial dilution. In specific aspects, the method further comprises expansion of the T cell clone by the rapid expansion protocol.

[0018] A further embodiment provides a method of treating cancer in a subject comprising administering an effective amount of the TCR-engineered cells of the present embodiments or aspects thereof to the subject. In some aspects, the subject is identified to have an HLA-A*0101 allele. In certain aspects, the TCR-engineered cell is a T cell or peripheral blood lymphocyte. In particular aspects, the T cell is a $CD8^+$ T cell, $CD4^+$ T cell, or Treg. In some aspects, the cancer is pancreatic cancer, ovarian cancer, gastric cancer, breast cancer, bladder cancer, uterine cancer, or cervical cancer. In certain aspects, the subject is a human. In some aspects, the TCR engineered cells are autologous. In certain aspects, the TCR engineered cells are allogeneic. In additional aspects, the method further comprises lymphodepletion of the subject prior to administration of the VGLL1-specific T cells. For example, lymphodepletion comprises administration of cyclophosphamide and/or fludarabine. In additional aspects, the method further comprises administering a second anticancer therapy. In some aspects, the therapy is a chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy. In certain aspects, the TCR-engineered 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. In some aspects, the subject is determined to have cancer cells which overexpress VGLL1.

[0019] 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

[0020] 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.

[0021] FIGS. 1A-1C: Immunopeptidome analysis reveals a VGLL1-derived peptide expressed by two PDAC patient- derived organoid lines. (FIG. 1A) Experimental strategy to identify PDAC tumor-specific, HLA class I-bound peptides from 39 tumor specimens derived from 35 M.D. Anderson PDAC patients. **(FIG. 1B)** Bioinformatics screening strategy to identify potentially targetable TAAs from amongst the eluted PDAC-associated peptides. Peptide-encoding genes were assessed for PDAC tumor RNAseq expression compared with transcript expression in 42 GTex Portal normal tissues. Excluding testis, normal tissues were separated into 4 categories (non-essential, caution, hazard, and danger tissues) that reflected the potential toxicities expected from off-tumor killing activity against different tissues. All peptide-encoding genes were filtered successively using four corresponding expression thresholds of increasing stringency (30, 10, 3, and 1 TPM, indicated by left dotted lines) to eliminate candidate TAAs most likely to elicit autoimmune toxicity in the context of CTL therapy (right dotted lines). Screening of high-confidence peptides isolated from tumor organoid cell lines of PDAC patients MP015 and MP081 is depicted, showing that few eluted peptides met these stringent criteria. **(FIG. 1C)** Mass spectra of an HLA-A*0101-restricted VGLL1-derived peptide isolated from two different PDAC organoid cell lines, MP015 and MP081 (top 2 panels). The patient-derived peptides co-eluted with and matched the MS fragmentation spectra of the synthetic isotope-labeled VGLL1 peptide LSELETPGKY (SEQ ID NO:93) (containing a $^{13}C/^{15}N$-labeled lysine residue), with the labeled y^+ fragment ion series demonstrating an expected shift of 8 atomic mass units (bottom panel).

[0022] FIG. 2: VGLL1 is overexpressed in multiple tumor types. VGLL1 transcript expression in normal tissues (left, GTex Portal database) and human cancers (right, TCGA database), as determined by RNAseq analyses. Each dot represents one normal donor or patient tumor sample. Colors correspond to the 4 normal tissue categories defined in FIG. 1: Green, non-essential tissues (adipose, cervix, ebv, fallopian tube, muscle, ovary, transformed fibroblasts, uterus, skin, testis, vagina, prostate, breast, salivary gland); Yellow, caution tissues (adrenal gland, nerve, spleen, thyroid, whole blood, pancreas, pituitary, bladder); Orange,

hazard tissues (colon, esophagus, liver, small intestine, stomach, kidney, lung); Red, danger tissues (artery, brain, heart). While >95% of normal GTex caution, hazard, and danger tissue samples fell below 3 transcripts per million (TPM, dotted line), significant numbers of TCGA cancer patients demonstrated VGLL1 transcript expression above this threshold (box).

[0023] FIG. 3: VGLL1 is associated with poor pancreatic patient survival. Top: Kaplan-Meier curves showing TCGA PDAC patient overall survival (OS) stratified by tumor VGLL1 transcript expression (n = 178). P-values indicate log-rank significance test results comparing the OS of 3 groups of VGLL1-expressing patients to those patients with low or absent VGLL1 expression. (>100 TPM showed lowest overall survival and <1.5 TPM showed highest overall survival). **Bottom:** Patient- derived xenografts (PDX) from an independent cohort of MD Anderson metastatic PDAC patient tumors (n =37) underwent RNAseq analysis after being grown in immunodeficient mice. Graph shows PDX specimens stratified into 3 groups corresponding to OS time and corresponding VGLL1 transcript expression.

[0024] FIGS. 4A-4D: Generation of VGLL1 antigen-specific CTLs from peripheral blood of Patient MP015. (FIG. 4A) Schematic outlining the experimental procedure for generating VGLL1-specific $CD8^+$ T-cells from human donor PBMCs. **(FIG. 4B)** PBMC isolated from PDAC Patient MP015 by leukapheresis were stimulated with autologous LSELETPGKY (SEQ ID NO: 93) peptide-pulsed dendritic cells (DCs). After two stimulations (top row), $CD8^+$ and VGLL1 tetramer-positive cells were sorted and expanded using a standard rapid expansion protocol (REP). VGLL1-specific T-cells were re-sorted and expanded a second time due to low numbers of antigen-specific cells following the first REP. The second REP yielded 19.6×10^9 VGLL1-specific CTLs, which Patient MP015 safely received as an infusion under a personalized ETC therapy Compassionate IND protocol. **(FIG. 4C)** TCR repertoire analysis of expanded VGLL1-specific CTLs was performed using Vβ antibodies corresponding to 24 different specificities. **(FIG. 4D)** VGLL1-specific T-cells expanded from Patient MP015 were tested for functionality in a standard ^{51}Cr release assay to assess specific lysis of Mel888 melanoma cells (VGLL1-negative HLA- A*0101-positive) pulsed with titrated amounts of LSELETPGKY (SEQ ID NO: 93) peptide at a 5:1 effector-to-target (E:T) ratio.

[0025] FIGS. 5A-5B: VGLL1-specific CTLs recognize and kill multiple allogeneic pancreatic cancer cell lines. (FIG. 5A) Expanded VGLL1-specific $CD8^+$ T cells from Patient

MP015 were co-cultured with a panel of HLA-A*0101- positive PDAC tumor cell lines in a standard ^{51}Cr release assay to measure cytotoxic activity at different effector-to-target (E:T) cell ratios. WM793 melanoma cells (VGLL1-negative HLA-A*0101-positive) were used as a negative control line. VGLL1-CTLs robustly killed the autologous organoid cell line MP015 from which the VGLL1 peptide was originally isolated, and also demonstrated cytotoxic activity against four allogeneic, HLA-A*0101-expressing PDAC cell lines. Results show the means and standard deviations of six replicate samples, and data is representative of a minimum of 4 replicate experiments. **(FIG. 5B)** Western blot analysis showing expression of VGLL1 protein in all five PDAC cell lines tested.

[0026] FIGS. 6A-6D: VGLL1-specific T cells recognize and kill multiple tumor types, but have reduced recognition of primary tissue cell lines. (FIG. 6A) VGLL1-specific CD8+ T cells were co-cultured with 13 different HLA-A*0101- expressing tumor cell lines derived from ovarian, lung, breast, bladder, or gastric cancer in a standard ^{51}Cr release assay to measure cytotoxic activity at different effector-to-target (E:T) cell ratios. Five HLA-A*0101- negative cell lines (EBC1, HT1197, HT1376, GT-5, and MKN74) were lentivirally transduced to stably express HLA-A*0101; VGLL1-CTL killing of the parental cell lines (grey lines) are shown in comparison to HLA- A*0101-transduced counterparts (black lines). **(FIG. 6B)** Western blot analysis showing VGLL1 protein expression in tumor cell lines derived from ovarian, lung, breast, bladder or gastric cancers. **(FIG. 6C)** VGLL1-specific CTLs were co-cultured with HLA-A*0101-expressing primary tissue cells derived from bladder, breast, lung airway, or skin melanocytes in a standard ^{51}Cr release assay to measure cytotoxic activity. VGLL1-CTL assay results show the means and standard deviations of six replicate samples, and data is representative of a minimum of 2 replicate experiments. **(FIG. 6D)** VGLL1 protein expression in primary cell lines as assessed by Western blot analysis.

[0027] FIGS. 7: Quantity of immunoprecipitated HLA class I correlates with PDAC tumor specimen weight. Surgical tumor resections from PDAC patients (n=34) or patient-derived xenografts (n=3) was weighed prior to tissue lysis and immunoprecipitation of total HLA class I using mAb W6/32. Recovered HLA class I was quantitated based on Western blot analysis by assessing the HLA class I band intensity (expected size 42 - 44 KD) on a scale of 0 (none detected) to 4 (highest level detected). Graph shows specimen weight plotted by Western blot band intensity; the dotted line delineates samples with lower than expected HLA class I recovery, indicating reduced tumor HLA expression.

[0028] FIG. 8: Total number of detected PDAC-associated peptides correlates with quantity of recovered HLA class I. HLA class I recovered from PDAC patient-derived surgical resections (n=34), xenografts (n=3), or organoid cell lines (n=2) was quantitated by Western blot analysis by assessing the HLA class I band intensity (size 42 - 44 KD) on a scale of 0 (none detected) to 4 (highest level detected). Peptides eluted from immunoprecipitated HLA class I were analyzed by tandem MS and searched against the SwissProt human proteome database. Graph shows number of unique, high quality peptide matches plotted against HLA class I intensity as analyzed by Western blot.

[0029] FIG. 9: VGLL1-derived peptide was eluted from the PANC-1005 cell line. Mass spectra of HLA- A*0101-restricted VGLL1-derived peptide isolated from PDAC cell line PANC10.05 (top panel). The native peptide co-eluted with and matched the MS fragmentation spectra of the synthetic isotope-labeled peptide LSELETPGKY (SEQ ID NO: 93) containing a $^{13}C/^{15}N$-labeled lysine residue (bottom panel).

[0030] FIG. 10: VGLL1 is preferentially expressed in basal-like breast cancer compared to other breast cancer subtypes. TCGA breast cancer patients were subdivided into 5 major sub-types (LumA, LumB, Basal-like, HER2 overexpressing, and normal-like) and analyzed for tumor VGLL1 expression by RNAseq analysis. Each dot represents one TCGA patient sample, and VGLL1 transcript expression is expressed in fragments per kilobase of transcript per million mapped reads (FPKM).

[0031] FIG. 11: VGLL1 gene expression in tumor cell lines derived from a variety of cancer types. Gene expression microarray analysis of a diverse array of tumor cell lines (n=679) from the Cancer Cell Line Encyclopedia (CCLE) showed that VGLL1 is expressed by a majority of PDAC and bladder cancer cell lines, in addition to a significant percentage of breast, gastric, ovarian, and lung cancer cell lines. No VGLL1 expression was found in cell lines derived from melanoma, thyroid, or hematopoietic cancers. Threshold for VGLL1 antigen positivity was 3-fold above background signal.

[0032] FIGS. 12A-12D: High tumor VGLL1 expression is associated with reduced survival in multiple cancer types. TCGA cancer patients were stratified into three groups according to tumor VGLL1 expression as determined by RNAseq analysis. Kaplan-Meier curves show overall survival (OS) of each group for **(FIG. 12A)** Stomach adenocarcinoma (>10 TPM showed lowest overall survival and <1.5 showed highest overall survival), **(FIG.**

12B) Breast carcinoma (>50 TPM showed lowest overall survival and <1.5 showed highest overall survival), **(FIG. 12C)** Ovarian serous adenocarcinoma, and **(FIG. 12D)** bladder urothelial carcinoma patients. P-values indicate log-rank significance test results comparing the OS of the groups with the lowest and highest VGLL1 expression.

[0033] FIGS. 13A-13B: Generation of HLA-A*0101-restricted VGLL1 antigen-specific CTLs from multiple normal donor PBMC. (FIG. 13A) Induction of VGLL1-specific CD8 T cells from PBMC of two healthy donors. HLA-A*0101-expressing donor PBMC were stimulated twice with LSELETPGKY (SEQ ID NO: 93) peptide-pulsed dendritic cells over 2 weeks. VGLL1 tetramer-positive $CD8^+$ T cells were sorted by ARIA sorter (top panels) and the sorted T cells were expanded using a standard rapid expansion protocol (REP). **(FIG. 13B)** TCR repertoire analysis of expanded VGLL1-specific CTLs was performed using Vβ antibodies corresponding to 24 different specificities.

[0034] FIG. 14: PDAC patient MP015 showed loss of VGLL1 antigen expression prior to VGLL1-CTL therapy. RNAseq analyses from lung tumor metastases of PDAC patient MP015 revealed loss of VGLL1 transcript expression between November 2013 and December 2015.

[0035] FIG. 15: HLA-A*0101 surface expression confirmed on target cell lines by flow cytometry. All tumor cell lines and normal primary cells used in this study were stained with fluorophore-labeled HLA-A*0101- specific mAb and analyzed by flow cytometry to confirm natural endogenous HLA-A*0101 surface expression (grey histograms) prior to use as targets in VGLL1-specific CTL assays. Five tumor cell lines were transduced to express HLA-A*0101 using a lentiviral expression vector.

[0036] FIG. 16: VGLL1-CTL killing is blocked with an HLA-class I-specific antibody. Expanded VGLL1- specific $CD8^+$ T cells were co-cultured with HLA-A*0101-positive PDAC tumor cell line PANC10.05 in a standard ^{51}Cr release assay to measure cytotoxic activity at different effector-to-target (E:T) cell ratios. Addition of the HLA class I blocking antibody W6/32 largely abrogated VGLL1-CTL killing, demonstrating that antitumor CTL activity is HLA class I-restricted

[0037] FIG. 17: TCR expressing T cells can efficiently kill Mel888HLAA1 cells pulsed with peptide. PBMCs were transduced with VGLL1 TCRs to target Mel888HLAA1 cells pulsed with peptide.

[0038] FIG. 18: Cloned VGLL1 TCRs can recognize endogenous expressed antigen. The Mel888A1 cells are $HLA^{+}VGLL1^{-}$ and the Panc cells are $HLAA1^{+}VGLL1^{+}$.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0039] Cytotoxic T lymphocyte (CTL)-based cancer immunotherapies have shown great promise for inducing clinical regression by targeting tumor-associated antigens (TAA). To expand the TAA landscape of pancreatic ductal adenocarcinoma (PDAC), tandem mass spectrometry analysis of HLA class I-bound peptides was performed from tumor specimens of 35 PDAC patients. This led to the identification of a shared HLA-A*0101 restricted peptide derived from co-transcriptional activator Vestigial-like 1 (VGLL1), a novel putative TAA demonstrating overexpression in multiple tumor types and low or absent transcript expression in essential normal tissues. VGLL1-specific CTLs were isolated and expanded from the blood of a male PDAC patient showed the capacity to recognize and kill in an antigen-specific manner a majority of HLA-A*0101 allogeneic tumor cell lines derived not only from PDAC, but also bladder, ovarian, gastric, lung and basal-like breast cancers. Gene expression profiling revealed that VGLL1 is a member of a unique group of cancer-placenta antigens (CPA) that may constitute immunotherapeutic TAA targets for patients with multiple different cancer types. The studies identified VGLL1 as a tumor-associated antigen from a pancreatic elution as described in International Patent Publication No. WO2018/067869; incorporated herein in its entirety.

[0040] 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. T cell receptors (TCRs) from these antigen-specific CTLs were cloned and sequenced, and can in various incarnations represent powerful tools with which to target cancer, such as pancreatic, ovarian, gastric, and breast tumors, in cancer patients. Thus, these VGLL1-specific TCRs provided herein may be used to target solid cancers (*e.g.*, pancreatic, ovarian, gastric, and breast cancer).

[0041] Accordingly, the present disclosure provides TCRs (e.g., SEQ ID NOs:1-92) that specifically bind VGLL1, such as the VGLL1 peptide epitope (LSELETPGKY: SEQ ID NO:93). The present disclosure also provides nucleotide sequences encoding these TCRs, expression vectors comprising the nucleotide sequences which can be used to modify naïve T cells and generate VGLL1-specific T cells. The present disclosure further provides the use of VGLL1-specific T cells for therapy, such as adoptive cell therapy for cancer patients, such as HLA-A*0101-positive cancer patients, whose malignant cells express VGLL1 antigen. The antigen-specific T cells, such as CTLs, provided herein may be used to target solid cancers.

I. Definitions

[0042] 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.

[0043] 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.

[0044] 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.

[0045] The term "essentially" is to be understood that methods or compositions include only the specified steps or materials and those that do not materially affect the basic and novel characteristics of those methods and compositions.

[0046] As used herein, a composition or media that is "substantially free" of a specified substance or material contains $\leq 30\%$, $\leq 20\%$, $\leq 15\%$, more preferably $\leq 10\%$, even more preferably $\leq 5\%$, or most preferably $\leq 1\%$ of the substance or material.

[0047] The terms "substantially" or "approximately" as used herein may be applied to modify any quantitative comparison, value, measurement, or other representation that could permissibly vary without resulting in a change in the basic function to which it is related.

[0048] The term "about" means, in general, within a standard deviation of the stated value as determined using a standard analytical technique for measuring the stated value. The terms can also be used by referring to plus or minus 5% of the stated value.

[0049] "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.

[0050] "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.

[0051] 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.

[0052] 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.

[0053] The phrases "pharmaceutical or pharmacologically acceptable" refers to 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, pyrogenicity, general safety, and purity standards as required by FDA Office of Biological Standards.

[0054] 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, 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 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.

[0055] 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 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.

[0056] 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.

[0057] 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 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 comprise domains for additional co-stimulatory signaling, such as CD3ζ, 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, homing receptors, chemokines, chemokine receptors, cytokines, and cytokine receptors.

[0058] 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 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 TCR Methods and Compositions

[0059] In some embodiments, the present disclosure provides VGLL1-specific TCRs. The TCR may comprise alpha chain CDRs of SEQ ID NOs:16-19, 39-41, 62-64, or 86-87 and/or beta chain CDRs of SEQ ID NOs:21-23, 44-46, 67-69, or 90-92. The TCR may comprise an alpha variable chain with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:15, 38, 61, or 84 and/or a beta variable chain with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:20, 43, 66, or 89. The TCR may comprise an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:13, 36, 59, or 82.

[0060] Also provided herein are polynucleotides encoding the alpha chain and/or beta chain of the VGLL1 TCRs provided herein. Polynucleotides encoding the present TCRs may comprise alpha chain CDRs of SEQ ID NOs:4-6, 27-29, 50-52, or 73-75 and/or beta chain CDRs of SEQ ID NOs:10-12, 33-35, 56-58, or 79-81. The TCR may be encoded by an alpha chain with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:1, 24, 47, or 70 and/or a beta chain with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:7, 30, 53, or 76. The TCR may be encoded by an alpha variable chain with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:3, 26, 49, or 72 and/or a beta variable chain with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity or similarity to SEQ ID NOs:9, 32, 55, or 78.

[0061] The antigen binding region of the present TCRs may be included in a chimeric antigen receptor (CAR) as the extracellular domain comprising an antigen binding region. The TCR may be transfected into cells (*e.g.*, autologous or allogeneic cells) that may be used in an adoptive cell transfer therapy. In some embodiments, the CAR is humanized to reduce immunogenicity (hCAR).

[0062] VGLL1 TCR sequences are provided below.

VGLL1 TCR #1 TRAV19*01 J56*01/ TRBVC1 5-6*01 J1-1 Alpha Chain

TCTAGACCGCCATGGGTCGACGCCACCATGAACATGCTGACTGCCAGCCTGTTGAGGGC
AGTCATAGCCTCCATCTGTGTTGTATCCAGCATGGCTCAGAAGGTAACTCAAGCGCAGAC
TGAAATTTCTGTGGTGGAGAAGGAGGATGTGACCTTGGACTGTGTGTATGAAACCCGTG
ATACTACTTATTACTTATTCTGGTACAAGCAACCACCAAGTGGAGAATTGGTTTTCCTTAT
TCGTCGGAACTCTTTTGATGAGCAAAATGAAATAAGTGGTCGGTATTCTTGGAACTTCCA
GAAATCCACCAGTTCCTTCAACTTCACCATCACAGCCTCACAAGTCGTGGACTCAGCAGT
ATACTTCTGTGCTCTGAGTCCTGGAGCCAATAGTAAGCTGACATTTGGAAAAGGAATAAC
TCTGAGTGTTAGACCAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTC
TAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTC
ACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTA
TGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCA
AACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCC
TGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAA
CCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCAT
GACGCTGCGGCTGTGGTCCAGC (SEQ ID NO:1)

VGLL1 TCR #1 Alpha Chain Signal peptide

ATGAACATGCTGACTGCCAGCCTGTTGAGGGCAGTCATAGCCTCCATCTGTGTTG
TATCCAGCATGGCT (SEQ ID NO:2)

VGLL1 TCR #1 Alpha Chain V-region

CAGAAGGTAACTCAAGCGCAGACTGAAATTTCTGTGGTGGAGAAGGAGGATGTG
ACCTTGGACTGTGTGTATGAAACCCGTGATACTACTTATTACTTATTCTGGTACAA
GCAACCACCAAGTGGAGAATTGGTTTTCCTTATTCGTCGGAACTCTTTTGATGAG
CAAAATGAAATAAGTGGTCGGTATTCTTGGAACTTCCAGAAATCCACCAGTTCCT
TCAACTTCACCATCACAGCCTCACAAGTCGTGGACTCAGCAGTATACTTCTGTGC
TCTGAGTCCTGGAGCCAATAGTAAGCTGACATTTGGAAAAGGAATAACTCTGAG
TGTTAGACCAG (SEQ ID NO:3)

VGLL1 TCR #1 Alpha Chain CDR1

ACCCGTGATACTACTTATTAC (SEQ ID NO:4)

VGLL1 TCR #1 Alpha Chain CDR2

CGGAACTCTTTTGATGAGCAAAAT (SEQ ID NO:5)

VGLL1 TCR #1 Alpha Chain CDR3

TGTGCTCTGAGTCCTGGAGCCAATAGTAAGCTGACATTT (SEQ ID NO:6)

VGLL1 TCR #1 Beta Chain

ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGC
AAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCA
ACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGA
GAGACAGAGAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTAT
AGCTCTGAGCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCT

GTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCA
GACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTT
TGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCCT
GGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAA
GGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGC
CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC
TGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGG
AGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCG
CCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAAG
GGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTA
TGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTTC
TGACTCGAGAAGCTTGCGGCCGCGGATCCGATAAAATAA (SEQ ID NO:7)

VGLL1 TCR #1 Beta Chain Signal peptide

ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCT (SEQ ID NO:8)

VGLL1 TCR #1 Beta Chain V-region

GGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGACT
CTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCAACAGGCCC
TGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGAGAGACAGA
GAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTATAGCTCTGA
GCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGC
AGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA
GTTGTAG (SEQ ID NO:9)

VGLL1 TCR #1 Beta Chain CDR1

TCTGGGCATGACACT (SEQ ID NO:10)

VGLL1 TCR #1 Beta Chain CDR2

TATTATGAGGAGGAAGAG (SEQ ID NO:11)

VGLL1 TCR #1 Beta Chain CDR3

TGTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTT (SEQ ID NO:12)

VGLL1 TCR #1 Amino Acid Sequence

MNMLTASLLRAVIASICVVSSMAQKVTQAQTEISVVEKEDVTLDCVYETRDTTYYLF
WYKQPPSGELVFLIRRNSFDEQNEISGRYSWNFQKSTSSFNFTITASQVVDSAVYFCA
LSPGANSKLTFGKGITLSVRPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQS
KDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC
DVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSSRAKRSGSGATN
FSLLKQAGDVEENPGPMGPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQVTL
RCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNV
NALLLGDSALYLCASSVGTGITEAFFGQGTRLTVVEDLNKVFPPEVAVFEPSEAEISH
TQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSR

LRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT
SVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO:13)

VGLL1 TCR #1 Alpha Chain Signal peptide
MNMLTASLLRAVIASICVVSSM (SEQ ID NO:14)

VGLL1 TCR #1 Alpha Chain V-region
AQKVTQAQTEISVVEKEDVTLDCVYETRDTTYYLFWYKQPPSGELVFLIRRNSFDEQ
NEISGRYSWNFQKSTSSFNFTITASQVVDSAVYFCALSPGANSKLTFGKGITLSVRPDI
Q (SEQ ID NO:15)

VGLL1 TCR #1 Alpha Chain CDR1
TRDTTYY (SEQ ID NO:16)

VGLL1 TCR #1 Alpha Chain CDR2
RNSFDEQN (SEQ ID NO:17)

VGLL1 TCR #1 Alpha Chain CDR3
CALS**PGANSKLTF** (SEQ ID NO:18)

VGLL1 TCR #1 Beta Chain Signal peptide
MGPGLLCWALLCLLG (SEQ ID NO:19)

VGLL1 TCR #1 Beta Chain V-region
AGLVDAGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEE
EERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSVGTGITEAFFGQGTRL
TVVE (SEQ ID NO:20)

VGLL1 TCR #1 Beta Chain CDR1
SGHDT (SEQ ID NO:21)

VGLL1 TCR #1 Beta Chain CDR2
YYEEEE (SEQ ID NO:22)

VGLL1 TCR #1 Beta Chain CDR3
CASSVGTGITEAFF (SEQ ID NO:23)

VGLL1 TCR#2 TRAV13-1*02 J10/ TRBVC1 5-6*01 J1-1 Alpha Chain
TCTAGACCGCCATGGGTCGACGCCACCATGACATCCATTCGAGCTGTATTTATAT
TCCTGTGGCTGCAGCTGGACTTGGTGAATGGAGAGAATGTGGAGCAGCATCCTTC
AACCCTGAGTGTCCAGGAGGGAGACAGCGCTGTTATCAAGTGTACTTATTCAGA
CAGTGCCTCAAACTACTTCCCTTGGTATAAGCAAGAACTTGGAAAAAGACCTCA
GCTTATTATAGACATTCGTTCAAATGTGGGCGAAAAGAAAGACCAACGAATTGC
TGTTACATTGAACAAGACAGCCAAACATTTCTCCCTGCACATCACAGAGACCCAA
CCTGAAGACTCGGCTGTCTACTTCTGTGCAGCAAGGGGACTCACGGGAGGAGGA
AACAAACTCACCTTTGGGACAGGCACTCAGCTAAAAGTGGAACTCAATATCCAG
AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTG
TCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTC

TGATGTGTATATCACGGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAAG
AGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCC
TTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCT
GTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTC
AAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAA
TCTGCTCATGACGCTGCGGCTGTGGTCCAGC (SEQ ID NO:24)

VGLL1 TCR #2 Alpha Chain Signal peptide
ATGACATCCATTCGAGCTGTATTTATATTCCTGTGGCTGCAGCTGGACTTGGTGA
AT (SEQ ID NO:25)

VGLL1 TCR #2 Alpha Chain V-region
GGAGAGAATGTGGAGCAGCATCCTTCAACCCTGAGTGTCCAGGAGGGAGACAGC
GCTGTTATCAAGTGTACTTATTCAGACAGTGCCTCAAACTACTTCCCTTGGTATA
AGCAAGAACTTGGAAAAAGACCTCAGCTTATTATAGACATTCGTTCAAATGTGG
GCGAAAAGAAAGACCAACGAATTGCTGTTACATTGAACAAGACAGCCAAACATT
TCTCCCTGCACATCACAGAGACCCAACCTGAAGACTCGGCTGTCTACTTCTGTGC
AGCAAGGGGACTCACGGGAGGAGGAAACAAACTCACCTTTGGGACAGGCACTC
AGCTAAAAGTGGAACTCA (SEQ ID NO:26)

VGLL1 TCR #2 Alpha Chain CDR1
GACAGTGCCTCAAACTAC (SEQ ID NO:27)

VGLL1 TCR #2 Alpha Chain CDR2
ATTCGTTCAAATGTGGGCGAA (SEQ ID NO:28)

VGLL1 TCR #2 Alpha Chain CDR3
TGTGCAGCAAGGGGACTCACGGGAGGAGGAAACAAACTCACCTTT (SEQ ID NO:29)

VGLL1 TCR #2 Beta Chain
ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGC
AAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCA
ACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGA
GAGACAGAGAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTAT
AGCTCTGAGCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCT
GTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCA
GACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTT
TGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCCT
GGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAA
GGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGC
CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC
TGGCAGAACCCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGG
AGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCG
CCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAAG

GGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTA
TGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTTC
TGA*CTCGAG*AAGCTT*GCGGCCGC*GGATCCGATAA (SEQ ID NO:30)

VGLL1 TCR #2 Beta Chain Signal peptide
ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCT (SEQ ID NO:31)

VGLL1 TCR #2 Beta Chain V-region
GGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGACT
CTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCAACAGGCCC
TGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGAGAGACAGA
GAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTATAGCTCTGA
GCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGC
AGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA
GTTGTAG (SEQ ID NO:32)

VGLL1 TCR #2 Beta Chain CDR1
TCTGGGCATGACACT (SEQ ID NO:33)

VGLL1 TCR #2 Beta Chain CDR2
TATTATGAGGAGGAAGAG (SEQ ID NO:34)

VGLL1 TCR #2 Beta Chain CDR3
TGTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTT (SEQ ID NO:35)

VGLL1 TCR #2 Amino Acid Sequence
MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPWYKQ
ELGKRPQLIIDIRSNVGEKKDQRIAVTLNKTAKHFSLHITETQPEDSAVYFCAARGLT
GGGNKLTFGTGTQLKVELNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKD
SDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV
KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSSRAKRSGSGATNFSL
LKQAGDVEENPGPMGPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQVTLRCS
PKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNAL
LLGDSALYLCASSVGTGITEAFFGQGTRLTVVEDLNKVFPPEVAVFEPSEAEISHTQK
ATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRV
SATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVS
YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO:36)

VGLL1 TCR #2 Alpha Chain Signal peptide
MTSIRAVFIFLWLQLDLVNG (SEQ ID NO:37)

VGLL1 TCR #2 Alpha Chain V-region
ENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPWYKQELGKRPQLIIDIRSNVGEKKD
QRIAVTLNKTAKHFSLHITETQPEDSAVYFCAARGLTGGGNKLTFGTGTQLKVELNI
Q (SEQ ID NO:38)

VGLL1 TCR #2 Alpha Chain CDR1
DSASNY (SEQ ID NO:39)

VGLL1 TCR #2 Alpha Chain CDR2
IRSNVGE (SEQ ID NO:40)

VGLL1 TCR #2 Alpha Chain CDR3
CAARGLTGGGNKLTF (SEQ ID NO:41)

VGLL1 TCR #2 Beta Chain Signal peptide
MGPGLLCWALLCLLGAGLVDA (SEQ ID NO:42)

VGLL1 TCR #2 Beta Chain V-region
GVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGN
FPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSVGTGITEAFFGQGTRLTVVE
(SEQ ID NO:43)

VGLL1 TCR #2 Beta Chain CDR1
SGHDT (SEQ ID NO:44)

VGLL1 TCR #2 Beta Chain CDR2
YYEEEE (SEQ ID NO:45)

VGLL1 TCR #2 Beta Chain CDR3
CASSVGTGITEAFF (SEQ ID NO:46)

VGLL1 TCR#3 TRAV13-1*02 J1301/ TRBVC1 5-6*01 J1-1 Alpha Chain
TCTAGACCGCCATGGGTCGACGCCACCATGACATCCATTCGAGCTGTATTTATAT
TCCTGTGGCTGCAGCTGGACTTGGTGAATGGAGAGAATGTGGAGCAGCATCCTTC
AACCCTGAGTGTCCAGGAGGGAGACAGCGCTGTTATCAAGTGTACTTATTCAGA
CAGTGCCTCAAACTACTTCCCTTGGTATAAGCAAGAACTTGGAAAAAGACCTCA
GCTTATTATAGACATTCGTTCAAATGTGGGCGAAAAGAAAGACCAACGAATTGC
TGTTACATTGAACAAGACAGCCAAACATTTCTCCCTGCACATCACAGAGACCCAA
CCTGAAGACTCGGCTGTCTACTTCTGTGCAGCAATTCCTAATTCTGGGGGTTACC
AGAAAGTTACCTTTGGAATTGGAACAAAGCTCCAAGTCATCCCAAATATCCAGA
ACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGT
CTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCT
GATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAAG
AGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCC
TTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCT
GTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTC
AAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAA
TCTGCTCATGACGCTGCGGCTGTGGTCCAGC (SEQ ID NO:47)

VGLL1 TCR #3 Alpha Chain Signal peptide
ATGACATCCATTCGAGCTGTATTTATATTCCTGTGGCTGCAGCTGGACTTGGTGA
AT (SEQ ID NO:48)

VGLL1 TCR #3 Alpha Chain V-region
GGAGAGAATGTGGAGCAGCATCCTTCAACCCTGAGTGTCCAGGAGGGAGACAGC
GCTGTTATCAAGTGTACTTATTCAGACAGTGCCTCAAACTACTTCCCTTGGTATA
AGCAAGAACTTGGAAAAAGACCTCAGCTTATTATAGACATTCGTTCAAATGTGG
GCGAAAAGAAAGACCAACGAATTGCTGTTACATTGAACAAGACAGCCAAACATT
TCTCCCTGCACATCACAGAGACCCAACCTGAAGACTCGGCTGTCTACTTCTGTGC
AGCAATTCCTAATTCTGGGGGTTACCAGAAAGTTACCTTTGGAATTGGAACAAAG
CTCCAAGTCATCCCAAA (SEQ ID NO:49)

VGLL1 TCR #3 Alpha Chain CDR1
GACAGTGCCTCAAACTAC (SEQ ID NO:50)

VGLL1 TCR #3 Alpha Chain CDR2
ATTCGTTCAAATGTGGGCGAA (SEQ ID NO:51)

VGLL1 TCR #3 Alpha Chain CDR3
TGTGCAGCAATTCCTAATTCTGGGGGTTACCAGAAAGTTACCTTT (SEQ ID NO:52)

VGLL1 TCR #3 Beta Chain
ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGC
AAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCA
ACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGA
GAGACAGAGAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTAT
AGCTCTGAGCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCT
GTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCA
GACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTT
TGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCCT
GGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAA
GGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGC
CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC
TGGCAGAACCCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGG
AGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCG
CCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAAG
GGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTA
TGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTTC
TGA*CTCGAG*AAGCTT*GCGGCCGC*GGATCCGATAAA (SEQ ID NO:53)

VGLL1 TCR #3 Beta Chain Signal peptide
ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCT (SEQ ID NO:54)

VGLL1 TCR #3 Beta Chain V-region
GGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGACT
CTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCAACAGGCCC
TGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGAGAGACAGA
GAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTATAGCTCTGA
GCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGC
AGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA
GTTGTAG (SEQ ID NO:55)

VGLL1 TCR #3 Beta Chain CDR1
TCTGGGCATGACACT (SEQ ID NO:56)

VGLL1 TCR #3 Beta Chain CDR2
TATTATGAGGAGGAAGAG (SEQ ID NO:57)

VGLL1 TCR #3 Beta Chain CDR3
TGTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTT (SEQ ID NO:58)

VGLL1 TCR #3 Amino Acid Sequence
MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPWYKQ
ELGKRPQLIIDIRSNVGEKKDQRIAVTLNKTAKHFSLHITETQPEDSAVYFCAAIPNSG
GYQKVTFGIGTKLQVIPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL
VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSSRAKRSGSGATNFSLLK
QAGDVEENPGPMGPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQVTLRCSPK
SGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLL
GDSALYLCASSVGTGITEAFFGQGTRLTVVEDLNKVFPPEVAVFEPSEAEISHTQKAT
LVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSA
TFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQ
QGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO:59)

VGLL1 TCR #3 Alpha Chain Signal peptide
MTSIRAVFIFLWLQLDLVNG (SEQ ID NO:60)

VGLL1 TCR #3 Alpha Chain V-region
ENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPWYKQELGKRPQLIIDIRSNVGEKKD
QRIAVTLNKTAKHFSLHITETQPEDSAVYFCAAIPNSGGYQKVTFGIGTKLQVIPNI
(SEQ ID NO:61)

VGLL1 TCR #3 Alpha Chain CDR1
DSASNY (SEQ ID NO:62)

VGLL1 TCR #3 Alpha Chain CDR2
IRSNVGE (SEQ ID NO:63)

VGLL1 TCR #3 Alpha Chain CDR3
CAAIPNSGGYQKVTF (SEQ ID NO:64)

VGLL1 TCR #3 Beta Chain Signal peptide
MGPGLLCWALLCLLGAGLVDA (SEQ ID NO:65)

VGLL1 TCR #3 Beta Chain V-region
GVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGN
FPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSVGTGITEAFFGQGTRLTVV (SEQ
ID NO:66)

VGLL1 TCR #3 Beta Chain CDR1
SGHDT (SEQ ID NO:67)

VGLL1 TCR #3 Beta Chain CDR2
YYEEE (SEQ ID NO:68)

VGLL1 TCR #3 Beta Chain CDR3
CASSVGTGITEAFF (SEQ ID NO:69)

VGLL1 TCR #4 TRAV23 J12/TRBV5-6*01 FJ1-1*01 F Alpha Chain
TCTAGACCGCCATGGGTCGACGCCACCATGGACAAGATCTTAGGAGCATCATTTT
TAGTTCTGTGGCTTCAACTATGCTGGGTGAGTGGCCAACAGAAGGAGAAAAGTG
ACCAGCAGCAGGTGAAACAAAGTCCTCAATCTTTGATAGTCCAGAAAGGAGGGA
TTTCAATTATAAACTGTGCTTATGAGAACACTGCGTTTGACTACTTTCCATGGTAC
CAACAATTCCCTGGGAAAGGCCCTGCATTATTGATAGCCATACGTCCAGATGTGA
GTGAAAAGAAAGAAGGAAGATTCACAATCTCCTTCAATAAAAGTGCCAAGCAGT
TCTCATTGCATATCATGGATTCCCAGCCTGGAGACTCAGCCACCTACTTCTGTGC
AGCCGTAAGATACAACTTCAACAAATTTTACTTTGGATCTGGGACCAAACTCAAT
GTAAAACCAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCT
AAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATG
TGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACAT
GAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGA
CTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTC
CCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACA
GATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCC
TGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGC (SEQ
ID NO:70)

VGLL1 TCR #4 Alpha Chain Signal peptide
ATGGACAAGATCTTAGGAGCATCATTTTTAGTTCTGTGGCTTCAACTATGCTGGG
TGAGTGGC (SEQ ID NO:71)

VGLL1 TCR #4 Alpha Chain V-region
CAACAGAAGGAGAAAAGTGACCAGCAGCAGGTGAAACAAAGTCCTCAATCTTTG
ATAGTCCAGAAAGGAGGGATTTCAATTATAAACTGTGCTTATGAGAACACTGCGT
TTGACTACTTTCCATGGTACCAACAATTCCCTGGGAAAGGCCCTGCATTATTGAT
AGCCATACGTCCAGATGTGAGTGAAAAGAAAGAAGGAAGATTCACAATCTCCTT
CAATAAAAGTGCCAAGCAGTTCTCATTGCATATCATGGATTCCCAGCCTGGAGAC
TCAGCCACCTACTTCTGTGCAGCCGTAAGATACAACTTCAACAAATTTTACTTTG
GATCTGGGACCAAACTCAATGTAAAACCAA (SEQ ID NO:72)

VGLL1 TCR #4 Alpha Chain CDR1
AACACTGCGTTTGACTAC (SEQ ID NO:73)

VGLL1 TCR #4 Alpha Chain CDR2
ATACGTCCAGATGTGAGTGAA (SEQ ID NO:74)

VGLL1 TCR #4 Alpha Chain CDR3
TGTGCAGCCGTAAGATACAACTTCAACAAATTTTACTTT (SEQ ID NO:75)

VGLL1 TCR #4 Beta Chain

ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGC
AAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCA
ACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGA
GAGACAGAGAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTAT
AGCTCTGAGCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCT
GTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCA
GACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTT
TGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCCT
GGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAA
GGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGC
CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC
TGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGG
AGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCG
CCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAAG
GGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTA
TGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTTC
TGACTCGAGAAGCTTGCGGCCGCGGATCCGATAAA (SEQ ID NO:76)

VGLL1 TCR #4 Beta Chain Signal peptide

ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAGCAGGCTTAG
TGGACGCT (SEQ ID NO:77)

VGLL1 TCR #4 Beta Chain V-region

GGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAGAGGACAGCAAGTGACT
CTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTCCTGGTACCAACAGGCCC
TGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGGAGGAAGAGAGACAGA
GAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTCCCTAACTATAGCTCTGA
GCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCTCTATCTCTGTGCCAGC
AGCGTCGGGACAGGTATCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACA
GTTGTAG (SEQ ID NO:78)

VGLL1 TCR #4 Beta Chain CDR1

TCTGGGCATGACACT (SEQ ID NO:79)

VGLL1 TCR #4 Beta Chain CDR2

TATTATGAGGAGGAAGAG (SEQ ID NO:80)

VGLL1 TCR #4 Beta Chain CDR3

TGTGCCAGCAGCGTCGGGACAGGTATCACTGAAGCTTTCTTT (SEQ ID NO:81)

VGLL1 TCR #4 Amino Acid Sequence

MDKILGASFLVLWLQLCWVSGQQKEKSDQQQVKQSPQSLIVQKGGISIINCAYENTA
FDYFPWYQQFPGKGPALLIAIRPDVSEKKEGRFTISFNKSAKQFSLHIMDSQPGDSAT

YFCAAVRYNFNKFYFGSGTKLNVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQT
NVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFP
SPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSSRAKRSG
SGATNFSLLKQAGDVEENPGPMGPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRG
QQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYS
SELNVNALLLGDSALYLCASSVGTGITEAFFGQGTRLTVVEDLNKVFPPEVAVFEPSE
AEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRY
CLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA
DCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID
NO:82)

VGLL1 TCR #4 Alpha Chain Signal peptide
MDKILGASFLVLWLQLCWVSG (SEQ ID NO:83)

VGLL1 TCR #4 Alpha Chain V-region
QQKEKSDQQQVKQSPQSLIVQKGGISIINCAYENTAFDYFPWYQQFPGKGPALLIAIR
PDVSEKKEGRFTISFNKSAKQFSLHIMDSQPGDSATYFCAAVRYNFNKFYFGSGTKL
NVKPNIQ (SEQ ID NO:84)

VGLL1 TCR #4 Alpha Chain CDR1
NTAFDY (SEQ ID NO:85)

VGLL1 TCR #4 Alpha Chain CDR2
IRPDVSE (SEQ ID NO:86)

VGLL1 TCR #4 Alpha Chain CDR3
CAAVRYNFNKFYF (SEQ ID NO:87)

VGLL1 TCR #4 Beta Chain Signal peptide
MGPGLLCWALLCLLGAGLVDA (SEQ ID NO:88)

VGLL1 TCR #4 Beta Chain V-region
GVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGN
FPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSVGTGITEAFFGQGTRLTVV (SEQ
ID NO:89)

VGLL1 TCR #4 Beta Chain CDR1
SGHDT (SEQ ID NO:90)

VGLL1 TCR #4 Beta Chain CDR2
YYEEEE (SEQ ID NO:91)

VGLL1 TCR #4 Beta Chain CDR3
CASSVGTGITEAFF (SEQ ID NO:92)

[0063] In some embodiments, host cells, such as T cells (*e.g.*, $CD4^+$ T cells, $CD8^+$ T cells, γδ T cells, and Tregs), NK cells, invariant NK cells, NKT cells, mesenchymal stem cells

(MSCs), or induced pluripotent stem (iPS) cells of the present disclosure can be genetically engineered to express antigen receptors such as engineered TCRs and/or CARs. Thus, further provided herein are cells, such as T cells, NK cells, invariant NK cells, NKT cells, MSCs, or iPS cells, engineered to express the VGLL1-specific TCRs provided herein. For example, the autologous or allogeneic cells (*e.g.*, isolated from an umbilical cord) are modified to express a TCR having antigenic specificity for a cancer antigen. These non-T cell effector immune cells may express a TCR together with CD3 molecules or other signaling domains linked to the TCR, which would initiate the signal transduction in these cells. Suitable methods of modification are known in the art. See, for instance, Sambrook and Ausubel, supra. For example, the T cells may be transduced to express a TCR having antigenic specificity for a cancer antigen using transduction techniques described in Heemskerk *et al. Hum Gene Ther.* 19:496-510 (2008) and Johnson *et al. Blood* 114:535-46 (2009).

[0064] In some embodiments, antigen-specific cells can be generated by using the VGLL1 TCRs provided herein (*e.g.*, SEQ ID NOs:1-92). In this method, the TCR sequence is inserted into a vector (*e.g.*, retroviral or lentiviral vector) which is introduced into host cells, such as T cells (*e.g.*, $CD4^+$ T cells, $CD8^+$ T cells, γδ T cells, and Tregs), NK cells, invariant NK cells, NKT cells, MSCs, or iPS cells to generate antigen-specific cells which can be used for adoptive cell therapy for cancer patients.

[0065] The engineered immune cells may be constructed using any of the many well-established gene transfer methods known to those skilled in the art. In certain embodiments, the engineered cells are constructed using viral vector-based gene transfer methods to introduce nucleic acids encoding a VGLL1-specific TCR. The viral vector-based gene transfer method may comprise a lentiviral vector, a retroviral vector, an adenoviral or an adeno-associated viral vector. In certain embodiments, the engineered cells are constructed using non-viral vector-based gene transfer methods to introduce nucleic acids encoding a VGLL1-specific TCR. The vector for the TCR may comprises the alpha chain polypeptide and the beta chain polypeptide, which may be linked by a linker domain or IRES sequence. In certain embodiments, the non-viral vector-based gene transfer method comprises a gene-editing method selected from the group consisting of a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TALENs), and a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) nuclease. In certain embodiments, the non-viral vector-based gene editing method comprises a transfection or transformation method

selected from the group consisting of lipofection, nucleofection, virosomes, liposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA.

[0066] Electroporation of RNA coding for the full length TCR α and β (or γ and δ) chains can be used as alternative to overcome long-term problems with autoreactivity caused by pairing of retrovirally transduced and endogenous TCR chains. Even if such alternative pairing takes place in the transient transfection strategy, the possibly generated autoreactive T cells will normally lose this autoreactivity after some time, because the introduced TCR α and β chain are only transiently expressed. When the introduced TCR α and β chain expression is diminished, only normal autologous T cells are left. This is not the case when full length TCR chains are introduced by stable retroviral transduction, which do not lose the introduced TCR chains, causing a constantly present autoreactivity in the patient.

[0067] Exemplary antigen receptors, including CARs and recombinant TCRs, as well as methods for engineering and introducing the receptors into cells, include those described, for example, in international patent application publication numbers WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013/166321, WO2013/071154, WO2013/123061 U.S. patent application publication numbers US2002131960, US2013287748, US20130149337, U.S. Patent Nos.: 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application number EP2537416, and/or those described by Sadelain *et al., Cancer Discov.* 2013 April; 3(4): 388-398; Davila *et al.* (2013) *PLoS ONE* 8(4): e61338; Turtle *et al., Curr. Opin. Immunol.*, 2012 October; 24(5): 633-39; Wu *et al., Cancer*, 2012 March 18(2): 160-75. In some aspects, the genetically engineered antigen receptors include a CAR as described in U.S. Patent No.: 7,446,190, and those described in International Patent Application Publication No.: WO/2014055668 Al.

A. T Cell Receptors

[0068] In some embodiments, the genetically engineered antigen receptors include recombinant TCRs and/or TCRs cloned from naturally occurring T cells. A "T cell receptor" or "TCR" refers to a molecule that contains a variable a and β chains (also known as TCRα and TCRβ, respectively) or a variable γ and δ chains (also known as TCRy and TCRδ, respectively) and that is capable of specifically binding to an antigen peptide bound to a MHC receptor. In some embodiments, the TCR is in the αβ form.

[0069] Typically, TCRs that exist in αβ and γδ forms are generally structurally similar, but T cells expressing them may have distinct anatomical locations or functions. A TCR can be found on the surface of a cell or in soluble form. Generally, a TCR is found on the surface of T cells (or T lymphocytes) where it is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. In some embodiments, a TCR also can contain a constant domain, a transmembrane domain and/or a short cytoplasmic tail (see, *e.g.*, Janeway *et al*, Immunobiology: The Immune System in Health and Disease, 3rd Ed., Current Biology Publications, p. 433, 1997). For example, in some aspects, each chain of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C- terminal end. In some embodiments, a TCR is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. Unless otherwise stated, the term "TCR" should be understood to encompass functional TCR fragments thereof. The term also encompasses intact or full-length TCRs, including TCRs in the αβ form or γδ form.

[0070] Thus, for purposes herein, reference to a TCR includes any TCR or functional fragment, such as an antigen-binding portion of a TCR that binds to a specific antigenic peptide bound in an MHC molecule, *i.e.* MHC-peptide complex. An "antigen-binding portion" or antigen- binding fragment" of a TCR, which can be used interchangeably, refers to a molecule that contains a portion of the structural domains of a TCR, but that binds the antigen (*e.g.* MHC-peptide complex) to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable a chain and variable β chain of a TCR, sufficient to form a binding site for binding to a specific MHC-peptide complex, such as generally where each chain contains three complementarity determining regions.

[0071] In some embodiments, the variable domains of the TCR chains associate to form loops, or complementarity determining regions (CDRs) analogous to immunoglobulins, which confer antigen recognition and determine peptide specificity by forming the binding site of the TCR molecule and determine peptide specificity. Typically, like immunoglobulins, the CDRs are separated by framework regions (FRs) (see, *e.g.*, Jores *et al., PNAS U.S.A.* 87:9138, 1990; Chothia *et al., EMBO J.* 7:3745, 1988; see also Lefranc *et al., Dev. Comp. Immunol.* 27:55, 2003). In some embodiments, CDR3 is the main CDR responsible for recognizing processed antigen, although CDR1 of the alpha chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the beta chain interacts with the C-terminal part

of the peptide. CDR2 is thought to recognize the MHC molecule. In some embodiments, the variable region of the β-chain can contain a further hypervariability (HV4) region.

[0072] In some embodiments, the TCR chains contain a constant domain. For example, like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, a-chain, β-chain) can contain two immunoglobulin domains, a variable domain (*e.g.*, V_a or Vp; typically amino acids 1 to 116 based on Kabat numbering Kabat *et al.*, "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.) at the N-terminus, and one constant domain (*e.g.*, a-chain constant domain or C_a, typically amino acids 117 to 259 based on Kabat, β-chain constant domain or Cp, typically amino acids 117 to 295 based on Kabat) adjacent to the cell membrane. For example, in some cases, the extracellular portion of the TCR formed by the two chains contains two membrane-proximal constant domains, and two membrane-distal variable domains containing CDRs. The constant domain of the TCR domain contains short connecting sequences in which a cysteine residue forms a disulfide bond, making a link between the two chains. In some embodiments, a TCR may have an additional cysteine residue in each of the α and β chains such that the TCR contains two disulfide bonds in the constant domains.

[0073] In some embodiments, the TCR chains can contain a transmembrane domain. In some embodiments, the transmembrane domain is positively charged. In some cases, the TCR chains contains a cytoplasmic tail. In some cases, the structure allows the TCR to associate with other molecules like CD3. For example, a TCR containing constant domains with a transmembrane region can anchor the protein in the cell membrane and associate with invariant subunits of the CD3 signaling apparatus or complex.

[0074] Generally, CD3 is a multi-protein complex that can possess three distinct chains (γ, δ, and ε) in mammals and the ζ-chain. For example, in mammals the complex can contain a CD3γ chain, a CD3δ chain, two CD3ε chains, and a homodimer of CD3ζ chains. The CD3γ, CD3δ, and CD3ε chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3γ, CD3δ, and CD3ε chains are negatively charged, which is a characteristic that allows these chains to associate with the positively charged T cell receptor chains. The intracellular tails of the CD3γ, CD3δ, and CD3ε chains each contain a single conserved motif known as an immunoreceptor tyrosine -based activation motif or ITAM, whereas each CD3ζ chain has three. Generally, ITAMs are involved in the signaling capacity of the TCR complex. These accessory

molecules have negatively charged transmembrane regions and play a role in propagating the signal from the TCR into the cell. The CD3- and ζ-chains, together with the TCR, form what is known as the T cell receptor complex.

[0075] In some embodiments, the TCR may be a heterodimer of two chains α and β (or optionally γ and δ) or it may be a single chain TCR construct. In some embodiments, the TCR is a heterodimer containing two separate chains (α and β chains or γ and δ chains) that are linked, such as by a disulfide bond or disulfide bonds. In some embodiments, a TCR for a target antigen (*e.g.*, a cancer antigen) is identified and introduced into the cells. In some embodiments, nucleic acid encoding the TCR can be obtained from a variety of sources, such as by polymerase chain reaction (PCR) amplification of publicly available TCR DNA sequences. In some embodiments, the TCR is obtained from a biological source, such as from cells such as from a T cell (*e.g.* cytotoxic T cell), T cell hybridomas or other publicly available source. In some embodiments, the T cells can be obtained from *in vivo* isolated cells. In some embodiments, a high-affinity T cell clone can be isolated from a patient, and the TCR isolated. In some embodiments, the T cells can be a cultured T cell hybridoma or clone. In some embodiments, the TCR clone for a target antigen has been generated in transgenic mice engineered with human immune system genes (*e.g.*, the human leukocyte antigen system, or HLA). See, *e.g.*, tumor antigens (see, *e.g.*, Parkhurst *et al.* (2009) *Clin Cancer Res.* 15: 169-180 and Cohen *et al.* (2005) *J Immunol.* 175:5799-5808). In some embodiments, phage display is used to isolate TCRs against a target antigen (see, *e.g.*, Varela-Rohena *et al.* (2008) *Nat Med.* 14: 1390-1395 and Li (2005) *Nat Biotechnol.* 23:349-354). In some embodiments, the TCR or antigen-binding portion thereof can be synthetically generated from knowledge of the sequence of the TCR.

B. Chimeric T Cell Receptors

[0076] In some embodiments, the engineered antigen receptors include CARs, including activating or stimulatory CARs, costimulatory CARs (see WO2014/055668), and/or inhibitory CARs (iCARs, see Fedorov *et al.*, Sci. Transl. Medicine, 5(215) (December, 2013). The CARs generally include an extracellular antigen (or ligand) binding domain linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). Such molecules typically mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone. In some embodiments, the CAR includes an

antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (VH) and variable light (VL) chains of a monoclonal antibody (mAb).

[0077] The arrangement of the antigen-binding domain of a CAR may be multimeric, such as a diabody or multimers. The multimers can be formed by cross pairing of the variable portions of the light and heavy chains into what may be referred to as a diabody. The hinge portion of the CAR may in some embodiments be shortened or excluded (*i.e.*, generating a CAR that only includes an antigen binding domain, a transmembrane region and an intracellular signaling domain). A multiplicity of hinges may be used with the present invention, *e.g.*, as shown in Table 1. In some embodiments, the hinge region may have the first cysteine maintained, or mutated by a proline or a serine substitution, or be truncated up to the first cysteine. The Fc portion may be deleted from scFv used to as an antigen-binding region to generate CARs according to the present invention. In some embodiments, an antigen-binding region may encode just one of the Fc domains, *e.g.*, either the CH2 or CH3 domain from human immunoglobulin. One may also include the hinge, CH2, and CH3 region of a human immunoglobulin that has been modified to improve dimerization and oligermerization. In some embodiments, the hinge portion of may comprise or consist of a 8-14 amino acid peptide (*e.g.*, a 12 AA peptide), a portion of CD8α, or the IgG4 Fc. In some embodiments, the antigen binding domain may be suspended from cell surface using a domain that promotes oligomerization, such as CD8 alpha. In some embodiments, the antigen binding domain may be suspended from cell surface using a domain that is recognized by monoclonal antibody (mAb) clone 2D3 (mAb clone 2D3 described, *e.g.*, in Singh *et al.*, 2008).

[0078] The endodomain or intracellular signaling domain of a CAR can generally cause or promote the activation of at least one of the normal effector functions of an immune cell comprising the CAR. For example, the endodomain may promote an effector function of a T cell such as, *e.g.*, cytolytic activity or helper activity including the secretion of cytokines. The effector function in a naive, memory, or memory-type T cell may include antigen-dependent proliferation. The terms "intracellular signaling domain" or "endodomain" refers to the portion of a CAR that can transduce the effector function signal and/or direct the cell to perform a specialized function. While usually the entire intracellular signaling domain may be included in a CAR, in some cases a truncated portion of an endodomain may be included. Generally,

endodomains include truncated endodomains, wherein the truncated endodomain retains the ability to transduce an effector function signal in a cell.

[0079] In some embodiments, an endodomain comprises the zeta chain of the T cell receptor or any of its homologs (*e.g.*, eta, delta, gamma, or epsilon), MB1 chain, B29, Fc RIII, Fc RI, and combinations of signaling molecules, such as CD3ζ and CD28, CD27, 4-1BB, DAP-10, OX40, and combinations thereof, as well as other similar molecules and fragments. Intracellular signaling portions of other members of the families of activating proteins can be used, such as FcγRIII and FcεRI. Examples of these alternative transmembrane and intracellular domains can be found, *e.g.*, Gross *et al.* (1992), Stancovski *et al.* (1993), Moritz *et al.* (1994), Hwu *et al.* (1995), Weijtens *et al.* (1996), and Hekele *et al.* (1996), which are incorporated herein be reference in their entirety. In some embodiments, an endodomain may comprise the human CD3ζ intracellular domain.

[0080] The antigen-specific extracellular domain and the intracellular signaling-domain are preferably linked by a transmembrane domain. Transmembrane domains that may be included in a CAR include, *e.g.*, the human IgG4 Fc hinge and Fc regions, the human CD4 transmembrane domain, the human CD28 transmembrane domain, the transmembrane human CD3ζ domain, or a cysteine mutated human CD3ζ domain, or a transmembrane domains from a human transmembrane signaling protein such as, *e.g.*, the CD16 and CD8 and erythropoietin receptor.

[0081] In some embodiments, the endodomain comprises a sequence encoding a costimulatory receptors such as, *e.g.*, a modified CD28 intracellular signaling domain, or a CD28, CD27, OX-40 (CD134), DAP10, or 4-1BB (CD137) costimulatory receptor. In some embodiments, both a primary signal initiated by CD3 ζ, an additional signal provided by a human costimulatory receptor may be included in a CAR to more effectively activate a transformed T cells, which may help improve *in vivo* persistence and the therapeutic success of the adoptive immunotherapy. As noted in Table 1, the endodomain or intracellular receptor signaling domain may comprise the zeta chain of CD3 alone or in combination with an Fcγ RIII costimulatory signaling domains such as, *e.g.*, CD28, CD27, DAP10, CD137, OX40, CD2, 4-1BB. In some embodiments, the endodomain comprises part or all of one or more of TCR zeta chain, CD28, CD27, OX40/CD134, 4-1BB/CD137, FcεRIγ, ICOS/CD278, IL-2Rbeta/CD122, IL-2Ralpha/CD132, DAP10, DAP12, and CD40. In some embodiments, 1, 2,

3, 4 or more cytoplasmic domains may be included in an endodomain. For example, in some CARs it has been observed that at least two or three signaling domains fused together can result in an additive or synergistic effect.

[0082] In some aspects, an isolated nucleic acid segment and expression cassette including DNA sequences that encode a CAR may be generated. A variety of vectors may be used. In some preferred embodiments, the vector may allow for delivery of the DNA encoding a CAR to immune such as T cells. CAR expression may be under the control of regulated eukaryotic promoter such as, *e.g.*, the MNDU3 promoter, CMV promoter, EF1alpha promoter, or Ubiquitin promoter. Also, the vector may contain a selectable marker, if for no other reason, to facilitate their manipulation *in vitro*. In some embodiments, the CAR can be expressed from mRNA *in vitro* transcribed from a DNA template.

[0083] Chimeric antigen receptor molecules are recombinant and are distinguished by their ability to both bind antigen and transduce activation signals *via* immunoreceptor activation motifs (ITAM's) present in their cytoplasmic tails. Receptor constructs utilizing an antigen-binding moiety (for example, generated from single chain antibodies (scFv)) afford the additional advantage of being "universal" in that they can bind native antigen on the target cell surface in an HLA-independent fashion. For example, a scFv constructs may be fused to sequences coding for the intracellular portion of the CD3 complex's zeta chain (ζ), the Fc receptor gamma chain, and sky tyrosine kinase (Eshhar *et al.*, 1993; Fitzer-Attas *et al.*, 1998). Re-directed T cell effector mechanisms including tumor recognition and lysis by CTL have been documented in several murine and human antigen-scFv: ζ systems (Eshhar *et al.*, 1997; Altenschmidt *et al.*, 1997; Brocker *et al.*, 1998).

[0084] In some embodiments, a TCR is included in a CAR as the antigen binding domain (e.g., as a scFv region) and the CAR further comprises a hinge region, a transmembrane region, and an endodomain.

C. Soluble TCRs and BiTEs

[0085] In addition, the present disclosure provides soluble TCRs which can be used to treat positive cancer patients directly. Soluble bispecific T cell-engaging molecules (BiTEs) can be generated by linking the VGLL1 TCR to CD3-specific Fab fragments. These bispecific molecules can bind the tumor cell surface via their VGLL1 TCR binding to the peptide/HLA complex, and the CD3-specific Fab fragments would crosslink the TCR. This would result in

cellular activation and elimination of the target cell. Thus, these soluble bispecific TCR constructs can be used for treating the cancer patients directly.

[0086] Finally, the soluble TCR can be used as a probe for diagnostic evaluation of peptide/MHC in tumor cells or to direct therapeutic molecules to the tumor site. This soluble TCR molecule also could be labeled with tracers such as a fluorescent probe or radioactive probe, and then used for diagnostic evaluation of the presentation of peptide/MHC in tumor cells. Furthermore, this soluble TCR molecule could be linked with therapeutic molecules such as toxin, and then direct these therapeutic molecules to the tumor sites for the treatment of cancer patients.

[0087] In some embodiments, the present disclosure provides soluble TCRs, such as a VGLL1-specific TCR provided herein. Soluble TCRs may be used for investigating specific TCR-pMHC interactions or as a diagnostic tool to detect infection, or to detect autoimmune disease markers. Soluble TCRs may have applications in staining, for example to stain cells for the presence of a particular peptide antigen presented in the context of the MHC. Similarly, soluble TCRs can be used to deliver a therapeutic agent, for example a cytotoxic compound or an immunostimulating compound, to cells presenting a particular antigen. Soluble TCRs may also be used to inhibit T cells, for example, those reacting to an auto-immune peptide antigen. In some aspects, the TCR is linked to another molecule that delivers a cell in proximity to the tumor. In further aspects, the TCR delivers a toxin, a cytokine, costimulatory ligand, or inhibitor ligand and directs the molecule, cell or compound to the target cells expressing the peptide-MHC.

[0088] In some aspects, the present disclosure provides a soluble T cell receptor (sTCR), which comprises (i) all or part of a TCR α chain (*e.g.*, SEQ ID NOs:1, 24, 47, or 70), except the transmembrane domain thereof, and (ii) all or part of a TCR β chain (*e.g.*, SEQ ID NOs:7, 30, 53, or 76), except the transmembrane domain thereof, wherein (i) and (ii) each comprise a functional variable domain and at least a part of the constant domain of the TCR chain, and are linked by a disulphide bond between constant domain residues which is not present in native TCR.

[0089] In some aspects, the soluble TCR comprises a TCR α or γ chain extracellular domain dimerized to a TCR β or δ chain extracellular domain respectively, by means of a pair of C-terminal dimerization peptides, such as leucine zippers.

[0090] A soluble TCR of the present disclosure may be provided in substantially pure form, or as a purified or isolated preparation. For example, it may be provided in a form which is substantially free of other proteins.

[0091] A plurality of soluble TCRs of the present disclosure may be provided in a multivalent complex. Thus, the present disclosure provides, in one aspect, a multivalent TCR complex, which comprises a plurality of soluble TCRs as described herein. Each of the plurality of soluble TCRs is preferably identical.

[0092] In its simplest form, a multivalent TCR complex according to the present disclosure comprises a multimer of two or three or four or more T cell receptor molecules associated (*e.g.* covalently or otherwise linked) with one another, preferably via a linker molecule. Suitable linker molecules include, but are not limited to, multivalent attachment molecules such as avidin, streptavidin, neutravidin and extravidin, each of which has four binding sites for biotin. Thus, biotinylated TCR molecules can be formed into multimers of TCRs having a plurality of TCR binding sites. The number of TCR molecules in the multimer will depend upon the quantity of TCR in relation to the quantity of linker molecule used to make the multimers, and also on the presence or absence of any other biotinylated molecules. Preferred multimers are dimeric, trimeric or tetrameric TCR complexes.

[0093] Suitable structures for use in the present methods include membrane structures such as liposomes and solid structures which are preferably particles such as beads, for example latex beads. Other structures which may be externally coated with T cell receptor molecules are also suitable. Preferably, the structures are coated with T cell receptor multimers rather than with individual T cell receptor molecules.

[0094] In the case of liposomes, the T cell receptor molecules or multimers thereof may be attached to or otherwise associated with the membrane. Techniques for this are well known to those skilled in the art.

[0095] A label or another moiety, such as a toxic or therapeutic moiety, may be included in a multivalent TCR complex of the present disclosure. For example, the label or other moiety may be included in a mixed molecule multimer. An example of such a multimeric molecule is a tetramer containing three TCR molecules and one peroxidase molecule. This could be achieved by mixing the TCR and the enzyme at a molar ratio of 3:1 to generate tetrameric complexes, and isolating the desired complex from any complexes not containing

the correct ratio of molecules. These mixed molecules could contain any combination of molecules, provided that steric hindrance does not compromise or does not significantly compromise the desired function of the molecules. The positioning of the binding sites on the streptavidin molecule is suitable for mixed tetramers since steric hindrance is not likely to occur.

[0096] The TCR (or multivalent complex thereof) of the present disclosure may alternatively or additionally be associated with (*e.g.* covalently or otherwise linked to) a therapeutic agent which may be, for example, a toxic moiety for use in cell killing, or an immunostimulating agent such as an interleukin or a cytokine. A multivalent TCR complex of the present disclosure may have enhanced binding capability for a TCR ligand compared to a non-multimeric T cell receptor heterodimer. Thus, the multivalent TCR complexes according to the present disclosure are particularly useful for tracking or targeting cells presenting particular antigens in vitro or in vivo, and are also useful as intermediates for the production of further multivalent TCR complexes having such uses. The TCR or multivalent TCR complex may therefore be provided in a pharmaceutically acceptable formulation for use *in vivo*.

[0097] The present disclosure also provides a method for delivering a therapeutic agent to a target cell, which method comprises contacting potential target cells with a TCR or multivalent TCR complex in accordance with the present disclosure under conditions to allow attachment of the TCR or multivalent TCR complex to the target cell, said TCR or multivalent TCR complex being specific for the TCR ligand and having the therapeutic agent associated therewith.

[0098] In particular, the soluble TCR or multivalent TCR complex can be used to deliver therapeutic agents to the location of cells presenting a particular antigen. This would be useful in many situations and, in particular, against tumors. A therapeutic agent could be delivered such that it would exercise its effect locally but not only on the cell it binds to. Thus, one particular strategy envisages anti-tumor molecules linked to T cell receptors or multivalent TCR complexes specific for tumor antigens.

[0099] Many therapeutic agents could be employed for this use, for instance radioactive compounds, enzymes (perforin for example) or chemotherapeutic agents (cisplatin for example). To ensure that toxic effects are exercised in the desired location the toxin could be inside a liposome linked to streptavidin so that the compound is released slowly. This will

prevent damaging effects during the transport in the body and ensure that the toxin has maximum effect after binding of the TCR to the relevant antigen presenting cells.

[00100] The soluble TCRs of the present disclosure may be used to modulate T cell activation by binding to specific TCR ligand and thereby inhibiting T cell activation. Autoimmune diseases involving T cell-mediated inflammation and/or tissue damage would be amenable to this approach, for example type I diabetes. Knowledge of the specific peptide epitope presented by the relevant pMHC is required for this use.

[00101] The use of the soluble TCRs and/or multivalent TCR complexes of the present disclosure in the preparation of a composition for the treatment of cancer or autoimmune disease is also envisaged.

[00102] Also provided is a method of treatment of cancer or autoimmune disease comprising administration to a patient in need thereof of an effective amount of the soluble TCRs and/or multivalent TCR complexes of the present disclosure.

[00103] As is common in anti-cancer and autoimmune therapy the soluble TCRs of the present disclosure may be used in combination with other agents for the treatment of cancer and autoimmune disease, and other related conditions found in similar patient groups.

III. Adoptive Cell Transfer Therapies

[00104] Provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount an antigen-specific cell (*e.g.*, autologous or allogeneic T cells (*e.g.*, regulatory T cells, $CD4^+$ T cells, $CD8^+$ T cells, or gamma-delta T cells), NK cells, invariant NK cells, NKT cells, MSCs, or iPS cells) therapy, such as a VGLL1-specific cell therapy. Adoptive T cell therapies with genetically engineered TCR-transduced T cells (*e.g.*, expressing a TCR comprising one of SEQ ID NOs: 1-92) are also provided herein. In some embodiments, the adoptive cell transfer therapy is provided to a subject (*e.g.*, a human patient) in combination with as second therapy, such as a chemotherapy, a radiotherapy, a surgery, or a second immunotherapy.

[00105] Embodiments of the present disclosure concern obtaining and administering TCR-engineered cells to a subject as an immunotherapy to target cancer cells. In particular, the TCR-engineered cells are antigen-specific cells (*e.g.*, VGLL1-specific cells).

Several basic approaches for the derivation, activation and expansion of functional anti-tumor effector cells have been described in the last two decades. These include: autologous cells, such as tumor-infiltrating lymphocytes (TILs); T cells activated *ex-vivo* using autologous DCs, lymphocytes, artificial antigen-presenting cells (APCs) or beads coated with T cell ligands and activating antibodies, or cells isolated by virtue of capturing target cell membrane; allogeneic cells naturally expressing anti-host tumor T cell receptor (TCR); and non-tumor-specific autologous or allogeneic cells genetically reprogrammed or "redirected" to express tumor-reactive TCR or chimeric TCR molecules displaying antibody-like tumor recognition capacity known as "T-bodies". These approaches have given rise to numerous protocols for T cell preparation and immunization which can be used in the methods described herein.

A. T Cell Preparation

[00106] In some embodiments, the T cells are derived from the blood, bone marrow, lymph, or lymphoid organs. In some aspects, the cells are human cells. The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, $CD4^+$ cells, $CD8^+$ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-specificity, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. In some aspects, such as for off-the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, as described herein, and re-introducing them into the same patient, before or after cryopreservation.

[00107] Among the sub-types and subpopulations of T cells (*e.g.*, $CD4^+$ and/or $CD8^+$ T cells) are naive T (T_N) cells, effector T cells (T_{EFF}), memory T cells and sub-types thereof, such as stem cell memory T (TSC_M), central memory T (TC_M), effector memory T (T_{EM}), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T

cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[00108] In some embodiments, one or more of the T cell populations is enriched for or depleted of cells that are positive for a specific marker, such as surface markers, or that are negative for a specific marker. In some cases, such markers are those that are absent or expressed at relatively low levels on certain populations of T cells (*e.g.*, non-memory cells) but are present or expressed at relatively higher levels on certain other populations of T cells (*e.g.*, memory cells).

[00109] In some embodiments, T cells are separated from a PBMC sample by negative selection of markers expressed on non-T cells, such as B cells, monocytes, or other white blood cells, such as CD14. In some aspects, a $CD4^+$ or $CD8^+$ selection step is used to separate $CD4^+$ helper and $CD8^+$ cytotoxic T cells. Such $CD4^+$ and $CD8^+$ populations can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations.

[00110] In some embodiments, $CD8^+$ T cells are further enriched for or depleted of naive, central memory, effector memory, and/or central memory stem cells, such as by positive or negative selection based on surface antigens associated with the respective subpopulation. In some embodiments, enrichment for central memory T (T_{CM}) cells is carried out to increase efficacy, such as to improve long-term survival, expansion, and/or engraftment following administration, which in some aspects is particularly robust in such sub-populations. See Terakura *et al.* (2012) *Blood.* 1:72- 82; Wang *et al.* (2012) *J Immunother.* 35(9):689-701.

[00111] In some embodiments, the T cells are autologous T cells. In this method, tumor samples are obtained from patients and a single cell suspension is obtained. The single cell suspension can be obtained in any suitable manner, *e.g.*, mechanically (disaggregating the tumor using, *e.g.*, a gentleMACS™ Dissociator, Miltenyi Biotec, Auburn, Calif.) or enzymatically (*e.g.*, collagenase or DNase). Single-cell suspensions of tumor enzymatic digests are cultured in interleukin-2 (IL-2). The cells are cultured until confluence (*e.g.*, about 2×10^6 lymphocytes), *e.g.*, from about 5 to about 21 days, preferably from about 10 to about 14 days.

[00112] The cultured T cells can be pooled and rapidly expanded. Rapid expansion provides an increase in the number of antigen-specific T cells of at least about 50-

fold (*e.g.*, 50-, 60-, 70-, 80-, 90-, or 100-fold, or greater) over a period of about 10 to about 14 days. More preferably, rapid expansion provides an increase of at least about 200-fold (*e.g.*, 200-, 300-, 400-, 500-, 600-, 700-, 800-, 900-, or greater) over a period of about 10 to about 14 days.

[00113] Expansion can be accomplished by any of a number of methods as are known in the art. For example, T cells can be rapidly expanded using non-specific T cell receptor stimulation in the presence of feeder lymphocytes and either interleukin-2 (IL-2) or interleukin-15 (IL-15), with IL-2 being preferred. The non-specific T-cell receptor stimulus can include around 30 ng/ml of OKT3, a mouse monoclonal anti-CD3 antibody (available from Ortho-McNeil®, Raritan, N.J.). Alternatively, T cells can be rapidly expanded by stimulation of peripheral blood mononuclear cells (PBMC) *in vitro* with one or more antigens (including antigenic portions thereof, such as epitope(s), or a cell) of the cancer, which can be optionally expressed from a vector, such as an human leukocyte antigen A1 (HLA-A1) binding peptide, in the presence of a T-cell growth factor, such as IL-2. The *in vitro*-induced T-cells are rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A1-expressing antigen-presenting cells. Alternatively, the T-cells can be re-stimulated with irradiated, autologous lymphocytes or with irradiated HLA-A1+ allogeneic lymphocytes and IL-2, for example.

[00114] The autologous T-cells can be modified to express a T-cell growth factor that promotes the growth and activation of the autologous T-cells. Suitable T-cell growth factors include, for example, interleukin (IL)-2, IL-7, IL-15, and IL-12. Suitable methods of modification are known in the art. See, for instance, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, NY, 1994. In particular aspects, modified autologous T-cells express the T-cell growth factor at high levels. T-cell growth factor coding sequences, such as that of IL-12, are readily available in the art, as are promoters, the operable linkage of which to a T-cell growth factor coding sequence promote high-level expression.

B. Methods of Treatment

[00115] Further provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount an antigen-specific T cell therapy, such as a VGLL1-specific T cell therapy. Adoptive T cell

therapies with genetically engineered TCR-transduced T cells (conjugate TCR to other bioreactive proteins (*e.g.*, anti-CD3) are also provided herein. In further embodiments, methods are provided for the treatment of cancer comprising immunizing a subject with a purified tumor antigen or an immunodominant tumor antigen-specific peptide.

[00116] Examples of cancers contemplated for treatment include lung cancer, head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, lymphomas, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer. Additional exemplary cancers include, but are not limited to, lung cancer, head and neck cancer, breast cancer, pancreatic cancer, prostate cancer, renal cancer, bone cancer, testicular cancer, cervical cancer, gastrointestinal cancer, lymphomas, pre-neoplastic lesions in the lung, colon cancer, melanoma, and bladder cancer. Further examples cancers include melanomas, malignant melanomas, colon carcinomas, lymphomas, sarcomas, blastomas, renal carcinomas, gastrointestinal tumors, gliomas, prostate tumors, bladder cancer, rectal tumors, stomach cancer, oesophageal cancer, pancreatic cancer, liver cancer, mammary carcinomas, uterine cancer, cervical cancer, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), leukaemia, hepatomas, various virus-induced tumors such as, for example, papilloma virus-induced carcinomas (*e.g.* cervical carcinoma), adenocarcino-mas, herpes virus-induced tumors (*e.g.* Burkitt's lymphoma, EBV-induced B cell lymphoma), heptatitis B-induced tumors (hepatocell carcinomas), HTLV-1- and HTLV-2-induced lym-phomas, acoustic neuroma, lung carcinomas, small-cell lung carcinomas, pharyngeal cancer, anal carcinoma, glioblastoma, rectal carcinoma, astrocy-toma, brain tumors, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, pancreatic cancer, testicular cancer, Hodgkin's syndrome, meningiomas, Schneeberger disease, hypophysis tumor, Mycosis fungoides, carcinoids, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, renal cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumors , oligodendrogli-oma, vulval cancer, intestinal cancer, colon carcinoma, oesophageal carcinoma, wart involvement, tumors of the small intestine, craniopharyngeomas, ovarian carci-noma, genital tumors, ovarian cancer, pancreatic carcinoma, endometrial carcinoma, liver metastases, penile cancer, tongue cancer, gall bladder cancer, leukaemia, plasmocytoma, lid tumor, and prostate cancer.

[00117] In some embodiments, T cells are autologous. However, the cells can be allogeneic. In some embodiments, the T cells are isolated from the patient themself, so that the cells are autologous. If the T cells are allogeneic, the T cells can be pooled from several donors. The cells are administered to the subject of interest in an amount sufficient to control, reduce, or eliminate symptoms and signs of the disease being treated.

[00118] In some embodiments, the subject can be administered nonmyeloablative lymphodepleting chemotherapy prior to the T cell therapy. The nonmyeloablative lymphodepleting chemotherapy can be any suitable such therapy, which can be administered by any suitable route. The nonmyeloablative lymphodepleting chemotherapy can comprise, for example, the administration of cyclophosphamide and fludarabine, particularly if the cancer is melanoma, which can be metastatic. An exemplary route of administering cyclophosphamide and fludarabine is intravenously. Likewise, any suitable dose of cyclophosphamide and fludarabine can be administered. In particular aspects, around 60 mg/kg of cyclophosphamide is administered for two days after which around 25 mg/m^2 fludarabine is administered for five days.

[00119] In certain embodiments, a T-cell growth factor that promotes the growth and activation of the autologous T cells is administered to the subject either concomitantly with the autologous T cells or subsequently to the autologous T cells. The T-cell growth factor can be any suitable growth factor that promotes the growth and activation of the autologous T-cells. Examples of suitable T-cell growth factors include interleukin (IL)-2, IL-7, IL-15, and IL-12, which can be used alone or in various combinations, such as IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, IL-2, IL-7 and IL-15, IL-12 and IL-7, IL-12 and IL-15, or IL-12 and IL2. IL-12 is a preferred T-cell growth factor.

[00120] The T cell may be administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. The appropriate dosage of the T cell therapy may be determined based on the type of disease to be treated, severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[00121] Intratumoral injection, or injection into the tumor vasculature is specifically contemplated for discrete, solid, accessible tumors. Local, regional or systemic

administration also may be appropriate. For tumors of >4 cm, the volume to be administered will be about 4-10 ml (in particular 10 ml), while for tumors of <4 cm, a volume of about 1-3 ml will be used (in particular 3 ml). Multiple injections delivered as single dose comprise about 0.1 to about 0.5 ml volumes.

C. Pharmaceutical Compositions

[00122] In select embodiments, it is contemplated that a cell expressing a TCR as disclosed herein, a protein containing the variable regions of a TCR, or a DNA encoding the variable regions of a TCR of the present disclosure may be comprised in a vaccine composition and administered to a subject to induce a therapeutic immune response in the subject towards a cancer, such as a cancer that expresses VGLL1. A vaccine composition for pharmaceutical use in a subject may comprise a tumor antigen peptide (*e.g.*, VGLL1) composition disclosed herein and a pharmaceutically acceptable carrier. A therapeutic composition for pharmaceutical use in a subject may comprise a TCR composition disclosed herein, such as a soluble TCR (optionally attached to an imaging agent), and a pharmaceutically acceptable carrier.

[00123] 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, increasing survival, *etc.*

[00124] A person having ordinary skill in the medical arts will appreciate that the actual dosage amount of a therapeutic composition administered to an animal or human patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. 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.

[00125] A therapeutic composition disclosed herein can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostaticaly, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, and by inhalation, injection, infusion,

continuous infusion, lavage, and localized perfusion. A therapeutic composition may also be administered to a subject *via* a catheter, in cremes, in lipid compositions, by ballistic particulate delivery, or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington: The Science and Practice of Pharmacy, 21st Ed. Lippincott Williams and Wilkins, 2005, incorporated herein by reference).

[00126] While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patents 4,897,268 and 5,075,109.

[00127] In some embodiments, the vaccine composition may be administered by microstructured transdermal or ballistic particulate delivery. Microstructures as carriers for vaccine formulation are a desirable configuration for vaccine applications and are widely known in the art (Gerstel and Place 1976 (U.S. Patent 3,964,482); Ganderton and McAinsh 1974 (U.S. Patent 3,814,097); U.S. Patents 5,797,898, 5,770,219 and 5,783,208, and U.S. Patent Application 2005/0065463). In these embodiments, a support substrate can include, but is not limited to, a microcapsule, a microparticle, a microsphere, a nanocapsule, a nanoparticle, a nanosphere, or a combination thereof.

[00128] Microstructures or ballistic particles that serve as a support substrate for an TCR, such as a soluble TCR, disclosed herein may be comprised of biodegradable material and non-biodegradable material, and such support substrates may be comprised of synthetic polymers, silica, lipids, carbohydrates, proteins, lectins, ionic agents, crosslinkers, and other microstructure components available in the art. Protocols and reagents for the immobilization of a peptide of the invention to a support substrate composed of such materials are widely available commercially and in the art.

[00129] In other embodiments, a vaccine composition comprises an immobilized or encapsulated TCR or soluble TCR disclosed herein and a support substrate. In these embodiments, a support substrate can include, but is not limited to, a lipid microsphere, a lipid nanoparticle, an ethosome, a liposome, a niosome, a phospholipid, a sphingosome, a surfactant, a transferosome, an emulsion, or a combination thereof. The formation and use of liposomes and other lipid nano- and microcarrier formulations is generally known to those of ordinary skill in the art, and the use of liposomes, microparticles, nanocapsules and the like have gained widespread use in delivery of therapeutics (*e.g.*, U.S. Patent 5,741,516, specifically incorporated herein in its entirety by reference). Numerous methods of liposome and liposome-like preparations as potential drug carriers, including encapsulation of peptides, have been reviewed (U.S. Patents 5,567,434; 5,552,157; 5,565,213; 5,738,868 and 5,795,587, each of which is specifically incorporated in its entirety by reference).

[00130] In addition to the methods of delivery described herein, a number of alternative techniques are also contemplated for administering the disclosed vaccine compositions. By way of nonlimiting example, a vaccine composition may be administered by sonophoresis (*i.e.*, ultrasound) which has been used and described in U.S. Patent 5,656,016 for enhancing the rate and efficacy of drug permeation into and through the circulatory system; intraosseous injection (U.S. Patent 5,779,708), or feedback-controlled delivery (U.S. Patent 5,697,899), and each of the patents in this paragraph is specifically incorporated herein in its entirety by reference.

[00131] Any of a variety of adjuvants may be employed in the vaccines of the present disclosure to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit, Mich.) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and quil A.

[00132] A soluble TCR may be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example,

hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[00133] In any case, the composition may comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[00134] Sterile injectable solutions are prepared by incorporating the active peptides in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The preparation of highly concentrated compositions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

[00135] The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein.

[00136] In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

D. Combination Therapies

[00137] In certain embodiments, the compositions and methods of the present embodiments involve an antigen peptide or antigen-specific T cell population in combination with at least one additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy.

[00138] In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (*e.g.*, agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, *etc.*). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PBK/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents known in the art.

[00139] A T cell therapy may be administered before, during, after, or in various combinations relative to an additional cancer therapy, such as immune checkpoint therapy. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the T cell therapy is provided to a patient separately from an additional therapeutic agent, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the antibody therapy and the anti-cancer therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[00140] Various combinations may be employed. For the example below an antigen-specific T cell therapy is "A" and an anti-cancer therapy is "B":

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B
B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A
B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[00141] Administration of any compound or therapy of the present embodiments to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

1. Chemotherapy

[00142] A wide variety of chemotherapeutic agents may be used in accordance with the present embodiments. The term "chemotherapy" refers to the use of drugs to treat cancer. A "chemotherapeutic agent" is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[00143] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclosphosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine,

chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics, such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaI1); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKpolysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine,plicomycin, gemcitabien, navelbine, farnesyl-protein

tansferase inhibitors, transplatinum, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

2. Radiotherapy

[00144] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

3. Immunotherapy

[00145] The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the embodiments. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (RITUXAN®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells

[00146] Antibody-drug conjugates have emerged as a breakthrough approach to the development of cancer therapeutics. Cancer is one of the leading causes of deaths in the world. Antibody–drug conjugates (ADCs) comprise monoclonal antibodies (MAbs) that are covalently linked to cell-killing drugs. This approach combines the high specificity of MAbs against their antigen targets with highly potent cytotoxic drugs, resulting in "armed" MAbs that deliver the payload (drug) to tumor cells with enriched levels of the antigen. Targeted delivery

of the drug also minimizes its exposure in normal tissues, resulting in decreased toxicity and improved therapeutic index. The approval of two ADC drugs, ADCETRIS® (brentuximab vedotin) in 2011 and KADCYLA® (trastuzumab emtansine or T-DM1) in 2013 by FDA validated the approach. There are currently more than 30 ADC drug candidates in various stages of clinical trials for cancer treatment (Leal *et al.*, 2014). As antibody engineering and linker-payload optimization are becoming more and more mature, the discovery and development of new ADCs are increasingly dependent on the identification and validation of new targets that are suitable to this approach and the generation of targeting MAbs. Two criteria for ADC targets are upregulated/high levels of expression in tumor cells and robust internalization.

[00147] In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present embodiments. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[00148] Examples of immunotherapies currently under investigation or in use are immune adjuvants, *e.g.*, Mycobacterium bovis, Plasmodium falciparum, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998); cytokine therapy, *e.g.*, interferons α, β, and γ, IL-1, GM-CSF, and TNF (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hollander, 2012; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

[00149] In some embodiments, the immunotherapy may be an immune checkpoint inhibitor. Immune checkpoints either turn up a signal (*e.g.*, co-stimulatory molecules) or turn down a signal. Inhibitory immune checkpoints that may be targeted by

immune checkpoint blockade include adenosine A2A receptor (A2AR), B7-H3 (also known as CD276), B and T lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin (KIR), lymphocyte activation gene-3 (LAG3), programmed death 1 (PD-1), T-cell immunoglobulin domain and mucin domain 3 (TIM-3) and V-domain Ig suppressor of T cell activation (VISTA). In particular, the immune checkpoint inhibitors target the PD-1 axis and/or CTLA-4.

[00150] The immune checkpoint inhibitors may be drugs such as small molecules, recombinant forms of ligand or receptors, or, in particular, are antibodies, such as human antibodies (*e.g.*, International Patent Publication WO2015016718; Pardoll, 2012; both incorporated herein by reference). Known inhibitors of the immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized or human forms of antibodies may be used. As the skilled person will know, alternative and/or equivalent names may be in use for certain antibodies mentioned in the present disclosure. Such alternative and/or equivalent names are interchangeable in the context of the present disclosure. For example it is known that lambrolizumab is also known under the alternative and equivalent names MK-3475 and pembrolizumab.

[00151] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PDL1 and/or PDL2. In another embodiment, a PDL1 binding antagonist is a molecule that inhibits the binding of PDL1 to its binding partners. In a specific aspect, PDL1 binding partners are PD-1 and/or B7-1. In another embodiment, the PDL2 binding antagonist is a molecule that inhibits the binding of PDL2 to its binding partners. In a specific aspect, a PDL2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. US8735553, US8354509, and US8008449, all incorporated herein by reference. Other PD-1 axis antagonists for use in the methods provided herein are known in the art such as described in U.S. Patent Application No. US20140294898, US2014022021, and US20110008369, all incorporated herein by reference.

[00152] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab,

pembrolizumab, and CT-011. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP- 224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PDL2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[00153] Another immune checkpoint that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[00154] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[00155] Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in: US 8,119,129, WO 01/14424, WO 98/42752; WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab), U.S. Patent No. 6,207,156; Hurwitz *et al.*,1998; Camacho *et al.*, 2004; and Mokyr *et al.*, 1998 can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are

hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No. WO2001014424, WO2000037504, and U.S. Patent No. US8017114; all incorporated herein by reference.

[00156] An exemplary anti-CTLA-4 antibody is ipilimumab (also known as 10D1, MDX-010, MDX-101, and Yervoy®) or antigen binding fragments and variants thereof (see, *e.g.*, WOO 1/14424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises the CDR1, CDR2, and CDR3 domains of the VH region of ipilimumab, and the CDR1, CDR2 and CDR3 domains of the VL region of ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on CTLA-4 as the above-mentioned antibodies. In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95%, or 99% variable region identity with ipilimumab).

[00157] Other molecules for modulating CTLA-4 include CTLA-4 ligands and receptors such as described in U.S. Patent Nos. US5844905, US5885796 and International Patent Application Nos. WO1995001994 and WO1998042752; all incorporated herein by reference, and immunoadhesins such as described in U.S. Patent No. US8329867, incorporated herein by reference.

4. Surgery

[00158] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[00159] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or

local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

5. Other Agents

[00160] It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment. These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present embodiments to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present embodiments. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present embodiments to improve the treatment efficacy.

IV. Articles of Manufacture or Kits

[00161] An article of manufacture or a kit is provided comprising antigen-specific T cells or TCRs is also provided herein. The article of manufacture or kit can further comprise a package insert comprising instructions for using the antigen-specific T cells to treat or delay progression of cancer in an individual or to enhance immune function of an individual having cancer. Any of the antigen-specific T cells described herein may be included in the article of manufacture or kits. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other

buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, the article of manufacture further includes one or more of another agent (*e.g.*, a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

V. Examples

[00162] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Vestigial-Like 1 is a Shared Targetable Cancer-Placenta Antigen Expressed by Pancreatic and Basal-Like Breast Cancers

[00163] **Immunopeptidome analysis of PDAC patient tumors identifies tumor-associated peptides:** To identify peptide targets for CTL-based immunotherapy of PDAC, 39 tumor specimens derived from 35 PDAC patients treated at M.D. Anderson Cancer Center were analyzed. This included 34 freshly-excised surgical specimens (20 metastatic and 14 primary tumors), in addition to 3 patient-derived xenografts (PDX) and 2 organoid cell lines derived from metastases. Tumor cells were lysed and subjected to total HLA class I immunoprecipitation and acid elution, followed by tandem mass spectrometry (MS) to analyze the HLA-bound peptides. Eluted peptide fragmentation spectra were searched against the Swiss-Prot database (updated 9/2018) to identify matches encoded within the human proteome. Individual peptide matches were assessed using several orthogonal parameters, including Mascot Ion score, MS1 mass differential (delta mass), and predicted binding to the patient's HLA allotypes as determined by high-resolution genetic sequencing. Further validation and potential suitability as therapeutic TAA targets was determined by evaluating all peptide-encoding genes for (1) patient tumor tissue transcript expression as determined by RNAseq, (2) normal tissue transcript expression (GTex Portal database), and (3) overall expression in tumor tissues (TCGA database) (FIG. 1A).

[00164] The amount of immunoprecipitated HLA class I correlated with the size of the fresh tumor specimens analyzed ($R^2 = 0.79$), with the exception of 8 tumors (21.6%) that showed relatively low HLA class I expression as assessed by Western blot analysis (FIG. 7, Table 1). As expected, HLA class I protein levels correlated with the number of Swis-Prot database matches to eluted peptides ($R^2 = 0.62$, FIG. 8). Overall, the 39 tumor specimens analyzed yielded a total of 23,245 unique, high confidence peptide identities, of which 7,966 peptides (34.3%) were 8- to 13-mer peptides predicted to bind to one or more patient HLA class I allotypes. Fresh tumor specimens yielded a highly variable number of peptides, ranging from 238 to 1657 (mean = 542). For 3 patients, PDX derivation resulted in larger tumor specimens, yielding an increased number of eluted peptides in all 3 cases. One of the two patient-derived organoid cell lines (MP015) yielded the highest number of eluted peptides overall (n = 1903), underscoring the quantitative advantage provided by expanding tumor specimens *in vitro* prior to MS analysis (Table 2, FIG. 8).

[00165] Table 2. VGLL1-derived peptide eluted from two HLA-A*0101+PDAC patient tumor organoids.

Patient Identifier	Eluted peptide	Source gene(s)	Match rank	Tumor RNA expression (RNAseq, TPM)	Predicted HLA binding affinity (nM)					
C(1203	LSELETPGKY	VGLL1	1	77.53	A*0101	A*2601	B*3502	B*3801	C*0401	C*1203
					51	12558	33903	29369	30164	6181
MP081	LSELETPGKY	VGLL1	1	56.39	A*0101	A*0101	B*3502	B*5701	C*0401	C*0602
					51	51	33903	11936	30164	35852

[00166] **Expression profiling of peptide-encoding genes identified VGLL1 as a novel pancreatic cancer TAA:** To evaluate if any of the eluted peptides constituted potential therapeutic CTL targets, peptide-encoding genes were individually assessed for normal tissue transcript expression with reference to the GTex Portal database containing RNAseq data derived from >50 different human tissues. Normal tissues (excluding testis) were categorized into 4 groups that reflected the potential toxicities expected from off-target killing activity by antigen-specific CTLs (Table 2). Peptide-encoding genes were then screened using four corresponding expression filters of increasing stringency in order to eliminate candidate TAAs

most likely to elicit autoimmune toxicity in the context of CTL therapy (FIG. 1B). Thus, while TAA transcript expression up to 30 TPM maximum was allowed in non-essential tissues (such as prostate, breast, and adipose tissues), a maximum expression threshold of 1 TPM was imposed for highly essential tissues such as heart and brain, for which CTL recognition can be lethal. Using these stringent criteria, 12 TAA peptides were deemed safest to target, the genes encoding these peptides being *MUC16* (encoding 5 unique peptides), *MUC19, ZNF717, EIF5AL1, RGPD1, SLC30A8, MIA2,* and *VGLL1* (each encoding 1 unique peptide). Peptides encoded by TAAs MSLN and IDO1 were also detected, but were excluded in the screening due to elevated RNA transcript expression in normal lung tissue (88 TPM and 16 TPM, respectively, FIG. 1B). Amongst the TAAs deemed safest to target, only 2 peptides (derived from *MIA2* and *VGLL1)* were found to be presented by tumors of more than one PDAC patient (Table 2).

[00167] The 10-mer peptide LSELETPGKY (SEQ ID NO:93), uniquely encoded by *VGLL1*, was eluted from both PDAC patient- derived organoid cell lines MP015-Org and MP081-Org. This peptide was predicted to bind with high affinity to HLA-A*0101 (51 nM), and RNAseq analysis confirmed high *VGLL1* transcript expression in both organoid lines (Table 2). Peptide identity was confirmed by targeted LC-MS, in which a synthetic peptide was analyzed as part of a mixture with organoid tumor-associated peptides. As shown in FIG. 1C, the synthetic isotope- labeled peptide LSELETPGK<u>Y</u> (SEQ ID NO: 93) generated a highly similar fragmentation spectra to the native VGLL1 peptide detected from PDAC organoid lines MP015-Org and MP081-Org, and was also detected at nearly identical LC- MS retention times. Targeted MS analysis on 2 additional HLA-A*0101-expressing cell lines (PANC10.05 and BXPC3) demonstrated that the same peptide could also be detected on PANC10.05, suggesting that LSELETPGKY (SEQ ID NO: 93) might constitute a widely shared TAA (FIG. 9).

[00168] **VGLL1 is expressed by multiple cancer types and is associated with poorer overall survival:** VGLL1, also known as TONDU, was first identified as the human homolog of the Vestigial (Vg) protein in *Drosophila*, a key regulator of wing development. Since VGLL1 is a transcriptional co-activator that binds to the TEA domain family of transcription factors (TEFs) implicated in cancer development, *VGLL1* transcript expression was further examined in the 31 cancer types listed in TCGA. As shown in FIG. 2A, in comparison to most normal tissues *VGLL1* is overexpressed in a number of different cancers,

including PDAC, bladder, ovarian, breast, lung, and stomach cancer. Interestingly, *VGLL1* appears to be preferentially expressed in basal-like breast cancers while demonstrating a relatively low prevalence in other breast cancer subtypes (FIG. 10). A similar profile was confirmed by gene expression analysis of tumor cell lines listed in the Cancer Cell Line Encyclopedia (CCLE, FIG. 11). According to the GTex RNAseq database, the highest median *VGLL1* transcript expression was found in 3 non-essential tissues: bladder (15.3 TPM), salivary gland (3.9 TPM), and breast (1.3 TPM). The highest level of *VGLL1* transcript expression in essential tissues was in normal lung (1.0 TPM), esophagus (0.73 TPM), and kidney (0.34 TPM), while *VGLL1* expression in heart and brain tissues was virtually undetectable (FIG. 2A). Collectively, this data suggested that VGLL1 may constitute a safe, targetable TAA for multiple cancer types.

[00169] It was next assessed if tumor *VGLL1* transcript expression was associated with cancer patient survival. As shown in FIG, 2B, TCGA PDAC patient survival (n = 179) was found to be inversely correlated with *VGLL1* expression: patients with high expression had a significantly shorter overall median survival compared to patients with low or absent expression (16 months vs. 37 months, p=0.001). This was confirmed in an independent cohort of 37 M.D. Anderson PDAC patients for whom PDX tissues could be derived: patients showing an overall survival of less 18 months demonstrated a significantly higher mean PDX *VGLL1* expression compared to patients that survived longer than 36 months (57.3 TPM vs. 9.6 TPM, p=0.003, Figure 2C). It is worth noting that *VGLL1* transcript expression was found to be considerably higher in PDAC tumor cell lines and PDX tissues compared with surgically resected PDAC tumors, perhaps due to the high stromal content of many PDAC tumors *in situ* (FIGS. 2A, 2C, Table 2). Highly elevated *VGLL1* expression was also associated with shorter overall survival time in breast cancer (p = 0.037) and stomach cancer (p = 0.047), but showed no association with survival in ovarian cancer (FIG. 13). Interestingly, low or absent *VGLL1* expression was associated with shorter survival time in bladder cancer (p = 0.036). One potential explanation is that loss a normal bladder tissue antigen like VGLL1 may indicate tumor dedifferentiation, which has been associated with poorer prognosis in bladder cancer and many other tumor types.

[00170] **VGLL1 is part of a unique group of Cancer-Placenta Antigens (CPAs) with therapeutic potential:** VGLL1 had been previously identified as having a regulatory role during early events in human placental development, and is a specific marker

of proliferative cytotrophoblast. In accordance with this, RNAseq gene expression data from 7 human placenta samples showed that *VGLL1* demonstrated the highest expression in this tissue by a large margin (mean = 302.7 TPM), nearly 20-fold higher than its expression normal bladder (FIG. 3A). This led to explore the notion that cancer-placenta antigens (CPA) may constitute a distinct category of targetable TAAs analogous to cancer-testis antigens (CTAs), which have been successfully targeted with CTL- based therapies. To identify other CPAs with similar expression profiles to VGLL1, the GTex, TCGA, and other RNAseq databases were searched for genes that demonstrated the following attributes: (1) highest normal tissue expression in placenta; (2) low to absent expression in other normal tissues; and (3) elevated expression in pancreatic, breast, bladder, and/or ovarian cancer. This search yielded 9 additional genes, including Placenta- specific 1 (*PLAC1*), previously identified as a target of humoral antitumor immunity in cancer patients.

[00171] Interestingly, Chorionic Gonadotropin (CG) Beta subunits 3 and 5 (*CGB3/CGB5*), components of the CG hormone complex produced by placental trophoblasts during pregnancy, were also identified as potential CPAs due to their overexpression in a subset of pancreatic, testicular, uterine, and bladder cancers (FIG. 3B). The other 6 putative CPAs demonstrated diverse expression profiles, ranging from those found only in a restricted set of cancer types (*IGF2BP3, ADAM12*), to those overexpressed in most cancer types but also demonstrating elevated expression in normal female reproductive tissues (*CAPN6, MMP11*) (FIG. 3, 14 to 23).

[00172] Although peptides derived from these genes were detected in this set of PDAC specimens, epitopes from several of these putative CPAs have been identified in multiple tumor types and are listed in the Immune Epitope Database (IEDB).

[00173] **VGLL1-specific cytotoxic T cells were expanded from the peripheral blood of PDAC patient MP015:** Patient MP015 was a 50-year old male when first diagnosed with primary PDAC in December 2011. Two years following surgical removal of the primary pancreatic tumor, a thorascopic wedge resection of a left lung lesion was performed in November 2013 and used to derive organoid cell line MP015-Org. The patient's disease was kept in check for nearly 2 more years through a series of chemotherapeutic regimens, but following progression he was enrolled in an IRB-approved cell therapy protocol at M.D. Anderson to receive autologous, expanded tumor-antigen-specific CTLs. Immunopeptidome analysis performed on the expanded organoid cell line

[00174] MP015-Org in May 2015 led to the identification of 6 HLA class I-bound peptides (4 derived from MUC16 and 1 each from ZNF717 and VGLL1) that met the criteria as safe, targetable TAAs (FIG. 1B and Table 2). Custom clinical-grade tetramers were available for 3 of the 6 potential targets: two HLA-B*3502-restricted MUC16 peptides and the single HLA-A*0101-restricted VGLL1 peptide.

[00175] Following leukapheresis, patient MP015 PBMCs were stimulated twice with individual peptide-pulsed DCs in the presence of IL-21, followed by tetramer-based sorting of antigen-specific CD8+ T cells (FIG. 4A). Although MUC16-specific CTLs failed to expand from patient PBMC, VGLL1 CTLs expanded successfully, with VGLL1 tetramer-positive T cells comprising 3.4% of CD8+ after 2 weeks of DC-peptide stimulation (FIG. 4B). Cell sorting followed by employment of the rapid expansion protocol (REP) was repeated twice, resulting in nearly 20 billion expanded CTLs, of which >90% were VGLL1 tetramer-positive and demonstrated restricted Vβ usage (FIGS. 4B and C). VGLL1-specific CTLs were also successfully expanded from 2 of 2 healthy HLA- A*0101-positive blood donors, demonstrating the general immunogenicity of the LSELETPGKY (SEQ ID NO: 93) peptide (FIG. 14).

[00176] Expanded CTLs from patient MP015 were tested functionally using standard ^{51}Cr release assays. Mel888 melanoma cells (VGLL1-negative, HLA-A*0101 positive) pulsed with titrated amounts of VGLL1 peptide elicited CTL recognition and killing at peptide concentrations as low as 10 nM, indicating relatively high affinity for cognate peptide (FIG. 4D). Importantly, expanded patient-derived CTLs also showed robust recognition of the autologous organoid cell line MP015-Org from which the VGLL1 peptide was originally detected by MS (FIG. 5A). In October 2015 following a pre-treatment regimen of Cytoxan, Patient MP015 was infused with 19.6 billion autologous, expanded VGLL1-specific CTL, subsequently receiving interleukin-2 and pembrolizumab. Although the patient experienced a transient fever (a frequent side effect of T-cell infusion- induced cytokine release), they experienced no adverse events indicating potential CTL-mediated toxicities.

[00177] Unfortunately, scans in late November 2015 showed rapid disease progression manifested as an interval increase in lung lesions and pleural-based metastatic disease. Surprisingly, a biopsy of a pleural-based nodule taken at this time revealed a poorly differentiated neuroendocrine tumor. DNA sequencing analysis of serial liquid biopsies collected over the previous 18 months provided evidence of an extremely rapid evolution of

Patient MP015's cancer due to numerous progressive genetic amplifications, deletions, re-arrangements, and epigenetic changes. RNAseq analysis of lung metastases also demonstrated that a dramatic reduction in VGLL1 transcript expression (35.1 TPM to 1.6 TPM) had occurred between November 2013 and December 2015, providing a potential explanation for the lack of clinical response to ETC therapy (FIG. 14). Patient MP015 expired in January 2016 due to extensive complications deriving from progression of his lung metastases.

[00178] VGLL1-specific CTLs demonstrate cytotoxicity against multiple allogeneic PDAC tumor cell lines: Although Patient MP015 did not experience clinical benefit from adoptive transfer of his own VGLL1-specific CTLs, the robust antitumor activity demonstrated by these T-cells *in vitro* led to explore whether they may have therapeutic potential for other PDAC patients. HLA-A*0101 was expressed by ~30% of the PDAC patient cohort, and RNAseq analysis of TCGA and MDACC PDAC surgical specimens and PDXs showed that 43.2% to 62.5% of patients express VGLL1 transcript at a level >5 TPM. From these data, it is estimated that 12% to 15% of PDAC patients present the LSELETPGKY (SEQ ID NO: 93) peptide target in the context of HLA-A*0101 and therefore could potentially benefit from VGLL1-CTL therapy.

[00179] To determine if VGLL1-CTLs derived from Patient MP015 could recognize allogeneic PDAC tumors, a panel of HLA-A*0101 expressing PDAC tumor cell lines were tested as targets for killing using a ^{51}Cr release assay. Western blot analysis was used to confirm VGLL1 protein expression, and flow cytometry confirmed surface expression of HLA-A*0101 in cell lines (FIG. 15). While control cell line WM793 (VGLL1- negative, HLA-A*0101-positive) was not recognized, VGLL1-specific CTLs recognized autologous MP015-Org cells and 4 out of 4 allogenic PDAC lines tested, including inducing robust killing of PANC-1005, CAPAN-1, and BXPC3 (FIGS. 5A and 5B). The PDAC organoid cells derived from Patient MP081 were also lysed by VGLL1-CTLs but with reduced efficiency, likely due to an outgrowth of VGLL1-negative cells within the culture. VGLL1-CTL specificity was demonstrated by co-incubation with the pan-MHC class I antibody W6/32, which resulted in blockade of PANC10.05 recognition and lysis (FIG. 27). Collectively, these results provide evidence that the LSELETPGKY peptide constitutes a shared PDAC tumor antigen that can be effectively targeted with VGLL1-specific CTLs.

[00180] VGLL1-CTLs show activity against multiple tumor types and reduced recognition of primary cells: TCGA RNAseq data analysis indicated that VGLL1 is

expressed by several cancer types (16 of 31), most notably in 75 - 80% of patients with bladder, ovarian, and basal-type breast cancers, and 15 - 20% of patients with lung and gastric cancers (FIG. 3B). It was therefore set out to determine whether cell lines derived from these cancer types could be targets for VGLL1-specific CTLs (FIG. 6A). Western blot analysis of a panel of ovarian, basal-type breast, bladder, gastric, and lung cancer cell lines showed high VGLL1 expression in 12 of 14 lines analyzed (FIG. 6B). Of the 8 cell lines that naturally expressed HLA-A*0101, VGLL1-CTLs killed 2 of 3 ovarian lines, 2 of 3 breast lines, and 2 of 2 bladder and lung cancer lines (FIG. 6A). Five additional HLA-A*0101-negative cell lines (2 gastric, 2 bladder, and 1 lung line) were transduced to express HLA-A*0101 prior to testing them as targets for VGLL1-CTLs. As shown in FIG. 6A, all five HLA-A*0101-transduced cell lines were rendered susceptible to killing by VGLL1-CTLs, indicating presentation of the LSELETPGKY (SEQ ID NO: 93) peptide from processed, endogenously-expressed VGLL1 protein. Taken together, these results suggest that VGLL1-CTLs have potential therapeutic value for at least five additional cancer types besides PDAC.

[00181] In order to assess the safety of VGLL1-CTLs for potential therapeutic use, they tested against a panel of normal primary cells most likely to elicit VGLL1-specific reactivity according to the GTex normal tissue expression profile (FIG. 2A). Since bladder demonstrated the highest normal tissue *VGLL1* transcript expression, two different HLA-A*0101 positive primary bladder cell lines were tested as targets for VGLL1-CTL killing. As shown in FIG. 6C, specific lysis was low, detectable in one bladder line but only at the highest E:T ratio. Since the GTex database indicated that *VGLL1* transcript is also expressed at low levels in normal breast and lung, VGLL1-CTL killing activity was tested against HLA-A*0101-expressing primary mammary and lung airway cells, along with primary melanocytes as a negative control. Of this panel, mammary cells elicited moderately high levels of killing by VGLL1-specific CTL, results that were consistent with VGLL1 protein levels as assessed by Western blot (FIG. 6D). By contrast, lung airway epithelial cells were not killed by VGLL1-CTLs, despite demonstrating ample HLA-A*0101 surface expression (FIG. 15). These results provide evidence that VGLL1-specific T cells are unlikely to recognize essential normal tissues; however, safety concerns may be warranted due to the potential for reactivity against some non-essential tissues.

[00182] Employing an unbiased immunopeptidome analysis of tumor specimens derived from 35 PDAC patients, VGLL1 was identified as a novel putative shared TAA, ranked

second only to MUC16 in terms of tumor expression in comparison to essential normal tissues. However, in contrast to MUC16 epitopes, the HLA- A*0101 restricted VGLL1 peptide was considerably more immunogenic, capable of eliciting antigen-specific CTLs from multiple PBMC donors, including one PDAC patient. Such immunogenicity provides a significant advantage in the context of developing endogenous T-cell (ETC) therapies for cancer patients. HLA-A*0101 is expressed at a relatively high prevalence (25 - 30%) in Western European and North American countries, suggesting that these patient populations would be most likely to benefit from targeting this epitope [52]. Expanded VGLL1-specific CTLs not only recognized and killed a panel of allogenic PDAC tumor lines, but also demonstrated reactivity against A*0101-expressing tumor cells derived from five other cancer types. It is estimated that targeting this single VGLL1 epitope could potentially benefit a large number of Western cancer patients, including over 20% of patients with ovarian, bladder, or basal-like breast cancers, ~12% of patients with PDAC, and 5 - 10% of patients with lung, stomach, cervical, uterine, or head and neck cancers (FIG. 3).

Example 2 - Materials and Methods

[00183] ***Cell Lines.*** Human cancer cell lines demonstrating *VGLL1* mRNA expression were identified using the Cancer Cell Line Encyclopedia (CCLE) microarray-based gene expression analysis. HLA-A*0101-expressing cancer cell lines PANC10.05, CAPAN-1 OAW28, HT1197, HT1376, BXPC3, UBCL-1, and primary cell lines were obtained from commercial sources (ATCC and Sigma-Aldrich). The patient-derived organoid cell line MP015- Org (hMIA2D) was generated by the Tuveson lab at Cold Spring Harbor Labs as previously described [33]. The patient-derived organoid cell line MP081-Org was generated by the Maitra lab from tumor tissue derived from a wedge biopsy. The gastric cancer cell lines GT-5 and MKN74 were a kind gift from Dr. Lee Ellis. WM793, MKN74, PANC1005, GT-5, and OAW28 cells were cultured in RPMI 1640 medium (GIBCO), containing 10% fetal bovine serum, 1% penicillin-streptomycin (Pen-Strep) (Cellgrow), and 1% Insulin-Transferrin-Seleum-A (GIBCO). BT20 and bladder cell lines were cultured in equal parts DMEM F12K and MEM Alpha, with FBS, Pen-Strep, and 1% sodium pyruvate (GIBCO). All other cell lines were cultured in RPMI 1640, FBS, and Penn- strep, with the addition of HEPES (GIBCO) and Glutamax (GIBCO).

[00184] ***Lentiviral Transductions.*** Some HLA-A*0101-negative tumor cell lines that naturally expressed VGLL1 protein were transduced with a lentiviral gene transfer

vector to express HLA-A*0101 driven by the human PGK promoter, as previously described (Bradley *et al.*, 2015). Ectopic cell surface expression of A*0101 was assessed by staining with anti-human HLA-A1-biotin and streptavidin-FITC (US Biological) and measuring fluorescence using a FACScanto II flow cytometer (BD Biosciences). Tumor cells expressing physiological and comparable levels of surface HLA-A*0101 were isolated by cell sorting and used in subsequent experiments.

[00185] ***VGLL1 Protein Expression.*** VGLL1 protein expression was confirmed in all cell lines by Western blot analysis. Cell lysates from tumor and primary cell lines were prepared and protein content normalized using the BCA method (Thermo-Fisher). Using standard Western blot techniques, cell lysates were run by polyacrylamide gel electrophoresis, transferred, and membranes probed with VGLL1-specific rabbit polyclonal antibody (TA322329, OriGene). VGLL1 protein was visualized using an enzyme-linked anti-rabbit mAb with the Scientific Pierce Fast Western Blot Kit, according to the manufacturer's instructions.

[00186] ***Peptide Identification, Selection and Validation.*** Patient-derived laparoscopic wedge biopsies, xenografts (PDX), or cell lines were lysed using Triton X-100 and cell lysates incubated overnight at 4°C with 1μg of pan-HLA-ABC specific mAb W6/32 for every 10 mg of protein. Protein A/G Ultralink resin beads were used to immunoprecipate HLA class I molecules and HLA-bound peptides were then eluted with 0.1M acetic acid. HLA-A, B, C isolation was confirmed by Western blot analysis, then HLA-positive elutes were analyzed by tandem mass spectrometry (MS/MS). HLA class I protein recovery was semi-quantitatively assessed by rating Western blot band intensity on a scale from 0 (not detectable) to 4 (highest intensity). Tumor-associated HLA- bound peptides were injected onto HPLC system (Dionex 3000 RSLC), and separated by reverse-phase chromatography in 0.1% formic acid water-acetonitrile on 1.8μm C18 (Agilent Technologies) in the MS/MS discovery phase. Peptides were analyzed on an Orbitrap Elite mass spectrometer (Thermo-Fisher) using data-dependent acquisition. To analyze the acquired MS/MS spectra, the Mascot algorithm was utilized to search the spectra against the SwissProt complete human protein database (updated 9/2018), which provided potential matches to conventionally annotated peptides.

[00187] Individual peptide matches underwent quality assessment by reference to multiple orthogonal parameters, including Mascot Ion score, MS1 measured differential to the calculated peptide mass (delta mass), and predicted binding to the patient's HLA allotypes

as determined by high-resolution genetic sequencing and the NetMHC and NetMHCpan algorithms. High-confidence peptide matches were analyzed by BLAST searches to identify all potential source genes, which were then cross-referenced to RNAseq data derived from individual tumor samples to provide further validation of peptide identity (validation requiring a minimum source gene expression of 0.3 transcripts per million, TPM). Eluted TAA peptides were screened for safety as potential CTL targets by applying sequential RNA transcript expression filters to eliminate peptides most likely to elicit autoimmune toxicities due to normal tissue expression (GTex Portal RNAseq data). Excluding testis and placenta, source gene transcript expression of 30 TPM maximum was allowed in non-essential tissues, 10 TPM in "caution" tissues, 3 TPM in "hazard" tissues and 1 TPM in highly essential "danger" tissues (such as heart and brain). Putative TAA genes were also screened for expression and prevalence in different cancer types through analysis of TCGA RNAseq data. In selected cases, targeted-MS/MS analysis was performed to confirm TAA peptide identity. For these analyses, retention-time windows for $^{13}C/^{15}N$ isotope-labeled synthetic peptide standards were pre-determined by MS analysis of the synthetic peptides, then targeted methods for searching TAA peptides were constructed using mass windows of 3 Da around each m/z.

[00188] ***Gene Expression Analysis and Patient Survival.*** Whole transcriptome sequencing (RNAseq) analysis was performed on RNA derived from all PDAC tumor specimens, xenografts, and organoid cell lines using the Illumina TruSeq Stranded Total RNA kit with Ribo-Zero Gold with approximately 200 million paired-end reads for each tumor RNA sample (Avera Institute for Human Genetics). Gene expression profiles of *VGLL1* and other cancer placenta antigens were determined by compiling RNAseq data derived from normal human primary tissues (GTex Portal) and tumor tissues (TCGA). Kaplan-Meier curves were generated from survival data of TCGA cancer patients when stratified by tumor *VGLL1* transcript expression.

[00189] ***Isolation and expansion of VGLL1-specific CD8 T cells.*** Tumor antigen–specific CTLs were generated as previously described (Li *et al.*, 2005). HLA-A*0101 positive patient- or healthy donor-derived PBMC were stimulated twice by autologous dendritic cells (DCs) pulsed with the $VGLL1_{231-240}$ peptide LSELETPGKY (SEQ ID NO: 93). Six days after the second DC stimulation, cultured cells were stained with $VGLL1_{231-240}$ peptide/HLA-A*0101–PE-conjugated custom tetramer (Fred Hutchinson Cancer Research Center), washed and then stained with APC-conjugated CD8 antibody. Cells were washed and

analyzed by flow cytometry (LSRFortessa X-20 Analyzer). CD8 and tetramer double-positive cells were sorted by ARIA II and the VGLL1-specific CD8 T cells were expanded using the Rapid Expansion Protocol (REP) with PBMC and LCL feeder cells. The TCR V_β repertoire of expanded CD8 T cells was assessed using the IOTest Beta Mark TCR-V_β Repertoire kit.

[00190] ***Cytotoxic T cell assays.*** Antitumor killing by VGLL1-specific CD8+ T cells was assessed using a standard chromium-51 (^{51}Cr) release assay. Target cells were labeled with 100μL of ^{51}Cr for 1 hour, then washed and plated at 2,000 target cells per well in triplicate. VGLL1-specific CD8+ T cells were incubated with target cells at various effector-to-target (E:T) cell ratios for four hours. After the incubation period, supernatant was collected from the wells and ^{51}Cr was measured with a gamma radiation counter. The percentage of specific target cell lysis was calculated, correcting for background ^{51}Cr release and relative to a maximum ^{51}Cr release as measured by Triton X-100 lysed target cells.

[00191] ***Statistical analysis.*** Data analysis was performed using GraphPad Prism version 7.03. Normally distributed data were analyzed using parametric tests (ANOVA or unpaired t test). Kaplan-Meier survival curves were analyzed by log-rank tests. Test differences were considered statistically significant if $P<0.05$.

Example 3 - Development and Characterization of T Cell Receptors

[00192] VGLL1 TCRs were cloned and characterized for killing efficiency of target cells. PBMCs were transduced with VGLL1 TCRs to target Mel888HLAA1 cells pulsed with peptide. It was found that TCR expressing T cells can efficiently kill Mel888HLAA1 cells pulsed with peptide (FIG. 1). In addition, the cloned VGLL1 TCRs were shown to recognize endogenous expressed antigen (FIG. 2).

[00193] To clone T cell receptors, total RNA was isolated from VGLL1 specific T cells by RNeasy kit (QIAGEN, 74104), and cDNA was synthesized using 5'-RACE technique (rapid amplification of cDNA ends, Takara, 634859). TCRα and TCRβ chains were then separately amplified by PCR using 3'-end primer that specifically bind to either TCRα or TCRβ constant region. The 5'-end primer complement to the common sequence was added during cDNA synthesis. PCR product was then cloned into TOPO cloning vector (Invitrogen, 450641) and 30 DNA clones were sequenced to determine each of the α or β chains. One TCRβ (TRBVC1 5-6*01 J1-1) and 4 major TCRα clones (TRAV19*01 J56*01, TRAV13-1*02 J10,

TRAV13-1*02 J1301, TRAV23 J12) were identified after gene alignment using IMGT database. For the functional test, 4 TCRα/β clones were designed by paring the TCRβ with different TCRα and each of the α and β chains was connected by a linker containing Furin and P2A cleavage sites. The full-length genes were synthesized by Twist company and then cloned into pMSGV1 vector which has been used in several clinical trials by inserting the TCR DNA fragment into the SalI and NotI sites.

[00194] ***Generation of retroviral particles for TCR transduction***: The pMSGV1-based on retroviral vectors encoding 4 different VGLL1-specific TCRs were used for generating retroviral particles using the packaging cell line 293GP with RT114 as envelop protein to enhance T cell transduction efficiency. Normal PBMCs obtained from MD Anderson blood bank were transduced and continually cultured for 5 days. VGLL1 peptide tetramer and CD8 double positive T cells were then sorted out by flow cytometry. These T cells were either directly used in cytotoxicity assay or subjected to T cell replication depending on the cell number after sorting.

[00195] ***VGLL1-TCR expression mediates CTL cytotoxicity*:** Melanoma cell line mel888 express endogenous HLAA1, but do not express detectable level of VGLL1 protein. Mel888 were pulsed with 10 microgram of peptides, and co-cultured with TCR-transduced effector T cells at different ratios. T cell-mediated target cell death was measured based on chromium-51 release. Effector T cells expressing TCR clones number 1 and 3 could lyse 20-30% target tumor cells at as low as 1.25:1 of effector: target ratio and 70-80 % target cells at 40:1 ratio within 4 hours of incubation. VGLL1 TCR clones number2 and 4 appeared to be slightly weaker, but the difference is not dramatic among all four TCR clones (FIG. 16).

[00196] The efficiency of VGLL1-TCR to recognize and lyse tumor cells expressing endogenous VGLL1 was further determined. In this assay, mel888 cell line and a pancreatic cell line expressing both HLAA1 and VGLL1 were co-cultured with TCR-4-transduced T cells at 40:1 and 5:1 ratios for different time points. 30% of pancreatic cells was lysed after 3.5 hours of incubation and 50% was killed after 5 hours (FIG. 17). Thus, VGLL1-TCR expression mediates cytotoxic activity of transduced T cell.

* * *

[00197] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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WHAT IS CLAIMED IS:

1. An engineered T cell receptor (TCR), wherein the TCR specifically binds Vestigial-like 1 (VGLL1) and comprises:

(a) alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23);

(b) alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46);

(c) alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69); or

(d) alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92).

2. The TCR of claim 1, wherein the TCR comprises alpha chain CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12) and beta chain CDR1 (SEQ ID NO:21), CDR2 (SEQ ID NO:22), and CDR3 (SEQ ID NO:23).

3. The TCR of claim 2, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:15 and a beta chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:20.

4. The TCR of claim 2 or 3, wherein the TCR comprises an alpha chain variable region of SEQ ID NO:15 and a beta chain variable region of amino acid sequence of SEQ ID NO:20.

5. The TCR of any of claims 1-4, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ

ID NO:3 and a beta chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:9.

6. The TCR of any of claims 1-5, wherein the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:3 and a beta chain comprising the nucleotide sequence of SEQ ID NO:9.

7. The TCR of any of claims 1-6, wherein the TCR other than the CDRs has at least 90% identity to SEQ ID NO:13.

8. The TCR of any of claims 1-7, wherein the TCR comprises SEQ ID NO:13.

9. The TCR of claim 1, wherein the TCR comprises alpha chain CDR1 (SEQ ID NO:33), CDR2 (SEQ ID NO:34), and CDR3 (SEQ ID NO:35) and beta chain CDR1 (SEQ ID NO:44), CDR2 (SEQ ID NO:45), and CDR3 (SEQ ID NO:46).

10. The TCR of claim 9, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:38 and a beta chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:43.

11. The TCR of claim 9 or 10, wherein the TCR comprises an alpha chain variable region of SEQ ID NO:38 and a beta chain variable region of amino acid sequence of SEQ ID NO:43.

12. The TCR of any of claims 9-11, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:26 and a beta chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:32.

13. The TCR of any of claims 9-12, wherein the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:26 and a beta chain variable region comprising the nucleotide sequence of SEQ ID NO:32.

14. The TCR of any of claims 9-13, wherein the TCR other than the CDRs has at least 90% identity to SEQ ID NO:36.

15. The TCR of any of claims 9-14, wherein the TCR comprises SEQ ID NO:36.

16. The TCR of claim 1, wherein the TCR comprises alpha chain CDR1 (SEQ ID NO:56), CDR2 (SEQ ID NO:57), and CDR3 (SEQ ID NO:58) and beta chain CDR1 (SEQ ID NO:67), CDR2 (SEQ ID NO:68), and CDR3 (SEQ ID NO:69).

17. The TCR of claim 16, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:61 and a beta chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:66.

18. The TCR of claim 16 or 17, wherein the TCR comprises an alpha chain variable region of SEQ ID NO:61 and a beta chain variable region of amino acid sequence of SEQ ID NO:66.

19. The TCR of any of claims 16-18, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:49 and a beta chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:55.

20. The TCR of any of claims 16-19, wherein the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:49 and a beta chain comprising the nucleotide sequence of SEQ ID NO:55.

21. The TCR of any of claims 16-20, wherein the TCR other than the CDRs has at least 90% identity to SEQ ID NO:59.

22. The TCR of any of claims 16-21, wherein the TCR comprises SEQ ID NO:59.

23. The TCR of claim 1, wherein the TCR comprises alpha chain CDR1 (SEQ ID NO:79), CDR2 (SEQ ID NO:80), and CDR3 (SEQ ID NO:81) and beta chain CDR1 (SEQ ID NO:90), CDR2 (SEQ ID NO:91), and CDR3 (SEQ ID NO:92).

24. The TCR of claim 23, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:84 and a beta chain variable region other than the CDRs having at least 90% identity to the amino acid sequence of SEQ ID NO:89.

25. The TCR of claim 23 or 24, wherein the TCR comprises an alpha chain variable region of SEQ ID NO:84 and a beta chain variable region of amino acid sequence of SEQ ID NO:89.

26. The TCR of any of claims 23-25, wherein the TCR comprises an alpha chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:72 and a beta chain variable region other than the CDRs having at least 90% identity to the nucleotide sequence of SEQ ID NO:78.

27. The TCR of any of claims 23-26, wherein the TCR comprises an alpha chain variable region comprising the nucleotide sequence of SEQ ID NO:72 and a beta chain comprising the nucleotide sequence of SEQ ID NO:78.

28. The TCR of any of claims 23-27, wherein the TCR other than the CDRs has at least 90% identity to SEQ ID NO:82.

29. The TCR of any of claims 23-28, wherein the TCR comprises SEQ ID NO:82.

30. The TCR of any of claims 1-29, wherein the engineered TCR binds HLA-A*0101.

31. The TCR of any of claim 1-30, wherein the TCR is further defined as a soluble TCR, wherein the soluble TCR does not comprise a transmembrane domain.

32. The TCR of any one of claims 1-31, further comprising a detectable label.

33. The TCR of any one of claims 1-32, wherein the TCR is covalently bound to a therapeutic agent.

34. The TCR of claim 33, wherein the therapeutic agent is an immunotoxin or a chemotherapeutic agent.

35. A multivalent TCR complex comprising a plurality of TCRs according to any one of claims 1-34.

36. The complex of claim 35, wherein the multivalent TCR comprises 2, 3, 4 or more TCRs associated with one another.

37. The complex of claim 35 or 36, wherein the multivalent TCR is present in a lipid bilayer, in a liposome, or is attached to a nanoparticle.

38. The complex of any of claims 36-38, wherein the TCRs are associated with one another via a linker molecule.

39. A polypeptide encoding the TCR of any one of claims 1-38.

40. A polynucleotide encoding the polypeptide of claim 39.

41. An expression vector encoding the TCR of any one of claims 1-38.

42. The expression vector of claim 41, wherein the sequence encoding the TCR is under the control of a promoter.

43. The expression vector of claim 41 or 42, wherein the expression vector is a viral vector.

44. The expression vector of claim 43, wherein the viral vector is a retroviral vector.

45. The expression vector of any of claims 41-44, wherein the vector further encodes a linker domain.

46. The expression vector of claim 45, wherein the linker domain is positioned between the alpha chain and beta chain.

47. A host cell engineered to express the TCR of any one of claims 1-34.

48. The host cell of claim 47, wherein the cell is a T cell, NK cell, invariant NK cell, NKT cell, mesenchymal stem cell (MSC), or induced pluripotent stem (iPS) cell.

49. The host cell of claim 47 or 48, wherein the host cell is an immune cell.

50. The host cell of any of claims 47-49, wherein the host cell is isolated from an umbilical cord

51. The host cell of any of claims 48-50, wherein the T cell is a $CD8^+$ T cell, CD4+ T cell, or γδ T cell.

52. The host cell of any of claims 48-51, wherein the T cell is a regulatory T cell (Treg).

53. The host cell of any of claims 47-53, wherein the cell is autologous.

54. The host cell of any of claims 47-53, wherein the cell is allogeneic.

55. A method for engineering the host cell of claim 47 comprising contacting said immune cell with the TCR of any one of claims 1-34 or the expression vector of any one of claims 41-33.

56. The method of claim 55, wherein the immune cell is a T cell, or a peripheral blood lymphocyte.

57. The method of claim 55 or 56, wherein contacting is further defined as transfecting or transducing.

58. The method of any of claims 55-57, wherein transfecting comprises electroporating RNA encoding the TCR of any one of claims 1-34 into the immune cell.

59. The method of any of claims 55-58, further comprising generating viral supernatant from the expression vector of claim 41 prior to transducing the immune cell.

60. The method of any of claims 55-59, wherein the immune cell is a stimulated lymphocyte.

61. The method of claim 60, wherein the stimulated lymphocyte is a human lymphocyte.

62. The method of claim 60 or 61, wherein stimulating comprises contacting the immune cell with or incubating the immune cell in OKT3 and/or IL-2.

63. The method of any of claims 55-62, further comprising sorting the immune cells to isolate TCR engineered T cells.

64. The method of claim 63, further comprising performing T cell cloning by serial dilution.

65. The method of claim 63 or 64, further comprising expansion of the T cell clone by the rapid expansion protocol.

66. A method of treating cancer in a subject comprising administering an effective amount of the TCR-engineered cells of any of claims 47-53 to the subject.

67. The method of claim 66, wherein the subject is identified to have an HLA-A*0101 allele.

68. The method of claim 66 or 67, wherein the TCR-engineered cell is a T cell or peripheral blood lymphocyte.

69. The method of claim 66, wherein the T cell is a $CD8^+$ T cell, $CD4^+$ T cell, or Treg.

70. The method of any of claims 66-69, wherein the cancer is pancreatic cancer, ovarian cancer, gastric cancer, breast cancer, bladder cancer, uterine cancer, or cervical cancer.

71. The method of any of claims 66-70, wherein the subject is a human.

72. The method of any of claims 66-71, wherein the TCR engineered cells are autologous.

73. The method of any of claims 66-72, wherein the TCR engineered cells are allogeneic.

74. The method of any of claims 66-73, further comprising lymphodepletion of the subject prior to administration of the VGLL1-specific T cells.

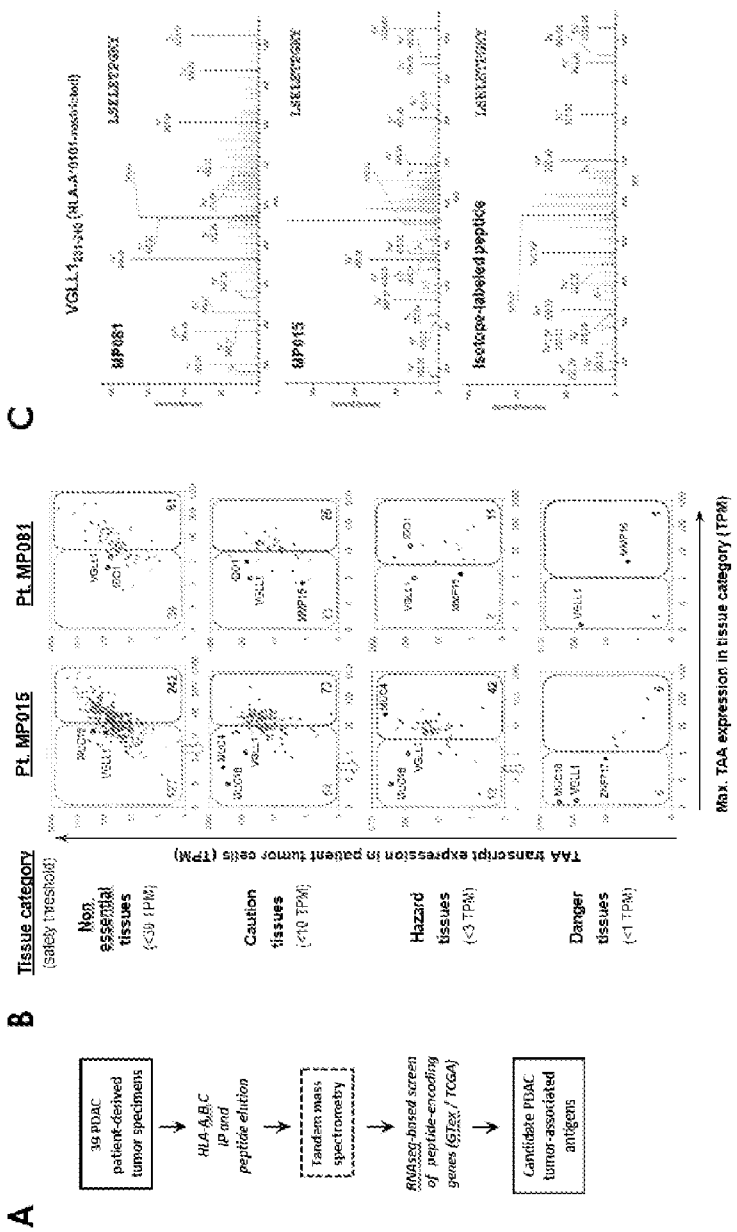
75. The method of claim 74, wherein lymphodepletion comprises administration of cyclophosphamide and/or fludarabine.

76. The method of any one of claims 66-75, further comprising administering a second anticancer therapy.

77. The method of claim 76, wherein the therapy is a chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy.

78. The method of any of claims 66-77, wherein the TCR-engineered 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.

79. The method of any of claims 66-78, wherein the subject is determined to have cancer cells which overexpress VGLL1.



FIGS. 1A-1C

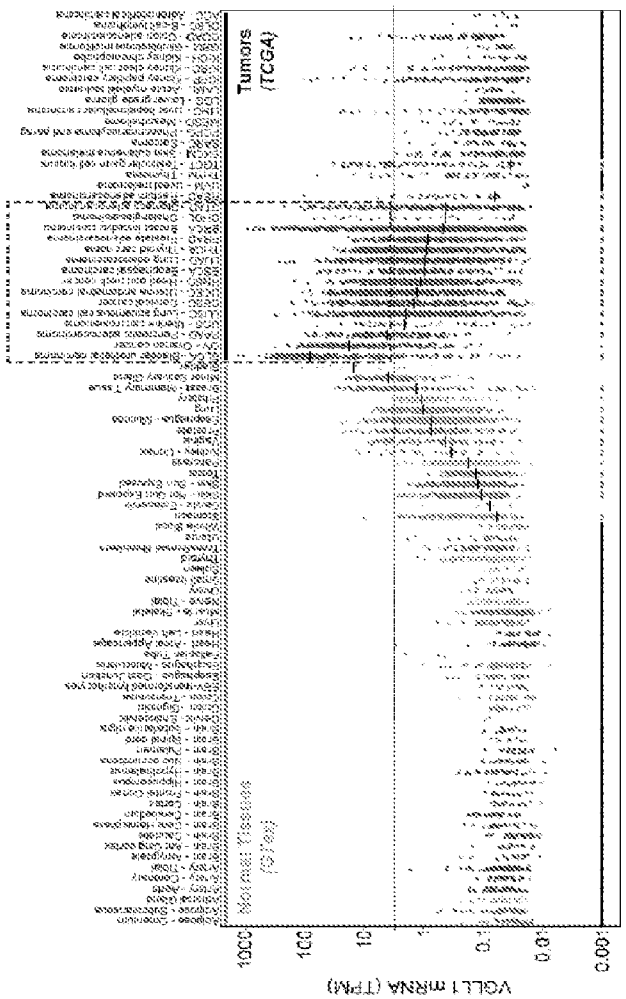


FIG. 2

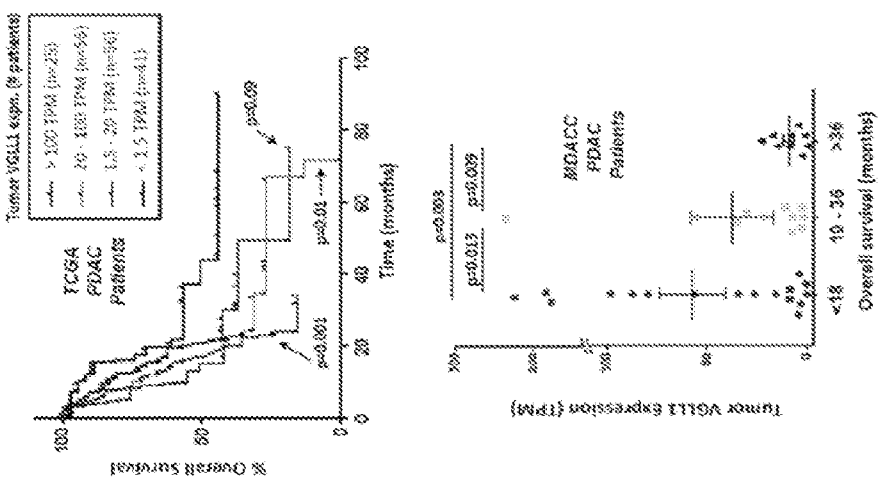
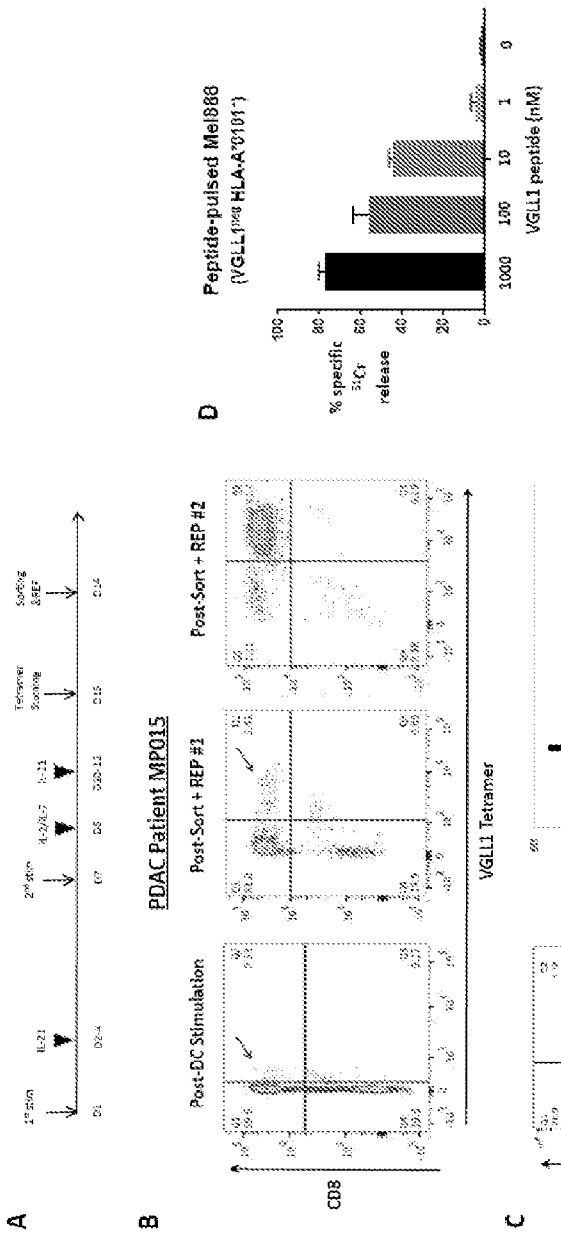
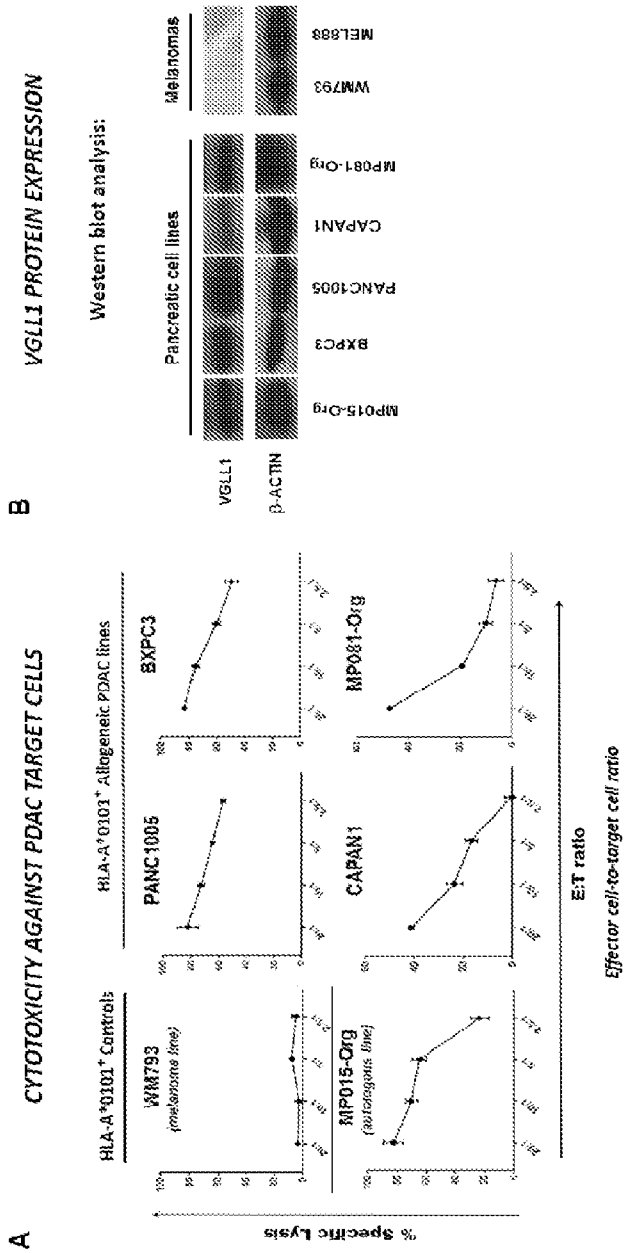


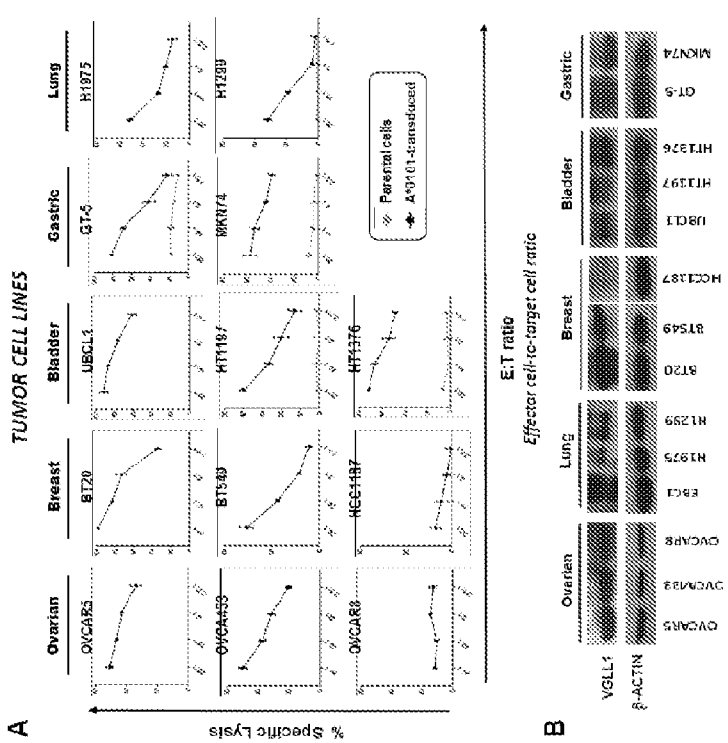
FIG. 3



FIGS. 4A-4D



FIGS. 5A-5B



FIGS. 6A-6D

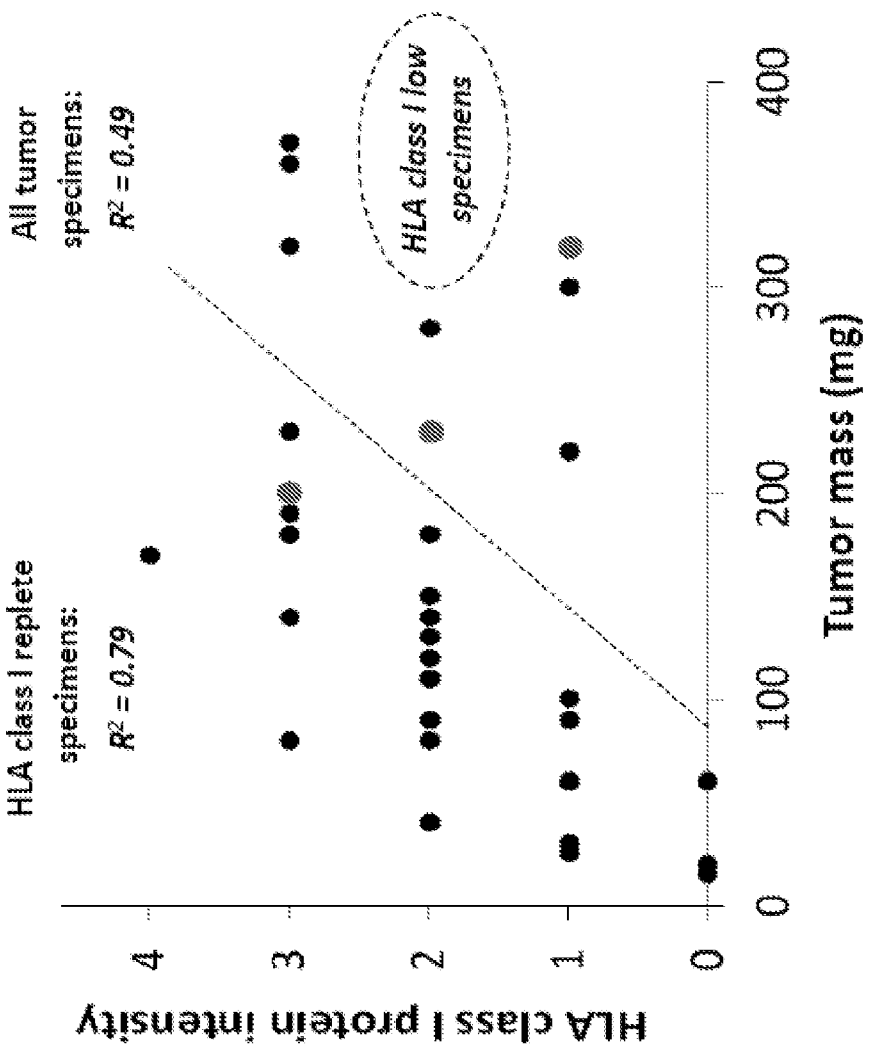


FIG. 7

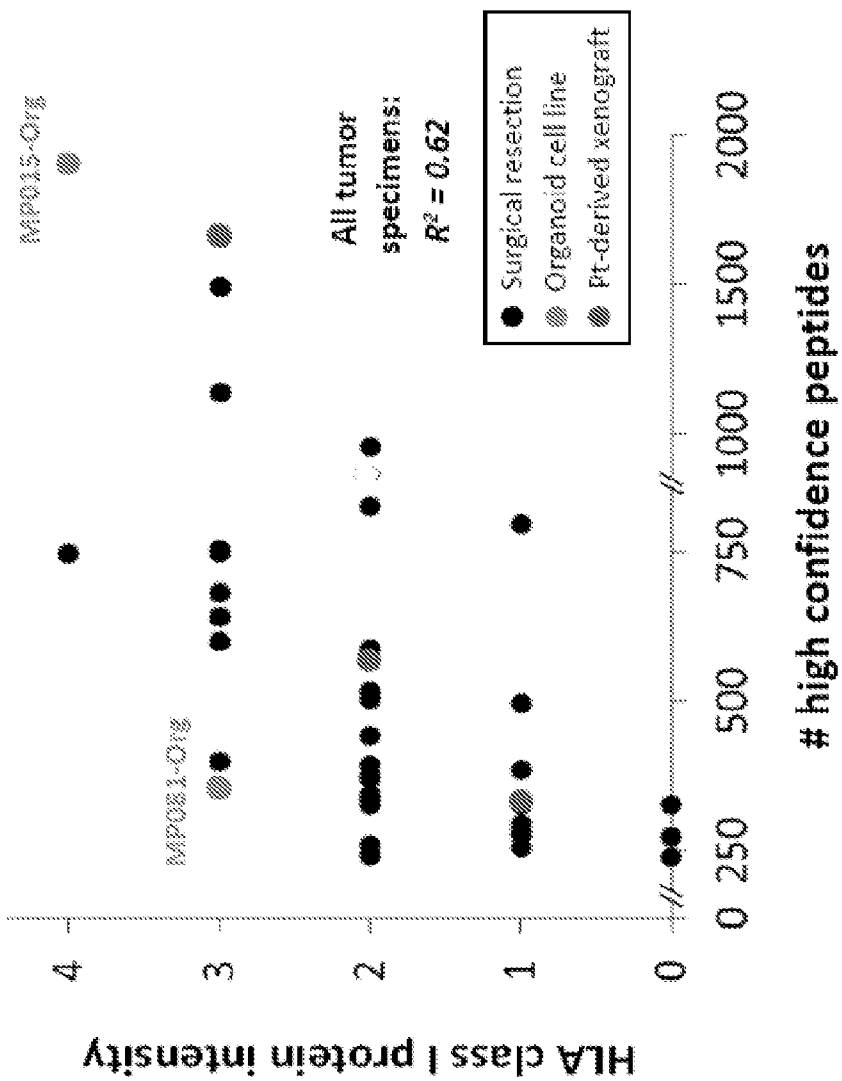


FIG. 8

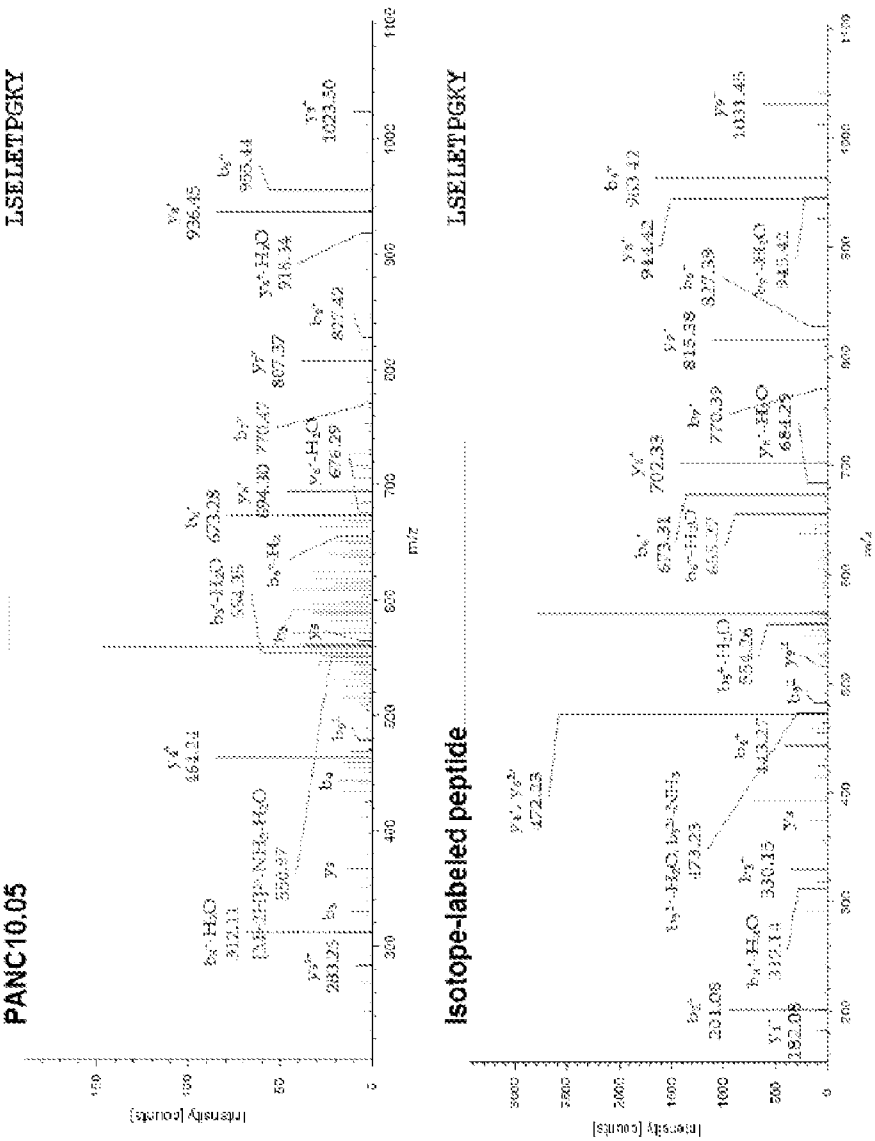
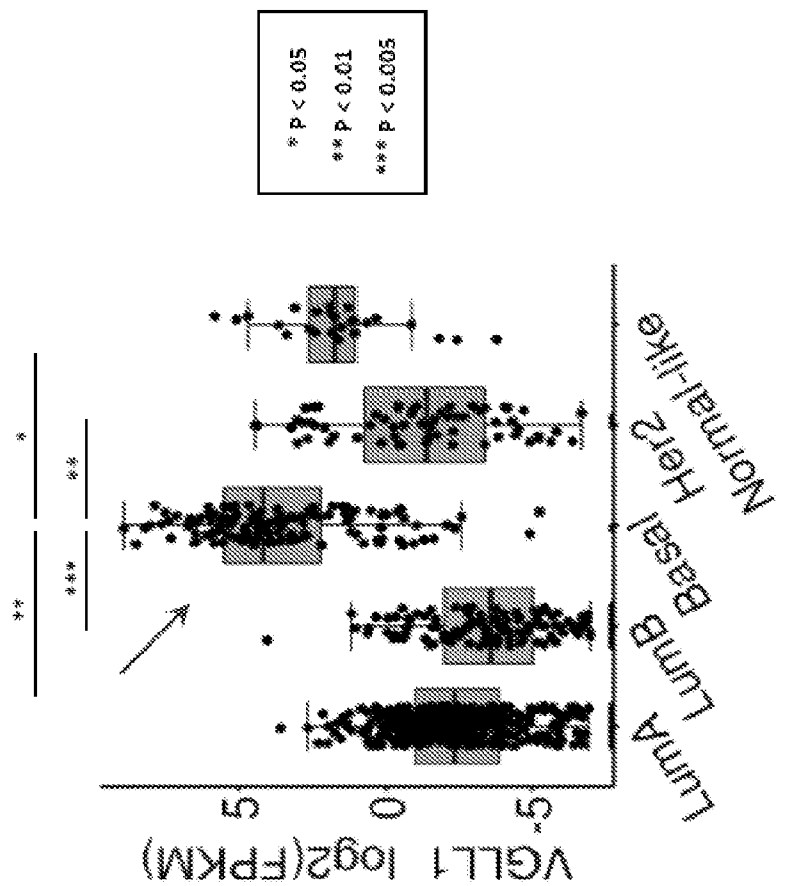


FIG. 9



The Cancer Genome Atlas (TCGA)

FIG. 10

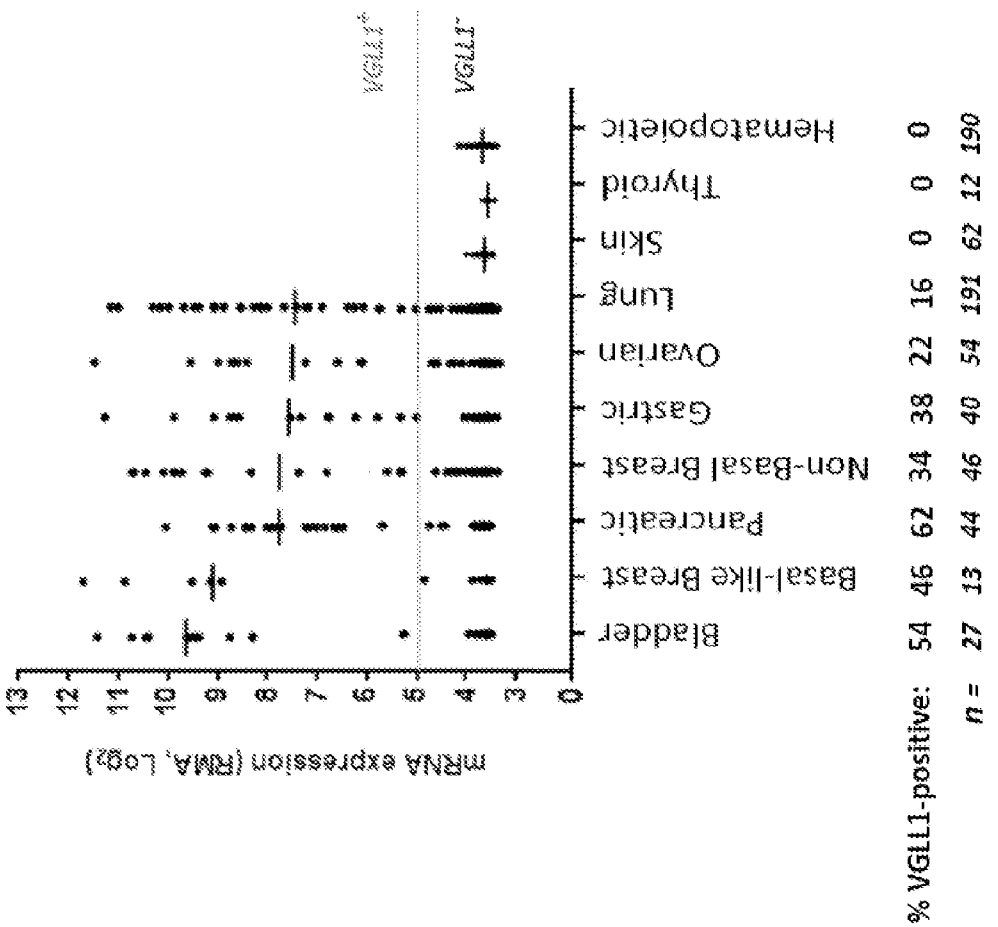
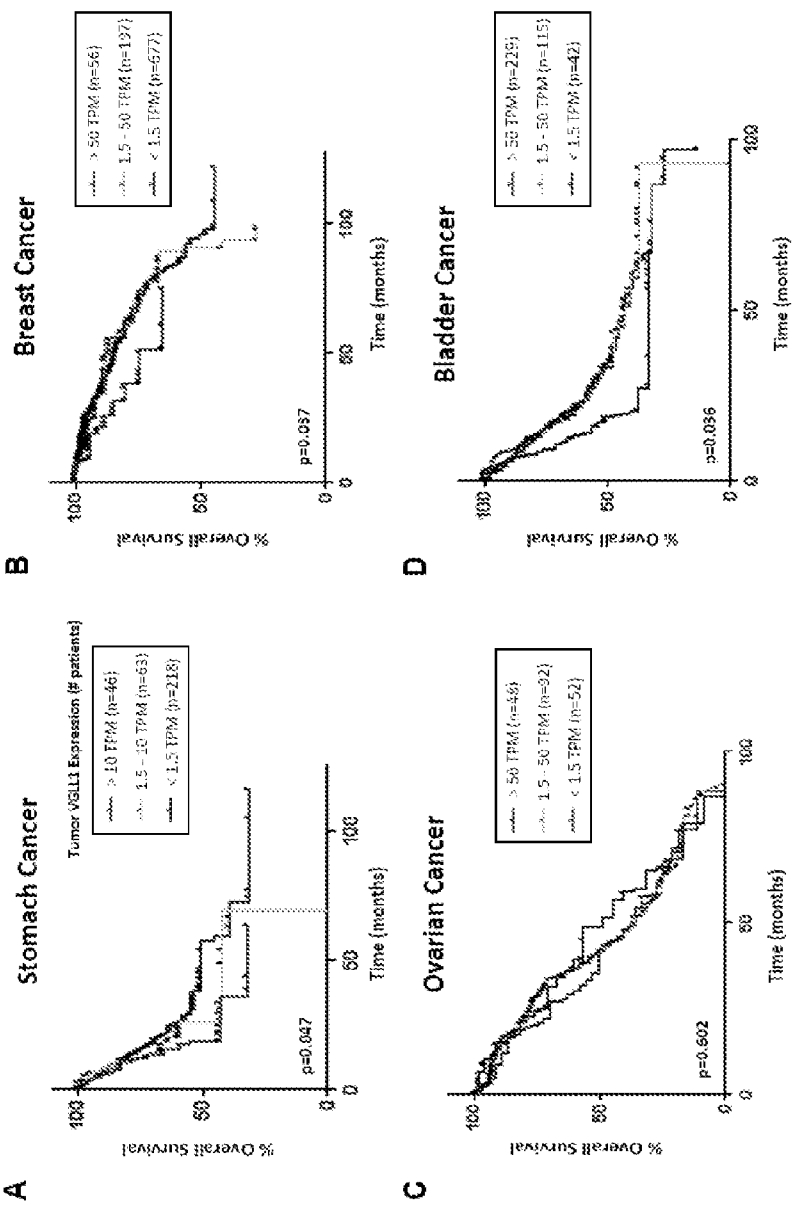
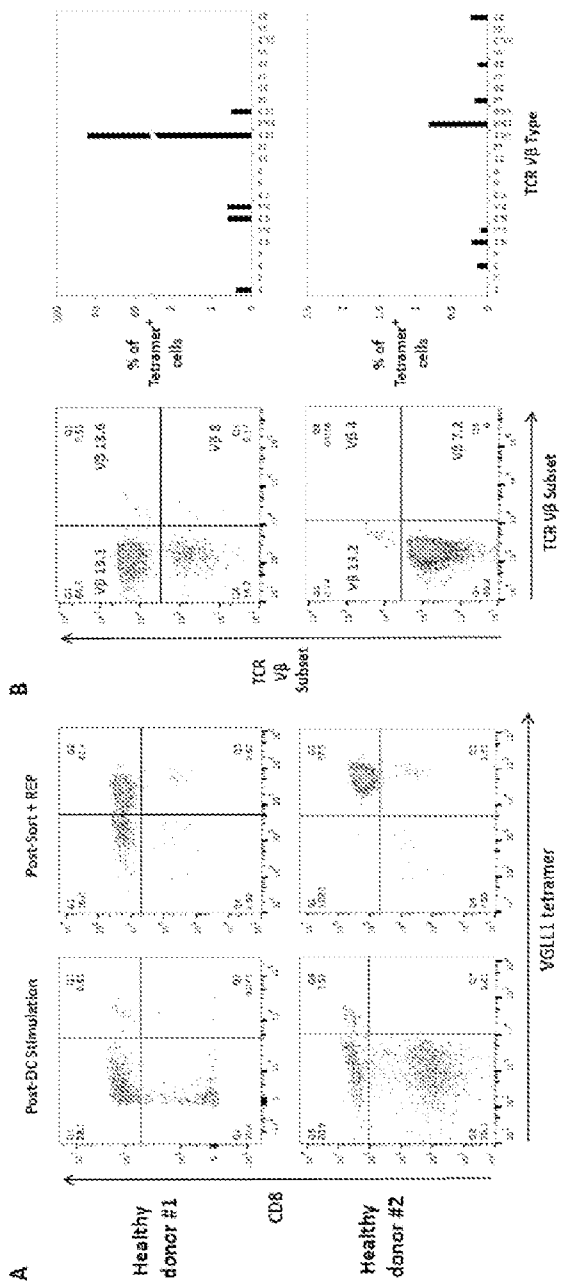


FIG. 11



FIGS. 12A-12D



FIGS. 13A-13B

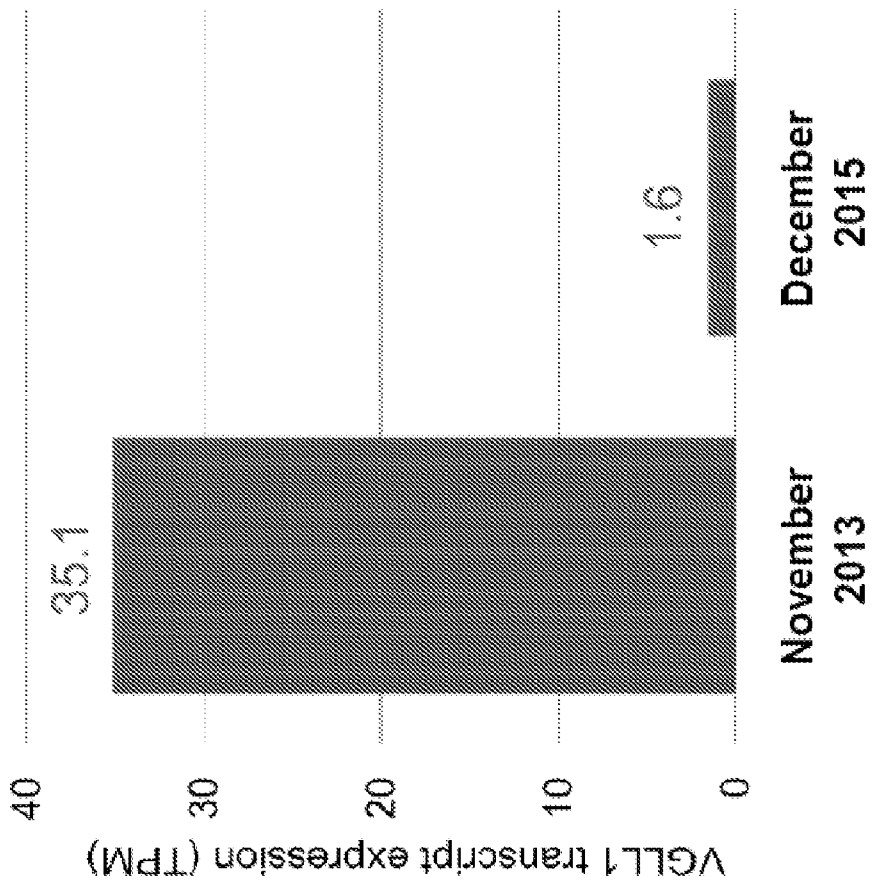


FIG. 14

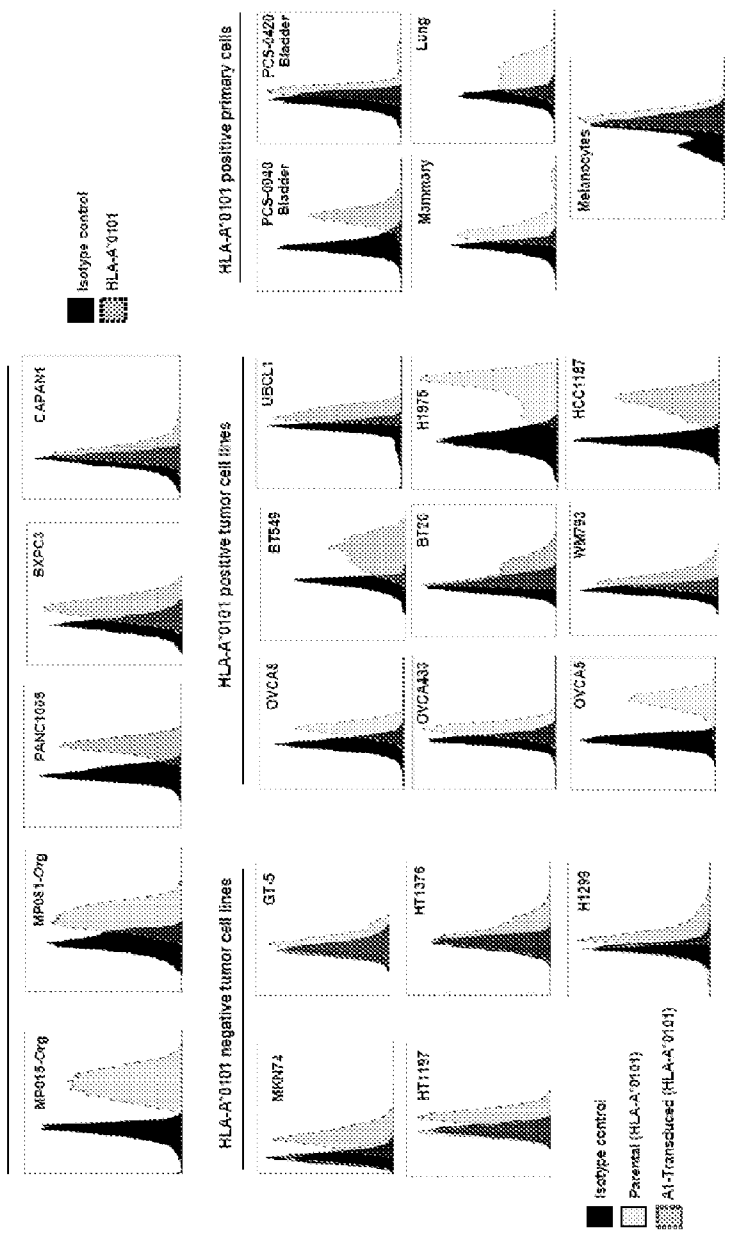


FIG. 15

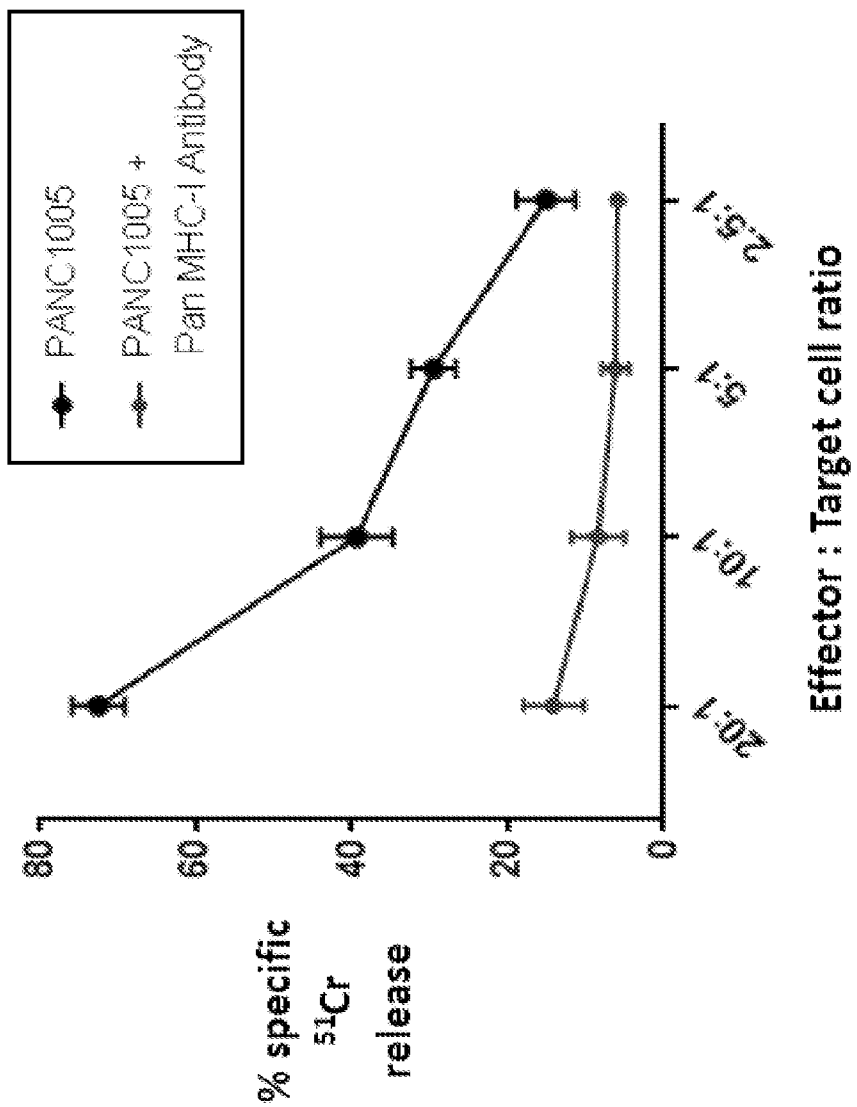


FIG. 16

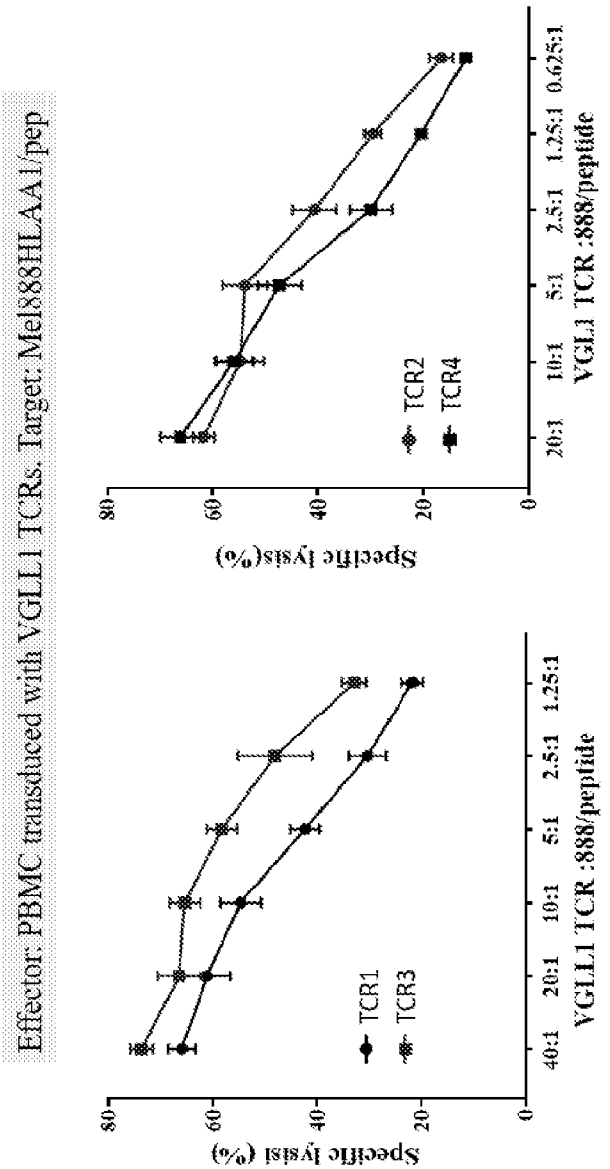


FIG. 17

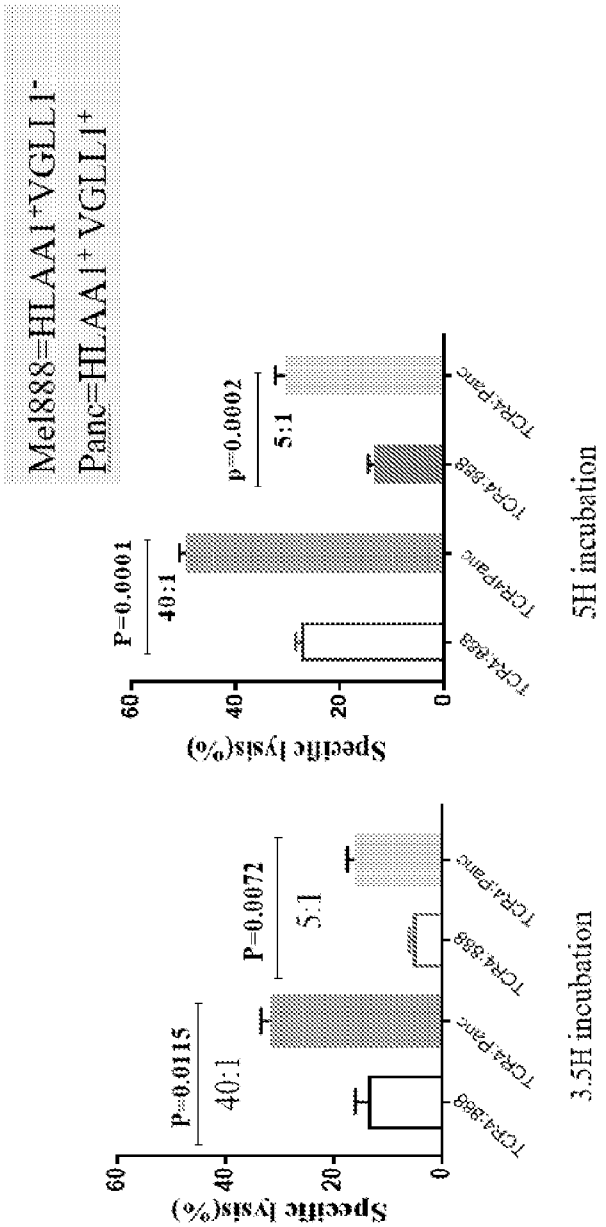


FIG. 18